年報 Annual Report 2010

近畿大学医学部消化器内科学教室

医局員集合写真

第5回　Kinki GUT Club　平成23年1月16日　於 帝国ホテル大阪

目　　次

2010 年 Annual Report の発刊にあたって

近畿大学消化器内科学教室主任教授　工藤正俊

1.はじめに

2010 年からは消化器内科の病床数は 85 床に増床となりました。少し発刊が遅れましたが 2010 年の教育、研究、診療の実績をお届けします。近畿大学医学部に消化器内科学教室が新設されたのは平成 11 年 4 月であります。従って平成 23 年 3 月でやっと丸 12 年が経過したことになります。開設当初は医局のスペースも 2 部屋のみでスタッフ 8 名、研修医 6 名、計 14 名での出発でありました。現在では狭山の本院に籍を置くスタッフは約 40 名、また堺病院や奈良病院もそれなりに人材・設備共に整いつつあります。

しかしながら、新設私立医大の宿命かもしれませんが、2004 年より始まった新臨床研修医制度の余波をまともに受けて消化器内科も毎年 5～6 人から 12～13 人入局していた入局者数が最近では激減し、また次第に開業あるいは結婚退職、郷里への U ターンなどの退職者も増え、現在は大変厳しい状況に置かれているというのが現状です。ただし、ここ 2-3 年は 3-4 人の入局がコンスタントに続いており、良い傾向が見え始めました。今後の更なる消化器内科学教室の発展のために医局員一同が一致団結して診療、研究、教育活動に専念していかなければならない重要な時期であると考えております。

2.診療活動

別添えの資料をご覧頂ければ一目瞭然でありますが、消化器内科の年間の入院及び外来収入、及びそれを合計した総収入は平成 11 年の開設初年度は約 8 億程度でありましたが、平成 22 年には 29 億 84 万円を超える収入となっており、病院経営にも多大の貢献をしております。平成 22 年度は病院全体も約 12 億の黒字決算となりましたが、消化器内科の貢献も大きいと考えております。また一日平均入院患者数も年間を通して 80 人前後、平均在院日数も 9 日を切っており極めて多忙な診療活動を行っていることがおわかり頂けると思います。腹部超音波検査の件数も確実に右肩上がりであり、内視鏡の件数も総件数が平成 22 年度は 20,125 例と着実に上昇を示しております。また、肝癌に対するラジオ波治療（RFA）の総件数も平成 22 年度は 239 例であり、日経新聞や朝日新

聞、読売新聞、週刊朝日等にも度々取り上げられ、総件数としては連続 6 年以上、日本国内の 2 位（内科と外科の件数、及び転移性肝癌を含めて）に位置づけられるという実績を残しております（600 ページ参照)。ラジオ波は平成 11 年 6 月より開始し、平成 22 年 12 月末の時点で総件数 3,500 例に達しており、5 年生存率は 70%強であり、手術とほぼ同等の治療成績が得られております。インターフェロン治療の実績でも PEG インターフェロン治療の実績は全国ランキング 5 位以内でした（2 社の合計分)。現在、C 型肝炎治療を積極的に行っており、大阪南部から C 型肝炎・肝癌を根絶したいと願っています。

平成 15 年度に導入した早期胃癌に対する内視鏡的粘膜下層切開剥離術（ESD）も確実に症例数が増え、平成 22 年は 109 症例に対し ESD を行っており今後益々増えていくものと考えております。もちろん、ESD 関連の研究論文も少しずつ増えていっております。平成 23 年度には内視鏡室が光学治療センターに格上げとなり、スペースの拡充も予定されております。さらには腹部超音波室も拡充が決定され、平成 23 年 10 月 3 日から稼働予定となっております。これらのことは病床数の増加とともに消化器内科にとっては今後ますます発展する素地ができあがりつつあると考えます。

御承知のように大和川以南は一般に「南大阪」と呼ばれておりますが、その南大阪の人口は約 260 万にも達しております。その 260 万人の医療圏の中で大学医学部は近畿大学のみであります。その意味でこの 260 万人の方々の健康を守り病からの回復の重責を我々こそが担っているのだという自負と自覚をもってこれからも良い医療を行っていきたいと考えております。

3.教育活動

教育は当然のことながら大学医学部の役割の極めて根幹を占める重要な部分であります。消化器内科学は消化器コースの内の肝臓の責任科であり、肝臓のユニットを 1 週間担当している他、上部消化管、下部消化管、胆膵のユニットや臨床腫瘍コースならびに画像診断のコースでも講義を担当しております。更には病因・病態のコースの 3 週間のうち 1 週間の責任科として大変多忙な教育活動を行っております。5 年生 6 年生のクリニカルクラークシップも例年 6 年生を常時 6 人程度受け入れており、講義や総括など充実した bed side 教育となるよう全力を尽くしております。

平成 20 年 10 月から（私の意志ではありませんでしたが）病院長に任ぜ

られましたのでその公務のために教育活動の多くの部分を北野准教授、松井講師はじめ多くの講師の先生方にご負担をおかけすることになってしまい、申し訳なく思っております。消化器コース及び病因・病態コースあるいは日々のクリニカルクラークシップ等の教育活動では決して手を抜かず積極的に行っていくつもりですので何卒ご容赦下さい。この紙面をお借りして感謝とお詫びを申し上げたいと思います。

3. 研究活動

(1) 論文業績

英文論文の発表は 1999 年消化器内科の設立当初は一桁台でありましたが、年と共に確実に増加し、3 年目からは平均 20 編以上の英文論文がコンスタントに出るようになりました。2010 年の英文論文数は 50 編に達しました。是非 2011 年も 50 編の大台を超える論文を書いて頂きたいと思います。12 年間の総インパクトファクターは 807.869 点であり英文総論文数は 285 編ですので、近畿大学消化器内科のような小さな所帯の教室としてはまずまずの結果を残せているのではないかと思っております。来年以降も最低、英文原著論文は 50 編以上を目標に頑張っていきたいと考えておりますので教室員の皆様の自覚と更なる奮闘を期待致しております。

(2) 厚生労働省科学研究費補助金事業研究班の活動

平成 22 年度に採択された厚労科研（がん臨床部門）「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究」（工藤班）の主任研究者として日本発のエビデンスを創出すべく、努力しています。またその他にも下記の厚労科研の分担研究者としてそれぞれの先生方に実務を担当して頂いております。この場をお借りして感謝申し上げます。

① 「血小板低値例へのインターフェロン治療法の確立を目指した基礎および臨床的研究」（西口班）
② 「抗悪性腫瘍薬による肝炎ウイルス再活性化の調査とその対応に関する研究」（池田班）
③ 「初発肝細胞癌に対する肝切除とラジオ波焼灼両方の有効性に関する多施設共同研究」（國土班）

④　「肝がんの新規治療法に関する研究」（本多班）
⑤　「多発肝のう胞症に対する治療ガイドライン作成と試料バンクの構築」（大河内班）
⑥　「進行肝胆膵がんの治療法の開発に関する研究」（奥坂班）
⑦　「C 型肝炎ウイルス（ＨＣＶ）陽性進行肝臓がん症例に対するテーラーメイドがんペプチドワクチンの第Ⅱ相単盲検無作為割付群間比較臨床試験」

（3）今後の研究の方向性

今年の消化器内科の論文も一覧するとやはりまだまだ Impact factor の高い雑誌に掲載されているのは少ないようです。やはり Impact factor 15 点以上の雑誌を目指すには prospective な比較試験など中・長期的な視野に立った研究計画を組んで質の高い臨床研究を進めて行くことが現時点での我々に課せられた最も大きな課題と考えております。臨床試験については 2008 年 9 月 11 日に大阪府より認証を受けた NPO 法人「日本肝がん臨床研究機構（JLOG）」を中心に現在 7 つの prospective study が走っております。そのうち 3 つが厚労科研に採択されたため現在では 4 つの前向き試験を行っております。これからも世界へ向けて発信できるような成果を出して行くつもりでおります。もちろん、retrospective な解析研究で新しいデータを publish していくという努力も今後も続けていかなければなりません。

もう一つの重要な点は私が常日頃申し上げておりますように症例観察の重要性であります。臨床においては一例一例がたとえ同じ病名であったとしても一例として同じ症例はありません。同じ病気でも一つとして全く同一であるということはなく、何か異なるメッセージを発信しているのです。そのことを的確にキャッチすることにこそ意味があるという目で一例一例の患者さんを注意深く診療し観察していくことこそが最も大事であると考えています。そのような注意深い観察から新しい臨床的な発見も生まれてきますし、また逆にそのような観察眼が生まれる素地としては臨床家として真面目に臨床と向き合って最高の level に到達している必要があります。そのような点で日々の臨床の現場には”clinical pearl”とでも言うべきものがあちこちに転がっている、まさに宝の山であります。そのような理由で症例観察に基づいたケースレポートを書くということも極めて、その本人の勉強になることはもちろんのこと、今後の新しい疾患概念の確立、新しい治療法の着想などに結びつき得る重要な姿勢である

と思われます。残念ながら、ケースレポートは最近の Impact factor 重視主義の多くの Journal から採用されない傾向にはありますが、それでも short report や Letter to the Editor などとしては採用されますので業績をあげるという目的ではなく、症例をキチンと観察・整理して document していくという姿勢に立つことは重要であります。すなわち症例の観察研究を報告することは我々、アカデミアに籍を置く者に課せられた使命であると自覚すべきと考えております。

ここまで読まれた方は最初に私が述べたような大規模な前向きな比較試験を行うべきということと症例の観察研究とでは全く正反対の次元の違うことを述べているように思われるかもしれません。しかしこの 2 つは臨床を知り尽くし、かつ、臨床をじっくり真面目にやっている医師にしかできないことであるという点で共通していることであります。基礎研究あるいは臨床に結びつくかもしれない基礎研究までは MD ではなくとも PhD でも実行可能なことであり、その field ではしばしば PhD の方が quality の高い研究成果を上げ得るかも知れません。しかしながら、臨床の疑問点にもとづいた基礎研究もしくは本当に臨床に直結するような基礎研究や症例の観察研究、および大規模臨床試験などはその価値を知り得る MD にしかできないことであることは間違いありませんし、それらを遂行し得るのは患者さんと日々正面から向き合っている最高水準の医師にしかできない研究であります。そのような点でこの二つは決して矛盾するものではありませんし、両方ともに臨床家こそがやるべき研究であります。

以上、述べた 2 つの異なったアプローチは、我々の教室の研究の方向性として今後も積極的に実行して行きたいと思っております。繰り返しになりますが、臨床的な発想に基づく、あるいは臨床に本当に必要な基礎的データを抑えるという研究は、大変重要ですのでそれらは引き続き継続していかなくてはなりません。

2009 年に立ち上げた日本肝がん分子標的治療研究会（第 1 回研究会: 2010 年 1 月 16 日）でも 450 人を越える参加者がありました。肝癌はこれからは分子標的治療の時代ですので永井先生をはじめ、2 名の大学院生が分子標的の研究でゲノム生物学教室で頑張ってくれているは大変私にとって嬉しい限りです。特許も出版することが出来ましたし、Impact Factor が 7 台の雑誌にも 2-3 編通りました。臨床的ニーズに基づいた基礎研究で成果を上げることほどエキサイティングなことはありません。是非とも近畿大学から肝がんに関して臨床に貢献できる基礎的エビデンスを次々と発信して行きたいと心から願っています。

(3) Research Conference

現在消化器内科では定期の各グループの臨床カンファレンスに加え、毎週火曜日の早朝の 1 時間みっちりと Research Conference を行っております。このカンファレンスでは全て英語で Presentation から Discussion までを行っております。ほとんど 1 年を通じて海外からの留学生がおりますし、特筆すべき点としてこれまではアジアの留学生が中心でしたが昨年はイタリア人の Dr. Lorenzo が apply してできたことです。これも日本における肝細胞癌研究の leading center としてヨーロッパの国からも認知され始めている証拠であると思いますので大変喜ばしいと思っております。平成 23 年には世界で最も古い歴史のあるイタリアボローニャ大学の Prof. Bolondi の教室から一名留学生（Dr. Alberto）がやってきます。また今年はエジプトから Alshimaa 先生が 5 か月間来られました。そのような留学生にも配慮して Research Conference は英語で行っておりますが、やはりこの English Research Conference というのが消化器内科が行っているカンファレンスの中でも最も重要であると考えております。もちろん、このカンファレンスへの出席は本人の自発的意欲に基づくものではありますが、毎週多くの教室員に参加して頂いております。以下にこの数年の出席率を示しますが、出席率の高い医局員ほどやはり研究に対する activity が高い傾向にあると感じておりますので今後も引き続き積極的に参加して頂きたいと思っております。

副次的な効果としてこのカンファレンスを通じて海外で英語で Discussion できる英語力や自信も自然と磨かれるものと確信しております。

English Reseach Conference 出席状況

教室員	2008		2009		2010	
	出席回数	出席率	出席回数	出席率	出席回数	出席率
工藤	27/27	100	20/20	100	29/29	100
樫田	-	-	-	-	12/19	63
北野	25/27	93	16/20	80	21/29	72
松井	22/27	81	18/20	90	23/29	79
上嶋	6/27	22	3/20	15	12/29	41
鄭	23/27	85	15/19	79	26/29	90

櫻井	-	-	-	-	17/19	89
南	19/24	79	1/2	50	-	-
石川	13/27	48	11/19	58	1/10	10
末富	5/27	19	2/20	10	0/1	0
梅原	19/27	70	12/20	60	4/8	50
坂本	19/27	70	1/2	50	12/19	63
井上	17/25	68	16/20	80	25/29	86
野田	27/27	100	4/5	80	-	-
萩原	11/24	46	5/20	25	9/29	31
矢田	7/11	64	14/19	74	26/29	90
畑中	9/27	33	1/20	5	1/17	6
高橋	22/27	81	1/5	20	-	-
北井	15/27	56	8/20	40	15/29	52
朝隈	16/27	59	12/20	60	11/29	38
峯	-	-	9/15	60	17/29	59
岡田	19/27	70	2/20	10	2/10	20
辰巳	12/27	44	3/20	15	3/10	30
小牧	18/27	67	13/20	65	20/29	69
永井	18/19	95	17/20	85	14/29	48
上田	15/27	56	6/20	30	11/29	38
川崎	23/27	85	6/20	30	4/29	14
早石	10/19	53	9/19	47	5/29	17
田北	1/2	50	13/20	65	15/29	52
有住	-	-	7/15	47	15/29	52
鎌田	-	-	11/15	73	15/29	52
高山	-	-	4/7	57	-	-
宮田	-	-	9/15	60	16/29	55
今井	-	-	9/15	60	18/29	62
永田	-	-	13/15	87	16/29	55

4.学会活動および海外における活動

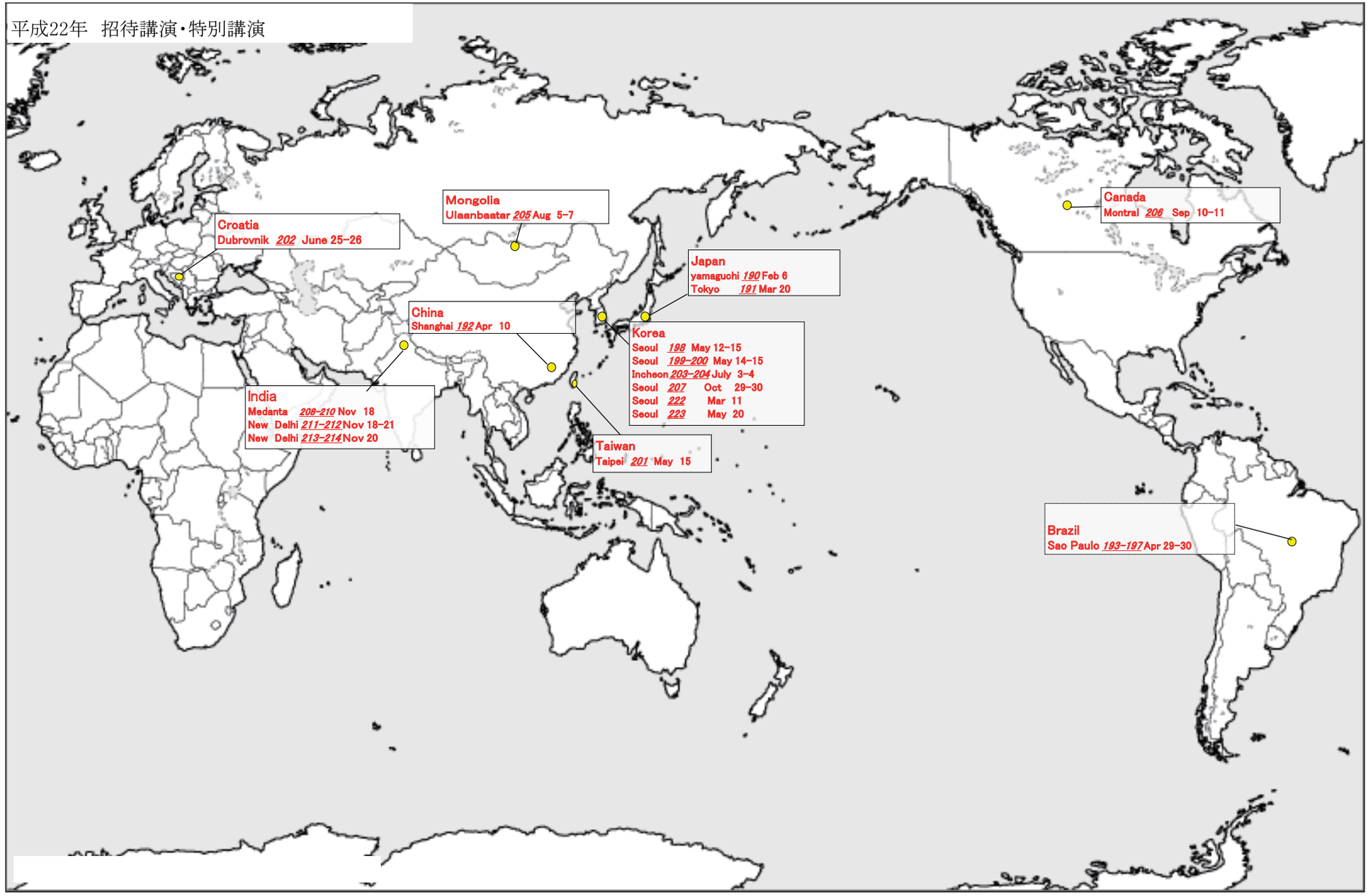
2010 年における国内の学会発表については 96 演題、国際学会の発表については 35 演題、海外特別講演は 44、国内特別講演は 92 と例年よりも増えつつあります。私自身の海外出張は 2010 年は 18 回とこれもやや例年より多くなっております。

2010 年

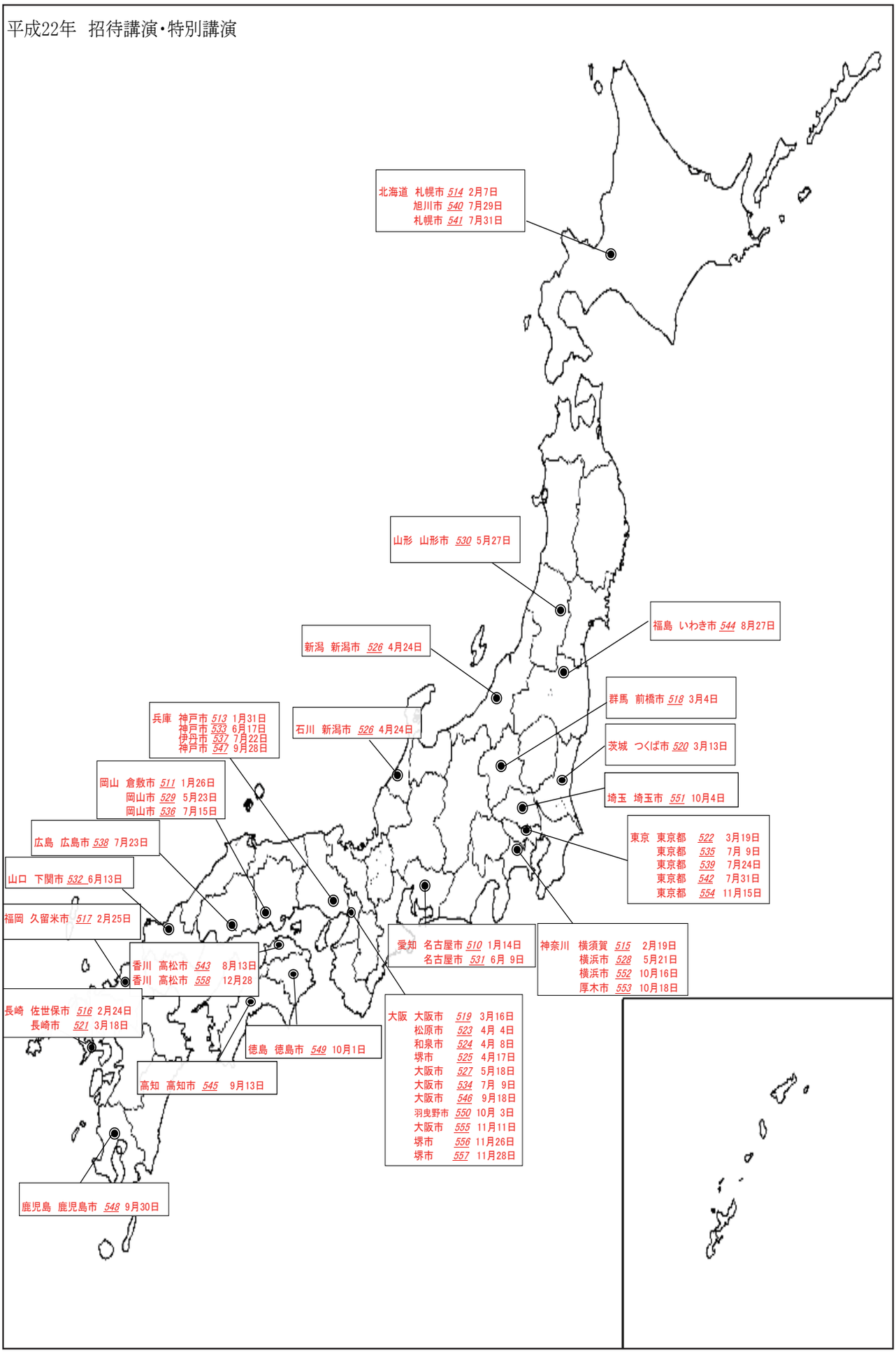
1. 1 月 21 日-25 日　ASCO-GI, Post TACE study の発表
   (Orlando, Florida, USA)
2. 1 月 28 日-30 日　Regorafenib Advisary Board Meeting
   (Berlin, Germany)
3. 3 月 28 日-29 日　International Liver Cancer Study Steering Committee
   (Beijing, China)
4. 4 月 9 日-11 日　ISR-WFUMB Joint Meeting, Special lecture
   (Shanghai, China)
5. 4 月 28 日-5 月 5 日　JPR（ブラジル放射線医学会）Invited Lecture
   サンパウロ大学で 2 つの Lecture
   (Sao Paulo, Brazil)
6. 5 月 12 日－13 日　Laennec Liver Pathology Meeting
   (Seoul, Korea)
7. 5 月 14 日-15 日　Korean Society of Ultrasound in Medicine (KSUM) Invited Lecture
   Jisan Lectureship
   (Seoul, Korea)
8. 6 月 3 日-8 日　ASCO, GIDEON Steering committee meeting
   (Chicago, USA
9. 6 月 24 日-28 日　EASL-AASLD-APASL Joint HCC meeting
   Invited Lecture
   (Dubrovnik, Croatia)
10. 7 月 3 日-4 日　1st APPLE Meeting
    Invited lecture, Chair, Co-chairman of Meeting
    (Seoul, Korea)

11. 8 月 4 日-6 日	AFSUMB Workshop, 3 invited lectures (Mongol)
12. 9 月 2 日-6 日	WFUMB Council Meeting President-elect として参加 (Paris, France)
13. 9 月 9 日-14 日	International Liver Cancer Association (ILCA) ① 1 Lecture ② 1 Lunche on Seminar ③ 1 Session Chair (Hong Kong, China)
14. 10 月 29 日-30 日	International MRI Forum (Seoul, Korea)
15. 10 月 31 日-11 月 4 日	AASLD (Boston, USA)
16. 11 月 18 日-23 日	AFSUMB India（アジア超音波医学会） ① 3 Lectures ② 2 Session Chair ③ Administrative council meeting ④ General Assembly ⑤ New administrative council meeting (New Delhi, India)
17. 12 月 1 日-2 日	Consensus conference on Liver Transplantation (Geneva, Switzerland)
18. 12 月 3 日-4 日	WFUMB CEUS guideline meeting (Chicago, USA)

平成22年　招待講演・特別講演



平成22年　招待講演・特別講演



5. 学会主催

今年の学会主催はとしては 5 月 29 日－31 日に「第 83 回日本超音波医学会学術集会」（演題数: 667 題、参加数: 3,426 名）を開催させて頂きました。11 月 14 日には第 44 回日本消化器病学会近畿支部市民公開講座を開催させて頂きました。

6. 留学生受け入れ

留学生の受け入れですが、1999 年から 2000 年にかけて中国上海から Ding Hong 先生（丁　紅）（上海医科大学）、2001 年には中国広州から Wen YL 先生（文　艶玲）（中山医科大学）、2002 年には中国広州から Zheng RQ 先生（鄭栄琴）（中山医科大学）、2003 年には中国重慶より Zhou Pei（周　佩）（人民解放軍重慶病院）、2004 年にはカンボジアより Ly Sokhey 先生、2005 年にはタイから Worawan Chinamnan 先生、同じく 2005 年に若干時期を違えてインドから Kaushal Madan 先生（All India Institute of Medical Science: AIIMS）、2007 年 Kunal Das 先生を受け入れました。2008 年 Yu Xia（北京、中国）、2009 年 Md. Nadiruzzaman（バングラディシュ）、2010 年 Lorenzo Andreana（イタリア）が来ていました。またエジプトから Alshimaa 先生も来られました。2011 年にはマレーシアから Hadzri 先生が来られる予定です。またイタリア　ボローニャ大学からも留学生を受け入れる予定です。このように毎年のように留学生が日中友好協会、笹川財団や日本消化器病学会、日本超音波医学会のフェローシップ留学生あるいは自国での fund をもって私どもの教室を希望して頂き、受け入れてきました。また来年度以降も先生方にはご迷惑をお掛けするかと思いますが、これも国際交流、アジアや世界への日本の貢献、各々の英語力に磨きをかけるという意味で有益と思いますので何卒御理解・御協力のほどお願い申し上げます。

7. 人事について

冒頭でも述べましたが、2003 年までの入局者は毎年 5、6 名～12、13 名と大学内でも最も多くの入局者がおりましたが、2004 年に新臨床研修医制度が開始されてからの入局者、すなわち 2006 年の入局者は 2 名に留まり、2007 年

の入局者も 1 名に留まりました。2008 年には 8 名もの入局者が入って来られました。2010 年には 3 名の新入局者とともに 4 月 1 日には樫田博史先生に臨床教授として来て頂きました。反面、2-3 人の方が医局を離れました。従いまして依然、医局としての体制は大変厳しい状況にあります。このような状況の中で南大阪では大阪大学や大阪市大、和医大、奈良医大などがそれぞれの大学に人を引き上げているという状況のため、消化器内科医が激減し、南大阪の多くの公的病院では消化器内科医がほとんどゼロの状態が続いております。そのあおりで近医からの紹介患者や外来患者数は激増し、消化器内科の診療にも大きな負担がかかっております。しばらくはこのような状況が続くものと思われるますので、本学ならびに分院の奈良病院、堺病院ともに結束して一人でも多くの人に入局して頂き、教育・研究・診療を円滑に行っていきたいと考えております。

8. NPO 法人「日本肝がん臨床研究機構（Japan Liver Oncology Group）」の活動

1. **JLOG 0801 trial**「肝癌早期診断のための多施設共同無作為化比較試験（Sonazoid-Enhanced LivEr Cancer Trial for Early Detection（SELECTED Study））」
2. **JLOG 0901 trial** 「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究（Randomized Controlled Trial Comparing Efficacy of Sorafenib versus Sorafenib In combination with Low dose cisplatin/fluorouracil hepatic arterial InfUSion chemotherapy in Patients with Advanced Hepatocellular Carcinoma And Explolatory Study of Biomarker Predicting Its Efficacy (SILIUS Phase III trial)）」

→**2010 年より厚労科研に移行**（厚生労働省科学研究費補助金　厚生労働省科学研究費補助金事業研究班（がん臨床部門）平成 23 年度「進行・再発肝細胞癌に対する動注化学療法と分子標的薬併用による新規治療法の確立を目指した臨床試験（Phase III）ならびに効果を予測する biomarker の探索研究」（工藤班））

3. **JLOG 0902 trial**「早期肝癌診断における EOB-MRI の有用性に関する多施設共同研究（Diagnosis of Early LIver Cancer Through EOB-MRI(DELICATE

Study))」

4. **JLOG 1001 trial** 「切除不能肝細胞癌に対する肝動脈化学塞栓療法（TACE）とソラフェニブの併用療法第 II 相臨床試験（Phase II study: Transcatheter Arterial Chemoembolization Therapy In Combination with Sorafenib (TACTICS Study))」
5. **JLOG 1002 trial**「慢性肝疾患における非侵襲的弾性検査法を用いた肝線維化評価予測に関する研究(Assessment of Liver FIBROsis by Real-time Tissue ELASTography in Chronic Liver Disease (FIBROELAST Study))」

→**2011 年より厚労科研に移行**（厚生労働省科学研究費補助金事業研究班（難病・がん等の疾患分野の医療の実用化部門）平成 23 年度「慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立」（工藤班））

6. **JLOG 1003 trial**「非侵襲的弾性検査法を用いた肝線維化度評価によるウイルス性肝炎患者における肝発癌・門脈圧亢進症の発現予測（Prediction of Incidence of Liver Cancer or porTal Hypertension in Patients with Viral Hepatitis by Use of Real-time Tissue Elastography (PICTURE Study))」

→**2011 年より厚労科研に移行**（厚生労働省科学研究費補助金事業研究班（難病・がん等の疾患分野の医療の実用化部門）平成 23 年度「慢性ウイルス性肝疾患の非侵襲的線化評価法の開発と臨床的有用性の確立」（工藤班））

7. **JLOG 1004 trial**「インスリン抵抗性を合併する C 型代償性肝硬変患者を対象とした BCAA 顆粒製剤の肝細胞癌抑制効果に関する第 III 相臨床試験（BCAA Granule for patients with Hepatitis C-related Liver Cirrhosis and Insulin Resistance On the Effect of Reduction of Carcinogenic RisK in the Liver(Phase III study)(BLOCK Study))」

9. おわりに

この年報を作成にあたりましては例年の如く、教授秘書、医局秘書の秘書連合軍の 8 名の皆様に全面的に編集をして頂き大変感謝を致しております。また、医局員の皆様にも大変この一年お世話になりました。この二年間は大変なハードワークではありましたが、無事皆様の頑張りにより乗り切ることができました。この場をお借りして深く感謝申し上げます。2010 年には病床数も 76 床から 85 床への増床、念願の一病棟まるまる消化器内科が占めるという状態が実現しましたし、内視鏡部、腹部超音波室も拡充計画は決定しておりますので

何卒昨年以上にモチベーションを上げて頂いて日本一、あるいは世界一の消化器内科学教室へ育つようにご尽力頂きたいと思います。2011 年も教育・診療・研究において、特に英文論文、新しい研究の立ち上げ（種蒔き）ということについては 2010 年以上に積極的に取り組んでいきたいと考えておりますので医局員全員が共通の価値観と消化器内科の将来の方向性に対するベクトルを共有し、心を一つにして邁進して頂きたいと祈念・期待しております。

2011 年 4 月　大阪狭山にて

2010 年度表彰式一覧

- **Highest Impact Factor Award 2010（最高インパクトファクター賞）**

1 位　北野雅之　6.713（Gastrointest Endosc）
2 位　坂本洋城　6.012（Am J Gastroenterol）
3 位　鄭　浩柄　5.865（J Infect Dis）

※　4 位　鎌田　研　5.545（Endoscopy）

- **Most Numbers of Paper Award 2010（最多英文論文発表賞）**

1 位　坂本洋城　3 本（World J Radiol×2, Am J Gastroenterol）
1 位　南　康範　3 本（World J Radiol×2, Liver Int）
3 位　鄭　浩柄　2 本（Intervirology, J Infect Dis）

※　工藤正俊　13 本

- **Total Highest Impact Factor Award 2010（累積最高インパクトファクター賞）**

1 位　鄭　浩柄　6.971（2 本）
2 位　北野雅之　6.713（1 本）
3 位　坂本洋城　6.012（3 本）

※　工藤正俊　24.293

- **最多入院受持患者賞**

1 位　井上達夫　195 人
2 位　今井　元　184 人
3 位　峯　宏昌　178 人

※　4 位　矢田典久、宮田　剛　170 人

- **最多緊急内視鏡賞**

1 位　松井繁長

- **最高しんどかったで賞**

1 位　井上達夫　15 人
1 位　今井　元　15 人
3 位　北井　聡　13 人

※　4 位　鄭　浩柄、小牧孝充、有住忠晃、鎌田　研　　8 人

- **最多外来患者診療賞**

1 位　萩原　智　3,752 人
2 位　上嶋一臣　3,004 人
3 位　鄭　浩柄　2,842 人

※ 工藤正俊　5,881 人　4 位　松井繁長　2,405 人

日本超音波医学会 第82回学術集会開催

5月22～24日、東京で

16題のシンポを中心に

診断・治療の将来展望を討議

日本超音波医学会第八十二回学術集会（会長＝椎名毅・京都大学大学院医学研究科人間健康科学系教授）が五月二十二～二十四の三日間、東京・千代田区丸の内の東京国際フォーラムにおいて盛大に開催される。今学術集会は「超音波診断・治療におけるInnovationとFusion」をメインテーマに掲げ、これに基づいて①異分野との融合による超音波イメージングの新展開②超音波検査と教育－超音波専門医と超音波検査士の育成について考える－③超音波診断と治療技術の融合④一目見ておけば診断できるまれな心血管疾患－を含む十六題のシンポジウムを中心に、日本発のカラードプラ法、経食道心エコー法の開発、さらに組織弾性映像法の実用化など、世界に誇れる技術開発を踏まえて、超音波診断・治療の将来展望などを討議することにしており、その成果が注目される。今学術集会のプログラムの概要は次のとおり。

社団法人日本超音波医学会第 83 回学術集会を終えて

会長　工藤　正俊
（近畿大学　消化器内科学教授）

本学会の第 83 回学術集会（**写真 1**）を 2010 年 5 月 29 日から 31 日の 3 日間，国立京都国際会館で開催致しました．おかげさまで 3, 426 名の参加者を得て盛会裏に終えることができました．御協力頂きました関係の先生方，皆様には御礼を申し上げます．本稿では開催準備から終了までの印象を振り返ってみたいと思います．

写真 1

写真 2　左から Prof. Byung Ihn Choi，Prof. Giovanni Cerri，岡井崇前理事長，Prof. Michel Claudon，工藤正俊先生，渡邉泱先生，Prof. David Evans，Prof. Seung Hyup Kim

1. テーマ

第 83 回のメインテーマは「サイエンス・テクノロジーのイノベーションからアートへ」と致しました．サイエンス，あるいはテクノロジーのイノベーションから実地臨床へ還元することは今や，超音波の進歩にとっては切っても切り離せないものになっております．最近の 3D や 4D あるいは超音波造影剤の進歩などはその最たるものであり，現在でも超音波造影剤の開発などは臨床そのものにつながっております．また，逆に臨床的なニーズ（State-of-the Art Practice）から工学系，あるいは企業への働きかけによって実現した技術（Technology）や Innovation も存在致します．そのような意味でメインテーマに加えてサブテーマとして “From the State-of-the Art Technology to Future Ultrasound Imaging, From the State-of-the-Art Ultrasound Practice to Future Technology” とさせて頂きました．このサブテーマは日本語にすると陳腐ではありますが，最先端のテクノロジーの進歩が将来の超音波診療の現場へと確実に反映されるべきで，さらには最先端の診療の現場にいるもののニーズから新しい将来のテクノロジーへのシーズが生まれるという意味であります．超音波医学会は「医」と「工」の両輪がうまく連携することが大変に重要であります．その意味で最先端の超音波臨床をやっている医師，及び技師こそが超音波技術の開発を正しい方向へと導き innovation へもつなげていく役割を担っているものと信じます．もちろん逆もまた真実で最先端の工学・基礎研究を極めることが超音波臨床のニーズに答えることができると考えております．

2. プログラムについて

プログラムについては実行委員の先生方に適宜メール会議をはさみながら数回お集まり頂き，決定致しました．この先生方には本当にお世話になりました．この場をお借りしましてもう一度感謝申し上げます．各領域からシンポジウムは 12 セッション，パネルディスカッションを 13 セッション，ワークショップを 7 セッションご用意致しました．特別企画として「診断基準を巡る諸問題」ということで「1. 血管エコー」，「2. 肝腫瘍」，「3. 胎児異常超音波スクリーニング」，「4. 結節性甲状腺腫」の四つを取り上げました．また，今回は日本超音波医学会の国

写真 3　Prof. Byung Ihn Choi

写真 5　展示会場

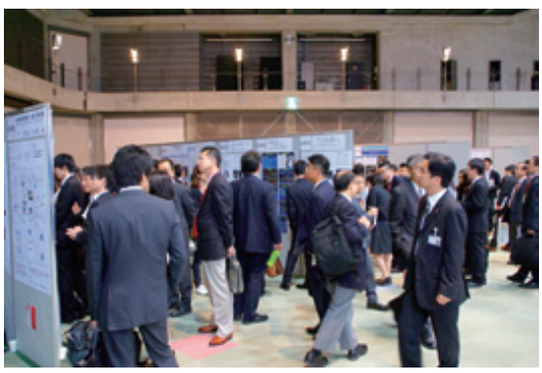


写真 4　ポスターセッション

写真 6　展示会場

際化ということも視野に入れて，世界超音波医学会（WFUMB），アジア超音波医学会（AFSUMB）のメンバーの方も Key note lecture としてお呼び致しました（**写真 2**）．従いまして 10 の Key note lecture を用意致しました．また，WFUM-AFSUMB-JSUM Joint Session で WFUMB の President である Michel Claudon 教授，AFSUMB の Immediate-past President の Byung Ihn Choi 教授（**写真 3**），及び JSUM の岡井理事長のお話を頂きました．Key note lecture では WFUMB，AFSUMB のそれぞれの Council Board member の方々にそれぞれの領域で貴重なお話を頂くことができました（**写真 2，3**）．特別講演と致しましては循環器の領域より，吉川純一先生，及び消化器領域より幕内雅敏先生に特別講演を頂くことができました．もちろんポスターセッションも充実していました（**写真 4**）．

3. 開催前に苦労したこと

前の椎名先生の第 82 回においても述べられていることでありますが，一昨年のいわゆるリーマンショックによる急速な景気の落ち込みがあり，本年の開催前の 4，5 ヵ月前になってもランチョンセミナーや展示などが本格的に埋まらない状態でありました．これについては私も大変に苦労し，教室の財務担当者だけでなく，私自ら多くの装置メーカー，医薬品メーカーの方々に直接電話でお願いし，何とか，ランチョンセミナーや展示などもほぼ目途が立ち，ほっとしたことを今でも鮮明に覚えています．この度のことで予想以上に装置メーカーにとっての不況の波は強いものであることを実感した次第であります．

しかしながら，その中でも御協力頂いた各社のメーカーの方々に大変感謝を申し上げる次第です．このご恩は一生忘れませんので今回ご協力頂いたメーカーの方も私に貸しがあると思って頂いて結構です（?）．おかげさまで本学会の名物となっている展示（**写真 5, 6**），イノベイティブテクノロジー，バーチャルライブも大変盛況でありましたことを付け加えさせて頂きたいと思います．

4. 国立京都国際会館について

会場については私の職場は近畿大学で大阪にあり

写真 7　国立京都国際会館

写真 9　吉川純一先生（特別講演演者）

写真 8　宝ヶ池から見た会場

写真 10　幕内雅敏先生（特別講演演者）

ますが，学生時代は京都で過ごしたこと，また 5 月という新緑の季節が京都は最も過ごしやすいということから京都に決定致しました．結果的に京都を会場に選んだことは多くの会員の方々にも好評でありました（**写真 7，8**）．また，それまで 1 週間以上降り続いた雨も会期中の 3 日間については快晴で，これも皆様方のお陰と感謝致しております．季節としては最高で新緑も美しく，湿度も高くなく，暖かくもなく，寒くもないという絶好のタイミングでの開催となりましたことは大変喜ばしいことと考えております．また，2 日目の Fire side talk においても屋外でのパーティーも行うことができ，心配していた雨による影響も避けることができました．

5. 特別企画の工夫

特別企画としては今回，これまでの会と差別化を意識したのはやはり WFUMB, AFSUMB のメンバーを招いた WFUMB-AFSUMB-JSUM Joint Session, 及び WFUMB，AFSUMB のメンバーによる Key note lecture，そして横断領域として「1. 専門医・Sonographer 養成のための教育システムを考える」というセッション，ならびに「2. CT，MRI 時代における超音波検査の在り方」,「3. 用語の誤用」,「4. マイクロバブルの基礎と臨床をめぐって」，という四つのセッションを設けたことであります．これらのセッションも大変好評で多くの医師，技師の先生方にご参加を頂きました．

6. 特別講演について

今回の学会の特別講演では私のひよっこの頃から叱咤激励をして頂き，常にご指導頂きました循環器と消化器の 2 大巨頭にお願いいたしました．お一人は神戸中市立央市民病院で 18 年間ご指導頂きました吉川純一先生（**写真 9**），ならびに肝臓内科医として本当にかけだしの頃から肝臓癌の手術見学，あるいは学問が何たるものかを教えて頂き，英文論文執筆のいろはから薫陶を頂いた幕内雅敏先生にお願い致しました（**写真 10**）．これらのお二人の先生は私が超音波に関わる機会を作って頂いた私が師と仰ぐ先生方であります．このお二人の講演を拝聴致しまして改めてお二人の偉大さに感じ入った次第であります．

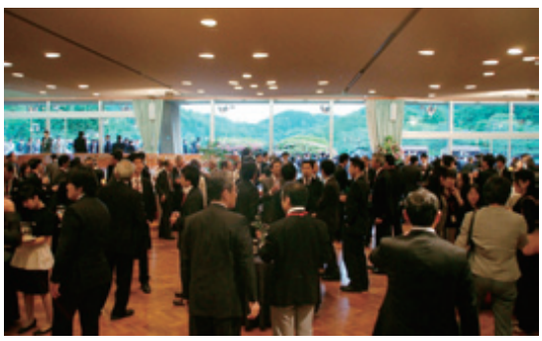


写真 11　ファイアーサイドトーク

写真 12　屋外でのファイアーサイドトーク

写真 13　夕刻のファイアーサイドトーク

7. ファイヤーサイドトーク

ファイヤーサイドトークも 500 名以上の参加を得て，盛会に行うことができました（**写真 11－13**）．特に，宝ヶ池を望む屋外を開放できたことは天候にも恵まれ，大変好評でありました．また，WFUMB，AFSUMB のメンバーも会員数 13, 000 にも及ぶ日本超音波医学会の実力，ならびに一回の学術集会で 3, 400 名を越える参加者があるということにも大変な感銘を受けたようです（**写真 14**）．その意味でも日本，アジア，及び世界における日本超音波医学会の底力を WFUMB，AFSUMB のメンバーにも印象付けることができたのも本学会の成功の一つである

写真 14　WFUMB, AFSUMB, JSUM のメンバー

写真 15　今回の学会のシンボルデザインの由来について解説する筆者

と考えております．

8. エッセイ集発行について

本学会の特別企画といたしまして「私と超音波」というエッセイ集を企画致しました（**写真 15**）．これはこれまでに同様の企画がなかったこと，及び日本超音波医学会は多岐にわたる専門の先生方で構成されており，「医」と「工」，及び「医」の中でも循環器内科，消化器内科，産婦人科，泌尿器科を始め，多くの専門の先生方がおられるため，大変著名な先生方がどのように超音波に携わってこられたのか，あるいはどのようなスタンスで超音波と接してこられたのか，過去にどの様な業績があるのかといったことが，あまり超音波医学会以外では接することがないため，「ピン」とこない方もおられるのではないかと思います．従いまして，この度は全てのこれまでの功労会員，名誉会員，及び理事経験者，学会長経験者，及び現在の代議員全ての方にエッセイの執筆をお願いし，多くの方々から快諾のお返事を頂き，1 冊の本にまとめることができました．この本は大変好評で，一つの日本超音波医学会のマイルス

写真 16　Prof. Cheng-Wen Chiang

写真 18　Prof. Michel Claudon

写真 17　Prof. Michel Claudon

写真 19　第 83 回学術集会会長挨拶

トーンともなるべき記念誌になると考えております．現在，まだ在庫に多少の余裕はありますのでぜひとも欲しいという方は無料でお送り致したいと思いますのでご連絡頂ければ幸いに存じます．

9. WFUMB，AFSUMB と日本超音波医学会

私は現在，日本超音波医学会理事会の御推挙を受け，アジア超音波医学会（Asian Federation of Society of Ultrasound in Medicine and Biology: AFSUMB）の vice-president，また世界超音波医学会（World Federation of Society of Ultrasound in Medicine and Biology: WFSUMB）の President-elect という大役を拝命しております．その関係で AFSUMB，及び WFUMB の Board member，あるいは Council member でアクティブに活動されている世界のトップクラスの先生方をお招きして，基調講演をお願い致しました．具体的には AFSUMB からは Secretary の Prof. Seung Hyup Kim，Immediate-past President の Prof. Byung Ihn Choi（**写真 3**），President の Prof. Cheng-Wen Chiang（**写真 16**），そして Treasurer の Prof. Yi-Hong Chou をお招きしました．さらに WFUMB からは現在の President である Prof. Michel Claudon（**写真 17，18**），Secretary の Prof. David Evans，Treasurer の Prof. Beryl Benacerraf さらに American Institute of Ultrasound in Medicine（AIUM），WFUMB Councilor である Prof. Joshua Copel（**写真 14 の中央**），Immediate-past President の Prof. Cerri（**写真 2**）などをお迎えしました．それぞれの領域の専門の立場から素晴らしい講演を拝聴することができました．今後は，日本の国際化ということも課題の一つでありますのでこれをきっかけに来年以降もこのような海外の先生方を交えたセッションを企画して頂ければと考えております．

10. 終わりに

今回開催準備がやや出遅れ気味であった本学会も最後の数ヵ月で多くの医局員，及び実行委員の先生方，及び企業の皆様方の御協力を得て成功裏に終えることができました（**写真 19**）．これもひとえに会員の先生方のお陰と考えておりますので，今後とも宜しくご指導の程，お願い申し上げます．

工藤正俊（くどうまさとし）

（平成 23 年 9 月 30 日更新）



昭和 29 年	愛媛県西条市生まれ
昭和 53 年	京都大学医学部 卒業
同	京都大学医学部附属病院 勤務（研修医）
昭和 54 年	神戸市立中央市民病院内科　勤務（研修医）
昭和 55 年	同　消化器内科　医員
昭和 60 年	同　消化器内科　副医長
昭和 62 年	カリフォルニア大学留学（デービスメデイカルセンター）
平成元年	神戸市立中央市民病院消化器内科　副医長　復職
平成　4 年	同　消化器内科　医長
平成　9 年	近畿大学医学部第２内科学　助教授
平成 11 年	近畿大学医学部消化器内科学 教授　現在に至る
	Website（ www.med.kindai.ac.jp/shoukaki/ ）
（現在の併任）	近畿大学医学部附属病院病院長（平成 20 年 10 月 1 日～現在）
	高度先端総合医療センター長（平成 22 年 10 月 1 日-現在）
	光学治療センター長（平成 22 年 10 月 1 日-現在）
	中央臨床検査部長（平成 22 年 10 月 1 日-現在）
	臨床研修センター長（平成 23 年 4 月 1 日-現在）
	心身医療センター長（平成 23 年 4 月 1 日-現在）
	近畿大学医学部奈良病院消化器内科　教授（兼務）
	近畿大学医学部堺病院消化器科　教授（兼務）
	神戸市立中央市民病院消化器内科　顧問（兼務）

主な所属学会

日本消化器病学会（財団評議員・指導医・専門医・国際委員会委員）、日本肝臓学会（理事・指導医・専門医・治験支援委員会委員長・倫理委員会副委員長・肝移植検討委員会委員）、日本消化器内視鏡学会（評議員・指導医・専門医）、日本超音波医学会（理事・指導医・専門医・国際交流委員会委員長）、日本内科学会(評議員・認定内科医)、日本核医学会(評議員・専門医)、日本肝癌研究会（常任幹事・追跡調査委員長・取扱規約委員長・肝癌治療効果判定基準作成委員会委員長・事務局代表）、日本臨床腫瘍学会、日本肝移植研究会（世話人）、肝血流動態イメージ研究会（幹事）、日本腹部造影エコー・ドプラ診断研究会（事務局・世話人）、肝癌治療シミュレーション研究会（副代表幹事・企画委員）、超音波治療研究会（常任世話人）、日本肝がん分子標的治療研究会（代表世話人・事務局代表）、日本消化器内視鏡財団（評議員）、日本臨床腫瘍学会（評議員）、日本癌学会（評議員）、米国肝臓学会（AASLD）（肝癌部門企画運営委員：Steering Committer hepatobiliary malignancy）、米国消化器病学会（AGA）、世界肝臓学会（IASL）、欧州肝臓学会（EASL）、米国消化器内視鏡学会（ASGE）など。

国際学会役員・委員

- 世界超音波医学会（WFUMB） President（理事長）
- アジア超音波医学会（AFSUMB）Vice-President（副理事長）
- 世界肝癌学会（ILCA）理事（Founding Board Member, Governing Board Council Member）
- 米国肝臓学会（AASLD）肝癌部門運営委員会委員（Steering Committee Member）
- 世界保健機構（WHO）Blue Book「Classification of the Tumor」改訂委員（平成 21 年 5 月 1 日）
- ウイルス肝炎研究財団　日米医学協力研究会肝炎専門部会研究員

- International Liver Thought Leadership Study (ILCS), Council member

受賞

- 米国核医学会 Berson-Yalow Award 受賞（平成元年 6 月）
- 日本対がん協会がん研究助成奨励賞 受賞（平成 4 年 3 月）
- 日本消化器病学会奨励賞 受賞（平成 4 年 4 月）
- 日本核医学会賞 受賞（平成 5 年 10 月）
- 米国超音波医学会（AIUM）学会賞受賞（平成 15 年 6 月 4 日）
- ボローニャ大学医学部医学会名誉会員賞（平成 18 年 9 月 15 日）
- フィリピン超音波医学会名誉会員（Honorary Member of PSUCMI）（平成 20 年 3 月 19 日）
- アジア太平洋消化器病学会（APDW）OKUDA Award 受賞（平成 20 年 9 月 13 日）
- 北米放射線学会 Certificate of Merit 受賞（平成 20 年）
- インド肝臓学会 Madangopalan Award 受賞（平成 21 年 3 月 28 日）
- 北米放射線学会 Cum Laude 賞受賞（平成 21 年 12 月）（7000 編の論文中上位 10 編に採択）
- 日本肝臓学会「日本肝臓学会機関誌 Highest Citation 賞」受賞（平成 22 年 6 月）
- JISAN Lecture Award Presented by Korean Society of Ultrasound in Medicine（平成 22 年 5 月）
- 米国超音波医学会名誉会員賞（AIUM Honorary Member Award）受賞（平成 22 年 4 月）
- 韓国超音波医学会名誉会員賞（KSUM honorary Award）受賞（平成 23 年 5 月）
- 日本肝臓学会「日本肝臓学会機関誌 Highest Citation 賞」受賞（平成 23 年 6 月）（2 回目）

著書（単著）

- Contrast Harmonic Imaging in the Diagnosis and Treatment of Liver Tumors （Springer-Verlag　2003）
- 肝腫瘍における造影ハーモニックイメージング（医学書院　2001）

編集

- 松井　修，**工藤正俊**,編集：消化器疾患の造影エコーUp Date．南江堂，東京，2003.
- **工藤正俊**，編集：肝細胞癌治療の最近の進歩，消化器病セミナー97，へるす出版，東京，2004.
- 河田純男，白鳥康史，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2004-2005，日本メディカルセンター，東京，2004.
- 河田純男，白鳥康史，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2006-2007,日本メディカルセンター，東京，2006.
- 河田純男，横須賀收，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2008-2009，日本メディカルセンター，東京，2008.
- 幕内雅敏，菅野健太郎，**工藤正俊**，編集：今日の消化器疾患治療指針　第3版，医学書院，東京，2010.
- **工藤正俊**，泉　並木，編集：症例から学ぶ ウイルス肝炎の治療戦略．　（株）診断と治療社，東京，2010.
- **工藤正俊**，編集：肝癌の分子標的治療，アークメディア，東京，2010.
- 山雄健次，**工藤正俊**，編集：見逃し、誤りを防ぐ!肝・胆・膵癌画像診断アトラス，羊土社，東京，2010.
- **工藤正俊**，編集：朝倉内科学，「肝胆膵疾患」，朝倉書店，東京，2010.
- 河田純男，横須賀收，**工藤正俊**，榎本信幸，編集，小俣政男，監修：肝疾患 Review 2010-2011，日本メディカルセンター，東京，2010.
- **工藤正俊**，編集：医学のあゆみ「肝癌の分子標的治療」，医歯薬出版株式会社，東京，2011.
- **工藤正俊**，編集:最新医学「肝細胞がん診療の進歩：Up-To-Data」，株式会社最新医学社，大阪，2011.

学術雑誌編集委員

- Editor-in-Chief

  World Journal of Hepatology (China)
  Liver Cancer

- Guest Editor-in-Chief

Intervirology	Vol 47,	2004
Intervirology	Vol 48,	2005
Oncology	Vol 72,	2007
Digestive Deases	Vol 25,	2007
Oncology		2008
Intervirology		2009
Oncology		2010
Pancreatology		2011
Digestive Disease		2011
Digestion		2011
Oncology		2011

- Associate Editor

Hepatology Research (Tokyo)
Journal of Gastroenterology (Tokyo)
Journal of Oncology (Germany)
Ultrasound in Medicine and Biology (New York)
World Journal of Gastroenterology (China)
World Journal of Roenterology (China)
肝胆膵画像編集幹事（医学書院, 東京）
肝胆膵編集委員（アークメディア, 東京）

- Editorial Board Member

International Journal of Clinical Oncology (Tokyo)
Ultrasound in Medicine and Biology (New York)
Hepatology International (New York)
Liver International (New York)
Open Hepatology Journal

- Journal Referee

J Clin Oncol(18.970), Lancet Oncol (17.764), Gastroenterology(12.032), Hepatology(10.885), J Hepatol(9.334), Oncologist(5.826), Am J Gastroenterol(6.882), Endoscopy(6.096), Clin Exp Metastas(4.113), Cancer Sci(3.846), Expert Rev Mol Diagn(4.652), Eur Radiol(3.594), Liver Int(3.840), J Gastroenterol(3.610), Eur J Clin Invest(2.736), J Nucl Med(7.022), J Gastroen Hepatol(2.410), Oncology-Basel (International Journal of Cancer Research and Treatment)(2.538), Ultrasound Med Biol(2.493), Acta Paediatr(1.955), Hepatol Int(2.963), Eur J Gastroen Hepat (1.598), J Hepato-Bil-Pan Scu (1.963), Hepatol Res(1.857), Int J Clin Oncol(1.437), Jpn J Clin Oncol(1.856), Internal Med(1.037), J Clin Ultrasound(0.808), Biomark Med(1.247), Hepato-Gastroenterol(0.677), Ann Nucl Med(1.386), Expert Review of Anticancer Treatment(0), J Cancer Res Ther (0.825), CSR National Registry(0), J Gastrointest Liver (1.434), Cancer Informatics(0), Expert Review of Proteomics and Future Oncology(0)
日本核医学会雑誌「核医学」(東京)
日本消化器病学会雑誌(東京), 日本肝臓学会誌「肝臓」(東京)
日本超音波医学会機関誌「超音波医学」 (東京)
日本内科学会英文誌 「Internal Medicine」(Tokyo)
日本老年医学会誌
日本消化器内視鏡学会誌
「最新医学」(大阪)

研究業績

- 発表論文（英文） 387 編（総 IF=1,189.262）（内、著書分担執筆 20 編）
- 発表論文（和文） 937 編（内、著書・分担執筆 230 編）
- 特別講演（国内学会） 570 件
- 特別講演（国際学会） 227 件
- 学会発表（国内学会） 1,594 件
- 学会発表（国際学会） 291 件

科学研究費等外部資金の獲得状況

- 文部科学省科学研究費補助金 基盤研究(A) 1 件（総額 1,000 万円）
  基盤研究(B) 6 件（総額 2,311 万円）

	基盤研究(C)	9 件（総額 1,290 万円）
	挑戦的萌芽研究	2 件（総額 350 万円）
知的クラスター創生事業（がんペプチドワクチン）		1 件（総額 100 万円）
・ 厚生労働省がん研究助成金	主任研究者	2 件（総額 2 億 8,000 万円）
	分担研究者	7 件（総額 1,690 万円）
・ 車両財団がん研究助成金		2 件（総額 600 万円）
・ 学会奨励研究補助金		3 件（総額 490 万円）
・ 医師会・民間医学振興財団等研究補助金		29 件（総額 1,872 万 5 千円）

ガイドライン策定委員会委員

- 「科学的根拠に基づく肝癌診療ガイドライン」（日本肝臓学会編），金原出版
- 「「慢性肝炎の治療ガイドライン」（日本肝臓学会編），文光堂
- 「肝癌診療マニュアル」（日本肝臓学会編），医学書院
- 「肝癌治療効果判定基準」（日本肝癌研究会取扱い規約委員会編），肝臓
- 臨床病理「肝癌取り扱い規約」（日本肝癌研究会編）
- Clinical Practice Guidelines for Hepatocellular Carcinoma, Japan Society of Hepatology, Hepatology Research
- General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 3rd English Version, Liver Cancer Study Group of Japan, Kanehara, Tokyo, 2010
- Response Evaluation criteria in the Cancer of the Liver (RECICL), Liver Cancer Study Group of Japan, Hepatology Research

特許取得

発明の名称：　ソラフェニブの効果予測方法
出願番号：　特願 2011-104275
出願日：　2011 年 5 月 9 日
発明者：　荒尾徳三、松本和子、西尾和人、工藤正俊
出願人：　学校法人近畿大学

発明の名称：　N 型糖鎖を利用した膵臓癌の診断方法
公開番号：　特許公開 2009-270996
公開日：　2009 年 11 月 19 日
発明者：　荒尾徳三、松本和子、西尾和人、坂本洋城、北野雅之、工藤正俊
出願人：　住友ベークライト株式会社

全国規模の学会・研究会事務局

・ 日本肝癌研究会（**事務局**・追跡調査委員長）
・ 日本腹部造影エコー・ドプラ診断研究会（**事務局**）
・ NPO 法人日本肝がん臨床研究機構（**理事長・事務局**）
・ 日本肝がん分子標的治療研究会（**代表世話人・事務局**）

全国規模の研究会世話人・役員

平成 6 年–平成 8 年　日本超音波医学会腹部造影エコー研究部会幹事
平成 7 年–現在　肝血流動態イメージ研究会世話人
平成 8 年–現在　日本腹部造影エコー・ドプラ造影研究会世話人（事務局兼務）
平成 9 年–現在　肝動脈塞栓療法研究会世話人
平成 10 年–現在　国際造影超音波研究会世話人
平成 11 年–現在　臨床消化器病研究会世話人
平成 11 年–現在　西日本肝臓研究会世話人
平成 13 年–現在　肝疾患フォーラム世話人
平成 14 年–現在　犬山シンポジウム会員
平成 14 年–現在　日本消化器画像診断研究会世話人
平成 16 年–現在　肝臓病研究会世話人
平成 16 年–現在　Liver Forum in Kyoto 世話人
平成 18 年–現在　肝癌治療シミュレーション研究会副代表世話人
平成 19 年–現在　日本超音波治療研究会常任世話人
平成 20 年–現在　日本肝がん分子標的治療研究会（**代表世話人**）

関西地区研究会代表世話人

・平成 11 年–現在　関西造影超音波研究会（**代表世話人**）
・平成 13 年–現在　関西 B 型肝炎研究会（**代表世話人**）
・平成 14 年–現在　肝癌局所治療研究会（**代表世話人**）
・平成 14 年–現在　大阪消化器化学療法懇話会（**代表世話人**）
・平成 15 年–現在　臨床消化器病フォーラム（**代表世話人**）
・平成 18 年–平成 22 年　Bay Area Gut Club（**代表世話人**）
・平成 18 年–平成 22 年　South Osaka Liver Club（**代表世話人**）
・平成 19 年–現在　関西肝血流動態イメージ研究会（**代表世話人**）
・平成 20 年–現在　Kinki Liver Club（**代表世話人**）
・平成 21 年–現在　南大阪肝疾患研究会（**代表世話人**）
・平成 21 年–現在　南大阪肝胆膵疾患研究会（**代表世話人**）

関西地区研究会世話人

・平成 2 年–現在　大阪肝穿刺生検治療研究会世話人
・平成 6 年–現在　兵庫インターベンショナルラディオロジー研究会世話人
・平成 8 年–現在　肝胆膵治療フォーラム・神戸世話人
・平成 9 年–現在　京都肝疾患懇話会世話人
・平成 9 年–現在　肝臓分子生物学研究会
・平成 11 年–平成 18 年　肝代謝コロキウム世話人
・平成 11 年–現在　大阪肝胆膵懇話会世話人
・平成 11 年–現在　南大阪肝胆膵疾患研究会世話人
・平成 11 年–現在　南大阪消化器病懇話会世話人
・平成 11 年–現在　南大阪肝疾患研究会世話人
・平成 11 年–現在　消化器ラウンドテーブルディスカッション世話人
・平成 11 年–平成 18 年　泉州肝臓病研究会世話人
・平成 11 年–平成 18 年　大阪肝炎ミーティング世話人
・平成 12 年–現在　大阪肝臓病談話会世話人
・平成 12 年–現在　関西経皮内視鏡的胃瘻造設術研究会世話人
・平成 12 年–現在　肝疾患座談会 in Kyoto 世話人
・平成 12 年–現在　近畿肝癌談話会常任幹事
・平成 13 年–現在　関西肝血流動態イメージ研究会世話人
・平成 16 年–現在　あおい肝臓研究会世話人
・平成 18 年–現在　大阪肝臓ミーティング世話人
・平成 19 年–現在　近畿・超音波内視鏡研究会顧問

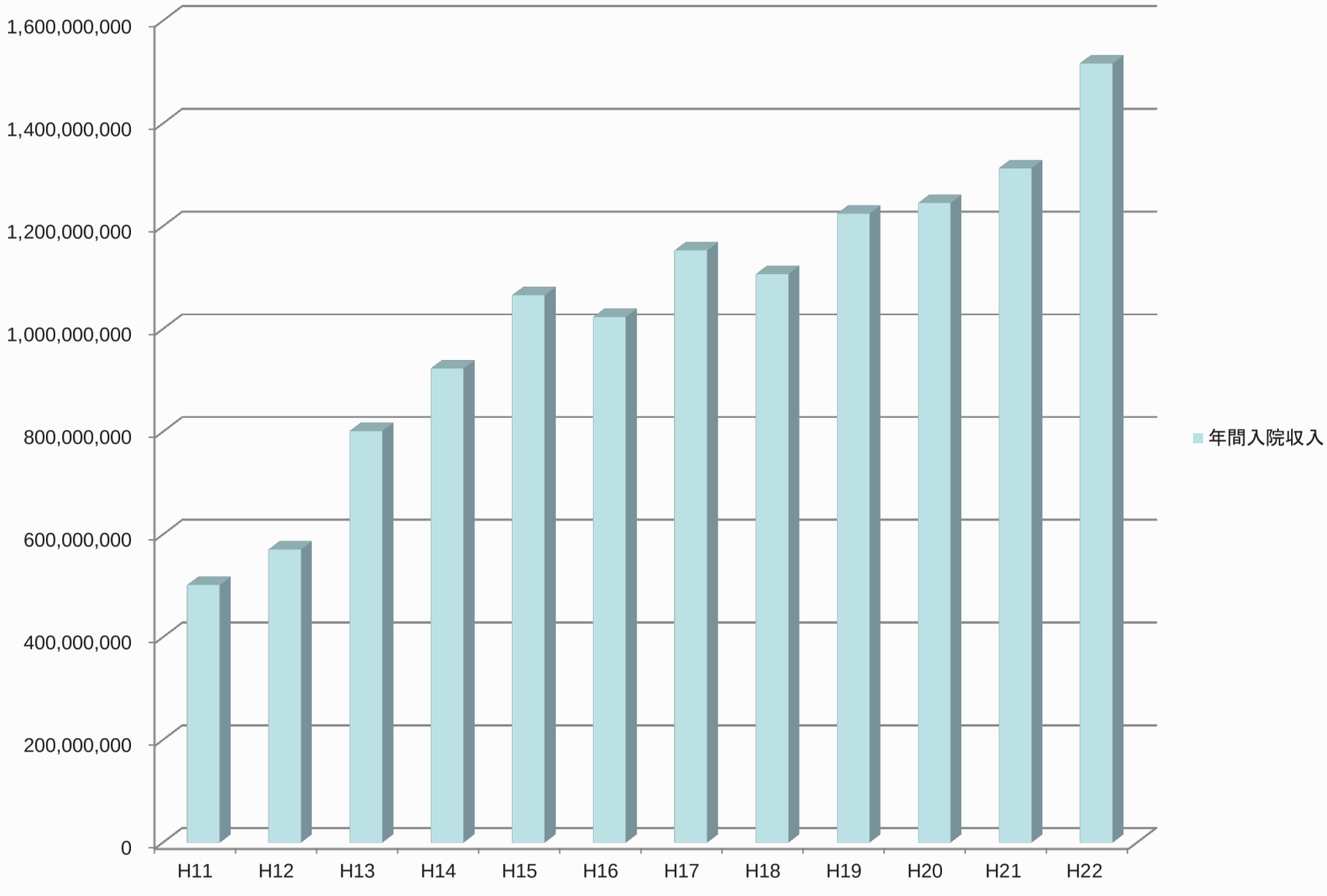
消化器内科学教室業績抜粋

	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011 (-10月)	計
英文論文 (Impact Factor)	7 40. 281	11 35. 318	23 55. 769	13 18. 596	21 48. 048	20 68. 517	25 54. 206	15 35. 094	31 93. 084	43 138.626	26 94.146	50 126.184	68 274.513	353 1082. 382
和文論文 (著書・分担執筆を含む)	37	41	43	34	31	54	45	39	45	74	81	124	102	750
海外学会発表	2	9	4	6	24	22	14	14	18	27	20	35	48	243
国内学会発表	46	56	71	113	105	79	69	52	79	87	65	96	77	995
海外特別講演	0	11	4	11	8	18	16	25	16	35	34	44	14	236
国内特別講演	37	40	40	52	37	38	39	27	36	39	62	92	34	573
単著教科書			1		1 (英文)									2

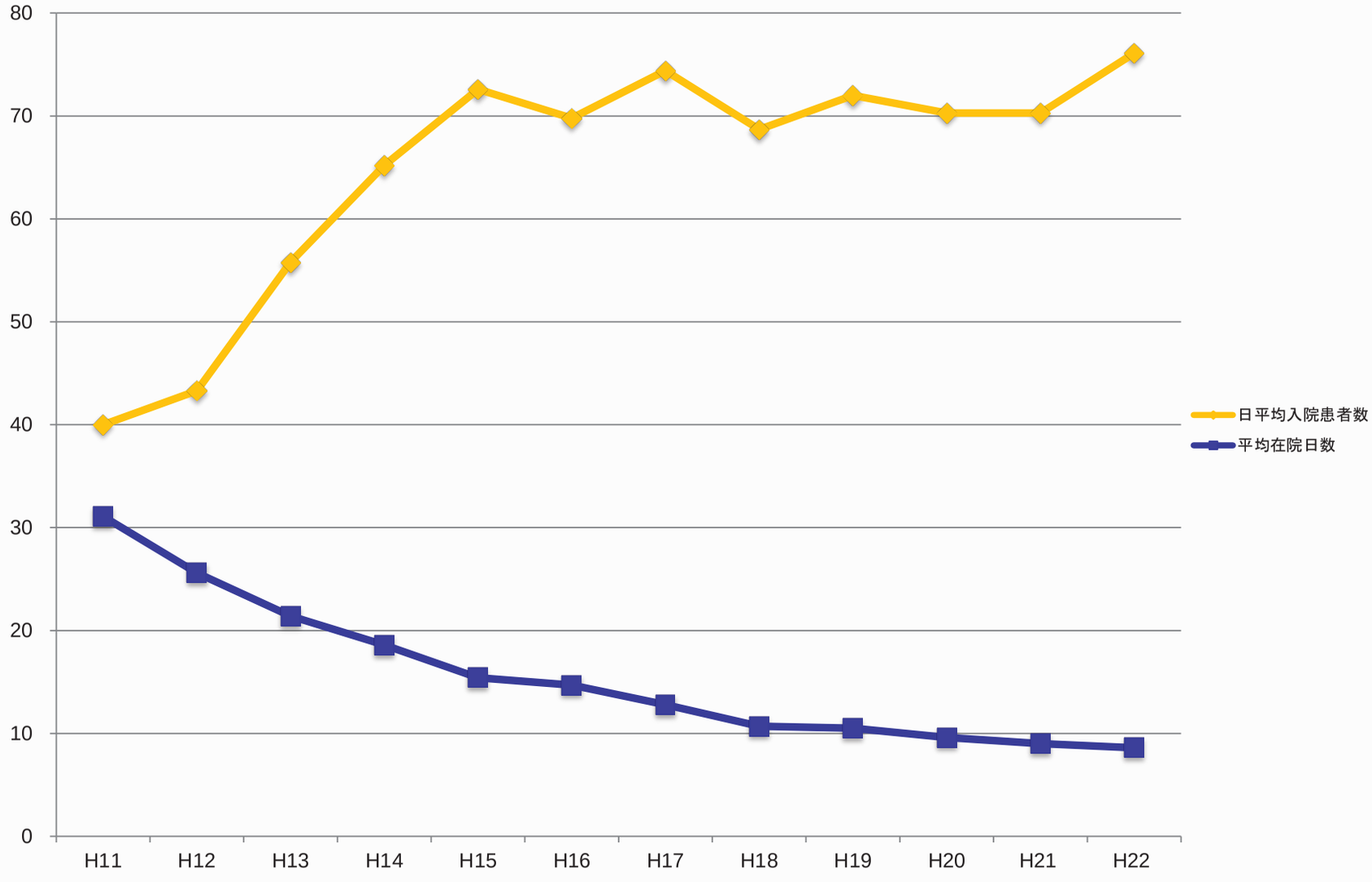
消化器内科年度別診療実績

	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22
稼働床	40	44	44	44	60	78	78	77	76	73	85	84
稼働率	107.2%	98.5%	126.7%	148.2%	121.0%	89.5%	95.3%	89.2%	94.7%	96.3%	91.8%	89.9%
日平均入院患者数	40	43.3	55.8	65.2	72.6	69.8	74.4	68.7	72.0	70.3	70.3	76.1
平均在院日数	31.1	25.6	21.4	18.6	15.4	14.7	12.8	10.7	10.5	9.6	9	8.6
年間入院収入	501,570,188	570,616,464	801,199,124	923,171,333	1,065,481,449	1,023,271,279	1,152,778,111	1,106,484,453	1,224,122,968	1,244,806,271	1,312,812,506	1,516,925,835
年間外来収入	314,641,639	334,517,979	386,084,329	530,035,297	635,562,806	649,876,475	818,049,485	966,247,389	1,013,910,559	1,257,804,553	1,432,350,698	1,464,645,183
消化器内科年間収入	816,211,827	905,134,443	1,187,283,453	1,453,206,630	1,701,044,255	1,673,147,754	1,970,827,596	2,072,731,842	2,238,033,527	2,502,610,824	2,745,163,204	2,981,571,018

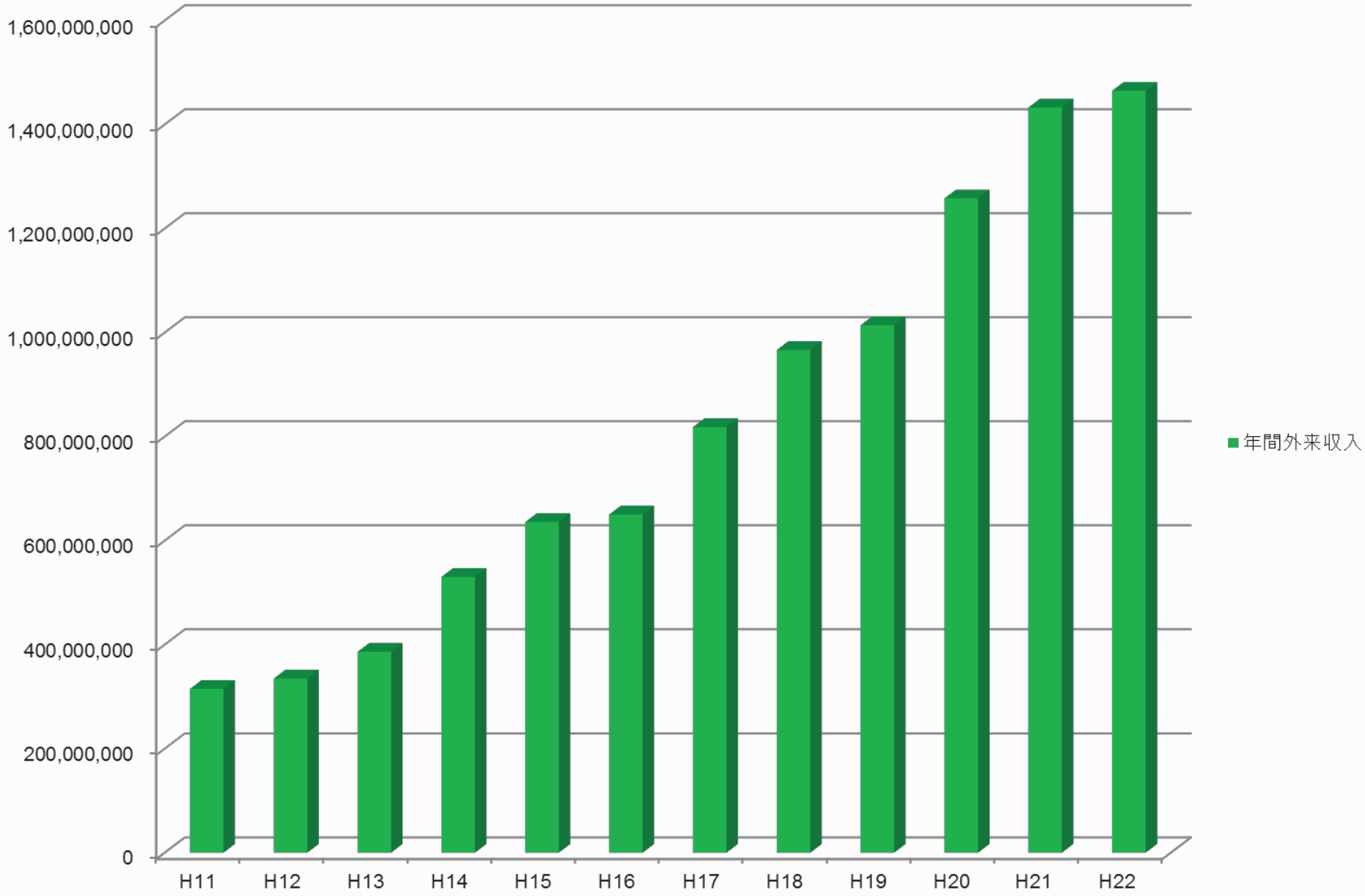
消化器内科年間入院収入



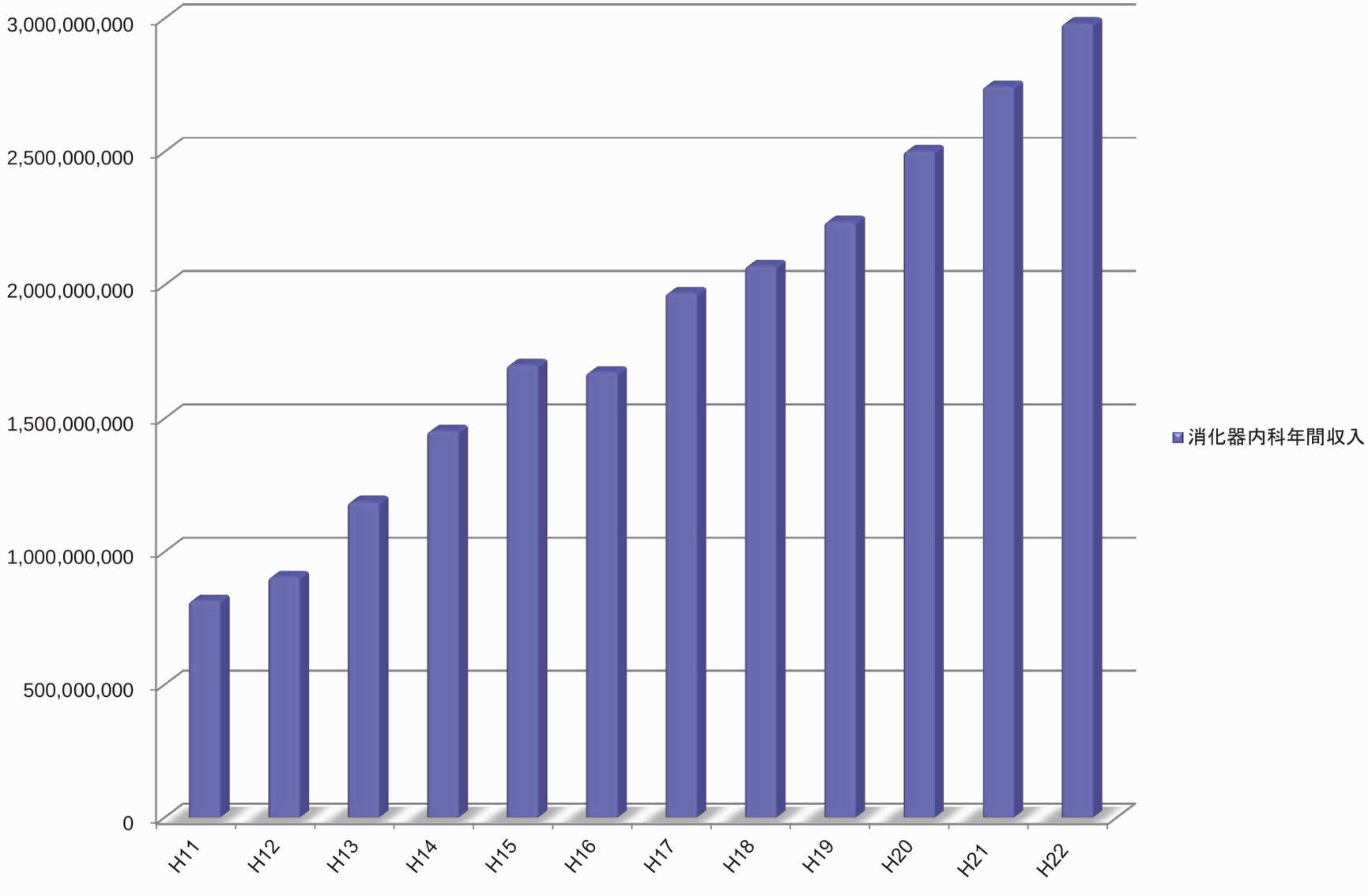
消化器内科入院診療実績



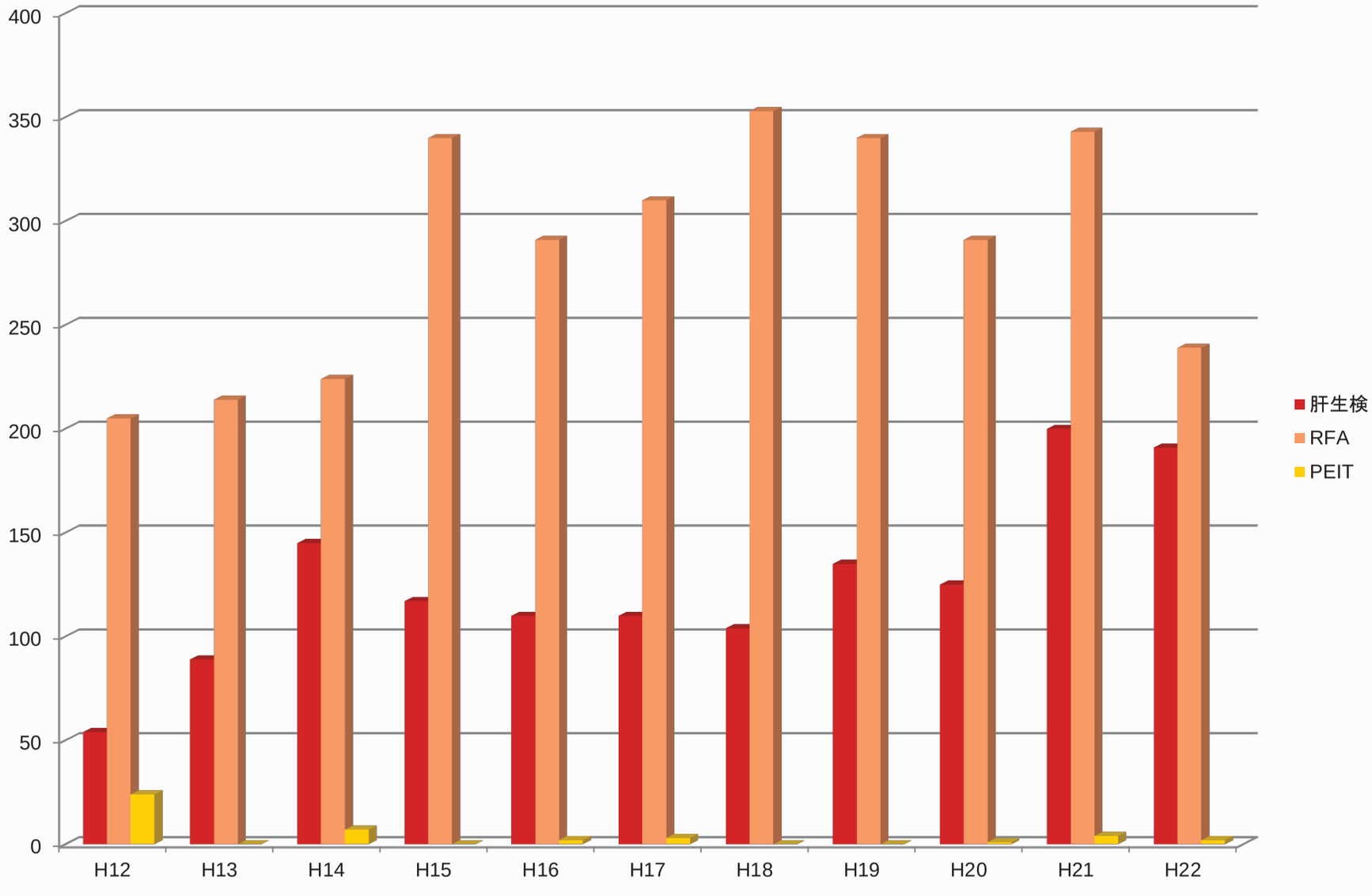
消化器内科年間外来収入



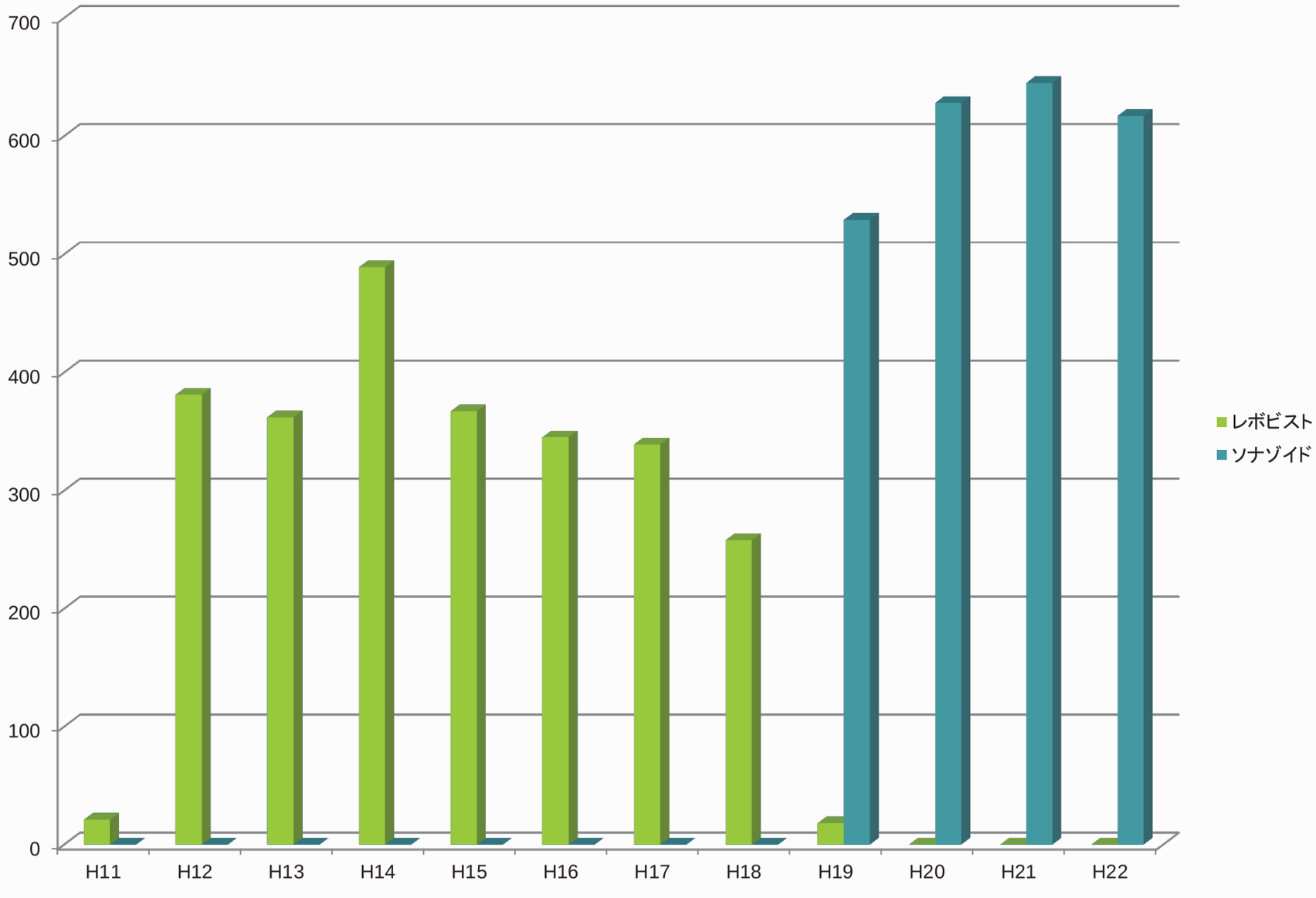
消化器内科年間総収入


3,000,000,000
2,500,000,000
2,000,000,000
1,500,000,000
1,000,000,000
500,000,000
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
消化器内科年間収入

経皮的局所治療・肝生検総件数



腹部造影エコー検査

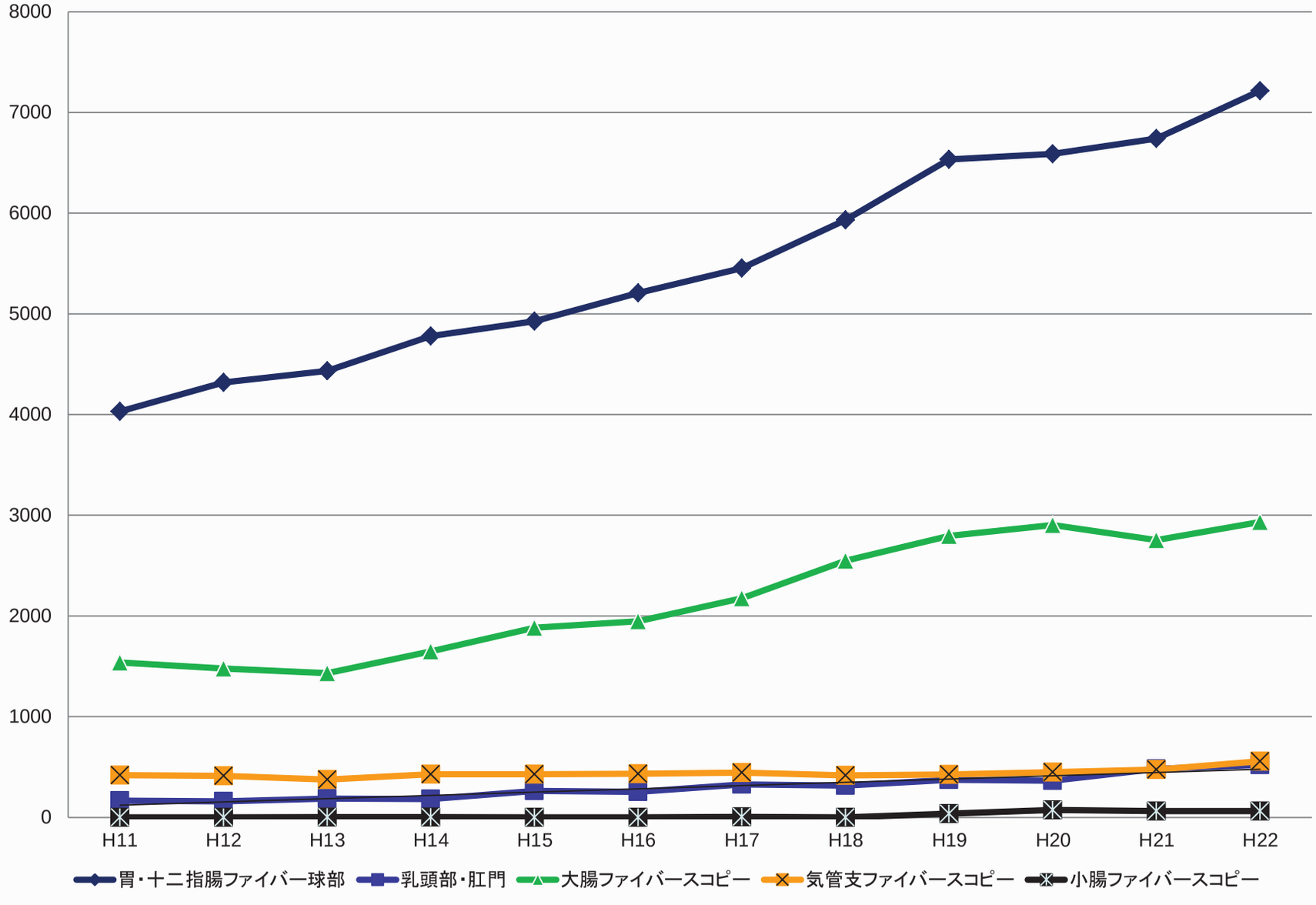

700
600
500
400
300
200
100
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
レボビスト
ソナゾイド

光学治療センター年報

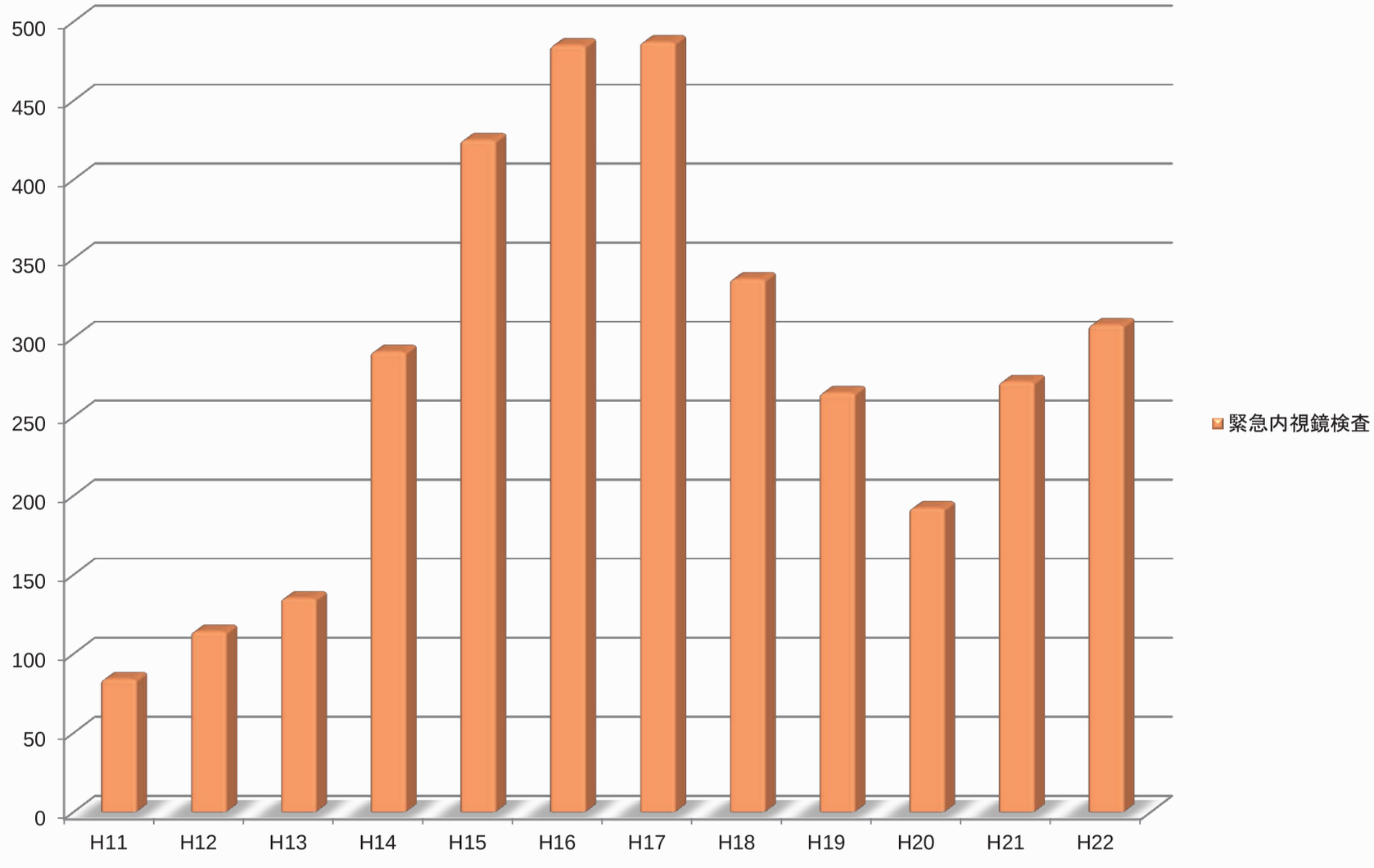
光学治療センター長 工藤正俊・消化器内視鏡部長 樫田博史

検査	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22
胃・十二指腸ファイバー球部	4032	4318	4434	4780	4926	5208	5453	5934	6534	6588	6742	7215
乳頭部・肛側	165	160	187	181	263	254	327	316	373	365	482	520
大腸ファイバースコピー	1537	1479	1433	1648	1883	1947	2175	2548	2796	2904	2754	2934
気管支ファイバースコピー	420	412	376	429	428	434	445	417	426	450	475	558
小腸ファイバースコピー	1	0	3	3	0	0	4	0	37	75	62	63
計（スクリーニング）	6155	6369	6433	7041	7500	7843	8404	9215	10166	10382	10515	11290
胃生検	1509	1650	1851	1908	2005	2101	2473	2533	2412	2261	2175	2134
大腸生検	1028	970	831	829	861	869	952	1143	1088	987	932	1001
気管支生検	245	222	227	265	260	296	281	314	302	314	345	393
小腸生検	1	0	0	2	0	0	1	0	11	8	12	18
計	2783	2842	2909	3004	3126	3266	3707	3990	3813	3570	3464	3546
胆道ドレナージ	43	43	60	66	121	96	151	122	141	142	234	225
乳頭切開	15	19	14	11	44	44	46	44	48	46	74	132
乳頭バルーン拡張術	0	6	8	2	1	0	9	11	13	5	5	9
結石除去	16	24	15	17	38	49	55	51	62	65	86	130
食道静脈瘤結紮術	72	79	51	62	97	100	75	58	81	83	62	69
硬化療法	2	9	13	13	23	12	7	4	5	12	11	7
EISL	-	22	52	56	47	54	40	27	22	11	26	31
食道ブジー	116	137	124	152	210	308	252	246	234	308	284	269
APC	6	8	22	23	25	32	24	52	72	65	68	32
異物除去	6	2	3	11	14	17	14	11	18	21	14	20
胃ポリペクトミー	12	18	18	16	7	12	9	1	2	0	1	2
大腸ポリペクトミー	257	300	296	344	397	111	89	66	41	20	22	42
EMR（胃）	27	24	64	73	70	73	90	100	52	127	52	39
ESD(胃)	-	-	-	-	36	32	52	51	71	82	98	109
EMR（大腸）	-	-	-	-	-	290	373	470	443	458	467	484
緊急内視鏡検査	84	114	135	291	425	485	487	337	265	192	272	308
凝固止血バイポーラ	35	48	50	91	91	68	89	52	51	32	63	63
色素撒布法	216	291	459	724	1194	1470	1582	1909	1753	1731	1891	1937
トロンビン被覆療法	81	88	113	193	198	236	294	369	387	319	326	282
アルト被覆療法	41	41	52	97	91	96	180	161	190	144	182	130
経皮内視鏡的胃瘻造設術	-	14	18	10	15	23	33	28	54	33	69	74
超音波内視鏡（胃）	48	77	181	170	205	249	293	379	505	523	622	895
超音波内視鏡（大腸）	-	17	28	6	8	5	5	0	1	0	1	0
計	1077	1381	1776	2428	3357	3836	4203	4498	4511	4419	4930	5289

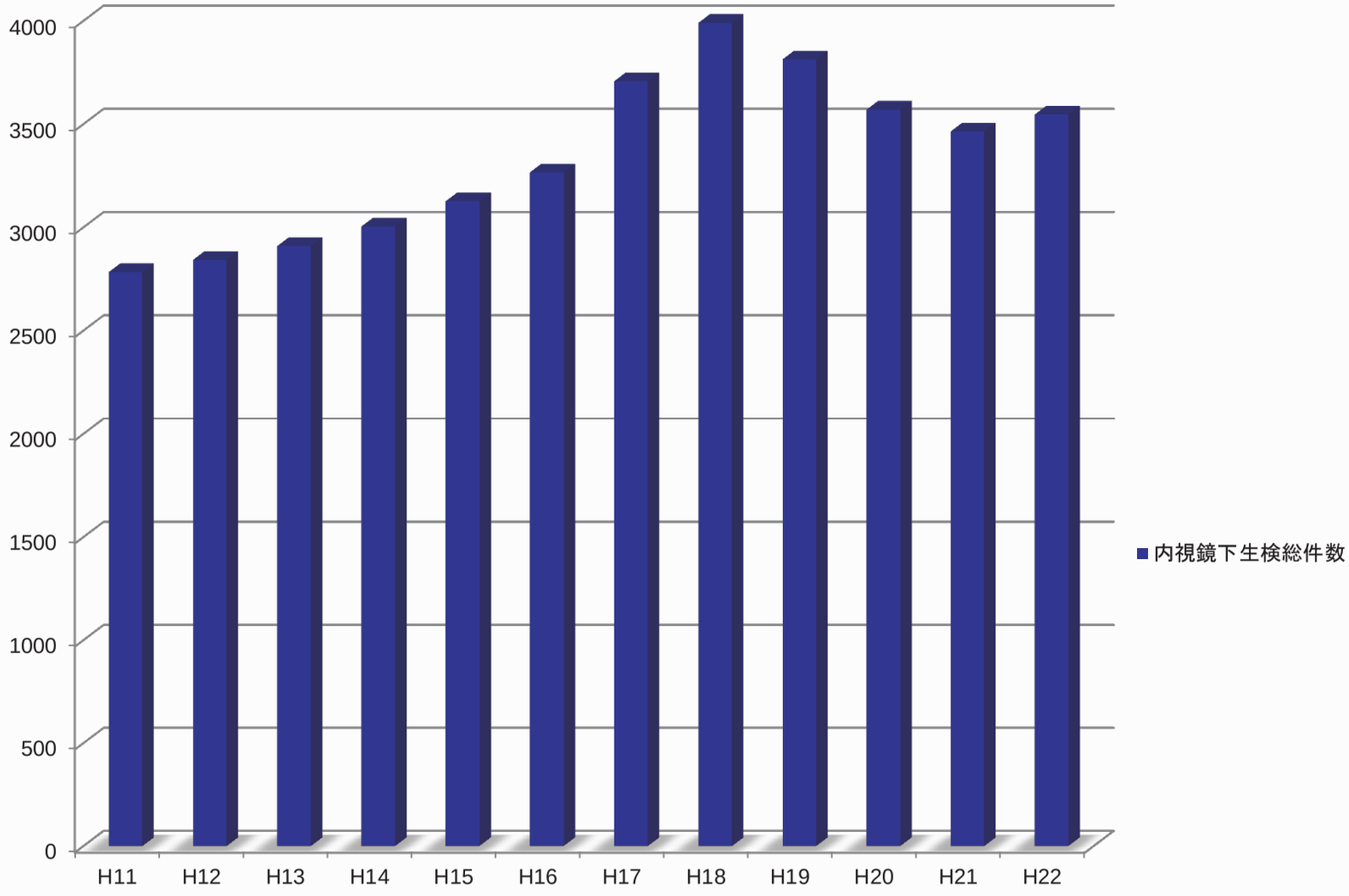
消化管内視鏡検査件数年次推移

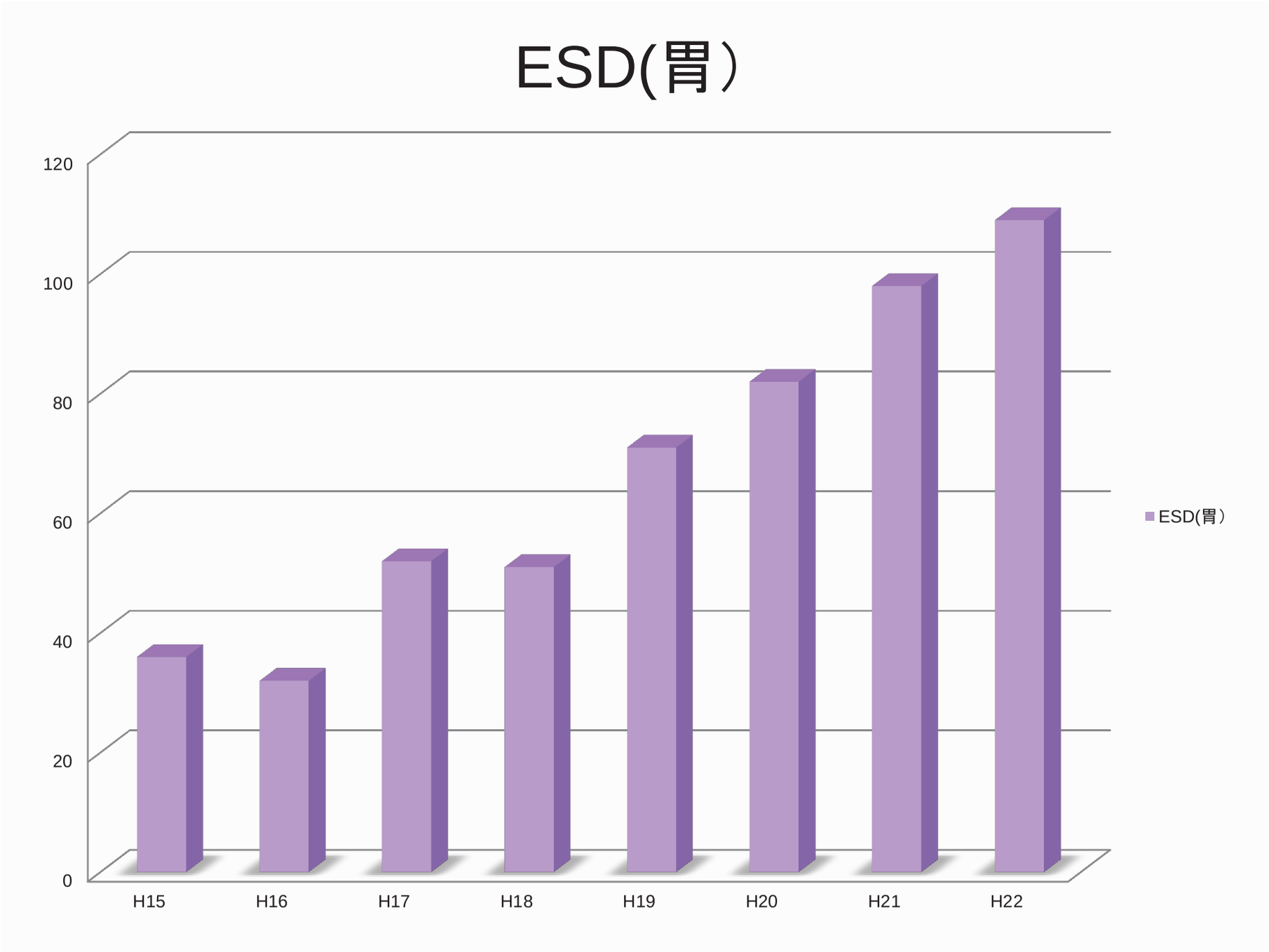


緊急内視鏡検査


緊急内視鏡検査
500
450
400
350
300
250
200
150
100
50
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
緊急内視鏡検査

内視鏡下生検総件数


4000
3500
3000
2500
2000
1500
1000
500
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
内視鏡下生検総件数


ESD(胃）
120
100
80
60
40
20
0
H15
H16
H17
H18
H19
H20
H21
H22
ESD(胃）


ERCP件数
12000
10000
8000
6000
4000
2000
0
H11
H12
H13
H14
H15
H16
H17
H18
H19
H20
H21
H22
ERCP件数

近畿大学　消化器内科学教室医局員

（平成 23 年 4 月現在）

主任教授	工藤正俊	S53	肝臓・消化器・肝癌の診断と治療
教授（内視鏡部）	樫田博史	S58	下部消化管
准教授	汐見幹夫	S55	上部・胆膵内視鏡（関空クリニック所長・教授兼務）
	北野雅之	H2	消化管全般・胆膵疾患
	西田直生志	S60	肝臓病学・肝癌の分子生物学
講師	松井繁長	H3	食道静脈瘤止血・上部消化管
	(医局長)		
医学部講師	上嶋一臣	H7	慢性肝炎・肝癌の治療
	(病棟医長)		
	櫻井俊治	H7	上部消化管・分子生物学
	南　康範	H9	肝疾患・消化器一般
	萩原　智	H10	肝疾患・消化器一般
	井上達夫	H11	肝疾患・消化器一般
	矢田典久	H11	肝疾患・消化器一般
	坂本洋城	H12	胆膵疾患・消化器一般
	朝隈　豊	H14	上部消化管・消化器一般
	北井　聡	H14	肝疾患・消化器一般
助教	小牧孝充	H7	胆膵疾患・消化器一般
	畑中絹世	H13	肝疾患の腹部超音波検査
	川崎正憲	H15	消化器内視鏡・消化器一般
	永井知行	H16	ゲノム生物学
	永田嘉昭	H16	消化器一般
	今井　元	H17	肝疾患・消化器一般
	早石宗右	H18	肝疾患・消化器一般
	有住忠晃	H19	肝疾患・消化器一般
	鎌田　研	H19	肝疾患・消化器一般
	峯　宏昌	H19	肝疾患・消化器一般
	宮田　剛	H19	肝疾患・消化器一般
	高山政樹	H19	肝疾患・消化器一般
	足立哲平	H20	肝疾患・消化器一般

	大本俊介	H20	肝疾患・消化器一般
	門阪薫平	H20	肝疾患・消化器一般
非常勤	黒田恵美	H12	肝疾患・消化器一般
	岡田無文	H13	消化器一般
	辰巳千栄	H15	肝疾患・消化器一般
	上田泰輔	H15	肝疾患・消化器一般
大学院 4 年	田北雅弘	H15	肝疾患・消化器一般
	早石宗右	H18	肝疾患・消化器一般
大学院 3 年	永田嘉昭	H16	肝疾患・消化器一般
	今井　元	H17	肝疾患・消化器一般
	有住忠晃	H19	肝疾患・消化器一般
	鎌田　研	H19	肝疾患・消化器一般
	宮田　剛	H19	肝疾患・消化器一般
	高山政樹	H19	肝疾患・消化器一般
大学院 2 年	峯　宏昌	H19	肝疾患・消化器一般
大学院 1 年	足立哲平	H20	肝疾患・消化器一般
	大本俊介	H20	肝疾患・消化器一般
	門阪薫平	H20	肝疾患・消化器一般
実験助手	原田八千代		
	安井章子		
臨床試験コーディネーター（CRC）			
	小川佳良子		
教授秘書	藤田真紀		
	井上真由美		
	村橋亜季		
	弓削公子		
日本肝癌研究会	田村利恵		
	前原なつみ		
医局秘書	胡桃由佳		
	朝隈　智		
	林　直子		

院外出張

辻　直子	S60	近畿大学堺病院　准教授・科長
山本典雄		近畿大学堺病院　臨床助教
奥村直巳	H21	近畿大学堺病院　臨床助教
高場雄久		近畿大学堺病院
梅原康湖	H12	近畿大学堺病院
川崎俊彦	S58	近畿大学奈良病院　准教授
岸谷　譲	S62	近畿大学奈良病院消化器内分泌内科　診療助教
加藤玲明	H11	
豊澤昌子	H13	近畿大学奈良病院消化器内分泌内科　診療助教
林　道友		近畿大学奈良病院消化器内分泌内科　診療助教
宮本容子	H12	近畿大学奈良病院消化器内分泌内科
宮部欽生	H14	近畿大学奈良病院消化器内分泌内科　診療助教
茂山朋広	H17	近畿大学奈良病院消化器内分泌内科　診療助教
奥田英之		近畿大学奈良病院消化器内分泌内科
鍋島紀滋	S61	天理よろづ相談所病院
山本健二		岡本クリニック
井上良一	S43	吉川病院内科
南野達夫	S55	なんの医院
中里　勝		上ヶ原病院
川端一史	H 元年	川端内科クリニック
米田　円	H 元年	米田内科
渡邉和彦	H3	結核予防会大阪府支部相談診療所
森村正嗣	H3	森村医院
遠田弘一	H7	
遠田由紀		
亀山千晴	H7	育和会記念病院
谷池聡子	H7	串本病院
工藤可苗	H12	近畿大学ゲノム生物学
仲谷達也	H3	仲谷・飯山クリニック
福永豊和	H4	北野病院消化器内科
由谷逸朗	S62	高石藤井病院
中岡良介	H8	山本病院内科
水野成人	S61	神戸薬科大学医療薬学講座
福田信宏	H10	朝日大学附属村上病院消化器内科

小川　力	H11	高松赤十字病院
坂口康浩	H11	河崎内科病院
永島美樹	H12	
冨田崇文	H14	冨田病院
坂本康明	H15	坂本医院
市川　勉	H13	内海町いちかわ診療所
齊藤佳寿	H14	
高橋俊介	H14	市立堺病院
西尾　健	H14	高石藤井病院
末冨洋一郎	H8	末冨放射線科医院
梅原　泰	H11	辻　腎太郎クリニック
鄭　浩柄	H8	神戸市立中央センター中央市民病院

略　歴

1983年　3月　京都大学医学部卒業
同年　　6月　神戸市立中央市民病院　　内科研修医
1985年　6月　　同　　消化器内科専攻医
1988年　6月　　同　　消化器内科医員
1990年　4月　　同　　消化器内科副医長
1997年　4月　　同　　消化器内科医長
2001年　8月　昭和大学助教授　横浜市北部病院消化器センター
（2007年より呼称変更　准教授）
2010 年　4月　近畿大学教授　医学部内科学教室（消化器内科部門）
2010 年10月　光学治療センター・消化器内視鏡部長

2010年4月1日付けで近畿大学消化器内科に赴任し、あっという間に月日が過ぎ去りました。今更ですが、自己紹介させて頂きます。

1959年京都市に生まれ、1983年3月京都大学医学部を卒業、神戸市立中央市民病院研修医として内科全般・救急・麻酔をローテートの後、消化器内科の道へ進みました。当時工藤正俊先生がおられたため、最初は肝癌の超音波（カラードプラ、造影）や血管造影などを得意としておりましたが、途中から胆膵領域の画像診断や内視鏡治療にも手を広げました。大腸疾患に本格的に取り組み始めたのは、かの有名な工藤進英先生に出会った1995年です。

2001年オープンの昭和大学横浜市北部消化器センターにお招きにあずかり、助(のち准)教授として赴任致しました。大所帯であるにも関わらず肝胆膵を担当する者が少なかったため、私は大腸を専門としながら、ERCPからRFA、インターフェロン治療まで担当していました。北部病院は世界的に有名になり、学会・研究会、班会議、ライブセミナーなどを、毎年5回くらいのペースで開催してきました。そのお陰で、研究会の運営にも多少慣れました。

縁あって2010年から近畿大学にお世話になることになりました。昔から尊敬していた工藤正俊先生と再び一緒に仕事をできるなんて、びっくりしました。上嶋・櫻井・鄭・矢田先生はかつての教え子（失礼）ですが、当時から光っていました。肝臓はいうまでもなく、胆膵領域でも活躍の目立つ医局の一員に加えて頂いて光栄です。

私は、早期大腸癌の診断、ESDを含む内視鏡治療、炎症性腸疾患などを中心に取り組んで参ります。未熟者ですが、好運にも2010年は海外での講演やライブデモンストレーションに招聘して頂く機会が多く、大変良い経験ができました。ぜひ若い医局員も海外へどんどん進出して欲しいと思っています。12月には高度先進医療としての大腸ESDに関し、

施行施設として厚生労働省の認定を受けることができました。病診連携の甲斐あって、近隣の先生方からの紹介も増えてきました。

10 月から突然、内視鏡室が「光学治療センター」と名称変更になり、ちょっと戸惑いました。設備が古く、大変手狭ですが、リニューアルの予定です。おそらく移転の上、スペースも広くなると期待しています。2011 年から本格的に計画が進むでしょう。近畿大学の内視鏡部を日本でも有数のセンターに発展させるべく、精一杯努力して参る所存です。御協力・御指導を、皆さま、宜しくお願い申し上げます。

汐見幹夫

【履歴】

1980年　3月　近畿大学医学部卒
1980年　5月　天理よろづ相談所病院　レジデント
1984年　4月　近畿大学医学部　第2内科　助手
1989年　4月　近畿大学医学部　第2内科　病院講師
1994年　4月　近畿大学医学部　第2内科　講師
1999年　4月　近畿大学医学部消化器内科　講師
1999年11月　近畿大学医学部附属病院内視鏡部　室長
2002年　3月　近畿大学医学部内科学教室消化器内科部門　助教授
2007年　4月　近畿大学医学部内科学教室消化器内科部門　准教授（職制移行）

1996年　9月　ドイツ・ハンブルグ・アルトナ総合病院　第1内科・内視鏡部留学
　～97年12月
現在に至る

頚椎症性神経根症の経験

頚椎は加齢とともに多かれ少なかれ進行性に変化し、変形性頚椎症をきたします。これは誰にでも起こることであって、このこと自体は病気ではありません。この変形性頚椎症が起こったために、神経根が圧迫されて、そのための痛みやしびれや麻痺が出てくる場合が頚椎症性神経根症です。頚椎症（椎間板ヘルニアや骨棘の形成など）によって、脊髄から別れて上肢へゆく「神経根」が直接圧迫されたり刺激されたり

して起こるわけですから、主症状は「耐え難い激痛」です。

僕の場合、初発症状は右肘から前腕にかけての痛みでした。特に重量物を持ったり、ぶつけたり誘因となるようなことは思い当たりません。筋肉痛・関節痛と思って、なるべく右手を使わないようにして湿布で様子をみていました。しかし、数日経っても一向に良くならないどころか、痛みは増強しました。NSAIDs を追加しても、痛みのためか肩甲骨から後頸部の筋肉はガチガチに凝って、頚も動かせない状況になりました。某整形外科医院を受診したところ、すぐさま肘ではなく、頚だと言われました。単純X線では骨棘形成が、数年前追突された時から軽度のものは指摘されていたのですが、増強していました。一部の Luschka 孔は左や右の上下と比べても明らかに狭小化していました。MRI を撮ると、軽度のヘルニアもありましたが、骨棘形成が多数にみられ、症状は Luschka 孔での脊髄神経根の圧排と炎症による「頚椎症性神経根症」との診断が確定しました。幸い、知覚・運動障害はありませんでしたが、それからの痛みとの闘いは壮絶でした。痛みは徐々に肩甲骨部から項部に移り、頚を傾けたり回すこと、右上肢を前方に挙上することができなくなりました。前屈みの姿勢、字を書いたり、キーボードの操作は最悪で、もちろん内視鏡もです。洗浄の水のシリンジが取れません。神経根が直接刺激されての神経痛ですからこれは激烈で、24時間持続します。膵癌の腹腔神経叢への浸潤による神経痛の経験はもちろんありませんが、恐らくそれに近いものだと思います。ボルタレンなども使いまくりましたが、胃がおかしくなっただけでほとんど効果はありませんし、トリガーポイント注射くらいではまず効きません。神経痛の新薬リリカも取り寄せて使いましたが効果なく、恐らく麻薬や神経ブロックでないと効かないのでしょう。最初の数日はステロイドの点滴と2週間程は内服で炎症を抑え、その間は少し効果がありましたが長くは続けられないので後は NSAIDs しかありません。痛みで眠れませんから眠剤も初めて連用しましたが、夜中に何度も痛くて眼が覚めます。そのうち、カラーを装着して頚を前屈して少し右を向き、身体は少し反り返ったような姿勢で右手を挙げて後頭部にあててじっとしていると痛みが少し和らぐことに気づきましたが、この姿勢は長く続けられるものでもありません。真剣に手術も考えました。整形よりも脳外科がいいとか、顕微鏡手術がいいとか色々助言をいただきました。矢田先生からはご家族が同じような治療を受けられた大津の名医（手術は2年待ちらしい）を紹介していただくことにもなっていましたが、なかなか決心はつきません。

整形外科医からは、マッサージは厳禁で安静に、2、3週休みなさいと言われましたが、なかなか休める消化器内科ではないでしょう・・・

一ヶ月待って、整体と牽引を開始しました。結局、これが一番効いた気がします。約一ヶ月であの痛みが嘘のように軽減しました。しかし、骨棘などはそのままですから再発がいつ起こるかわからない状況に変りはありません。原因のひとつは永年の内視鏡操作での無理な姿勢にあったことは間違いなさそうです。松井先生もどうぞ気をつけて下さいね。

まつい　しげなが
松井　　繁長
昭和４１年１２月２７日生まれ
出身地　東大阪市
平成３年　　近畿大学医学部卒業
平成３年　　近畿大学第２内科入局
平成５年　　近畿大学第２内科助手
平成１１年　近畿大学消化器内科助手
平成１４年　近畿大学消化器内科講師
専門領域
消化器病、消化器内視鏡、門脈圧亢進症、食道、胃静脈瘤
早期胃癌の内視鏡診断・治療
所属学会　資格など
近畿大学医学博士
日本内科学会（認定医）
日本消化器病学会（評議員・近畿支部評議員、専門医）
日本消化器内視鏡学会（学術評議員・近畿地方会評議員、指導医）
日本門脈圧亢進症学会（評議員）

みなさんお疲れ様です。医局長の松井です。医局長になって早くも 6 年目になりますが、医局長の仕事とは何であるか考えてもよくわからないまま現在に至っています。ただ言えることは医局内の問題、工藤先生から与えられた課題などすべて医局長の仕事であることです。今年も一年頑張りたいと思いますが、なかなか消化器内科は平穏とはいかないかもしれません。みなさんのご協力の程宜しくお願いします。
また、消化管グループにおいては、樫田教授、櫻井講師がメンバーに
加わり、充実した布陣となり、これから肝臓、胆・膵グループに負けないように臨床、研究面でさらに精進していきたいと思います。

氏名　鄭　浩柄（てい　ひろし）

学歴・職歴

平成 8 年	東京慈恵会医科大学卒業	
平成 8 年	神戸市立中央市民病院	内科研修医
平成 10 年	同	消化器内科専攻医
平成 12 年	近畿大学医学部附属病院	消化器内科　助手
平成 15 年	同	同　講師
	現在に至る	

主な所属学会

日本内科学会（認定医）
日本消化器病学会（専門医、近畿支部評議員）
日本肝臓学会（専門医）
日本超音波医学会（専門医、指導医）
日本消化器内視鏡学会
日本肝癌研究会
日本肝がん分子標的治療研究会

究極の選択

毎年、４月から翌２月にかけて医学部５回生が病棟実習（いわゆるクリクラ）のため２週毎に消化器内科をローテイトしており、私は毎週月曜朝に彼らのオリエンテーションを担当している。といってもごく簡単にスケジュールを説明し、グループ分けを行った後に各グループへ引き渡す、程度で大した仕事ではない。唯一、気に喰わない、というか憂鬱なのはグループ分けの際、「肝臓グループを志願する学生がきわめて少ない」事である。人気順は消化管＞胆膵＞肝臓で、たいていジャンケンで負けた学生が「じゃあ肝臓で・・」となる。紛れもなくここは「近畿大学消化器内科」のはずである。自分なりに理由を考えてみた事がある。「消化管グループは最も楽らしい、という認識が学生間で確立している」「回診前の症例呈示で、肝疾患だと教授に厳しく突っこまれる事が稀にある、ことがバレている」など。しかしリサーチした限り、どうも事実とは異なるようである。純粋に「肝疾患への興味が薄い」ことが理由のように思われた。確かに昨年久しぶりに市内への遊びついでに某メーカーが主催する消化管の研究会へふと出向いたところ、いつも参加するような肝臓の研究会とは明らかに雰囲気が異なっていた。内容が異なるので面子が違うのは当然であるが、とにかく若手が多いのである（女医も多かった）。きちんとした統計をとった訳ではないが、「肝臓内科医を志す若手消化器内科医師は明らかに減っている」ように思う。そういえば当科の現状を鑑みてもそうであった（理由は違うかも知れない）。今後Ｃ型肝炎患者、肝細胞癌患者の減少と共にさらに肝臓専門医志望者が減少するのではなかろうか？そう考えると「肝癌撲滅運動」微妙に複雑な思いである。スマートフォンには全く興味はないが流行を多少気にする自分と

しては、ESD・小腸内視鏡を川崎（正）に教わるか、EUS を宮田に教わるか、クールチップ片手に渡航延期勧告（平成 23 年 1 月 31 日現在）を無視してエジプト（C 型肝炎罹患率最多国）に渡るか、究極の選択を迫られている。

上嶋一臣（うえしまかずおみ）

略　　歴：

平成 7 年 3 月	神戸大学医学部卒	
平成 7 年～平成 9 年	神戸市立中央市民病院	内科研修医
平成 9 年～平成 12 年	神戸市立中央市民病院	救急部専攻医
平成 12 年～平成 14 年	神戸市立中央市民病院	消化器内科医員
平成 14 年～平成 16 年	神戸市立中央市民病院	消化器内科副医長
平成 17 年～	近畿大学医学部　消化器内科講師	

所属学会：　日本内科学会
日本消化器病学会
日本救急医学会
日本臨床救急医学会
日本肝臓学会
日本消化器内視鏡学会
日本超音波医学会
日本 IVR 学会
日本臨床腫瘍学会

研究会：　日本肝癌研究会
リザーバー研究会
日本肝がん分子標的治療研究会

資　格：　日本内科学会認定医
日本救急医学会専門医
日本消化器病学会専門医
日本消化器内視鏡学会専門医
日本肝臓学会専門医
日本医師会認定産業医

最近の私の業務において治験（臨床試験）の占める割合が大きくなってきている。臨床試験について私が思っていることを少し述べてみたいと思う。

臨床試験は新薬が世に出てくるためには必ず行わなければいけないものである。その実施施設については大学病院はいうまでもなく、日本全国の症例豊富な施設が対象になる。我々の施設も例外ではない。このような臨床試験を行うことは大学病院の重要な役割のひとつといえる。実際の業務としては確かに CRF の記入であるとか、Visit 毎のデータチェック、有害事象チェックなど多忙な外来業務にさらに負担がかかる内容ばかりである。このためか若いドクターからは臨床試験業務が敬遠されることが多い。

臨床試験にはご存知のように、第 I 相試験から第 IV 相試験まで 4 つの段階がある。第 I 相試験は薬剤の安全性、薬物動態を明らかにするために行われ、対象患者数は通常十数人である。第 II 相試験は探索的試験と位置づけられ、薬剤の効果をみるための試験であり、対象患者数は通常数十人である。そして第 III 相試験は、検証的試験と呼ばれ、薬剤の有効性と安全性について既存薬あるいは既存の治療との比較を行い検証する目的で行われる。通常ランダム化比較試験が行われ、対象患者数は数百人規模となる。第 IV 相試験はいわゆる市販後に実施される臨床試験であり、対象者は数千人規模になる。

われわれ医師の責務は患者さんを治療し尊い命を救うことである。しかし、一人の医師が一生で治療できる患者さんの数にはおのずと限りがある。さて臨床試験に携わればもっとたくさんの患者さんのためになるとは考えられないであろうか。すなわち第 I 相試験で一人の患者さんをエントリーし治療を行ったとすると、その 1 例は数年から十数年先の数百、数千、もっと多くの患者さんを治療していることにつながると考えられはしないであろうか。確かに目の前の患者さんを救うことはなによりも優先すべき尊いことであるが、臨床試験に参加することもまたより多くの患者さんを救う尊い業務であるわけである。そのように考えればまた臨床試験に対する見方も変わるのではないかと思っている。もし治験が面倒くさい業務と感じている先生がおられたなら、いちどこのように考えてみてもらえないだろうか。おのずとモチベーションが湧いてくると思う。

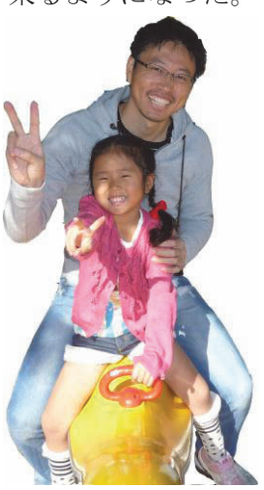
現在、近畿大学消化器内科は肝細胞癌をはじめ、膵癌、胆道癌、ウイルス性肝炎に対する臨床試験に多数参加している。これほど多くの臨床試験に参加している施設はそうそうない。非常に恵まれた環境であるといえる。特に肝臓領域においては、工藤教授がこの分野のオピニオンリーダーであることから、かなり特殊な臨床試験にも携わることが可能である。是非、この恵まれた環境を生かし、積極的に臨床試験に参加してほしいと思っている。

矢田　典久

経歴
1974 年 滋賀県甲賀郡（現　甲賀市）に生まれる
1999 年 滋賀医科大学卒業

1999 年 神戸市立中央市民病院 内科研修医
樫田先生・上嶋先生・鄭先生に指導を受ける。

2001 年 市立岸和田市民病院 消化器内科嘱託医
萩原先生に指導を受ける。
当時、TOSHIBA Aplio を用いて Levovist 造影・腸炎・小児エコーを得意とした。
思い出の症例：
小児の腎細胞癌・噴門狭窄症 2 例・十二指腸狭窄症 1 例を US で診断。無事に手術を受けてもらえた。お陰で小児科から直接エコー依頼が来るようになった。

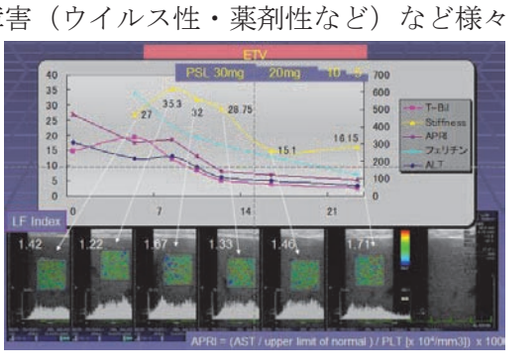


2004 年 京都大学大学院 消化器内科学に入り基礎研究に励む。
櫻井先生に指導を受ける。

2008 年 近畿大学医学部消化器内科 助教 B
2009 年 近畿大学医学部消化器内科 助教 A
2010 年 近畿大学医学部消化器内科 学内講師

近大に来た当初は、造影エコーを中心に研究するつもりだった。
2010 年から Elastography を担当する機会を得た。
更に NPO 法人日本肝がん臨床研究機構（JLOG）での研究（FIBROELAST Study と PICTURE Study）の事務局を担当させてもらえることになった。

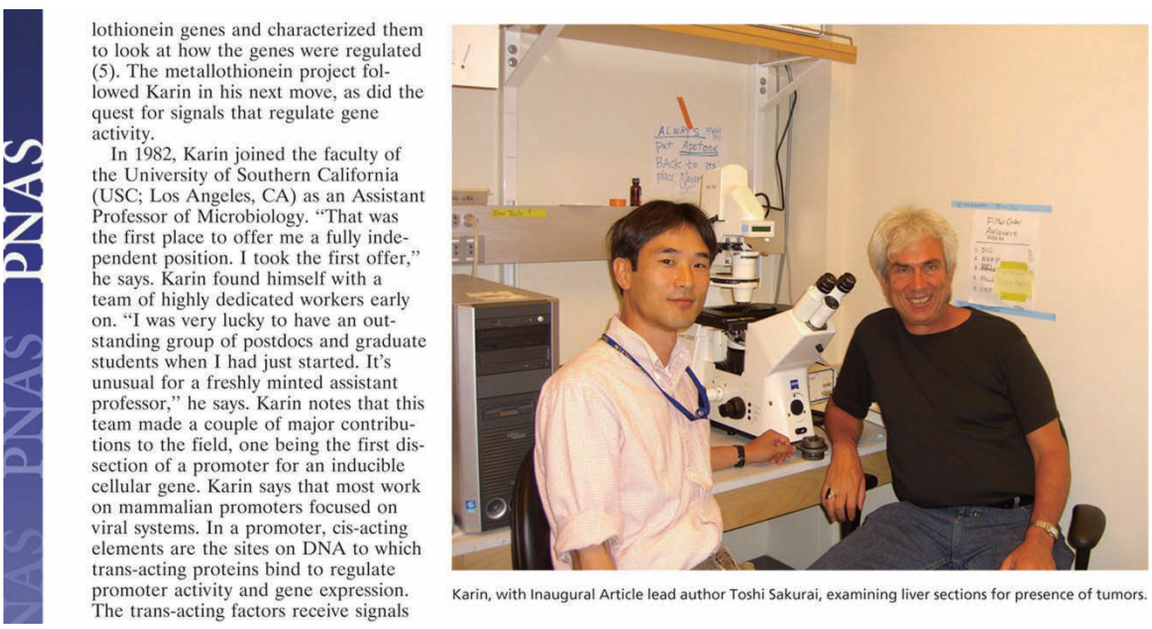
現在、B 型および C 型慢性肝炎だけでなく、非アルコール性脂肪肝、アルコール性肝障害、自己免疫性肝炎、原発性胆汁性肝硬変、急性肝障害（ウイルス性・薬剤性など）など様々な患者でデータを収集している。



症例を提供して下さっている皆様には、感謝しております。
これからもご協力お願い致します。

略歴

1989 年 (平成元年)　私立淳心学院高等学校　　卒業
1989 年　　京都大学　医学部　入学
1995 年　　神戸市立中央市民病院　内科研修医
1997 年　　天理よろづ相談所病院　消化器内科シニアレジデント
2004 年　　京都大学　医学博士
2004 年　　京都大学　医学部　助手
2005 年　　米国カリフォルニア大学サンディエゴ校　留学
2010 年　　近畿大学　医学部　講師



lothionein genes and characterized them to look at how the genes were regulated (5). The metallothionein project followed Karin in his next move, as did the quest for signals that regulate gene activity.

In 1982, Karin joined the faculty of the University of Southern California (USC; Los Angeles, CA) as an Assistant Professor of Microbiology. "That was the first place to offer me a fully independent position. I took the first offer," he says. Karin found himself with a team of highly dedicated workers early on. "I was very lucky to have an outstanding group of postdocs and graduate students when I had just started. It's unusual for a freshly minted assistant professor," he says. Karin notes that this team made a couple of major contributions to the field, one being the first dissection of a promoter for an inducible cellular gene. Karin says that most work on mammalian promoters focused on viral systems. In a promoter, cis-acting elements are the sites on DNA to which trans-acting proteins bind to regulate promoter activity and gene expression. The trans-acting factors receive signals

Karin, with Inaugural Article lead author Toshi Sakurai, examining liver sections for presence of tumors.

2010 年 4 月より近畿大学医学部消化器内科にて勤務させていただいております。

思い返せば、非常に自然な流れでここに赴任させていただいたような気がします。平成 7 年に卒業後すぐに神戸中央市民病院に赴任しました。そこでは工藤正俊教授、樫田博史教授に直接ご指導を受け消化器内科学について多くのことを学ばせていただき、その大きな影響のもの消化器内科医になりたいと心に決めました。その後、天理よろづ相談所病院にて消化器内科シニアレジデントとして勤務し、羽白清先生の下で多くの実践的な修練を積ませていただくと同時に、疾患の病態解明、サイエンスとしての医学の神髄に触れることができたように思います。学術的な雰囲気に染まった天理よろづ相談所病院を退職後、京都大学に戻り博士号を取得し、同助手を経て、米国カリフォルニア大学 Michael Karin 教授のもとで留学しました。これからも“臨床に基づく医学の探求”を目指して精進していきたいと存じます。よろしくお願いいたします。

櫻井俊治

上田　泰輔(ウエダ　タイスケ)

略歴

2003 年 4 月　近畿大学医学部消化器内科　入局
2005 年 6 月　市立岸和田市民病院　出向
2007 年 4 月　近畿大学医学部大学院　入学
2011 年 3 月　同上　修了予定

目標

・JDDW2010 横浜で発表した物を論文に。
・Skill　up、Blush up。

永井　知行

略歴）

04 年　　近畿大学医学部　卒業

04 年～06 年　　神戸労災病院　臨床研修医

06 年～08 年　　市立岸和田市民病院　消化器内科

08 年～現在　　近畿大学医学部附属病院　消化器内科　大学院（11 年 3 月修了見込み）

所属学会）

日本内科学会、日本消化器病学会、日本消化器内視鏡学会、日本肝臓学会

日本癌学会、日本分子標的学会

米国癌学会(AACR)

認定医、専門医）

日本内科学会認定医

日本消化器病学会専門医

2011 年度の目標

近畿大学　医学部附属病院　消化器内科　大学院生　永井　知行

去年はゲノム生物学教室で研究に従事し、多くの先生方のおかげで論文を書き上げることができました。 研究内容は単純で、特に目新しい事項ではないものの、夜遅くまで失敗を繰り返しながら初めて論文を作成したことから感慨深いものがありました。先日、研修医時代の同期の先生方と飲みに行く機会がありましたが、海外留学をされている先生や臨床の第一線で活躍されており、刺激を受けましたし、同期の先生方のようにアクティブにいきたいと思いました。

今年の目標として内視鏡学会専門医の取得を目指していたのですが、医師4年目に内視鏡学会に入会したため、受験資格がまだないことが判明したため断念しました。（若手の先生方はこのようなことがないように学会には早く入りましょう！）

そのため、今年は癌治療認定医の取得を目指すと同時に、分野は全く違いますが、今年の 10 月に大阪マラソンが開催されるようなので、それを目標にランニングも頑張っていきたいと思います。

有住忠晃　徳島県出身
略歴
昭和 57 年 2 月 6 日：出生（同じ誕生日にはベーブ・ルース、豊臣秀吉、アンパンマン）
昭和と平成にかけてはサッカー少年
平成 19 年 4 月：近畿大学医学部附属病院　研修医
平成 21 年 4 月：近畿大学医学部付属病院　消化器内科入局
平成 21 年 4 月：近畿大学医学部大学院内科学系　入学
現在に至る

入局後２年間、肝臓・胆膵・消化管グループを回らせていただきました。やはり消化器内科疾患は奥が深いため各グループもっと回りたいという気持ちが出てきています。出て来ると言えば今年の高校総体の全国大会に僕の母校の徳島北高校のサッカー部が出てきました。後輩達の活躍に感動をしました。今度は自分が全国へ羽ばたく番だと思い日々努力をしていこうと思います。

今年はいい魚が釣れず魚を載せることができませんでした・・・。

峯　宏昌

平成 19 年 3 月：近畿大学医学部卒業

平成 19 年 4 月：近畿大学医学部付属病院　研修医

平成 21 年 4 月：近畿大学医学部消化器内科　助教

平成 22 年 4 月：近畿大学医学部大学院内科学系　入学

同期より 1 年遅れで、今年より大学院入学となりました。1 年間助教をして得た経験もあれば、1 年間助教をしたから遅れていることもあり、日々精進の生活でした。

去年は「出産より痛かったです！」と温かいお言葉をいただきましたが、今年は「痛くないですか？」と聞くと、黙ったままハンカチがちぎれるほど噛まれている方がおられました。もう言葉にもならない痛みだったようです・・・

今年は多くの CF をこなすことができましたが、経験すればするほど、課題が見え、樫田教授をはじめ、先輩 Dr のご指導をいただいています。また今年は初の海外学会の参加や（ほとんど観光ばかり！？）、いろいろな研究会に参加させていただきました。

来年度からは消化管グループにお世話になり、内視鏡診断、治療の技術向上に努めたいと思います。今後ともどうぞご指導のほどよろしくお願いします。

中岡　良介

略歴

１９９６年　近畿大学医学部医学科　卒業
１９９６年　近畿大学医学部附属病院　第二内科入局（研修医）
１９９７年　ベルランド総合病院　内科（消化器）
１９９８年　近畿大学医学部附属病院　第二内科
１９９９年　近畿大学医学部附属病院　内科学　消化器内科学部門
２００５年　～　博寿会　山本病院　消化器内科

そのとき歴史は動いた

私のような立場の者が、近畿大学消化器内科の 2010 年年報に執筆して良いのかと自問しながらこの文章を作成しております。もう十年以上前の 1997 年、私は、当時イケイケの市中病院であったベルランド総合病院に消化器内科医として勤務していました。ベルランド総合病院は救急患者も多く、硫黄島の戦いのような過酷な職場でしたが先輩方にも恵まれ暖かく支えていただき充実した時間を過ごしておりました。工藤先生が、近大病院に赴任されたのもこの年です。非常に都会的で洗礼され、今まで近大にいないタイプのイケてる先生というのが始めて耳にした工藤先生の噂でした。実際、生工藤先生の印象は物静かですが熱い何かを内に秘めたナイスガイでした。しかも、当時の偉い先生の中では珍しく私の悪ノリも軽くいなす懐の深さも持ち合わせておられました。翌年、第二内科に帰ることになった私ですが、職場は硫黄島よりもずっと恵まれていたわけではありませんでした。ナンバー内科の臨床は様々な疾患を扱うことが多く決して消化器専門ではなかったこと、大学院の研究テーマはほとんどが内分泌・代謝だったこともあり私自身は悶々とした一年を過ごしておりました。そんな、1998 年の暮れに当時の第二内科教授の青木教授より呼び出しがありました。「来年、工藤君を中心とした消化器内科が独立することになるから。君は、消化器の方に進んでもらうから。」と言われました。よく考えると第二内科からは三行半を突きつけられたわけですが工藤消化器内科の誕生は、悶々といや粛々と仕事をしてい

た私にとって明日への光りの様な存在だったことを今でも覚えています。立ち上げは 7 人の侍のごとく、まさかの 7 人でした。今では考えられませんが医局会で工藤先生は「僕も何日かは、当直を覚悟しているよ」とおっしゃっておられました。これが、和を強調した工藤政権の誕生です。最年少医局員であった私は、いつも工藤先生の passion に目頭を熱くしておりました。そんな私も年を重ねました、2011 年の私の目標には、若い医局員が将来を夢見られる様な医局作りを外から応援することなどと奇麗ごとを掲げ脱稿させていただこうと思います。

石川恵美

略歴: 平成　11　年　近畿大学医学部消化器内科　入局
平成　13　年　近畿大学医学部消化器内科　助手
平成　22　年　近畿大学医学部消化器内科　非常勤医師

南大阪の C 型肝炎ウイルスを全駆除するべく毎日奔走しております。それとともに毎週グルメ班を設立し、おいしいものを食べにも行っています。これからもがんばりたいと思います。

川崎俊彦

略歴

昭和 58 年	京都大学医学部医学科専門課程卒業
昭和 58 年	京都大学医学部附属病院（研修医）
昭和 59 年	大阪府済生会野江病院（内科医員）
昭和 61 年	京都大学医学部附属病院（第一内科医員）
平成２年	京都桂病院（内科医員）
平成５年	Diagnostic Radiology, Yale University School of Medicine, (Visiting Scientist)
平成６年	神戸中央市民病院（内科副医長）
平成６年	西神戸医療センター（内科副医長）
平成９年	西神戸医療センター（内科医長）に昇進
平成 12 年	近畿大学医学部附属病院（講師）
平成 16 年	大阪北逓信病院第１内科（部長）
平成 22 年	近畿大学医学部奈良病院消化器・内分泌内科（准教授）

2010 年の総括、2011 年の抱負。

４月に赴任して思ったのは、６年前に３ヶ月だけ出向した時と比べてずいぶん忙しい病院に変わっていた事と、その割にはスタッフの数が増えていない事です。１年前までは後２名いたスタッフが、１年前に１人抜け、今年になりさらに１人抜けて、２人欠員の状態でのスタートでした。そして、この状態は未だに続いています。

２人欠員はさすがに厳しく、学会活動も自粛しないといけない状態でしたが、夜の１０時頃まで皆で残って ERCP や ESD をするのは一体感があり、それなりに楽しい経験でした。

来年の４月には、現在研修２年目の２人が消化器内科に入ってくれる事が決まったので、漸くまともな消化器内科として活動できるのではないかと思います。

近畿大学医学部奈良病院　消化器・内分泌内科　2010 年年報（1～12 月）

１．スタッフ

准教授	川崎俊彦（昭和 58 年卒）
講師	岸谷　譲（昭和 62 年卒）
診療助教	豊澤昌子（平成 12 年卒）
診療助教	宮部欽生（平成 14 年卒）
診療助教	茂山朋広（平成 17 年卒）
臨床助教	奥田英之（平成 19 年卒）

非常勤医師	水野成人
非常勤医師	加藤玲明
非常勤医師	宮本容子
非常勤医師	林　道友

２．臨床業績

1 日平均外来患者	101.1 人
1 日平均在院患者	30.0 人
平均在院日数	10.3 日

上部内視鏡検査	2940 件（含 ESD 57 件）
下部内視鏡検査	1680 件（含 EMR 262 件）
ERCP	214 件
腹部超音波	2405 件
腹部血管造影	110 件
ラジオ波治療	60 件

３．学会業績（地方会）

（１）日本消化器病学会近畿支部第 92 回例会「サイトメガロウイルス検査が陰性を示したがガンシクロビル投与により軽快した潰瘍性大腸炎も一例」

（２）日本消化器病学会近畿支部第 93 回例会「十二指腸に穿破し IVR にて止血しえた多発腹部内臓動脈瘤」

４．学会業績（研究会）

（１）第 8 回 R24 肝臓カンファレンス「集学的治療が奏効した巨大肝細胞癌の一例」

辻　直子

昭和 60 年　京都府立医科大学卒業
昭和 60 年　神戸市立中央市民病院　内科系研修医
昭和 62 年　明石市立市民病院　消化器内科　医員
平成　元年　大阪府立成人病センター　病理検査科　レジデント
平成　5 年　同　診療主任
平成　7 年　京都第一赤十字病院　胃腸科（現　消化器センター内科）　医長
平成　8 年　佑生会みどりヶ丘病院　消化器内科および臨床検査科　医長
平成 13 年　近畿大学医学部堺病院　消化器科　科長・講師
平成 18 年　同　助教授（現　消化器内科　准教授）　現在にいたる

所属学会

日本内科学会（専門医）、日本消化器内視鏡学会（指導医・近畿支部評議員）、日本消化器病学会（指導医）、日本肝臓学会、米国消化器病学会、日本癌学会、日本胃癌学会、日本食道学会、日本超音波医学会、日本病理学会（専門医）、日本臨床細胞学会、日本臨床検査医学会（専門医）、日本乳癌学会

日本がん治療認定医機構　暫定教育医、日本医師会認定産業医、日本医師会認定健康スポーツ医

受賞

平成 11 年度　日本消化器内視鏡学会　学会賞
平成 17 年度　日本消化器内視鏡学会　学会賞
平成 18 年度　日本胃癌学会　学会賞（西記念賞）

2011 年度の目標

2010年には後期研修2年目の奥村・山本先生がそろってJDDWのポスター優秀演題賞を受賞し、また奥村先生が UEGW2010 で Travel Grant を受賞しました。思わぬサプライズで award というのは日ごろの忙しさを労いモチベーションを上げてくれるものだとつくづく感じました。
2011 年度は南先生が本院へ帰るため堺病院のスタッフは私を除き卒後 5 年目 2 人、4 年目1人、3 年目1人と非常に若いメンバーとなり、目標はまず事故なく無事に1年を終えることです。若い医師の教育のみが堺病院に残された使命ですので、教育目標としてルーチン検査が問題なくこなせるようになった5年目となる奥村・山本先生は内科認定医の取得（梅原康湖先生のお尻たたきのもとサマリーは提出済み）と 6 年目以降の臨床研究に向けての英文ペーパーの読み書きの練習を開始、4 年目となる高場先生は引き続き内視鏡やベッドサイドのトレーニング、春から迎える3年目の松本先生はいちからの消化器内科や内視鏡の手ほどきです。余力があれば私自身は以前から興味をもっていた胆汁酸逆流と胃や食道胃接合部病変との関連の研究を始めてみたいのですが、本当に 1 日があと 2 時間ほど長くならないかと思う毎日です。ころころ方向転換する堺病院に心乱されることなく平常心を保って今年も細々と情熱をつないでいこうと考えています。

2010 年度　堺病院消化器内科診療実績ならびに業績

スタッフ

辻　直子（科長・准教授）南　康範（診療講師）山本典雄・奥村直己・高場雄久（臨床助教）

診療実績

① 外来　件数　7711 件、延件数　12524 件、診療額　218987982 円
② 入院　件数　1006 件、延入院　8338、診療額　339416250 円
③ 紹介患者数　775 人、救急搬送患者数　132 人、初診患者数　1315 人
④ 内視鏡検査数(2009 年度) 上部消化管　2039 件、下部消化管　1319 件、ERCP　87 件、EUS 10 件、治療内視鏡　406 件

業績

国際学会

① Okumura N, et.al. Percutaneous endoscopic gastrosotmy with Funada-style gastropexy, an easy and safe technique, greatly reduce the risk of peristomal infection UEGW2010 Barcelona 2010. 10 （Travel Grant 受賞）
② Yamamoto N,　et al. Colonoscopic polypectomy in the very elderly, is it safe? UEGW2010 Barcelona 2010.10

国内総会

① 南　康範 他　造影超音波による血流定量化の試み-肝細胞癌に対する TACE の早期治療効果判定-　第 14 回日本肝臓学会大会　2010.10
② 梅原康湖　他　高齢者の外来下部消化管内視鏡検査におけるプロポフォール至適導入量の検討.　第 80 回日本消化器内視鏡学会総会　2010.10
③ 山本典雄 他　消化管悪性リンパ腫の初回内視鏡診断と病理診断の問題点. 第 80 回日本消化器内視鏡学会総会　2010.10 （優秀演題賞受賞）
④ 奥村直己　他　経皮内視鏡的胃瘻増設術（PEG）における胃壁固定の有用性と問題点. 第 80 回日本消化器内視鏡学会総会　2010.10 （優秀演題賞受賞）
⑤ 高場雄久　他　胃壁固定併用の経皮内視鏡的胃瘻増設術（PEG）における pull 法と direct 法の比較検討. 第 80 回日本消化器内視鏡学会総会　2010.10

国内地方会

① 山本典雄　他　Malignant gastric outlet obstruction (MGOO)に対するステント留置術と胃空腸吻合術の比較検討.パネルディスカッション「消化管ステント留置の苦痛と限界」 第 86 回日本消化器内視鏡学会近畿地方会パネルディスカッション　2011.3
②　 他　一般演題6題

消化器内科学教室業績一覧（2010年）

I. 英文論文

1. 2010 **Kudo M***: Viral hepatitis A to E: An update in 2010. **Intervirology** 53: 5-9, 2010. **(IF=1.756)**

2. 2010 Chung H, Ueda T, **Kudo M***: Changing trends in hepatitis C interfection over the past 50 years in Japan. **Intervirology** 53: 39-43, 2010. **(IF=1.756)**

3. 2010 Kim SR, Imoto S, **Kudo M**, Mita K, Taniguchi M, Kim KI, Sasase N, Shouji I, Ngano M, El-Shamy A, Hotta H, Nagai T, Nagata Y, Hayashi Y: Double-filtration plasmapheresis plus IFN for non-sustained virological response to previous combination therapy: early viral dynamics. **Intervirology** 53: 44-48, 2010. **(IF=1.756)**

4. 2010 Sasase N, Kim SR, **Kudo M**, Kim KI, Taniguchi M, Imoto S, Mita K, Hayashi Y, Shouji I, El-Shamy A, Hotta H: Outcome and early viral dynamics with viral mutation in PEG-IFN/RBV therapy for chronic hepatitis in patients with high viral loads of serum HCV RNA genotype 1b. **Intervirology** 53: 49-54, 2010. **(IF=1.756)**

5. 2010 Ueda T, Chung H, **Kudo M***, Ishikawa E, Hayaishi S, Tatsumi C, Inoue T, Yada N, Hagiwara S, Minami Y, Ueshima K: Prolonged PEG-IFN and RBV is effective in patients with HCV genotype 1 and high viral load who achieved virological response later than 24 weeks. **Intervirology** 53: 55-59, 2010. **(IF=1.756)**

6. 2010 Yada N, **Kudo M***, Chung H, Hayaishi S, Takita M, Ueda T, Tatsumi C, Hatanaka K, Kitai S, Ishikawa E, Inoue T, Hagiwara S, Ueshima K: PEG-IFN α/RBV combination therapy for choronic hepatitis C patients increases serum ferritin level while it improves sustained viral response rate. **Intervirology** 53: 60-65, 2010. **(IF=1.756)**

7. 2010 Tatsumi C, **Kudo M***, Ueshima K, Kitai S, Ishikawa E, Yada N, Hagiwara S, Inoue T, Minami Y, Chung H, Maekawa K, Fujimoto

K, Kato M, Tonomura A, Mitake T, Shiina T: Non-invasive evaluation of hepatic fibrosis for type C chronic hepatitis. **Intervirology** 53: 76-81, 2010. **(IF=1.756)**

8. 2010 Kitano M, Sakamoto H, Das K, Komaki T, **Kudo M**: EUS-guided in vivo microdialysis of the pancreas: a novel technique with potential diagnostic and therapeutic application. **Gastrointest Endosc** 71: 176-179, 2010. **(IF=5.608)**

9. 2010 Takayasu K, Arii S, Ikai I, **Kudo M**, Matsuyama Y, Kojiro M, Makuuchi M; Liver Cancer Study Group of Japan: Overall survival after transarterial lipiodol infusion chemotherapy with and without embolization for unresectable hepatocellular carcinoma: propensity score analysis. **AJR Am J Roentgenol** 194: 830-837, 2010. **(IF=2.797)**

10. 2010 Izumi N, Nishiguchi S, Hino K, Suzuki F, Kumada H, Itoh Y, Asahina Y, Tamori A, Hiramatsu N, Hayashi N, **Kudo M**: Management of hepatitis C: Report of the consensus meeting at the 45th annual meeting of the Japan Society of Hepatology (2009). **Hepatol Res** 40: 347-368, 2010. **(IF=1.857)**

11. 2010 **Kudo M***: The 2008 Okuda lecture: Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. **J Gastroen Hepatol** 25: 439-452, 2010. **(IF=2.410)**

12. 2010 Sakamoto H, Kitano M, kamata K, El-Masry M, **Kudo M**: Diagnosis of pancreatic tumors by endoscopic ultrasonography. **World J Radiol** 2: 122-134, 2010. **(IF=0.000)**

13. 2010 Minami Y, **Kudo M***, Hatanaka K, Kitai S, Inoue T, Hagiwara S, Chung H, Ueshima K: Radiofrequency ablation guided by contrast harmonic sonography using perfluorocarbon microbubbles (Sonazoid) for hepatic malignancies: an initial experience. **Liver Int** 30: 759-764, 2010. **(IF=3.840)**

14. 2010 Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, **Kudo M**, Lee JM, Choi BI, Poon RTP, Shiina S, Cheng AL, Jia JD, Obi S, Han KH, Jafri W, Chow P, Lim SG, Chawla YK, Budihusodo U, Gani RA, Lesmana CR, Putranto TA, Liaw YF, Sarin SK: Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. **Hepatol Int** 4: 439-474, 2010. **(IF=2.963)**

15. 2010 Miura N, Osaki Y, Nagashima M, Kohno M, Yorozu K, Shomori K, Kanbe T, Oyama K, Kishimoto Y, Maruyama S, Noma E, Horie Y, **Kudo M**, Sakaguchi S, Hirooka Y, Ito H, Kawasaki H, Hasegawa J, Shiota G: A novel biomarker TERTmRNA is applicable for early detection of hepatoma. **BMC Gastroenterol** 10: 46-57, 2010. **(IF=2.468)**

16. 2010 Lencioni R, Marrero J, Venook A, Ye SL, **Kudo M**: Design and rationale for the non-interventional Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib (GIDEON) study. **Int J Clin Pract** 64: 1034-1041, 2010. **(IF=2.309)**

17. 2010 **Kudo M***: Current status of molecularly targeted therapy for hepatocellular carcinoma: clinical practice. **Int J Clin Oncol** 15: 242-255, 2010. **(IF=1.437)**

18. 2010 **Kudo M***: Management of hepatocellular carcinoma: from the prevention to molecular targeted therapy. **Oncology** 78: S1-6, 2010. **(IF=2.538)**

19. 2010 **Kudo M***, Hatanaka K, Maekawa K: Newly developed novel ultrasound technique, defect reperfusion ultrasound imaging, using Sonazoid in the management of hepatocellular carcinoma. **Oncology** 78: S40-45, 2010. **(IF=2.538)**

20. 2010 Hatanaka K, Chung H, **Kudo M***, Haji S, Minami Y, Maekawa K, Hayaishi S, Nagai T, Takita M, Kudo K, Ueda T, Tatsumi C, Kitai S, Ishikawa E, Yada N, Inoue T, Hagiwara S, Ueshima K: Usefulness of the post-vascular phase of contrast-enhanced ultrasonography with Sonazoid in the evaluation of gross types of Hepatocellular carcinoma. **Oncology** 78: S53-59, 2010. **(IF=2.538)**

21. 2010 **Kudo M***, Hatanaka K, Inoue T, Maekawa K: Depiction of portal supply in early hepatocellular carcinoma and dysplastic nodule: value of pure arterial ultrasound imaging in hepatocellular carcinoma. **Oncology** 78: S60-67, 2010. **(IF=2.538)**

22. 2010 Andreana L, **Kudo M***, Hatanaka K, Chung H, Minami Y, Maekawa K, Ruggiero G: Contrast-enhanced ultrasound techniques for

guiding and assessing response to locoregional treatments for hepatocellular carcinoma. **Oncology** 78: S68-77, 2010. **(IF=2.538)**

23. 2010 **Kudo M***: Will Gd-EOB-MRI change the diagnostic algorithm in hepatocellular carcinoma? **Oncology** 78: S87-93, 2010. **(IF=2.538)**

24. 2010 Inoue T, Minami Y, Chung H, Hayaishi S, Ueda T, Tatsumi C, Takita M, Kitai S, Hatanaka K, Ishikawa E, Yada N, Hagiwara S, Ueshima K, **Kudo M***: Radiofrequency ablation for hepatocellular carcinoma: assistant techniques for difficult cases. **Oncology** 78: S94-101, 2010. **(IF=2.538)**

25. 2010 **Kudo M***: Radiofrequency ablation for hepatocellular carcinoma: updated review in 2010. **Oncology** 78: S113-124, 2010. **(IF=2.538)**

26. 2010 Ueshima K, **Kudo M***, Takita M, Nagai T, Tatsumi C, Ueda T, Kitai S, Ishikawa E, Yada N, Inoue T, Hagiwara S, Minami Y, Chug H: Hepatic arterial infusion chemotherapy using low-dose 5-fluorouracil and cisplatin for advanced hepatocellular carcinoma. **Oncology** 78: S148-153, 2010. **(IF=2.538)**

27. 2010 **Kudo M***, Ueshima K: Positioning of a molecular-targeted agent, Sorafenib, in the treatment algorithm for hapatocellular carcinoma and implication of many complete remission cases in Japan. **Oncology** 78: S154-166, 2010. **(IF=2.538)**

28. 2010 **Kudo M***: Real practice of hepatocellular carcinoma in Japan: conclusions of the Japan Society of Hepatology 2009 Kobe Congress. **Oncology** 78: S180-188, 2010. **(IF=2.538)**

29. 2010 Ishikawa E, **Kudo M**, Minami Y, Ueshima K, Kitai S, Ueda K: Cecal intussusception in an adult with cronkhite-Canada syndrome relieved by colonoscopy. **Intern Med** 49: 1123-1126, 2010. **(IF=1.037)**

30. 2010 Kim SR, Imoto S, **Kudo M**, Nakajima T, Ando K, Mita K, Fukuda K, Hong HS, Lee YH, Nakashima K, Shoji I, Nagano-Fujii M, Hotta H, Hayashi Y: Autoimmune thrombocytopenic purpura during pegylated interferon α treatment for chronic hepatitis C. **Intern Med** 49: 1119-1122, 2010. **(IF=1.037)**

31. 2010 Makuuchi M, Kokudo N, Arii S, Igaki H, Ikai I, Kaneko S, Kawasaki S, **Kudo M**, Matsuyama Y, Ohtomo K, Okazaki M, Omata M, Takayama T, Takayasu K, Tateishi R: Clinical practice guidelines for hepatocellular carcinoma -The Japan Society of Hepatology 2009 update. **Hepatol Res** 40: S1-144, 2010. (IF=1.857)

32. 2010 **Kudo M***, Makuuchi M, Kokudo N, Arii S, Igaki H, Ikai I, Kaneko S, Kawasaki S, Matsuyama Y, Ohtomo K, Okazaki M, Omata M, Takayama T, Takayasu K, Tateishi R: Local ablation therapy. **Hepatol Res** 40: S113-119, 2010. (IF=1.857)

33. 2010 Arii S, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, Kokudo N, Tanaka M, Takayama T, **Kudo M***: Management of hepatocellular carcinoma: Report of consensus meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009). **Hepatol Res** 40: 667-685, 2010. (IF=1.857)

34. 2010 **Kudo M***, Kubo S, Takayasu K, Sakamoto M, Tanaka M, Ikai I, Furuse J, Nakamura K, Makuuchi M, for The Liver Cancer Study Group of Japan (Committee for response evaluation criteria in cancer of the liver, Liver cancer study group of Japan): Response evaluatin criteria in cancer of the liver (RECICL) proposed by the liver cancer study group of Japan (2009 revised version). **Hepatol Res** 40: 686-692, 2010. (IF=1.857)

35. 2010 Das K, Kitano M, Komaki T, Sakamoto H, Noda K, Suetomi Y, **Kudo M**: Pancreatic ductal drainage by endoscopic ultrasound-assisted rendezvous technique for pain caused by ductal stricture with chronic pancreatitis. **Digest Endosc** 22: 217-219, 2010. **(IF=0.946)**

36. 2010 Umehara Y, **Kudo M***, Kawasaki M: Endoscopic findings of intestinal Behcet's disease complicated with toxic megacolon. **Endoscopy** 42: E173-174, 2010. **(IF=6.096)**

37. 2010 Minami Y, **Kudo M**: Hepatic malignancies: Correlation between sonographic findings and pathological features. **World J Radiol** 2: 249-256, 2010. **(IF=0.000)**

38. 2010 Mita K, Kim SR, **Kudo M**, Imoto S, Nakajima T, Ando K, Fukuda

K, Matsuoka T, Maekawa Y, Hayashi Y: Diagnostic sensitivity of imaging modalities for hepatocellular carcinoma smaller than 2cm. **World J Gastroenterol** 16: 4187-4192, 2010. **(IF=2.240)**

39. 2010 Chung H, Watanabe T, **Kudo M***, Chiba T: Hepatitis C virus core protein induces homotolerance cross-tolerance to Toll-like receptor ligands by activation of Toll-like receptor 2. **J Infect Dis** 202: 853-861, 2010. **(IF=6.288)**

40. 2010 **Kudo M**, Han KH, Kokudo N, Cheng AL, Choi BI, Furuse J, Izumi N, Park JW, Poon RT, Sakamoto M: Liver cancer working group report. **Jpn J Clin Oncol** 40: *i*19- *i* 27, 2010. **(IF=1.856)**

41. 2010 Ikai I, **Kudo M**, Arii S, Omata M, Kojiro M, Sakamoto M, Takayasu K, Hayashi N, Makuuchi M, Matsuyama Y, Monden M: Report of the 18th follow-up survey of primary liver cancer in Japan. **Hepatol Res** 40: 1043-1059, 2010. **(IF=1.857)**

42. 2010 Minami Y, **Kudo M**: Radiofrequency ablation of hepatocellular carcinoma: Current status. **World J Radiol** 2: 417-424, 2010. **(IF=0.000)**

43. 2010 Marrero J, **Kudo M**, Bronowicki JP: The challenge of prognosis and staging for hepatocellular carcinoma. **Oncologist** 4: 23-33, 2010. **(IF=5.826)**

44. 2010 Sakamoto H, Kitano M, Kamata K, Komaki T, Imai H, Chikugo T, Taketyama Y, **Kudo M**: EUS-guided broad plexus-neurolysis over the superior mesenteric artery using a 25 gauge needle. **Am J Gastroenterol** 105: 2599-2606, 2010. **(IF=6.882)**

45. 2010 Xia Y, Kitano M, **Kudo M**, Imai H, Kamata K, Sakamoto H, Komaki T: Characterization of intra-abdominal lesions of undetermined origin by contrast-enhanced harmonic EUS (with videos). **Gastrointest Endosc** 72: 637-642, 2010. **(IF=5.608)**

46. 2010 Furuse J, Okusaka T, Kaneko S, **Kudo M**, Nakachi K, Ueno H, Yamashita T, Ueshima K: Phase I/II study of the pharmacokinetics, safety, and efficacy of S-1 in patients with advanced hepatocellular carcinoma. **Cancer Sci** 101: 2606-2611, 2010. **(IF=3.846)**

47. 2010 Sakamoto H, Kitano M, **Kudo M**: Diagnosis of subepithelial tumors in the upper gastrointestinal tract by EUS. **World J Radiol** 2: 289-297, 2010. **(IF=0.000)**

48. 2010 Kamata K, Kitano M, **Kudo M**, Imai H, Sakamoto H, Komaki T: Endoscopic ultrasound (EUS)-guided transluminal endoscopic removal of gallstones. **Endoscopy** 42: E331-332, 2010. **(IF=6.096)**

49. 2010 Wada Y, Kashida H, Kudo S, Misawa M, Ikehara N, Hamatani S: Diagnostic accuracy of pit pattern and vascular pattern analysis in colorectal lesions. **Digest Endosco** 22: 192-199, 2010. **(IF=0.946)**

50. 2010 Hirose M, Fukui H, Igarashi Y, Fujimori Y, Katake Y, Sekikawa A, Ichikawa K,Tomita S, Imura J, Ajioka Y, Ueno H, Hase K, Ohkura Y, Kashida H, Togashi K, Nishigami T, Matsui T, Yao T, Wada R, Matsuda K, Watanabe T, Ochiai A, Sugai T, Sugihara K, Fujimori T: Detection of desmoplastic reaction in biopsy specimens is useful for predicting the depth of invasion of early colorectal cancer: a Japanese collaborative study. **J Gastroenterol** 45: 1212-1218, 2010. **(IF=3.610)**

51. 2011 Nagai T, Arao T, Furuta K, Sakai K, Kudo K, Kaneda H, Tamura D, Aomatsu K, Kimura H, Fujita Y, Matsumoto K, Saijo N, **Kudo M**, Nishio K: Sorafenib inhibits the hepatocyte growth factor-mediated epithelial mesenchymal transition in hepatocellular carcinoma. **Mol Cancer Ther** 10: 169-177, 2011. **(IF=5.225)**

52. 2011 **Kudo M**, Hatanaka K, Kumada T, Toyoda H, Tada T: Double-contrast ultrasoud: a novel surveillance tool for hepatocellular carcinoma. **Am J Gastroenterol** 106: 368-370, 2011. **(IF=6.882)**

53. 2011 Kudo K, Arao T, Tanaka K, Kaneda H, Matsumoto K, Tamura D, Aomatsu K, Velasco M, Fujita Y, Saijo N, **Kudo M**, Nishio K: Antitumor activity of BIBF 1120, a vascular endothelial

growth factor-2 inhibitor and use of VEGR2+pTYR+leucocyte as a pharmacodynamic biomarker. **Clin Cancer Res** 17: 1373-1381, 2011. **(IF=7.338)**

54. 2011 Hagiwara S, **Kudo M***, Ueshima K, Chung H, Yamaguchi M, Takita M, Haji S, Kimura M, Arao T, Nishio K, Park AM, Munakata H: The cancer stem cell marker CD133 is a predictor of the effectiveness of S1+pegylated interferon α-2b therapy against advanced hepatocellular carcinoma. **J Gastroenterol** 46: 212-221, 2011. **(IF=3.610)**

55. 2011 Sakamoto H, Kitano M, Matsui S, Kamata K, Komaki T, Imai H, Dote K, **Kudo M**: Estimation of malignant potential of GI stromal tumors by contrast-enhanced harmonic EUS. **Gastrointest Endosc** 73: 227-237, 2011. **(IF=5.608)**

56. 2011 Kagawa Y, Okada M, Kumano S, Katsube T, Imaoka I, Tanigawa N, Ishii K, **Kudo M**, Murakami T: Optimal scanning protocol of arterial dominant phase for hypervascular hepatocellular carcinoma with gadolinium-ethoxybenzyl -diethylenetriamine pentaacetic acid-enhanced MR. **J Magn Reson Imaging** 33: 864-872, 2011. **(IF=2.747)**

57. 2011 Yokosuka O, Kurosaki M, Imazeki F, Arase Y, Tanaka Y, Chayama K, Tanaka E, Kumada H, Izumi N, Mizokmi M, **Kudo M**: Management of hepatitis B: Consensus of Japan Society of Hepatology 2009. **Hepatol Res** 41: 1-21, 2011. **(IF=1.857)**

58. 2011 Katsube T, Okada M, Kumano S, Hori M, Imaoka I, Ishii K, **Kudo M**, Kitagaki H, Murakami T: Estimation of liver function using T1 mapping onGd-EOB-DTPA-enhanced magnetic resonance imaging. **Invest Radiol** 46: 277-283, 2011. **(IF=4.665)**

59. 2011 **Kudo M**, Yamao K, Shimosegawa T: The prognosis of patients with pancreatic cancer is extremely poor. Prefece. **Pancreatology** 11: 1-2, 2011. **(IF=2.128)**

60. 2011 Sakurai T, **Kudo M**, Fukuta N, Nakatani T, Kimura M, Park AM, Munakata H: Involvement of angiotensin II and reactive oxygen species in pancreatic fibrosis. **Pancreatology** 11: 7-13, 2011. **(IF=2.128)**

61. 2011 Kitano M, **Kudo M**, Sakamoto H, Komaki T: Endoscopic ultrasonography and contrast-enhanced endoscopic. **Pancreatology** 11: 28-33, 2011. **(IF=2.128)**

62. 2011 Komaki T, Kitano M, Sakamoto H, **Kudo M**: Endoscopic ultrasonography-guided biliary drainage: evaluation of a choledochoduodenostomy technique. **Pancreatology** 11: 47-51, 2011. **(IF=2.128)**

63. 2011 Sakamoto H, Kitano M, Komaki T, Imai H, Kamata K, **Kudo M**: Endoscopic ultrasound-guided neurolysis in pancreatic cancer. **Pancreatology** 11: 52-58, 2011. **(IF=2.128)**

64. 2011 Minami Y, **Kudo M**: Radiofrequency ablation of hepatocellular carcinoma: A literature review. **Int J Hepatol** 2011: 9pages (104685), 2011. **(IF=0.000)**

65. 2011 Eguchi S, Kanematsu T, Arii S, Omata M, **Kudo M**, Sakamoto M, Takayasu K, Makuuchi M, Matsuyama Y, Monden M, for the Liver Cancer Study Group of Japan: Recurrence-free survival more than 10 years after liver resection for hepatocellular carcinoma. **Brit J Surg** 98: 552-557, 2011. **(IF=4.444)**

66. 2011 Kitano M, Sakamoto H, Komaki T, **Kudo M**: New techniques and future perspective of EUS for the differential diagnosis of pancreatic malignancies; Contrast harmonic imaging. **Digest Endosc** 23: 46-50, 2011. **(IF=0.946)**

67. 2011 **Kudo M**: Molecular targeted therapy for hepatocellular carcinoma: bench to bedside. **Digest Dis** 29: 273-277, 2011. **(IF=1.000)**

68. 2011 **Kudo M**: Signaling pathway and molecular-taegeted therapy for hepatocellular carcinoma. **Digest Dis** 29: 289-302, 2011. **(IF=1.000)**

69. 2011 **Kudo M**: mTOR inhibitor for the treatment of hepatocellular carcinoma. **Digest Dis** 29: 310-315, 2011. **(IF=1.000)**

70. 2011 **Kudo M**: Future treatment option for hepatocellular carcinoma: a focus on brivanib. **Digest Dis** 29: 316-320, 2011. **(IF=1.000)**

71. 2011 Ueshima K, **Kudo M**, Takita M, Nagai T, Tatsumi C, Ueda T, Kitai S, Ishikawa E, Yada N, Inoue T, Hagiwara S, Minami Y, Chung H, Sakurai T: Des-γ-carboxyprothrombin may be a promising biomarker to determine the therapeutic efficacy of Soraenib for hepatocellular carcinoma. **Digest Dis** 29: 321-325, 2011. **(IF=1.000)**

72. 2011 Hayaishi S, Chung H, **Kudo M**, Ishikawa E, Takita M, Ueada T, Kitai S, Inoue T, Yada N, Hagiwara S, Minami Y, Ueshima K: Oral branched-chaun amino acid granules reduce the incidence of hepatocellular carcinoma and improve event-free survival in patients with liver cirrhosis. **Digest Dis** 29: 326-332,

2011. **(IF=1.000)**

73. 2011 **Kudo M**, Izumi N, Kokudo N, Matsui O, Sakamoto M, Nakashima O, Kojiro M, Makuuchi M: Management of hepatocellular carcinoma in Japan: Consensus-based clinical practice guideline proposed by the Japan Society of Hepatology (JSH) 2010 updated version. **Digest Dis** 29: 339-364, 2011. **(IF=1.000)**

74. 2011 Yoshida H, Shiratori Y, **Kudo M**, Shiina S, Mizuta T, Kojiro M, Yamamoto K, Koike Y, Saito K, Koyanagi N, Kawabe T, Kawazoe S, Kobashi H, Kasugai H, Osaki Y, Araki Y, Izumi N, Oka H, Tsuiji K, Toyota J, Seki T, Osawa T, Masaki N, Ichinose M, Seike M, Ishikawa A, Ueno Y, Tagawa K, Kuromatsu R, Sakisaka S, Ikeda H, Kuroda H, Hokuryu H, Yamashita T, Sakaida I, Katamoto T, Kikuchi K, Nomoto M, Omata M: Effect of vitamin K2 on the recurrence of hepatocellular carcinoma. **Hepatology** 54: 532-540, 2011. **(IF=1.857)**

75. 2011 Chung H, Watanabe T, **Kudo M**, Chiba T: Correlation between hyporesponsiveness to Toll-like receptor ligands and liver dysfunction in patients with chronic hepatitis C virus infection. **J Viral Hepat** 18: e561-567, 2011. **(IF=3.502)**

76. 2011 Kashida H, Ikehara N, Hamatani S, Kudo S, **Kudo M**: Endoscopic characteristics of colorectal serrated lesions. **Hepato gastroenterology** 58: 1163-1167, 2011. **(IF=0.677)**

77. 2011 **Kudo M**, Imanaka K, Chiba N, Nakachi K, Tak WT, Takayama T, Yoon JH, Hori T, Kumada H, Hayashi N, Kaneko S, Tsubouchi H, Suh DJ, Furuse J, Okusaka T, Tanaka K, Matsui O, Wada M, Yamaguchi I, Ohya T, Meinhardt G, Okita K: Phase III study

of Sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. **Eur J Cancer** 47: 2117-2127, 2011. **(IF=4.944)**

78. 2011 Llovet JM, Paradis V, **Kudo M**, Zucman-Rossi J: Tissue biomarkers as predictors of outcome and selection of transplant candidates with hepatocellular carcinoma. **Liver Transplant** 17: S67-71, 2011. **(IF=3.068)**

79. 2011 **Kudo M**: Tailor-made therapy for viral hepatitis: Recent advances. **Digestion** 84: 1-4, 2011. **(IF=2.146)**

80. 2011 Kim SR, Saito J, Imoto S, Komaki T, Nagata Y, Nakajima T, Ando K, Fukuda K, Otono Y, Kim KI, Ohtani A, Sugimoto K, Hasegawa Y, Fujinami A, Ohta M, Hotta H, Maekawa Y, Hayashi Y, **Kudo M**: Correlation between insulin resistance and outcome of pegylated interferon and ribavirin therapy, hepatic steatosis, hepatic fibrosis in chronic hepatitis C-1b and high viral load. **Digestion** 84: 5-9, 2011. **(IF=2.146)**

81. 2011 Kim SR, Saito J, Imoto S, Komaki T, Nagata Y, Kim KI, Sasase N, Kimura N, Sasatani K, Konishi E, Hasaegawa Y, Fujinami A, Ohta M, Ei-Shamy A, Tanaka Y, Sugano M, Sakashita M, Nakamura A, Tsuchida S, Makino T, Kawada T, Nakajima T, Morikawa T, Muramatsu A, Hotta H, **Kudo M**: Double-filtration plasmapheresis plus interferon-β for HCV-1b patients with non-sustained virological response to previous combination therapy. **Digestion** 84: 10-16, 2011. **(IF=2.146)**

82. 2011 Kim SK, Marusawa H, Eso Y, Nishikawa H, Ueda Y, Kita R, Kimura T, Chiba T, Osaki Y, **Kudo M**: Clinical characteristics of non-B

non-C hepatocellular carcinoma: A single-center retrospective study. **Digestion** 84: 43-49, 2011. **(IF=2.146)**

83. 2011 Takita M, Hagiwara S, Arizumi T, Hayaishi S, Ueda T, Kitai S, Yada N, Inoue T, Minami Y, Chung H, Ueshima K, Sakurai T, **Kudo M**: Association of interleukin-28B and hepatitis C genotype 1 with a high viral load and response to pegylated plus ribavirin therapy. **Digestion** 84: 56-61, 2011. **(IF=2.146)**

84. 2011 Katsube T, Okada M, Kumano S, Imaoka I, Kagawa Y, Hori M, Ishii K, Tanigawa N, Imai Y, **Kudo M**, Murakami T: Estimation of liver function using T2* mapping on gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid enhanced magnetic resonance imaging. **Eur J Radiol**, 2011(in press, Epub ahead of print) **(IF=2.941)**

85. 2011 Minami Y, Kitai S, **Kudo M**: Treatment response assessment of radiofrequency ablation for hepatocellular carcinoma: Usefulness of virtual CT sonography with magnetic navigation. **Eur J Radiol**, 2011(in press, Epub ahead of print) **(IF=2.941)**

86. 2011 Suzuki H, Murata K, Gotoh T, Kusano M, Okano H, Oyamada T, Yasuda Y, Imamura M, **Kudo M**, Mizokami M, Sakamoto A: Phenotype-dependent production of des-γ-carboxy prothrombin in hepatocellular carcinoma. **J Gastroenterol,** 2011 (in press, Epub ahead of print) **(IF=3.610)**

87. 2011 Watanabe T, Yamashita K, Fujikawa S, Sakurai T, **Kudo M**, Shiokawa M, Kodama Y, Uchida K, Okazaki K, Chiba T: Activation of toll-like receptors and NOD-like receptors is involved in enhanced IgG4 responses in autoimmune pancreatitis.

Arthritis Rheum-US, 2011 (in press, Epub ahead of print) **(IF=8.435)**

88. 2011 Higashi T, Hasegawa K, Kokudo N, Makuuchi M, Izumi N, Ichida T, **Kudo M**, Ku Y, Sakamoto M, Nakashima O, Matsui O, Matsuyama Y, Sobue T; the Liver Cancer Study Group of Japan: Demonstration of quality of care measurement using the Japanese liver cancer rigstry. **Hepatol Res**, 2011 (in press, Epub ahead of print) **(IF=1.857)**

89. 2011 **Kudo M**: Introduction. **Oncology**, 2011 (in press) **(IF=2.538)**

90. 2011 Sakurai T, **Kudo M**: Signaling pathways governing tumor angiogenesis. **Oncology**, 2011 (in press) **(IF=2.538)**

91. 2011 **Kudo M**: Adjubant therpy after curative treatment for hepatocellular carcinoma. **Oncology**, 2011 (in press) **(IF=2.538)**

92. 2011 Alaboudy A, Inoue T, Hatanak K, Chung H, Hyodo T, Kumano S, Murakami T, Fawzy E, Moustafa A, **Kudo M**: Usefulness of combination of imaging modalities in diagnosis of hepatocellular carcinoma using Sonazoid enhanced ultrasound, gadolinium Diethylene-Triamine-Pentaacetic Acid enhanced magnetic resonance imaging and contrast enhanced computed tomography. **Oncology**, 2011 (in press) **(IF=2.538)**

93. 2011 **Kudo M**: Diagnostic imaging of hepatcocellular carcinoma: Recent progress. **Oncology**, 2011 (in press) **(IF=2.538)**

94. 2011 Inuzuka T, Nishikawa H, Sekikawa A, Takeda H, Henmi S, Sakamoto A, Saito S, Kita R, Kimura T, Osaki Y, **Kudo M**:

Complete response of advanced hepatocellular carcinoma with multiple lung metastases treated with Sorafenib: A case report. **Oncology**, 2011 (in press) **(IF=2.538)**

95. 2011 Han KH, **Kudo M**, Ye SL, Choi JY, Poon RTP, Seong J, Park JW, Ichida T, Chung JW, Chow P, Cheng AL: Asian consensus workshop report: Expert consensus guideline for the management of intermediate and advanced hepatocellular carcinoma in Asia. **Oncology**, 2011 (in press) **(IF=2.538)**

96. 2011 **Kudo M**, Tateishi R, Yamashita T, Ikeda M, Furuse J, Ikeda K, Kokudo N, Izumi N, Matsui O: Current status of hepatocellular carcinoma treatment in Japan: Case study and discussion-voting system. **Clin Drug Invest**, 2011 (in press) **(IF=1.622)**

97. 2011 Kim SR, Imoto S, Nakajima T, Ando K, Mita K, Fukuda K, Nishikawa R, Koma Y, Matsuoka T, **Kudo M**, Hayashi Y: Utility of Gd-EOB-DTPA-enhanced MRI in diagnosing small hepatocellular carcinoma. **Case Report Gastroenterology** 2011 (in press) **(IF=0.000)**

98. 2011 Kim SR, Taniguchi M, Sasase N, Kim KI, Ninomiya T, Imoto S, Ando K, Mita K, Fuki S, Fukuda K, **Kudo M**, Sakamoto H, Inui K, Hayashi Y: Multicentric occurrence of HCC detected 3-4 yerars after AFP-L3 positivity. **Intern Med** 2011 (in press) **(IF=1.037)**

99. 2011 **Kudo M**: Molecular targeted therapy for hepatocellular carcinoma: Sorafenib and beyond. **Curr Cancer Drug Tar**, 2011 (in press) **(IF=4.771)**

100. 2011 Yang J, Kim SR, **Kudo M**, Hino O: Recent advance in the management of chronic hepatitis B. **Hepat Res Treat**, 2011 (in press) **(IF=0.000)**

101. 2011 Sakamoto H, Kitano M, **Kudo M**: EUS-guided broad plexus neurolysis technique and its indications. **Diagnostic Imaging Asia-Pacific**, 2011 (in press) **(IF=0.000)**

102. 2011 Inoue T, **Kudo M**, Komuta M, Hayaishi S, Ueda T, Takita M, Kitai S, Hatanaka K, Yada N, Hagiwara S, Minami Y, Chung H, Ueshima K, Sakamoto M, Maenishi O, Okada M, Kumano S, Murakami T: Assessment of hepatobiliary phase Gd-EOB-DTPA-enhanced MR imaging for disctriminating between hepatocellular carcinoma and borderline lesions and comparison of detection ability versus multi-detector raw helical CT. **J Gastroenterol**, 2011 (in press) **(IF=3.610)**

103. 2011 Kitano M, **Kudo M**, Yamao K, Takagi T, Sakamoto H, Komaki T, Kamata K, Imai H, Murakami T, Takeyama Y: Characterization of small pancreatic neoplasms by contrast-enhanced harmonic endosonography. **Am J Gastroenterol**, 2011 (in press) **(IF=6.882)**

104. 2011 Sakurai T, **Kudo M**, Itoh K, Ryu U, Higashitsuji H, Fujita J: Adriamycin enhances proteasome-mediated generation of the proapoptotic processed form of MAGE-A4 in hepatoma cells. **Oncology**, 2011 (in press) **(IF=2.538)**

II. 和文論文（著書・分担執筆）

1. 2010 樫田博史：便潜血反応． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p66-p67，2010（分担執筆）

2. 2010 梅原 泰，工藤正俊：大腸内視鏡検査． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p104-106，2010（分担執筆）

3. 2010 松井繁長，工藤正俊：緊急内視鏡． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p108-109，2010（分担執筆）

4. 2010 畑中絹世，工藤正俊：造影エコー検査． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p153-159，2010（分担執筆）

5. 2010 井上達夫，工藤正俊：肝細胞癌のスクリーニングと診断．今日の消化器疾患治療指針 第 3 版，医学書院，東京，p646-648，2010（分担執筆）

6. 2010 上嶋一臣，工藤正俊：肝細胞癌の治療方針． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p649-652，2010（分担執筆）

7. 2010 井上達夫，工藤正俊：肝結核．今日の消化器疾患治療指針 第 3 版，医学書院，東京，p706-707，2010（分担執筆）

8. 2010 坂本洋城，工藤正俊：腫瘤形成性膵炎．今日の消化器疾患治療指針 第 3 版，医学書院，東京，p752-754，2010（分担執筆）

9. 2010 北野雅之，工藤正俊：膵癌の診断と治療方針・疼痛対策． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p773-776，2010（分担執筆）

10. 2010 南 康範，工藤正俊：科学的根拠に基づく肝癌診療ガイドライン． 今日の消化器疾患治療指針 第 3 版，医学書院，東京，p997-1003，2010（分担執筆）

11. 2010 工藤正俊：肝悪性腫瘍． 今日の診断指針 第 6 版，医学書院，東京，p768-777，2010（分担執筆）

12. 2010 辰巳千栄，工藤正俊，上嶋一臣：非侵襲的肝線維化測定法 -Real-time Tissue Elastography と Fibroscan はどちらが優るか． 肝疾患 Review，日本メディカルセンター，東京，p225-229，

2010（分担執筆）

13. 2010 建石良介，泉　並木，金子周一，工藤正俊：肝癌早期発見のためのスクリーニング法．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，＊，p29-31，2010（分担執筆）

14. 2010 工藤正俊，井上達夫，村上卓道：肝癌の診断 画像診断 どのようなときにGd-EOB-MRIを行うか．肝癌診療マニュアル 第2版，日本肝臓学会編集，医学書院，東京，p38-40，2010（分担執筆）

15. 2010 工藤正俊：肝癌の診断 画像診断 どのようなときに造影超音波を行うか．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p43-49，2010（分担執筆）

16. 2010 松井　修，工藤正俊，高安賢一，神代正道：肝癌の診断 画像診断 早期肝癌の画像的特徴．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p49-52，2010（分担執筆）

17. 2010 泉　並木，工藤正俊，松井　修：肝癌の診断 肝癌診断のアルゴリズム 多血性肝細胞癌の診断アルゴリズム．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p53-56，2010（分担執筆）

18. 2010 工藤正俊，泉　並木，松井　修：肝癌の診断 肝癌診断のアルゴリズム 乏血性肝細胞性結節（境界病変，異型結節，早期肝癌）はどのような場合に治療すべきか．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p56-59，2010（分担執筆）

19. 2010 工藤正俊：肝癌の治療 肝癌診療のためのステージングシステム．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p65-67，2010（分担執筆）

20. 2010 南　康範，工藤正俊：肝癌の治療 肝癌治療の実際 内科的局所治療 ラジオ波焼灼療法 RFA の適応と除外基準 造影超音波下 RFA．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p86-88，2010（分担執筆）

21. 2010 工藤正俊：肝癌の治療 肝癌治療の実際 全身化学療法と分子標的治療．肝癌診療マニュアル 第 2 版，日本肝臓学会編集，医学書院，東京，p109-113，2010（分担執筆）

22. 2010 工藤正俊：肝癌治療のアルゴリズム TACE不応例に対する治療指

針. 肝癌診療マニュアル 第 2 版, 日本肝臓学会編集, 医学書院, 東京, p118-121, 2010（分担執筆）

23. 2010 工藤正俊: 肝癌治療のアルゴリズム 肝動注化学療法と分子標的治療薬をどう使い分けるか. 肝癌診療マニュアル 第 2 版, 日本肝臓学会編集, 医学書院, 東京, p121-122, 2010（分担執筆）

24. 2010 工藤正俊, 幕内雅敏, 國土典宏, 田中正俊, 川崎誠治, 高安賢一, 松井 修, 泉 並木, 大崎往夫: 肝癌治療のアルゴリズム 肝癌全体の治療アルゴリズム. 肝癌診療マニュアル 第 2 版, 日本肝臓学会編集, 医学書院, 東京, p122-127, 2010（分担執筆）

25. 2010 工藤正俊: 肝癌治療後のフォローアップの仕方 肝癌根治後の再発抑制治療. 肝癌診療マニュアル 第 2 版, 日本肝臓学会編集, 医学書院, 東京, p143-144, 2010（分担執筆）

26. 2010 工藤正俊, 泉 並木: 肝癌治療後のフォローアップの仕方 肝癌根治的治療後の再発の早期発見. 肝癌診療マニュアル 第 2 版, 日本肝臓学会編集, 医学書院, 東京, p145-147, 2010（分担執筆）

27. 2010 工藤正俊, 泉 並木: 序文. 症例から学ぶウイルス肝炎の治療戦略, 診断と治療社, 東京, p*vi-vii*, 2010（分担執筆）

28. 2010 鄭 浩柄, 工藤正俊: B 型肝炎 Child-Pugh C の非代償性肝硬変で核酸アナログ投与によって肝機能が改善した症例. ウイルス肝炎の治療戦略. 診断と治療社, 東京, p108-112, 2010.（分担執筆）

29. 2010 萩原 智, 工藤正俊: B 型肝炎 ペグインターフェロンとエンテカビル投与によって薬剤フリーが得られている B 型肝炎症例. ウイルス肝炎の治療戦略. 診断と治療社, 東京, p113-115, 2010.（分担執筆）

30. 2010 上田泰輔, 鄭 浩柄, 工藤正俊: C 型肝炎 肝癌治癒後にインターフェロン投与行ったが肝癌の再発が認めた症例. ウィルス肝炎の治療戦略. 診断と治療社, 東京, p154-157, 2010.（分担執筆）

31. 2010 工藤正俊: 序説. 肝細胞癌の分子標的治療, アークメディア, 東京, p2-3, 2010.（分担執筆）

32. 2010 工藤正俊, 池田公史, 古瀬純司, 沖田 極, 有井滋樹: 特別座談会 肝細胞癌の分子標的治療. 肝細胞癌の分子標的治療, ア

ークメディア，東京，p9-31，2010.（分担執筆）

33. 2010 工藤正俊：ソラフェニブによる進行肝癌の治療：その有効性・副作用対策と将来展望. 肝細胞癌の分子標的治療，アークメディア，東京，p88-100，2010.（分担執筆）

34. 2010 工藤正俊：肝細胞癌のシグナル伝達系と分子標的治療. 肝細胞癌の分子標的治療，アークメディア，東京，p101-117，2010.（分担執筆）

35. 2010 工藤正俊：第 1 回日本肝がん分子標的治療研究会を振り返って. 肝細胞癌の分子標的治療，アークメディア，東京，p129-131，2010.（分担執筆）

36. 2010 上嶋一臣，工藤正俊：ソラフェニブにより CR となった進行肝細胞癌の 2 症例. 肝細胞癌の分子標的治療，アークメディア，東京，p135-141，2010.（分担執筆）

37. 2010 工藤正俊：序. 見逃し、誤りを防ぐ!肝・胆・膵癌画像診断アトラス，羊土社，東京，p7，2010.（分担執筆）

38. 2010 工藤正俊：肝癌. 見逃し、誤りを防ぐ!肝・胆・膵癌画像診断アトラス. 羊土社，東京，p14-18，2010（分担執筆）.

39. 2010 井上達夫，工藤正俊：早期肝細胞癌. 見逃し、誤りを防ぐ!肝・胆・膵癌画像診断アトラス，羊土社，東京，p86-90，2010.（分担執筆）

40. 2010 樫田博史：大腸の pit pattern 分類. 斉藤裕輔，田中信治，渡邉聡明 編集，大腸疾患診療の Strategy，日本メディカルセンター，東京，pp236-237，2010（分担執筆）

41. 2010 樫田博史：表面型鋸歯状腺腫. 丹羽寛文 監修，田尻久雄，田中信治，加藤元嗣，斎藤 豊 編集，画像強調観察による内視鏡診断 Image-Enhanced Endoscopy アトラス，日本メディカルセンター，東京，pp216，2010（分担執筆）

42. 2010 樫田博史：拡大鏡観察. 田中信治編：スキルアップ大腸内視鏡診断編，中外医学社，東京，pp58-67，2010（分担執筆）

43. 2010 樫田博史：EMR/EPMR（一括/分割切除）. 田中信治編：スキルアップ大腸内視鏡 治療編，中外医学社，東京，pp44-57，2010（分担執筆）

44. 2010　　樫田博史: 消化器内視鏡総論. 医療情報科学研究所編 : 病気がみえる　vol. 1 消化器　第 4 版, メディックメディア, 東京, pp20-25, 2010（分担執筆）

45. 2010　　樫田博史: イレウス（腸閉塞）. 医療情報科学研究所編 : 病気がみえる　vol. 1 消化器　第 4 版, メディックメディア, 東京, pp114-121, 2010（分担執筆）

46. 2010　　樫田博史: 大腸癌. 医療情報科学研究所編 : 病気がみえる　vol. 1 消化器　第 4 版, メディックメディア, 東京, pp130-139, 2010（分担執筆）

47. 2010　　樫田博史: 消化管ポリポーシス. 医療情報科学研究所編 : 病気がみえる　vol. 1 消化器　第 4 版, メディックメディア, 東京, pp140-143, 2010（分担執筆）

48. 2010　　樫田博史: 消化管カルチノイド. 医療情報科学研究所編 : 病気がみえる　vol. 1 消化器　第 4 版, メディックメディア, 東京, pp144-145, 2010（分担執筆）

49. 2010　　樫田博史: グリセリン浣腸. 和田　攻, 南　裕子, 小峰光博　総編集　看護学大事典　第２版, 医学書院, 東京, p828, 2010（分担執筆）

50. 2010　　樫田博史: 消化器内視鏡手術. 和田　攻, 南　裕子, 小峰光博　総編集　看護学大事典　第２版, 医学書院, 東京, p1425, 2010（分担執筆）

51. 2010　　樫田博史: 内視鏡的食道拡張術. 和田　攻, 南　裕子, 小峰光博　総編集　看護学大事典　第２版, 医学書院, 東京, p2181, 2010（分担執筆）

52. 2010　　樫田博史: ステント留置法. 和田　攻, 南　裕子, 小峰光博　総編集　看護学大事典　第２版, 医学書院, 東京, p1646, 2010（分担執筆）

53. 2010　　樫田博史: ゼングスターケン･ブレークモア管. 和田　攻, 南　裕子, 小峰光博　総編集　看護学大事典　第２版, 医学書院, 東京, pp1753-1754, 2010（分担執筆）

54. 2010　　樫田博史: 注射硬化療法（痔核の）. 和田　攻, 南　裕子, 小峰光博　総編集　看護学大事典　第２版, 医学書院, 東京, p1989,

2010（分担執筆）

55. 2010　樫田博史：電気凝固止血法．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版　，医学書院，東京，2078，2010（分担執筆）

56. 2010　樫田博史：内視鏡手術．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，p2180，2010（分担執筆）

57. 2010　樫田博史：内視鏡的狭窄拡張術．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，pp2180-2181，2010（分担執筆）

58. 2010　樫田博史：内視鏡的硬化療法．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，p2181，2010（分担執筆）

59. 2010　樫田博史：内視鏡的食道静脈瘤結紮術．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，p2181，2010（分担執筆）

60. 2010　樫田博史：内視鏡的膵管ドレナージ．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，p2181，2010（分担執筆）

61. 2010　樫田博史：内視鏡的胆管ステント留置法．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，pp2181-2182，2010（分担執筆）

62. 2010　樫田博史：内視鏡的粘膜下腫瘍摘出術．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，p2182，2010（分担執筆）

63. 2010　樫田博史：内視鏡的粘膜切除術．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，pp2182-2183，2010（分担執筆）

64. 2010　樫田博史：内視鏡的ポリペクトミー．和田　攻，南　裕子，小峰光博　総編集　看護学大事典　第２版，医学書院，東京，p2183，2010（分担執筆）

65. 2010　樫田博史，南　ひとみ，佐藤嘉高，加賀まこと，亀田　亮，山村

冬彦，井上晴洋，工藤進英：十二指腸腫瘍に対する治療法選択，偶発症予防，経過観察の方法．　桑山　肇　編　消化管癌内視鏡治療　40 の工夫とコツ，ヴァンメディカル，東京，pp107-112，2010（分担執筆）

66. 2010　　樫田博史，蟹江　浩，塩飽洋生，和田祥城，林　武雅，細谷寿久，若村邦彦，宮地英行，池原伸直，工藤進英：IIc 病変の診断と治療．武藤徹一郎監修，杉原健一，藤盛孝博，五十嵐正広，渡邉聡明編集　大腸疾患 NOW 2010，日本メディカルセンター，東京，pp67-79，2010（分担執筆）

67. 2010　　樫田博史，中村大樹，久行友和，児玉健太，林　武雅，和田祥城，宮地英行，池原伸直，工藤進英，浜谷茂治：欧米と日本とで大腸癌の病理診断は違うか　臨床の立場から．武藤徹一郎監修，杉原健一，藤盛孝博，五十嵐正広，渡邉聡明編集　大腸疾患 NOW 2010 特別号，日本メディカルセンター，東京，　pp66-74，2010（分担執筆）

III. 和文論文

1. 2010 工藤正俊: 発刊にあたって. きずな 3: 1, 2010.

2. 2010 工藤正俊: 生涯一臨床医のつぶやき「炉辺閑話 2010」. 日本医事新報 4471: 55-56, 2010.

3. 2010 工藤正俊: 肝細胞癌の診断・治療アルゴリズムにおける画像の役割. 特集「消化器領域の画像診断: 肝胆膵を中心に」, 映像情報メディカル 42: 245-249, 2010.

4. 2010 北野雅之, 坂本洋城, 小牧孝充, 工藤正俊: 胆膵疾患における超音波内視鏡検査: 造影ならびに FNA の有用性. 特集「消化器領域の画像診断: 肝胆膵を中心に」, 映像情報メディカル 42: 277-282, 2010.

5. 2010 工藤正俊: 病院長からのメッセージ「世界への発信と同時に地域医療への貢献もめざす」 「臨床研修指定病院紹介」, DOCTOR'S MAGAZINE 124: 20-21, 2010.

6. 2010 上嶋一臣, 工藤正俊: こう変わった・こう変わる！肝がん化学療法. 消化器外科 NURSING 臨時増刊: 128-129, 2010.

7. 2010 工藤正俊: わが国の肝がん治療のガイドラインを解釈する. 肝胆膵 60: 271-277, 2010.

8. 2010 工藤正俊: 肝癌の診断のアルゴリズム. 臨牀消化器内科 25: 443-451, 2010.

9. 2010 有井滋樹, 森安史典, 工藤正俊, 廣岡芳樹: 座談会 造影超音波は臨床を変えるか. 肝胆膵 60: 479-499, 2010.

10. 2010 北野雅之, 小牧孝充, 鎌田 研, 今井 元, 坂本洋城, 工藤正俊: 造影 EUS. 肝胆膵 60: 457-464, 2010.

11. 2010 工藤正俊: 超音波診断の技術的到達点と臨床的価値の最新座標装置・造影剤利用法を含めて. 新医療 5: 92-97, 2010.

12. 2010 猪飼伊和夫, 工藤正俊: 臓器がん登録の現状と将来展望-臨床へのフィードバックを目指して-. 外科治療 102: 372-377, 2010.

13. 2010 工藤正俊: 早期肝細胞がんの診断・治療におけるアルゴリズム-境界病変の鑑別の可能性 1)内科の立場から. INNERVISION 25: 23-24, 2010.

14. 2010 前川 清, 工藤正俊, 上硲俊法: 造影超音波検査による肝腫瘍の質的診断. 近畿大学医学雑誌 35: 47-53, 2010.

15. 2010 坂本洋城, 北野雅之, 松井繁長, 工藤正俊: 超音波内視鏡. 臨牀と研究 87: 683-687, 2010.

16. 2010 坂本洋城, 北野雅之, 工藤正俊: 胆・膵における US・EUS 診断. 臨牀消化器内科 25: 963-970, 2010.

17. 2010 工藤正俊: 日本超音波医学会第 83 回学術集会の開催にあたって. 病院新聞 2124: 3, 2010.

18. 2010 工藤正俊: 肝細胞癌. 消化器の臨床 13: 113-123, 2010.

19. 2010 工藤正俊, 久保正二, 高安賢一, 坂元亨宇, 田中正俊, 猪飼伊和夫, 古瀬純司, 中村健治, 幕内雅敏: 肝癌治療効果判定基準（2009 年改訂版）. 肝臓 51: 261-266, 2010.

20. 2010 工藤正俊, 上田泰輔, 土谷 薫, 橋元 悟, 井上泰輔, 稲生実枝, 田中 篤, 垣内雅彦, 今関文夫, 西口修平: ペグインターフェロンα-2b/リバビリン併用療法の無効・再燃例に対するペグインターフェロンα-2a/リバビリン併用療法の再治療. 肝胆膵 61: 127-133, 2010.

21. 2010 工藤正俊: 肝細胞癌画像診断の進歩 肝細胞癌サーベイランス各国の現状. The Liver Cancer Journal 2: 100-108, 2010.

22. 2010 前川 清, 工藤正俊, 畑中絹世, 井上達夫, 鄭 浩柄, 上嶋一臣, 石川 原, 土師誠二, 佐藤隆夫: 出血を伴った胆管嚢胞腺癌の一例. Modern Physician 30: 1113-1118, 2010.

23. 2010 工藤正俊: 肝癌の内科治療の将来展望. クリニシアン 57: 72-79, 2010.

24. 2010 工藤正俊: 小分子物質 3)Sorafenib. 腫瘍内科 5: 617-629, 2010.

25. 2010 宮田 央, 宮田 学, 工藤正俊: C 型慢性肝炎に対する PEG-IFN 療法施行中にみられる ALT およびフェリチンの上昇と瀉血療法

の有用性. 肝臓 51: 371-378, 2010.

26. 2010 工藤正俊, 有井滋樹, 猪飼伊和夫, 小俣政男, 神代正道, 坂元亨宇, 高安賢一, 林 紀夫, 幕内雅敏, 松山 裕, 門田守人: 第 18 回全国原発性肝癌追跡調査報告(2004～2005). 肝臓 51: 460-484, 2010.

27. 2010 荒尾徳三, 工藤正俊, 西尾和人: Liver, Pancreas, Biliary Tract Cancer 肝・胆・膵癌 肝癌分子標的治療の基礎と臨床 肝癌分子標的治療におけるバイオマーカーの探索. 癌と化学療法 (Jpn J Cancer Chemother) 37: 1879-1882, 2010.

28. 2010 工藤正俊: 特集にあたって. Pharma Medica 28: 9-11, 2010.

29. 2010 南 康範, 工藤正俊: 肝細胞癌に対するペルフルブタン造影エコー法. Pharma Medica 28: 13-17, 2010.

30. 2010 北野雅之, 工藤正俊: 膵腫瘍に対する造影超音波内視鏡. Pharma Medica 28: 45-49, 2010.

31. 2010 樫田博史, 川崎正憲, 梅原 泰, 峯 宏昌, 永田嘉昭, 朝隈豊, 櫻井俊治, 松井繁長, 工藤正俊: 下部消化管疾患に対する内視鏡の進歩. Pharma Medica 28: 57-63, 2010.

32. 2010 松井繁長, 樫田博史, 朝隈 豊, 永田嘉昭, 川崎正憲, 櫻井俊治, 工藤正俊: 十二指腸静脈瘤の診断と治療法. 消化器内視鏡 22: 1835-1841, 2010.

33. 2010 工藤正俊: ネクサバール. 肝胆膵 61: 1155-1165, 2010.

34. 2010 坂本洋城, 北野雅之, 工藤正俊: 膵嚢胞性腫瘍診断. 胆と膵 31: 1175-1180, 2010.

35. 2010 北野雅之, 小牧孝充, 坂本洋城, 鎌田 研, 今井 元, 工藤正俊: 早期膵癌. 消化器内視鏡 22: 1933-1940, 2010.

36. 2010 工藤正俊: 肝がん根治治療後の補助療法. 腫瘍内科 6: 455-464, 2010.

37. 2010 工藤正俊: 近畿大学医学部消化器内科における肝細胞癌治療の取り組み 根治治療も延命治療もベストをめざし、エビデンス集積に尽力. The Liver Cancer Journal 2: 219-225, 2010.

38. 2010 工藤正俊: 肝生検: 肝診療との接点 非典型画像を呈する肝腫瘍の画像診断. 病理と臨床 28: 1269-1273, 2010.

39. 2010 溝上雅史, 田中榮司, 茶山一彰, 田中靖人, 黒崎雅之, 泉 並木, 荒瀬康司, 熊田博光, 今関文夫, 横須賀收, 工藤正俊: 日本肝臓学会コンセンサス神戸 2009: B 型肝炎の診断と治療. 肝臓 51: 243-260, 2010.

40. 2010 上嶋一臣, 工藤正俊: PIVKA-II は Sorafenib の治療効果予測因子になりうる. 肝臓 51: 681-683, 2010.

41. 2010 汐見幹夫: 近畿大学医学部附属病院内視鏡部. Gastroenterological Endoscopy 52: 1451-1453, 2010.

42. 2010 北野雅之: EUS-FNA 検査. 消化器 now 51: 8, 2010.

43. 2010 井上達夫, 北野雅之: 腹部超音波検査法. 見逃し、誤りを防ぐ! 肝・胆・膵癌画像診断アトラス, 羊土社, 東京, p28-36, 2010. (分担執筆)

44. 2010 北野雅之: 超音波内視鏡ガイド下 *in vivo* マイクロダイアリシス法による膵疾患の局所病態評価. 膵臓病研究財団第 17 回研究報告書: 65-70, 2010.

45. 2010 樫田博史, 請川淳一, 吉松軍平, 尾松睦子: S状結腸捻転症に対する内視鏡の役割. 消化器内視鏡 22: 1483-1488, 2010.

46. 2010 樫田博史: 大腸ポリープの取り扱い－摘除すべきポリープの鑑別と摘除後のサーベイランス－. Medical Practice 27: 1317-1323, 2010.

47. 2010 樫田博史, 小形典之, 大塚和朗, 池原伸直, 工藤進英: 単純性潰瘍・腸型ベーチェット病. 消化器内視鏡 22: 1240-1244, 2010.

48. 2010 樫田博史, 大塚和朗, 工藤進英: 潰瘍性大腸炎内視鏡診断のポイント－画像強調観察の意義も含めて. Modern Physician 30: 897-903, 2010.

49. 2010 樫田博史, 林 武雅, 細谷寿久, 若村邦彦, 和田祥城, 宮地英行, 池原伸直, 山村冬彦, 大塚和朗, 工藤進英: 深達度診断からみた側方発育型大腸腫瘍 (LST) の分類と意義 内視鏡診断の立場から: 拡大観察・NBI からみた LST. 胃と腸 45: 969-980, 2010.

50. 2010 樫田博史, 林　武雅, 細谷寿久, 若村邦彦, 和田祥城, 宮地英行, 池原伸直, 山村冬彦, 大塚和朗, 工藤進英: 早期大腸癌の治療 1) EMR/EPMR. 胃と腸　45: 873-881, 2010.

51. 2010 樫田博史: スコープ挿入法の基本手技. 消化器内視鏡 22: 605-610, 2010.

52. 2010 樫田博史, 林　武雅, 細谷寿久, 若村邦彦, 和田祥城, 西脇裕高, 池原伸直, 山村冬彦, 工藤進英: 分割 EMR の位置づけと実際. Intestine 14: 145-154, 2010.

53. 2010 大塚和朗, 児玉健太, 池田晴夫, 小形典之, 樫田博史, 工藤進英: 十二指腸・小腸の内視鏡. 臨牀消化器内科 25: 1139-1144, 2010.

54. 2010 大塚和朗, 児玉健太, 池田晴夫, 小形典之, 樫田博史, 横山顕礼, 豊嶋直也, 若村邦彦, 工藤進英: 小腸内視鏡―診断能向上に向けて　バルーン内視鏡挿入困難例に対する対応　シングルバルーン内視鏡. Intestine 14: 311-315, 2010.

55. 2010 大塚和朗, 工藤進英, 児玉健太, 池田晴夫, 小形典之, 樫田博史, 三澤将史, 山村冬彦, 池原伸直, 宮地英行, 若村邦彦, 横山顕礼: 小腸出血性疾患に対する診断手技　シングルバルーン小腸内視鏡. 胃と腸 45 : 328-332, 2010.

56. 2010 工藤進英, 三澤将史, 樫田博史, 和田祥城, 細谷寿久, 若村邦彦, 林　武雅, 宮地英行, 池原伸直, 山村冬彦, 大塚和朗, 石田文生, 遠藤俊吾, 田中淳一:高齢者の大腸内視鏡時の注意点と対策. 外科　72: 272-275, 2010.

57. 2010 工藤進英, 池原伸直, 林　武雅, 及川裕将, 小形典之, 塩飽洋生, 和田祥城, 樫田博史, 浜谷茂治: LST の病態 / 術前診断所見からみた治療法選択. Intestine 14: 115-124, 2010.

IV. 招待講演・特別講演（海外）

1. **Kudo M**: Special Lecture “Imaging diagnosis of early HCC: Recent advance.” Choshu International Liver Smposium 2010, Yamaguchi, Japan, February 6, 2010.

2. Kitano M: Update endosonographie 2010. Fortbildung Gastroenterologie, Hanau, Germany, February 11, 2010.

3. **Kudo M**: Special Lecture “The use of TACE in the treatment of hepatocellular carcinoma.” The 8th ASIA PACIFIC ONCOLOGY SUMMIT Customized Cancer Therapy and Management, Tokyo, Japan, March 20, 2010.

4. **Kudo M**: Special Lecture “Contrast-enhanced US: its role in the management of HCC.” 26th International Congress of Radiology, Shanghai, China, April 10, 2010.

5. **Kudo M**: Special lecture “Imaging diagnosis of every stage HCC.” Liver Group Research Meeting at the Pathology Division of the University of Sao Paulo School of Medicine General Hospital, Sao Paulo, Brazil, April 29, 2010.

6. **Kudo M**: Special lecture “Molecular targeted therapy for hepatocellular carcinoma.” Liver Group Research Meeting at the Pathology Division of the University of Sao Paulo School of Medicine General Hospital, Sao Paulo, Brazil, April 29, 2010.

7. **Kudo M**: Special lecture “Enhanced sonography of hepatic nodules.” JPR 2010, Sao Paulo, Brazil, April 30, 2010.

8. **Kudo M**: Special lecture “Advanced in US techniques for treatment guidance for liver tumours.” JPR 2010, Sao Paulo, Brazil, April 30, 2010.

9. **Kudo M**: Special lecture “Earlier HCC diagnosis: US, CT and MRI aspects -anatomopathological correlation-US aspects-.” JPR 2010, Sao Paulo, Brazil, April 30, 2010.

10. **Kudo M**: Special lecture “Imaging diagnosis of very early stage HCC.” Seoul Laennec meeting 2010, Seoul, Korea, May 12-15, 2010.

11. **Kudo M**: Special lecture “Sonazoid-enhanced US as a treatment-guidance for HCC.” Korean Society of Ultrasound in Medicine 2010 Open, Seoul, Korea, May 14-15, 2010.

12. **Kudo M**: Special lecture “Defect re-perfusion imaging for HCC.” Korean Society of Ultrasound in Medicine 2010 Open, Seoul, Korea, May 14-15, 2010.

13. **Kudo M**: Special lecture “Management of HCC in Japan.” Global HCC investigator’s meeting in Taiwan, Taipei, Taiwan, May 15, 2010.

14. Kashida H: Training system of colonoscopic techniques including endoscopic submucosal dissection. Symposium “training sustem and registration of endoscopic submucosal dissection” 9th Japan-Korea joint Symposium on Gastrointestinal Endoscopy, May 15, 2010, Tokyo, Japan.

15. Kashida H: Japanese practice and teaching of the diagnosis and treatment for early colorectal neoplasias. Endopathology-Endomicroscopy Course, June 10, 2010, Mainz, Germany.

16. Kashida H: live demonstration. Forum Gastroenterologie Mainz-Wiesbaden, June 11-12, 2010, Mainz, Germany.

17. Kashida H: live demonstration. Techniche Diagnostiche Avanzate in Gastroenterologia, June 14-15, 2010, Bologna, Italy.

18. Kashida H: Mucosectomy (EMR) & Submucosal Dissection (ESD) of early colorectal cancers and large flat adenomas. Update in Gastroenterologia, June 16, 2010, Bologna, Italy.

19. **Kudo M**: Special lecture “Clinical classification in Asia.” Europian Association for the Study of the Liver, Dubrovnik, Croatia, June 25-26, 2010.

20. Kashida H: Tips and tricks of the treatment of early colorectal neoplasia based on the precise diagnosis. ESGE endorsed international endoscopy workshop “Advanced Digestive Endoscopy and Gastroenterology”, July 1-2, 2010, Yaroslavl, Russia.

21. Kashida H: live demonstration. ESGE endorsed international endoscopy workshop “Advanced Digestive Endoscopy and Gastroenterology”, July 1-2, 2010, Yaroslavl, Russia.

22. **Kudo M**: Workshop "Report from working group." The 1st Asia-Pacific Primary Liver Cancer Expert Meeting, Incheon, Korea, July 3-4, 2010.

23. **Kudo M**: Workshop "Treatment algorithm for intermediate and advanced stage of HCC: Japan." The 1st Asia-Pacific Primary Liver Cancer Expert Meeting, Incheon, Korea, July 3-4, 2010.

24. Kamata K: Management of IPMNs by endoscopic ultrasonogtaphy. Jouint Meeting of the International Association of Pancreatology and the Japan Pancreas Society 2010, July 11-13, 2010, Fukuoka, Japan.

25. **Kudo M**: Special lecture "Ultrasound diagnosis of pancreatic tumors." 8 AFSUMB Workshop: 2010, Ulaanbaatar, Mogolia, August 5-7, 2010.

26. Kashida H: special lecture "The image-enhanced endoscopy for early colorectal neoplasm." ACID Meeting 2010, August 6, 2010, Osaka, Japan.

27. Kashida H: Screening colonoscopy in the 21st century. 31st National Congress of Gastroenterology, August 30-September 3, 2010, Caracas, Venezuela.

28. Kashida H: Chromoendoscopy vs electronic chromoendoscopy for the diagnosis of colorectal polyps. 31st National Congress of Gastroenterology, August 30-September 3, 2010, Caracas, Venezuela.

29. Kashida H: Endoscopic submucosal dissection (ESD) for colon. 31st National Congress of Gastroenterology, August 30-September 3, 2010, Caracas, Venezuela.

30. Kashida H: New classifications concerning colorectal neoplastic lesions. 31st National Congress of Gastroenterology, August 30-September 3, 2010, Caracas, Venezuela.

31. **Kudo M**: Special Lecture "Management and outcome of HCC in Japan: Analysis of 51,430 HCC cases registerd in nationwide survey program of Liver Cancer Study Group of Japan." 4th Annual Conference International Liver Cancer Association (ILCA), Montral, Canada, September 10, 2010.

32. Kashida H: Endoscopy in the anticoagulated patient. Endoscopy Directors' Workshop, Asia Pacific Digestive Week (APDW 2010), September 19-22, 2010, Kuala Lumpur, Malaysia.

33. Kitano M: Contrast agents and elastgraphy. New generation of EUS? Endoscopic Ultrasonography Live 2010, October 8-10, 2010, Chicago, USA.

34. **Kudo M**: Special lecture "Imaging diagnosis of early HCC." 4th International Forum for Liver MRI, Seoul Korea, October 29-30, 2010.

35. Kitano M: Metal stenting for malignant biliary strictures (Bare &Covered). BONASTENT Summit 2010, Jeju-Do, Korea.

36. Kitano M: State of the Art I; Contrast harmonic imaging. 17th International symposium on endoscopic ultrasonography (EUS) 2010, November 12-14, 2010, Shanghai International Conventional Center, Shanghai, China.

37. **Kudo M**: Special lecture "Molecular targeted therapy for HCC: Current situation and future prospective." Medanta University Hospital, India, November 18, 2010.

38. **Kudo M**: Special lecture "Imaging diagnosis of early-stage HCC: Role of EOB-MRI." Medanta University Hospital, India, November 18, 2010.

39. **Kudo M**: Special lecture "Sonazoid-enhanced US in the management of HCC." Medanta University Hospital, India, November 18, 2010.

40. **Kudo M**: Special lecture "Endoscopic CEUS for pancreatic lesions." 9th Congress of Asian Federation of Societies for Ultrasound in Medicine and Biology, New Delhi, India, November 18-21, 2010.

41. **Kudo M**: Special lecture "Interventional US for GI & pancreatico biliary disease." 9th Congress of Asian Federation of Societies for Ultrasound in Medicine and Biology, New Delhi, India, November 18-21, 2010.

42. **Kudo M**: Special lecture "Current role of sorafenib in the management of HCC." All India Institute of Medical Science (AIIMS), New Delhi, India, November 20, 2010.

43. **Kudo M**: Special lecture "On going trial and future role of molecular targeted agent in HCC." All India Institute of Medical Science (AIIMS), New Delhi, India, November 20, 2010.

V. 招待講演・特別講演（国内）

1. 工藤正俊: 特別講演「肝細胞癌に対するネクサバール治療: 副作用対策の成功が治療の成功」, 名古屋肝癌セミナー, 愛知, 平成 22 年 1 月 14 日.

2. 工藤正俊: 特別講演「肝癌診療の新しいパラダイム」, 第 70 回倉敷肝臓臨床談話会, 岡山, 平成 22 年 1 月 26 日.

3. 工藤正俊: 特別講演「Sonazoid 造影エコー法の新しい展開」, 日本超音波医学会第 29 回中部地方会学術集会, 石川, 平成 22 年 1 月 31 日.

4. 工藤正俊: 特別講演「異型結節・早期肝癌の診断、多血性腫瘍への移行 US」, 第 16 回肝血流動態イメージ研究会 シンポジウム「肝細胞癌多段階発癌の診断: 慢性肝炎、異型結節、進行肝癌の個別化診断に向けて」, 神戸ポートピアホテル,兵庫, 平成 22 年 1 月 31 日.

5. 工藤正俊: 特別講演「肝癌治療アルゴリズムにおける分子標的治療の位置づけ」, 第 24 回冬季札幌がんセミナー, ロイトン札幌, 北海道, 平成 22 年 2 月 7 日.

6. 工藤正俊: 特別講演「肝細胞癌に対する最新の話題: ネクサバールの有用性とその位置づけ」, 学術講演会, メルキュールホテル横須賀, 神奈川, 平成 22 年 2 月 19 日.

7. 工藤正俊: 特別講演「肝癌診療ガイドラインと最新治療: 分子標的治療の位置付けも含めて」, 第 22 回県北 DSC, ホテルリソル佐世保, 長崎, 平成 22 年 2 月 24 日.

8. 工藤正俊: 特別講演「肝癌治療の新しいパラダイム」, 第 12 回久留米消化器癌セミナー, 久留米大学筑水会館, 福岡, 平成 22 年 2 月 25 日.

9. 工藤正俊: 特別講演「HCC 治療における分子標的治療への期待と課題」, 群馬県 HCC 分子標的治療セミナー, 前橋マーキュリーホテル, 群馬, 平成 22 年 3 月 4 日.

10. 工藤正俊: 特別講演「肝癌の分子標的治療: Up date」, 第 12 回関西肝癌局所療法研究会, 阪急電鉄本社ビル, 大阪, 平成 22 年 3 月 6 日.

11. 北野雅之: 基調講演「標準化に基づくコンベックス描出法 EUS-FNA の基本手技」,第 4 回 EUS ミニライブ, 平成 22 年 3 月 6 日,JA 尾道総合病院, 広島.

12. 北野雅之: ミニライブ「コンベックススコープを用いた EUS-FNA」, 第 4 回 EUS ミニライブ, 平成 22 年 3 月 6 日, JA 尾道総合病院, 広島.

13. 萩原　智: B 型肝炎の最新治療.　南大阪肝臓病セミナー, 平成 22 年 3 月 11 日, スイスホテル南海大阪, 大阪.

14. 南　康範: C 型肝炎の最新治療.　南大阪肝臓病セミナー, 平成 22 年 3 月 11 日, スイスホテル南海大阪, 大阪.

15. 上嶋一臣:肝炎・肝癌診療連携について～連携パス導入の試み～.　南大阪肝臓病セミナー, 平成 22 年 3 月 11 日, スイスホテル南海大阪, 大阪.

16. 工藤正俊: 特別講演「肝がん診療ガイドラインの現状と問題点」, 第 2 回肝疾患地域連携の会総会「肝疾患診療ネットワーク」, 筑波大学附属病院, 茨城, 平成 22 年 3 月 13 日.

17. 北野雅之: 消化器全般における PPI 処方に関する考え方 ～食道から大腸まで～.　臨床消化器談話会, 平成 22 年 3 月 15 日, 梅田スカイビルタワー, 大阪.

18. 工藤正俊: 特別講演「肝癌診療の新しいパラダイム」, 第 12 回長崎肝癌研究会学術講演会, 長崎全日空ホテルグラバーヒル, 長崎, 平成 22 年 3 月 18 日.

19. 工藤正俊: 特別講演「肝細胞癌の治療」, 第 8 回日本臨床腫瘍学会学術集会, 東京ビッグサイト, 東京, 平成 22 年 3 月 19 日.

20. 工藤正俊: 特別講演「ウイルス性肝炎の治療」, 肝がん撲滅の為の肝臓病市民公開講座, 松原市民文化会館, 大阪, 平成 22 年 4 月 4 日.

21. 上嶋一臣:肝がんの診断と治療.　肝がん撲滅の為の肝臓病市民公開講座, 平成 22 年 4 月 4 日, 松原市文化会館, 大阪.

22. 工藤正俊: 特別講演「本当は怖い B 型慢性肝疾患」, 第 11 回府中臨床セミナー, 府中病院, 大阪, 平成 22 年 4 月 8 日.

23. 工藤正俊: 特別講演「肝癌診療ガイドラインをめぐる最新の話題」, 肝癌診療の最前線～ミリプラ新発売記念講演会～, リーガロイヤルホテル堺, 大阪, 平成 22 年 4 月 17 日.

24. 工藤正俊: ポストグラデュエイトコース「肝腫瘍の診断」, 第 96 回日本消化器病学会総会, 新潟市民プラザ, 新潟, 平成 22 年 4 月 24 日.

25. 工藤正俊: 特別講演「肝細胞癌治療における分子標的治療への期待と課題」, 大阪外科 HCC 分子標的治療セミナー, ホテル阪急インターナショナル, 大阪, 平成 22 年 5 月 18 日.

26. 工藤正俊: 特別講演「肝癌の最新の治療: RFA 治療困難例対策から分子標的治療まで」, 第 9 回神奈川肝炎若手の会, 横浜ベイシェラトンホテル, 神奈川, 平成 22 年 5 月 21 日.

27. 工藤正俊: 特別講演「肝細胞癌診療の最新の話題」, 第 42 回生涯教育講演会, 岡山コンベンションセンター, 岡山, 平成 22 年 5 月 23 日.

28. 工藤正俊: 特別企画「肝細胞癌の画像診断 up-to-date」, 第 46 回日本肝臓学会総会, ホテルメトロポリタン山形, 山形, 平成 22 年 5 月 27 日.

29. 川崎正憲: 特別講演「*Helicobacter pylori* 除菌治療が ESD 後潰瘍治癒に及ぼす影響の検討」, 日本人の消化管疾患を考える会, 平成 22 年 5 月 27 日, リーガロイヤルホテル堺, 大阪.

30. 工藤正俊: 特別講演「肝癌に対する分子標的治療への期待と今後の展望」, 肝細胞癌ソラフェニブ治療研究会, 名古屋マリオットアソシアホテル, 愛知, 平成 22 年 6 月 9 日.

31. 工藤正俊: ランチョンセミナー「造影超音波は肝癌診療をどう変えたか？」, 日本消化器病学会中国支部例会 第 12 回教育講演会, 山口県国際総合センター, 山口, 平成 22 年 6 月 13 日.

32. 工藤正俊: 特別講演「肝癌診療の新しいパラダイム」, 第 4 回消化器疾患地域連携フォーラム, ホテルオークラ神戸, 兵庫, 平成 22 年 6 月 17 日.

33. 樫田博史：講演「NBI の有用性と大腸病変の治療方針決定における NBI の位置づけ」, 第 3 回大阪 NBI セミナー, 平成 22 年 6 月 19 日, 大阪.

34. 北野雅之: 特別講演「EUS-FNA の現況とコツ」, 第 3 回北里 EUS トレーニングコース, 平成 22 年 6 月 19 日, 北里大学東病院, 神奈川.

35. 樫田博史：講演「内視鏡診断・治療の進歩―大腸腫瘍を中心に」, 第 15 回 Shin-yokohama Digestive Disease Meeting (SDDM), 平成 22 年 6 月 23 日, 横浜, 神奈川.

36. 北野雅之: シンポジウム「EUS-FNA を中心に」, 第 45 回近畿消化器内視鏡懇談会, グランキューブ, 大阪.

37. 井上達夫: Pros and Cons Debates Session 「ソナゾイドはスクニーニン

グに使えるか」，第 10 回関西肝血流動態イメージ研究会，平成 22 年 7 月 3 日，オーバルホール，大阪.

38. 工藤正俊: ランチョンセミナー「コンセンサスに基づく肝細胞癌診断アルゴリズム」，第 46 回日本肝癌研究会，大阪国際会議場，大阪，平成 22 年 7 月 9 日.

39. 工藤正俊: 教育セミナー「肝細胞癌 内科の立場から-肝癌の内科治療の将来展望-」，第 13 回日本高齢消化器病学会，六本木アカデミーヒルズ，東京，平成 22 年 7 月 9 日.

40. 工藤正俊: 特別講演「肝癌診療の最新の話題」,OK7KK（岡山市中基幹 7 病院肝疾患研究会），ホテルグランヴィア岡山，岡山，平成 22 年 7 月 15 日.

41. 工藤正俊: 特別講演「肝細胞癌の分子標的治療」，伊丹市医師会内科医会 第 7 回消化器勉強会，伊丹シティホテル，兵庫，平成 22 年 7 月 22 日.

42. 工藤正俊: 特別講演「進行性肝細胞癌に対するソラフェニブの使用経験」，広島ネクサバール承認 1 周年記念セミナー，リーガロイヤルホテル広島，広島，平成 22 年 7 月 23 日.

43. 工藤正俊: 特別講演「ペグインターフェロン・リバビリン併用療法無効・再燃例に対するペグインターフェロン・リバビリン併用療法による再治療」，第 8 回肝臓病研究会シンポジウム，六本木アカデミーヒルズ 49，東京，平成 22 年 7 月 24 日.

44. 松井繁長: 講演「Helicobacter pylori 感染の最近の話題」，堺市医師会内科医会学術講演会，平成 22 年 7 月 27 日，堺市産業振興センター，大阪.

45. 工藤正俊: 特別講演「肝細胞癌に対する分子標的治療の現状と今後の展望」，日本医師会生涯教育講座 第 34 回肝臓を診る会，旭川グランドホテル，北海道，平成 22 年 7 月 29 日.

46. 工藤正俊: 特別講演「肝癌診療の新しいパラダイム」，第 21 回北海道肝がん研究会，ホテルニューオータニ札幌，北海道，平成 22 年 7 月 31 日.

47. 工藤正俊: 特別講演「肝癌診療ガイドライン 2009 年版 改訂のポイント」，第 11 回臨床消化器病研究会，グランドプリンスホテル新高輪，東京，平成 22 年 7 月 31 日.

48. 工藤正俊: 特別講演「肝細胞癌の最新の話題」，KBNC の会，全日空ホテルクレメント高松，香川，平成 22 年 8 月 13 日.

49. 北野雅之: 特別講演「超音波内視鏡診療の新潮流」, 第 7 回岡山胆膵診療技術向上研究会, 平成 22 年 8 月 21 日, アークホテル岡山, 岡山.

50. 北野雅之: 特別講演「超音波内視鏡ハンズオンセミナー」, 第 7 回岡山胆膵診療技術向上研究会, 平成 22 年 8 月 21 日, アークホテル岡山, 岡山.

51. 上嶋一臣: 講演「肝細胞癌に対する化学療法-分子標的薬を中心に-」, 第 5 回 Kinki Liver Club, 平成 22 年 8 月 26 日, スイスホテル南海大阪, 大阪

52. 工藤正俊: 特別講演「造影超音波は肝癌診療をどう変えたか？」, 第 52 回いわき肝疾患研究会, いわきワシントンホテル, 福島, 平成 22 年 8 月 27 日.

53. 井上達夫: 特別企画「特異な経過をたどったアルコール性肝硬変に合併した肝細胞癌の一例」, 第 53 回日本消化器画像診断研究会, 平成 22 年 9 月 3 日, 門司港ホテル, 福岡.

54. 工藤正俊: 特別講演「肝細胞癌診療における新しいパラダイム」, 高知肝癌診断治療セミナー, 高知新阪急ホテル, 高知, 平成 22 年 9 月 7 日.

55. 工藤正俊: 特別講演「肝癌治療の新しいパラダイム」, H22 八尾徳洲会医療連携の会, リーガロイヤルホテル大阪, 大阪, 平成 22 年 9 月 18 日.

56. 工藤正俊: 特別講演「肝細胞癌に対する分子標的治療の現状と今後の展望」, 兵庫 HCC 分子標的治療セミナー, 神戸ポートピアホテル, 兵庫, 平成 22 年 9 月 28 日.

57. 工藤正俊: 特別講演「肝細胞癌治療に対する分子標的治療の現状と今後の展望」, 第 1 回鹿児島肝細胞がん分子標的治療研究会, 城山観光ホテル, 鹿児島, 平成 22 年 9 月 30 日.

58. 工藤正俊: 特別講演「肝細胞癌診療の新しいパラダイム」, 学術講演会, 徳島グランヴィリオホテル, 徳島, 平成 22 年 10 月 1 日.

59. 工藤正俊: 特別講演「ウイルス性肝炎の治療」, 肝がん撲滅の為の肝臓病市民公開講座, 羽曳野市市民会館, 大阪, 平成 22 年 10 月 3 日.

60. 鄭　浩柄: 講演「肝がんの診断と治療」, 肝がん撲滅の為の肝臓病市民公開講座, 平成 22 年 10 月 3 日, 羽曳野市市民会館, 大阪.

61. 工藤正俊: 特別講演「コンセンサスに基づく肝細胞癌診断アルゴリズム」, 第 4 回肝癌の診断・治療に関する病診連携セミナー, ソニックシティ, 埼玉, 平成 22 年 10 月 4 日.

62. 鎌田　研: IPMN 診療における EUS の有用性.　第 18 回若手膵臓研究会, 平成 22 年 10 月 13 日, 横浜エクセルホテル東急, 神奈川.

63. 2010 樫田博史: 講演「大きい大腸腫瘍に対する EMR」, ランチョンセミナー「次世代につなぐ大腸 EMR ～ピットフォールかわすコツ・乗り越える技～」, 第 80 回日本消化器内視鏡学会総会（18 回日本消化器関連学会週間 JDDW 2010）, 平成 22 年 10 月 15 日, 横浜, 神奈川.

64. 北野雅之: EUS-FNA と Interventional EUS ～更なる普及に向けて～.　オリンパスサテライトシンポジウム「膵胆道領域の内視鏡戦略 ～ERCP から EUS まで～ 第 80 回日本消化器内視鏡学会総会, 平成 22 年 10 月 15 日, パシフィコ横浜, 神奈川.

65. 工藤正俊: 教育講演「消化器癌の治療戦略-海外との比較も含めて-」, 第 18 回日本消化器関連学会週間（第 28 回日本医学会総会共催）, パシフィコ横浜, 神奈川, 平成 22 年 10 月 16 日.

66. 工藤正俊: 特別講演「肝細胞癌診療の最新の話題: 発癌抑制から分子標的治療まで」, 西神奈川肝炎学術講演会, ロワジールホテル厚木, 神奈川, 平成 22 年 10 月 18 日.

67. 南　康範: Pros and Cons セッション「消化器領域 II　肝細胞癌の治療に造影剤が必須か？」, 社団法人日本超音波医学会第 37 回関西地方会学術集会, 平成 22 年 10 月 23 日, 神戸国際会議場, 兵庫.

68. 矢田典久: Pros and Cons セッション「消化器領域 III 超音波は肝線維化の診断に有用化？」, 社団法人日本超音波医学会第 37 回関西地方会学術集会, 平成 22 年 10 月 23 日, 神戸国際会議場, 兵庫.

69. 樫田博史: 講演「最新の大腸内視鏡－診断と治療」, 西神戸医療センター消化器オープンカンファレンス, 平成 22 年 10 月 28 日, 神戸, 兵庫.

70. 樫田博史: 講演「大腸疾患に対する最近の話題 - 内視鏡診断・治療を中心に - 」, 第 4 回関西消化器・肝疾患懇話会, 平成 22 年 10 月 30 日, 大阪.

71. 樫田博史: ショートレクチャー「最新の早期食道・胃がんの内視鏡診断と治療」, 消化器疾患地域連携フォーラム, 平成 22 年 11 月 4 日, 堺, 大阪.

72. 樫田博史: 特別講演「大腸疾患に対する最新の内視鏡診断・治療」, 消化器疾患地域連携フォーラム, 平成 22 年 11 月 4 日, 堺, 大阪.

73. 工藤正俊: 特別講演「肝細胞癌診療の最新の話題～IFN 治療から分子標的治

療まで～」，第 3 回渋谷消化器病ゼミナール，セルリアンタワー東急ホテル，東京，平成 22 年 11 月 5 日.

74. 北野雅之：超音波内視鏡の新たな潮流-造影と穿刺術-．第 5 回長野県超音波内視鏡研究会，平成 22 年 11 月 6 日，信州大学医学部附属病院，信州.

75. 坂本洋城：超音波内視鏡（EUS）を用いたインターベンション． 第 3 回膵・胆道がんセミナー in 大阪，平成 22 年 11 月 6 日，TKP 新大阪会議室，大阪.

76. 工藤正俊：特別講演「肝癌診療の新しいパラダイム」，阪神肝臓病治療研究会 第三回学術講演会，ホテル阪急インターナショナル，大阪，平成 22 年 11 月 11 日.

77. 朝隈　豊：講演「早期食道・胃がんの内視鏡診断と治療」，第 44 回日本消化器病学会近畿支部市民公開講座 最新の消化器の病気のお話，平成 22 年 11 月 14 日，藤井寺パープルホール，大阪.

78. 川崎正憲：講演「小腸・大腸の内視鏡診断と治療」，第 44 回日本消化器病学会近畿支部市民公開講座 最新の消化器の病気のお話，平成 22 年 11 月 14 日，藤井寺パープルホール，大阪.

79. 萩原　智：講演「肝炎の診断と治療」，第 44 回日本消化器病学会近畿支部市民公開講座 最新の消化器の病気のお話，平成 22 年 11 月 14 日，藤井寺パープルホール，大阪.

80. 井上達夫：講演「肝がんの診断と治療」，第 44 回日本消化器病学会近畿支部市民公開講座 最新の消化器の病気のお話，平成 22 年 11 月 14 日，藤井寺パープルホール，大阪.

81. 坂本洋城：講演「胆・膵がんの診断と治療」，第 44 回日本消化器病学会近畿支部市民公開講座 最新の消化器の病気のお話，平成 22 年 11 月 14 日，藤井寺パープルホール，大阪.

82. 樫田博史：講演「大腸腫瘍に対する EMR/ESD」，第 1 回 広島 EMR/ESD ハンズオンセミナー，平成 22 年 11 月 20 日，広島.

83. 工藤正俊：特別講演「B 型慢性肝疾患治療の最近の話題～肝がん抑止を目指して～」，OSAKA HBV SEMINAR ～de novo HEPATITIS & Latest CHB treatment, For Hepatologist and Hematologist～，リーガロイヤルホテル堺，大阪，平成 22 年 11 月 26 日.

84. 松井繁長：講演「上部消化管疾患の診断と治療」，近大消化器疾患病診連携の会，平成 22 年 11 月 27 日，近畿大学医学部小講堂，大阪.

85. 上嶋一臣：講演「肝炎・肝がんの最新の動向」，近大消化器疾患病診連携の会，平成 22 年 11 月 27 日，近畿大学医学部小講堂，大阪.

86. 北野雅之：講演「胆膵疾患における病診連携」，近大消化器疾患病診連携の会，平成 22 年 11 月 27 日，近畿大学医学部小講堂，大阪.

87. 樫田博史：講演「どこまで進んだ下部消化管内視鏡？」，近大消化器疾患病診連携の会，平成 22 年 11 月 27 日，近畿大学医学部小講堂，大阪.

88. 工藤正俊：特別講演「ウイルス性肝炎の治療」，肝がん撲滅の為の肝臓病市民公開講座，堺市民会館，大阪，平成 22 年 11 月 28 日.

89. 上嶋一臣：講演「肝がんの診断と治療」，肝がん撲滅の為の肝臓病市民公開講座，平成 22 年 11 月 28 日，堺市民会館，大阪.

90. 樫田博史：講演「大腸腫瘍：正確な診断に基づいた、確実・安全な治療」，第 5 回枚方消化器疾患談話会，平成 22 年 12 月 18 日，枚方，大阪.

91. 北野雅之：講演「Interventional EUS」，第 24 回日本消化器内視鏡学会近畿セミナー，平成 22 年 12 月 18 日，大阪国際交流センター，大阪.

92. 松井繁長：ショートレクチャー「早期食道・胃癌の最新の診断と治療」，堺市医師会外科医会役員研修会，平成 22 年 12 月 25 日，ホテル第一堺，大阪.

93. 工藤正俊：特別講演「世界から見た日本の肝癌治療の現状」，KBNC の会，全日空ホテルクレメント高松，香川，平成 22 年 12 月 28 日.

VI. 学会発表（海外シンポジウム）

1. 2010 Kitano M, Sakamoto H, Kudo M: Contrast-enhanced harmonic EUS for diagnosis of pancreatic tumors. Symposium “Therapeutic and diagnostic EUS for pancreatobiliary diseases -Current practices and new frontiers-, 第 79 回日本内視鏡学会総会, Tokyo, Japan, May 13-15.

2. 2010 Kitano M, Komaki T, Imai H, Sakamoto H, Takeyama Y, Kudo M: Contrast-enhanced harmonic endosonography in diagnosing pancreatic diseases. JPS Symposium 1 “Recent advances in the imaging studies of pancreatic diseases”, Joint Meeting of the International Association of Pancreatology and the Japan Pancreas Society 2010, Fukuoka, Japan, July 11-13.

1. 2010 Kamata K, Kitano M, Komaki T, Imai H, Sakamoto H, Takeyama Y, Kudo M: Endoscopic ultrasound-guided drainage for pancreatic diseases. JPS Video Symposium 1 “Cutting edge endoscopic procedures for diagnosis and treatment of panvreatic diseases”, Joint Meeting of the International Association of Pancreatology and the Japan Pancreas Society 2010, Fukuoka, Japan, July 11-13.

VII. 学会発表(海外一般演題)

1. 2010 Kitano M, Komaki T, Sakamoto H, Kamata K, Imai H, Kudo M: EUS-guided choledochoduodenostomy followed by endoscopic antegrade biliary stenting via the fistula for treatment of obstructive jaundice with duodenal stenosis. 2010 Digestive Disease Week, Louisiana, USA, May 1-5.

2. 2010 Kudo S, Mori Y, Miyachi H, Ikehara N, Yamamura F, Ohtsuka K, Kashida H, Hamatani S: Characteristics of type 0-Is+IIc colorectal neoplasms: protruded lesions derived from depressed type colorectal neoplasms. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

3. 2010 Kudo S, Hayashi T, Ikehara N, Yamamura F, Miyachi H, Ohtsuka K, Kashida H, Hamatani S, Yamano H: The submucosal invasive rate of depressed type colorectal lesions over the past decade. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

4. 2010 Kodama K, Ohtsuka K, Kashida H, Miyachi H, Wakamura K, Yamamura F, Kudo S: Therapeutic and diagnostic ERCP using the single balloon enteroscope in patients with reconstructed upper gastrointestinal tract. ASGE (DDW 2010) , May 1-5, 2010, New Orleans, USA.

5. 2010 Misawa M, Wada Y, Kashida H, Wakamura K, Hayashi T, Hosoya T, Miyachi H, Ikehara N, Yamamura F, Ohtsuka K, Hamatani S, Kudo S: Magnifying endoscopy with narrow-band imaging for diagnosis of colorectal lesions. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

6. 2010 Wada Y, Kudo S, Kashida H, Misawa M, Hayashi T, Miyachi H, Ikehara N, Ohtsuka K, Hamatani S: Diagnostic accuracy of pit pattern and vascular pattern analysis in colorectal lesions. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

7. 2010 Nakamura H, Ikeda H, Kutsukawa M, Kashida H, Ohtsuka K, Kudo S: Surveillance colonoscopy of neoplasm associated with ulcerative colitis by chromoendoscopy and magnifying endoscopy. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

8. 2010 Kudo S, Wakamura K, Kashida H, Ikehara N, Ohtsuka K, Miyachi H, Kobayashi Y, Kutsukawa M, Wada Y, Hosoya T, Hayashi T, Inoue H, Hamatani S: Real time diagnosis in the colorectum with integrated type endocytoscope. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

9. 2010 Kutsukawa M, Kudo S, Wakamura K, Kashida H, Miyachi H, Ikehara N, Wada Y: Diagnosis of massively invasive T1 cancers with endocytoscopy. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

10. 2010 Nishiwaki H, Kudo S, Kashida H, Ikehara N, Hayashi T, Ohtsuka K, Miyachi H: Complications associating endoscopic treatment of colorectal neoplasm. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

11. 2010 Hayashi T, Kashida H, Hosoya T, Wada Y, Miyachi H, Ikehara N, Yamamura F, Ohtsuka K, Kudo S: Colorectal ESD; treatment results and management of complications. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

12. 2010 Ikehara N, Kudo S, Kashida H, Hamatani S, Wakamura K, Kutsukawa M, Miyachi H, Kobayashi Y, Wada Y, Hosoya T, Hayashi T, Yamamura F, Ohtsuka K: Colorectal serrated lesions; clinicopathological features and magnifying endoscopic findings including endocytoscopy. ASGE (DDW 2010), May 1-5, 2010, New Orleans, USA.

13. 2010 Hayashi T, Kashida H, Kudo S: Colorectal ESD; treatment result and management of complications. International Symposium “ESD-current status and future perspectives; colorectal ESD” 79th General Meeting of Japan Gastroenterological Endoscopy Society, May 13-15, 2010, Tokyo, Japan.

14. 2010 Kamata K, Kitano M, Imai H, Komaki T, Sakamoto H, Takeyama Y, Kudo M: Management of IPMNs by endoscopic ultrasonography. Joint Meeting of the International Association of Pancreatology and the Japan Pancreas Society 2010, Fukuoka, Japan, July 11-13.

15. 2010 Sakamoto H, Kitano M, Komaki T, Imai H, Kamata K, Takeyama

Y, Nakai T, Ishida G, Yasuda T, Kamei K, Kudo M: EUS-guided broad plexus-neurolysis over the superior mesenteric artery using a 25 gauge needle. Joint Meeting of the International Association of Pancreatology and the Japan Pancreas Society 2010, Fukuoka, Japan, July 11-13.

16. 2010 Hayaishi S, Chung H, Kudo M: Branched-chain amino acid granules reduce the incidence of hepatocellular carcinoma in patients with liver cirrhosis. The 7th Japan-Korea Liver Symposium, Kyoto, Japan, July 18.

17. 2010 Bruix J, Kudo M: Luncheon workshop "Novel concepts in HCC staging." 4th Annual Conference International Liver Cancer Association (ILCA), Montral, Canada, September 11, 2010.

18. 2010 Bruix J, Kudo M: Luncheon workshop "Novel concepts in HCC staging." 4th Annual Conference International Liver Cancer Association (ILCA), Montral, Canada, September 11, 2010.

19. 2010 Kawasaki M, Asakuma Y, Mine H, Nagata Y, Sakurai T, Matsui S, Kashida H, Kudo M: The usefulness of helicobacter pylori eradication therapy for the healing of artificial gastric ulcer after endoscopic submucosal dissection for early gastric cancer. Asian Pacific Digestive Week (APDW) 2010, Kuala Lumpur, Malaysia, September 19-22.

20. 2010 Lencioni R, Lim HY, Stål P, Marrero J, Kudo M, Venook A, Nakajima K, Ye S-L: First Interim Results of The Global Investigation of Therapeutic DEcisions in Hepatocellular Carcinoma and Of its Treatment with SorafeNib (GIDEON) Study. Europian Society for Medical Oncology (ESMO) congress, Milan, Italy, October 8-12.

21. 2010 Kitai S, Kudo M, Arii S, Ichida T, Omata M, Sakamoto M, Takayasu K, Nakashima O, Makuuchi M, Matsuyama Y, Monden M, for The Liver Cancer Study Gourp of Japan: Non-liver transplantation treatment for hepatocellular carcinoma within the Milan criteria in child-pugh score 10-11 cirrhotic patients has a survival benefit. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

22. 2010 Matsui S, Kudo M, Okada M, Asakuma Y, Kawasaki M, Nagata Y, Kashida H: Utility of evaluation of the response to chemotherapy in advanced gastric cancer by contrast-enhanced harmonic EUS using Sonazoid. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

23. 2010 Kitano M, Takagi T, Sakamoto H, Komaki T, Imai H, Kamata K, Yamao K, Kudo M: Characterization of small pancreatic neoplasms by contrast-enhanced harmonic EUS. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

24. 2010 Inoue T, Kudo M, Hayaishi S, Ueda T, Takita M, Kitai S, Yada N, Hagiwara S, Chung H, Ueshima K: Usefulness of hepatocyte phase imaging of Gd-EOB-DTPA-MRI in detecting borderline lesions which are difficult to detect other imaging modalities. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

25. 2010 Inoue T, Kudo M, Komuta M, Hayaishi S, Ueda T, Takita M, Kitai S, Hatanaka K, Yada N, Hagiwara S, Minami Y, Chung H, Ueshima K, Sakamoto M, Okada M, Kumano S, Murakami T: Can Gd-EOB-DTPA-enhanced MRI discriminate between dysplastic nodules and early-to- well-differentiated HCC? 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

26. 2010 Sakamoto H, Kitano M, Komaki T, Kamata K, Imai H, Kudo M: Estimation of malignant potential gist by contrast-enhanced harmonic endoscopic ultrasonography. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

27. 2010 Asakuma Y, Matsui S, Mine H, Nagata Y, Kitai S, Sakamoto H, Inoue T, Sakurai T, Kashida H, Kudo M: Is the combination therapy of ecabet sodium and proton pump inhibitor (PPI) useful for treating the artificial ulcer after endoscopic submucosal dissection (ESD) treatment of early gastric cancer? : Prospective randomized study. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

28. 2010 Asakuma Y, Matsui S, Mine H, Nagata Y, Kawasaki M, Kitai S, Sakamoto H, Inoue T, Sakurai T, Kashida H, Kudo M: The efficacy of helicobacter pylori eradication therapy for the healing of artificial gastric ulcer after endoscopic submucosal dissection early gastric cancer: Prospective randomized study. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

29. 2010 Asakuma Y, Matsui S, Mine H, Nagata Y, Kawasaki M, Kitai S, Sakamoto H, Inoue T, Sakurai T, Kashida H, Kudo M: Examination of factors of delayed bleeding after endoscopic submucosal dissection (ESD) for gastric tumors. 18th United European Gastroenterology Week (UEGW) 2010, Barcelona, Spain, October 23-27.

30. 2010 Hatanaka K, Chung H, Kudo M, Kitai S, Inoue T, Yada N, Hagiwara S, Ueshima K: The usefulness of the post-vascular phase of contrast-enhanced ultrasonography with Sonazoid in the evaluation of gross type of hepatocellular carcinoma. American Association for the Study of Liver Diseases (AASLD) The Liver Meeting 2010, Massachusetts, USA, October 29-November 2.

31. 2010 Marrero J, Lim HY, Stål P, Lencioni R, Kudo M, Venook A, Nakajima K, Ye S-L: Sorafenib treatment and safety profile in Child Pugh B patients characterized in first interim results of GIDEON (Global Investigation Of Therapeutic Decisions In Hepatocellular Carcinoma And Of Its Treatment With Sorafenib). American Association for the Study of Liver Diseases (AASLD) The Liver Meeting 2010, Massachusetts, USA, October 29-November 2.

VIII. 学会発表

(国内シンポジウム・パネルディスカッション・ワークショップ)

1. 2010 鎌田　研, 北野雅之, 工藤正俊: EUS を主とした IPMN の診断とフォローアップ方法. ワークショップ「嚢胞性膵疾患の鑑別診断と治療法の選択」, 第84回日本消化器内視鏡学会近畿地方会, 平成 22 年 3 月 13 日, 大阪国際交流センター, 大阪.

2. 2010 鎌田　研, 北野雅之, 工藤正俊: EUS を用いた IPMN の診断とフォローアップ.　シンポジウム「膵 IPMN の手術適応の見直し」, 第 96 回日本消化器病学会総会, 平成 22 年 4 月 22 日-24 日, 新潟市民プラザ, 新潟.

3. 2010 鎌田　研, 北野雅之, 工藤正俊: 胆膵疾患に対する EUS ガイド下ステント治療の成績.　パネルディスカッション「消化器ステント治療の進歩と現状」, 第 96 回日本消化器病学会総会, 平成 22 年 4 月 22 日-24 日, 新潟市民プラザ, 新潟.

4. 2010 小牧孝充, 北野雅之, 工藤正俊: Sonazoid を用いた造影 EUS 検査による膵腫瘍性病変の診断.　ワークショップ「胆膵画像診断の進歩」, 第 96 回日本消化器病学会総会, 平成 22 年 4 月 22 日-24 日, 新潟市民プラザ, 新潟.

5. 2010 今井　元, 北野雅之, 工藤正俊: 切除不能悪性胆道狭窄に対する胆管ステンティングの検討①.　特別シンポジウム「胆道ステントの適応と選択」, 第 79 回日本消化器内視鏡学会総会, 平成 22 年 5 月 13 日-15 日, グランドプリンスホテル新高輪, 東京.

6. 2010 今井　元, 北野雅之, 工藤正俊: 切除不能悪性胆道狭窄に対する胆管ステンティングの検討②.　特別シンポジウム「胆道ステントの適応と選択」, 第 79 回日本消化器内視鏡学会総会, 平成 22 年 5 月 13 日-15 日, グランドプリンスホテル新高輪, 東京.

7. 2010 坂本洋城, 北野雅之, 工藤正俊: 造影ハーモニック EUS による SMT の鑑別および GIST の悪性度評価の試み.　シンポジウム「上部消化管 SMT のマネージメント-GIST との鑑別と取り扱い」, 第 79 回日本消化器内視鏡学会総会, 平成 22 年 5 月 13 日-15 日, グランドプリンスホテル新高輪, 東京.

8. 2010 鎌田　研, 北野雅之, 工藤正俊: EUS を用いた IPMN の診断～診断ハーモニック法を含めて～.　ワークショップ「国際診療ガイ

ドラインを踏まえたIPMNの内視鏡診断の現状と問題点」，第79回日本消化器内視鏡学会総会，平成22年5月13日-15日，グランドプリンスホテル新高輪，東京.

9. 2010 樫田博史，山村冬彦，工藤進英：大腸内視鏡挿入の基本は軸保持短縮法である．ビデオワークショップ「エキスパートに学ぶ大腸内視鏡挿入法のコツ」第79回日本消化器内視鏡学会総会，平成22年5月13日-15日，東京.

10. 2010 横山顕礼，樫田博史，工藤進英：当院における緊急大腸内視鏡の実際．パネルディスカッション「救急医療における内視鏡の現状と問題点－消化管疾患を中心に－」，第79回日本消化器内視鏡学会総会 ，平成22年5月13日-15日，東京.

11. 2010 森川吉英，樫田博史，工藤進英：超高齢者の大腸腫瘍に対する内視鏡治療の安全性．パネルディスカッション「超高齢者に対する内視鏡治療－適応と限界－」，第79回日本消化器内視鏡学会総会，平成22年5月13日-15日，東京.

12. 2010 塩飽洋生，樫田博史，工藤進英：大腸内視鏡検査に関連した偶発症の予防と対策．パネルディスカッション「内視鏡の安全管理」 第79回日本消化器内視鏡学会総会，平成22年5月13日-15日，東京.

13. 2010 林　武雅，樫田博史，工藤進英：大腸 ESD 標準化についての検討．ビデオシンポジウム「下部消化管 ESD の手技と工夫」 第79回日本消化器内視鏡学会総会，平成22年5月13日-15日，東京.

14. 2010 和田祥城，樫田博史，工藤進英，浜谷茂治：NBI 拡大観察における大腸病変の質的・深達度診断を用いた大腸病変の微小血管構造観察．特別シンポジウムコンセンサス2010 ①「大腸腫瘍に対する NBI 拡大観察所見分類の統一を目指して」，第79回日本消化器内視鏡学会総会，平成22年5月13日-15日，東京.

15. 2010 上嶋一臣，<u>工藤正俊</u>：HCCに対するソラフェニブの治療効果予測について．　シンポジウム「肝細胞癌の分子標的探索と臨床応用」，第46回日本肝臓学会総会，平成22年5月27日-28日，ホテルメトロポリタン山形，山形.

16. 2010 畑中絹世，鄭　浩柄，<u>工藤正俊</u>，土師誠二，熊野正士，岡田真広：肝細胞癌の肉眼敬体とSonazoid造影超音波における defect 像の比較．　シンポジウム「肝腫瘍の造影エコーの最先端

（術中超音波含む）」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

17. 2010 岡田無文，松井繁長，工藤正俊：造影EUS検査による進行胃癌の化学療法効果判定. シンポジウム「消化管疾患における超音波診断」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

18. 2010 藤本研治，外村明子，辰巳千栄，石田哲士，上嶋一臣，三竹毅，椎名 毅，工藤正俊，加藤道夫：慢性肝疾患における Real-time Tissue Elastographyの精度の検討. シンポジウム「組織エラストグラフィーの現況と展望」，日本超音波医学会第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

19. 2010 鎌田 研，北野雅之，工藤正俊，坂本洋城，小牧孝充，今井元：EUSを用いたIPMN診療. シンポジウム「膵疾患の超音波診断」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

20. 2010 小牧孝充，北野雅之，今井 元，鎌田 研，工藤正俊：Sonazoidを用いた造影EUS検査による膵腫瘍性病変の診断. パネルディスカッション「超音波内視鏡の新展開」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

21. 2010 北野雅之，小牧孝充，坂本洋城，今井 元，鎌田 研，工藤正俊：膵疾患に対する超音波内視鏡ガイド下ドレナージ術. ワークショップ「消化器疾患における Interventional Sonography」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

22. 2010 坂本洋城，北野雅之，工藤正俊：癌性疼痛におけるEUS下広範囲腹腔神経叢融解術の有用性の検討：preliminary study. ワークショップ「消化器疾患における Interventional sonography」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

23. 2010 坂本洋城，北野雅之，工藤正俊：造影ハーモニックEUSによるGISTの悪性度評価の試み. ワークショップ「胆・膵・消化管疾患による造影エコー法の位置づけ」，日本超音波医学会 第83回学術集会，平成22年5月29日-31日，京都国際会議場，京都.

24. 2010 矢田典久，辰巳千栄，上嶋一臣，藤本研治，加藤道夫，椎名毅，外村明子，三竹　毅，工藤正俊：C 型慢性肝疾患患者に対する非侵襲的肝線維化評価の有用性に関する検討．　ワークショップ「びまん性肝疾患の Ultrasound Functional Imaging」，日本超音波医学会 第 83 回学術集会，平成 22 年 5 月 29 日-31 日，京都国際会議場，京都.

25. 2010 南　康範，畑中絹世，工藤正俊：造影エコー撮像法の工夫 - Defect Re-perfusion imaging-．　特別企画「肝腫瘍の超音波診断基準の検証」，日本超音波医学会 第 83 回学術集会，平成 22 年 5 月 29 日-31 日，京都国際会議場，京都.

26. 2010 森　秀明，畠　二郎，樫田博史，関根智紀，西田　睦，西川かおり，長谷川雄一，藤井康友，本田伸行，山田博康，宮本幸夫：消化管診断基準小委員会からの報告．シンポジウム「消化管疾患における超音波診断」，日本超音波医学会第 83 回学術集会，平成 22 年 5 月 29 日-31 日，京都.

27. 2010 永井知行，荒尾徳三，坂井和子，工藤可苗，金田裕靖，田村大介，青松圭一，木村英晴，藤田至彦，松本和子，西條長宏，工藤正俊，西尾和人：Sorafenib inhibits the hepatocyte growth factor-mediated epithelial mesenchymal transition in hepatocellular carcinoma．ソラフェニブは肝細胞癌株において、HGF 起因の上皮間葉移行（Epithelial mesenchymal transition）を阻害する．　ワークショップ，第 69 回日本癌学会学術総会，平成 22 年 9 月 22 日-24 日，大阪国際会議場，大阪.

28. 2010 今井　元，北野雅之，工藤正俊：経乳頭的アプローチ困難例に対する EUS 下胆道ドレナージの有用性．　ビデオワークショップ「私が薦める胆道内視鏡のコツ～安全性を目指して～」，第 46 回日本胆道学会学術集会，平成 22 年 9 月 24 日-25 日，リーガロイヤルホテル広島，広島.

29. 2010 北野雅之，坂本洋城，小牧孝充，今井　元，鎌田　研，工藤正俊：EUS-FNA 穿刺針の使い分けとコツ．　特別企画講演「先端施設における膵の EUS-FNA」，第 8 回 FNA-Club Japan，平成 22 年 9 月 25 日，三井ガーデンホテル広島，広島.

30. 2010 今井　元，北野雅之，工藤正俊：悪性胆道狭窄に対する EUS 下胆道ドレナージ術の有用性．　ビデオワークショップ「胆膵疾患における治療の進歩と今後の展開」，第 85 回日本消化器内視鏡

学会近畿地方会，平成 22 年 10 月 2 日，大阪国際交流センター，大阪.

31. 2010 坂本洋城，北野雅之，工藤正俊：癌性疼痛における超音波内視鏡下広範囲腹腔神経叢融解術（EUS-BPN）の有用性. シンポジウム「胆道・膵臓癌に対する Interventional oncology-現在そして将来を展望する-」，第 18 回日本消化器関連学会週間（第 80 回日本消化器内視鏡学会総会・第 8 回日本消化器外科学会大会合同），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

32. 2010 鎌田 研，北野雅之，工藤正俊：胆膵疾患に対する EUS ガイド下ドレナージ術. シンポジウム「Interventional EUS の評価」，第 18 回日本消化器関連学会週間（第 80 回日本消化器内視鏡学会総会・第 52 回日本消化器病学会大会合同），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

33. 2010 矢田典久，鄭 浩柄，工藤正俊：PEG-IFNα/RBV 併用療法における血清フェリチン値と SVR との関係. パネルディスカッション「代謝異常（金属代謝を含む）からみた C 型肝炎の病態解析」，第 18 回日本消化器関連学会週間（第 14 回日本肝臓学会大会・第 52 回日本消化器病学会大会合同），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

34. 2010 畑中絹世，工藤正俊，熊野正士：肝細胞癌の肉眼分類評価におけるソナゾイド造影超音波の有用性-造影 dynamic CT との比較. ワークショップ「肝細胞癌に対する画像診断の進歩と新たな治療戦略」，第 18 回日本消化器関連学会週間（第 52 回日本消化器病学会大会・第 14 回日本肝臓学会大会合同），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

35. 2010 鎌田 研，北野雅之，工藤正俊：EUS を主とした IPMN、IPNB の診療ストラテジー. ワークショップ「肝胆膵での上皮内腫瘍：病態解明と治療戦略」，第 18 回日本消化器関連学会週間（第 14 回日本肝臓学会大会・第 52 回日本消化器病学会・第 8 回日本消化器外科学会大会合同），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

36. 2010 坂本洋城，北野雅之，工藤正俊：造影ハーモニック EUS による GIST の悪性度評価. ワークショップ「GIST の基礎と臨床」，第 18 回日本消化器関連学会週間（第 52 回日本消化器病学会・第 80 回日本消化器内視鏡学会総会・第 8 回日本消化器外科学会大会合同），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈

川.

37. 2010 北野雅之，小牧孝充，工藤正俊：EUS ガイド下治療のコツと工夫． ワークショップ「胆膵内視鏡治療のエキスパートテクニック＜ビデオ＞」，第 18 回日本消化器関連学会週間（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

38. 2010 山村冬彦，樫田博史，工藤進英：苦痛なく安全な大腸内視鏡検査の挿入と工夫．ワークショップ「トータルコロノスコピーを巡って～挿入法匠の技～」，第 80 回日本消化器内視鏡学会総会（18 回日本消化器関連学会週間 JDDW 2010），平成 22 年 10 月 13 日-16 日，横浜，神奈川.

IX. 学会発表（国内一般演題）

1. 2010 今井　元，北野雅之，末冨洋一郎，小牧孝充，鎌田　研，坂本洋城，工藤正俊：十二指腸ステント留置後に EUS 下胆嚢ドレナージ術を行った閉塞性黄疸の一例．　日本消化器病学会近畿支部第 92 回例会，平成 22　年 2 月 27 日，大阪国際交流センター，大阪.

2. 2010 萩原　智，上嶋一臣，鄭　浩柄，工藤正俊：癌幹細胞のマーカーである CD133 は進行肝細胞癌に対する S1+PEG-IFNalpha2b 治療における効果予測因子である．　シンポジウム「消化器癌化学療法の適応と限界-肝胆膵領域-」，日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪.

3. 2010 有住忠晃，石川恵美，宮田　剛，峯　宏昌，早石宗右，田北雅弘，上田泰輔，辰巳千栄，北井　聡，畑中絹世，矢田典久，井上達夫，萩原　智，鄭　浩柄，上嶋一臣，工藤正俊，金井良高：慢性C型肝炎に対してPEG-IFN+Ribavirin併用療法中にITPを発症した 1 例．　日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪.

4. 2010 高場雄久，宮田　剛，峯　宏昌，鎌田　研，有住忠晃，田北雅弘，早石宗右，永井知行，上田泰輔，辰巳千栄，北井　聡，畑中絹世，矢田典久，井上達夫，石川恵美，萩原　智，鄭　浩柄，上嶋一臣，工藤正俊：C 型慢性肝炎 SVR 後に悪性リンパ腫を発症した一例．　日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪.

5. 2010 足立哲平，萩原　智，有住忠晃，峯　宏昌，宮田　剛，早石宗右，辰巳千栄，上田泰輔，田北雅弘，畑中絹世，北井　聡，石川恵美，矢田典久，井上達夫，鄭　浩柄，上嶋一臣，工藤正俊，梅原　泰：肝機能障害を認めたエルシニア腸炎の一例．　日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪.

6. 2010 湯本妙子，今井　元，鎌田　研，坂本洋城，末冨洋一郎，小牧孝充，北野雅之，工藤正俊：癌性疼痛に対し EUS 下腹腔神経叢ブロックが有用であった 1 症例．　日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪.

7. 2010 奥村直己，山本典雄，富田崇文，梅原康湖，南　康範，森村正嗣，米田　円，山田　哲，辻　直子，工藤正俊：潰瘍性大腸炎経過中に発症した Clostridium difficile 関連腸病変の一例．日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪．

8. 2010 林　道友，奥田英之，茂山朋広，宮部欽生，豊澤昌子，岸谷譲，鍋島紀滋，工藤正俊：サイトメガロウイルス検査が陰性を示したガンシクロビル投与により軽快した潰瘍性大腸炎の一例．日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪．

9. 2010 宮田　剛，鄭　浩柄，有住忠晃，早石宗右，田北雅弘，上田泰輔，辰巳千栄，北井　聡，畑中絹世，石川恵美，矢田典久，井上達夫，萩原　智，上嶋一臣，工藤正俊，土師誠二，山崎満夫：遊走脾の捻転により脾梗塞をきたした一例．　日本消化器病学会近畿支部第 92 回例会，平成 22 年 2 月 27 日，大阪国際交流センター，大阪．

10. 2010 南　康範，奥村直己，山本典雄，辻　直子，工藤正俊：造影超音波による血流定量化の試み-肝細胞癌に対する TACE の早期治療効果判定-．　第 12 回関西肝癌局所療法研究会，平成 22 年 3 月 6 日，阪急電鉄本社ビル，大阪．

11. 2010 土師誠二，山崎満夫，北口博士，中多靖幸，亀井敬子，安田武生，石川　原，中居卓也，竹山宜典，畑中絹世，工藤正俊：肝細胞癌外科治療における術中造影エコーの意義．　第 12 回関西肝癌局所療法研究会，平成 22 年 3 月 6 日，阪急電鉄本社ビル，大阪．

12. 2010 山本典雄，奥村直己，冨田崇文，梅原康湖，南　康範，森村正嗣，米田　円，山田　哲，辻　直子，工藤正俊，村上春郎，浦瀬文明：閉塞性黄疸で発見された悪性リンパ腫の一例．　第 84 回日本消化器内視鏡学会近畿地方会，平成 22 年 3 月 13 日，大阪国際交流センター，大阪．

13. 2010 小牧孝充，北野雅之，工藤正俊，末冨洋一郎，今井　元，鎌田研：難治性胆管炎を伴った胆管癌に対する低容量ジェムザール治療．　第 96 回日本消化器病学会総会，平成 22 年 4 月 22 日-24 日，新潟市民プラザ，新潟．

14. 2010 小牧孝充，北野雅之，工藤正俊，末冨洋一郎，今井　元，鎌田研：当院における根治手術不能な膵小細胞癌の治療成績．　第

96 回日本消化器病学会総会，平成 22 年 4 月 22 日-24 日，新潟市民プラザ，新潟.

15. 2010 鎌田　研，北野雅之，今井　元，小牧孝充，坂本洋城，末冨洋一郎，工藤正俊：経乳頭的アプローチ困難例に対する EUS 下胆道ドレナージ術の有用性．第 79 回日本消化器内視鏡学会総会，平成 22 年 5 月 13 日-15 日，グランドプリンスホテル新高輪，東京.

16. 2010 三澤将史，和田祥城，樫田博史，工藤進英：横行結腸 LST-NG(PD)の 1 例．第 5 回大腸拡大内視鏡研究会，第 79 回日本消化器内視鏡学会総会，平成 22 年 5 月 15 日，東京.

17. 2010 和田祥城，三澤将史，森　悠一，細谷寿久，若村邦彦，池原伸直，山村冬彦，大塚和朗，樫田博史，工藤進英，浜谷茂治：NBI 併用 endocytoscope（EC-NBI）を用いた大腸病変の微小血管構造観察．第 2 回内視鏡画像標準化のための研究会　第 79 回日本消化器内視鏡学会総会，平成 22 年 5 月 15 日，東京.

18. 2010 工藤正俊，今中和穂，千田信之，仲地耕平，高山忠利，金子周一，坪内博仁，林　紀夫，熊田博光，和田道彦，沖田　極：根治的治療不能の肝細胞癌に対して肝動脈塞栓化学療法（TACE）を施行した患者を対象としたソラフェニブの日韓共同第 III 相臨床試験．　第 46 回日本肝臓学会総会，平成 22 年 5 月 27 日-28 日，ホテルメトロポリタン山形，山形.

19. 2010 上田泰輔，鄭　浩柄，工藤正俊：肝細胞癌根治後 C 型肝癌に対するインターフェロン少量長期維持療法の生命予後改善効果に関する検討．　第 46 回日本肝臓学会総会，平成 22 年 5 月 27 日-28 日，ホテルメトロポリタン山形，山形.

20. 2010 藤本研治，外村明子，辰巳千栄，石田哲士，上嶋一臣，三竹毅，山本圭司，椎名　毅，工藤正俊，加藤道夫：Real-time Tissue Elastography による非侵襲的肝線維化評価法は炎症の影響を受けない．　第 46 回日本肝臓学会総会，平成 22 年 5 月 27 日-28 日，ホテルメトロポリタン山形，山形.

21. 2010 坂本洋城，北野雅之，小牧孝充，今井　元，鎌田　研，工藤正俊：造影ハーモニック EUS による上部消化管粘膜下腫瘍の鑑別の試み．　日本超音波医学会 第 83 回学術集会，平成 22 年 5 月 29 日-31 日，京都国際会議場，京都.

22. 2010 井上達夫，畑中絹世，前川　清，工藤正俊：造影超音波検査に

よる肝細胞癌の診断能-Gd-EOB-MRI, Dynamic CT との比較検討-. 日本超音波医学会 第 83 回学術集会, 平成 22 年 5 月 29 日-31 日, 京都国際会議場, 京都.

23. 2010 今井 元, 北野雅之, 鎌田 研, 小牧孝充, 坂本洋城, 工藤正俊: 膵腫瘍に対する腹部超音波, 超音波内視鏡, MDCT の部位別検出率の比較検討. 日本超音波医学会 第83回学術集会, 平成22年5月29日-31日, 京都国際会議場, 京都.

24. 2010 岡田真広, 熊野正士, 香川祐毅, 塚部明大, 工藤正幸, 上嶋一臣, 矢田典久, 井上達夫, 工藤正俊, 村上卓道: 進行型肝細胞癌症例に対するSorafenib治療前後の肝CT perfusion検査. 第2回日本肝がん分子標的治療研究会, 平成22年6月19日, 大手町サンケイプラザ, 東京.

25. 2010 櫻井俊治, 萩原 智, 矢田典久, 井上達夫, 鄭 浩柄, 上嶋一臣, 工藤正俊: 発癌分子機序に基づく新しい肝がん治療薬の可能性. 第 2 回日本肝がん分子標的治療研究会, 平成 22 年 6 月 19 日, 大手町サンケイプラザ, 東京.

26. 2010 荒尾徳三, 松本和子, 工藤可苗, 永井知行, 工藤正俊, 西尾和人: 分子標的薬によるがん幹細胞マーカーCD133 の発現制御. 第2回日本肝がん分子標的治療研究会, 平成22年6月19日, 大手町サンケイプラザ, 東京.

27. 2010 永井知行, 荒尾徳三, 工藤可苗, 工藤正俊, 西尾和人: Sorafenibは肝細胞がんの上皮間葉移行を阻害する. 第 2 回日本肝がん分子標的治療研究会, 平成 22 年 6 月 19 日, 大手町サンケイプラザ, 東京.

28. 2010 南 康範, 奥村直己, 山本典雄, 辻 直子, 工藤正俊, Kono Y: 造影超音波による血流定量化の試み-肝細胞癌に対する TACE の早期治療効果判定- Early response of transcatheter arterial chemoembolization for hepatocellular carcinoma: Quantification of tumor vascularity with contrast-enhanced sonography. 第 10 回関西肝血流動態イメージ研究会, 平成 22 年 7 月 3 日, オーバルホール, 大阪.

29. 2010 岡田真広, 熊野正士, 香川祐毅, 工藤正幸, 上嶋一臣, 矢田典久, 井上達夫, 工藤正俊, 村上卓道: 進行型肝細胞癌に対する Sorafenib 治療効果判定における肝 CT Perfusion 検査. 第 10 回関西肝血流動態イメージ研究会, 平成22年7月3日, オーバルホール, 大阪.

30. 2010 上嶋一臣，工藤正俊：HCC に対するソラフェニブを用いた血管新生抑制治療の効果予測因子としてのPIVKA-IIの有用性に関する検討. 第10回関西肝血流動態イメージ研究会，平成22年7月3日，オーバルホール，大阪.

31. 2010 櫻井俊治：新しい分子標的治療薬の可能性. 第23回南大阪肝疾患研究会,平成22年7月16日，リーガロイヤル堺，大阪.

32. 2010 奥村直己，高場雄久，山本典雄，富田崇文，梅原康湖，南 康範，森村正嗣，米田 円，山田 哲，辻 直子，工藤正俊：十二指腸狭窄を契機に診断された十二指腸悪性リンパ腫の一例. 日本消化器病学会近畿支部第93回例会，平成22年9月18日，大阪国際交流センター，大阪.

33. 2010 有住忠晃，萩原 智，早石宗右，田北雅弘，上田泰輔，北井聡，畑中絹世，矢田典久，井上達夫，鄭 浩柄，上嶋一臣，樫田博史，工藤正俊：発熱、及び軽度の肝機能障害に発症した肝サルコイドーシスの1例. 日本消化器病学会近畿支部第93回例会，平成22年9月18日，大阪国際交流センター，大阪.

34. 2010 小牧孝充，北野雅之，工藤正俊，坂本洋城：自己免疫性膵炎の検討：EUSによる膵実質所見. 日本消化器病学会近畿支部第93回例会，平成22年9月18日，大阪国際交流センター，大阪.

35. 2010 宮田 剛，樫田博史，峯 宏昌，川崎正憲，永田嘉昭，朝隈豊，櫻井俊治，松井繁長，工藤正俊：特発性腸間膜静脈硬化症の1例. 日本消化器病学会近畿支部第93回例会，平成22年9月18日，大阪国際交流センター，大阪.

36. 2010 峯 宏昌，朝隈 豊，川崎正憲，永田嘉昭，櫻井俊治，松井繁長，樫田博史，工藤正俊，筑後孝章：早期胃癌に内分泌細胞巣を併発した一例. 日本消化器病学会近畿支部第93回例会，平成22年9月18日，大阪国際交流センター，大阪.

37. 2010 鎌田 研，北野雅之，工藤正俊，今井 元，小牧孝充，坂本洋城：Sonazoidを用いた造影EUSによる胆嚢病変の診断. 第46回日本胆道学会学術集会，平成22年9月24日-25日，リーガロイヤルホテル広島，広島.

38. 2010 小牧孝充，北野雅之，坂本洋城，今井 元，鎌田 研，工藤正俊，中居卓也，竹山宜典：EUSによる肝外胆管癌の進展度診断. 第46回日本胆道学会学術集会，平成22年9月24日-25日，リ

ーガロイヤルホテル広島，広島.

39. 2010 宮田　剛，井上達夫，有住忠晃，早石宗右，上田泰輔，辰巳千栄，田北雅弘，北井　聡，石川恵美，矢田典久，萩原　智，鄭浩柄，上嶋一臣，工藤正俊：非閉塞性腸管虚血を発症した悪性リンパ腫の一例.　第 18 回日本消化器関連学会週間 JDDW 2010（第 52 回日本消化器病学会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

40. 2010 川崎正憲，松井繁長，峯　宏昌，永田嘉昭，朝隈　豊，工藤正俊：ヘリコバクターピロリ陽性早期胃癌における除菌治療が ESD 後人工潰瘍治癒過程に及ぼす影響の検討.　第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

41. 2010 山本典雄，辻　直子，高場雄久，奥村直己，冨田崇文，梅原康湖，南　康範，森村正嗣，米田　円，山田　哲，本庶　元，藤田淳也，浦瀬文明，前倉俊治，工藤正俊：消化管悪性リンパ腫の初回内視鏡診断と病理診断の問題点.　第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

42. 2010 永田嘉昭，松井繁長，朝隈　豊，川崎正憲，岡田無文，工藤正俊：治療成績からみら胃腫瘍に対する ESD の検討.　第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

43. 2010 奥村直己，辻　直子，高場雄久，山本典雄，冨田崇文，梅原康湖，南　康範，森村正嗣，米田　円，山田　哲，本庶　元，工藤正俊：経皮内視鏡的胃瘻増設術（PEG）における胃壁固定の有用性と問題点.　第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

44. 2010 高場雄久，辻　直子，奥村直己，山本典雄，冨田崇文，梅原康湖，南　康範，森村正嗣，米田　円，山田　哲，本庶　元，工藤正俊：胃壁固定併用の経皮内視鏡的胃瘻増設術（PEG）における quli 法と direct 法の比較検討.　第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川.

45. 2010 梅原康湖，高場雄久，奥村直己，山本典雄，冨田崇文，南　康範，

森村正嗣，米田　円，山田　哲，辻　直子，工藤正俊：高齢者の外来下部消化管内視鏡検査におけるプロポフォール至適導入量の検討．　第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

46.　2010　今井　元，北野雅之，小牧孝充，鎌田　研，坂本洋城，工藤正俊：胆管挿入困難例に対する EUS 下ドレナージ術の位置づけ．第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

47.　2010　上田泰輔，土谷　薫，橋元　悟，今関文夫，垣内雅彦，工藤正俊：PEG-IFN α2b/RBV 併用療法の無効・再燃例に対する PEG-IFN α2a/RBV 併用療法の再治療の検討-他施設共同研究 RETRY study．　第 18 回日本消化器関連学会週間 JDDW 2010（第 14 回日本肝臓学会大会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

48.　2010　井上達夫，畑中絹世，早石宗右，上田泰輔，田北雅弘，北井聡，矢田典久，萩原　智，鄭　浩柄，上嶋一臣，工藤正俊：造影エコーによる肝細胞癌の診断能、Gd-EOB-MRI、Dynamic CT との比較検討．　第 18 回日本消化器関連学会週間 JDDW 2010（第 14 回日本肝臓学会大会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

49.　2010　鄭　浩柄，上田泰輔，早石宗右，田北雅弘，北井　聡，畑中絹世，矢田典久，井上達夫，萩原　智，上嶋一臣，工藤正俊，土師誠二：線維化進行 C 型肝炎患者における脾摘後のインターフェロン導入における問題点-好中球数の変化について-．　第 18 回日本消化器関連学会週間 JDDW 2010（第 14 回日本肝臓学会大会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

50.　2010　土師誠二，畑中絹世，竹山宜典，工藤正俊：肝細胞癌治療における術中造影エコーの有用性．　第 18 回日本消化器関連学会週間 JDDW 2010（第 14 回日本肝臓学会大会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

51.　2010　南　康範，奥村直己，山本典雄，辻　直子，工藤正俊：造影超音波による血流定量化の試み-肝細胞癌に対する TACE の早期治療効果判定-．　第 18 回日本消化器関連学会週間 JDDW 2010（第 14 回日本肝臓学会大会），平成 22 年 10 月 13 日-16 日，パシフィコ横浜，神奈川．

52. 2010 及川裕将，工藤進英，三澤将史，小形典之，森川吉英，細谷寿久，和田祥城，出口義雄，山村冬彦，樫田博史，塩川　章：腹腔鏡下組織生検により診断が確定した腹部原発悪性中皮腫の１例．第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，横浜，神奈川．

53. 2010 垣本哲宏，工藤進英，樫田博史，大塚和朗，山村冬彦，池原伸直，若村邦彦，和田祥城，林　武雅，細谷寿久，小林泰俊，宮地英行，西脇裕高：大腸腫瘍の内視鏡治療における偶発症の検討．第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，横浜，神奈川．

54. 2010 池原伸直，工藤進英，樫田博史，三澤将史，森　悠一，小形典之，畑　英行，西脇裕高，林　武雅，和田祥城，細谷寿久，若村邦彦，宮地英行，山村冬彦，大塚和朗，浜谷茂治：大腸鋸歯状病変における拡大内視鏡診断の有用性－SSA/P の取り扱いを含めて－．第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，横浜，神奈川．

55. 2010 和田祥城，三澤将史，林　武雅，細谷寿久，森　悠一，若村邦彦，池原伸直，山村冬彦，大塚和朗，樫田博史，工藤進英，浜谷茂治：NBI 併用 Endocytoscope（EC-NBI）を用いた大腸病変の微小血管構造観察．第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，横浜，神奈川．

56. 2010 矢川裕介，工藤進英，池原伸直，塩飽洋生，大塚和朗，樫田博史，山村冬彦，細谷寿久，若村邦彦，和田祥城，西脇裕高，三澤将史，小形典之，及川裕将，豊嶋直也，工藤孝毅，松平真吾，石垣智之，中村大樹，浜谷茂治：超拡大内視鏡（endocytoscopy）により質的診断が可能であった回腸陥凹型腺腫．第 18 回日本消化器関連学会週間 JDDW 2010（第 80 回日本消化器内視鏡学会総会），平成 22 年 10 月 13 日-16 日，横浜，神奈川．

57. 2010 木畑　穣，樫田博史，三澤将史，森　悠一，小形典之，横山顕良，久行友和，児玉健太，西脇裕高，小林芳生，小林泰俊，林武雅，和田祥城，細谷寿久，若村邦彦，宮地英行，池原伸直，山村冬彦，大塚和朗，工藤進英：Ｃ型肝炎に対するペグインターフェロン・リバビリン併用療法の当院における治療成績．　第

18 回日本消化器関連学会週間 JDDW 2010（第 13 回日本肝臓学会大会），平成 22 年 10 月 13 日-14 日，横浜，神奈川.

58. 2010 横川 美加，木原 里江子，桑口 愛，前野 知子，前川 清，鄭 浩柄，樫田 博史，工藤 正俊：腹部超音波検査で特発性腸間膜静脈硬化症が疑われた 1 例. 日本超音波医学会第 37 回関西地方会学術集会，平成 22 年 10 月 23 日，兵庫.

写真で綴る消化器内科
2010年

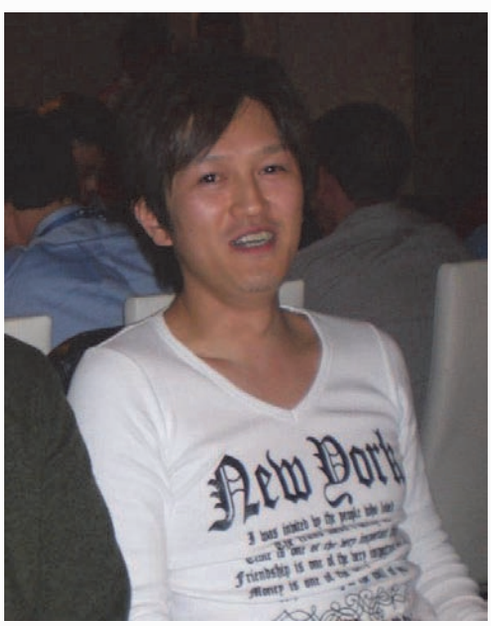
CEUS Guideline Meeting, Chicago

CEUS Guideline Meeting, Chicago


4th International Forum for Liver MRI • 2010 • Seoul, Korea

忘年会＆新年会

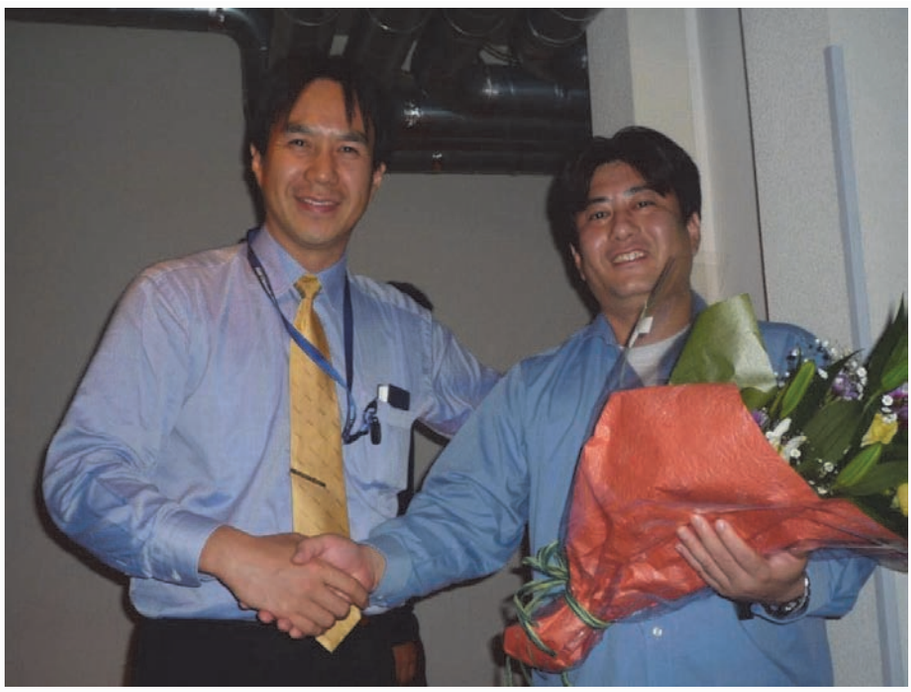
平成22年1月5日
堺東にて


New York


CERTIFICATE
Platinum Price


朝隈先生


Pioneer
PS-W1
PRACIA

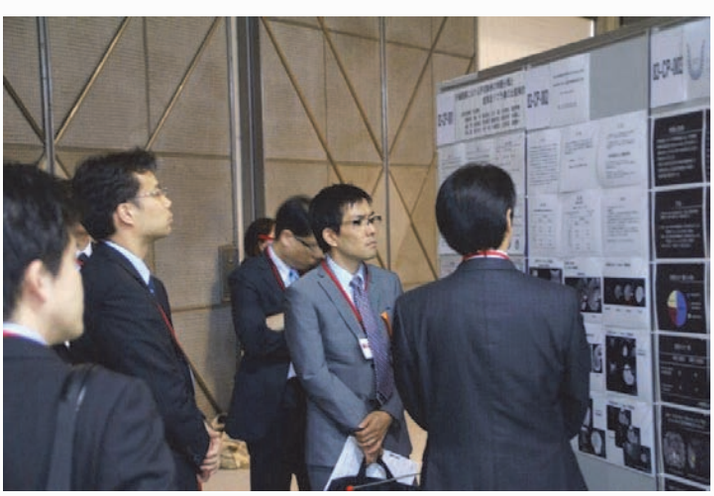


日本超音波医学会第83回学術集会

平成22年5月29日-31日
国立京都国際会館にて


一日不作一日不食


花だより
高台寺塔頭全景図




座　長
松谷
鄭浩柄


LOGIQ E9
肝臓領域
工藤正俊 先生

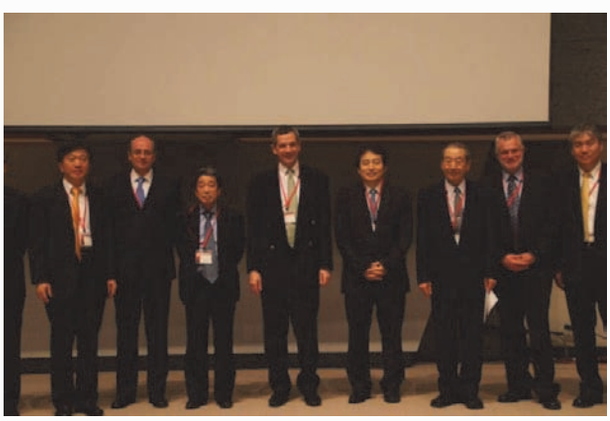

工藤 正俊

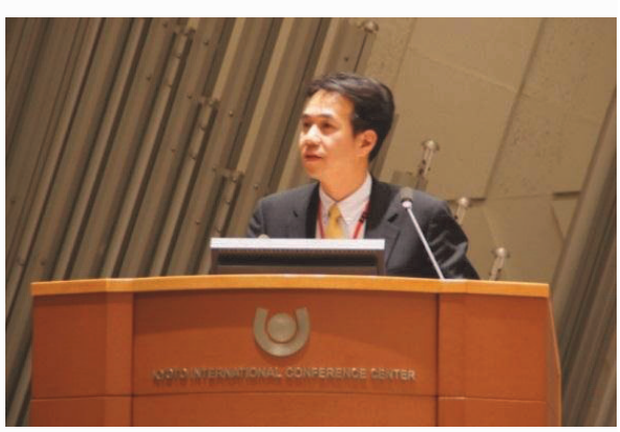

坂本洋城
塩澤克


座 長
工藤 正俊

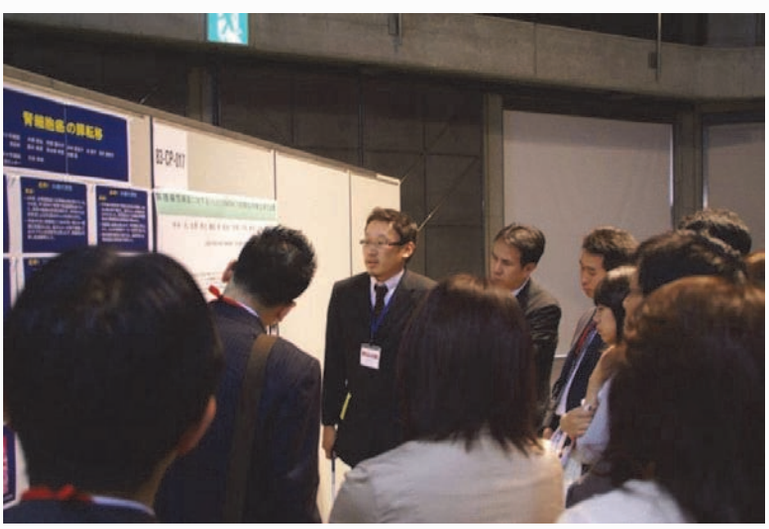

lasatoshi Kudo


吉川純一



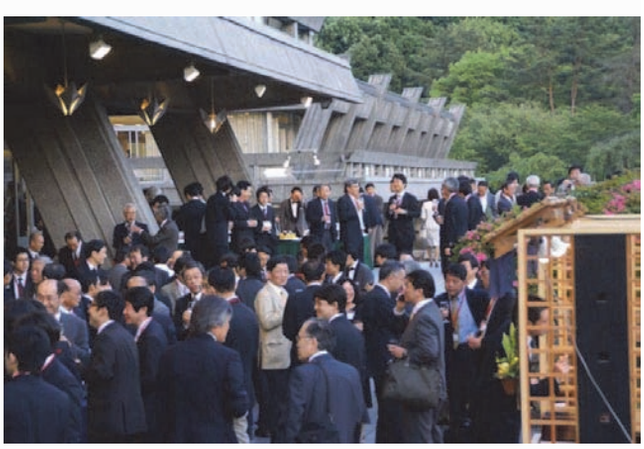

KOITO INTERNATIONAL CONFERENCE CENTER

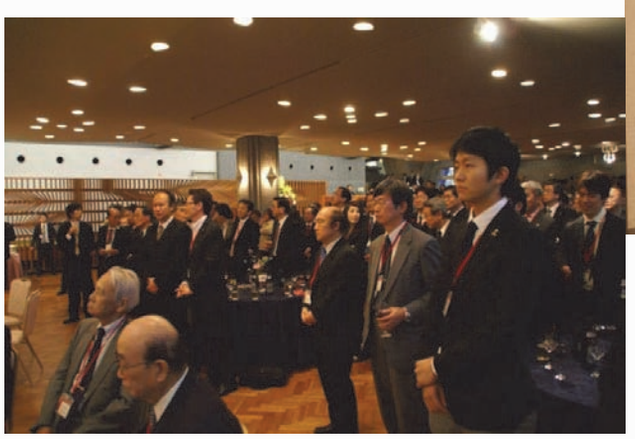

TOSHIBA



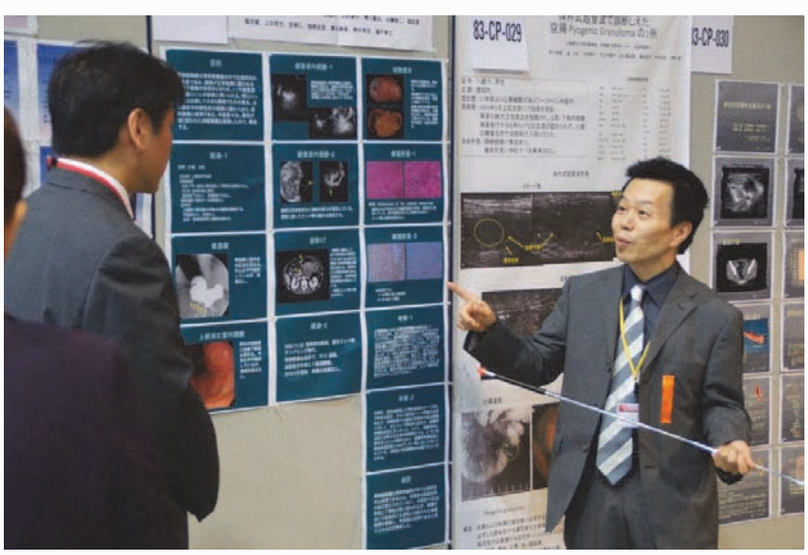

座長


次座長席
司会






VAIO


83-CP-029
83-CP-030


山田 昌彦
南 康範
昱永 圭子


日本超音波医学会第83回学術集会
The 83rd Annual Scientific Meeting of the Japan Society of Ultrasonics in Medicine
サイエンス・テクノロジーのイノベーションからアートへ


日本超音波医学会第83回学術集会
The 83rd Annual Scientific Meeting of the Japan Society of Ultrasonics in Medicine
サイエンス・テクノロジーのイノベーションからアートへ

歓送迎会

平成22年4月20日
堺東にて

Intervirology

Preface

Intervirology 2010;53:5–9
DOI: 10.1159/000252777

Published online: January 5, 2010

Viral Hepatitis A to E: An Update in 2010

Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Viral hepatitis is still one of the major health-care problems worldwide, since hepatitis B and C can cause the potentially fatal conditions of hepatocellular carcinoma (HCC) and/or liver failure. Similarly, hepatitis A is still a serious problem in Asia and, interestingly, hepatitis E is re-emerging as a topic of medical discussion, since it causes fulminant hepatitis even in developed countries like Japan.

The 6th Korea-Japan Liver Symposium was held in Kyongju (Korea) on July 18 and 19, 2009, to focus on and discuss current and emerging topics related to viral hepatitis. The symposium began with eye-opening lectures by some of the world's leading researchers, followed by extensive discussion. This issue of *Intervirology* selects the most important articles presented to this congress.

Hepatitis A

Due to improved living conditions and subsequent changes in hepatitis A epidemiology, the burden of this disease is increasing in many regions. Recently, Korea has faced a large, community-wide outbreak of hepatitis A, which has prompted a vaccination program [1].

Hepatitis A infection is caused by the hepatitis A virus (HAV), which is transmitted through the fecal-oral route. Lifelong protective antibodies are present after infection. The prevalence of anti-HAV in the 10–50-year age range has declined rapidly during the last three decades. As a result, this age group has a high risk for HAV infection, and clinically overt acute hepatitis A is increasing in adolescents and adults. It is well established that the severity of the disease is related to the age of the patients. The clinical features and the epidemiological shift of HAV underscores the importance in Korea, as well as in other countries with similar issues, of childhood vaccination and consideration of catch-up vaccination for adolescents and adults as well as targeted vaccination for individuals at increased risk for infection or its complications.

An active campaign for universal childhood HAV vaccination should be continued and catch-up vaccination, which is directed at people between 10 and 50 years of age, should be considered. Additionally, conventional high-risk groups and persons more vulnerable to developing fulminant hepatitis – such as travelers to highly endemic areas, patients medicated with clotting factors and patients with chronic liver disease – should be vaccinated. To provide evidence-based recommendations for HAV vaccination, an urgent nationwide survey of HAV seroepidemiology as well as regional surveys and studies of the cost-effectiveness of vaccination of each vaccination strategy are needed.

The clinical spectrum of HAV infection ranges from asymptomatic infection to fulminant hepatitis. Clinical manifestations depend on the age of the host; in other words, less than 30% of infected young children are symptomatic, while about 80% of infected adults manifest as severe hepatitis with remarkably elevated serum aminotransferases. Fulminant hepatitis is rare, with a reported incidence from 0.015 to 0.5%. Atypical manifesta-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0005$26.00/0

Accessible online at:
www.karger.com/int

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

tions include relapsing hepatitis and prolonged cholestasis, and complicated cases with acute kidney injury have been reported [2].

Management of hepatitis A includes general supportive care, and critical decisions regarding liver transplantation await further studies on prognostic predictors. Fundamental management of hepatitis A is active vaccination. However, a vaccination program should be adapted to the regional situation, according to differing epidemiology and disease burden [2].

Hepatitis B

Hepatitis B virus (HBV) vaccination has effectively reduced the acute and chronic infection rates in recent years. Since 1983, HBV vaccination has been recommended for all neonates in many countries, including Korea. Before the introduction of the HBV vaccination program, approximately 8% of the general Korean population tested positive for HBsAg. The percentage of vaccinated infants has surpassed 98.9% since 1990. The HBsAg carrier rate in the general population decreased to 3.7% in 2007. In particular, the prevalence of HBsAg decreased to 0.44% in teenagers and 0.2% in children younger than 10 years. In addition, administration of the HBV vaccine may have reduced the risk of HCC among adults. Despite the administration of hepatitis B immunoglobulin and the HBV vaccine to children with HBsAg-positive mothers, the failure rate of HBV immunoprophylaxis was 4.2% in 2008. In Korea, there have been no reported cases of HBV surface gene variants such as G145R.

HBV vaccination has effectively reduced the infection and chronicity rates of HBV and related complications. The overall prevalence of HBV vaccination has exceeded 99% since 1990. However, acute HBV infection may still occur in unvaccinated and uninfected adults. In those cases, catch-up vaccination will be needed. Despite considerable effort to reduce HBV infection via the universal vaccination of all newborn and school-age children, a large proportion of the population was previously infected with HBV and still harbors the virus. The prevalence of HBV carriage in Korea declined after the introduction of a universal HBV vaccination program. Korea is now classified as an area of intermediate endemicity for HBV. The prevention of complications such as cirrhosis and HCC in infected individuals requires appropriate therapeutic agents [3].

The goals of antiviral therapy in patients with chronic hepatitis B are long-lasting suppression of HBV DNA, normalization of serum alanine aminotransferase (ALT) and prevention of progression of chronic liver disease to liver cirrhosis, HCC and liver-related death. Even though substantial advances have been made in the treatment of chronic hepatitis B in the past decade with the use of oral nucleoside/nucleotide analogues (NAs), emergence of antiviral resistance is the most important factor in treatment failure for chronic hepatitis B. Therefore, to prevent antiviral resistance, development of antiviral agents that act with different mechanisms and at different sites (the paradigm of combination therapy for HIV management) is needed. However, such antiviral agents are unlikely to become available in the near future. Therefore, an understanding of the molecular basis of NA resistance and an optimal use of NAs are important for the time being. To minimize the emergence of drug resistance using currently available antiviral agents, physicians should avoid unnecessary therapy. Once initiated, an antiviral agent should suppress viral replication as quickly and completely as possible. Drug resistance should be continuously monitored. However, once antiviral resistance develops, prompt combination therapy should be initiated [4].

Hepatitis C

HCC is one of the leading cause of death from cancer worldwide. Hepatitis C virus (HCV) is a major cause of HCC, accounting for 70% of all HCC cases in Japan. HCV genotype 1b, the most prevalent subtype in Japan, started to spread in the 1930s among injecting drug users or through medical procedures such as blood transfusion and use of contaminated syringes. The prevalence of HCV infection is much lower in the current younger generation compared with that in the older generation, particularly those aged >55 years old (0.1–0.2 vs. ≥2%). Therefore, the total number of patients with HCV infection is expected to decrease, even though sporadic HCV transmission is mainly seen among young injecting drug users. Of note, HCV genotype 2 seems to be spreading among young drug users, but the response to antiviral therapy in these patients is better than that in older patients, irrespective of the genotype. Although the number of patients who die because of HCC has steadily increased over the last 50 years, the incidence of HCC is now decreasing, mainly because of the decreased prevalence of HCV-related HCC [5].

The spread of HCV infection in Japan started in the 1930s, and widespread dissemination of the virus has occurred since then. The risk of iatrogenic HCV transmission has been almost eliminated; however, sporadic HCV transmission still occurs. The prevalence of HCV infection in the younger generation is extremely low and the total number of HCV patients is expected to decrease. Although the number of patients who die of HCC has steadily increased because of the dissemination of HCV infection in the past, it is estimated to decrease in the near future because of the decrease in rates of HCV-related HCC [5].

New drugs that can be used in combination with interferon (IFN) are being actively developed. Also, attempts are being made to physically remove HCV particles from the blood. Granulocyte apheresis, plasma exchange and hemofiltration have been applied to HCV-infected patients for the treatment of cryoglobulinemia and vasculitis, modalities shown to reduce HCV RNA in the blood during treatment. The mechanisms of the clinical results of plasmapheresis have been described, whereby HCV in the blood is related to the effects of IFN therapy that could be enhanced by removing the virus from the blood. Low-density lipoprotein cholesterol apheresis and plasma exchange in hypercholesteremic patients with HCV infection reduces the quantity of HCV RNA in the blood of some patients. Hemodialysis, hemofiltration and peritoneal dialysis in chronic dialysis patients infected with HCV significantly lower HCV RNA levels in the blood. Combined granulocyte apheresis with IFN therapy for chronic hepatitis C and the prerequisite for early reduction of the virus in the treatment of chronic hepatitis C are essential. Thus, the potential effectiveness of IFN therapy combined with early physical removal of the virus is of particular interest [5].

Double-filtration plasmapheresis (DFPP) was approved in April 2008 in Japan for the retreatment of chronic hepatitis C patients with genotype 1b and high viral loads, whose HCV was not eradicated by earlier IFN therapy or by pegylated IFN plus ribavirin (PEG-IFN/RBV) combination therapy. In the current issue, Kim et al. [6] assessed the early viral dynamics of 9 patients with non-sustained virological response (non-SVR) to combination therapy. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks showed a reduction of $\geq$1 log in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9) and 77.8% (7/9) in the viral load at 24 h, 1 week, 2 weeks and 4 weeks after the start of treatment. In contrast, DFPP plus consecutive intravenous IFN-β for 4 weeks reduced the viral load by $\geq$1 log in 33% (2/6), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1 week, 2 weeks and 4 weeks. The viral load declined by $\geq$2 log in 50% (3/6) at 4 weeks after the start of treatment. DFPP plus consecutive intravenous IFN-β for 4 weeks is a promising treatment for non-SVR patients [6].

The prerequisite for early virological response (EVR: indicating negative HCV RNA at 12 weeks) has been emphasized in predicting SVR and non-SVR in chronic hepatitis C patients undergoing IFN treatment; those who do not reach EVR fail to respond to further therapy. Treatment discontinued in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for chronic hepatitis C is highly desirable [6].

From the above considerations, DFPP plus consecutive intravenous IFN-β treatment for 4 weeks is a promising regimen for non-SVR patients with genotype 1b and high viral loads who have been previously treated with PEG-IFN/RBV therapy [6].

Sasase et al. [7] investigated whether SVR and non-SVR by chronic hepatitis C patients to PEG-IFN/RBV combination therapy are distinguishable by viral factors such as the IFN/RBV resistance-determining region (IRRDR) and by on-treatment factors through new indices such as the rebound index (RI). The first RI (RI-1st: the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd: the viral load at week 2 divided by the viral load at 24 h) were calculated, and the subject patients were divided into 3 groups based on RI-1st and RI-2nd: an RI-A group (RI-1st $\leq$1.0), an RI-B group (RI-1st $>$1.0 and RI-2nd $<$0.7) and an RI-C group (RI-1st $>$1.0 and RI-2nd $\geq$0.7). The SVR rate was 71.4% (10/14) in the RI-A group, 46.2% (6/13) in the RI-B group and 20.0% (3/15) in the RI-C group ($p = 0.005$ between the RI-A group and the RI-C group). In IRRDR $\geq$6 and IRRDR $\leq$5, the SVR rate was 81.3% (13/16) and 23.1% (6/26) ($p = 0.0002$), respectively. By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group (IRRDR $\geq$6) and 7.7% (1/13) in the RI-C group (IRRDR $\leq$5) ($p = 0.0003$). Therefore, IRRDR combined with RIs is the quite promising predictor for SVR and non-SVR. With the aid of RIs and IRRDR, a more effective PEG-IFN/RBV treatment could be within reach [7].

Another approach to increase the SVR rate, the extension of treatment duration, has been proposed for late virological responders infected with HCV genotype 1 and high viral load. However, the effectiveness of extended treatment in patients whose serum HCV RNA becomes undetectable later than 24 weeks of treatment (ultra-late

virological responder; ULVR) has not yet been determined. Ueda et al. [8] reported that among a total of 130 patients infected with HCV genotype 1 and high viral load treated with PEG-IFN/RBV combination therapy, 10 ULVR received extended combination treatment beyond 48 weeks. The duration of the combination treatment for ULVR ranged between 59 and 119 weeks and the mean duration was 80 weeks. Although the majority of ULVR were older female patients (≥60 years; older age and female sex both being factors related to poor therapeutic response), 8 patients (80%) achieved SVR. The SVR rate correlated well with the duration of the treatment. Five ULVR achieved SVR when treatment was continued until serum HCV RNA remained undetectable for longer than 48 weeks. From these observations it is concluded that the extended duration of PEG-IFN/RBV combination treatment is a possible strategy to improve treatment response in HCV genotype 1 infection, even for ULVR. The extension of the treatment does not seem to increase side effects or the rate of dose reductions, and treatment should be continued until the serum HCV RNA remains undetectable for at least 24 weeks and, if possible, for longer than 48 weeks during the course of treatment [8].

Recently, the significance of serum ferritin levels in PEG-IFN/RBV combination therapy for chronic hepatitis C has become of interest. Yada et al. [9] examined the correlation of serum ferritin level with serum ALT levels during therapy and response to the therapy. A total of 175 patients with chronic hepatitis C received the combination therapy. Correlation between serum ferritin levels and serum ALT levels at 12 and 24 weeks of therapy were examined. Differences in serum ferritin levels during therapy between patients with SVR and non-SVR were also examined. The authors found that only 24 (13.7%) and 20 (11.4%) patients showed elevated serum ALT levels (≥70 IU/l) at 12 and 24 weeks of therapy, respectively. There was no correlation between serum ferritin levels and ALT levels. Ninety-five (54.3%) of 175 patients achieved SVR. Serum ferritin levels increased dramatically in both SVR and non-SVR groups after starting the therapy and were significantly higher in the SVR group throughout the therapy. Therefore, they concluded that serum ferritin level increases during PEG-IFN/RBV combination therapy; however, it did not correlate with either serum ALT level or the total dose of RBV. Higher serum ferritin levels during combination therapy are associated with a favorable therapeutic response, which has not been reported before.

Enomoto et al. [10] searched HCV genetic elements determining the early response to PEG-IFN/RBV therapy using HCV genome-wide analysis. From a total of 88 chronic hepatitis C patients with HCV-1b treated with PEG-IFN/RBV, the whole HCV amino acid sequence was determined and analyzed according to the viral response during the treatment. Mutations in NS5A-ISDR are associated with rapid viral response at week 4, and the core arginine70glutamine (R70Q) mutation is associated with no early viral response at week 12, revealing that core 70 and NS5A are the most important factors determining the virological kinetics during PEG-IFN/RBV therapy. Viral genome-wide analysis is a promising tool for elucidating the unknown viral factors involved in different pathological pictures, such as disease progression [10].

Hepatitis E

The epidemiology of acute viral hepatitis (AVH) is dynamic and is affected by many factors including hygiene, socioeconomic status and vaccination coverage. A total of 4,302 cases of AVH were sequentially studied on a nationwide scale between 1980 and 2008. Acute hepatitis A (AHA), acute hepatitis B (AHB), acute hepatitis C (AHC), and non-A, non-B and non-C (non-ABC) hepatitis accounted for 1,583 (36.8%), 1,197 (27.8%), 359 (8.3%) and 1,163 (27.0%) of all AVH. The proportions of AHA, AHB, AHC and non-ABC were approximately 40, 25, 10 and 25%, respectively, between 1980 and 1995. The proportions were approximately 30, 30, 10 and 30% between 1996 and 2003, and shifted to approximately 10, 40, 10, and 40% in the last 5 years. The number of AHB caused by genotype A, which is not indigenous to Japan, was 6.0% between 1991 and 1996 and markedly increased after 2000 to reach 52% in 2008. Autochthonous acute hepatitis E (AHE) accounted for 10–15% of non-ABC hepatitis after 2002. The etiology of AVH in Japan has been drastically changing. A marked increase of AHB genotype A and constant occurrence of autochthonous AHE require attention, and necessary measures should be taken [11].

HEV is a major cause of acute hepatitis in many developing countries where AHE is an important public health concern. However, cases of sporadic AHE in people with no history of recent travel have been reported in developed regions such as North America, Europe, Japan and Australia. The reporting of such infections together with the availability of more comprehensive molecular and serological data has led to the re-evaluation of HEV epidemiology and the acceptance that autochthonous AHE is a clinical problem in developed countries. Information

on AHE in non-ABC hepatitis populations in Japan is limited, although there are many reports of sporadic or epidemic occurrence of AHE. The current study also showed the trend of AHE in Japan. AHE constituted 4.9% (44/896) of non-ABC hepatitis. Although the number of AHE cases (1–6 cases per year) and its ratio to non-ABC hepatitis (0–14.5%) are not very high and are insignificant, the occurrence of AHE became constant after 2002. Surprisingly, AHE constituted as much as 11.0% (25/228) of non-ABC hepatitis after 2002. The clinical course was generally modest and none of the patients showed severe type of hepatitis, probably because most domestic cases were caused by HEV genotype 3, which has been associated with milder clinical outcome compared to HEV genotype 4. This phenomenon may reflect the fact that their sentinels involve only a few institutes in Hokkaido, where HEV genotype 4 is endemic. Nevertheless, the trend of AHE requires particular attention, because modes of transmission are still often unknown, even after taking very careful history of eating particular foods such as raw meat of deer, pigs and boars [11].

Fibrosis and Hepatocarcinogenesis by Viral Hepatitis

FibroScan has many limitations. It cannot be used in patients with ascites, thick subcutaneous fat, narrow intercostals spaces and hepatic atrophy. Real-time elastography (RTE) does not have such limitations and can be used in almost all patients including those with conditions mentioned above, who have difficulty in using FibroScan. However, RTE requires training to analyze the data and also to scan the patients to obtain reproducible images. To address these issues in RTE, Tatsumi et al. [12] investigated easy-to-use acquisition techniques to reduce inter-observer variability and also to simplify RTE image acquisition.

FibroScan and RTE both correlate highly with the F staging of the liver using biopsy. In particular, RTE is very useful for the differential diagnosis and staging of the liver fibrosis.

More cases need to be evaluated by FibroScan and RTE for liver fibrosis staging and to establish guidelines to minimize unnecessary liver biopsies, which will significantly benefit the patients with chronic liver diseases. Furthermore, RTE might be a very good indicator to predict the incidence rate of HCC in the near future [12].

Disclosure Statement

The author declares that he has no financial conflict of interest.

References

1 Kim YJ, Lee HS: Increasing incidence of hepatitis A in Korean adults. Intervirology 2010; 53:10–14.
2 Jeong SH, Lee HS: Hepatitis A: clinical manifestations and management. Intervirology 2010;53:15–19.
3 Park NH, Chung YH, Lee HS: Impacts of vaccination on hepatitis B viral infections in Korea over a 25-year period. Intervirology 2010; 53:20–28.
4 Song BC: How to overcome antiviral-resistant hepatitis B virus? Intervirology 2010; 53:29–38.
5 Chung H, Ueda K, Kudo M: Changing trends in hepatitis C infection over the past 50 years in Japan. Intervirology 2010;53:39–43.
6 Kim SR, Imoto S, Kudo M, et al: Double-filtration plasmapheresis plus IFN for HCV-1b patients with non-sustained virological response to previous combination therapy: early viral dynamics. Intervirology 2010; 53:44–48.
7 Sasase N, Kim SR, Kudo M, et al: Outcome and early viral dynamics with viral mutation in PEG-IFN/RBV therapy for chronic hepatitis in patients with high viral loads of serum HCV RNA genotype 1b. Intervirology 2010;53:49–54.
8 Ueda T, Chung H, Kudo M, et al: Prolonged PEG-IFN and RBV is effective in patients with HCV genotype 1 and high viral load who achieved virological response later than 24 weeks. Intervirology 2010;53:55–59.
9 Yada N, Kudo M, Chung H, et al: PEG-IFNα/RBV combination therapy for chronic hepatitis C patients increases serum ferritin level while it improves sustained viral response rate. Intervirology 2010;53:60–65.
10 Enomoto N, Maekawa S: HCV genetic elements determining the early response to peginterferon and ribavirin therapy. Intervirology 2010;53:66–69.
11 Yano K, Tamada Y, Yatsuhashi H, et al: Dynamic epidemiology of acute viral hepatitis in Japan. Intervirology 2010;53:70–75.
12 Tatsumi C, Kudo M, Ueshima K, et al: Non-invasive evaluation of hepatic fibrosis for type C chronic hepatitis. Intervirology 2010; 53:76–81.

Review Article

Intervirology

Intervirology 2010;53:39–43
DOI: 10.1159/000252782

Published online: January 5, 2010

Changing Trends in Hepatitis C Infection over the Past 50 Years in Japan

Hobyung Chung Taisuke Ueda Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

Key Words

Chronic hepatitis · Hepatitis C virus · Hepatocellular carcinoma · Injecting drug user · Mortality rate · Post-transfusion hepatitis

Abstract

In Japan, hepatocellular carcinoma (HCC) is the fourth leading cause of death in males and the fifth in females. Hepatitis C virus (HCV) is a major cause of HCC in Japan, with 70% of cases being HCV related. HCV genotype 1b, the most prevalent subtype in Japan, started to spread in the 1930s among injecting drug users (IDUs) during and after World War II or through medical procedures such as blood transfusion and use of contaminated syringes. The prevalence of HCV infection is much lower in the current younger generation compared with that in the older generation, particularly those aged >55 years (0.1–0.2% vs. ≥ 2%). Therefore, the total number of patients with HCV infection is estimated to decrease, even though sporadic HCV transmission is mainly seen among young IDUs. Of note, HCV genotype 2 seems to be spreading among IDUs, but the response to antiviral therapy in these patients seems to be better than that in older patients, irrespective of the genotype. Although the number of patients who die because of HCC has steadily increased over the last 50 years, the incidence of HCC is now decreasing, mainly because of the decreased prevalence of HCV-related HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer death. The prevalence of HCC in Japan has increased over the past 50 years and more than 30,000 patients die because of HCC every year, accounting for rates of death of 36.3 and 17.5 per 100,000 males and females, respectively [1]. The main causes of HCC in Japan are hepatitis C virus (HCV) and hepatitis B virus (HBV); nearly 70% of HCC cases are caused by HCV [1]. This situation differs from that in other Asian countries where HBV-related HCC is more common, and the situation in Japan is more similar to that in Western countries [2, 3].

Japan has one of the highest endemic rates of HCV infection. The number of patients with HCV infection is estimated to be about 2,000,000 in Japan; 70% of patients are infected with HCV genotype 1b, 20% with genotype 2a, and the rest with genotype 2b. The prevalence of HCV infection is closely related to age: the number of HCV carriers increases with age and the prevalence of HCV infection is much higher in people aged over 55 years [4].

Here, we review the history and current status of HCV infection in Japan and predict the future changes in rates of infection and HCV-related mortality.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0039$26.00/0

Accessible online at:
www.karger.com/int

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

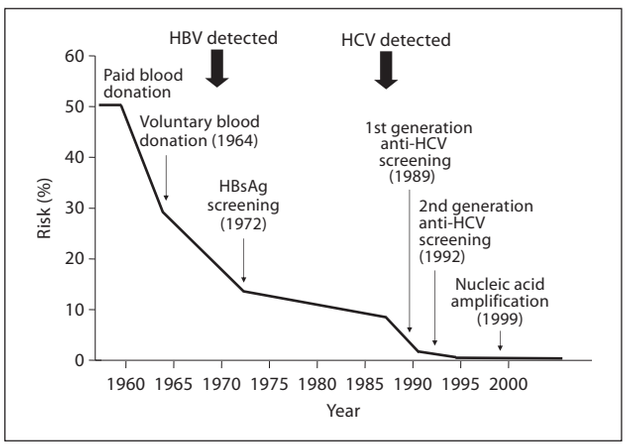


Fig. 1. Risk of post-transfusion hepatitis in Japan. Post-transfusion hepatitis occurred in more than half of transfused cases until the start of the 1960s. However, the risk has decreased significantly over the past 50 years because of the introduction of several preventive measures.

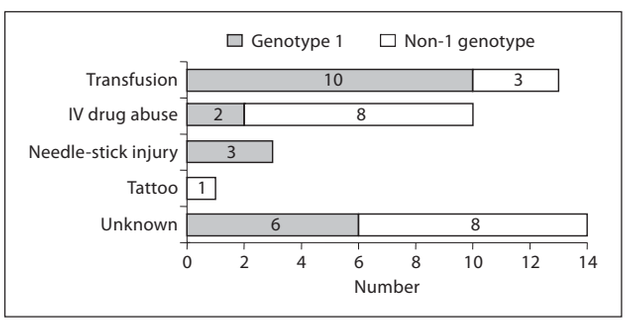


Fig. 2. Differences in transmission routes of HCV infection between genotype 1 and non-1 genotypes among patients aged less than 40 years. Intravenous drug abuse was the major route of HCV transmission in the non-1 genotypes, while blood transfusion was the major route in genotype 1.

When and How Did HCV First Spread in Japan?

Mizokami et al. [5] demonstrated based on molecular clock analysis that HCV genotype 1b, which is the most dominant genotype in Japan, started to spread in the 1930s. They also revealed that HCV genotype 1a started to spread in the United States in the 1960s, at least 30 years later than the spread of HCV genotype 1b in Japan. Furthermore, HCV infection is still increasing exponentially in the United States whereas it has been decreasing since approximately 1995 in Japan [5]. The main causes of HCV dissemination in Japan included intravenous stimulant drug (methamphetamine) abuse among the young generation during and after World War II, blood transfusion from paid blood donors, and injections using contaminated syringes and needles, particularly for the treatment of schistosomiasis japonica. Schistosomiasis japonica was previously an endemic disease in Japan before the introduction of intravenous antimony in 1921. Interestingly, HCV infection is more prevalent in south-western regions of Japan compared with north-eastern regions [4], and areas endemic for schistosomiasis japonica, such as Fukuoka and Hiroshima prefectures, are located in south-west Japan. It seems likely that the geographical distribution of schistosomiasis japonica is at least partly attributable to differences in the prevalence of HCV infection.

Resolution of HCV Transmission

Tanaka et al. [4] investigated the age-specific prevalence of HCV infection among first-time blood donors in Japan, and found that the prevalence of HCV infection is closely correlated with age. The number of HCV carriers increased with age and an exponential increase was seen in blood donors aged more than 55 years, irrespective of the area of Japan. On the other hand, the prevalence of HCV infection in blood donors younger than 30 years is quite low: 0.13% at 16–19 years and 0.21% at 20–29 years [4]. This suggests that the rate of HCV transmission has decreased significantly and that the total number of patients with HCV is likely to decrease [6–8].

Paid blood donation was commonly performed in Japan and post-transfusion hepatitis occurred in more than half of transfused cases until the beginning of the 1960s (fig. 1). After the introduction of voluntary blood donation in 1964 and HBsAg screening in 1972, the risk of post-transfusion hepatitis dramatically decreased (to around 10%). After the discovery of HCV RNA, screening tests for first- and second-generation HCV antibodies were started in 1989 and 1992, respectively. These screening tests further decreased the risk of post-transfusion hepatitis. Furthermore, nucleic acid amplification tests, introduced in 1999, almost eliminated the risk [8]. Although sporadic HCV transmission still occurs through other routes, such as intravenous drug abuse and accidental exposure in medical procedures (e.g. needlestick injury), measures to prevent post-transfusion hepatitis have significantly reduced the rate of HCV transmission.

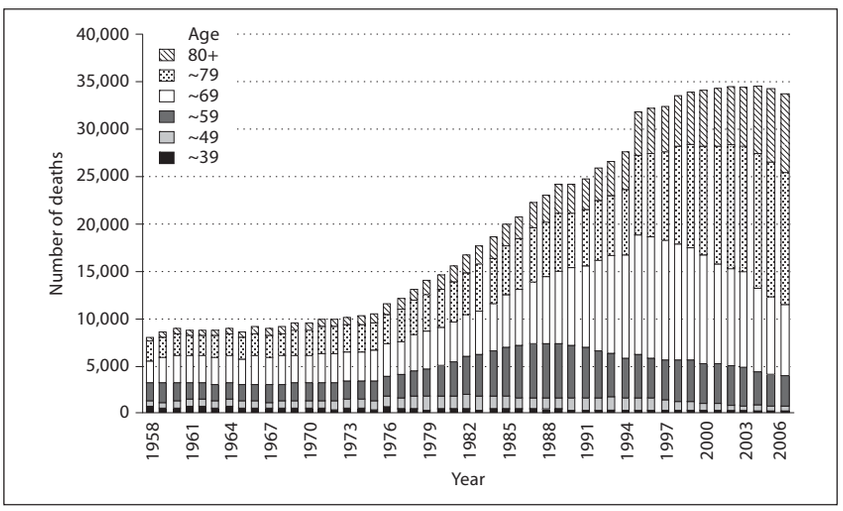


Fig. 3. Trends in mortality rates of patients with primary liver cancer. The mortality rate has steadily increased over the past 50 years, and more than 30,000 patients die of primary liver cancer every year. However, the age at death from liver cancer is also increasing.

Features of Recent HCV Transmission in Japan

As described above, HCV transmission through blood transfusion is now an extremely rare event. Instead, intravenous drug abuse has become the main cause of HCV transmission in developed areas such as Europe and the USA [9, 10]. The estimated prevalence of HCV infection among injecting drug users (IDUs) is between 30 and 80% [11–13]. The number of IDUs in Japan is steadily increasing, particularly among young people, according to the data published by the Ministry of Health, Labour and Welfare. Therefore, intravenous drug abuse has become an important route of HCV transmission among young people [8, 14]. Satoh et al. [15] investigated the HCV genotypes that are spreading among Japanese IDUs and found that non-1b genotypes of HCV, particularly genotype 2b, seemed to be most prevalent among IDUs. We also examined the HCV genotypes in 42 young (less than 40 years old) chronic hepatitis patients with HCV who were treated by peginterferon and ribavirin combination therapy at the Kinki University Hospital between 2006 and 2008. Twenty-one patients (50%) were infected with non-1 genotypes of HCV, 20 patients had genotype 2, and 1 patient had genotype 3. The proportion of non-1 genotypes in these patients is higher than that in the whole Japanese population with HCV infection, which is estimated to be about 30%. Possible transmission routes that were assessed by interviewing each patient were compared between those with genotype 1 and those with non-1 genotypes. As shown in figure 2, the major transmission routes appeared to be different between the 2 types: intravenous drug abuse was the major route of non-1 genotype HCV transmission, which is consistent with the results of previous reports [15, 16]. The sustained virological response rates in these patients, 72% for genotype 1 and 95% for non-1 genotype, are better than those in previous reports, probably due to the shorter duration of infection (associated with less advanced disease) and younger age, both of which are factors associated with favorable outcomes of IFN therapy [17, 18].

Accidental exposure to HCV during medical procedures is another important route of transmission, although the risk is believed to be very low (0.3–2.7%) [19–21]. These cases are usually monitored carefully after the accident and, even if they present with acute hepatitis, most cases can be cured by IFN therapy during the early phase of infection [22].

In summary, even though sporadic HCV infections are still occurring, particularly among young people, it seems that this population will not increase the total population of HCV infection in Japan.

Future Perspectives on HCV-Related Diseases and Mortality

In Japan, the number of HCC patients and their mortality rate remain extremely high in comparison with other developed countries [8]. Accordingly, a nationwide health screening program for viral hepatitis was launched to identify new patients with HCV or HBV infection who were unaware of their diseases. The target population of

this program included citizens aged over 40 years, and the program was carried out between 2002 and 2006. A total of 6,280,111 people (26% of the target population) were tested in this program, and 99,950 patients with HCV infection were newly detected, and some of them have already been treated with IFN therapy. Therefore, this program will provide a significant contribution to decrease the number of patients who suffer from and die of HCV-related liver diseases.

Peginterferon and ribavirin combination therapy is the current standard treatment for chronic hepatitis C infection and improves the sustained virological response rate even in patients infected with HCV genotype 1 and who have high viral load [23]. In addition, several new-generation drugs, such as protease inhibitors and polymerase inhibitors, further enhance the efficacy of IFN therapy and will be available soon [24, 25]. The improved efficacy of antiviral therapy for HCV will inevitably contribute to decrease the HCV-related mortality rate.

Although the number of patients who die of HCC has steadily increased over the past 50 years, as shown in figure 3, the incidence of HCC has recently started to decrease in Japan, mainly because of the decrease in rates of HCV-related HCC [26, 27]. In addition, the age at diagnosis of HCC has increased in parallel with the increased proportion of older patients with HCV infection [27, 28]. These trends can be explained by an estimation of the decreased number of patients with HCV infection because of the extremely low prevalence of infection in the younger generation in conjunction with improved efficacy of antiviral therapy.

Conclusion

The spread of HCV infection in Japan started in the 1930s, and widespread dissemination of the virus has occurred since then. The risk of iatrogenic HCV transmission has been almost eliminated; however, sporadic HCV transmission still occurs. The prevalence of HCV infection in the younger generation is extremely low and the total number of HCV patients is expected to decrease. Although the number of patients who die of HCC has steadily increased because of the dissemination of HCV infection in the past, it is estimated to decrease in the near future because of the decrease in rates of HCV-related HCC.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M, Kudo M: Report of the 17th nationwide follow-up survey of primary liver cancer in Japan. Hepatol Res 2007;37:676–691.

2 Yu MC, Yuan JM, Govindarajan S, Ross RK: Epidemiology of hepatocellular carcinoma. Can J Gastroenterol 2000;14:703–709.

3 El-Serag HB: Hepatocellular carcinoma: recent trends in the United States. Gastroenterology 2004;127:27–34.

4 Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H: Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.

5 Mizokami M, Tanaka Y, Miyakawa Y: Spread times of hepatitis C virus estimated by the molecular clock differ among Japan, the United States and Egypt in reflection of their distinct socioeconomic backgrounds. Intervirology 2006;49:28–36.

6 Sasaki F, Tanaka J, Moriya T, Katayama K, Hiraoka M, Ohishi K, Nagakami H, Mishiro S, Yoshizawa H: Very low incidence rates of community-acquired hepatitis C virus infection in company employees, long-term inpatients, and blood donors in Japan. J Epidemiol 1996;6:198–203.

7 Tanaka J, Mizui M, Nagakami H, Katayama K, Tabuchi A, Komiya Y, Miyakawa Y, Yoshizawa H: Incidence rates of hepatitis B and C virus infections among blood donors in Hiroshima, Japan, during 10 years from 1994 to 2004. Intervirology 2008;51:33–41.

8 Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62(suppl 1):8–17.

9 Sy T, Jamal MM: Epidemiology of hepatitis C virus (HCV) infection. Int J Med Sci 2006; 3:41–46.

10 Lavanchy D: The global burden of hepatitis C. Liver Int 2009;29(suppl 1):74–81.

11 Villano SA, Vlahov D, Nelson KE, Lyles CM, Cohn S, Thomas DL: Incidence and risk factors for hepatitis C among injection drug users in Baltimore, Maryland. J Clin Microbiol 1997;35:3274–3277.

12 Touzet S, Kraemer L, Colin C, Pradat P, Lanoir D, Bailly F, Coppola RC, Sauleda S, Thursz MR, Tillmann H, Alberti A, Braconier JH, Esteban JI, Hadziyannis SJ, Manns MP, Saracco G, Thomas HC, Trepo C: Epidemiology of hepatitis C virus infection in seven European Union countries: a critical analysis of the literature. Hencore group. (Hepatitis C European Network for Co-operative Research). Eur J Gastroenterol Hepatol 2000;12:667–678.

13 Mathei C, Robaeys G, van Damme P, Buntinx F, Verrando R: Prevalence of hepatitis C in drug users in Flanders: determinants and geographic differences. Epidemiol Infect 2005;133:127–136.

14 Wada K, Greberman SB, Konuma K, Hirai S: HIV and HCV infection among drug users in Japan. Addiction 1999;94:1063–1069.

15 Satoh Y, Hino K, Kato T, Mizokami M, Yamashita S, Nakamura H, Okita K: Molecular epidemiologic analysis of hepatitis C virus infection in injecting drug users with acute hepatitis C in Japan. J Gastroenterol Hepatol 2004;19:1305–1311.
16 Kato H, Maeno Y, Seko-Nakamura Y, Monma-Ohtaki J, Sugiura S, Takahashi K, Zhe LX, Matsumoto T, Kurvanov F, Mizokami M, Nagao M: Identification and phylogenetic analysis of hepatitis C virus in forensic blood samples obtained from injecting drug users. Forensic Sci Int 2007;168:27–33.
17 Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H: Predictive values of amino acid sequences of the core and ns5a regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol 2009;44:952–963.
18 Kau A, Vermehren J, Sarrazin C: Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol 2008;49:634–651.
19 Chung H, Kudo M, Kumada T, Katsushima S, Okano A, Nakamura T, Osaki Y, Kohigashi K, Yamashita Y, Komori H, Nishiuma S: Risk of HCV transmission after needlestick injury, and the efficacy of short-duration interferon administration to prevent HCV transmission to medical personnel. J Gastroenterol 2003;38:877–879.
20 Takagi H, Uehara M, Kakizaki S, Takahashi H, Takezawa J, Kabeya K, Satoh K, Kojima A, Saito S, Matsumoto T, Hashimoto Y, Abe T, Yamada T, Konaka K, Shimoda R, Takayama H, Takehara K, Nagamine T, Mori M: Accidental transmission of HCV and treatment with interferon. J Gastroenterol Hepatol 1998;13:238–243.
21 Kiyosawa K, Sodeyama T, Tanaka E, Nakano Y, Furuta S, Nishioka K, Purcell RH, Alter HJ: Hepatitis C in hospital employees with needlestick injuries. Ann Intern Med 1991;115:367–369.
22 Maheshwari A, Ray S, Thuluvath PJ: Acute hepatitis C. Lancet 2008;372:321–332.
23 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peg-interferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
24 Asselah T, Benhamou Y, Marcellin P: Protease and polymerase inhibitors for the treatment of hepatitis C. Liver Int 2009;29(suppl 1):57–67.
25 Thompson A, Patel K, Tillman H, McHutchison JG: Directly acting antivirals for the treatment of patients with hepatitis C infection: a clinical development update addressing key future challenges. J Hepatol 2009;50:184–194.
26 Umemura T, Ichijo T, Yoshizawa K, Tanaka E, Kiyosawa K: Epidemiology of hepatocellular carcinoma in Japan. J Gastroenterol 2009;44(suppl 19):102–107.
27 Tanaka H, Imai Y, Hiramatsu N, Ito Y, Imanaka K, Oshita M, Hijioka T, Katayama K, Yabuuchi I, Yoshihara H, Inoue A, Kato M, Takehara T, Tamura S, Kasahara A, Hayashi N, Tsukuma H: Declining incidence of hepatocellular carcinoma in Osaka, Japan, from 1990 to 2003. Ann Intern Med 2008;148:820–826.
28 Taura N, Hamasaki K, Nakao K, Ichikawa T, Nishimura D, Goto T, Fukuta M, Kawashimo H, Miyaaki H, Fujimoto M, Kusumoto K, Motoyoshi Y, Shibata H, Inokuchi K, Eguchi K: Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. Oncol Rep 2006;16:837–843.

Original Article

Intervirology

Intervirology 2010;53:44–48
DOI: 10.1159/000252783

Published online: January 5, 2010

Double-Filtration Plasmapheresis plus IFN for HCV-1b Patients with Non-Sustained Virological Response to Previous Combination Therapy: Early Viral Dynamics

Soo Ryang Kim[a] Susumu Imoto[a] Masatoshi Kudo[d] Keiji Mita[a]
Miyuki Taniguchi[a] Ke Ih Kim[a] Noriko Sasase[a] Ikuo Shoji[b]
Motoko Nagano-Fujii[b] Ahmed El-Shamy[b] Hak Hotta[b] Tomoyuki Nagai[d]
Yoshiaki Nagata[d] Yoshitake Hayashi[c]

[a]Department of Gastroenterology, Kobe Asahi Hospital, [b]Department of Microbiology, and
[c]Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, and
[d]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

Key Words
Chronic hepatitis C · Double-filtration plasmapheresis · Early viral dynamics · Genotype 1b · High viral load · Interferon β · Non-sustained virological responder · Peginterferon plus ribavirin combination therapy

Abstract
Double-filtration plasmapheresis (DFPP) was approved in Japan in April 2008 for the retreatment of chronic hepatitis C patients with genotype 1b and high viral loads, whose hepatitis C virus was not eradicated by earlier IFN therapy or by pegylated IFN plus ribavirin (PEG-IFN/RBV) combination therapy. In this study, we assessed the early viral dynamics of 9 patients with non-sustained virological response to the combination therapy. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks showed a reduction of ≥1 log in the viral load in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9) and 77.8% (7/9) at 24 h, 1, 2 and 4 weeks after the start of treatment. By contrast, DFPP plus consecutive intravenous IFN-β for 4 weeks reduced the viral load by ≥1 log in 33% (2/6), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1, 2 and 4 weeks. The viral load declined by ≥2 log in 50% (3/6) at 4 weeks after the start of treatment. DFPP plus consecutive intravenous IFN-β for 4 weeks is a promising treatment for non-sustained virolgical response patients.

Introduction

Hepatitis C virus (HCV) infection is the major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) in industrialized countries. HCV infection is manageable, however, and its complications can be prevented by antiviral therapy [1, 2]. Currently, the most effective treatment for chronic HCV infection is based on pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy [3]. Nonetheless, sustained

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0044$26.00/0

Accessible online at:
www.karger.com/int

Soo Ryang Kim, MD
Department of Gastroenterology
Kobe Asahi Hospital
3-5-25 Bououji-cho, Nagata-ku, Kobe 653-0801 (Japan)
Tel. +81 78 612 5151, Fax +81 78 612 5152, E-Mail asahi-hp@arion.ocn.ne.jp

virological response (SVR) rates for those infected with the most resistant genotypes (HCV-1a and HCV-1b) still hover around 50% [3, 4].

To surmount this SVR rate with combination therapy, several trials have been undertaken, two of which are: (1) retreatment with combination therapy and (2) double-filtration plasmapheresis (DFPP). By the protocol-defined primary analysis of the former, the SVR rate has been 16% at most, even for a 72-week induction group [5].

The use of DFPP [approved in Japan in April 2008 for the retreatment of chronic hepatitis C (CHC) patients with genotype 1b and high viral loads] together with IFN administration has produced a substantial reduction in the viral load during the early stages of treatment and has effected a high SVR [6], suggesting that this treatment is a new modality for CHC patients in difficult-to-treat states. In this study, we used DFPP plus IFN to enhance the efficacy of the treatment of CHC patients whose HCV was not eradicated by earlier PEG-IFN/RBV combination therapy, and we assessed early viral dynamics associated with SVR.

Patients and Methods

Patients

Nine patients (aged 43–66 years) whose HCV had not been eradicated by earlier PEG-IFNα-2b plus RBV combination therapy carried out between 2008 and 2009 were enrolled in this study. The patients were divided into 2 groups: partial responders (PR; relapse after the end of therapy) and non-responders (NR; no disappearance of HCV RNA during therapy). All the patients were confirmed to be HCV RNA positive with high transaminase levels persisting for 6 months or longer, and with HCV RNA genotype 1b at levels exceeding 10^5 log IU/ml in blood (as determined before the start of therapy by real-time PCR). Also, the patients were negative for hepatitis B surface antigen. Patients with platelet counts of $\leq 10 \times 10^4/\mu l$, leukocyte counts of $\leq$3,000/μl, or hemoglobin levels of ≤12 g/dl were excluded from the study.

Each patient gave written informed consent and agreed to receive concomitant DFPP, and the study was approved by the review board of the Kobe Asahi Hospital.

DFPP and Blood Collection

Blood collected from the peripheral vein for DFPP by a Plasmaflo™ OP-18W filter (Asahi Kasei Medical, Tokyo, Japan) was separated into plasma and cell components. The virus was then removed from the plasma by a second filter (Cascadeflo™ EC-50W; Asahi Kasei Medical) of an average pore size of 30 nm. For each session, the final volume of treated plasma was 50 ml/kg; the number of sessions was 5 over 2 weeks, and the time of DFPP, based on the reduced plasma fibrinogen levels during DFPP, was decided by the physicians and as required by the patients.

Types of IFN for 4 Weeks with DFPP

During DFPP, the patients were treated with different kinds of IFN: patient 1 with PEG-IFNα-2b plus RBV for 4 weeks; patients 2 and 3 with IFN-β 3 MU twice daily for 2 weeks and PEG-IFNα-2a plus RBV for 2 weeks; patients 4 and 9 with IFN-β 3 MU twice daily for 2 weeks and IFN-β 6 MU daily for 2 weeks; patient 5 with IFN-β 3 MU twice daily for 10 days and IFN-β 6 MU daily for 18 days, and patients 6, 7 and 8 with IFN-β 3 MU twice daily for 4 weeks. The dose of PEG-IFNα-2b was 1.5 μg/kg and 180 μg of α-2a per week. The RBV dose was 800 mg/day with α-2b and 600–800 mg/day with α-2a. After DFPP plus IFN treatment for 4 weeks, all patients were scheduled to receive PEG-IFN/RBV combination therapy (patient 1: PEG-IFNα-2b 1.5 μg/kg per week plus RBV 800 mg/day; patients 2–9: PEG-IFNα-2a 180 μg per week plus RBV 600–800 mg/day).

Amino Acid Substitutions in the Core Region (aa 30 and aa 91) and Number of IFN Sensitivity-Determining Region Mutations

We measured pre-treatment factors such as prediction of clinical outcome of therapy, amino acid sequence variation in the NS5A region (referred to as IFN sensitivity-determining regions) and in the core protein regions (aa 70 and aa 91) of HCV with a given genotype, and the viral load.

HCV RNA Measurement

The quantity of HCV RNA was measured by real-time PCR (detection limit 1.2 log IU/ml), by HCV core antigen (detection limit 20 fmol/l), and by RT-PCR (Amplicor HCV monitor v 2.0; Roche; detection limit 50 IU/ml).

Virus Removal at Second Filter Inlet and Outlet

Plasma was collected twice from the inlet and outlet of the second filter during 1 session of DFPP: once when the treated plasma volume reached half of the target quantity, and once when DFPP was completed. The change in the quantity of HCV RNA was evaluated through the plasma samples collected.

Viral Reduction and Viral Response Rate

The quantity of HCV RNA was converted to a log value at the beginning of the treatment (A) and at each of the virus measurement points (B). Δlog was then calculated: Δlog = logA – logB = log (A/B).

Evaluation of DFPP Safety

The subjective and objective adverse events of DFPP were observed, and five clinical factors were measured (platelet and lymphocyte counts, and hemoglobin, albumin and fibrinogen levels) before the first session of DFPP, before successive sessions on the second, third, fourth, fifth and sixth days, and 2 weeks after the last session.

Statistical Analysis

Statistical analysis consisted of analysis of variance for patient background factors, and the paired t test for quantities of HCV RNA at the second filter inlet during DFPP. The t test was used for viral load reductions and Fisher's exact test for viral response rates among the groups. The t test was 2-tailed, and differences of $p < 0.05$ were considered significant.

Table 1. Early viral dynamics with DFPP plus IFN treatment

Case	Age/ sex	Type of IFN for 4 weeks with DFPP	Viral dynamics after DFPP+IFN						Viral dynamics of previous treatment (PEG-IFN/RBV)				Viral mutation		
			before treatment	log drop				unit	before treatment	log drop	unit	outcome	aa 70	aa 91	ISDR
				24 h	1 wk	2 wks	4 wks			4 wks					
1	66/M	PEG-IFNα-2b/RBV 4 wks	6,510	0.5	0.6	0.6	1.1	fmol/l	452	0.7	KIU/ml	NR	wild	wild	0
2	65/F	IFN-β (3 MU 2/day) 2 wks → PEG-IFNα-2a/RBV 2 wks	7.5	0.4	1.3	2.6	1.0	log IU/ml	2,800	ND	KIU/ml	PR	wild	wild	0
3	52/F	IFN-β (3 MU 2/day) 2 wks → PEG-IFNα-2a/RBV 2 wks	5.8	0.4	1.0	1.6	+0.2	log IU/ml	6.3	0.2	log IU/ml	NR	wild	wild	1
4	47/F	IFN-β (3 MU 2/day) 2 wks → IFN-β (6 MU 1/day) 2 wks	6.8	0.6	0.3	0.4	0.4	log IU/ml	2,900	0.3	KIU/ml	NR	mutant	mutant	1
5	52/F	IFN-β (3 MU 2/day) 10 days → IFN-β (6 MU 1/day) 18 days	5.5	1.4	1.5	1.2	1.9	log IU/ml	782	0.6	fmol/l	NR	wild	mutant	1
6	61/F	IFN-β (3 MU 2/day) 4 wks	6.5	1.2	3.4	5.0	4.8	log IU/ml	8,450	2.6	fmol/l	NR	wild	wild	0
7	66/F	IFN-β (3 MU 2/day) 4 wks	5.3	0.0	0.8	1.2	1.3	log IU/ml	11,500	0.8	fmol/l	NR	mutant	wild	1
8	43/F	IFN-β (3 MU 2/day) 4 wks	3,460	0.5	0.2	1.3	2.2	fmol/l	745	0.1	fmol/l	NR	wild	mutant	1
9	43/M	IFN-β (3 MU 2/day) 2 wks → IFN-β (6 MU 1/day) 2 wks	7.2	0.6	1.4	2.5	2.9	log IU/ml	426	0.1	KIU/ml	NR	wild	wild	0

PEG-IFN/RBV: PEG-IFNα-2a (180 μg per week) plus RBV (600–800 mg/day) or PEG-IFNα-2b (1.5 μg/kg per week) plus RBV (800 mg/day). IFN-β: 3 MU twice daily or 6 MU daily. ND = Not done; aa = amino acid; ISDR = interferon sensitivity-determining region.

Results

Of the 9 patients, 1 was PR and 8 were NR. Virus mutation in the core region was as follows: wild type (7 patients) and mutant type (2 patients) at aa 70; wild type (6 patients) and mutant type (3 patients) at aa 91. IFN sensitivity-determining regions demonstrated mutation 1 (5 patients) and mutation 0 (4 patients), while mutation 2 was not seen in any patient. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks showed a reduction in the viral load of ≥1 log in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9) and 77.8% (7/9) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively. The early viral dynamics after DFPP plus consecutive intravenous IFN-β treatment for 4 weeks showed a reduction in the viral load of ≥1 log in 33% (2 of 6 patients), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively. The reduction of the viral load by ≥2 log was observed in 50% (3 of 6 patients) at 4 weeks after the start of treatment (table 1).

Discussion

New drugs to replace IFN as well as drugs that can be used in combination with IFN are being actively developed. Also, attempts are being made to find ways to physically remove HCV particles from the blood. Granulocyte apheresis, plasma exchange and hemofiltration have been applied to HCV-infected patients for the treatment of cryoglobulinemia and vasculitis, modalities which have been shown to reduce HCV RNA in the blood during treatment [6–11]. The mechanisms of the clinical results of plasmapheresis have been described, whereby HCV in the blood is related to the effects of IFN therapy that could be enhanced by removing the virus from blood [12–14]. Low-density lipoprotein-cholesterol apheresis and plasma exchange in hypercholesteremic patients with HCV infection reduces the quantity of HCV RNA in the blood of some patients [15]. Hemodialysis, hemofiltration and peritoneal dialysis in chronic dialysis patients infected with HCV significantly lower HCV RNA levels in the blood [16]. Combined granulocyte apheresis with IFN therapy for CHC [17–19] and the prerequisite for early reduction of the virus in the treatment of CHC [20, 21] are essential. Thus, the potential effectiveness of IFN therapy combined with early physical removal of the virus is of particular interest.

Asahina et al. [22] studied HCV dynamics in both serum and peripheral blood mononuclear cells in 44 patients, with HCV genotype 1b and high viral loads, randomly assigned to 4 treatment groups: (1) combination therapy with 6 MU daily of IFNα-2b plus 800 mg of RBV; (2) monotherapy with 6 MU daily of IFNα-2b; (3) monotherapy with twice-daily intravenous administration of 3 MU of IFN-β, and (4) monotherapy with daily intravenous administration of 6 MU of IFN-β. HCV RNA levels measured serially by highly sensitive real-time PCR and HCV dynamics in both serum and peripheral blood mononuclear cells have demonstrated a 'biphasic' pattern. The exponential decay slopes of the second phase have been significantly higher in the combination or the twice-daily dose regimen groups than in group 2 or 4 (0.10 ± 0.08 vs. 0.02 ± 0.09 or 0.16 ± 0.09 vs. 0.02 ± 0.04 day^{-1}; $p < 0.05$ and $p < 0.0005$, respectively) [22]. Kim et al. [23] observed that a daily dose of IFN-β 6 MU for 4 weeks effects a 2 log decrease in the HCV RNA load in 7 patients with genotype 1b and high viral loads.

In this study, early viral dynamics were assessed in the 9 patients non-SVR to the combination therapy. The overall viral dynamics of DFPP plus IFN treatment with or without RBV for 4 weeks reduced the viral load by ≥1 log in 22% (2 of 9 patients), 55.6% (5/9), 77.8% (7/9), and 77.8% (7/9) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively. DFPP plus consecutive intravenous IFN-β treatment for 4 weeks reduced the viral load by ≥1 log in 33% (2/6), 50% (3/6), 83.3% (5/6) and 83.3% (5/6) at 24 h, 1, 2 and 4 weeks after the start of treatment, respectively.

The prerequisite for early virological response (EVR; indicating negative HCV RNA at 12 weeks) has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those who do not reach EVR fail to respond to further therapy. Treatment discontinued in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is highly desirable [24].

To be able to predict SVR with PEG-IFN/RBV treatment, reduction of the HCV RNA viral load by week 4 is considered essential. A 2 log reduction in the HCV RNA viral load by week 4 is a prerequisite to achieving SVR with PEG-IFN/RBV treatment [25]. In our study of DFPP plus consecutive intravenous IFN-β treatment for 4 weeks, a reduction in the viral load of ≥2 log was achieved in 50% (3 of 6 patients) at 4 weeks after the start of treatment.

From the above considerations, DFPP plus consecutive intravenous IFN-β treatment for 4 weeks is a promising regimen for non-SVR patients with genotype 1b and high viral loads, previously treated with PEG-IFN/RBV therapy. Further study is needed to elucidate the SVR rate in a larger number of patients given DFPP plus IFN treatment, especially with consecutive intravenous IFN-β.

Acknowledgment

We are indebted to Yoshiko Kawamura for assistance in the preparation of the manuscript.

Disclosure Statement

No conflict of interest exists.

References

1 Hoofnagle JH, Seeff LB: Peginterferon and ribavirin for chronic hepatitis C. N Engl J Med 2006;355:2444–2451.

2 Pawlotsky JM: Therapy of hepatitis C: from empiricism to eradication. Hepatology 2006; 43:S207–S220.

3 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.

4 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347:975–982.

5 Jensen DM, Freilich B, Andreone P, et al: Pegylated interferon alfa-2A (40KD) plus ribavirin (RBV) in prior non-responders to pegylated interferon alfa-2B (12KD)/RBV: final efficacy and safety outcomes of the repeat study. Hepatology 2007;46(suppl 1):291–292.

6 Fujiwara K, Kaneko S, Kakumu S, et al: Double filtration plasmapheresis and interferon therapy for chronic hepatitis C patients with genotype 1 and high viral load. Hepatol Res 2007;37:701–710.

7 Fabrizi F, Martin P, Dixit V, et al: Biological dynamics of viral load in hemodialysis patients with hepatitis C virus. Am J Kidney Dis 2000;35:122–129.

8 Manzin A, Candela M, Solforosi L, Gabrielli A, Clementi M: Dynamics of hepatitis C viremia after plasma exhange. J Hepatol 1999; 31:389–393.

9 Ramratnam B, Bonhoeffer S, Binley J, et al: Rapid production and clearance of HIV-1 and hepatitis C virus assessed by large volume plasma apheresis. Lancet 1999;354: 1782–1785.

10 Schettler V, Monazahian M, Wieland E, Thomssen R, Muller GA: Effect of heparin-induced extracorporeal low-density lipoprotein precipitation (HELP) apheresis on hepatitis C plasma virus load. Ther Apher 2001;5: 384–386.

11 Schettler V, Monazahian M, Wieland E, et al: Reduction of hepatitis C virus load by H.E.L.P.-LDL apheresis. Eur J Clin Invest 2001;31:154–155.
12 Sakai A, Kaneko S, Matsushita E, Kobayashi K: Floating density of hepatitis C virus particles and response to interferon treatment. J Med Virol 1998;55:12–17.
13 Sakai A, Kaneko S, Kobayashi K: Immunoadsorption therapy for HCV infected chimpanzee. Nippon Rinsho 2001;59:1374–1378.
14 Yamashita T, Arai K, Sakai A, et al: Virological effects and safety of combined double filtration plasmapheresis (DFPP) and interferon therapy in patients with chronic hepatitis C: a preliminary study. Hepatol Res 2006;36:167–175.
15 Marson P, Boschetto R, De Silvestro G, et al: Changes in HCV viremia following LDL apheresis in a HCV positive patient with familial hypercholesterolemia. Int J Artif Organs 1999;22:640–644.
16 Ishida H, Tanabe K, Tokumoto T, et al: Hepatitis C virus decrease in patients with maintenance hemofiltration therapy. Artif Organs 2004;28:316–318.
17 Diepolder HM, Kashiwagi N, Teuber G, et al: Leucocytapheresis with Adacolumn enhances HCV-specific proliferative responses in patients infected with hepatitis C virus genotype 1. J Med Virol 2005;77:209–215.
18 Sawada K, Masaki N, Hayashi S, et al: Immunomodulatory effects of selective leucocytapheresis as a new adjunct to interferon-α2b plus ribavirin combination therapy: a prospective study in patients with high plasma HCV viraemia. J Viral Hepat 2005;12:274–282.
19 Moriyama M, Kaneko M, Matsumura H, et al: Removal of hepatitis C virus by G-1 beads in sera from patients with chronic hepatitis C. Intervirology 2005;48:84–88.
20 Hayashi N, Takehara T: Antiviral therapy for chronic hepatitis C: past, present, and future. J Gastroenterol 2006;41:17–27.
21 Ballesteros AL, Fuster D, Planas R, Clotet B, Tural C: Role of viral kinetics under HCV therapy in HIV/HCV-coinfected patients. J Antimicrob Chemother 2005;55:824–827.
22 Asahina Y, Izumi N, Uchihara M, et al: A potent antiviral effect on hepatitis C viral dynamics in serum and peripheral blood mononuclear cells during combination therapy with high-dose daily interferon alfa plus ribavirin and intravenous twice-daily treatment with interferon beta. Hepatology 2001;34:377–384.
23 Kim KI, Sasase N, Taniguchi M, et al: Interferon-β induction/interferon-α2b plus ribavirin therapy in patients with chronic hepatitis C. Int J Clin Pharm Res 2005;25:71–76.
24 Davis GL: Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002;36:S145–S151.
25 Nomura H, Miyagi Y, Tanimoto H, Higashi M, Ishibashi H: Effective prediction of outcome of combination therapy with pegylated interferon alpha 2b plus ribavirin in Japanese patients with genotype-1 chronic hepatitis C using early viral kinetics and new indices. J Gastroenterol 2009;44:338–345.

Original Article

Intervirology

Intervirology 2010;53:49–54
DOI: 10.1159/000252784

Published online: January 5, 2010

Outcome and Early Viral Dynamics with Viral Mutation in PEG-IFN/RBV Therapy for Chronic Hepatitis in Patients with High Viral Loads of Serum HCV RNA Genotype 1b

Noriko Sasase[a] Soo Ryang Kim[b] Masatoshi Kudo[e] Ke Ih Kim[a]
Miyuki Taniguchi[b] Susumu Imoto[b] Keiji Mita[b] Yoshitake Hayashi[c]
Ikuo Shoji[d] Ahmed El-Shamy[d] Hak Hotta[d]

Departments of [a]Pharmacy and [b]Gastroenterology, Kobe Asahi Hospital, [c]Center for Infectious Diseases and [d]Division of Microbiology, Kobe University Graduate School of Medicine, Kobe, and [e]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

Key Words

Chronic hepatitis · Early viral dynamics · IFN/RBV resistance-determining region · HCV RNA genotype 1b · High viral load · PEG-IFN/RBV combination therapy · Virological response, prediction

Abstract

We investigated whether sustained virological response (SVR) and non-SVR by chronic hepatitis C patients to pegylated interferon plus ribavirin (PEG-IFN/RBV) combination therapy are distinguishable by viral factors such as the IFN/RBV resistance-determining region (IRRDR) and by on-treatment factors through new indices such as the rebound index (RI). The first RI (RI-1st; the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd; the viral load at week 2 divided by the viral load at 24 h) were calculated. The subject patients were divided into 3 groups based on RI-1st and RI-2nd: an RI-A group (RI-1st $\leq$1.0), an RI-B group (RI-1st >1.0 and RI-2nd <0.7) and an RI-C group (RI-1st >1.0 and RI-2nd $\geq$0.7). The SVR rate was 71.4% (10/14) in the RI-A group, 46.2% (6/13) in the RI-B group and 20.0% (3/15) in the RI-C group (p = 0.005 between the RI-A group and the RI-C group). In IRRDR $\geq$6 and IRRDR $\leq$5 the SVR rate was 81.3% (13/16) and 23.1% (6/26) (p = 0.0002), respectively. By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group ($\geq$6 mutations in the IRRDR) and 7.7% (1/13) in the RI-C group ($\leq$5 IRRDR mutations) (p = 0.0003).

Introduction

Recently, global consensus has obtained that a combination of IFN or pegylated IFN plus ribavirin (PEG-IFN/RBV) is the treatment of choice for chronic hepatitis C (CHC). Notwithstanding this treatment regimen, sustained virological response (SVR) rates of those infected with the most resistant genotypes [hepatitis C virus (HCV)-1a and -1b] still hover at ~50% [1, 2]. It is therefore worthwhile to identify the predictive factors that allow the selection of patients who would achieve eradication

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0049$26.00/0

Accessible online at:
www.karger.com/int

Soo Ryang Kim, MD
Department of Gastroenterology
Kobe Asahi Hospital
3-5-25 Bououji-cho, Nagata-ku, Kobe 653-0801 (Japan)
Tel. +81 78 612 5151, Fax +81 78 612 5152, E-Mail asahi-hp@arion.ocn.ne.jp

of HCV RNA either before or during therapy, especially since IFN/RBV combination therapy is costly and has several side effects [3].

Predictors of the effectiveness of IFN-based therapy can be classified into pretreatment and on-treatment factors. Pretreatment factors comprise: (1) host factors such as age, gender, obesity, alcohol consumption, hepatic iron overload, fibrosis, immune responses and co-infection with other viruses, and (2) viral factors that mainly include viral genotypes and loads, particular amino acid sequence variations in the NS5A region [4, 5] and in the core protein region of HCV [6] within a given genotype. Moreover, the mean number of mutations in variable region 3 (V3) plus its upstream flanking region of NS5A [amino acid 2334–2379, referred to as IFN/RBV resistance-determining region (IRRDR)] is significantly higher in HCV isolates obtained from patients who later achieve SVR by PEG-IFN/RBV than in those from non-SVR patients. On-treatment factors are mainly related to viral kinetics within the first few weeks of treatment [7].

In the current study, with the aim of investigating whether SVR and non-SVR can be distinguished by viral factors such as IRRDR and by on-treatment factors through new indices such as the rebound index (RI), we calculated the first RI (RI-1st; the viral load at week 1 divided by the viral load at 24 h) and the second RI (RI-2nd; the viral load at week 2 divided by the viral load at 24 h), as proposed by Nomura et al. [8].

Patients and Methods

The 42 patients included in this study, who all demonstrated high viral loads (>100 KIU/ml) of serum HCV RNA of genotype 1b, had been diagnosed with CHC on the basis of abnormal serum alanine aminotransferase persisting for at least 6 months, and of positive HCV RNA assessed by RT-PCR. None of the patients was positive for hepatitis B surface antigen or other liver diseases (autoimmune hepatitis, alcoholic liver disease). All the patients received a regimen of PEG-IFNα-2b (peginterferon alpha-2b; PegIntron; Schering-Plough, Kenilworth, N.J., USA) (1.5 μg/kg/week, subcutaneously) in combination with RBV (ribavirin; Rebetol; Schering-Plough) 600–1,000 mg/day for 48 weeks. RBV was administered at a dose of 600 mg/day (3 capsules) to patients weighing <60 kg, 800 mg/day (4 capsules) to those weighing <80 kg and 1,000 mg/day (5 capsules) to those weighing ≥80 kg.

The efficacy of the combination therapy was evaluated by HCV RNA negativity determined by qualitative RT-PCR analysis at the end of therapy (end of therapy response) and 6 months after the completion of therapy (SVR). The amount of HCV RNA was also measured quantitatively by RT-PCR (Amplicor HCV monitor v. 2.0; Roche) before therapy. The lower detection limit of the assay was 5 KIU/ml. Samples collected during and after therapy were also determined by qualitative RT-PCR (Amplicor; Roche), which has a higher sensitivity than quantitative analysis, and the results were labeled as positive or negative. The lower limit of the assay was 50 IU/ml.

SVR was defined as undetectable serum HCV RNA at 24 weeks after the cessation of treatment, and non-SVR as detectable HCV RNA at 24 weeks after the discontinuation of treatment. Informed consent was obtained from all patients enrolled in the study after thoroughly explaining the aims, risks and benefits of the therapy.

The amount of HCV core antigen was assessed by the IRM assay (Ortho Clinical Diagnostics, Tokyo, Japan), which provides a good correlation between the amount of HCV core antigen and the amount of HCV RNA, as shown in our previous study [9]. The HCV core antigen was measured on days 0, 1 (24 h), 7 (1 week) and 14 (2 weeks) according to the detection limit of 20 fmol/l established by the manufacturer.

RI-1st was defined as the coefficient derived by dividing the viral load of HCV core antigen at week 1 by that at 24 h, and RI-2nd was defined as the coefficient derived by dividing the viral load at week 2 by that at 24 h [8].

The patients were divided into 3 groups based on RI-1st and RI-2nd: group A (RI-1st ≤1.0), group B (RI-1st >1.0 and RI-2nd <0.7) and group C (RI-1st >1.0 and RI-2nd ≥0.7).

NS5A sequence analysis (IRRDR) was performed as described [4]. Briefly, the sequences of the amplified fragments were determined by direct sequencing without subcloning with the use of a Big Dye Deoxy Terminator cycle sequencing kit and an ABI 337 DNA sequencer (Applied Biosystems, Japan). The aa sequences were deduced and aligned with Genetyx Win software v. 7.0 (Genetyx Corp., Tokyo, Japan). Numbering of aa throughout the manuscript is according to the polyprotein of HCV genotype 1b prototype HCV-J.

Statistical Analysis

Differences between the groups were assessed by the χ^2 test, Fisher's exact test or Student's t test, the Mann-Whitney test and the Kruskal-Wallis test. $p < 0.05$ was considered statistically significant.

Results

Of the 42 patients treated with combination therapy, 19 (45.2%) achieved SVR and 23 (54.8%) were still HCV RNA positive (non-SVR) 6 months after therapy. No significant differences were observed in patient characteristics between SVR and non-SVR, except in platelet counts and the degree of fibrosis (table 1), or among the RI-A, -B and -C groups (table 2).

The SVR rate was 71.4% (10/14), 46.2% (6/13) and 20.0% (3/15) in the RI-A, -B and -C groups, respectively, with a significant difference between the RI-A and -C groups ($p = 0.005$), but not significant between the RI-A and -B groups and the RI-B and -C groups (fig. 1). In the 14 patients of the RI-A group, HCV RNA turned negative

Table 1. Host-dependent, virus-related profile by response (SVR and non-SVR)

	SVR	Non-SVR	p value
Gender (M/F), n	11/8	13/10	NS
Age, years	56.7 ± 8.8	59.3 ± 10.5	NS
HCV RNA level, KIU/ml	1,685 ± 1,477	1,660 ± 1,363	NS
HCV core antigen, fmol/l	7,044 ± 6,763	9,343 ± 12,563	NS
Body weight, kg	59.9 ± 11.5	59.8 ± 13.6	NS
Treatment history (retreatment/naïve)	6/13	13/10	NS
Platelet count ($\times 10^4/mm^3$)	18.7 ± 4.4	14.8 ± 5.4	0.02
F0, 1/F2, 3	12/2	5/10	0.004

Table 2. Host-dependent, virus-related profile by response (RI-A, -B and -C groups)

	RI-A	RI-B	RI-C	p value
Gender (M/F), n	7/7	9/4	8/7	NS
Age, years	60.0 ± 5.9	58.5 ± 9.4	56.1 ± 12.8	NS
HCV RNA level, KIU/ml	1,401 ± 1,014	2,053 ± 1,286	1,593 ± 1,772	NS
HCV core antigen, fmol/l	6,084 ± 5,106	7,674 ± 5,038	11,000 ± 15,837	NS
Body weight, kg	62.1 ± 16.6	59.5 ± 10.4	58.2 ± 10.1	NS
Treatment history (retreatment/naïve)	3/11	7/6	9/6	NS
Platelet count ($\times 10^4/mm^3$)	15.3 ± 3.5	18.3 ± 5.9	16.3 ± 6.0	NS
F0, 1/F2, 3	7/3	5/4	5/5	NS

Table 3. SVR rate between IRRDR ≤5 and IRRDR ≤6 in RI-A, -B and -C groups

	RI-A		RI-B		RI-C	
	IRRDR ≤5	IRRDR ≥6	IRRDR ≤5	IRRDR ≥6	IRRDR ≤5	IRRDR ≥6
SVR	3	7	2	4	1	2
Non-SVR	3	1	5	2	12	0
SVR rate, %	50.0	87.5	28.6	66.7	7.7	100
p value	NS		NS		0.0024	
	0.0003					

by week 4 in 3 patients, week 8 in 5 patients, week 12 in 5 patients and was positive in 1 patient throughout the treatment. In the 13 patients of the RI-B group, HCV RNA was negative by week 4 in 1 patient, week 8 in 2 patients, week 12 in 4 patients, at and after week 16 in 5 patients and remained positive throughout the treatment in 1 patient. In the 15 patients of the RI-C group, HCV RNA was negative by week 12 in 1 patient, on and after week 16 in 6 patients and remained positive throughout the treatment in 8 patients (fig. 2).

The SVR rate was 81.3% (13/16) in the group with ≥6 mutations in IRRDR, and 23.1% (6/26) in those with ≤5 (fig. 3), with a significant difference between the 2 groups (p = 0.0002).

By combining RI and IRRDR, the SVR rate was 87.5% (7/8) in the RI-A group (IRRDR ≥6) and 7.7% (1/13) in the RI-C group (IRRDR ≤5) (table 3), with a significant difference between the 2 groups (p = 0.0003).

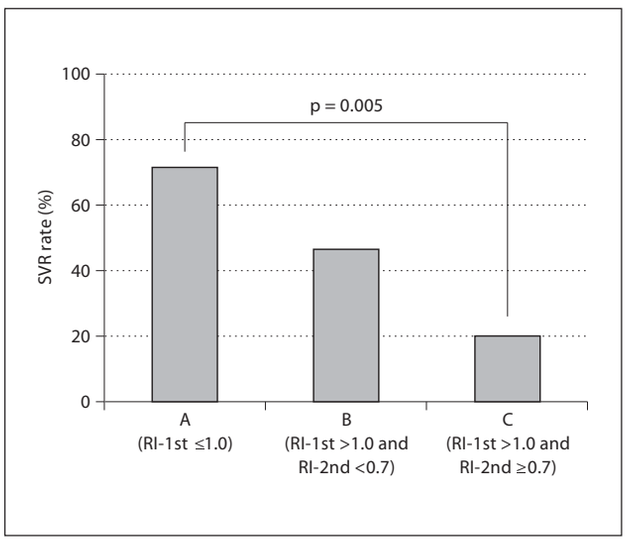


Fig. 1. SVR rate in RI-A, -B and -C groups. The overall SVR rate was 71.4, 46.2 and 20.0%, respectively. Significant difference in SVR rate is indicated.

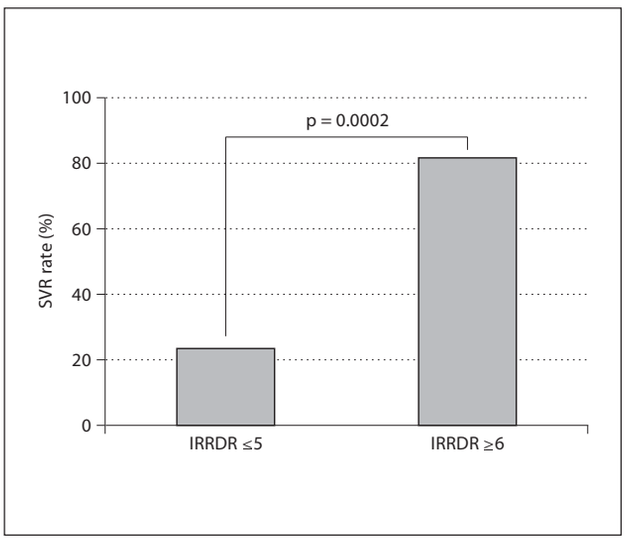


Fig. 3. SVR rate and IRRDR number. The SVR rate was 23.1% in IRRDR ≤5 and 81.3% in IRRDR ≥6, which was significantly different.

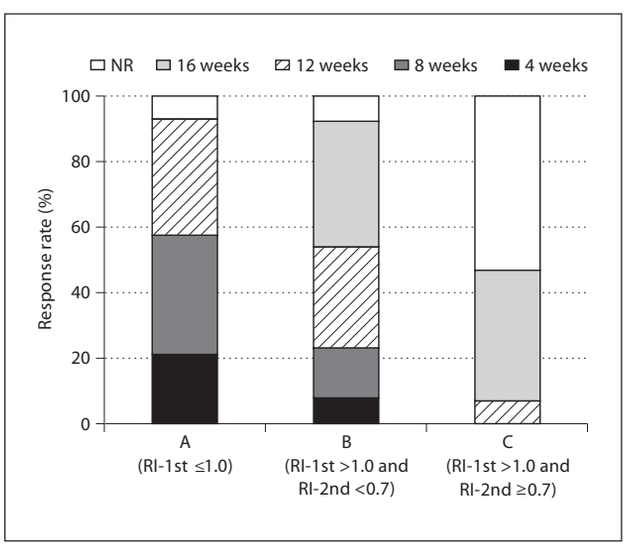


Fig. 2. Relation between response time and virus dynamics. In the 14 patients of the RI-A group, HCV RNA turned negative by week 4 in 3 patients, week 8 in 5 patients, week 12 in 5 patients and remained positive throughout the treatment in 1 patient. In the 13 patients of the RI-B group, HCV RNA was negative by week 4 in 1 patient, week 8 in 2 patients, week 12 in 4 patients, at and after week 16 in 5 patients and remained positive throughout the treatment in 1 patient. In the 15 patients of the RI-C group, HCV RNA was negative by week 12 in 1 patient, at and after week 16 in 6 patients and remained positive throughout the treatment in 8 patients.

Discussion

The importance of early virological response (EVR; signifying HCV RNA negative at 12 weeks) has been emphasized in predicting SVR and non-SVR in CHC patients undergoing IFN treatment; those not reaching EVR do not respond to further therapy. Discontinuation of treatment in patients not reaching EVR would reduce drug costs by more than 20%; consequently, early confirmation of viral reduction after initiating antiviral therapy for CHC is worth investigating [10].

Treatment with IFN induces a decline in HCV RNA levels that can be mathematically measured in 2 phases. The decline in the first phase, usually measured at 24 or 48 h, probably reflects direct inhibition of intracellular production and release of HCV [11], with IFN efficacy ranging from about 70% (approx. 0.7 log units) for standard IFN (given 3 times a week) to more than 90% (1 log unit) for high daily doses of standard IFN or PEG-IFN (given once a week) [12, 13]. The decline in the second phase, beginning after 24–48 h, is slower and more variable than that in the first phase, and is thought to reflect continued inhibition of replication and the gradual elimination of virus-infected cells [11]. The decay in the first phase has little correlation with the IFN dose, but is more rapid with PEG-IFN than with standard IFN preparations [10].

Lowering HCV RNA during the first phase is essential for efficient elimination of HCV during the second phase. Decreases in HCV RNA titers within the first 24–48 h after the start of IFN would, therefore, be a dependable estimate of antiviral efficacy [12, 13].

Early viral kinetics, determined up to week 2, are believed to express the therapeutic effect of PEG-IFN. The concentration of PEG-IFNα-2b in serum peaks after 24 h, then declines gradually [14, 15]. The viral load is thus reduced by 24 h but increases in week 1 [16, 17]; with a large dose of PEG-IFN at each administration, it decreases markedly at 24 h but then increases in week 1 regardless of the dose. In the responder group, however, the viral load continues to decline each week thereafter [17].

In this study, we used new indices proposed by Nomura et al. [8]: RI-1st and RI-2nd calculated from early viral kinetics. RI-1st was defined as the coefficient derived by dividing the viral load of HCV core antigen at week 1 by that at 24 h, and the RI-2nd was defined as the coefficient derived by dividing the viral load at week 2 by that at 24 h. In the SVR group, a number of patients demonstrated no increase in the viral load at week 1. Patients with a high RI-2nd were regarded as poor responders or non-responders to PEG-IFN. The RI-2nd of those other than non-responders was below 0.7; therefore, 0.7 was adopted as the reference value for RI-2nd, and the patients were divided into 3 groups based on RI-1st and RI-2nd: the RI-A group (RI-1st $\leq$1.0), the RI-B group (RI-1st $>$1.0 and RI-2nd $<$0.7) and the RI-C group (RI-1st $>$1.0 and RI-2nd $\geq$0.7). The SVR rate of the RI-A, RI-B and RI-C groups was 71.4% (10/14), 46.2% (6/13) and 20% (2/10), respectively ($p = 0.005$ between the RI-A group and the RI-C group). RIs are also associated with the early clearance of HCV RNA that is related to SVR.

In the RI-A group 21.4% (3/14), 35.7% (5/14) and 35.7% (5/14) became HCV RNA negative by weeks 4, 8 and 12, respectively. In the RI-B group 7.7% (1/13), 15.4% (2/13), 30.8% (4/13) and 38.5% (5/13) became HCV RNA negative by weeks 4, 8, 12, and at and after week 16, respectively. In the RI-C group 6.7% (1/15) and 40.0% (6/15) became HCV RNA negative by week 12, and at and after week 16, respectively. It is believed that the simplified RI-1st and RI-2nd are evidential indices for determining the therapeutic efficacy of PEG-IFN/RBV treatment.

We have previously reported that the high degree of sequence variation in IRRDR (IRRDR $\geq$6) significantly correlates with SVR, whereas the low degree of sequence variation in this region (IRRDR $\leq$5) correlates with non-SVR [4]. A significant correlation between the rapid reduction of HCV core antigen titers and the degree of sequence variation in IRRDR has been observed. This, in particular, suggests a possible influence of IRRDR $\geq$6 on HCV replication kinetics during IFN-based therapy, especially that the direct effect of IFN begins a few hours after the first dose.

In this study, the SVR rate was 81.2% (13/16) with IRRDR $\geq$6 and 23.1% (6/26) with IRRDR $\leq$5 ($p = 0.0002$), strongly suggesting that IRRDR $\geq$6 would be a useful marker for the prediction of SVR.

By combining RI and IRRDR as a predicting factor, the SVR rate was 87.5% (7/8) in the RI-A group (RI-1st $\leq$1.0) with IRRDR $\geq$6, signifying that about 90% of these patients turned SVR and were, thus, believed to be very good responders. An SVR rate of 7.7% (1/13) was obtained in the RI-C group with IRRDR $\leq$5 ($p = 0.0003$).

In conclusion, we propose that IRRDR combined with RIs is the most sensitive predictive factor for SVR and non-SVR. With the aid of RIs and IRRDR, a more effective PEG-IFN/RBV treatment could be within reach. A more detailed investigation with a larger number of subjects is needed to confirm the current results in patients given PEG-IFN/RBV combination therapy.

Acknowledgment

We are indebted to Yoshiko Kawamura for assistance in the preparation of the manuscript.

Disclosure Statement

No conflict of interest exists.

References

1 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.

2 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347:975–982.

3 Nakamura H: Early prediction of sustained viral responder and non-responder during interferon and ribavirin combination therapy in chronic hepatitis C. Hepatol Res 2005; 33:269–271.

4 El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H: Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. Hepatology 2008;48:38–47.
5 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996; 334:77–81.
6 Akuta N, Suzuki S, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Miyakawa Y, Kumada H: Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphism in the viral core protein and very early dynamics of viremia. Intervirology 2007;50:361–368.
7 Ferenci P: Predictors of response to therapy for chronic hepatitis C. Semin Liver Dis. 2004;24:S25–S31.
8 Nomura H, Miyagi Y, Tanimoto H, Higashi M, Ishibashi H: Effective prediction of outcome of combination therapy with pegylated interferon alpha 2b plus ribavirin in Japanese patients with genotype-1 chronic hepatitis C using early viral kinetics and new indices. J Gastroenterol 2009;44:338–345.
9 Sasase N, Kim SR, Kim KI, Taniguchi M, Imoto S, Hotta H, Shouji I, El-Shamy A, Kawada N, Kudo M, Hayashi Y: Usefulness of a new immunoradiometric assay of HCV core antigen to predict virological response during PEG-IFN/RBV combination therapy for chronic hepatitis with high viral load of serum HCV RNA genotype 1b. Intervirology 2008;51:S70–S75.
10 Davis GL: Monitoring of viral levels during therapy of hepatitis C. Hepatology 2002;36: S145–S151.
11 Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, Perelson AS: Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-α therapy. Science 1998;282:103–107.
12 Lam NP, Neumann AU, Gretch DR, Wiley TE, Perelson AS, Layden TJ: Dose-dependent acute clearance of hepatitis C genotype 1 virus with interferon alfa. Hepatology 1997;26:226–231.
13 Zeuzem S, Herrmann E, Lee JH, Fricke J, Neumann AU, Modi M, Colucci G, Roth WK: Viral kinetics in patients with chronic hepatitis C treated with standard or peginterferon alfa-2a. Gastroenterology 2001;120: 1438–1447.
14 Silva M, Poo J, Wagner F, Jackson M, Cutler D, Grace M, et al: A randomized trial to compare the pharmacokinetic, pharmacodynamic, and antiviral effects of peginterferon alfa-2b and peginterferon alfa-2a in patients with chronic hepatitis C (COMPARE). J Hepatol 2006;45:204–213.
15 Asahina Y, Izumi N, Umeda N, Hosokawa T, Ueda K, Doi F, et al: Pharmacokinetics and enhanced PKR response in patients with chronic hepatitis C treated with pegylated interferon alpha-2b and ribavirin. J Viral Hepat 2007;14:396–403.
16 Izumi N, Asahina Y, Kurosaki M, Uchihara M, Nishimura Y, Inoue K, et al: A comparison of the exponential decay slope between PEG-IFN alfa-2b/ribavirin and IFN alfa-2b/ribavirin combination therapy in patients with chronic hepatitis C genotype 1b infection and a high viral load. Intervirology 2004;47:102–107.
17 Buti M, Sanchez-Avila F, Lurie Y, Stalgis C, Valdes A, Martell M, et al: Viral kinetics in genotype 1 chronic hepatitis C patients during therapy with 2 different doses of peginterferon alfa-2b plus ribavirin. Hepatology 2002;35:930–936.

Original Article

Intervirology

Intervirology 2010;53:55–59
DOI: 10.1159/000252785

Published online: January 5, 2010

Prolonged PEG-IFN and RBV Is Effective in Patients with HCV Genotype 1 and High Viral Load Who Achieved Virological Response Later than 24 Weeks

Taisuke Ueda Hobyung Chung Masatoshi Kudo Emi Ishikawa Sousuke Hayaishi Chie Tatsumi Tatsuo Inoue Norihisa Yada Satoru Hagiwara Yasunori Minami Kazuomi Ueshima

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

Key Words

Genotype 1 · Hepatitis C virus · High viral load · Late virological responder · Peginterferon · Ribavirin · Sustained virological response

Abstract

Objective: The extension of treatment duration has been proposed in late virological responders with hepatitis C virus (HCV) genotype 1 and high viral load. However, the effectiveness of extended treatment in patients whose serum HCV RNA become undetectable later than 24 weeks of treatment (ultra-late virological responder; ULVR) has not yet been determined. ***Methods:*** A total of 130 patients infected with HCV genotype 1 and who had high viral load were treated with pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy. We retrospectively analyzed 10 ULVR who received extended combination treatment beyond 48 weeks. ***Results:*** The duration of the combination treatment for ULVR ranged between 59 and 119 weeks, and the mean duration was 80 weeks. Although the majority of ULVR were older female patients (≥60 years) with factors related to poor therapeutic response, 8 patients (80%) achieved sustained virological response (SVR). The SVR rate correlated well with the duration of the treatment. Five ULVR achieved SVR when treatment was continued until serum HCV RNA remained undetectable for longer than 48 weeks. ***Conclusion:*** The extended duration of PEG-IFN and RBV combination treatment is a possible strategy to improve treatment response in HCV genotype 1 infection, even for ULVR.

Introduction

Peginterferon (PEG-IFN) and ribavirin (RBV) combination treatment is the standard treatment for chronic hepatitis C infection that results in improved sustained virological response (SVR), even in patients with hepatitis C virus (HCV) genotype 1 and high viral load [1, 2]. Recently, an extension of the treatment duration to 72 weeks has been proposed for late virological responders who have a 2-log decrement in HCV RNA from baseline at 12 weeks of the treatment and undetectable serum HCV RNA after 13–24 weeks of the treatment [3, 4]. However, the benefit of the extended combination treat-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0055$26.00/0

Accessible online at:
www.karger.com/int

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

ment in patients whose serum HCV RNA become undetectable later than 24 weeks of the treatment (ultra-late virological responder; ULVR) has not yet been determined.

In the present study, we addressed the effectiveness and feasible duration of the extended PEG-IFN and RBV combination treatment in ULVR infected with HCV genotype 1 and who had a high viral load.

Patients and Methods

A total of 130 patients infected with HCV genotype 1 and who had a high viral load were treated with PEG-IFNα-2b (PegIntron; Schering-Plough, Kenilworth, N.J., USA) and RBV (Rebetol; Schering-Plough) combination treatment between January 2005 and August 2008 at the Kinki University Hospital. PEG-IFNα-2b was administered at 1.5 μg/kg subcutaneously each week and RBV was administered orally at a dose of 600 mg to patients weighing <60 kg, 800 mg to those weighing 60–80 kg, and 1,000 mg to those weighing ≥80 kg daily. The dose reduction and discontinuation of the combination treatment was determined according to standard protocols. The total duration of the treatment was determined by the attending physicians or by patient request. The serum HCV RNA was measured before commencement of treatment and every month during the treatment by quantitative Amplicor HCV monitor assay (version 2.0, Roche Diagnostics; detection limit 500 IU/ml). When the quantitative assay showed undetectable levels of HCV RNA, a qualitative Amplicor HCV assay (version 2.0, Roche Diagnostics; detection limit 50 IU/ml) was applied. We defined patients that had undetectable HCV RNA at later than 24 weeks of treatment as ULVR.

Patient characteristics assessed at baseline included age, sex, body mass index (BMI), histological results of pre-treatment liver biopsy (Metavir scoring: F0 = no fibrosis; F1 = portal fibrosis; F2 = few septa; F3 = many septa without cirrhosis; F4 = cirrhosis), platelet count, serum HCV RNA before treatment, viral amino acid (aa) substitutions, and 2-log decrement in serum HCV RNA from baseline at 12 and 24 weeks of the treatment. Viral aa substitutions were determined for the IFN sensitivity-determining region (ISDR) in the HCV NS5A and substitutions at positions 70 and 91 in the HCV core region, as previously described [5–7]. The ISDR was defined as wild type when there were 1 or no aa substitutions, and defined as mutant type when there were 2 or more.

Results

Overall Response in Patients with HCV Genotype 1 and High Viral Load

The SVR rate for combination treatment in all the patients with genotype 1 and high viral load was 41.5% (54/130) by intention-to-treat analysis, and 54.3% (51/94) by per-protocol-study analysis.

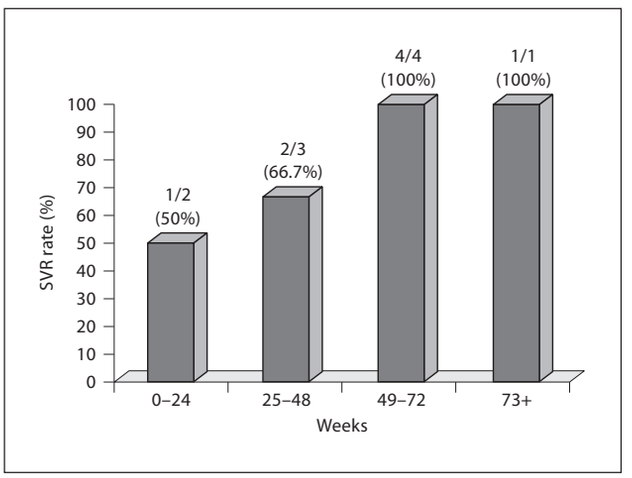


Fig. 1. The duration of maintaining undetectable HCV RNA while undergoing PEG-IFN/RBV combination treatment (maintenance treatment) was correlated with SVR rate. The horizontal axis represents the duration between the time serum HCV RNA was undetectable and the termination of the treatment.

Efficacy and Side Effects of Extended Treatment in ULVR

Among 10 ULVR, 8 patients achieved SVR by the extended treatment. The characteristics for ULVR are shown in table 1. Eight patients (80%) were older than 60 years of age and 7 (70%) were female. Four of the 8 patients with SVR showed severe fibrosis (F3) or cirrhosis (F4). Among 5 patients with SVR who were assessed for aa substitutions in HCV RNA, 3 patients showed a mutant type at ISDR and 4 showed wild type at both aa 70 and aa 91 in the HCV core region, while 2 patients with non-SVR showed substitutions at either aa 70 or aa 91. Six of 10 (60%) ULVR patients achieved a 2-log decrement of serum HCV RNA from baseline at 12 weeks and 8 patients (80%) at 24 weeks. Eight patients (80%) had undetectable HCV RNA within 36 weeks of treatment and the remaining 2 patients (1 each of the non-SVR and SVR groups) had undetectable HCV RNA at 48 and 60 weeks, respectively. The total duration of the combination treatment ranged from 59 to 119 weeks, with a mean duration of 80 weeks. All patients with SVR received extended treatment beyond 72 weeks with the exception of 1 patient, while treatment was stopped before 72 weeks for 2 patients with non-SVR.

Figure 1 shows the SVR rates according to the duration of maintaining undetectable serum HCV RNA while undergoing treatment (maintenance treatment). The SVR

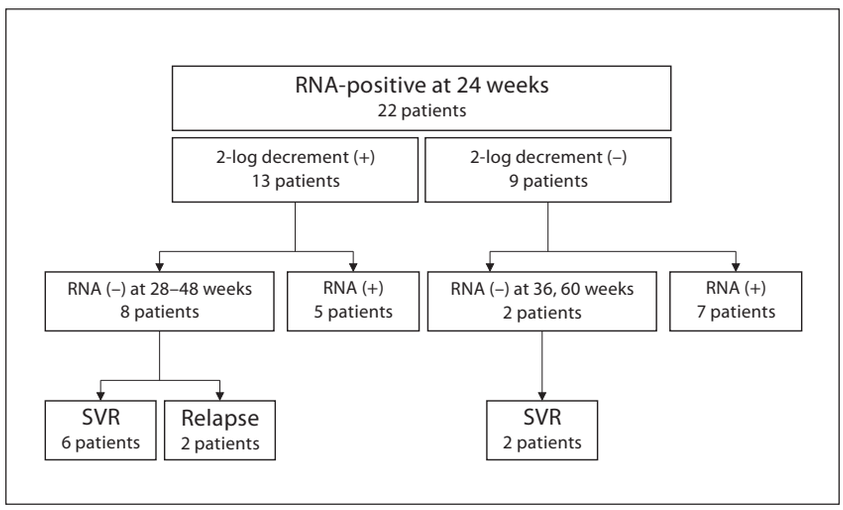


Fig. 2. Outcomes of the extended combination treatment in 22 patients who had detectable HCV RNA at 24 weeks of treatment. wks = Weeks.

Table 1. Patient characteristics of ULVR

Case No.	Age years	Gender	BMI	F stage	Platelet count/μl	HCV RNA KIU/ml	ISDR	HCV core mutation		HCV RNA 2-log decrease		RNA negative weeks	Total treatment duration weeks	SVR
								aa 70	aa 91	12 weeks	24 weeks			
1	56	F	22.5	2	16.2	975	M	W	W	yes	yes	28	77	yes
2	64	F	26.7	3	18.9	2,400	W	W	W	yes	yes	28	83	yes
3	71	F	18.5	1	9.9	1,380	M	W	W	yes	yes	28	83	yes
4	77	M	23.7	4	7.8	166	ND	ND	ND	yes	yes	32	59	yes
5	62	F	19.6	1	23.6	252	M	W	W	–	yes	32	85	yes
6	63	F	19.9	3	8.9	895	ND	ND	ND	–	yes	32	119	yes
7	65	F	21.2	1	23.2	445	W	M	W	–	–	26	82	yes
8	60	F	22.1	3	13.6	1,350	ND	ND	ND	–	–	60	81	yes
9	73	M	20.2	1	8.2	943	M	M	W	yes	yes	48	62	–
10	64	M	26.6	1	16.8	1,750	W	W	M	yes	yes	32	68	–

M = mutant type; W = wild type; ND = not determined.

rate correlated well with the duration of the maintenance treatment. All 5 ULVR achieved SVR when maintenance treatment was continued for longer than 48 weeks.

The PEG-IFN dose was reduced in 4 of 10 (40%) ULVR within 8 weeks of the treatment, and RBV dose was reduced in 3 of 10 ULVR within 28 weeks of the treatment. Dose reduction of both PEG-IFN and RBV was required for 2 patients. Dose reduction was not required during the extended treatment duration and none of the treatments were terminated because of side effects.

Response to the Extended Treatment in Patients with Detectable HCV RNA at 24 Weeks

The outcomes of the extended combination treatment for 22 patients, including 10 ULVR, who had detectable HCV RNA at 24 weeks, are shown in figure 2. Among 13 patients who achieved a 2-log decrement in serum HCV RNA at 24 weeks, eight patients achieved undetectable HCV RNA between 28 and 48 weeks, including 6 with SVR. Five patients remained positive for serum HCV RNA despite extended treatment for 55–105 weeks with a mean duration of 88 weeks. Even in 9 patients who did not achieve a 2-log decrement in HCV RNA at 24 weeks,

2 patients (22%) achieved SVR as a result of extended treatment beyond 72 weeks, while the remaining 7 patients did not.

Discussion

For the treatment of patients infected with HCV genotype 1 and who have high viral load, a 72-week course of PEG-IFN and RBV treatment has become a standard treatment regimen for late virological responders [3, 4]. However, the benefit of extended treatment in patients who had undetectable serum HCV RNA later than 24 weeks of the treatment remains to be elucidated. In the present study, the extended combination treatment attained 80% (8/10) SVR in ULVR and 46% (6/13) SVR in patients who achieved 2-log decrement in HCV RNA from baseline at 24 weeks but still had detectable HCV RNA. Furthermore, here we describe 2 cases with SVR that failed to achieve a 2-log decrement in serum HCV RNA from baseline at 24 weeks.

Several host and viral factors contribute to SVR in PEG-IFN and RBV combination treatment for Japanese patients infected with HCV genotype 1 and who have high viral load. The host factors include younger age, male gender, mild liver fibrosis, platelet count, LDL cholesterol values and γ-glutamyl transpeptidase values. The viral factors include aa substitutions in the ISDR of HCV NS5A and aa substitutions in the HCV core region [5–9]. In addition, the total dose of PEG-IFN or RBV is another important factor that can affect the treatment outcome [8, 9]. In the present study, most ULVR were difficult-to-treat patients with respect to host factors, 8 patients (80%) were older than 60 years of age and 7 patients (70%) were female. Furthermore, 4 of 8 patients with SVR (50%) showed severe fibrosis (F3) or cirrhosis (F4) which is another well-known refractory factor to IFN treatment. These patient characteristics were significantly different from those of patients who achieved early virological response, having undetectable HCV RNA within 12 weeks of the treatment (data not shown). On the other hand, ULVR with SVR had positive viral factors regarding the aa substitutions in the ISDR and HCV core regions. Although we cannot exclude the possibility that these viral factors contributed to SVR rate even in ULVR, an extended combination treatment regimen is still considered a feasible treatment strategy for ULVR.

There are several limitations to this study. First, the Amplicor HCV assay method was used to measure serum HCV RNA in this study. The results presented here are likely to be different if patients were monitored by TaqMan qPCR, a state-of-the-art method with higher sensitivity. Second, this study is retrospective and the duration and dose of the treatment varied considerably. Further prospective studies are necessary to confirm an optimal treatment regimen for ULVR.

In conclusion, the extended duration of PEG-IFN and RBV combination treatment is beneficial in terms of virological response, even for ULVR. The extension of the treatment does not seem to increase side effects or the rate of dose reductions, and treatment should be continued until the serum HCV RNA remains undetectable for at least 24 weeks and, if possible, for longer than 48 weeks during the course of treatment.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Manns MP, McHutchinson JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet 2001;358:958–965.

2 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

3 Berg T, von Wagner M, Nasser S, Sarrazin C, Heintges T, Gerlach T, Buggisch P, Goeser T, Rasenack J, Page GR, Schmidt WE, Kallinowski B, Klinker H, Spengler U, Martus P, Alshuth U, Zeuzem S: Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-alfa-2a plus ribavirin. Gastroenterology 2006;130:1086–1097.

4 Pearlman BL, Ehleben C, Saifee S: Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis C genotype 1-infected slow responders. Hepatology 2007;46:1688–1694.

5 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C: Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96:224–230.
6 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996; 334:77–81.
7 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.
8 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.
9 Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyoda J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H: Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol 2009;44:952–963.

Original Article

Intervirology

Intervirology 2010;53:60–65
DOI: 10.1159/000252786

Published online: January 5, 2010

PEG-IFNα/RBV Combination Therapy for Chronic Hepatitis C Patients Increases Serum Ferritin Level while It Improves Sustained Viral Response Rate

Norihisa Yada Masatoshi Kudo Hobyung Chung Sosuke Hayaishi
Masahiro Takita Taisuke Ueda Chie Tatsumi Kinuyo Hatanaka Satoshi Kitai
Emi Ishikawa Tatsuo Inoue Satoru Hagiwara Kazuomi Ueshima

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

Key Words
Alanine aminotransferase · Chronic hepatitis C · Combination therapy · Hemolytic anemia · Hepatic iron overload · Pegylated interferon · Ribavirin · Serum ferritin · Sustained viral response

Abstract
Objectives: We investigated the significance of serum ferritin levels in pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy for chronic hepatitis C (CHC) and examined its correlation with serum alanine aminotransferase (ALT) levels during therapy and response to the therapy. ***Methods:*** A total of 175 patients with CHC received the combination therapy. Correlations between serum ferritin levels and serum ALT levels at 12 and 24 weeks of therapy were examined. Differences in serum ferritin levels during therapy between patients with sustained viral response (SVR) and non-SVR were also examined. ***Results:*** Only 24 (13.7%) and 20 (11.4%) patients showed elevated serum ALT levels (≥70 IU/l) at 12 and 24 weeks of therapy, respectively. There was no correlation between serum ferritin levels and ALT levels. Ninety-five (54.3%) of 175 patients achieved SVR. Serum ferritin levels increased dramatically in both SVR and non-SVR groups after starting the therapy and were significantly higher in the SVR group throughout the therapy. ***Conclusions:*** Serum ferritin level increases during PEG-IFN and RBV combination therapy; however, it did not correlate with either serum ALT level or the total dose of RBV. Higher serum ferritin levels during combination therapy appear to be associated with favorable therapeutic response.

Introduction

Pegylated interferon (PEG-IFN) and ribavirin (RBV) combination therapy is the current standard treatment for chronic hepatitis C (CHC) infection, demonstrating an improved sustained viral response (SVR) rate even in patients infected with hepatitis C virus (HCV) genotype 1 and who had a high viral load [1, 2]. Several host and viral factors contribute to SVR in the combination treatment for Japanese patients infected with HCV genotype 1 with high viral load [3–7]. The host factors include younger age, male gender, mild liver fibrosis, platelet count, LDL cholesterol values and γ-glutamyl transpeptidase values. The viral factors include amino acid substitutions in the IFN sensitivity-determining region of the HCV nonstructural 5A (NS5A) protein and in the HCV core region.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0060$26.00/0

Accessible online at:
www.karger.com/int

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

Hepatic iron overload is frequently observed in patients with HCV infection and has been considered to be associated with disease progression and hepatocarcinogenesis [8, 9]. However, the association between hepatic iron overload and therapeutic response to IFN therapy for CHC remains controversial [10–12]. We previously reported that elevation of serum alanine aminotransferase (ALT) levels during PEG-IFNα-2a monotherapy for CHC seems to be caused by hepatic iron overload which may be induced by the therapy itself [13].

In this study, we investigated the correlation between serum ferritin levels and serum ALT levels during PEG-IFN and RBV combination therapy for CHC, and also investigated whether the elevated serum ferritin level during therapy is associated with a therapeutic response.

Patients and Methods

Patients

This retrospective study was conducted at the Kinki University of Medical Science. Eligible subjects were: CHC patients who had received weekly injections of PEG-IFNα-2a or -2b for 24–72 weeks; who had been followed for more than 24 weeks after treatment, and who had been examined serially for quantitative and qualitative HCV RNA, serum ferritin, serum iron, ALT and complete blood cell counts.

Patients with a high load (≥100 kIU/ml) of HCV genotype 1 were treated for 48–72 weeks, those with a low load (<100 kIU/ml) of genotype 1 for 24–48 weeks and those with genotype 2 were treated for 24 weeks. PEG-IFNα-2a was administered once a week at a daily dose of 90 or 180 μg, PEG-IFNα-2b was administered once a week at a daily dose of 1.5 μg/kg body weight. RBV was orally administered daily in 2 divided doses. The doses of RBV were adjusted based on the subject's body weight (600 mg for ≤60 kg, 800 mg for 60–80 kg, 1,000 mg for ≥80 kg). Doses were adjusted during therapy according to standard indications.

Patients with hepatitis B virus infection, HIV infection, autoimmune hepatitis, primary biliary cirrhosis, alcoholic liver disease, non-alcoholic steatohepatitis, hemochromatosis, Wilson's disease and hemoglobinopathy were excluded. Patients were classified as responders if they achieved SVR (defined as undetectable HCV RNA at 24 weeks after the completion of therapy). The remaining patients were categorized as non-SVR. Written informed consent was obtained from all patients before treatment and the protocol was approved by the ethics committee of the Kinki University School of Medicine.

Biochemical and Virological Assay

Laboratory tests including serum ALT levels, serum iron, serum ferritin and complete blood cell counts were assessed in a centralized laboratory using automated methods.

Quantitative HCV testing was performed using the Cobas® Amplicor HCV Monitor Test v.2.0 (Roche Diagnostics, Australia) on the Roche Cobas Amplicor Analyzer (Roche Diagnostics) according to the manufacturer's instructions. HCV RNA qualitative determination was performed by real-time PCR on a Cobas TaqMan 48 Analyzer or by Cobas Amplicor HCV Test, v.2.0 (Roche Diagnostics) on the Roche Cobas Amplicor Analyzer with a sensitivity limit of at least 50 IU/ml. Laboratory tests and HCV RNA were analyzed before treatment and at 4, 12 and 24 weeks after initiation of therapy, at the end of the treatment period and 4, 12, 24 and 48 weeks following completion of therapy.

Table 1. Demographics and baseline characteristics

Patients, n	175
Age	60.2 ± 0.8
Sex (male/female), n	85/90
BMI, kg/m^2	22.9 ± 0.3
Genotype, n	
1	151 (86.3%)
2	24 (13.7%)
Viral load, kIU/ml	2,200 ± 200
WBC, × 10^3/μl	4.9 ± 0.1
Hb, g/dl	13.8 ± 0.1
PLT, × 10^4/μl	16.3 ± 0.5
Serum ferritin, ng/ml	115.6 ± 12.5
ALT, IU/l	71.5 ± 4.3
PEG-IFN, n	
α-2a	42 (24%)
α-2b	133 (76%)

BMI = Body mass index; WBC = white blood cell count; Hb = hemoglobin; PLT = platelets.

Statistical Analysis

Data were expressed as the means ± standard errors. Differences between groups were determined by Wilcoxon's signed rank test and confirmed by the non-parametric Mann-Whitney U test between groups. Correlation between data was tested using the non-parametric Spearman rank correlation analysis. Differences were considered statistically significant at $p < 0.05$. Statistical calculations were performed using the commercially available software SPSS v.11.5 (SPSS, Chicago, Ill., USA).

Results

Demographics and Baseline Features

Of the 187 patients, 12 discontinued treatment due to adverse events. The remaining 175 patients, consisting of 90 (51.4%) women and 85 men (48.6%), who met the requirements were enrolled in the study. HCV genotype 1 was prevalent in 151 (86.3%) patients with the remaining 24 (13.7%) positive for type 2. PEG-IFNα-2b was administered to 133 (76%) of the patients (table 1).

Table 2. Univariate analysis for SVR

	SVR	Non-SVR	p value
Patients, n	95	80	
Age, years	60.2 ± 1.2	60.1 ± 9.0	0.418
Sex (male/female), n	50/45	35/45	0.243
BMI, kg/m^2	22.6 ± 4.4	23.2 ± 0.3	0.173
Genotype			<0.05
1	74 (49.0%)	77 (51.0%)	
2	21 (87.5%)	3 (12.5%)	
Viral load, kIU/ml	1,700 ± 200	2,700 ± 400	<0.05
WBC, $\times 10^3/\mu l$	5.1 ± 0.2	4.6 ± 0.2	<0.05
Hb, g/dl	14.0 ± 0.1	13.6 ± 0.2	<0.05
PLT, $\times 10^4/\mu l$	17.8 ± 0.6	14.6 ± 0.7	<0.05
Serum ferritin, ng/ml	135.9 ± 14.6	174.1 ± 19.9	0.214
ALT, IU/l	73.6 ± 6.5	69.0 ± 5.3	0.494
PEG-INF			0.321
α-2a	20 (47.6%)	22 (52.4%)	
α-2b	75 (56.4%)	58 (43.6%)	

BMI = Body mass index; WBC = white blood cell count; Hb = hemoglobin; PLT = platelets

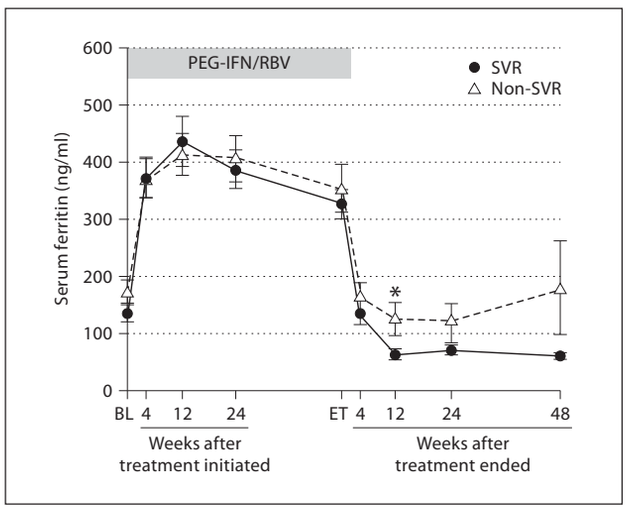


Fig. 1. Serum ferritin levels during and after treatment in patients with or without a SVR. Changes over time of serum ferritin levels in SVR and non-SVR patients after antiviral therapy are displayed. BL = Baseline; ET = end of treatment. * $p < 0.05$, comparing between groups at each time point.

Viral Response

SVR was achieved by 95 (54.3%) of the 175 patients, with the remaining 80 patients not responding even at 24 weeks after completion of the treatment. Of the 95 patients achieving SVR, 50 (52.6%) were male and 45 (47.4%) were female. There were no significant differences in the rate of SVR between the 2 sexes (p = 0.243). For HCV genotype 2, 21 (87.5%) of 24 patients exhibited SVR. Only 74 (49.0%) of 151 patients infected with HCV genotype 1 demonstrated SVR. Univariate analysis showed that factors significantly associated with SVR were low viral load, elevated white blood cell count, high hemoglobin and high platelet count. Serum ferritin levels tended to be lower in patients with SVR. There was no significant difference in age, sex, body mass index, serum ferritin level and serum ALT level between SVR and non-SVR patients (table 2).

Correlation between Serum ALT and Serum Ferritin Level

Only 24 (14%) and 20 (11%) patients showed elevated serum ALT levels (≥70 IU/l) at 12 and 24 weeks of the therapy, respectively. Among the subjects whose ALT levels were ≥70 IU/l at weeks 12 and 24, the relationship between the serum ALT and ferritin levels was investigated using Pearson's correlation coefficient test. There was no significant relation between serum ALT and ferritin levels at each time point (p = 0.838, r = –0.049 at week 12; p = 0.142, r = 0.340 at week 24).

Dynamics of Serum Ferritin Level during and after Treatment

Serum ferritin levels increased and peaked between 4 and 12 weeks after commencement of therapy, remained high until the end of the treatment period, and returned to baseline levels after completion of the treatment. If SVR did not occur, serum ferritin levels increased again following completion of therapy (fig. 1). The 'increasing ratio of serum ferritin' was calculated as the ratio of serum ferritin level to baseline serum ferritin level. The increasing ratio of serum ferritin in patients with a SVR was significantly higher than that in non-SVR individuals during treatment (fig. 2).

Correlation between Dosage of PEG-IFN or RBV and Increasing Ratio of Serum Ferritin

We analyzed the relationship between administered doses and serum ferritin during the first 4 and 12 weeks of treatment by Pearson's correlation coefficient test. There was no significant correlation between the administered dose of RBV and increasing ratio of serum ferritin (p = 0.110, r = 0.166 during the first 4 weeks; p = 0.071,

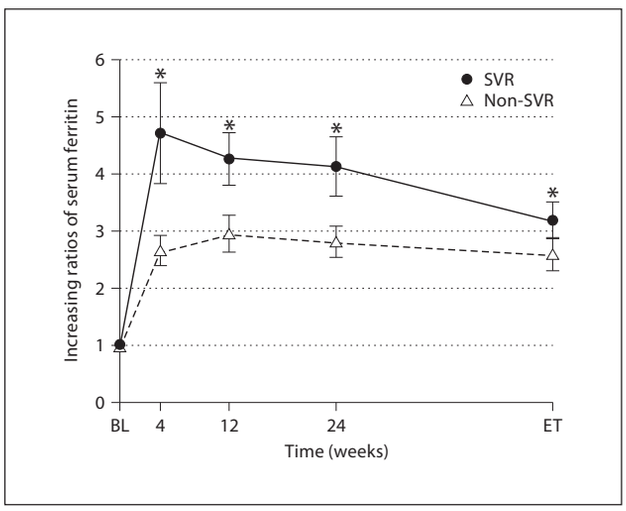
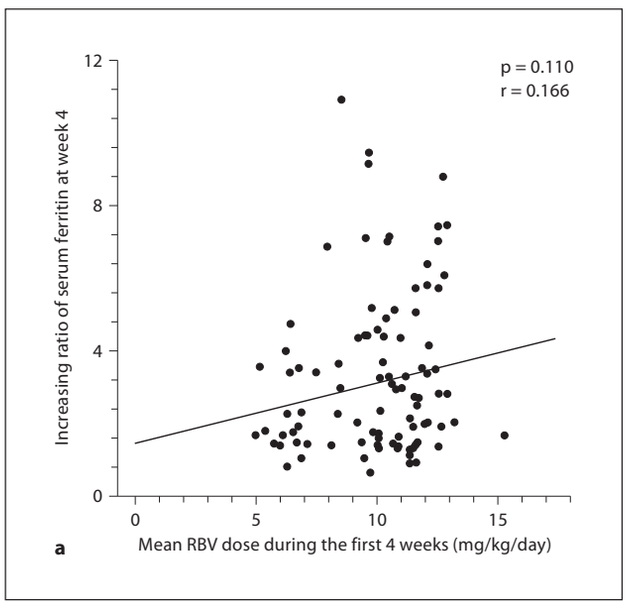


Fig. 2. Increasing ratios of serum ferritin during treatment in patients with or without a SVR. The 'increasing ratio of serum ferritin' was calculated as the ratio of serum ferritin level to baseline serum ferritin level. BL = Baseline; ET = end of treatment. * $p < 0.05$, comparing between groups at each time point.



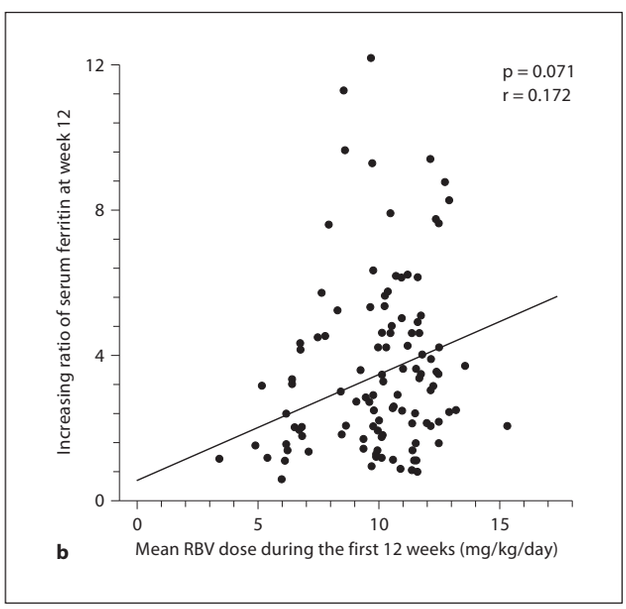


Fig. 3. Correlation between administered doses of RBV and increasing ratio of serum ferritin. The 'increasing ratio of serum ferritin' was calculated as the ratio of serum ferritin level to baseline serum ferritin level. **a** Correlation between the mean dose of RBV and increasing ratio of serum ferritin at week 4. **b** Correlation between the mean dose of RBV and increasing ratio of serum ferritin at week 12.

r = 0.172 during the first 12 weeks; fig.3). There was also no correlation between PEG-IFNα-2a or -2b and increasing ratio of serum ferritin (PEG-IFNα-2a: p = 0.856, r = 0.037 at week 4; p = 0.752, r = –0.58 at week 12; PEG-IFNα-2b: p = 0.692, r = 0.049 at week 4; p = 0.243, r = 0.132 at week 12).

Discussion

We previously reported that elevation of serum ALT levels correlated to serum ferritin levels during PEG-IFNα-2a monotherapy for CHC seems to be caused by hepatic iron overload, which may be induced by the therapy itself [13]. In this paper, 28 (44.4%) of 63 patients exhibited elevated ALT levels (≥70 IU/l). Also, serum ALT levels were elevated (≥70 IU/l) in 24 (13.7%) of 175 patients at week 12, and in 20 (11.4%) of 175 patients at week 24. In our present study, during PEG-IFN and RBV combination therapy, there was no elevation of serum ALT level in almost all patients, and there was no significant correlation between serum ferritin and ALT levels.

RBV, a guanosine analogue which has a broad antiviral spectrum, is known as an immunomodulator inhibiting the viral RNA polymerase, balancing Th1 and Th2 cell responses and acting by direct cytoprotection [14, 15].

Although HCV RNA levels and hepatic fibrosis scores did not change significantly in patients with CHC who were treated by RBV alone, serum ALT levels and inflammatory features of liver histology were improved [16, 17]. One possible reason why the correlation between serum ALT and serum ferritin levels was not seen in PEG-IFN and RBV combination therapy, distinct from PEG-IFNα-2a monotherapy, is the efficacy of RBV to improve inflammation of the liver as described above.

In this study, whether SVR was reached or not, serum ferritin levels significantly increased after initiation of therapy, peaked at an early stage (4–12 weeks) after initiation and decreased slowly. In SVR patients, serum ferritin levels after treatment were lower than the initial baseline. In non-SVR patients, serum ferritin levels increased again after completion of therapy with a corresponding increase of HCV-RNA load (data not shown). The increased ratio of serum ferritin (serum ferritin/baseline ferritin ratio) in SVR subjects was significantly higher than in non-SVR individuals. A similar report has been previously published, and its results correspond with what we are reporting here [18].

In general, it is thought that an adequate dose of RBV in the first stages of treatment correlates to SVR [19]. It is expected that the more RBV is administered, the more strongly hemolysis occurs and serum ferritin levels rise. In this study, there was no significant correlation between dose of RBV and serum ferritin levels. Even if the same dose of RBV was administrated, metabolism and concentration of RBV differ between individuals. Though we did not investigate parameters involving hemolysis or RBV concentration, the correlation between rising levels of ferritin and the rate of SVR may be related to RBV-induced hemolysis and/or RBV concentration. Ferrara et al. [18] considered that the rise of serum ferritin correlated to RBV-induced hemolysis at earlier phases of treatment, but the correlation between serum ferritin levels and hemolysis is lost at later phases of treatment. Therefore, they considered that elevated serum ferritin at a later phase might be caused by a reactive response from activated macrophages to interferon [18]. Their hypothesis that elevation of serum ferritin level during the PEG-IFN and RBV combination therapy might be caused not only by RBV-induced hemolysis but also by IFN is quite reasonable, because elevation of serum ferritin levels is also observed in PEG-IFNα-2a monotherapy [13]. Further studies are necessary to confirm these hypotheses.

In conclusion, serum ferritin levels increase during PEG-IFN and RBV combination therapy; however, this did not correlate with either serum ALT levels or the total dose of RBV administered. Higher serum ferritin levels during combination therapy appeared to be associated with favorable therapeutic response.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.

2 Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.

3 Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46:403–410.

4 Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Kobayashi M, Arase Y, Ikeda K, Kumada H: Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48:372–380.

5 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Izumi N, Marumo F, Sato C: Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b: sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96:224–230.

6 Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C: Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996;334:77–81.

7 Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, Tanaka E, Onji M, Toyota J, Chayama K, Yoshioka K, Izumi N, Akuta N, Kumada H: Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol 2009;44:952–963.

8 Kew MC: Hepatic iron overload and hepatocellular carcinoma. Cancer Lett 2008. DOI: 10.1016/j.canlet.2008.11.001.

9 Furutani T, Hino K, Okuda M, Gondo T, Nishina S, Kitase A, Korenaga M, Xiao SY, Weinman SA, Lemon SM, Sakaida I, Okita K: Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. Gastroenterology 2006;130:2087–2098.

10 Akiyoshi F, Sata M, Uchimura Y, Suzuki H, Tanikawa K: Hepatic iron stainings in chronic hepatitis C patients with low HCV RNA levels: a predictive marker for IFN therapy. Am J Gastroenterol 1997;92:1463–1466.

11 Arber N, Moshkowitz M, Konikoff F, Halpern Z, Hallak A, Santo M, Tiomny E, Baratz M, Gilat T: Elevated serum iron predicts poor response to interferon treatment in patients with chronic HCV infection. Dig Dis Sci 1995;40:2431–2433.

12 Fujita N, Sugimoto R, Urawa N, Araki J, Mifuji R, Yamamoto M, Horiike S, Tanaka H, Iwasa M, Kobayashi Y, Adachi Y, Kaito M: Hepatic iron accumulation is associated with disease progression and resistance to interferon/ribavirin combination therapy in chronic hepatitis C. J Gastroenterol Hepatol 2007;22:1886–1893.

13 Nagashima M, Kudo M, Chung H, Ishikawa E, Inoue T, Nakatani T, Dote K: Elevated serum ALT levels during pegylated interferon monotherapy may be caused by hepatic iron overload. Intervirology 2008;51(suppl 1):76–85.

14 Lau JY, Tam RC, Liang TJ, Hong Z: Mechanism of action of ribavirin in the combination treatment of chronic HCV infection. Hepatology 2002;35:1002–1009.

15 Abonyi ME, Lakatos PL: Ribavirin in the treatment of hepatitis C. Anticancer Res 2005;25:1315–1320.

16 Bodenheimer HC Jr , Lindsay KL, Davis GL, Lewis JH, Thung SN, Seeff LB: Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. Hepatology 1997;26:473–477.

17 Dusheiko G, Main J, Thomas H, Reichard O, Lee C, Dhillon A, Rassam S, Fryden A, Reesink H, Bassendine M, Norkrans G, Cuypers T, Lelie N, Telfer P, Watson J, Weegink C, Sillikens P, Weiland O: Ribavirin treatment for patients with chronic hepatitis C: results of a placebo-controlled study. J Hepatol 1996;25:591–598.

18 Ferrara F, Ventura P, Vegetti A, Guido M, Abbati G, Corradini E, Fattovich G, Ferrari C, Tagliazucchi M, Carbonieri A, Orlandini A, Fagiuoli S, Boninsegna S, Minola E, Rizzo G, Belussi F, Felder M, Massari M, Pozzato G, Bonetto S, Rovere P, Sardini C, Borghi A, Zeneroli ML, Toniutto P, Rossi E, Pietrangelo A: Serum ferritin as a predictor of treatment outcome in patients with chronic hepatitis C. Am J Gastroenterol 2009;104:605–616.

19 McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK: Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002;123:1061–1069.

Original Article

Intervirology

Intervirology 2010;53:76–81
DOI: 10.1159/000252789

Published online: January 5, 2010

Non-Invasive Evaluation of Hepatic Fibrosis for Type C Chronic Hepatitis

Chie Tatsumi[a] Masatoshi Kudo[a] Kazuomi Ueshima[a] Satoshi Kitai[a] Emi Ishikawa[a] Norihisa Yada[a] Satoru Hagiwara[a] Tatsuo Inoue[a] Yasunori Minami[a] Hobyung Chung[a] Kiyoshi Maekawa[b] Kenji Fujimoto[c] Michio Kato[c] Akiko Tonomura[d] Tsuyoshi Mitake[d] Tsuyoshi Shiina[e]

[a]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, and [b]Division of Abdominal Ultrasound, Department of Laboratory Medicine, Kinki University School of Medicine, Osaka-Sayama, [c]Department of Internal Medicine, National Hospital Organization Minami-Wakayama Medical Center, Tanabe, [d]Hitachi Medical Corporation, Tokyo, and [e]Human Health Science, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Key Words
Biopsy, liver · FibroScan · Fibrosis, liver · Real-time tissue elastography

Abstract
Objective: The aim of this study was to investigate liver fibrosis using non-invasive Real-time Tissue Elastography® (RTE) and transient elastography (FibroScan®) methods. ***Methods:*** RTE, FibroScan and percutaneous liver biopsy were all performed on patients with chronic liver disease, particularly hepatitis C, to investigate liver fibrosis. ***Results:*** FibroScan and RTE were compared for fibrous liver staging (F stage), which was pathologically classified using liver biopsy. In FibroScan, significant differences were observed between F1/F3 and F2/F4, but no such differences were observed between F1/F2, F2/F3 and F3/F4. In RTE, significant differences were observed between F1/F2, F2/F3 and F2/F4. But for F3/F4, no significant differences were observed. ***Conclusion:*** FibroScan and RTE correlated well with F staging of the liver. In particular RTE was more successful than FibroScan in diagnosing the degree of liver fibrosis.

Introduction

Currently, percutaneous liver biopsy is considered to be the gold standard for determining the index of the liver fibrosis in a patient with chronic liver disease including hepatitis type C. However, liver biopsy is associated with risks of complications, lacks accuracy due to sampling error, and is physically and psychologically uncomfortable for the patient. Transient elastography (FibroScan®) and Real-Time Tissue Elastography® (RTE) have been developed as non-invasive methods to evaluate the degree of liver fibrosis, potentially providing alternatives to liver biopsy. FibroScan detects the propagation speed of a shear wave transmitted from a probe through the liver and calculates the shear modulus of the liver to evaluate the degree of liver fibrosis [1–3]. On the other hand, RTE visualizes a 2-dimensional strain image induced by external freehand compression with the probe or by internal heartbeats. To evaluate the degree of liver fibrosis, it is reported that the pattern of strain image induced by compression becomes patchy as fibrosis progresses [4]. To increase objectivity,

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0300–5526/10/0531–0076$26.00/0

Accessible online at:
www.karger.com/int

Masatoshi Kudo, MD, PhD, Division of Gastroenterology and Hepatology
Department of Internal Medicine, Kinki University School of Medicine
377-2, Ohno-Higashi, Osaka-Sayama 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3149, Fax +81 72 367 2880
E-Mail m-kudo@med.kindai.ac.jp

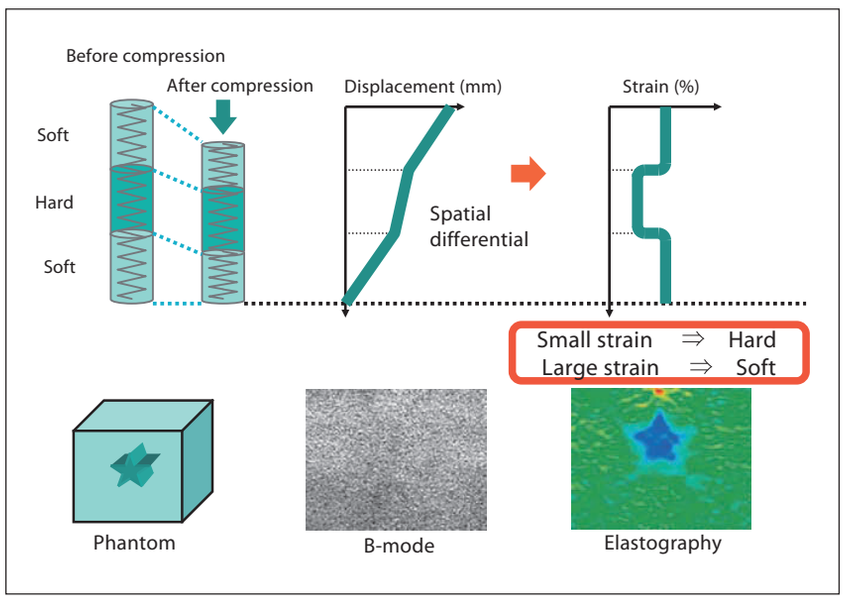


Fig. 1. Principle of elasticity imaging.

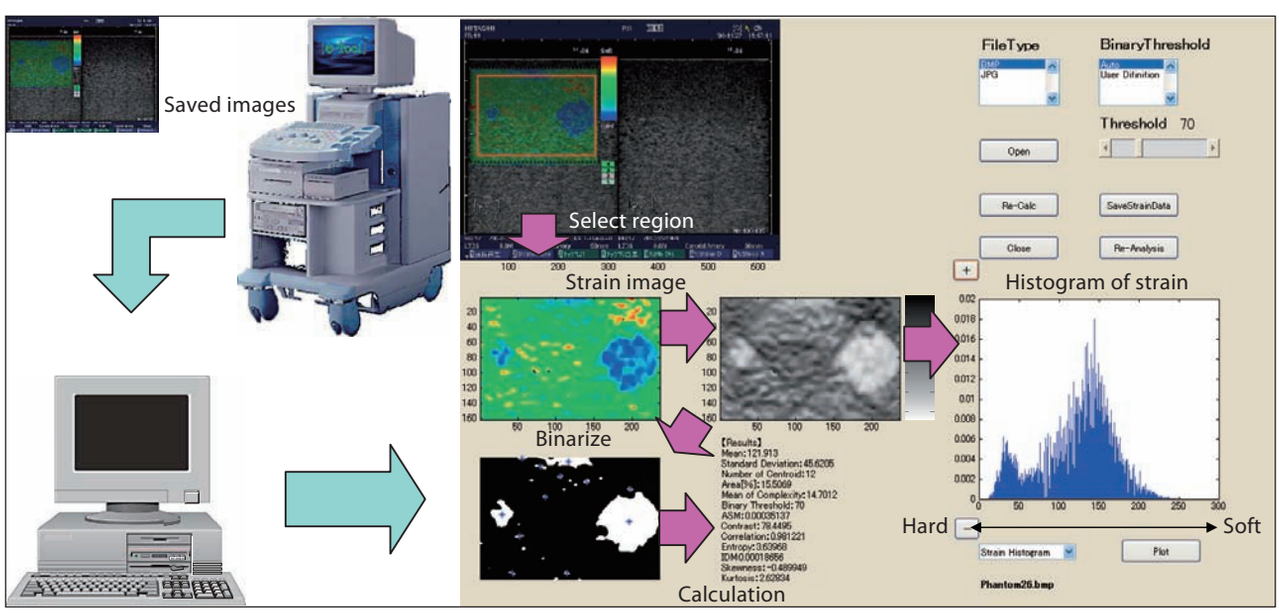


Fig. 2. Analysis tool. Mean = Mean of relative strain value; SD = standard deviation of relative strain value; area[%] = ratio of blue area in the analyzed region; complexity = complexity of blue area [calculated as (boundary length)^2/area].

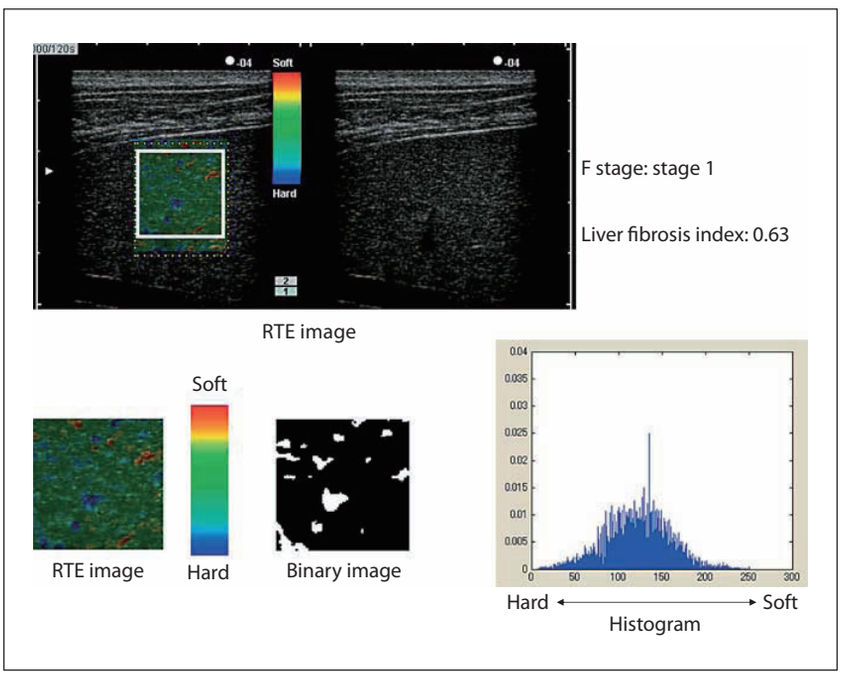


Fig. 3. Example of liver RTE image and result of liver fibrosis index (F stage 1).

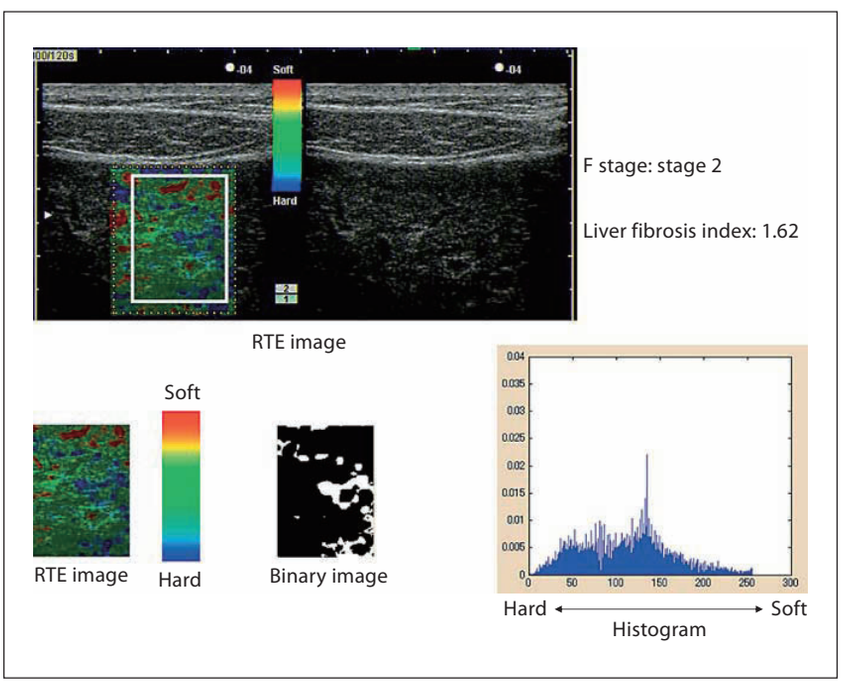


Fig. 4. Example of liver RTE image and result of liver fibrosis index (F stage 2).

we proposed an image analysis method to evaluate strain image features [5]. In this paper, we propose a new algorithm for RTE to deliver an index which corresponds to the liver fibrosis stage (F stage), and report our clinical experience.

Methods

Before IFN treatment, percutaneous liver biopsy, FibroScan and RTE examinations were performed on 44 patients with chronic hepatitis C. All patients gave their consent for this study. Of these 44 patients, 12 had F1, 9 had F2, 10 had F3, and 13 had F4, all diagnosed pathologically using percutaneous liver biopsy. FibroScan and RTE were performed before the liver biopsy for comparison with the F stage of the liver biopsy specimen. Measurements with FibroScan (EchoSens, Paris, France) were performed on the right lobe of the liver through intercostal spaces. The mean of 10 valid measurements was used as the index of liver stiffness. RTE was performed with EUB-8500 (Hitachi Medical Corp., Tokyo, Japan) through the right intercostal spaces to obtain the elastography images of the liver.

RTE displays elastic information of the tissue calculated from the tissue displacement. The tissue displacement is caused either by manual compression and relaxation of the probe or by the internal compression and relaxation with the heart. The principles underlying RTE are shown in figure 1 using a spring model with the hard and soft springs connected in series. When the spring is compressed, a hard spring is displaced to a lesser extent than a soft spring, thus the strain calculated from the displacement is small in hard springs and large in soft springs. RTE visualizes the relative strain using color gradations, similar to ultrasound color Doppler imaging. RTE is being clinically used and studied in various regions, such as breast, [6], thyroid gland, and prostate [7]. As discussed in the previous study, the RTE image shows a patchy pattern of colors as liver fibrosis progresses from hepatitis to cirrhosis [4, 5]. This is because the fibrotic region is harder than the normal liver parenchyma and does not spread uniformly. For evaluation of liver, the strain induced by heartbeats (diastole) is used to perform RTE to reduce interobserver variability by compressing with an external probe. Six RTE images were collected for each patient and analyzed with the prototype analysis software shown in figure 2 to calculate 9 image features: mean of relative strain value; standard deviation of relative strain value; ratio of blue area in the analyzed region; complexity of blue area; kurtosis of strain histogram; skewness of strain histogram; entropy; inverse difference moment, and angular second moment. Multiple regression analysis was then performed with these 9 image features to quantify the index of liver fibrosis. Examples of liver RTE images and index of liver fibrosis results are shown in figures 3–6. As can be seen from these figures, as F stage progresses, the liver fibrosis index increases.

Results

FibroScan and RTE findings were compared against pathologically classified F-staged patients using liver biopsy. In FibroScan, significant differences were observed between F1/F3 and F2/F4, but no significant differences were observed between F1/F2, F2/F4 and F3/F4 (fig. 7).

In RTE, significant differences were observed between F1/F2, F2/F3 and F2/F3, but no significant differences were recognized between F3/F4 (fig. 8).

Discussion

The percutaneous liver biopsy is most reliable but it is invasive and cannot be performed frequently to study the progress of fibrosis. Thus non-invasive techniques, such as FibroScan and RTE, are more desirable.

FibroScan is simple and easy to use and displays the results on a monitor immediately. The result is based on 1-dimensional information (1-line) only. In the case of RTE, it visualizes 2-dimensional elastic information in real time and can be used in most patients.

FibroScan has many limitations. It cannot be used in patients with ascites, thick subcutaneous fat, narrow intercostal spaces, and hepatic atrophy. RTE does not have such limitations and it can be used in almost all patients, including those with the conditions mentioned above. However, RTE requires training to scan patients to obtain reproducible images and to analyze the data. To address these issues in RTE, we are investigating easy-to-use acquisition techniques to reduce interobserver variability and also to simplify RTE image acquisition.

FibroScan and RTE both correlate highly with the F staging of the liver using biopsy. In particular RTE is very useful for the differential diagnosis and staging of the liver fibrosis.

In future, we plan to study more cases to evaluate FibroScan and RTE for liver fibrosis staging and to establish guidelines to minimize unnecessary liver biopsies, which will significantly benefit patients with chronic liver diseases.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

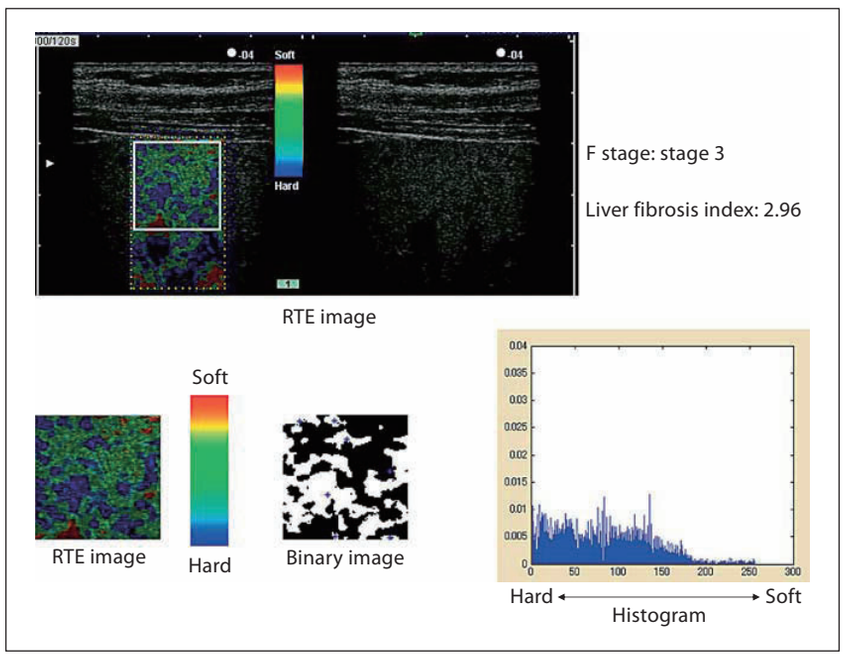


Fig. 5. Example of liver RTE image and result of liver fibrosis index (F stage 3).

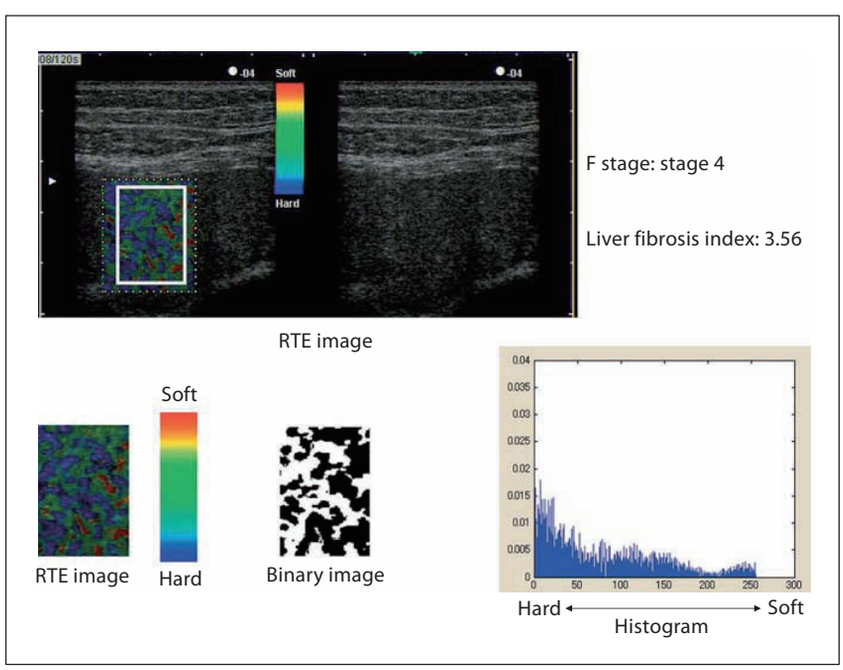


Fig. 6. Example of liver RTE image and result of liver fibrosis index (F stage 4).

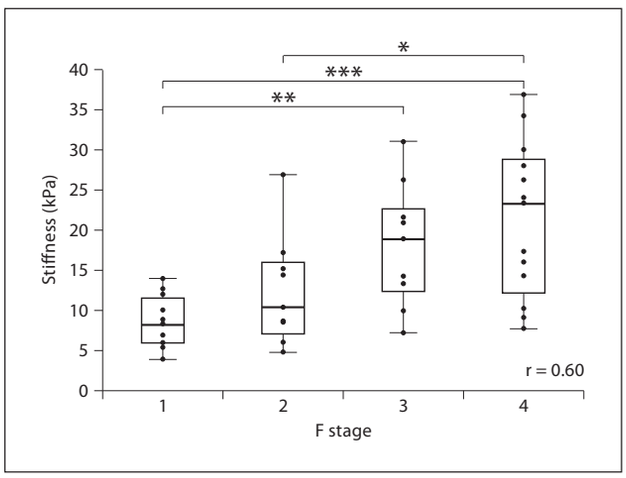


Fig. 7. FibroScan and F stage. * $p < 0.05$; ** $p < 0.005$; *** $p < 0.001$.

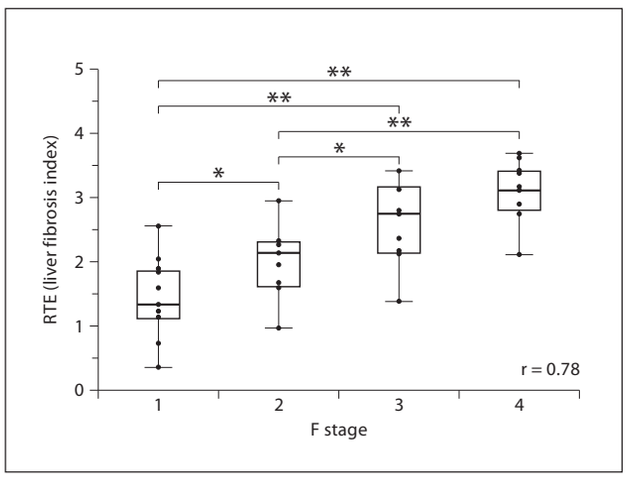


Fig. 8. RTE (liver fibrosis index) and F stage. * $p < 0.05$; ** $p < 0.001$.

References

1 Sandrin L, Fourquet B, Hasquenoph JM, et al: Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705–1713.

2 Foucher J, Chanteloup E, Vergniol J, et al: Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 2006;55:403–408.

3 Fraquelli M, Rigamonti C, Casazza G, et al: Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. Gut 2007;56:968–973.

4 Fujimoto K, Wada S, Oshita M, et al: Non-invasive evaluation of hepatic fibrosis in patients with chronic hepatitis C using elastography. Medix Suppl 2007;24–27.

5 Tatsumi C, Kudo M, Ueshima K, et al: Non-invasive evaluation of hepatic fibrosis using serum fibrotic markers, transient elastography (FibroScan) and real-time tissue elastography. Intervirology 2008;51(suppl 1):27–33.

6 Itoh A, Ueno E, Tohno E, et al: Breast disease: clinical application of US elastography for diagnosis. Radiology 2006;239:341–350.

7 Tsutsumi M, Miyagawa T, Matsumura T, et al: The impact of real-time tissue elasticity imaging (elastography) on the detection of prostate cancer: clinicopathological analysis. Int J Clin Oncol 2007;12:250–255.

EUS-guided in vivo microdialysis of the pancreas: a novel technique with potential diagnostic and therapeutic application

Masayuki Kitano, MD, Hiroki Sakamoto, MD, Kshaunish Das, MD, Takamitsu Komaki, MD, Masatoshi Kudo, MD

Osaka-sayama, Japan

Background: Microdialysis has been used in vivo to measure dynamic temporal variations in extracellular or interstitial concentrations of non-protein–bound substances that are unstable in the systemic circulation.

Objective: To evaluate the technical feasibility and possible complications of EUS-guided in vivo microdialysis of the pancreas.

Design and Intervention: Under the guidance of an echoendoscope inserted into the stomach of each dog, the pancreatic parenchyma was punctured by using a 19-gauge needle. A specially developed microdialysis probe threaded through the lumen of the 19-gauge needle was positioned in the pancreas. The probe was constantly perfused with saline solution at a flow rate of 1.0 μL/minute.

Setting: Experiments on 8 beagle dogs.

Main Outcome Measurements: The concentration of 5-fluorouracil (5-FU) in the microdialysate was measured at 10-minute intervals, once before and for 8 times after a single (20 mg/kg) bolus intravenous infusion of 5-FU.

Results: Following the administration of 5-FU, the concentration of 5-FU in all macrodialysate samples exceeded the cut-off value by more than 100-fold. The 5-FU levels in the microdialysate increased rapidly, peaked by 10 minutes (13.9 μg/mL), and gradually declined thereafter. No local bleeding or accumulation of fluid around the pancreas was observed.

Limitation: Sampling was unsuccessful in 2 of the 8 dogs because the probe broke while being inserted into the pancreatic parenchyma.

Conclusion: EUS-guided pancreatic microdialysis is feasible and has multiple potential clinical/therapeutic applications, including monitoring pharmacokinetics focally and detecting novel biomarkers that are unstable or undetectable in the plasma.

Since its first preclinical application,[1] in vivo microdialysis using a probe with a semipermeable membrane has been used to measure and monitor the levels of neuromodulators and neurotransmitters in the nervous system and the GI tract and to study the local tissue pharmacokinetics of chemotherapeutic agents.[2-10] The advantages of microdialysis are that it can be used in vivo in conscious or unconscious animals or humans to measure dynamic temporal variations in the extracellular or interstitial concentrations of unstable, low-molecular-weight (<20-50 kDa), non-protein–bound substances present in the tissues (referred to as analytes).[2]

Although chemotherapy with gemcitabine or S-1 (containing a prodrug of 5-fluorouracil [5-FU]) has been used recently for inoperable pancreatic cancers,[11-13] the efficacy and toxicity of these drugs vary significantly among patients. Variation in the activity of the relevant catabolic enzyme at the tissue level may explain resistance to these drugs.[14-16] However, the local concentrations of drugs in pancreatic cancers in vivo have not yet been studied. Because of its deep location, the pancreas is poorly accessible for routine in vivo tissue analysis compared with other organs. EUS-FNA has been particularly useful for diagnosing pancreatic diseases, including pancreatic cancer.[17] We performed a preclinical study with a large mammal model (beagle dogs) to determine the technical feasibility and possible complications of in vivo pancreatic microdialysis when using a specially

Abbreviations: 5-FU, 5-fluorouracil; GC-MS, gas chromatography-mass spectrometry.

DISCLOSURE: *All authors disclosed no financial relationships relevant to this publication. The microdialysis probe was made for this study by Eicom Co. Ltd. according to our specifications, but there was no financial relationship. The microdialysis probe and the microinfusion pump are commercially available. This study was supported by grants from the Japan Society for Promotion of Science, the Japan Research Foundation for Clinical Pharmacology, and the Japanese Foundation for Research and Promotion of Endoscopy.*

0016-5107/$36.00
doi:10.1016/j.gie.2009.05.040

developed microdialysis probe placed in the pancreatic parenchyma through an EUS-FNA needle, thereby delineating the tissue pharmacokinetic profile of a chemotherapeutic agent.

MATERIALS AND METHODS

Microdialysis

Microdialysis can monitor the concentration of any low-molecular-weight substance around the tip of the probe after it is implanted into the tissue (Fig. 1). The low-molecular-weight substance (the analyte) diffuses through the semipermeable membrane at the tip of the microdialysis probe, but no catabolic enzymes pass the membrane (Fig. 1). We used a new microdialysis probe (Eicom Co Ltd, Kyoto, Japan; membrane length, 3 mm; whole length, 2 m; outer diameter, 0.22 mm; molecular cutoff, 50 kDa) that can be inserted via the lumen of a 19-gauge needle (Echo Tip, Cook Endoscopy Co Ltd, Winston-Salem, NC) conventionally used for EUS-FNA (Fig. 2A).

Implantation of the microdialysis probe

Eight male beagle dogs (mean weight 10.8 ± 1.3 kg) were used. Under anesthesia, the dogs were mechanically ventilated. An echoendoscope was then inserted into the dog's stomach, and the pancreas was observed by EUS from the distal part of the gastric lumen. The microdialysis probe was threaded through the lumen of the 19-gauge puncture needle. With the scanning plane of the echoendoscope displaying the pancreas, the 19-gauge needle was inserted into the pancreatic parenchyma after puncturing the gastric wall (Fig. 2B). We allowed the microdialysis probe tip to protrude by approximately 5 mm by retracting only the needle. The inlet tube was connected to a microinfusion pump (Microdialysis pump/CMA/102, CMA Microdialysis AB, Stockholm, Sweden), and the probe was then continually perfused with saline solution at a flow rate of 1.0 μL/minute via the inlet tube. The dialysate in the outlet tube was allowed to drain into 1-mL, polypropylene vials.

Sampling of the microdialysate and evaluation of pharmacokinetics after 5-FU administration

After an equilibration period (10 minutes), the first microdialysate sample was collected for 10 minutes, and a blood sample was obtained. Immediately after collection of these first samples, 5-FU (Kyowa Kirin Co Ltd, Tokyo, Japan) was administered as a single intravenous bolus at a dose of 20 mg/kg. Thereafter, microdialysate samples were collected every 10 minutes until 80 minutes after 5-FU administration, whereas blood was drawn at 1, 10, 30, and 60 minutes after 5-FU administration. The 5-FU concentrations in the microdialysate and plasma samples were measured by gas chromatography-mass spectrometry (GC-MS). The detection limit was 10 ng/mL for a 5-μL sample.[18,19]

Capsule Summary

What is already known on this topic

- Microdialysis can be used in vivo in conscious or unconscious patients to measure dynamic temporal variations in the extracellular or interstitial concentrations of unstable non-protein–bound substances in tissue.

What this study adds to our knowledge

- EUS-guided pancreatic microdialysis was successful in 6 of 8 dogs with concentrations of 5-fluorouracil increasing rapidly, reaching a peak in 10 minutes, and declining gradually within 60 minutes.

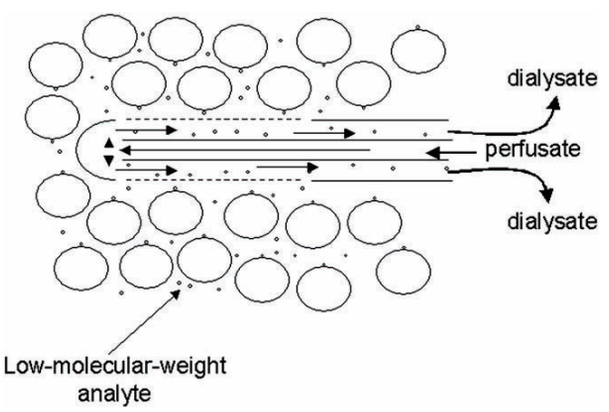


Figure 1. Principle of microdialysis. The semipermeable membrane at the tip of the probe allows diffusion of low-molecular-weight substances (*open black dots*) into the dialysate. The latter is collected in aliquots for analysis.

RESULTS

Microdialysis was unsuccessful in 2 of the 8 dogs because the probe broke during insertion into the pancreatic parenchyma. Therefore, the 5-FU concentrations in the microdialysate and plasma samples from 6 dogs were measured. 5-FU could not be detected in the microdialysate samples taken immediately prior to the administration of 5-FU (Fig. 3A). However, after the intravenous administration of 5-FU, the microdialysate concentrations of 5-FU increased rapidly, reaching a peak by the first 10-minute sampling time. The peak was followed by a gradual decline (Fig. 3A). Additionally, 5-FU was undetectable in the plasma before administration of the drug, but plasma 5-FU levels peaked immediately after injection. The plasma levels then gradually declined in a manner similar to that of the 5-FU levels in the microdialysates (Fig. 3B).

After collection of the microdialysis samples, we opened the abdomens of the dogs to check for any complications associated with the EUS-guided microdialysis.

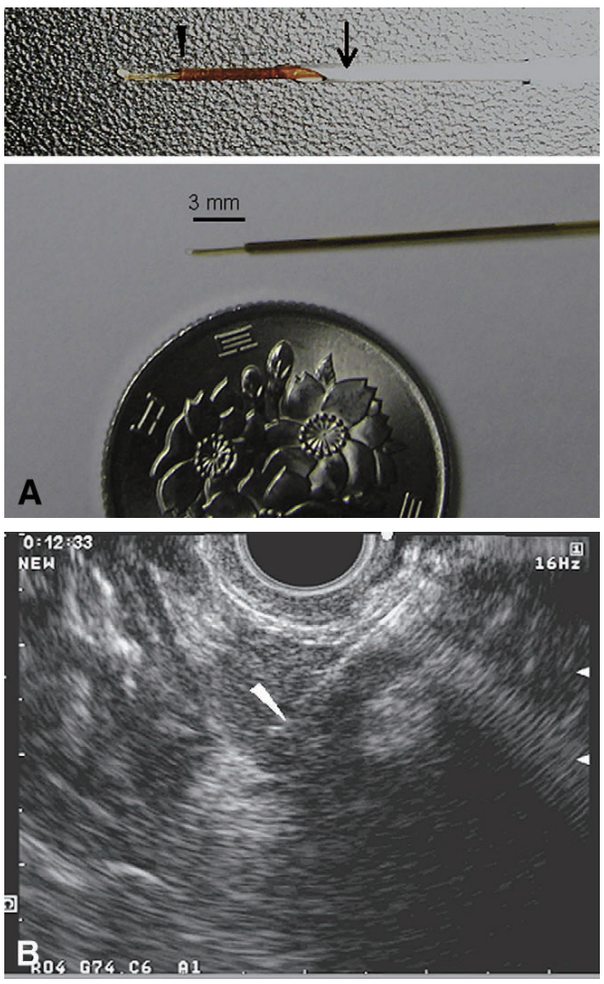


Figure 2. A, Microdialysis probe (*arrowhead*) exposed at the tip of a 19-gauge EUS-FNA needle (*arrow*). **B,** Implantation of the microdialysis probe (*arrowhead*) under EUS guidance into the pancreas of a dog.

No local bleeding or abnormal accumulation of fluid was observed (Fig. 4).

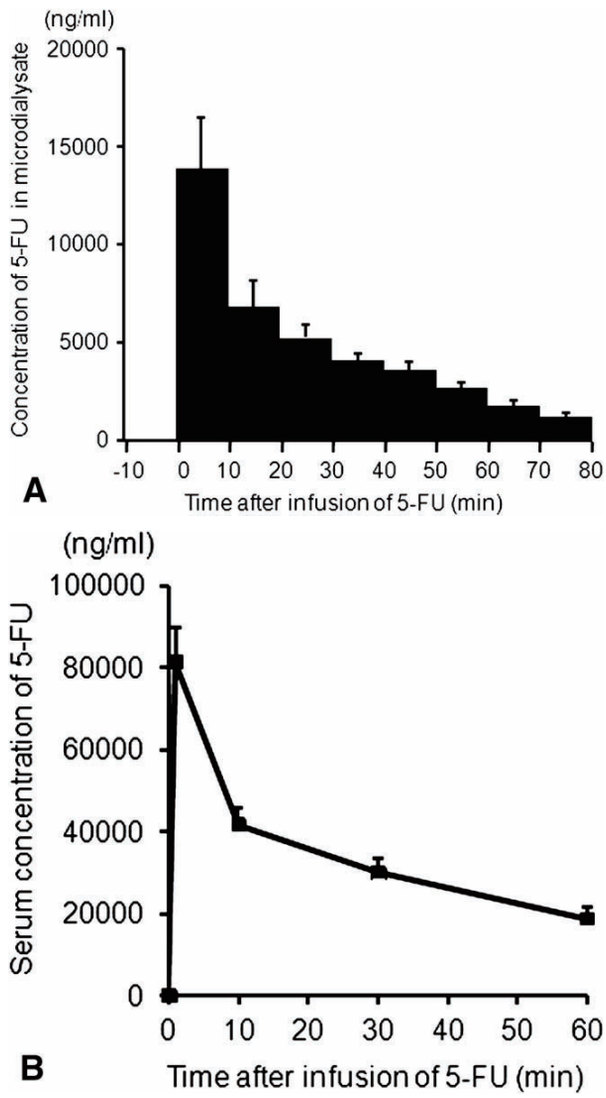


Figure 3. The concentration of 5-fluorouracil in the microdialysate (**A**) and the serum (**B**) over time after the infusion of 20 mg/kg 5-fluorouracil. The data are expressed as means ± standard error of the mean.

DISCUSSION

By 10 minutes after its administration, the level of 5-FU in the microdialysate had increased, then it declined gradually over the next hour. Thus, the interstitial concentrations of 5-FU in the pancreas followed a time course similar to that seen in the plasma. The 5-FU level in the microdialysate was very high (13.9 μg/mL) within 10 minutes after it was delivered intravenously. Because GC-MS has a detection limit of 10 ng/mL, the concentration of 5-FU could be measured every minute or at even shorter intervals within the first 10 minutes after drug administration. Although the microdialysate does not reflect the concentration of the analyte in the whole tissue but only in the interstitial fluid, this technique may thus be useful for estimating drug delivery to the target organ. Studies on microdialysis in breast cancer and melanoma patients have shown that concentrations of chemotherapeutic drugs in the tumor may correlate with clinical response to treatment, suggesting that this method may be more accurate and useful than measuring serum concentrations.[5-7] When pancreatic microdialysis becomes available for clinical purposes, it will be of great interest to investigate how the local concentration of 5-FU in pancreatic carcinomas changes over time. In animal experiments, in vivo microdialysis was also used for evaluating the interstitial concentration of islet hormones and vascular permeability to proteins in the pancreas.[20,21] This raises the possibility of discovering new local biomarkers for pancreatic diseases. Moreover, this technique can be used in other organs in which needle puncturing is possible, such as the

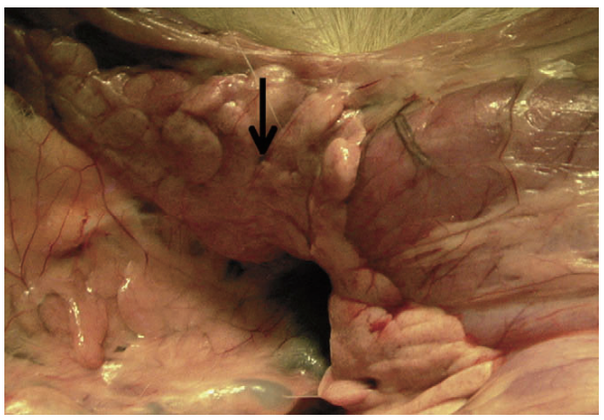


Figure 4. The pancreas of a dog after removal of the microdialysis probe. No bleeding was detected around the puncture (*arrowhead*).

stomach. Thus, EUS-guided microdialysis may have multiple potential uses in clinical pharmacology and oncology.

Because the inner volume of the perfusate fluid in the outlet tube (2 m) may cause a substantial time delay in sample collection, a fast perfusion rate is desirable. However, a flow rate of 2 μL/minute or more resulted in the leakage of the perfusate out of the membrane. Therefore, we used a flow rate of 1.0 μL/minute. The in vitro recovery rate of 5-FU by the microdialysis probe was 21% when the flow rate was 1.0 μL/minute. The interstitial concentration of 5-FU surrounding the tip of the probe can be estimated from that in the microdialysate with the in vitro recovery rate of the probe.

Microdialysis, being an invasive technique that involves probe implantation, could set off an acute inflammatory response that may influence drug level measurement and interpretation.[4] In addition, the microdialysis probe used in the present study has an exposed tip and uses a glass fiber in the perfusion route. These parts are fragile and can be broken when the probe is implanted in the tissue. Indeed, we could not collect microdialysates from 2 of the 8 dogs because the probe broke during implantation. Thus, for clinical use of this technique, it will be necessary to develop less fragile probes that can be combined with a needle. These probes could possibly be made with a smaller-gauge needle. Many EUS examinations are currently being conducted with smaller-gauge needles (25 or 22 gauge). Development of smaller gauge needles combined with in vivo microdialysis (in vivo microdialysis needle) would be useful for clinical application of this technology.

REFERENCES

1. Delgado JM, DeFeuds FU, Roth RH, et al. Dialytrode for long term intracerebral perfusion in awake monkeys. Arch Int Pharmacodyn Ther 1972;198:9-21.
2. Müller M. Science, medicine, and the future: Microdialysis. BMJ 2002;324:588-91.
3. Lee GJ, Park HJ, Park HK. Microdialysis applications in neuroscience. Neurol Res 2008;30:661-8.
4. Zhou Q, Gallo JM. In vivo microdialysis for PK and PD studies of anticancer drugs. AAPS J 2005;7:E659-67.
5. Müller M, Mader RM, Steiner B, et al. 5-fluorouracil kinetics in the interstitial tumor space: clinical response in breast cancer patients. Cancer Res 1997;57:2598-601.
6. Mader RM, Schrolnberger C, Rizovski B, et al. Penetration of capecitabine and its metabolites into malignant and healthy tissues of patients with advanced breast cancer. Br J Cancer 2003;88:782-7.
7. Joukhadar C, Klein N, Mader RM, et al. Penetration of dacarbazine and its active metabolite 5-aminoimidazole-4-carboxamide into cutaneous metastases of human malignant melanoma. Cancer 2001;92:2190-6.
8. Schmidt S, Banks R, Kumar V, et al. Clinical microdialysis in skin and soft tissues: an update. J Clin. Pharmacol 2008;48:351-64.
9. Kitano M, Norlén P, Håkanson R. Gastric submucosal microdialysis: a method to study gastrin- and food-evoked mobilization of ECL cell histamine in conscious rats. Regul Pept 2000;86:113-23.
10. Deeba S, Corcoles EP, Hanna BG, et al. Use of rapid sampling microdialysis for intraoperative monitoring of bowel ischemia. Dis Colon Rec 2008;51:1408-13.
11. Gan SI, Rajan E, Adler DG, et al. Role of EUS. Gastrointest Endosc 2007;66:425-34.
12. Burris HA III, Moore MJ, Andersen J, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. J Clin Oncol 1997;15:455-65.
13. Ueno H, Okusaka T, Ikeda M, et al. A phase I study of combination chemotherapy with gemcitabine and oral S-1 for advanced pancreatic cancer. Oncology 2005;69:421-7.
14. Nakamura K, Yamaguchi T, Ishihara T, et al. Phase II trial of oral S-1 combined with gemcitabine in metastatic pancreatic cancer. Br J Cancer 2006;94:1575-9.
15. Tatsumi K, Fukushima M, Shirasaka T, et al. Inhibitory effects of pyrimidine, barbituric acid and pyrimidine derivatives on 5-fluorouracil degradation in rat liver extracts. Jpn J Cancer 1987;78:748-55.
16. Takechi T, Fujioka A, Matsushima E, et al. Enhancement of the antitumour activity of 5-fluorouracil (5-FU) by inhibiting dihydropyrimidine dehydrogenase activity (DPD) using 5-chloro-2,4-dihydroxypyrimidine (CDHP) in human tumour cells. Eur J Cancer 2002;38:1271-7.
17. Shirasaka T, Shimamoto Y, Fukushima M, et al. Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumour activity in rats. Cancer Res 1993;53:4004-9.
18. Marunaka T, Umeno Y. Gas chromatographic-mass fragmentographic determination of 5-fluorouracil as a metabolic of new 5-fluorouracil derivatives. Chem Pharm Bull 1982;30:1868-71.
19. Odagiri H, Ichihara S, Semura E, et al. Determination of 5-fluorouracil in plasma and liver after oral administration of 5′-deoxy-5-fluorouridine using gas chromatography-mass spectrometry. J Pharmacobiodyn 1988;11:234-40.
20. Nakagawa A, Samols E, Stagner JI. Exocrine interstitial insulin and somatostatin in the perfused dog pancreas. Am J Physiol (Gastrointest Liver Physiol) 1993;264:G728–GG34.
21. Meriläinen S, Mäkelä J, Anttila V, et al. Acute edematous and necrotic pancreatitis in a porcine model. Scand J Gastroenterol 2008;43:1259-68.

Received February 11, 2009. Accepted May 29, 2009.

Current affiliations: Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, Osaka-Sayama, Japan.

Reprint requests: Masayuki Kitano, MD, PhD, Divison of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-sayama 589-8511, Japan.

If you would like to chat with an author of this article, you may contact Dr. Kitano at m-kitano@med.kindai.ac.jp.

Overall Survival After Transarterial Lipiodol Infusion Chemotherapy With or Without Embolization for Unresectable Hepatocellular Carcinoma: Propensity Score Analysis

Kenichi Takayasu[1]
Shigeki Arii[2]
Iwao Ikai[3]
Masatoshi Kudo[4]
Yutaka Matsuyama[5]
Masamichi Kojiro[6]
Masatoshi Makuuchi[7]
for the Liver Cancer Study Group of Japan

OBJECTIVE. Although iodized oil transarterial chemoembolization (TACE) has been found to have survival benefit in the care of patients with unresectable hepatocellular carcinoma, iodized oil infusion chemotherapy without embolization has not been clearly found inferior to or equal to TACE. The purpose of this study was to determine whether one of these therapies is superior to the other or the two are equal in survival benefit and whether embolization with gelatin sponge particles is indispensable to prolonging survival.

SUBJECTS AND METHODS. A prospective nonrandomized observational cohort study was conducted over 8 years. Among 11,030 patients with unresectable hepatocellular carcinoma, 8,507 underwent TACE, and 2,523 underwent transarterial infusion therapy with an emulsion of iodized oil and an anticancer agent as initial treatment. Patients with extrahepatic metastasis or any previous treatment were excluded. The primary end point was all-cause mortality. To minimize selection bias, propensity score analysis was used to compare the two groups.

RESULTS. During the follow-up period, 5,044 patients (46%) died. In the analysis of all patients, TACE was associated with a significantly higher survival rate than infusion therapy without embolization (hazard ratio, 0.60; 95% CI, 0.56–0.64; $p = 0.0001$). The propensity score analysis showed that the hazard ratio for death in the TACE group ($n = 1,699$ patients) compared with the group who underwent infusion therapy without embolization ($n = 1,699$) was 0.70 (95% CI, 0.63–0.76; $p = 0.0001$). The median survival time of the TACE group was 2.74 years, and the 1-, 3-, and 5-year survival rates were 81%, 46%, and 25%. The corresponding values for the group who underwent transarterial infusion therapy without embolization were 1.98 years and 71%, 33%, and 16%.

CONCLUSION. Propensity score analysis showed that in the treatment of patients with unresectable hepatocellular carcinoma, TACE was associated with significantly better overall survival rates than was transarterial infusion therapy without embolization. TACE can be recommended as initial treatment of these patients.

Keywords: chemotherapy, hepatocellular carcinoma, iodized oil infusion, propensity analysis, transarterial chemoembolization

DOI:10.2214/AJR.09.3308

Received July 8, 2009; accepted after revision August 24, 2009.

[1]Department of Diagnostic Radiology, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Address correspondence to K. Takayasu.

[2]Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Graduate School of Medicine, Tokyo, Japan.

[3]Department of Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

[4]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan.

[5]Department of Biostatistics, School of Health Sciences and Nursing University of Tokyo, Tokyo, Japan.

[6]Department of Pathology, Kurume University School of Medicine, Kurume, Japan.

[7]Department of Surgery, Japanese Red Cross Medical Center, Tokyo, Japan.

AJR 2010; 194:830–837

0361–803X/10/1943–830

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer and the third most common cause of cancer mortality in the world [1]. The incidence of HCC is increasing in Japan [2], the United States [3], and other Western countries [4]. However, the number of patients who can undergo curative therapy such as resection, transplantation, and percutaneous ablation remains low. A 2005 report by the Liver Cancer Study Group of Japan showed transarterial chemotherapy, including transarterial chemoembolization with iodized oil and gelatin sponge particles (TACE) and transarterial iodized oil infusion chemotherapy without embolization, accounted for the initial treatment of 36.4% of 16,941 patients with HCC [5].

Randomized controlled trials [6, 7] and meta-analyses [8, 9] have shown that TACE is widely performed and recognized as having survival benefit in the treatment of patients with unresectable HCC accompanied by well-compensated cirrhosis. However, TACE is not always indicated, especially for patients with poor liver function and those with cancer in an advanced stage, because of the risk of hepatic failure and death after treatment [10, 11]. Instead, transarterial infusion therapy with an emulsion of iodized oil and an anticancer agent, also known as lipiodolization [12], has been performed for patients in poor condition [13–19].

A few reports have appeared on comparisons of the survival associated with transarterial iodized oil infusion therapy without

embolization and that associated with TACE, but no consensus has been reached. Two studies [18, 19] showed no significant difference between the two therapies, another study [14] showed infusion without embolization was associated with better survival than was TACE in a subgroup of patients at high risk, and another study [16] showed the reverse. We conducted a prospective nonrandomized observational cohort study to determine whether one of the therapies is superior to the other or whether the therapies are equal in survival benefit. We also evaluated whether gelatin sponge particles are indispensable to prolonging survival.

Subjects and Methods

Patient Characteristics

During the 8 years January 1994–December 2001, the Liver Cancer Study Group of Japan prospectively collected and biannually registered clinicopathologic data on 72,836 patients with primary liver cancer at nearly 800 medical institutions. Data were collected with a registration and questionnaire sheet with more than 180 questions. From that population, 11,030 patients (15.1%) with unresectable HCC were assigned to the current study cohort. Among these patients, 8,507 (77%) underwent TACE and 2,523 (23%) underwent iodized oil transarterial infusion therapy without embolization as initial treatment. These patients did not receive any other therapy during the first investigation period of no more than 2 years. Exclusion criteria were extrahepatic metastasis to lymph nodes and other organs and any previous treatment before the one studied. The 8,507 patients who underwent TACE in the current study were among 8,510 patients who participated in another study [20].

The diagnosis of HCC was based mainly on findings with imaging techniques such as sonography, dynamic CT, MRI, and angiography or on findings at pathologic study of biopsy specimens (4.7%). Abnormal elevation of levels of tumor markers also was found: α-fetoprotein greater than 400 ng/mL (normal, < 20 ng/mL) and des-γ-carboxyl prothrombin more than 100 mAU/mL (normal, < 40 mAU/mL). Typical HCC was visualized as high attenuation or signal intensity in the arterial phase and low attenuation or signal intensity or washout in the delayed phase (≈ 3 minutes after the initiation of contrast injection) of dynamic CT [21, 22] and dynamic MRI and as a hypervascular lesion at hepatic arteriography. Extrahepatic metastatic lesions were routinely examined with sonography, CT, and chest radiography.

The baseline characteristics of the 11,030 patients who underwent TACE (n = 8,507) and transarterial infusion therapy without embolization (n = 2,523) are shown in Table 1. The hepatic functional reserve was evaluated as liver damage in grade A, B, or C in the classification proposed by the Liver Cancer Study Group of Japan in 2000 and published in English in 2003 [23] (Table 2). This classification consists of five clinical and laboratory findings: ascites, serum bilirubin concentration, serum albumin concentration, indocyanine green retention rate at 15 minutes, and prothrombin activity. The severity of each clinical finding is evaluated separately. Degree of liver damage is based on the highest grade that contains at least two findings. This classification is closely related to the Child-Pugh classification and is more precise for discriminating whether patients with Child-Pugh A disease, that is, good candidates for surgical resection, have liver damage grade A or B [5, 24]. Concerning hepatitis B and C virus infection, four groups were categorized: negative result for hepatitis B virus surface antigen and positive result for hepatitis C virus antibody, positive result for hepatitis B virus surface antigen and negative result for hepatitis C virus antibody, positive results for both, and negative results for both. Maximum tumor size had four subgroups, and number of tumors had three subgroups.

Tumor Characteristics

The degree of vascular invasion of the portal vein consisted of the following four categories: Vp0, no invasion; Vp1, invasion to a third-order branch; Vp2, invasion to a second-order or segmental portal vein; and greater than Vp3, first-order portal vein including Vp4, main portal trunk. The degree of hepatic vein invasion was Vv0, no invasion, and greater than Vv1, any hepatic vein invasion, including the main hepatic veins and the inferior vena cava.

The TNM staging adopted in this study was proposed and revised by the Liver Cancer Study Group of Japan in 2000 (Table 3) and published in English in 2003 [23]. This revised TNM system was proposed as a new concordant TNM classification of primary liver cancer by the International Hepato-Pancreato-Biliary Association [25]. Namely, the T category is determined on the basis of the following three criteria: single lesion, tumor diameter 2 cm or less, and no vascular or biliary invasion (Table 3). Category T1 is determined when three criteria are fulfilled; T2, two criteria; T3, one criterion; and T4, no criteria. Stages I–IVA are determined mainly by the corresponding T category from T1 to T4.

Technique

A 5-French catheter was advanced to the superior mesenteric artery to confirm the patency of the portal vein trunk at postmesenteric portography. Common hepatic or celiac arteriography was performed to discern the number and location of lesions, tumor size, feeding artery, and presence of anatomic variation. A coaxial microcatheter (2.7 or 3.0 French) was selectively inserted through a 5-French catheter into the feeding artery as close to the lesion as possible. For multiple foci occupying the hepatic lobes, the right or left or both hepatic arteries were treated. For transarterial infusion therapy without embolization, an emulsion of iodized oil and an anticancer agent dissolved in contrast medium was injected with a three-way stopcock. For TACE, the emulsion was followed by injection of 0.5- to 1-mm-diameter gelatin sponge particles until cessation of blood flow was recognized under radiographic monitoring.

The following anticancer agents, in order of frequency used, were administered mostly as single agents but in some instances as part of multiple-drug therapy: doxorubicin (20–40 mg/m^2), epirubicin (30–60 mg/m^2), analogue of doxorubicin, mitomycin C, cisplatin, or zinostatin stimalamer (4–6 mg/kg body weight) [26]. The common dose of iodized oil was 5 mL/kg body weight (range, 3–10 mL). The entire dose of iodized oil and gelatin sponge particles was based on tumor size and the extent of the tumor. Follow-up consisted of dynamic CT or MRI with measurement of a tumor marker such as α-fetoprotein or des-γ-carboxyl prothrombin every 3–4 months. Therapy was repeated on demand when local recurrence (regrowth of the treated tumor), intrahepatic metastasis, or a second primary HCC was found and the patient would tolerate the therapy.

Statistical Analysis

The survival rates of patients who underwent TACE or transarterial infusion therapy without embolization were calculated from the date of diagnosis of HCC. Follow-up was ended on December 31, 2003. The primary end point was all-cause mortality. For the analysis of the patient characteristics of the TACE and therapy without embolization groups, chi-square or Mantel Trend chi-square tests were used. All-cause mortality was analyzed with univariate and multivariate Cox proportional hazards regression models.

Because this study was nonrandomized and observational, potential confounding (selection) bias was accounted for with propensity score analysis [27–29] and a multivariate Cox proportional hazards model. The propensity score is the probability that a patient with specific prognostic factors will receive treatment. It is a scalar summary of all observed prognostic factors. Within propensity score strata, prognostic factors in treated and control groups are similarly distributed, so that stratifying on propensity score strata removes overt selection bias due to the prognostic factors. We computed the propensity

TABLE 1: Baseline Characteristics of Patients With Unresectable Hepatocellular Carcinoma Who Underwent Transarterial Chemoembolization With Iodized Oil and Transarterial Iodized Oil Infusion Chemotherapy Without Embolization (*n* = 11,030)

Background Factor	Transarterial Chemoembolization With Iodized Oil (*n* = 8,507)		Transarterial Iodized Oil Infusion Chemotherapy Without Embolization (*n* = 2,523)		*p*
	No. of Patients	%	No. of Patients	%	
Age (y)					0.0144
< 60	1,845	22	604	24	
≥ 60	6,645	78	1,908	76	
Sex					0.4076
Men	6,120	72	1,836	73	
Women	2,385	28	686	27	
Degree of liver damage					< 0.0001
A	4,000	51	1,046	45	
B	3,052	39	964	41	
C	768	10	332	14	
Hepatitis B and C virus status					0.664
Hepatitis B surface antigen negative, hepatitis C virus antibody positive	6,063	74	1,795	74	
Hepatitis B surface antigen positive, hepatitis C virus antibody negative	895	11	266	11	
Both positive	212	3	58	2	
Both negative	972	12	311	13	
Maximum tumor size (cm)					0.0004
< 2	1,986	24	597	24	
2.1–3	1,980	24	577	24	
3.1–5	2,319	28	584	24	
> 5.1	2,072	25	684	28	
No. of tumors					0.0016
1	3,645	43	1,040	42	
2–3	2,676	32	689	28	
≥ 4	2,065	25	722	29	
Degree of portal vein invasion					< 0.0001
Vp0	6,881	88	1,777	77	
Vp1	322	4	90	4	
Vp2	305	4	130	6	
≥ Vp3	347	4	297	13	
Degree of hepatic vein invasion					< 0.0001
Vv0	7,246	97	1,936	95	
≥ Vv1	243	3	106	5	
α-Fetoprotein level (ng/mL)					< 0.0001
< 20	2,745	34	724	30	
21–400	3,393	42	994	41	
> 401	2,001	25	700	29	
TNM stage					< 0.0001
I (T1N0M0)	915	12	280	13	
II (T2N0M0)	2,908	39	719	34	
III (T3N0M0)	2,972	40	775	37	
IVA (T4N0M0)	639	9	318	15	

Note—Numbers in the sections do not equal those in the number columns because of missing values on the questionaire. Some percentages do not total 100 due to rounding.

TABLE 2: Degree of Liver Damage According to the Classification of the Liver Cancer Study Group of Japan

Clinical or Laboratory Finding	Grade of Liver Damage		
	A	B	C
Ascites	None	Controllable	Uncontrollable
Serum bilirubin concentration (mg/dL)	< 2.0	2.0–3.0	> 3.0
Serum albumin concentration (g/dL)	> 3.5	3.0–3.5	< 3.0
Indocyanine green retention rate at 15 minutes (%)	< 15	15–40	> 40
Prothrombin activity (%)	> 80	50–80	< 50

Note—Degree of liver damage is based on the highest grade containing at least two findings. For example, grade C applies if a patient has three clinical findings, one in column B and two in column C.

TABLE 3: Definitions of TNM Stage Proposed by the Liver Cancer Study Group of Japan

Classification	Criteria
T category	Single lesion, tumor diameter 2 cm or less, and no vascular or biliary invasion
T1	Fulfilling 3 criteria
T2	Fulfilling 2 criteria
T3	Fulfilling 1 criterion
T4	Fulfilling no criteria
TNM stage	
I	T1N0M0
II	T2N0M0
III	T3N0M0
IVA	T4N0M0, any T N1M0
IVB	Any T, N0–1M1

score by using multiple logistic regression with the dependent variable receiving TACE. The independent variables (prognostic factors) were the first nine variables (all but TNM stage) in Table 1.

To provide optimal control for confounding, propensity-based matching was used to select control patients similar to patients undergoing TACE. Using a macro (available at http://www2.sas.com/proceedings/sugi26/p214-26.pdf), we used propensity scores to match TACE patients to unique patients undergoing transarterial infusion therapy without embolization. We tried to match the background characteristics of the patient in the two groups by using propensity scores identical to five digits. If we could not make the match, we proceeded to four-, three-, two- and one-digit matches. We were able to match 1,699 TACE patients to 1,699 patients undergoing transarterial therapy without embolization.

For the 3,398-patient propensity score–matched sample, the survival curves were obtained with the Kaplan-Meier method and compared by log-rank test. Although performed with a nonrepresentative sample of patients undergoing treatment, matched analyses may yield a more valid estimate of treatment effect because patients with similar observed characteristics are compared, all of whom are candidates for selection of the treatment. All significance tests were two-tailed, and a value of $p < 0.05$ was considered statistically significant. All analyses were performed with statistical software (SAS version 9.1.3, SAS).

Results

Patient Characteristics in the Whole Sample

In the baseline characteristics of patients with unresectable HCC who underwent TACE (n = 8,507) and those who underwent iodized oil infusion chemotherapy without embolization (n = 2,523) (Table 1), there was a significant difference between the two groups in the following variables: age (p = 0.0144), liver function ($p < 0.0001$), maximum tumor size (p = 0.0004), number of tumors (p = 0.0016), portal and hepatic vein invasion ($p < 0.0001$), α-fetoprotein value ($p < 0.0001$), and TNM stage ($p < 0.0001$).

Crude Survival of TACE Patients and Patients Undergoing Therapy Without Embolization

During an 8-year follow-up period, 3,671 patients (43%) in the TACE group died, and data on the other 4,836 (57%) were censored; 1,373 patients (54%) in the therapy without embolization group died, and the data on 1,150 patients (46%) were censored. The median follow-up period was 1.39 years (range, 0.003–7.99 years) for the TACE group and 0.95 year (range, 0.003–7.97 years) for the therapy without embolization group. The median time and overall survival rates at 1-, 2-, 3-, 4-, 5-, and 7-years were 2.76 years and 82%, 62%, 46%, 34%, 25%, and 15% for the TACE group and 1.69 years and 66%, 45%, 31%, 23%, 15%, and 7% for the therapy without embolization group. There was a significant difference between two therapies (hazard ratio [HR], 0.60; 95% CI, 0.56–0.64; p = 0.0001).

Multivariate analysis of factors affecting time to death of patients who underwent TACE and iodized oil infusion chemotherapy without embolization showed that the following seven covariates were independent factors (Table 4): treatment (HR, 0.63; 95% CI, 0.59–0.68; p = 0.0001), degree of liver damage (p = 0.0001), maximum tumor size (p = 0.0001), number of tumors (p = 0.0001), portal vein invasion (p = 0.0001), hepatic vein invasion (p = 0.001), and α-fetoprotein value (p = 0.0001).

Survival of TACE Patients and Patients Undergoing Therapy Without Embolization Matched by Propensity Score

The baseline characteristics of 1,699 patients treated with TACE and 1,699 treated with transarterial iodized oil infusion chemotherapy without embolization matched by propensity score are shown in Table 5. Unlike the population as a whole, these two propensity-matched groups were well balanced. Regarding portal vein invasion, a significant difference seen among four subgroups was not seen in two subgroups categorized as Vp0–Vp1 and greater than Vp3.

The median follow-up periods for the TACE and infusion chemotherapy without embolization groups were 1.82 and 1.06 years, respectively. The patients with TACE had a lower risk of death than those who underwent treatment without embolization (HR, 0.70; 95% CI, 0.63–0.76; p = 0.0001). The median survival time and overall survival rates at 1-, 2-, 3-, 4, 5-, and 7-years were 2.74 years and 81%, 62%, 46%, 34%, 25%, and 15% for TACE versus 1.98 years and 71%, 49%, 33%, 23%, 16%, and 7% for therapy without embolization (Fig. 1).

Discussion

Infusion therapy of an emulsion of iodized oil and an anticancer agent without gelatin

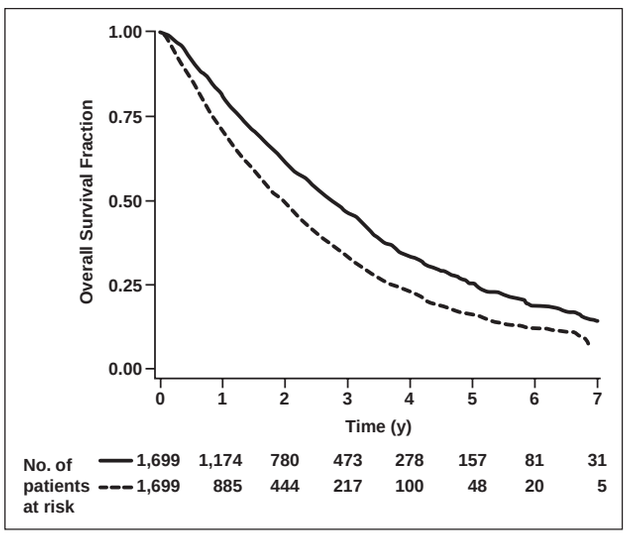


Fig. 1—Graph shows comparison of survival rates among patients with unresectable hepatocellular carcinoma treated with iodized oil transarterial chemoembolization (TACE) (*n* = 1,699 patients) (*solid line*) and those treated with iodized oil transarterial infusion therapy without embolization (*n* = 1,699) (*dotted line*) and matched by propensity score. TACE had significantly higher survival rate than therapy without embolization (hazard ratio, 0.70; 95% CI, 0.63–0.76; *p* = 0.0001).

TABLE 4: Results of Cox Proportional Hazards Model Multivariate Analysis of Factors Affecting Time to Death (*n* = 11,030)

Variable	Estimate	Standard Error	*p*	Hazard Ratio	
				Ratio	95% CI
Treatment (TACE vs no embolization)	−0.4556	0.0385	0.0001	0.63	0.59–0.68
Sex (male vs female)	0.0731	0.0383	0.056	1.08	0.99–1.16
Age (y) (≥ 60 vs < 60)	0.0551	0.0386	0.15	1.06	0.98–1.14
Liver damage					
Grade B vs A	0.3711	0.0358	0.0001	1.45	1.35–1.56
Grade C vs A	0.8566	0.0508	0.0001	2.36	2.13–2.60
Maximum tumor size (cm)					
2.1–3 vs ≤ 2	0.2076	0.0523	0.0001	1.23	1.11–1.36
3.1–5 vs ≤ 2	0.3802	0.0499	0.0001	1.46	1.33–1.61
≥ 5.1 vs ≤ 2	0.6689	0.0533	0.0001	1.95	1.76–2.17
No. of tumors					
2–3 vs 1	0.2593	0.0396	0.0001	1.30	1.20–1.40
≥ 4 vs 1	0.4990	0.0416	0.0001	1.65	1.52–1.79
Vascular invasion					
Vp1–≥ 3 vs Vp0	0.6137	0.0520	0.0001	1.85	1.67–2.05
≥ Vv1 vs Vv0	0.2649	0.0806	0.001	1.30	1.11–1.53
α-Fetoprotein (ng/mL)					
21–400 vs ≤ 20	0.2562	0.0412	0.0001	1.29	1.19–1.40
≥ 401 vs ≤ 20	0.7338	0.0454	0.0001	2.08	1.91–2.28

Note—TACE = transarterial iodized oil chemoembolization; no embolization = transarterial iodized oil infusion chemotherapy without embolization.

sponge particles was developed as a variation of TACE in the mid-1980s in Japan mainly to prevent posttherapeutic hepatic failure and to delay death among patients with poorer liver function and a more advanced stage of cancer than would be managed with TACE. Therapy without embolization continues to account for approximately one fourth of transarterial chemotherapeutic procedures [5].

The survival of patients who have undergone TACE and transarterial infusion therapy without embolization has stood in delicate balance between therapeutic effect against HCC and inadvertent injury to the noncancerous hepatic parenchyma. Pathologic study of resected specimens of HCC managed with TACE and with therapy without embolization revealed that TACE was associated with significantly more extensive tumor necrosis than was therapy without embolization [30, 31], whereas injury to noncancerous hepatic parenchyma has seldom been reported pathologically and clinically. An animal study [32] showed that intraarterial injection of iodized oil followed by gelatin sponge particles caused necrosis in the normal hepatic parenchyma that occurred in parallel with an increased dose of iodized oil, whereas injection of iodized oil alone did not induce necrosis. These findings are consistent with our impression of these therapies. TACE causes postembolization syndrome more frequently than does iodized oil infusion chemotherapy without embolization [19]. One serial clinical study of emulsion of iodized oil and zinostatin stimalamer, a lipophilic chemotherapeutic agent, with and without gelatin sponge particles showed that the former induced a higher response rate for HCC and more frequent impairment of hepatic function [33] than did the latter [34].

In our study of crude survival, TACE had a significantly higher overall survival rate than did therapy without embolization (HR, 0.60; 95% CI, 0.56–0.64; $p = 0.0001$). The median survival time and overall survival rates of therapy without embolization at 1-, 2-, 3-, and 5 years were 1.69 years and 66%, 45%, 31%, and 15%. The results in the literature are widely different from one series to another: a median survival time of 45 days [35], a 1-year survival rate of 25–82% [15, 19], a 2-year survival rate of 6–54% [15, 17], a 3-year survival rate of 24–40% [13, 19], and a 5-year survival rate of 18% [16]. The 1- to 5-year survival rates in our study were not inconsistent with those in other studies. In our study, patients who underwent TACE had better survival rates than patients in European [10, 11] and other Asian [7] series. The results may be due to the more preferable patient characteristics in our study for undergoing either transarterial therapy than was found in the other studies. More than 40% of patients in our study had a solitary HCC, and one fourth of them had HCCs smaller than 2 cm in diameter (Table 1).

Adjustment with multivariate analysis and the Cox proportional hazards model showed that TACE was associated with a better survival rate than was therapy without embolization (HR, 0.63; 95% CI, 0.59–0.68). We

TABLE 5: Baseline Characteristics of Patients in Two Groups Matched by Propensity Score (*n* = 3,398)

Background Factor	Transarterial Chemoembolization With Iodized Oil (*n* = 1,699)		Transarterial Iodized Oil Infusion Chemotherapy Without Embolization (*n* = 1,699)		*p*
	No. of Patients	%	No. of Patients	%	
Age (y)					0.75
< 60	422	25	414	24	
≥ 60	1,277	75	1,285	76	
Sex					0.52
Men	1,232	73	1,215	72	
Women	467	27	484	28	
Degree of liver damage					0.81
A	782	46	778	46	
B	696	41	694	41	
C	221	13	227	13	
Hepatitis B and C virus status					0.95
Hepatitis B surface antigen negative, hepatitis C virus antibody positive	1,282	75	1,269	75	
Hepatitis B surface antigen positive, hepatitis C virus antibody negative	165	10	172	10	
Both positive	36	2	39	2	
Both negative	216	13	219	13	
Maximum tumor size (cm)					0.59
< 2	475	28	463	27	
2.1–3	431	25	422	25	
3.1–5	394	23	413	24	
≥ 5.1	399	24	401	24	
No. of tumors					0.77
1	772	45	754	44	
2–3	472	28	494	29	
≥ 4	455	27	451	27	
Degree of portal vein invasion					0.03
Vp0	1,432	84	1,428	84	
Vp1	91	5	47	3	
Vp2	81	5	68	4	
≥ Vp3	95	6	156	9	
Degree of hepatic vein invasion					0.25
Vv0	1,630	96	1,616	95	
≥ Vv1	69	4	83	5	
α-Fetoprotein level (ng/mL)					0.19
< 20	560	33	533	31	
21–400	724	43	720	42	
> 401	415	24	446	26	
TNM stage					0.44
I (T1N0M0)	259	15	252	15	
II (T2N0M0)	636	37	628	37	
III (T3N0M0)	616	36	626	37	
IVA (T4N0M0)	188	11	193	11	

Note—Some percentages do not total 100 due to rounding.

compared the survival rates by performing patient-to-patient matching and computing the propensity score by logistic regression of the independent prognostic factors with all of the variables in Table 1 except TNM stage. As a result, the hazard ratio for death in the TACE compared with the therapy without embolization group was 0.70 (95% CI, 0.63–0.76; p = 0.0001), suggesting that TACE significantly reduced the overall risk of death 30%. This finding means embolization may be indispensable to better survival among patients with unresectable HCC. That is, the more intensive therapeutic effect of TACE may take precedence over the lower risk of inadvertent liver injury associated with therapy without embolization. Caturelli et al. [36] reported that the worsening of liver function expected in the long term with TACE did not occur. Results of phase 2 studies of transcatheter arterial therapy for HCC with drug-eluting beads with doxorubicin [37] and ^{90}Y-microspheres [38] and a cohort study of bland embolization with trisacryl gelatin microspheres without an anticancer agent and iodized oil [39] have been reported.

There were limitations to our study. The propensity score analysis might have matched the background of patients to have the same possibility of receiving one of the two therapies. This method, however, includes factors for insufficiency of treatment protocol among institutions and laboratory data that might affect survival. Another limitation was incomplete information about the doses of anticancer agents and iodized oil used, the total number of treatments, and Child-Pugh class because questions were overlooked on the questionnaire of the registration sheet.

Although a randomized controlled trial remains the reference standard, our analysis of an entire sample and of matched patients with a propensity score showed that in the care of patients with unresectable HCC, the survival rate associated with TACE was significantly higher than that associated with iodized oil infusion chemotherapy without embolization. These results may enhance or change decision-making about the strategy for transcatheter arterial therapy for HCC.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; 94:153–156
2. Kiyosawa K, Umemura T, Ichijo T, et al. Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; 127:S17–S26
3. El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. *Ann Intern Med* 2003; 139:817–823
4. Taylor-Robinson SD, Foster GR, Arora S, Hargreaves S, Thomas HC. Increase in primary liver cancer in the UK, 1979–94. *Lancet* 1997; 350:1142–1143
5. Ikai I, Arii S, Ichida T, et al. Report of the 16th follow-up survey of primary liver cancer. *Hepatol Res* 2005; 32:163–172
6. Llovet JM, Real MI, Montana X, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; 359:1734–1739
7. Lo CM, Ngan H, Tso WK, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; 35:1164–1171
8. Camma C, Schepis F, Orlando A, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. *Radiology* 2002; 224:47–54
9. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003; 37:429–442
10. [No authors listed]. A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. *N Engl J Med* 1995; 332:1256–1261
11. Pelletier G, Ducreux M, Gay F, et al. Treatment of unresectable hepatocellular carcinoma with lipiodol chemoembolization: a multicenter randomized trial. *J Hepatol* 1998; 29:129–134
12. Kanematsu T, Inokuchi K, Sugimachi K, et al. Selective effects of lipiodolized antitumor agents. *J Surg Oncol* 1984; 25:218–226
13. Ikeda K, Inoue H, Yano T, Kobayashi H, Nakajo M. Comparison of the anticancer effect of ADMOS alone and ADMOS with CDDP in the treatment of hepatocellular carcinoma by intra-arterial injection. *Cancer Chemother Pharmacol* 1992; 31[suppl]:S65–S68
14. Lu CD, Qi YG, Peng SY. Lipiodolization with or without gelatin sponge in hepatic arterial chemoembolization for hepatocellular carcinoma. *Chin Med J (Engl)* 1994; 107:209–215
15. Bhattacharya S, Novell JR, Dusheiko GM, Hilson AJ, Dick R, Hobbs KE. Epirubicin-lipiodol chemotherapy versus 131iodine-lipiodol radiotherapy in the treatment of unresectable hepatocellular carcinoma. *Cancer* 1995; 76:2202–2210
16. Hatanaka Y, Yamashita Y, Takahashi M, et al. Unresectable hepatocellular carcinoma: analysis of prognostic factors in transcatheter management. *Radiology* 1995; 195:747–752
17. Kawai S, Tani M, Okamura J, et al. Prospective and randomized trial of lipiodol-transcatheter arterial chemoembolization for treatment of hepatocellular carcinoma: a comparison of epirubicin and doxorubicin (second cooperative study). The Cooperative Study Group for Liver Cancer Treatment of Japan. *Semin Oncol* 1997;24[2 suppl 6]:S6-38–S6-45
18. Maeda S, Fujiyama S, Tanaka M, Ashihara H, Hirata R, Tomita K. Survival and local recurrence rates of hepatocellular carcinoma patients treated by transarterial chemolipiodolization with and without embolization. *Hepatol Res* 2002; 23:202–210
19. Ikeda M, Maeda S, Shibata J, et al. Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. *Oncology* 2004; 66:24–31
20. Takayasu K, Arii S, Ikai I, et al. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; 131:461–469
21. Takayasu K, Furukawa H, Wakao F, et al. CT diagnosis of early hepatocellular carcinoma: sensitivity, findings, and CT-pathologic correlation. *AJR* 1995; 164:885–890
22. Baron RL, Oliver JH 3rd, Dodd GD 3rd, Nalesnik M, Holbert BL, Carr B. Hepatocellular carcinoma: evaluation with biphasic, contrast-enhanced, helical CT. *Radiology* 1996; 199:505–511
23. The Liver Cancer Study Group of Japan. *The general rules for the clinical and pathological study of primary liver cancer.* Tokyo, Japan: Kanehara, 2003
24. Nanashima A, Sumida Y, Morino S, et al. The Japanese integrated staging score using liver damage grade for hepatocellular carcinoma in patients after hepatectomy. *Eur J Surg Oncol* 2004; 30: 765–770
25. Makuuchi M, Belghiti J, Belli G, et al. IHPBA concordant classification of primary liver cancer: working group report. *J Hepatobiliary Pancreat Surg* 2003; 10:26–30
26. Murakami T. *Local therapy: radiological intervention (transcatheter arterial embolization)*. Tokyo: Arcmedia, 2004:182–192
27. Rosenbaum PR. Rubin DB. The central role of the propensity score in observational studies for causal effects. *Biometrika* 1983; 70:41–55
28. Gum PA, Thamilarasan M, Watanabe J, Blackstone EH, Lauer MS. Aspirin use and all-cause mortality among patients being evaluated for known or suspected coronary artery disease: a propensity analysis. *JAMA* 2001; 286:1187–1194
29. Lindenauer PK, Pekow P, Wang K, Gutierrez B, Benjamin EM. Lipid-lowering therapy and in-

hospital mortality following major noncardiac surgery. *JAMA* 2004; 291:2092–2099

30. Takayasu K, Shima Y, Muramatsu Y, et al. Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. *Radiology* 1987; 163:345–351
31. Okayasu I, Hatakeyama S, Yoshida T, et al. Selective and persistent deposition and gradual drainage of iodized oil, lipiodol, in the hepatocellular carcinoma after injection into the feeding hepatic artery. *Am J Clin Pathol* 1988; 90:536–544
32. Kan Z, Sato M, Ivancev K, et al. Distribution and effect of iodized poppyseed oil in the liver after hepatic artery embolization: experimental study in several animal species. *Radiology* 1993; 186: 861–866
33. Okusaka T, Okada S, Ueno H, et al. Transcatheter arterial embolization with zinostatin stimalamer for hepatocellular carcinoma. *Oncology* 2002; 62:228–233
34. Okusaka T, Okada S, Ishii H, et al. Transarterial chemotherapy with zinostatin stimalamer for hepatocellular carcinoma. *Oncology* 1998; 55:276–283
35. Madden MV, Krige JE, Bailey S, et al. Randomised trial of targeted chemotherapy with lipiodol and 5-epidoxorubicin compared with symptomatic treatment for hepatoma. *Gut* 1993; 34: 1598–1600
36. Caturelli E, Siena DA, Fusilli S, et al. Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with cirrhosis: evaluation of damage to nontumorous liver tissue—long-term prospective study. *Radiology* 2000; 215:123–128
37. Varela M, Real MI, Burrel M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. *J Hepatol* 2007; 46:474–481
38. Kulik LM, Carr BI, Mulcahy MF, et al. Safety and efficacy of ^{90}Y radiotherapy for hepatocellular carcinoma with and without portal vein thrombosis. *Hepatology* 2008; 47:71–81
39. Maluccio MA, Covey AM, Porat LB, et al. Transcatheter arterial embolization with only particles for the treatment of unresectable hepatocellular carcinoma. *J Vasc Interv Radiol* 2008; 19:862–869

Hepatology Research

Hepatology Research 2010; **40**: 347–368 doi: 10.1111/j.1872-034X.2010.00642.x

Special Report

Management of hepatitis C; Report of the Consensus Meeting at the 45th Annual Meeting of the Japan Society of Hepatology (2009)

Izumi Namiki,[1] Shuhei Nishiguchi,[2] Keisuke Hino,[3] Fumitaka Suzuki,[4] Hiromitsu Kumada,[4] Yoshihito Itoh,[5] Yusuhiro Asahina,[1] Akihiro Tamori,[6] Naoki Hiramatsu,[7] Norio Hayashi[7] and Masatoshi Kudo[8]

[1]*Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Musashinoshi, Tokyo,* [2]*Department of Hepatobiliary and Pancreas Disease, Hyogo Medical University, Nishinomiya,* [3]*Department of Hepatobiliary and Pancreas Disease, Kawasaki Medical University, Kurashiki,* [4]*Department of Hepatology, Toranomon Hospital, Tokyo,* [5]*Molecular Gstroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, and* [6]*Department of Hepatology, Osaka City University Graduate School of Medicine, Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009),* [7]*Department of Gastroenterology, Osaka University and* [8]*Department of Gastroenterology, Kinki University, Osaka, Japan*

The consensus meeting for the diagnosis, management and treatment for hepatitis C was held in 45th annual meeting for the Japan Society of Hepatology (JSH) in June 2009 where the recommendations and informative statements were discussed including organizers and presenters. The Several important informative statements and recommendations have been shown. This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. Thus, this is the first report of JSH consensus of hepatitis C. The rate of development of hepatocellular carcinoma (HCC) in HCV-infected patients in Japan is higher than in the USA, because the average age of the HCV-infected patients is greater and there are more patients with severe fibrosis of the liver than in the USA. In Japan, more than 60% of HCV-infected patients are genotype 1b infection, and they show lower response to perinterferon and ribavirin combination treatment. To improve the response rate is also an important issue in our country. To establish the original recommendations and informative statements to prevent the development of HCC is a very important issue in Japan.

Key words: chronic hepatitis C, peginterferon, ribavirin, fibrosis of the liver, hepatocellular carcinoma, HCV mutation

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a major public health problem and a leading course of death from liver disease in Japan. Two million people are infected, and more than 30 000 patients die from hepatocellular carcinoma (HCC) and/or liver cirrhosis every year. HCC is the fourth leading cause of death from malignant neoplastic disease, and prevention of the development of HCC is an urgent issue in Japan. The purpose of this consensus is to provide clinicians with consensus-based approaches to diagnosis and treatment of HCV infection.

The consensus meeting for the diagnosis, management and treatment for hepatitis C was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M. Kudo), where the recommendations and informative statements were discussed and compared with AASLD practice guidelines which has been published in *Hepatology*.[1] This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. This is the first report of the JSH consensus of hepatitis C. Established information regarding the pathogenesis and contributing factors for disease

Correspondence: Mr Namiki Izumi, Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, 1-26-1 Kyonancho, Musashinoshi, Tokyo 180-8610, Japan. Email: nizumi@musashino.jrc.or.jp

Disclaimer Statement: The view expressed in these consensus do not necessarily represent the view of the National Health Insurance of Japan, or the Japanese Government.

This article was previously published in Japanese in *Kanzo* 50:11, pp 665–677 (November 2009).

Received 24 September 2009; revision 1 December 2009; accepted 1 December 2009.

Table 1 Grading system for recommendations

	Description
Classification	
Class I	Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation procedure or treatment is beneficial, useful and effective
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a diagnostic evaluation, procedure or treatment
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
Class IIb	Usefulness/efficacy is less well established by evidence/opinion
Class III	Conditions for which there is no evidence and/or general agreement that a diagnostic evaluation, procedure/ treatment is not useful/effective and in some cases may be harmful
Level of evidence	
Level A	Data derived from multiple randomized clinical trials or meta-analysis
Level B	Data derived from a single randomized trial, or non-randomized studies
Level C	Only consensus opinion of experts, case studies or standard of care

progression which were agreed by the organizers and presenters are shown as informative statements, and clinically useful consensuses are shown as "Recommendations". The rate of development of HCC in HCV-infected patients in Japan is higher than that in the USA, because the average age of the patient is greater and there are more patients with severe fibrosis of the liver than in the USA. To establish original recommendations and informative statements to prevent the development of HCC is a very important issue in our country. The quality of recommendations or informative statements is required to show a "class" (reflecting benefit vs risk) and "level" (assessing strength or certainty) of evidence according to AASLD practice guidelines (Table 1).[1,2]

PATHOGENESIS OF HEPATITIS C

HEPATITIS C VIRUS infection causes acute and chronic hepatitis (CH), cirrhosis and HCC. The severity and rate of progression of the disease are highly variable and may reflect both host and viral factors, but the mechanisms of pathogenesis are incompletely understood. Thus, understanding the mechanisms of HCV pathogenesis is an important goal of HCV research.

Entry pathway of HCV

For the virus, the first step in propagation is enter into hepatocytes. A decade ago, the HCV envelop protein E2 was shown to bind human CD81, a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes.[3] Next, two other essential proteins, scavenger receptor class B type I (SR-B1)[4] and claudin-1 (CLDN1),[5] and potentially additional accessory factors such as glycosaminoglycans and low-density protein receptors[6] were identified as receptors involved in HCV entry. Finally, the crucial factor was identified as the tight junction protein occludin (OCLN).[7] Interestingly, both CLDN1 and OCLN are components of tight junctions which are structures forming firm seals between adjacent cells. The initial adhesion of HCV to hepatocytes may be mediated by accessory factors and/or direct interaction with SR-B1 and CD81 proteins. On transfer to a tight junction complex, HCV may interact directly with CLDN1 and/or OCLN, allowing viral uptake into the cell.

Hepatitis C virus infects only humans and chimpanzees. Once these HCV entry factors were identified, the next concern was to determine which factors dictate species-specific tropism. CD81 proteins from other mammals, such as the mouse, are used inefficiently by HCV.[8] Although HCV does not discriminate between human and mouse SR-B1 and CLDN1, mouse OCLN like CD81 cannot substitute for the related human protein in aiding viral entry. These findings indicate that CD81 and OCLN represent minimal human-specific entry factors.

Informative statement: CLDN1 and OCLN in addition to CD81 and SR-B1 are required for entering of HCV into hepatocytes, and especially CD81 and OCLN represent minimal human-specific entry factors. (Grade A.)

Evasion of intracellular host defense by HCV

One of the mechanisms by which HCV infection is likely to lead to be persistent is evasion of intracellular host defense through a complex combination of processes that include interference of interferon (IFN) signaling, modulation of its effectors and continual viral genetic variation. The HCV genome contains pathogen-associated molecular pattern (PAMP) signatures which

are recognized by the retinoic-inducible gene I (*RIG-I*) and specific Toll-like receptors when introduced into naïve cells.[9–11] Viral signaling through *RIG-I* and its adaptor protein, IFN promoter-stimulator 1 (IPS-1), activates IFN regulatory factor-3 (IRF-3) and the host IFN-α/β response that limits virus infection.[12,13] HCV NS3/4A protease cleaves IPS-1, releasing IPS-1 from the mitochondrial membrane.[14] Cleavage results in subcellular redistribution of IPS-1 and loss of interaction with *RIG-I*, thereby preventing downstream activation of IRF-3 and induction of IFNβ.[15]

Secreted IFNβ engages the local tissue through the autocrine and paracrine processes of binding the IFN-α/β receptors. This results in activation of the Jak-signal transducer and activator of transcription (STAT) pathway, in which the receptor-associated Jak and Tyk1 protein kinases catalyze the phosphorylation of STAT proteins. The resulting IFN-stimulated gene factor-3 (ISGF3) transcription factor complex localizes in the cell nucleus, where it binds to the IFN-stimulated response element (ISRE) within the promoter/enhancer region of IFN-stimulated genes (ISG). Jak-STAT signaling leads to a second wave of transcriptional activity stimulating ISG expression in the infected cells. Expression of the HCV core protein has been associated with increased expression levels of suppressor of cytokine signaling (SOCS)-3.[16] The SOCS proteins are known for their role as negative regulators and inhibitors of Jak-STAT signaling, where they mediate a classic negative feedback loop on IFN-α/β receptor signaling events.[17] The HCV NS5A protein has been shown to induce interleukin (IL)-8 production leading to partial inhibition of the IFN-induced antiviral response, probably through the alteration of ISG expression.[18] The HCV NS5A and E2 proteins also bind double-strand RNA-activated protein kinase (PKR) and inhibit its catalytic activity,[19,20] which allows HCV to evade in part the translational-suppressive actions of IFN. Thus, HCV evasion of the host response includes various strategies directed by viral proteins to control IFN signaling, ISG expression or function.

Informative statement: HCV evades intracellular host defenses through a complex combination of processes that include IFN signaling, modulation of its effectors and continual viral genetic variation. These mechanisms include cleavage of IPS-1 by the NS3/4A protease, inhibition of Jak-STAT signaling by HCV-induced SOCS3, inhibition of the IFN-induced antiviral response by NS5A-induced IL-8, and/or inhibition of catalytic activity of PKR by the NS5A and E2 proteins. (Grade A.)

Oxidative stress induced by HCV

Oxidative stress is well known to be present in CH-C to a greater degree than in other inflammatory liver diseases. Although the mechanisms underlying oxidative stress induced by HCV have not been elucidated fully, there are several lines of evidence suggesting that HCV directly generates reactive oxygen species (ROS) *in vitro* and *in vivo*. Hepatic ROS production is significantly higher in HCV core transgenic mice than in normal control mice in the absence of hepatic inflammation.[21] HCV core protein also produces ROS in human hepatoma Huh-7 cells and HeLa cells.[22] Analysis of the interaction of HCV core protein with mitochondria in transgenic mice and direct interaction of recombinant core protein and isolated mitochondria indicated oxidation of the mitochondrial glutathione pool and an increase in ROS production by the mitochondrial electron transport complex I, suggesting that direct interaction of core protein with mitochondria is an important cause of the oxidative stress seen in CH-C.[23]

Informative statement: Mitochondrial dysfunction induced by HCV leads to ROS generation that causes the oxidative stress seen in CH-C. (Grade A.)

Metabolic disorders caused by HCV

Epidemiological studies have suggested a link between type 2 diabetes and chronic HCV infection, which implies HCV-induced insulin resistance. A high level of tumor necrosis factor (TNF)-α and disturbance of tyrosine phosphorylation of the insulin receptor substrate (IRS)1 by TNF-α has been demonstrated in HCV core transgenic mice.[24] Another possible mechanism is that HCV core-induced SOCS3 promotes proteosomal degradation of IRS1 and IRS2 through ubiquitination.[25] Hepatic steatosis is one of the histopathological features in CH-C. HCV core protein inhibits microsomal triglyceride transfer protein activity and secretion of very low density lipoprotein.[26] HCV core protein also upregulates the sterol regulatory element binding protein (SREBP)1c promoter activity through the enhanced binding of the LXRα/RXRα to LXR-response element,[27] which leads to an increase in transcription of genes involved in hepatic fatty acid synthesis. Hepatic iron accumulation is also a histopathological feature of CH-C, even though its levels are not extremely high. HCV-induced ROS downregulates the transcriptional activity of hepcidin, a negative regulator in iron homeostasis, in transgenic mice expressing the HCV polyprotein[28] and in HCV replicon cells[29] (Fig. 1).

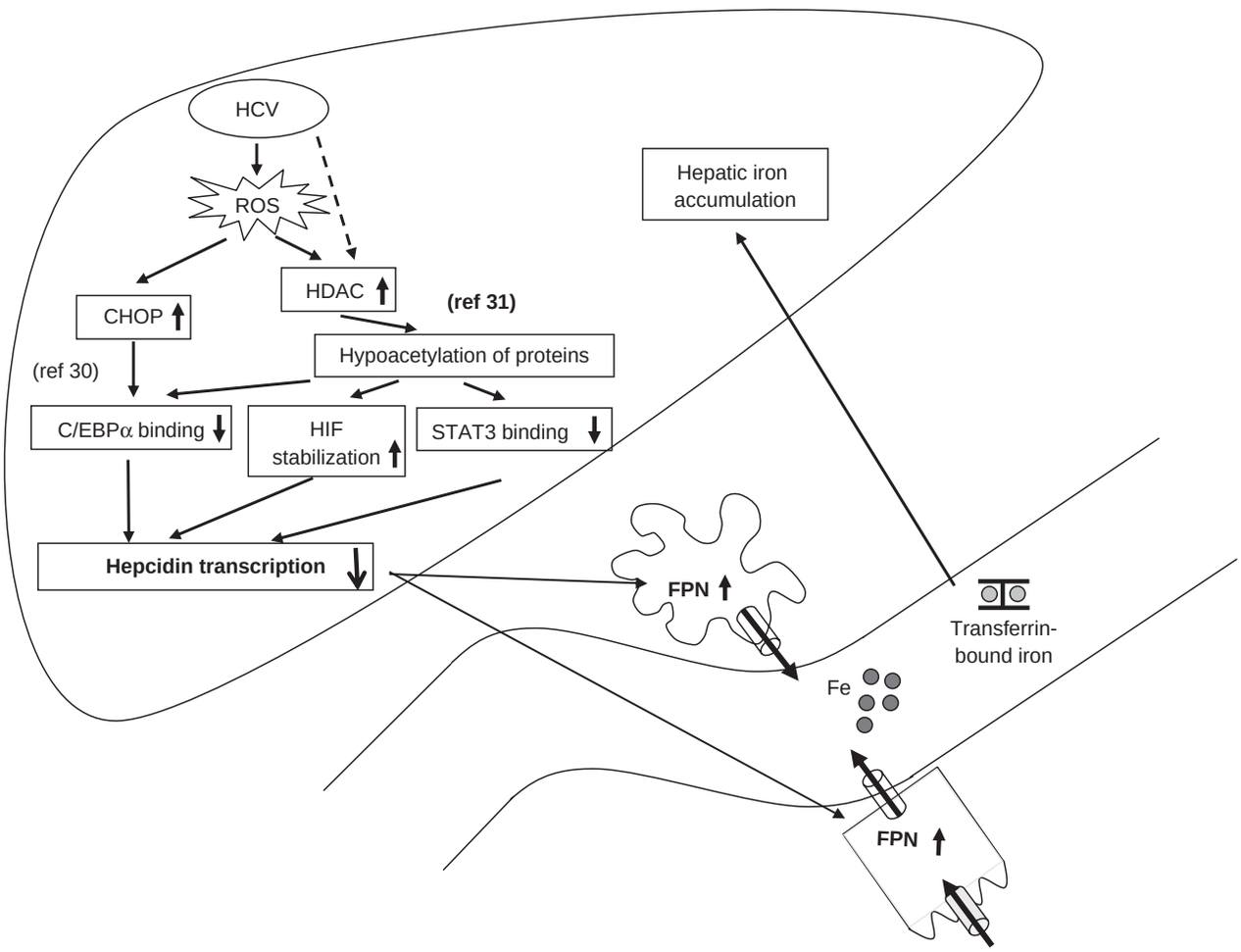


Figure 1 Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation induced by HCV. HCV-induced ROS reduces hepcidin transcription through the inhibited binding of CHOP and/or STAt3 to the hepcidin promoter, and/or stabilization of HIF that is negative hepcidin regulator. C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP homology protein; FPN, ferroportin; HCV, hepatitis C virus; HDAC, histone deacetylase; HIF, hypoxia inducible factor; ROS, reactive oxygen species; STAT, signal transducer and activation of transcription.

Metabolic disorders caused by HCV such as insulin resistance, hepatic steatosis and iron accumulation are clinically important in terms of amplification of oxidative stress and involvement in hepatocarcinogenesis in CH-C.[30–33] In addition, these metabolic disorders are related to the response to antiviral therapy. Insulin resistance[34] and hepatic steatosis[35] seem to be negatively correlated with response to antiviral therapy in CH-C.

Informative statement: HCV induces insulin resistance, hepatic steatosis, and/or hepatic iron accumulation, which is associated with hepatocarcinogenesis in CH-C. (Grade A.)

Recommendation 1: Insulin resistance and hepatic steatosis seem to be negatively correlated with response to antiviral therapy in CH-C, whereas it remains controversial whether hepatic iron accumulation is related to a poor response to therapy. (Level 2a, Grade C.)

Liver biopsy for evaluating pathogenesis of hepatitis C

Assessment of the extent of liver fibrosis is still of great importance in terms of predicting the response to antiviral therapy and hepatocarcinogenesis in CH-C. It is also apparent that as many as a quarter of CH-C patients with persistently normal aminotransferase values have significant fibrosis.[36] The recently developed transient elastography that uses ultrasound and low-frequency elastic waves to measure liver elasticity has

Table 2 Definitions of virological responses to interferon therapy

RVR (rapid virological response)	Undetectable HCV RNA at week 4
cEVR (complete early virological response)	Undetectable HCV RNA at week 12
pEVR (partial early virological response)	Two log drop of HCV RNA without undetectable level at week 12
LVR (late virological response)	Undetectable HCV RNA between week 13 and 24 week
NVR (null virological response)	Positive HCV RNA during treatment
Relapse	Undetectable HCV RNA at end of treatment followed by detectable level after treatment
SVR (sustained virological response)	Undetectable HCV RNA at 24 weeks after treatment

improved the ability to define the extent of fibrosis without a liver biopsy, particularly when combined with other non-invasive markers,[37] but it is not yet ready to replace liver biopsy. Among the pathological features, steatosis and excess hepatocellular iron that affect disease progression and possibly impede treatment response are difficult to diagnose without liver biopsy. Thus, a liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment.

Recommendation 2: A liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment. (Level 1, Grade C.)

VIRAL LOAD, GENOTYPE, VIRAL MUTATIONS

DEFINITIONS OF VIROLOGICAL responses to IFN therapy are summarized in Table 2.

HCV RNA assay and genotype

In clinical practice, the usual approach is to test initially for antibodies to HCV (anti-HCV), then to use HCV RNA to document viremia. The quantity of HCV RNA is useful to know before providing and monitoring HCV treatment. For HCV RNA determination, quantitative tests based on target amplification (reverse transcriptase polymerase chain reaction [RT–PCR]) and signal amplification (branched DNA [bDNA]) techniques with differing sensitivity and linear measuring ranges are commercially available. The COBAS Amplicor HCV Monitor Test v2.0 (Roche Molecular Systems, Branchburg, NJ, USA), however, requires sample dilutions for accurate quantification of high-titer specimens. In addition, the assay displays relatively low sensitivities of approximately 600 IU/mL. Recently, the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems) and AccuGene m-HCV (Abbott Molecular, Des Plaines, IL, USA) have become available. These meet the requirements for highly sensitive detection and reliable quantification of HCV in clinical samples.

There are six major HCV genotypes. Genotype specificity predicts the likelihood of treatment response and determines the duration of treatment. Therefore, HCV genotype should be determined in all HCV-infected persons prior to treatment in order to determine the duration of therapy and likelihood of response.[38]

Many reports showed that sustained virological response (SVR) rates in IFN monotherapy and IFN plus ribavirin (RBV) combination therapy were higher in patients who had lower pretreatment RNA levels and genotype 2 infections.[39–41]

Recommendation 3: HCV RNA level and genotype should be determined in all HCV-infected persons prior to treatment in order to predict the efficacy of response of therapy. SVR rate in IFN therapy are higher in patients who had lower pretreatment RNA levels and genotype 2 HCV infections in IFN therapy. (Level 1, Grade A.)

HCV mutation

IFN sensitivity determining region (ISDR)

Enomoto *et al.* were able to demonstrate a strong correlation between the number of mutations within the carboxy terminal region of the NS5A gene, the ISDR spanning codons 2209–2248, and response to IFN therapy.[42] Thus, no patient infected with HCV with a wild-type ISDR sequence (identical to the prototype Japanese HCV strain [HCV-J]) responded to IFN therapy whereas all patients infected with the "mutant type", defined by four or more amino acid substitutions in this region, showed an SVR.[43] These initial findings have been confirmed by other Japanese studies but controversial data were reported from other parts of the world, particularly from Europe and the

USA. This may indicate that geographical factors account for different sensitivities of HCV genotype 1b infection to antiviral therapy. Pascu *et al.* reported that the distribution of wild-, intermediate- and mutant-type ISDR sequences differed significantly between Japanese ($n = 655$) (44.1%, 37.6% and 18.3%, respectively) and European patients ($n = 525$) (24.8%, 63.4% and 11.8%, respectively; $P = 0.001$). However, there was a significant positive correlation between the number of ISDR mutations and SVR rate, irrespective of geographical region.[44]

Moreover, Shirakawa *et al.* reported that a logistic regression model that includes the sequence of ISDR of HCV, and other factors (T-helper cell [Th]1/Th2 ratio, bodyweight and neutrophil count) can be useful for accurately predicting accurately the SVR rate before pegylated (PEG)-IFN and RBV combination therapy.[45]

Recommendation 4: The ISDR should be evaluated before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)

IFN/RBV resistance-determining region (IRRDR)

El-Shamy *et al.* have reported recently that a high degree of sequence variation in the V3 and the pre-V3 regions (amino acid [aa]2334-2355) of NS5A, which they refer to collectively as the IRRDR (aa2334-2379), was closely correlated with virological response in HCV-1b-infected patients treated with PEG-IFN and RBV.[46] A high degree (>6 aa substitutions) of sequence variation in the IRRDR should be a useful marker for predicting SVR, whereas a less diverse (<5) IRRDR sequence predicts non-SVR.

Amino acid substitutions in the HCV core region

Akuta *et al.* identified pretreatment substitutions of aa70 and aa91 in the core region as independent and significant pretreatment factors associated with virological non-response, based on 48-week combination therapy of IFN plus RBV.[47] Moreover, they identified aa70 and aa91 substitutions in the core region as predictors of response to PEG-IFN-RBV therapy in Japanese patients infected with HCV genotype 1b[48] (Table 3). Donlin *et al.* reported sequencing the complete pretreatment genotype 1 HCV open reading frame using samples from 94 participants in the Virahep-C study to assess the effects of viral diversity on response to therapy.[49] Genotype 1b sequences from patients with more than 3.5 log declines in viral RNA levels by day 28 (marked responders) were more variable than those from patients with declines of less than 1.4 log (poor responders) in core and NS3. Moreover, arginine (R) at aa70 in the core region was related to a marked response.

Recently evaluations were made of the impact of aa substitutions in HCV core region on hepatocarcinogenesis. Akuta *et al.* reported that cumulative hepatocarcinogenesis rates in double wild-type (arginine at aa70/leucine at aa91) of the HCV core region were significantly lower than those in the non-double wild type in CH-C patients.[50] Moreover, another report showed that a logistic regression model developed

Table 3 Factors associated with sustained virological response to 48-week pegylated interferon plus ribavirin combination therapy in patients infected with hepatitis C virus genotype 1b, identified by multivariate analysis ($n = 114$) 52)

Factor	Category	Risk ratio (95% confidence interval)	*P*
Amino acid substitution in core region	1: double wild	1	
	2: non-double wild	0.102 (0.022–0.474)	0.004
Low-density lipoprotein cholesterol (mg/dL)	1: <86	1	
	2: ≥86	12.87 (2.177–76.09)	0.005
Sex	1: male	1	
	2: female	0.091 (0.017–0.486)	0.005
ICG R15 (%)	1: <10	1	
	2: ≥10	0.107 (0.017–0.678)	0.018
γ-Glutamyltransferase	1: <109	1	
	2: ≥109	0.096 (0.0011–0.819)	0.032
Ribavirin dose (mg/kg)	1: <11.0	1	
	2: ≥11.0	5.173 (1.152–23.22)	0.032

through analysis of full-length core gene sequences identified seven polymorphisms significantly associated with increased HCC risk (36G/C [aaK12N], 209A [aaR70Q], 271U/C [aaL91M], 309A/C, 435A/C, 481A and 546A/C).[51] HCV core gene sequence data might provide useful information about HCC risk.

Recommendation 5: Amino acid substitutions in the HCV core region (aa70 and aa91) should be determined before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)

NS3 protein secondary structure

Recently, Ogata *et al.* reported that HCV-1b strains can be classified into different groups based on the secondary structure of an amino-terminal portion of the NS3 protein and that specific strains are more prevalent among patients with HCC.[52] Moreover, the cumulative incidence of HCC was highest among patients infected with specific group HCV-1b, in whom the risk of HCC significantly increased compared with that among patients infected with another group (hazard ratio = 4.95 [95% confidence interval = 1.43–17.11]) after adjustment for age and histological stage.[53]

Informative statement: NS3 protein secondary structure may be related to hepatocarcinogenesis. (Grade B.)

NATURAL HISTORY OF CH-C

Progression to cirrhosis and HCC

PREVIOUS PUBLICATIONS REPORTED that approximately 60–80% of patients with acute hepatitis C develop chronic infection in the natural course.[54–57] Because it is difficult to ascertain precisely when the HCV infection occurred except for patients who had blood transfusions, and because chronic infection progresses slowly and asymptomatically, the natural entity of the disease has not been elucidated fully. Seeff *et al.* compared the long-term prognosis of HCV antibody-positive and -negative young men and reported that liver disease-related death was very rare in HCV antibody-positive patients.[58,59] Kenny-Walsh studied the liver histology of 363 young women 17 years after HCV infection and showed that 83% had no or mild hepatic fibrosis whilst 2% had liver cirrhosis.[60] These results demonstrate that progression to serious liver disease is a rare event two decades after infection of young people with HCV.

On the other hand, in blood transfusion-associated CH-C patients the mean interval to liver cirrhosis is estimated to be approximately 20–30 years and that to HCC approximately 30–40 years.[61,62] Because HCC is the most serious complication of HCV-infected people, it is desirable to predict the overall incidence of HCC in each patient. Up to now, many investigators have reported a close relationship between the stage of hepatic fibrosis and incidence of HCC. According to reports from Japan, the annual incidence of new HCC in liver cirrhosis is estimated to be approximately 5–8%.[63–65]

Informative statement: The natural history of CH-C is highly variable. HCV infection does not have much impact on the overall mortality of all the infected people, whereas progression to liver cirrhosis is observed 20–30 years and to HCC 30–40 years after infection. In Japan, the annual incidence of HCC in liver cirrhosis is estimated to be 5–8%. (Level 2b, Grade B.)

Recommendation 6: Treatment of HCV-infected people should be determined in consideration of the higher annual incidence of HCC in patients with liver cirrhosis in Japan as compared to Western countries. (Level 2b/3, Grade B.)

Progression of fibrosis

The rate of progression of fibrosis varies among patients with CH-C. Poynard *et al.*[66] calculated the average progression rate of hepatic fibrosis in CH-C to be 0.133 fibrosis units/year. In Japan, Shiratori *et al.*[67] reported this to be 0.10 fibrosis units/year. In HCV carriers with persistently normal aminotransferase levels (PNALT), progression of hepatic fibrosis is slower. Persico *et al.*[68] reported that median histological scores did not differ after 5 years of follow up in PNALT and Okanoue *et al.*[69] calculated the average progression rate of hepatic fibrosis in PNALT to be 0.05 fibrosis units/year.

Informative statement: On average, progression of hepatic fibrosis in CH-C is 0.10–0.13 fibrosis score units/year. The hepatic stage/grade score of HCV carriers with PNALT *are generally low and the progression of hepatic fibrosis is slow. Excessive alcohol intake, insulin resistance and hepatic steatosis are the major factors which induce the progression of hepatic fibrosis. (Level 2b, Grade B.)*

Alanine aminotransferase (ALT) levels

Alanine aminotransferase is an easy tool to evaluate hepatocellular damage in liver diseases. In the past, a higher incidence of HCC was reported in liver cirrhotic patients with elevated ALT levels.[70] The normal range of serum ALT level varies according to the institutions or hospitals, but it is likely to be located between 30 IU/L

and 40 IU/L. Recently, Kumada *et al.*[71,72] demonstrated that the cumulative incidence of hepatocarcinogenesis increased in parallel with the increase in ALT average integration value in CH-C even in patients with normal ALT levels. In a community-based study, an elevated ALT level (>35 IU/l) was shown to be a significant risk factor of HCC development.[73]

Recommendation 7: To prevent the occurrence of HCC, levels of serum ALT should be controlled at below 30 IU/l. (Level 3, Grade A.)

IFN administration

More than two decades have passed since IFN began to be used to treat CH-C patients. Nowadays, more than 70% of HCV-infected people can be cured by the combination therapy of PEG-IFN plus RBV. However, even in patients who were cured of HCV infection and attained an SVR, the occurrence of HCC may be reported long after completion of IFN therapy. The risk factor of HCC occurrence after IFN therapy is a combination of advanced hepatic fibrosis score before therapy, older age and male sex.[74–76] Bruno *et al.*[75] reported that annual incidence of HCC occurrence in liver cirrhosis after attaining SVR was 0.66%, which was one-third of the incidence of HCC in liver cirrhosis without a virological response (non-SVR).

Recommendation 8: Surveillance is required for the occurrence of HCC in patients with CH-C and liver cirrhosis. Even if IFN-based therapy is successful in attaining SVR, screening for the detection of HCC by computed tomography (CT), magnetic resonance imaging or ultrasonography and measurement of the serum tumor markers should be carried out routinely, especially for patients with advanced hepatic fibrosis, older age and male sex, because they are at high risk for the occurrence of HCC. (Level 2b, Grade A.)

Indication of IFN therapy for CH-C

Interferon-based therapy is used to treat chronic HCV-infected patients worldwide and PEG-IFN plus RBV is the first choice indication for CH-C patients. Because IFN and RBV have a variety of adverse effects including depression and thyroid dysfunction, "who and how" to treat should be determined with caution. The AALSD practice guideline advocates that treatment decision should be individualized based on the severity of liver disease, the potential for serious side-effects, the likelihood of treatment response, the presence of comorbid condition and the patient's readiness for treatment.[1]

Recommendation 9: Treatment decision of IFN therapy for CH-C should be individualized based on the body/mental condition, probability of successful therapy and prolonged survival, and likelihood of provoking serious adverse effects. Scores of hepatic stage/grade should be considered as well. For aged patients, in whom HCV infection is regarded as the major determinant of survival, IFN-based therapy should be considered with caution. (Level 3, Grade A.)

PEG-IFN AND RBV COMBINATION THERAPY

Factors associated with virological response to PEG-IFN and RBV combination therapy

TREATMENT WITH PEG-IFN-α-2A or -2b together with RBV has been evaluated in two nationwide phase III registration trials in Japan.[77,78] In one trial, which determined efficacy of PEG-IFNα-2b and RBV,[79] the SVR rate to 48-week combination therapy was 48% (121/254) in patients with HCV genotype 1b and a high viral load (≥100 KIU/mL). Another trial using PEG-IFN-α-2a and RBV demonstrated an SVR rate to 48-week combination therapy of 59% (57/96) in patients with HCV genotype 1b and a high viral load (≥100 KIU/mL).[80] Based on these results, the currently recommended standard therapy for the patients with CH-C in Japan is the combination of a PEG-IFN together with RBV, except for the treatment naïve patients with a low viral load for whom a PEG-IFN monotherapy is recommended.

These clinical trials identified the following factors that are associated with non-SVR in patients with HCV genotype 1b and a high viral load: (i) older patients; (ii) non-responders to previous IFN therapy; (iii) advanced fibrosis; (iv) female sex; and (v) poor adherence below 80%. In marked contrast to the data from Europe and the USA, the SVR rate in Japanese female patients is lower than that in the male patients. Several community-based retrospective studies in Japan also demonstrated that female patients, especially older female patients, are more difficult to treat compared with other patients.[81,82] Other factors associated with virological response reported from Japan include the low-density lipoprotein cholesterol level,[83] α-fetoprotein (AFP) level,[83] whole-body insulin sensitivity index,[84] single nucleotide polymorphisms of MAPKAPK3,[85] *RIG-I*/IPS-1 ratio,[86] Th1/Th2 ratio[45] and PKR response.[87] Association between viral mutations and treatment response is discussed in depth above.

Recommendation 10: Predictors associated with a non-SVR to PEG-IFN and RBV include: (i) age older than 60 years, particularly older women; (ii) advanced fibro-

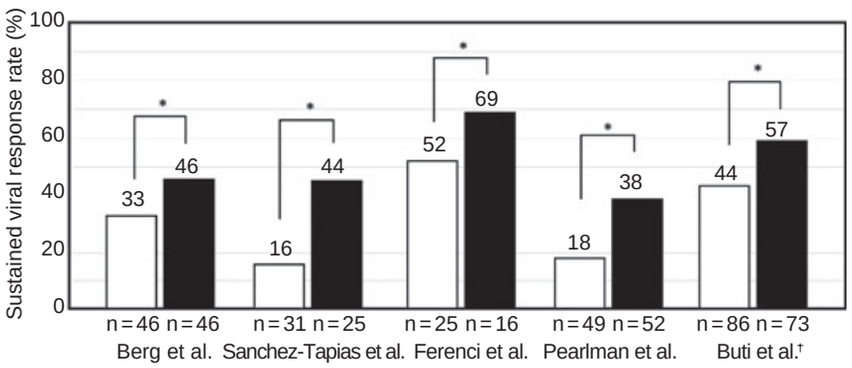


Figure 2 Comparison of sustained virological response rate between 48-week (open column) and 72-week (closed column) treatment with pegylated interferon and ribavirin in patients with partial early virological responder, which is defined as ≥2 log reduction in hepatitis C virus (HCV) RNA level compared to baseline HCV RNA level but detectable HCV RNA at treatment week 12. *Statistical significance between two treatment groups. †Comparison in patients with ≥80% adherence is shown.

sis; (iii) non-responder to previous IFN therapy; and (iv) poor adherence below 80%. (Level 2a, Grade B.)

Response-guided therapy for patients with HCV genotype 1

Measuring the rate of viral clearance from serum is helpful in predicting the likelihood of a response to PEG-IFN and RBV, and useful for determining the optimal duration of therapy. In two nationwide registration trials conducted in Japan,[77,78] the SVR rate was high, from 76–100% in patients whose HCV RNA was cleared rapidly from serum by week 4, and 71–73% in patients who achieved undetectable HCV RNA from week 5 to week 12. In contrast, the SVR rate in patients with late clearance of HCV RNA from week 13 to week 24 was low at 29–36%. No patients without clearance of HCV RNA by week 24 achieved SVR. It should be noted that time point of HCV clearance was determined by measurement of serum HCV RNA utilizing the Ampricor HCV method in these trials.

Recommendation 11: Measuring the time of viral clearance from serum is helpful in predicting the likelihood of a response to PEG-IFN and RBV. Measurement of HCV RNA is recommended at weeks 4, 12 and 24. (Level 1, Grade A.)

As mentioned above, patients whose HCV RNA measured by Amplicor HCV had not cleared by week 24 were unable to achieve SVR with 48-week standard PEG-IFN and RBV therapy. However, in a retrospective study conducted in 52 patients without HCV RNA clearance from serum by week 24, the rate of ALT normalization 6 months after the completion of therapy (so-called biochemical response) was 56% (5/9) and 62% (8/13) of patients achieved ALT normalization up to 2 years after the completion of therapy (sustained biochemical response).[88] Therefore, the proposal that recommends a continuation of PEG-IFN and RBV therapy for 48 weeks in biochemical responders at week 24 even without HCV clearance has been accepted widely in Japan. This proposal is in marked contrast to the AASLD practice guideline,[1] in which treatment discontinuation is strongly recommended in patients whose HCV RNA remains positive at week 24.

Recommendation 12: It is impossible to achieve SVR in patients without HCV RNA clearance by week 24 measured by Amplicor HCV. (Level 1, Grade A.) However, it is recommended to continue the therapy for 48 weeks even in patients without HCV RNA clearance by week 24 if ALT normalizes at week 24, because a sustained biochemical response can be obtained in these patients. (Level 4, Grade C.)

The strategy of extending therapy in patients with delayed virological responses, defined as clearance of HCV RNA between weeks 12 and 24, was evaluated in five studies.[89–93] These results cannot be compared directly with each other because of the heterogeneous study populations, differences in the baseline characteristics and the different regimens utilized amongst them. Nevertheless, the results showed a trend toward a higher SVR rate by extending therapy from 48 to 72 weeks in patients with delayed virological response (Fig. 2).[89–93]

In Japan, a randomized controlled trial was conducted in 113 patients with HCV genotype 1b and a high viral load, comparing a 48-week treatment group and extended treatment group where patients were treated for an additional 44 weeks after clearance of

HCV RNA from serum. In this trial, the SVR rate was 36% in the 48-week treatment group and 53% in the extended treatment group, and the SVR rate was significantly higher in patients in the extended treatment group who became HCV RNA-negative during the period week 16–24 (9% vs 78%, $P = 0.005$).[94] In addition, in a case–control study matched for age, sex and the timing of HCV RNA clearance from serum, the SVR rate was high at 62% in the 72-week treatment group ($n = 65$) compared to 33% in the 48-week treatment group ($n = 130$), and the extended treatment was particularly effective in patients with HCV core mutations at aa70 and aa91 as well as patients a with wild type of ISDR sequence.[79] Accordingly, 72-week extended treatment is recommended for patients who are slow to clear of HCV RNA between weeks 12 and 24.

Currently, HCV RNA clearance from serum is determined by real-time PCR detection, although most of former studies utilized the Amplicor HCV method for this purpose. Because real-time PCR is highly sensitive, it should be reevaluated in terms of who gains benefit from extended therapy. Currently, there is no sufficient evidence to determine this. Nevertheless, substantial number of community-based Japanese study using real-time PCR detection suggested that SVR could be obtained by 72-week treatment if HCV RNA became undetectable by week 36. Accordingly, when determining the timing of HCV RNA clearance using real-time PCR detection, 72-week treatment could be recommended for patients who achieve HCV RNA clearance between weeks 12 and 36.

Recommendation 13: 72-week extended therapy should be considered for patients with HCV genotype 1 who have delayed HCV RNA clearance from serum between weeks 12 and 24. (Level 2a, Grade B.)

Recommendation 14: When using a real-time detection PCR method for measurement of HCV RNA, SVR can be obtained by 72-week extended treatment in patients who have achieved HCV RNA clearance by week 36. (Level 2b, Grade C.)

Response-guided therapy for patients with HCV genotype 2

Six trials have evaluated a shortening of the duration of therapy from 24 weeks to 12–16 weeks for patients with chronic HCV genotype 2 and 3.[80,95–99] Although the data from some of these trials suggest that patients with genotype 2 and 3 infection who achieve viral clearance from serum by week 4 can shorten their treatment duration to 12–16 week,[80,95,99] the benefit of a shortening the duration of therapy remains controversial.[96] In a recent study by Mangia *et al.*, the factors associated with relapse after shorter duration of therapy are identified as age over 45 years, pre-treatment platelet count of less than 140×10^9/L, and body mass index over 30 kg/m^2,[100] suggesting shortening the duration of therapy can be considered only in particular patients without predictors associated with relapse. Because most Japanese patients have risk factors for relapse such as older age and advanced fibrosis, shortening the duration of the therapy is not generally recommended for Japanese patients with genotype 2, even if they achieve viral clearance by week 4.

PEG-IFN and RBV combination therapy in patients with compensated cirrhosis

In the early Western registration trials, patients with HCV-related compensated cirrhosis did achieve SVR but at lower rates than did those without cirrhosis.[101–103] Subsequently, there was one treatment study that focused exclusively on patients with compensated cirrhosis.[104] In this study, 124 patients with compensated cirrhosis were assigned randomly to an RBV 1000/1200-mg (standard dose) group and 600/800-mg (low dose) group to determine the efficacy of PEG-IFN and RBV combination therapy. The SVR was achieved in 52% of patients who received the standard RBV dose and in 38% of those treated with the low dose. Serious adverse events developed in 14% and 18% of recipients of the standard and low RBV doses, respectively, while dose reduction was necessary in 78% and 57% of the two groups, respectively. HCV genotype 2/3 and platelet count over 150×10^9/L were identified as factors contributing to SVR. Thus, patients with HCV-related compensated cirrhosis can be treated successfully with PEG-IFN and RBV but careful observation is needed because of an anticipated higher rate of adverse effects. Although PEG-IFN and RBV for patients with compensated cirrhosis has not been approved yet in Japan, the following recommendation is reasonable.

Recommendation 15: Patients with HCV-related compensated cirrhosis can be treated successfully with PEG-IFN and RBV but careful observation is needed because of an anticipated higher rate of adverse effects. (Level 3, Grade B.)

Retreatment with PEG-IFN and RBV combination therapy for patients who failed to respond to previous IFN treatment

Seven randomized controlled trials have been reported so far that examine the efficacy of PEG-IFN and RBV

combination therapy in patients who failed to respond to previous standard IFN therapy with or without RBV.[105–111] The SVR rate varies among these trials ranging 6–45%, and was lower among non-responders to previous IFN therapy compared with relapsers. In a study using PEG-IFNα-2b and RBV at two different doses (1.5 μg/kg per week of PEG-IFNα-2b together with 800 mg/day of RBV or 1.0 μg/kg per week of PEG-IFN together with 1000–1200 mg/day of RBV), the SVR rate was low at 10% and 6% in non-responders to previous treatment, but was high at 50% and 32% in relapsers, respectively.[109] In a phase III clinical trial in Japan, the SVR rate was also low in non-responders but sufficiently high in relapsers.[77] Accordingly, PEG-IFN and RBV combination therapy is well indicated for patients who relapse after standard IFN therapy with or without RBV.

Data on retreatment of patients who failed to respond to previous PEG-IFN plus RBV therapy have been evaluated in two trials.[112,113] In a randomized controlled trial that used two different doses of PEG-IFN-α-2a (360 or 180 μg/week) with two different durations of therapy (72- or 48-week),[112] an SVR was achieved in 7–14% of patients. It should be noted, however, that the SVR was favorable at 52% in patients who achieved HCV RNA clearance from serum by week 12 in the 72-week treatment arm.[112] In the other trial that used PEG-IFN-α-2b and RBV in 2333 patients who failed to respond to previous PEG or standard IFN together with RBV, an SVR was achieved in 56% of patients whose HCV RNA was cleared from serum by week 12 and in 48% of those with genotype 1.[113] Accordingly, it is reasonable to propose that SVR could be obtained by retreatment with PEG-IFN and RBV in patients who achieve HCV RNA clearance by week 12 of retreatment, even if they failed to respond to previous PEG-IFN and RBV combination therapy.[112,113] In contrast, in the AASLD practice guideline, retreatment with PEG-IFN and RBV is not recommended for patients who did not achieve an SVR after a prior full course of PEG-IFN and RBV. Because it is still unclear who is more likely to respond to retreatment with PEG-IFN and RBV, and new drugs such as protease inhibitors may be indicated in the near future for patients who failed to respond to previous PEG-IFN and RBV therapy, data with retreatment of PEG-IFN and RBV should be accumulated to enable a conclusive recommendation.

Recommendation 16: Retreatment with PEG-IFN and RBV can be considered for non-responders and relapsers who were treated previously with IFN-based therapy with or without RBV. An SVR could be obtained in these patients whose HCV RNA is cleared from serum by week 12 of retreatment with PEG-IFN and RBV. (Level 2b, Grade B.)

MONOTHERAPY WITH IFN OR PEG-IFN

IN JAPAN, IFN monotherapy has been used to treat HCV infection since 1992. Today, IFN monotherapy is used only in patients with specific characteristics because combination therapy with PEG-IFN and RBV has achieved a high rate of SVR. Recently, a large randomized control trial (RCT) of maintenance therapy with a low dose of PEG-IFN was reported.[114] There were no differences in progression of liver disease between a PEG-IFN group and a control group. However, Japanese studies of elderly patients or patients who received maintenance therapy for longer periods showed that IFN can improve outcomes in advanced hepatic fibrosis.

Naïve patients with low viral loads

Previous studies showed that 3 MIU of IFN monotherapy achieved SVR rates of 15–45% in patients with fewer than 2×10^6 copies of HCV.[115–118] Monotherapy with 180 μg/week of PEG-IFN-α-2a or 1.5 μg/kg per week of PEG-IFN-α-2b produced SVR rates of 16–46% in patients with fewer than 2×10^6 copies.[119–121] In Japanese patients with fewer than 1×10^5 copies of HCV, 6 MIU of IFN treatment for 24 weeks achieved an SVR rate of 86% (127/148).[122] PEG-IFN monotherapy for 48 weeks similarly achieved an SVR rate of 86% (106/123). A recent RCT showed that PEG-IFN monotherapy for 24 weeks produced the same SVR rate as similar treatment for 48 weeks in patients with fewer than 1×10^5 copies of HCV. On the basis of these results, monotherapy with IFN or PEG-IFN is considered to be an effective treatment for naïve patients with fewer than 5.0 log copies/mL of HCV.[123]

Recommendation 17: Monotherapy with IFN or PEG-IFN can be considered for naïve patients with low viral loads (<5.0 log copies/mL). (Level 2a, Grade B.)

Patients with chronic kidney disease

Patients with chronic kidney disease (CKD) who undergo hemodialysis have a high prevalence of HCV infection. In Japan, one study reported that HCV RNA was detected in 117 (22%) of 543 patients who underwent maintenance hemodialysis.[124] Hemodialysis patients infected with HCV have a higher mortality rate than uninfected hemodialysis patients.[125] This higher

mortality is attributed to the frequent progression to cirrhosis and/or HCC in HCV-infected patients who receive hemodialysis.

Because RBV is excreted renally, it is currently contraindicated in patients with CKD who have a creatinine clearance of less than 50 mL/min. In addition, pharmacokinetic studies have shown that the clearance of IFN is lower in patients who undergo hemodialysis than in patients who have normal renal function.[126]

Studies of antiviral therapy in patients who undergo hemodialysis suggest that IFN monotherapy is generally well tolerated and that SVR rates are higher than those in patients with normal renal function.[127] The overall SVR rate was reported to be 33–37% in hemodialysis patients.[128] However, the number of subjects in these trials was too low to support confident conclusions. Adverse events are common in this population, and many patients discontinue therapy prematurely because of such events. A recent RCT showed in EASL 2008 that 135 μg/week of PEG-IFN-α-2a for 48 weeks achieved an SVR rate of 39% (23/38), whereas a dose of 90 μg/week produced an SVR rate of 35% (16/43). In 74% of the patients, treatment was completed as scheduled.

Another important point is when to initiate antiviral therapy in hemodialysis patients. IFN might induce allograft rejection and renal failure.[129] Therefore, IFN therapy should be considered before renal transplantation. The next issue to be resolved is the efficacy and safety of low-dose RBV combination therapy in hemodialysis patients.

In 2008, KDIGO proposed guidelines for the treatment of patients with CKD.[130] In Japan, a committee including hepatologists and specialists for CKD is planning a clinical trial for HCV-infected patients with CKD.

Recommendation 18: 3 MIU of IFN thrice weekly or 90 or 135 μg of PEG-IFN-α-2a weekly is recommended for patients with CKD. (Level 2a, Grade B.)

Patients with acute HCV infection

Acute HCV infection progresses to chronic infection in approximately 70% of patients.[131] Antiviral treatment should therefore be considered for this group of patients. On the other hand, it is difficult to identify patients with self-limited disease not requiring therapy. The results of previous studies indicate that anti-HCV treatment should be initiated if HCV RNA is detected continuously for more than 12–16 weeks. If treatment is initiated within this period, monotherapy with IFN or PEG-IFN achieves an SVR rate of more than 80% in patients with acute HCV infection.[132] Reliable evidence showing that additional treatment with RBV improves the SVR rate in such patients is not available.

Recommendation 19: Patients with acute HCV infection should be considered as candidates for antiviral therapy. If HCV RNA is detected continuously for 12 or 16 weeks from the onset, treatment with 6 MIU of IFN or 180 μg of PEG-IFN monotherapy should be initiated. (Level 2a, Grade B.)

Patients who receive curative treatment for HCC

Hepatocellular carcinoma frequently recurs in HCV-infected patients, even after curative therapy for HCC. Prevention of the recurrence of HCC is essential in such patients. Several RCT showed that the incidence of HCC was low in an IFN-treated group, compared to a control group (Table 4).[133,134] For example, Kubo *et al.* reported that 3 MIU IFN monotherapy thrice weekly for 96 weeks inhibited the recurrence of HCC in patients who had undergone a curative resection.[134] Furthermore, Shiratori *et al.* performed an RCT in 74 patients who had received curative percutaneous ethanol injection therapy for HCC. They reported that second and third recurrences of HCC were less frequent in patients who received IFN.[135] In an Italian study of 150 patients who had undergone curative resection, the recurrence rate of HCC 2 years after operation was significantly lower among patients who received IFN.[136]

Japanese studies showed that the survival rate was also improved by IFN treatment owing to the suppression of HCC and/or the progression of hepatic failure.[137,138]

Recommendation 20: IFN therapy should be considered for patients after curative treatment for HCC. (Level 1, Grade A.)

Maintenance therapy for patients with advanced hepatic fibrosis

Previous studies of patients with advanced hepatic fibrosis, defined as a fibrosis score 3 or 4, showed that IFN monotherapy inhibited the occurrence of HCC, compared to patients who did not receive IFN.[64,139,140] In Japanese studies, IFN was effective not only in SVR patients, but also in non-SVR patients.[139,141] On the other hand, an Italian study showed that the incidence of HCC decreased only in cirrhotic patients in whom HCV was eradicated by IFN therapy.[75]

Case–control studies in patients older than 60 years showed that a low dose of IFN reduced ALT and AFP levels and decreased the incidence of HCC, compared to a control group.[142,143] RCT for IFN monotherapy non-

Table 4 Interferon monotherapy for patients after curative treatment for hepatocellular carcinoma

Author	Study design	No. of patients (IFN group vs non-IFN group)	Age (IFN group vs non-IFN group)	Interferon	Sustained virological response	Follow-up duration (months)	HCC recurrence (IFN group vs non-IFN group)	Survival (IFN group vs non-IFN group)
Ikeda *et al.*	RCT	10 vs 10	60 vs 65	beta	0	25	10% vs 70% $P = 0.0004$	
Kubo *et al.*	RCT	15 vs 15	62 vs 60	alpha	2 (13%)	54	60% vs 87% $P = 0.055$	80% vs 50% $P = 0.041$
Suou *et al.*	Pilot study	18 vs 22	61 vs 62	alpha	6 (33%)	60	28% vs 82% $P < 0.001$	100% vs 73% $P < 0.05$
Shiratori *et al.*	RCT	49 vs 25	61 vs 63	alpha	14 (29%)	60	80% vs 92%	68% vs 48%
Lin *et al.*	RCT	8 vs 6	61 vs 59	alpha	no data	27	63% vs 83% $P = 0.34$	
Jeong *et al.*	Prospective case–control study	16 vs 16	69 vs 68	alpha	2 (13%)	36	69% vs 80% $P = 0.157$	100% vs 88% $P = 0.45$
Sakaguchi *et al.*	Case–control study	24 vs 33	69 vs 67	alpha	1 (4%)	36	14% vs 73% $P = 0.011$	100% vs 94% $P = 0.25$
Mazzaferro *et al.*	RCT	76 vs 74	65 vs 67	alpha	2 (3%)	45	76% vs 94% $P = 0.49$	64% vs 52% $P = 0.47$
Akamatsu *et al.*	Retrospective study	53 vs 399	60 vs 68	no data	17 (32%)	72		88%, 71% vs 53.2% $P = 0.025$
Kudo *et al.*	Case–control study	43 vs 84	65 vs 66	alpha or pegylated IFN	2 (5%)	60	56% s 71% $P = 0.04$	86% vs 56% $P = 0.004$

IFN, interferon; HCC, hepatocellular carcinoma; RCT; randomized control study.

responders showed that histological fibrosis and activity was improved in the assigned IFN-treated group. In contrast, in the untreated group, the fibrosis score did not decline.[144] In Japan, several studies support the effectiveness of low-dose IFN maintenance therapy.[145–147] In the USA, an RCT of 53 patients in whom a histological response, but not a viral response was induced by 6 MIU of IFN showed that 3 MIU of IFN for 24 months improved the degree of hepatic fibrosis.

However, the Hepatitis C Antiviral Long-Term Treatment against Cirrhosis (HALT-C) trial found no difference in the progression of liver disease between a low-dose PEG-IFN group and a control group.[114] The large discrepancy in the effectiveness of IFN maintenance therapy between the HALT-C trial and Japanese trials might be attributed to several factors. First, the study designs differed. One of the most important differences was related to the patients' clinical characteristics. For example, patients enrolled in Japanese studies were older than those in the HALT-C trial. Elderly patients have a higher incidence of HCC than younger patients. It is suggested that the tumor-suppressive effect of IFN maintenance therapy might be more clearly demonstrated in a high-risk group, including elderly patients.[138]

Until more data become available, the decision to perform IFN maintenance therapy should be made on an individual basis.

Recommendation 21: IFN maintenance therapy is a treatment option that can inhibit the progression of liver disease in patients with advanced hepatic fibrosis, especially in those who are elderly. However, the effect of monotherapy with IFN or PEG-IFN remains uncertain in non-responders to combination therapy with PEG-IFN plus RBV. (Level 2a, Grade C.)

CONSENSUS ON THERAPEUTIC STRATEGY FOR CH-C

Indication of antiviral therapy

IKEDA *ET AL.* elucidated the necessities of antiviral therapy for elderly patients with chronic HCV infection.[132] At 5 and 10 years, hepatocarcinogenesis rates in the intermediate ($100–140 \times 10^9$/L) and low platelet ($<100 \times 10^9$/L) groups were 10.9% and 21.6% in the IFN group ($n = 217$) and 19.5% and 43.0% in the untreated group ($n = 459$), respectively ($P = 0.0005$). IFN independently decreased the risk of carcinogenesis risk with a hazard ratio of 0.56 ($P = 0.035$). On the other hand, in the high platelet ($\geq 150 \times 10^9$/L) group, no significant difference was found in 5- and 10-year carcinogenesis rates between the IFN-treated group ($n = 228$) and the untreated group ($n = 585$) ($P = 0.69$). Furthermore, IFN treatment significantly increased cumulative survival in the lower platelet subgroup ($P = 0.0001$) but did not affect the higher platelet subgroup ($P = 0.08$). Thus, the necessities of antiviral therapy are shown to be greater in elderly patients with advanced fibrosis, although adverse effects of IFN are reported to be more frequent and the efficacy of IFN to be lower in such patients.[148–150]

Therefore, the indication of antiviral therapy should be considered in the following order: the necessity of treatment, first; safety of treatment, second; and efficacy of treatment for a patient, last. Antiviral therapy should not be given up because the expected SVR rate is low.

Recommendation 22: Antiviral therapy should be offered even to CH-C patients whose SVR rates are expected to be low if type C chronic liver disease is the prognostic determinant (prognosis is improved by HCV elimination) for the individual patient, and the expected adverse effects are tolerable to the patients. (Level 6, Grade B/C.)

Effect of drug adherence of PEG-IFN and RBV on virological response

The relationship between drug exposure and antiviral effect of PEG-IFN plus RBV combination therapy has been reported in several papers.[101,151–155] McHutchison *et al.* revealed that the SVR rate in patients who received 80% or more of their total planned doses of PEG-IFN-α-2b and RBV for 80% or more of the scheduled duration of therapy was significantly higher than that of patients who received less than 80% of one or both drugs (51% vs 34%) and also suggested that the impact of dose reduction was greatest in patients for whom the dose had to be decreased within the first 12 weeks of treatment.[152]

Recently, Oze *et al.* evaluated how reducing drug doses affects complete early virological response (c-EVR) defined as HCV RNA negativity at week 12, using 984 patients with CH-C genotype 1.[156] As a result, the mean dose of PEG-IFN-α-2b, and not RBV, during the first 12 weeks was the independent factor for c-EVR ($P = 0.02$), not RBV.

Hiramatsu *et al.* reported on whether dose reduction of RBV (or PEG-IFN) has an effect on virological relapse in PEG-IFN plus RBV treatment for patients with CH-C genotype 1.[157] In the analysis of 472 patients responding to PEG-IFN-α-2b plus RBV, stepwise reduction of the

RBV dose was associated with a stepwise increase in relapse rate from 11% to 60% (Fig. 3).

Improving the treatment tolerability for genotype 2 or 3 patients has focused on dose reduction of treatment drugs. Weiland *et al.* examined low-dose PEG-IFN-α-2a (135 μg/week) with a weight-based standard dose of RBV (11 mg/kg daily) for genotype 2 and 3 patients.[158] Recently, Inoue *et al.* reported neither PEG-IFN nor RBV drug exposure were critical in reaching rapid virological response and SVR.[159]

Recommendation 23: In genotype 1 patients, PEG-IFN is dose-dependently correlated with c-EVR, independent of RBV dose. The administration over 80% of the scheduled dose of PEG-IFN-α-2a or over 1.2 μg/kg per week of PEG-IFN-α-2b should be chosen as a starting dose: a marked dose reduction of PEG-IFN should not be risked at the start even for patients with disadvantage (e.g. aged patients). (Level 2b/3, Grade B.)

Recommendation 24: In genotype 1 patients, RBV shows a dose-dependent correlation with the relapse after treatment. Maintaining the RBV dose over 80% of the scheduled dose or over 10 mg/kg per day (12 mg/kg per day, if possible) during the complete treatment period can lead to suppression of the relapse in HCV genotype 1 patients responding to PEG-IFN-α-2b plus RBV, especially in c-EVR patients. (Level 2b/3, Grade B.)

Recommendation 25: In genotype 2/3 patients, reducing drug doses of PEG-IFN and RBV (down to 400 mg/day) has no significant effect on virological responses. (Level 2a, Grade B.)

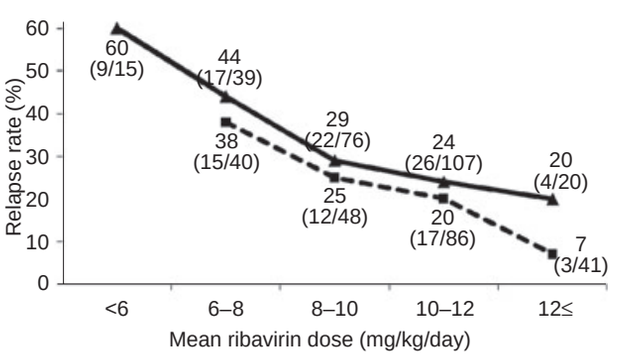


Figure 3 Relapse rate according to pegylated interferon (PEG-IFN)-α-2b and ribavirin doses during treatment of patients who completed treatment, which was stratified with the mean ribavirin doses (–▲–). Group with the mean PEG-IFN dose <1.4 μg kg/week (···■···). Group with the mean PEG-IFN dose ≥1.4 μg kg/week. There was no significant difference between the two PEG-IFN-α-2b-dose groups ($P = 0.17$).

Treatment for patients without elimination of HCV

Tarao *et al.* showed the rate of HCC appearance was significantly higher in HCV-related cirrhotic patients with a high ALT value (≥80 IU/mL) than in those with a lower ALT value (<80 IU/mL).[70] This suggested that suppression of inflammation in the liver with HCV infection is very important to prevent the hepatocarcinogenesis in patients with HCV-related cirrhosis.

Omata *et al.* assessed the effects of oral ursodeoxycholic acid (UDCA) on serum biomarkers. CH-C patients with elevated ALT were assigned randomly to 150 ($n = 199$), 600 ($n = 200$) or 900 mg/day ($n = 197$) UDCA intake for 24 weeks. As a result, the median changes in serum ALT at the end of treatment were shown to be −15.3, −29.2 and −36.2%, respectively, although serum HCV RNA did not change in any group.[160]

A glycyrrhizin product, Stronger Neo-Minophagen C (SNMC; Minophagen Pharmaceutical, Tokyo, Japan), is used widely in Japan and has been reported to improve ALT levels and liver inflammation.[161,162] Furthermore, Ikeda *et al.* reported liver carcinogenesis was suppressed by long-term administration of glycyrrhizin, using a cohort of 1249 patients, and its favorable effect on hepatocellular carcinogenesis in those patients with IFN-resistant CH-C.[163,164]

Repeated phlebotomy has been shown to be effective for the improvement of serum ALT as well as progression of fibrosis,[32] however, it remains controversial whether the effects of IFN improve with extensive phlebotomy.[165–169]

In Japan, Yano *et al.* showed the iron removal by repeated phlebotomy improved serum ALT levels in patients with CH-C.[170]

Recommendation 26: Patients whose HCV RNA was not eradicated by PEG-IFN plus RBV and whose ALT and/or AFP levels were not improved by IFN monotherapy or those without indication for IFN therapy should be treated with the liver-supporting therapy (SNMC, UDCA), and if the effect of this medication is inadequate, phlebotomy can be used in combination. (Level 3/6, Grade B/C.)

Treatment of patients with decompensated cirrhosis

The compensated patients who failed to eradicate HCV by antiviral therapy and decompensated patients should be referred for consideration of liver transplantation and liver supporting therapy should be performed. Long-

term nutritional supplementation with oral branched-chain amino acid (BCAA) has been shown to be useful to prevent progressive hepatic failure and to improve surrogate markers.[171,172] Early interventional with oral BCAA was shown to prolong the liver transplant waiting period by preserving hepatic reserve in cirrhosis.

Recommendation 27: Patients with compensated cirrhosis for the prevention of hepatocellular carcinogenesis, should be treated by not only IFN but also with liver supporting therapy (SNMC, UDCA) and/or phlebotomy and/or BCAA in order to improve the liver inflammation and AFP levels. (Level 3, Grade C.)

Novel antiviral drugs

Telaprevir, a protease inhibitor specific to the HCV nonstructural 3/4A serine protease, reduced HCV RNA levels rapidly in early studies. McHuthison *et al.* reported the improved SVR rate with triple therapy for 12 weeks followed by PEG-IFN-α-2a and RBV for 12 weeks.

Thus, the treatment for CH-C is progressing. Therefore, as a treatment strategy, PEG-IFN plus RBV combination therapy should be performed early for aged patients and the patients with the advanced fibrosis. However, the novel antiviral drugs, such as protease inhibitors and polymerase inhibitors, should be taken into account as a candidate of treatment for the patients who can wait for the oncoming drugs.

Recommendation 28: Novel antiviral drugs, such as a protease inhibitor or a polymerase inhibitor, in combination with PEG-IFN plus RBV, can improve the SVR rates in genotype 1 CH-C patients. (Level 2a, Grade A.)

REFERENCES

1 Ghany MG, Strader DB, Thomas DL *et al.* AASLD practice guidelines. Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology* 2009; **49:** 1335–74.
2 Shiffman RN, Shekelle P, Overhage J *et al.* Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standadization. *Ann Intern Med* 2003; **139:** 493–98.
3 Pileri P, Uematsu Y, Campagnoli S *et al.* Binding of hepatitis C virus to CD81. *Science* 1998; **282:** 938–41.
4 Scarselli E, Ansuini H, Cerino R *et al.* The human scavenger receptor class B type I is a novel candidate receptor for the hepatitis C virus. *EMBO J* 2002; **21:** 5017–25.
5 Evans MJ, von Hahn T, Tscherne DM *et al.* Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007; **446:** 801–5.
6 Dubuisson J, Helle F, Cocquerel L. Early steps of the hepatitis C virus life cycle. *Cell Microbiol* 2008; **10:** 821–7.
7 Ploss A, Evans MJ, Gaysinskaya VA *et al.* Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009; **457:** 882–6.
8 Flint M, von Hahn T, Zhang J *et al.* Diverse CD81 proteins support hepatitis C virus infection. *J Virol* 2006; **80:** 11331–42.
9 Sumpter R Jr, Loo YM, Foy E *et al.* Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, *RIG-I*. *J Virol* 2005; **79:** 2689–99.
10 Yoneyama M, Kikuchi M, Natsukawa T *et al.* The RNA helicase *RIG-I* has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol* 2004; **5:** 730–7.
11 Li K, Chen Z, Kato N, Gale M Jr, Lemon SM. Distinct poly(I-C) and virus-activated signaling pathways leading to interferon-beta production in hepatocytes. *J Biol Chem* 2005; **280:** 16739–47.
12 Kawai T, Takahashi K, Sato S *et al.* IPS-1, an adaptor triggering *RIG-I*- and Mda5-mediated type I interferon induction. *Nat Immunol* 2005; **6:** 981–8.
13 Sen GC. Viruses and interferons. *Annu Rev Microbiol* 2001; **55:** 255–81.
14 Loo YM, Owen DM, Li K *et al.* Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. *Proc Natl Acad Sci USA* 2006; **103:** 6001–6.
15 Gale M Jr, Foy EM. Evasion of intracellular host defense by hepatitis C virus. *Nature* 2005; **436:** 939–45.
16 Bode JG, Ludwig S, Ehrhardt C *et al.* IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. *FASEB J* 2003; **17:** 488–90.
17 Alexander WS. Suppressors of cytokine signalling (SOCS) in the immune system. *Nat Rev Immunol* 2002; **2:** 410–6.
18 Polyak SJ, Khabar KS, Paschal DM *et al.* Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. *J Virol* 2001; **75:** 6095–106.
19 Taylor DR, Shi ST, Romano PR *et al.* Inhibition of the interferon-inducible protein kinase PKR by HCV E2 protein. *Science* 1999; **285:** 107–10.
20 Noguchi T, Satoh S, Noshi T *et al.* Effects of mutation in hepatitis C virus nonstructural protein 5A on interferon resistance mediated by inhibition of PKR kinase activity in mammalian cells. *Microbiol Immunol* 2001; **45:** 829–40.
21 Moriya K, Nakagawa K, Santa T *et al.* Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. *Cancer Res* 2001; **61:** 4365–70.
22 Okuda M, Li K, Beard MR *et al.* Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; **122:** 366–75.

23 Korenaga M, Wang T, Li Y *et al.* Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *J Biol Chem* 2005; **280:** 37481–8.
24 Shintani Y, Fujie H, Miyoshi H *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126:** 840–8.
25 Kawaguchi T, Yoshida T, Harada M *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165:** 1499–508.
26 Perlemuter G, Sabile A, Letteron P *et al.* Hepatitis C virus core protein inhibits microsomal triglyceride transfer protein activity and very low density lipoprotein secretion: a model of viral-related steatosis. *FASEB J* 2002; **16:** 185–94.
27 Moriishi K, Mochizuki R, Moriya K *et al.* Critical role of PA28gamma in hepatitis C virus-associated steatogenesis and hepatocarcinogenesis. *Proc Natl Acad Sci USA* 2007; **104:** 1661–6.
28 Nishina S, Hino K, Korenaga M *et al.* Hepatitis C virus-induced reactive oxygen species raise hepatic iron level in mice by reducing hepcidin transcription. *Gastroenterology* 2008; **134:** 226–38.
29 Miura K, Taura K, Kodama Y, Schnabl B, Brenner DA. Hepatitis C virus-induced oxidative stress suppresses hepcidin expression through increased histone deacetylase activity. *Hepatology* 2008; **48:** 1420–9.
30 Veldt BJ, Chen W, Heathcote EJ *et al.* Increased risk of hepatocellular carcinoma among patients with hepatitis C cirrhosis and diabetes mellitus. *Hepatology* 2008; **47:** 1856–62.
31 Ohata K, Hamasaki K, Toriyama K *et al.* Hepatic steatosis is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis C virus infection. *Cancer* 2003; **97:** 3036–43.
32 Kato J, Kobune M, Nakamura T *et al.* Normalization of elevated hepatic 8-hydroxy-2′-deoxyguanosine levels in chronic hepatitis C patients by phlebotomy and low iron diet. *Cancer Res* 2001; **61:** 8697–702.
33 Furutani T, Hino K, Okuda M *et al.* Hepatic iron overload induces hepatocellular carcinoma in transgenic mice expressing the hepatitis C virus polyprotein. *Gastroenterology* 2006; **130:** 2087–98.
34 Romero-Gomez M, Del Mar Viloria M, Andrade RJ *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128:** 636–41.
35 Harrison SA, Brunt EM, Qazi RA *et al.* Effect of significant histologic steatosis or steatohepatitis on response to antiviral therapy in patients with chronic hepatitis C. *Clin Gastroenterol Hepatol* 2005; **3:** 604–9.
36 Martinot-Peignoux M, Boyer N, Cazals-Hatem D *et al.* Prospective study on anti-hepatitis C virus-positive patients with persistently normal serum alanine transaminase with or without detectable serum hepatitis C virus RNA. *Hepatology* 2001; **34:** 1000–5.
37 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128:** 343–50.
38 Strader DB, Wright T, Thomas DI, Seeff LB. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004; **38:** 1147–71.
39 Tsubota A, Chayama K, Ikeda K *et al.* Factors predictive of response to interferon-α therapy in hepatitis C virus infection. *Hepatology* 1994; **19:** 1088–94.
40 Lau JY, Davis GL, Kniffen J *et al.* Significance of serum hepatitis C virus RNA in chronic hepatitis C. *Lancet* 1993; **341:** 1501–4.
41 Yamada G, Takatani M, Kishi F *et al.* Efficacy of interferon alfa therapy in chronic hepatitis C patients depends primarily on hepatitis C virus RNA level. *Hepatology* 1995; **22:** 1351–4.
42 Enomoto N, Sakuma I, Asahina Y *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. *J Clin Invest* 1995; **96:** 224–30.
43 Enomoto N, Sakuma I, Asahina Y *et al.* Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334:** 77–81.
44 Pascu M, Martus P, Hohne M *et al.* Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. *Gut* 2004; **53:** 1345–54.
45 Shirakawa H, Matsumoto A, Joshita S *et al.* Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. *Hepatology* 2008; **48:** 1753–60.
46 El-Shamy A, Nagano-Fujii M, Sasase N *et al.* Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; **48:** 38–47.
47 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; **48:** 372–80.
48 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; **46:** 403–10.
49 Donlin MJ, Cannon NA, Yao E *et al.* Pretreatment sequence diversity differences in the full-length hepatitis

C virus open reading frame correlate with early response to therapy. *J Virol* 2007; **81:** 8211–24.
50 Akuta N, Suzuki F, Kawamura Y *et al.* Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; **46:** 1357–64.
51 Fishman SL, Factor SH, Balestrieri C *et al.* Mutations in the hepatitis C virus core gene are associated with advanced liver disease and hepatocellular carcinoma. *Clin Cancer Res* 2009; **15:** 3205–13.
52 Ogata S, Florese RH, Nagano-Fujii M *et al.* Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. *J Clin Microbiol* 2003; **41:** 2835–41.
53 Nishise Y, Saito T, Sugahara K *et al.* Risk of hepatocellular carcinoma and secondary structure of hepatitis C virus (HCV) NS3 protein amino-terminus, in patients infected with HCV subtype 1b. *J Infec Dis* 2007; **196:** 1006–9.
54 Mattsson L, Sonnerborg A, Weiland O. Outcome of acute symptomatic non-A, non-B hepatitis: a 13-years follow-up study of hepatitis C virus markers. *Liver* 1993; **13:** 274–8.
55 Barrera JM, Bruguera M, Ercilla MG *et al.* Persistent hepatitis C viremia after self-limitng posttransfusion hepatitis C. *Hepatology* 1995; **21:** 639–44.
56 Amoroso P, Rapicetta M, Tosti ME *et al.* Correlation between virus genotype and chronicity rate in acute hepatitis C. *J Hepatol* 1998; **28:** 939–44.
57 Tanaka E, Kiyosawa K. Natural history of acute hepatitis C. *J Gastroenterol Hepatol* 2000; **15:** E97–104.
58 Seeff LB, Buskell-Bales Z, Wright EC *et al.* Long-term mortality after transfusion-associated non-A, Non B hepatitis. *N Engl J Med* 1992; **327:** 1906–11.
59 Seeff LB, Miller RN, Rabkin CS *et al.* 45-year follow-up of hepatitis C virus infection in healthy young adults. *Ann Intern Med* 2000; **132:** 105–11.
60 Kenny-Walsh E, the Irish Hepatology Research Group. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. *N Eng J Med* 1999; **340:** 1228–33.
61 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology* 1990; **12:** 671–5.
62 Tong MJ, El-Farra NS, Reikes AR *et al.* Clinical outcomes after transfusion-associated hepatitis C. *N Eng J Med* 1995; **332:** 1463–6.
63 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatojogy* 1993; **18:** 47–53.
64 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Intern Med* 1999; **131:** 174–81.
65 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. *J Hepatol* 1999; **30:** 653–9.
66 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METEVIR, CLINIVIR and DOSVIRC groups. *Lancet* 1997; **349:** 825–32.
67 Shiratori Y, Imazeki F, Moriyama M *et al.* Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132:** 517–24.
68 Persico M, Persico E, Suozzo R *et al.* Natural history of hepatitis C virus carriers with persistently normal aminotransferase. *Gastroenterology* 2000; **118:** 760–4.
69 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatol* 2005; **43:** 599–605.
70 Tarao K, Rino Y, Ohkawa S *et al.* Association between high serum ALT and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients wit5h hepatitis C virus-associated cirrhosis. *Cancer* 1999; **86:** 589–94.
71 Kumada T, Toyoda H, Kiriyama S *et al.* Relation between incidence of hepatic cardcinhogenesis and integration value of alanine aminmotransferase in patients with hepatitis C virus infection. *Gut* 2007; **56:** 738–9.
72 Kumada T, Toyoda H, Kiriyama S *et al.* Long-term follow-up of patients with hepatitis C with a normal alanime aminotransferase. *J Med Virol* 2009; **81:** 446–51.
73 Suruki R, Hayashi K, Kusumoto K *et al.* Alanine aminotransferase level as a predictor of hepatitis C virus-associated hepatocellular carcinoma incidence in a community-based population in Japan. *Int J Cancer* 2006; **119:** 192–5.
74 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after a sustained response to interferon therapy. *Cancer* 2004; **101:** 1616–22.
75 Bruno S, Stroffolini T, Colombo M *et al.* Sustained virological response to interferon-alpha is associated with improved outcome in HCV-related cirrhosis: a retrospective study. *Hepatology* 2007; **45:** 579–87.
76 George S, Bacon BR, Brunt EM *et al.* Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49:** 729–38.

77 Iino S, Okita K, Omata M *et al.* Clinical efficacy of PEG-Interferon alfa-2b and ribavirin combination therapy for 48 weeks in chronic hepatitis C patients with genotype 1 and high viral load –retrospective comparison with Interferon alfa-2b and ribavirin combination therapy for 24 weeks. *Kantansui* 2004; **49:** 1099–121.
78 Yamada G, Iino S, Okuno T *et al.* Virological response in patients with hepatitis C virus genotype 1b and a high viral load: impact of peginterferon-alpha-2a plus ribavirin dose reductions and host-related factors. *Clin Drug Investig* 2008; **28:** 9–16.
79 Akuta N, Suzuki F, Hirakawa M *et al.* A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* 2009; **81:** 452–8.
80 Mangia A, Santoro R, Minerva N *et al.* Peginterferon alfa-2b and ribavirin for 12 vs. 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2005; **352:** 2609–17.
81 Sezaki H, Suzuki F, Kawamura Y *et al.* Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. *Dig Dis Sci* 2009; **54:** 1317–24.
82 Kogure T, Ueno Y, Fukushima K *et al.* Pegylated interferon plus ribavirin for genotype Ib chronic hepatitis C in Japan. *World J Gastroenterol* 2008; **14:** 7225–4230.
83 Akuta N, Suzuki F, Kawamura Y *et al.* Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2007; **79:** 1686–95.
84 Mizuta T, Kawaguchi Y, Eguchi Y *et al.* Whole-body insulin sensitivity index is a highly specific predictive marker for virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients with genotype 1b and high viral load. *Dig Dis Sci* 2010; **55:** 183–9.
85 Tsukada H, Ochi H, Maekawa T *et al.* A Polymorphism in MAPKAPK3 Affects Response to Interferon Therapy for Chronic Hepatitis C. *Gastroenterology* 2009; **136:** 1796–805.
86 Asahina Y, Izumi N, Hirayama I *et al.* Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response. *Gastroenterology* 2008; **134:** 1396–405.
87 Asahina Y, Izumi N, Umeda N *et al.* Pharmacokinetics and enhanced PKR response in patients with chronic hepatitis C treated with pegylated interferon alpha-2b and ribavirin. *J Viral Hepat* 2007; **14:** 396–403.
88 Sezaki H, Suzuki F, Kawamura Y *et al.* Evaluation of long-term biochemical responses to combination therapy of interferon plus ribavirin in those infected with hepatitis C virus genotype 1b and high baseline viral load. *Hepatol Res* 2007; **37:** 787–92.
89 Berg T, von Wagner M, Nasser S *et al.* Extended treatment duration for hepatitis C virus type 1: comparing 48 versus 72 weeks of peginterferon-Alfa-2a plus ribavirin. *Gastroenterology* 2006; **130:** 1086–109.
90 Sanchez-Tapias JM, Diago M, Escartin P *et al.* Peginterferon-alfa2a plus ribavirin for 48 versus 72 weeks in patients with detectable hepatitis C virus RNA at week 4 of treatment. *Gastroenterology* 2006; **131:** 451–60.
91 Ferenci P, Laferl H, Scherzer TM *et al.* Customizing treatment with peginterferon alfa-2a (40kD) (PEGASYS®) plus ribavirin (COPEGUS®) in patient with HCV genotype 1 or 4 infection: interim results of a prospective randomized trial. *Hepatology* 2006; **44:** 336a.
92 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; **46:** 1688–94.
93 Buti M, Lurie Y, Zakharova NG *et al.* Extended treatment duration in chronic hepatitis C genotype 1-infected slow responders: final results of the SUCCESS study (abstract #141). *J Hepatol* 2009; **50** (Suppl 1)**:** S58.
94 Ide T, Hino T, Ogata K *et al.* A randomized study of extended treatment with peginterferon alpha-2b plus ribavirin based on time to HCV RNA negative-status in patients with genotype 1b chronic hepatitis C. *Am J Gastroenterol* 2009; **104:** 70–5.
95 von Wagner M, Huber M, Berg T *et al.* Peginterferon-alpha-2a (40KD) and ribavirin for 16 or 24 weeks in patients with genotype 2 or 3 chronic hepatitis C. *Gastroenterology* 2005; **129:** 522–7.
96 Shiffman ML, Suter F, Bacon BR *et al.* Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. *N Engl J Med* 2007; **357:** 124–34.
97 Dalgard O, Bjøro K, Ring-Larsen H *et al.* Pegylated interferon alfa and ribavirin for 14 versus 24 weeks in patients with hepatitis C virus genotype 2 or 3 and rapid virological response. *Hepatology* 2008; **47:** 35–42.
98 Lagging M, Langeland N, Pedersen C *et al.* Randomized comparison of 12 or 24 weeks of peginterferon alpha-2a and ribavirin in chronic hepatitis C virus genotype 2/3 infection. *Hepatology* 2008; **47:** 1837–45.
99 Yu ML, Dai CY, Huang JF *et al.* A randomised study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut* 2007; **56:** 553–9.
100 Mangia A, Minerva N, Bacca D *et al.* Determinants of relapse after a short (12 weeks) course of antiviral therapy and re-treatment efficacy of a prolonged course in patients with chronic hepatitis C virus genotype 2 or 3 infection. *Hepatology* 2009; **49:** 358–63.
101 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358:** 958–65.

102 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347:** 975–82.
103 Hadziyannis SJ, Sette H Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140:** 346–55.
104 Helbling B, Jochum W, Stamenic I *et al.* HCV-related advanced fibrosis/cirrhosis: randomized controlled trial of pegylated interferon alpha-2a and ribavirin. *J Viral Hepat* 2006; **13:** 762–9.
105 Bergmann JF, Vrolijk JM, van der Schaar P *et al.* Gamma-glutamyltransferase and rapid virological response as predictors of successful treatment with experimental or standard peginterferon-alpha-2b in chronic hepatitis C non-responders. *Liver Int* 2007; **27:** 1217–25.
106 Diago M, Crespo J, Olveira A *et al.* Clinical trial: pharmacodynamics and pharmacokinetics of re-treatment with fixed-dose induction of peginterferon alpha-2a in hepatitis C virus genotype 1 true non-responder patients. *Aliment Pharmacol Ther* 2007; **26:** 1131–8.
107 Carr C, Hollinger FB, Yoffe B *et al.* Efficacy of interferon alpha-2b induction therapy before retreatment for chronic hepatitis C. *Liver Int* 2007; **27:** 1111–8.
108 Mathew A, Peiffer LP, Rhoades K *et al.* Sustained viral response to pegylated interferon alpha-2b and ribavirin in chronic hepatitis C refractory to prior treatment. *Dig Dis Sci* 2006; **51:** 1956–61.
109 Jacobson IM, Gonzalez SA, Ahmed F *et al.* A randomized trial of pegylated interferon alpha-2b plus ribavirin in the retreatment of chronic hepatitis C. *Am J Gastroenterol* 2005; **100:** 2453–62.
110 Herrine SK, Brown RS Jr, Bernstein DE *et al.* Peginterferon alpha-2a combination therapies in chronic hepatitis C patients who relapsed after or had a viral breakthrough on therapy with standard interferon alpha-2b plus ribavirin: a pilot study of efficacy and safety. *Dig Dis Sci* 2005; **50:** 719–26.
111 Shiffman ML, Di Bisceglie AM, Lindsay KL *et al.* Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004; **126:** 1015–23.
112 Jensen DM, Marcellin P, Freilich B *et al.* Re-treatment of patients with chronic hepatitis C who do not respond to peginterferon-alpha2b: a randomized trial. *Ann Intern Med* 2009; **150:** 528–40.
113 Poynard T, Colombo M, Bruix J *et al.* Peginterferon alfa-2b and ribavirin: effective in patients with hepatitis C who failed interferon alfa/ribavirin therapy. *Gastroenterology* 2009; **136:** 1618–28.
114 Di Bisceglie AM, Shiffman ML, Everson GT *et al.* Prolonged therapy of advanced chronic hepatitis C with low-dose peginterferon. *N Engl J Med* 2008; **359:** 2429–41.
115 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339:** 1485–92.
116 Poynard T, Marcellin P, Lee SS *et al.* Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). *Lancet* 1998; **352:** 1426–32.
117 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339:** 1493–9.
118 Reichard O, Norkrans G, Frydén A *et al.* Randomised, double-blind, placebo-controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. The Swedish Study Group. *Lancet* 1998; **351:** 83–7.
119 Zeuzem S, Feinman SV, Rasenack J *et al.* Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; **343:** 1666–72.
120 Lindsay KL, Trepo C, Heintges T *et al.* A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34:** 395–403.
121 Pockros PJ, Carithers R, Desmond P *et al.* Efficacy and safety of two-dose regimens of peginterferon alpha-2a compared with interferon alpha-2a in chronic hepatitis C: a multicenter, randomized controlled trial. *Am J Gastroenterol* 2004; **99:** 1298–305.
122 Akuta N, Suzuki F, Tsubota A *et al.* Efficacy of interferon monotherapy to 394 consecutive naive cases infected with hepatitis C virus genotype 2a in Japan: therapy efficacy as consequence of tripartite interaction of viral, host and interferon treatment-related factors. *J Hepatol* 2002; **37:** 831–6.
123 Iwasaki Y, Shiratori Y, Hige S, NIshiguchi S *et al.* A randomized trial of 24 versus 48 weeks of peginterferon a-2a in patients infected with chronic hepatitis C virus genotype 2 or low viral load genotype 1: a multicenter national study in Japan. *Hepatol Int* 2009; **3:** 468–79.
124 Masuko K, Okuda K, Meguro T *et al.* Hepatitis C virus antibodies, viral RNA and genotypes in sera from patients on maintenance haemodialysis. *J Viral Hepat* 1994; **1:** 65–71.
125 Fabrizi F, Martin P, Dixit V, Bunnapradist S, Dulai G. Meta-analysis: effect of hepatitis C virus infection on mortality in dialysis. *Aliment Pharmacol Ther* 2004; **20:** 1271–7.
126 Rostaing L, Chatelut E, Payen JL *et al.* Pharmacokinetics of alphaIFN-2b in chronic hepatitis C virus patients undergoing chronic hemodialysis or with normal renal function: clinical implications. *J Am Soc Nephrol* 1998; **9:** 2344–8.

127 Russo MW, Goldsweig CD, Jacobson IM, Brown RS Jr. Interferon monotherapy for dialysis patients with chronic hepatitis C: an analysis of the literature on efficacy and safety. *Am J Gastroenterol* 2003; **98:** 1610–5.
128 Fabrizi F, Dixit V, Messa P, Martin P. Interferon monotherapy of chronic hepatitis C in dialysis patients: meta-analysis of clinical trials. *J Viral Hepat* 2008; **15:** 79–88.
129 Kamar N, Ribes D, Izopet J, Rostaing L. Treatment of hepatitis C virus infection (HCV) after renal transplantation: implications for HCV-positive dialysis patients awaiting a kidney transplant. *Transplantation* 2006; **82:** 853–6.
130 KDIGO clinical practice guidelines for the prevention, diagnosis, evaluation, and treatment of hepatitis C in chronic kidney disease. Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int Suppl* 2008; **109:** S1–99.
131 Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; **13:** 34–41.
132 Ikeda K, Arase Y, Kawamura Y *et al.* Necessities of Interferon Therapy in Elderly Patients with Chronic Hepatitis C. *Am J Med* 2009; **122:** 479–86.
133 Ikeda K, Arase Y, Saitoh S *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000; **32:** 228–32.
134 Kubo S, Nishiguchi S, Hirohashi K *et al.* Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med* 2001; **134:** 963–7.
135 Shiratori Y, Shiina S, Teratani T *et al.* Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 2003; **138:** 299–306.
136 Mazzaferro V, Romito R, Schiavo M *et al.* Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; **44:** 1543–54.
137 Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002; **89:** 418–22.
138 Shiratori Y, Ito Y, Yokosuka O *et al.* Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. *Ann Intern Med* 2005; **142:** 105–14.
139 Nishiguchi S, Kuroki T, Nakatani S *et al.* Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet* 1995; **346:** 1051–5.
140 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; **29:** 1124–30.
141 Imai Y, Kawata S, Tamura S *et al.* Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; **129:** 94–9.
142 Arase Y, Ikeda K, Suzuki F *et al.* Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. *J Med Virol* 2007; **79:** 1095–102.
143 Nomura H, Kashiwagi Y, Hirano R *et al.* Efficacy of low dose long-term interferon monotherapy in aged patients with chronic hepatitis C genotype 1 and its relation to alpha-fetoprotein: A pilot study. *Hepatol Res* 2007; **37:** 490–7.
144 Shiffman ML, Hofmann CM, Contos MJ *et al.* A randomized, controlled trial of maintenance interferon therapy for patients with chronic hepatitis C virus and persistent viremia. *Gastroenterology* 1999; **117:** 1164–72.
145 Saito Y, Saito H, Tada S *et al.* Effect of long-term interferon therapy for refractory chronic hepatitis c: preventive effect on hepatocarcinogenesis. *Hepatogastroenterology* 2005; **52:** 1491–6.
146 Arase Y, Ikeda K, Suzuki F *et al.* Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. *J Med Virol* 2007; **79:** 1485–90.
147 Akuta N, Suzuki F, Kawamura Y *et al.* Efficacy of low-dose intermittent interferon-alpha monotherapy in patients infected with hepatitis C virus genotype 1b who were predicted or failed to respond to pegylated interferon plus ribavirin combination therapy. *J Med Virol* 2008; **80:** 1363–9.
148 Imai Y, Kasahara A, Tanaka H *et al.* Interferon therapy for aged patients with chronic hepatitis C: improved survival in patients exhibiting a biochemical response. *J Gastroenterol* 2004; **39:** 1069–77.
149 Iwasaki Y, Ikeda H, Araki Y *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; **43:** 54–63.
150 Hiramatsu N, Oze T, Tsuda N *et al.* Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? *Hepatol Res* 2006; **35:** 185–9.
151 Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38:** 645–52.
152 McHutchison JG, Manns M, Patel K *et al.* Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123:** 1061–9.

153 Shiffman ML, Ghany MG, Morgan TR *et al.* Impact of reducing peginterferon alfa-2a and ribavirin dose during retreatment in patients with chronic hepatitis C. *Gastroenterology* 2007; **132:** 103–12.
154 Reddy KR, Shiffman ML, Morgan TR *et al.* Impact of ribavirin dose reductions in hepatitis C virus genotype 1 patients completing peginterferon alfa-2a/ribavirin treatment. *Clin Gastroenterol Hepatol* 2007; **5:** 124–9.
155 Shiffman ML, Salvatore J, Hubbard S *et al.* Treatment of chronic hepatitis C virus genotype 1 with peginterferon, ribavirin, and epoetin alpha. *Hepatology* 2007; **46:** 371–9.
156 Oze T *et al.* Pegylated interferon alpha-2b affects early virologic response dose-dependently in patients with chronic hepatitis C genotype 1 during treatment with Peg-IFN alpha-2b plus ribavirin. *J Viral Hepat* 2009; **16:** 578–85.
157 Hiramatsu N *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; **16:** 586–94.
158 Weiland O, Hollamder A, Mattsson L *et al.* Lower-than standard dose peg-IFN alfa-2a for chronic hepatitis C caused by genotype 2 and 3 is sufficient when given in combination with weight-based ribavirin. *J Viral Hepat* 2008; **15:** 641–5.
159 Inoue Y, Hiramatsu N, Oze T *et al.* Factors affecting efficacy in patients with genotype 2 chronic hepatitis C treated by pegylated interferon alpha-2b and ribavirin: reducing drug doses has no impact on rapid and sustained virological responses. *J Viral Hepat* (in press).
160 Omata M, Yoshida H, Toyota J *et al.* A large-scale, multicentre, double-blind trial of ursodeoxycholic acid in patients with chronic hepatitis C. *Gut* 2007; **56:** 1747–53.
161 Suzuki H, Ohta Y, Takino T *et al.* Effects of glycyrrhizin on biochemical tests in patients with chronic hepatitis. Double blind trial. *Asian Med J* 1983; **26:** 423–38.
162 Wildhirt E. Experience in Germany with glycyrrhizinic acid for the treatment of chronic viral hepatitis. In: Nishioka K, Suzuki H, Mishiro S, Oda T, eds. *Viral Hepatitis and Liver Disease*. Tokyo, Springer-Verlag, 1994; 658–61.
163 Arase Y, Ikeda K, Murashima N *et al.* The long term efficacy of glycyrrhizin in chronic hepatitis C patients. *Cancer* 1997; **79:** 1494–500.
164 Ikeda K, Arase Y, Kobayashi M *et al.* A long-term glycyrrhizin injection therapy reduces hepatocellular carcinogenesis rate in patients with interferon-resistant active chronic hepatitis C: a cohort study of 1249 patients. *Dig Dis Sci* 2006; **51:** 603–9.
165 Piperno A, Sampietro M, D'Alba R *et al.* Iron stores, response to alpha-interferon therapy and effects of iron depletion in chronic hepatitis C. *Liver* 1996; **16:** 248–54.
166 Fong TL, Han SH, Tsai NC *et al.* A pilot randomized, controlled trial of the effect of iron depletionon long-term response to alpha-interferon in patients with chronic hepatitis C. *J Hepatol* 1998; **28:** 369–74.
167 Herrera JL. Iron depletion is not effective in inducing a virologic response in patients with chronic hepatitis C who failed to respond to interferon therapy. *Am J Gastroenterol* 1999; **94:** 3571–5.
168 Fontana RJ, Israel J, LeClair P *et al.* Iron reduction before and during interferon therapy of chronic hepatitis C: results of a multicenter, randomized, controlled trial. *Hepatology* 2000; **31:** 730–6.
169 Di Bisceglie AM, Bonkovsky HL, Chopra S *et al.* Iron reduction as an adjuvant to interferon therapy in patients with chronic hepatitis C who previously not responded to interferon: a multicenter, prospective randomized, controlled trail. *Hepatology* 2000; **32:** 135–8.
170 Yano M, Hayashi H, Yoshioka K *et al.* A significant reduction in serum alanine aminotransferase levels after 3-month iron reduction therapy for chronic hepatitis C: a multicenter, prospective, randomized, controlled trial in Japan. *J Gastroenterol* 2004; **39:** 570–4.
171 Marchesini G, Bianchi G, Merli M *et al.* Nutritional supplementation with branched-chain amino acids in advanced cirrhosis: a double-blind, randomized trial. *Gastroenterology* 2003; **124:** 1792–801.
172 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360:** 1827–38.

JGH Journal of Gastroenterology and Hepatology

JGHF

doi:10.1111/j.1440-1746.2009.06207.x

REVIEW

The 2008 Okuda lecture: Management of hepatocellular carcinoma: From surveillance to molecular targeted therapy

Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Osaka, Japan

Key words
contrast enhanced ultrasound, early hepatocellular carcinoma, Gd-EOB-DTPA, hepatocellular carcinoma, molecular targeted agent, sonazoid, staging system, surveillance, tumor marker.

Accepted for publication 25 November 2009.

Correspondence
Professor Masatoshi Kudo, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Osaka 589-8511, Japan.
Email: m-kudo@med.kindai.ac.jp

Abstract

Hepatocellular carcinoma (HCC) is responsible for approximately 600 000–700 000 deaths worldwide. It is highly prevalent in the Asia-Pacific region and Africa, and is increasing in Western countries. Alpha fetoprotein (AFP) alone is insufficient for HCC screening. A combination with other tumor markers, such as PIVKA-II and AFP-L3, and periodical ultrasound surveillance is necessary. Sensitivity of AFP in depicting HCC is highest, followed by PIVKA-II and AFP-L3, but the order of the specificity is inverse, AFP-L3, PIVKA-II, and AFP. Sonazoid-enhanced ultrasound (US) is extremely useful to characterize hepatic tumors equal to or more than multidetector row computed tomography (MDCT). Sonazoid-enhanced US with defect re-perfusion imaging is a breakthrough technique in the treatment of HCC. Defect re-perfusion imaging will markedly change the therapeutic strategy for liver cancer. Gd-EOB-DTPA-magnetic resonance imaging is a newly developed imaging technique in the detection and diagnosis of HCC. It is the most sensitive tool in the differentiation of early HCC from dysplastic nodules. Regarding the treatment strategy, there has been no established systemic chemotherapy for advanced HCC, except for Sorafenib. Empirically, intrahepatic arterial infusion chemotherapy using implanted reservoir port is known to be effective in response rate and overall survival for advanced HCC with vascular invasion. Sorafenib in combination with transcatheter arterial chemoembolization or adjuvant use after ablation or resection will significantly prolong the life expectancy if ongoing clinical trials provide positive results. In conclusion, it is expected that readers will gain deeper insight into the latest progress and updated diagnosis and treatment of HCC described in this review.

Surveillance for early detection of HCC

Definition of the population at high-risk for HCC

Hepatocellular carcinoma (HCC) is responsible for approximately 600 000–700 000 deaths worldwide. It is highly prevalent in the Asia-Pacific region and Africa, and is increasing in Western countries.[1] Persistent infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are the highest risk factors for hepatocarcinogenesis. The carcinogenesis risk for HBV-infected persons is about 200 times higher than for those non-infected, and the risk may be higher by approximately fivefold in patients with HCV-related cirrhosis compared with those with HBV-related cirrhosis. The characteristics of HCV-associated carcinogenesis are fibrosis stage 4 (F4), in which liver cirrhosis is complete in most cases, male gender and age 60 years or older. The yearly carcinogenesis rate of cirrhosis type C is 7–8% in Japan, which is higher than in Europe, Australia and North America (1–3% per year),[2] this difference might be attributed to the higher mean age of carriers.

Liver cirrhosis induced by causes other than HBV and HCV is also a risk. Thus, HCC occurs in some cases of liver cirrhosis associated with nonalcoholic steatohepatitis (NASH), alcoholic liver disease, primary biliary cirrhosis (PBC), hemochromatosis, alpha-1 antitrypsin deficiency and autoimmune hepatitis (AIH). For patients with any of these disorders, the course of the disease should be followed with close attention to hepatocarcinogenesis. In addition, alcohol increases the risk of chronic hepatitis B- and C-associated liver carcinogenesis, and obesity increases the risk of HCV-related hepatocellular carcinoma (HCC). In summary, patients with chronic hepatitis B and C and non-viral liver cirrhosis are defined as high-risk populations for HCC in both Evidence-Based Practice Guidelines,[3] the Consensus-Based Clinical Practice Manual[4] proposed by the Japan Society of Hepatology (JSH), and the Practice Guideline published by the American Association of Study of the Liver (AASLD).[5] Patients with liver cirrhosis from HBV or HCV are defined as a super high-risk population.[3,4]

Surveillance protocol for early detection of HCC

For HCC detection, sensitivity of ultrasonography is higher than serum alpha fetoprotein (AFP) measurement alone, but the specificities are not markedly different. For liver cirrhosis, a combination of the two methods has been reported to increase detection rate compared with detection by ultrasonography or AFP.[3]

There is not yet clear evidence to determine the optimal interval for screening, but HCCs detected in periodic screening by AFP, a protein induced by vitamin K absence or antagonist-II (PIVKA-II), AFP lectin fraction (AFP-L3) measurement, and ultrasonography are solitary and small in many cases, as compared with those detected in symptomatic patients. Thus, the Japanese Evidence-Based Clinical Practice Guidelines[3] and Consensus-Based Clinical Practice Manual[4] propose ultrasonography and tumor marker measurement every 3–4 months in the super high-risk population, and every 6 months in high-risk populations. Based on HCC doubling times, these intervals appear to be appropriate in Japan, which is different from Western Countries, where screening is done every 6–12 months.[5] At present, all three tumor markers, including AFP, PIVKA-II, and AFP-L3, are covered under the Japanese national health insurance as HCC tumor markers. Measurement of two or more tumor markers increases the sensitivity, while minimizing the specificity reduction, for small liver cancer. For patients with a very nodular background liver parenchyma because of cirrhosis or obesity, and therefore difficult to evaluate ultrasonographically, periodic imaging screening by dynamic computed tomography (CT) (MDCT) or dynamic magnetic resonance imaging (MRI) every 6–12 months is proposed by JSH,[4] which is identical to the protocol in the Japanese Evidence-Based Clinical Practice Guidelines.[3]

Result of early detection of HCC in Japan

In Japan, approximately 65% of the patients are detected at an early stage, for which curative treatment intervention is possible according to the Nationwide survey in 198 000 patients[6] (Fig. 1). This can be attributed to the establishment of a nationwide surveillance system across Japan.

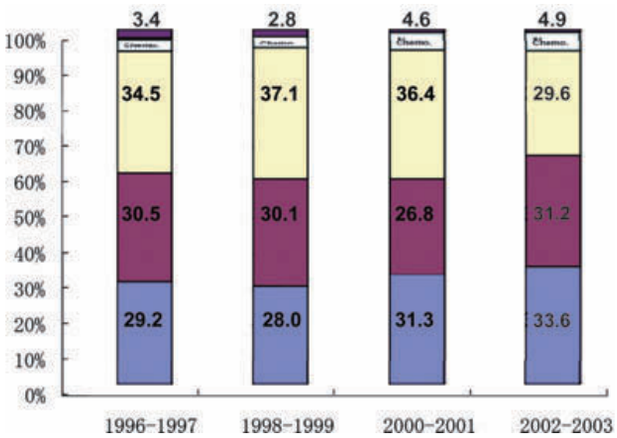


Figure 1 Treatment for newly diagnosed hepatocellular carcinoma (HCC) from 1996–2003 according to Nation-wide survey of Liver Cancer Study Group of Japan. 17th Nationwide survey clearly shows 64.8% of newly diagnosed HCCs receive potentially curative treatment such as operation or ablation. In other words, approximately 65% of HCCs are detected at early stage. ■, others; □, Chemo.; □, transcatheter arterial chemoembolization (TACE); ■, Ablation; ■, Ope.

Markers of HCC tumor biology

Alpha fetoprotein

Alpha fetoprotein is a tumor marker for HCC used worldwide. In Japan, according to the 17th Nationwide Follow-up Survey of Primary HCC by the Liver Cancer Study Group of Japan (LCSGJ),[6] most HCC patients were AFP-positive when the cutoff value was set at 15 ng/mL; however, AFP is positive in some patients with chronic hepatitis, particularly at the stage of liver cirrhosis, and in liver regeneration following necrosis. Therefore, AFP specificity is low depending on the cutoff value, and is considered inappropriate for screening HCC in the USA.[7] Accordingly, to effectively use AFP in clinical practice, it is important to recognize that sensitivity and specificity vary depending on the cutoff value.

Lens-culinaris agglutinin-reactive fraction of alpha fetoprotein

Lens-culinaris agglutinin-reactive fraction of alpha fetoprotein (AFP-L3) fraction was developed as a tumor marker in Japan. When the cutoff value was set to 10%, the sensitivity was approximately 30% (the 17th nationwide Follow-up Survey of Primary HCC by LCSGJ). Hence its clinical usefulness as an HCC surveillance marker is not appreciated in Western countries;[8] however, AFP-L3 is widely used, mainly in Japan, as a marker representing the degree of biological malignancy of HCC. Negative conversion of this marker after treatment is meaningful, although it is only approximately 50% after curative treatments.[9] Conversely, the prognosis of cases remaining positive after treatment is poor, and the rate of distant metastases is high; the possibility of early metastasis within the liver and to other organs should be kept in mind, in such cases, which require careful follow up for early detection of recurrence or intervention (such as interferon [IFN] treatment).

Protein induced by vitamin K absence-II

The sensitivity of protein induced by vitamin K absence-II (PIVKA-II) was 59% for a cutoff value of 40 mAU/mL according to the 17th Nationwide Survey by LCSGJ. The specificity is > 95%, but the positivity rate for 3 cm or smaller HCC is low (~40%). For HCCs larger than 5 cm, the positivity rate was 97%, indicating that this marker is superior to AFP. Further, the incidence of portal tumor thrombosis is high in PIVKA-II-positive cases (annual rate: 21%), and the risk ratio relative to negative cases is reportedly 5.65.[10] Although PIVKA-II is routinely used for HCC surveillance in Japan, the 2003 Single Topical Conference of the American Association of Study of the Liver (AASLD) posi-

tioned it as a diagnostic method,[11] rather than a screening method, because of its low sensitivity nature.

Other tumor markers

In addition to the above three tumor markers, glypican-3[12] and human telomerase reverse transcriptase (hTERT)[13] are attracting attention as HCC markers. Glypican-3 is a cell membrane protein; its positivity rate in HCC patients and specificity were reported to be 40–50 and 95–100%, respectively, showing its usefulness as a tumor marker. Further, the positivity rate is particularly high in the early stage, and the sensitivity rises to more than 80% when used in combination with AFP. In the future, it seems likely that glypican-3 may be used for clinical practice, such as diagnosis and screening for HCC.

hTERT is a telomerase-containing protein that has attracted attention as a cancer marker since the late 1990s. Sensitivity at the time of blood mRNA measurement was 88%, but specificity was lower, 70%. It may be clinically applicable by setting an optimum cutoff value based on a receiver operator curve (ROC).

Newly introduced diagnostic techniques

Contrast-enhanced ultrasound with a new contrast agent, Sonazoid

Clinical significance of contrast-enhanced ultrasound

In the management of HCC, despite advances in diagnostic imaging techniques such as ultrasound (US), CT or MRI, there remain many limitations, such as screening, staging, evaluation of treatment response, treatment guidance, localization of local recurrence after radiofrequency ablation (RFA), and detection of recurrence. Among these problems, Levovist-enhanced US has made a contribution to differential diagnosis,[14,15] evaluation of malignancy grade,[16] evaluation of therapeutic response to transcatheter arterial chemoembolization (TACE),[17–19] and needle insertion guidance.[20,21] However, there are still limitations in the evaluation of the therapeutic response to RFA,[22] screening or staging.

Sonazoid (GE HealthCare, Milwaukee, WI, USA) is a newly introduced second generation ultrasound contrast agent exclusively approved in Japan in 2007. The important characteristics of Sonazoid are that it facilitates real-time imaging in blood flow images at low acoustic power and stable Kupffer phase imaging, tolerable for multiple scanning from 10 to 120 min after its injection. Sonazoid is considered to be more effective and easier to use than Levovist in vascular imaging, and allows visualization, even using non-high-end equipment, and therefore, dependence on operator's skill/equipment is decreased, which may facilitate the widespread use of contrast-enhanced US. Sonazoid-enhanced US provides very stable post-vascular phase images for up to 60–120 min,[23] which resulted in the invention of the breakthrough method, defect reperfusion imaging. Thus, sonazoid-enhanced US with defect reperfusion imaging is an innovative technology that should greatly change the daily clinical practice of HCC investigation.

Development of defect reperfusion imaging (dual phase fusion imaging)

We recently developed defect reperfusion imaging[24–26] using the properties of very stable Kupffer images and real-time fine blood flow images obtained with Sonazoid for typical HCC, which is depicted by CT but not by B mode scanning. This method is a breakthrough for accurate localization and treatment guidance.[25] Until recently, diagnosis in dynamic studies was usually based on enhancing patterns according to a time sequence or phase; however, by introducing the novel idea of dual phase imaging with the re-injection method, both Kupffer and arterial phase images are obtained at the same slice of the ultrasound plane, which is really an innovative technique. Namely, this method is performed as follows: re-injection of Sonazoid is performed into areas that show defects in the post-vascular phase.[23–26] The introduction of this method has solved several limitations in the diagnosis and treatment of HCC, such as detection of small HCCs,[27] evaluation of treatment response,[28] or needle insertion guidance. Detection rate of small HCCs by Sonazoid-enhanced US is even more sensitive than that by MDCT (Fig. 2),[27] and it seems likely that this novel technique will eventually be used worldwide.

MRI using a new contrast agent, Gd-EOB-DTPA in the diagnosis of early HCC

Hepatocellular carcinoma is known to show multistep progression from the hyperplastic nodule to early HCC and finally to moderately/poorly differentiated HCC (Fig. 3). It is important to differentiate between premalignant nodules and early HCC. The imaging diagnosis of HCC by CT/MRI has been made by dynamic acquisition (hemodynamic diagnosis) using extracellular contrast medium, such as iodine contrast agent or gadolinium-diethylene-triamine-pentaacetic acid (GD-DTPA). HCC is supplied solely from arterial, not portal blood flow. Super paramagnetic iron oxide (SPIO) is specifically taken up by Kupffer cells and has been used as a liver-specific contrast agent for MRI since 1997; Kupffer cells are not present in overt HCC.

A newly introduced contrast agent, Gd-EthOxyBenzl-DTPA (Gd-EOB-DTPA), approved in 2008 in Japan, is a hepatocyte-specific MRI contrast medium with a different mechanism, using both dynamic and Kupffer cell imaging. This new contrast medium is useful to diagnose cases that would have been difficult using previous techniques such as dynamic MRI or SPIO-MRI. Gd-EOB-DTPA consists of the extracellular contrast medium, Gd-DTPA, and the lipid-soluble EOB group. Acquisition of both water and lipid solubility increases cell membrane permeability and the agent is therefore taken up by hepatocytes. Although the mechanism for hepatocellular uptake has not been fully clarified, it may involve organic anion transporting polypeptide (OATP1)[29] (Fig. 4). Recently, it was reported that uptake of Gd-DTPA-EOB is regulated by OATP1B3 in humans.[30] For excretion into bile, the active transport out of hepatocytes is by multidrug resistant protein (MRP2) system[31] (Fig. 4). Active transport is indicated by the high biliary excretion rate (~50%) of Gd-EOPB-DTPA. Imaging diagnosis of HCC can be made within 10–20 min after Gd-EOB-DTPA injection.

Typical HCCs show high intensity of Gd-EOB-DTPA in the arterial-dominant phase and low intensity in the portal-dominant

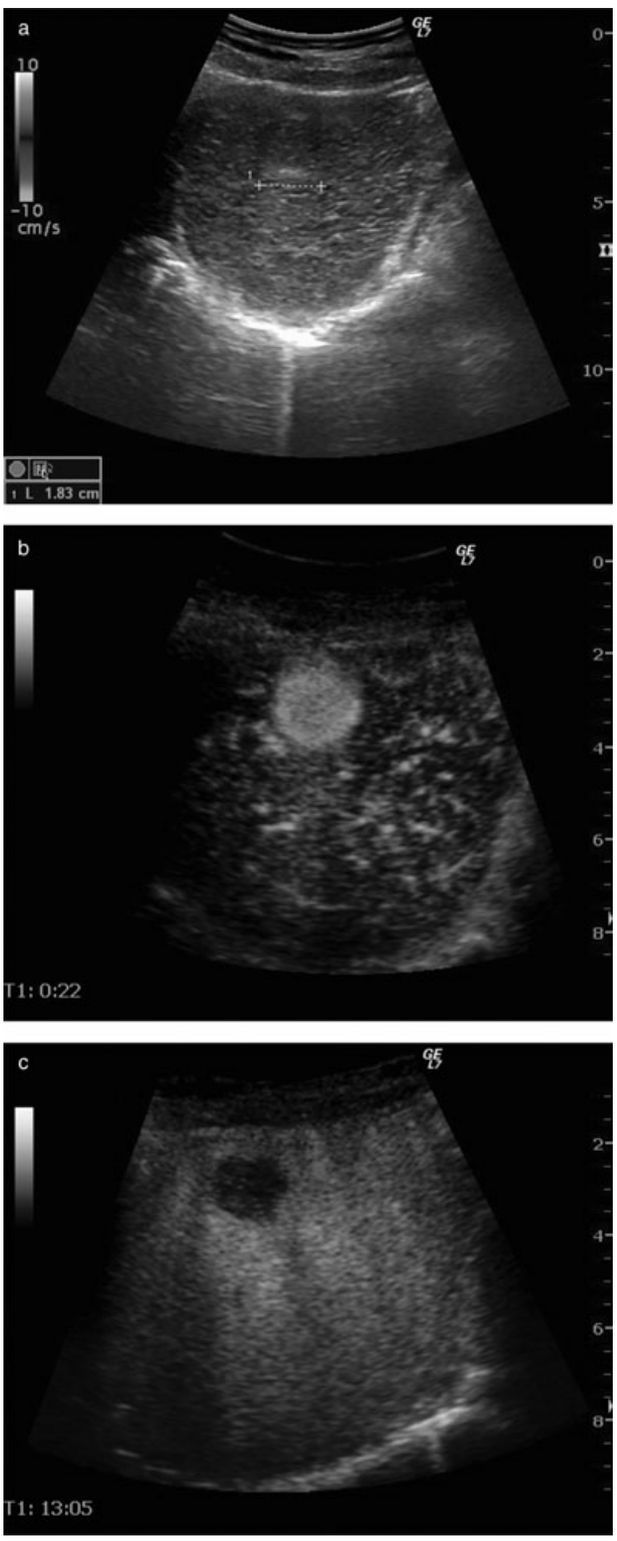


Figure 2 A case of hepatocellular carcinoma (HCC) demonstrated by Sonazoid-enhanced ultrasound. (a) B-mode image shows ill-defined isoechoic nodule measuring 1.83 cm in size. (b) Sonazoid-enhance ultrasound (US) clearly demonstrates this nodule as a hypervascular tumor. (c) Kupffer phase image shows this nodule as a clear defect, suggesting typical HCC.

phase and thereafter. In the arterial-dominant phase, Gd-EOB-DTPA is not taken up by normal hepatocytes, and thus, HCC nodules are intensely stained in the arterial dominant phase. In the portal-dominant phase and thereafter, Gd-EOB-DTPA is gradually taken up by normal hepatocytes, increasing the clear contrast between normal liver parenchyma and HCC nodules (Fig. 5).[29,32] After 20 min, the liver/tumor contrast is as high as or superior to that in CT during arterial portography (CTAP) except for approximately 5% of overt HCC cases, which show high or iso-intense on hepatocyte phase image (Table 1). Hepatocyte phase image of Gd-EOB-DTPA MRI is speculated to be regulated by the balance of OATP1B3 and MRP2 expression (Table 1).

In well-differentiated early HCC, some nodules may not be completely shown as defective areas on CTAP, but Gd-EOB-DTPA uptake is apparently lower than that in the surrounding normal liver parenchyma, being imaged as a low-intensity nodule. Well-differentiated early HCCs having Kupffer cells with enhanced SPIO uptake and receiving portal blood flow on CTAP have been difficult to characterize by SPIO-MRI or CTAP. However, they can be imaged clearly as hypointense nodules using Gd-EOB-DTPA hepatocyte phase MRI in many early HCC cases due to differences in the biological characteristics. This indicates that this new contrast agent may lead to a breakthrough in the diagnosis of early HCC (Table 2) (Fig. 6),[32,33] which has been clinically difficult and difficult even by pathological diagnosis in biopsy samples. It could be that this technique may be the most sensitive tool for detection of the phenotypic change of early hepatocarcinogenesis, much more sensitive than CTAP, computed tomography hepatic arteriography (CTHA), or SPIO-MRI (Fig. 7).

There are two reasons why pathological diagnosis of early HCC is sometimes difficult using biopsy: (i) possibility of sampling error; and (ii) stromal invasion, an important clue of pathological diagnosis of early HCCs,[34] can occasionally not be found in the biopsy sample compared with the resected specimen. Recently, a consensus on pathological diagnosis of early HCC has been established between 'East and West'.[34] Diagnosis of early HCC by Gd-EOB-DTPA-MRI may be the most comparable tool with that by expert liver specialized pathologist compared with pre-existing imaging modalities according to multicenter trials (Table 2). Accuracy in diagnosing early HCC is as high as 93%, which is much better than CTAP (Table 2). If so, this will change the diagnostic algorithm by introducing Gd-EOB-DTPA MRI in hypervascular and hypovascular liver nodules[4] (Figs 8,9).

Value of an integrated staging system

Various staging systems have been proposed for HCC and are used in the different regions, such as: (i) Okuda stage; (ii) Barcelona Clinic Liver Cancer (BCLC) stage;[35,36] (iii) Cancer of Liver Italian Program (CLIP) score;[37] (iv) Japan Integrated Staging (JIS)

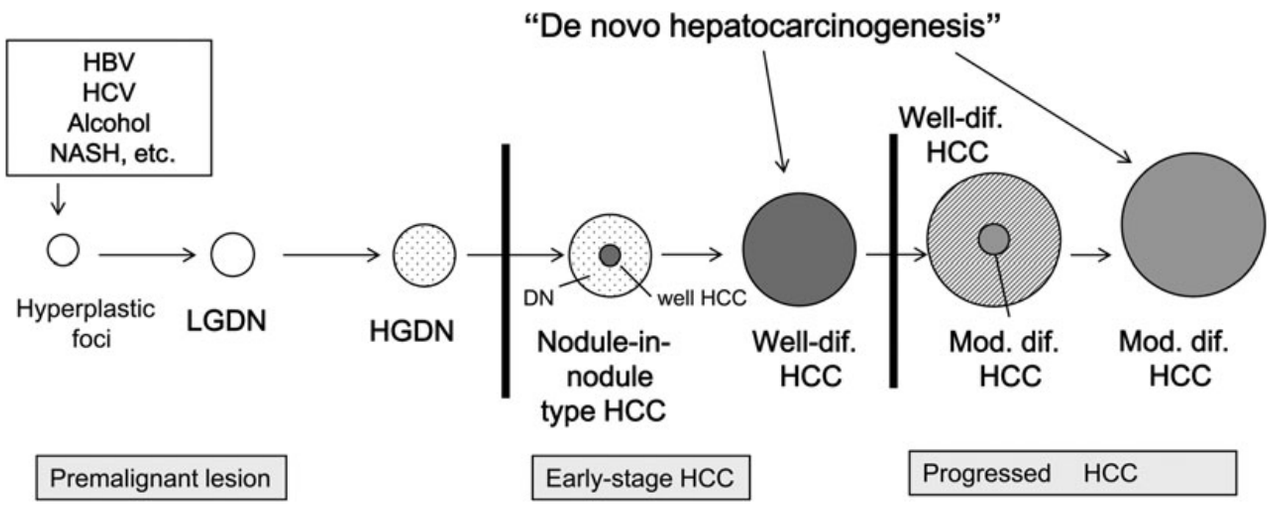


Figure 3 Schematic representation of multistep progression of human hepatocarcinogenesis. Differentiation between early-stage hepatocellular carcinoma (HCC) and premalignant lesion is extremely important. ○, Hyperplastic foci or low grade dysplastic nodule (LGDN); ◌, High grade dysplastic nodule (HGDN); ●, Well-differentiated HCC (well HCC); ●, Moderately differentiated HCC (classical overt HCC). HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis.

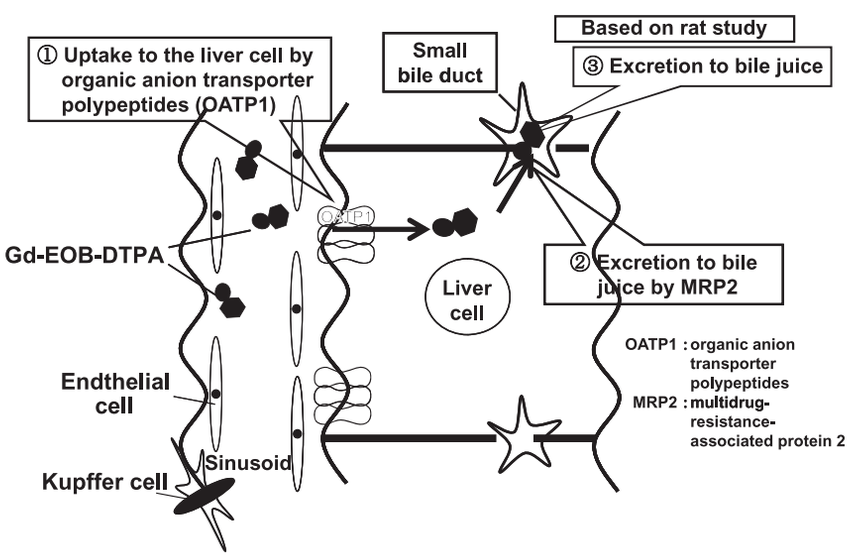


Figure 4 Pharmacokinetics of Gd-EOB-DTPA. Gd-EOB-DTPA is uptaken to the hepatocyte by organic anion transporter peptides (OATP1). Excretion to bile juice is believed to be regulated by multidrug resistance-associated protein (MRP)2.

score;[38,39] and (v) Tokyo Score.[40] In Japan, the JIS score, using both the LCSGJ TNM[41] and Child-Pugh stages, is considered to be the most useful for integrated staging of HCC. The CLIP score has several disadvantages: specification of the tumor-spreading degree is approximate, only AFP is used as a biological malignancy marker, and stratification ability is poor in advanced cases (many cases cluster to a score of 0–2).

The original JIS score used Child-Pugh staging, but the modified JIS score using liver damage instead is frequently used by liver surgeons.[42] The modified JIS score may be useful in planning hepatectomy because LCSGJ liver damage is more strictly classified. Recently, new staging systems for predicting prognosis have been developed; for example, the BALAD score,[43] which consists of the albumin level, bilirubin level, and three tumor markers (AFP, AFP-L3, PIVKA-II). The reported advantages of the BALAD score are that it does not require a tumor-spreading stage. The second method is the biological marker-combined JIS score,[44] which is a combination of the original JIS score and three tumor markers (AFP, PIVKA-II, AFP-L3). This staging system seems to be superior to the original JIS score and BALAD score.[44]

Globally, CLIP scores and BCLC stage are used in Europe and North America as staging systems; however, they have different characteristics: the BCLC stage is basically a treatment-selection system for deciding on a therapeutic strategy, whereas CLIP and JIS scores are prognostic predictors for staging. The CLIP score and BCLC stage tend to predict the prognosis of only large HCCs,

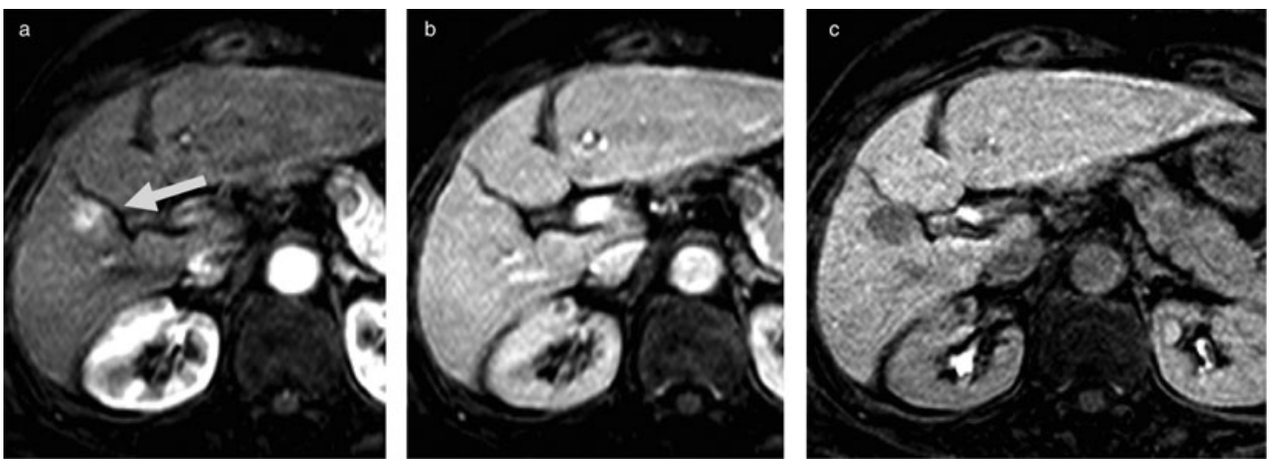


Figure 5 Typical findings of hepatocellular carcinoma (HCC) on Gd-EOB-DTPA magnetic resonance imaging (MRI). (a) Arterial enhancement (arrow) is evident or arterial phase. (b) Slight washout is seen on portal phase. (c) Clean defect is seen on hepatocyte specific phase 20 min later.

Table 1 Relationship between expression of OATP1B3/MRP2 and findings on hepatocyte phase

	Uptake transporter (OATP 1B3)	Excretory transporter (MRP2)	Hepatocyte phase imaging
Dysplastic nodule	+	+	Iso-high intense
Early HCC	(+)	(+)	(Low-intense)
	–	+	Low-intense
Well~Mod.dif.HCC	+ (5%)	+	Iso-high intense
		–	Iso-high intense (green hepatoma)
	– (95%)	–	Low-intense
Poorly dif.HCC	–	–	Low-intense

OATP1, organic anion transporter polypeptides, MRP2, multidrug-resistance-associated protein 2.

Table 2 Accuracy of the differentiation of early hepatocellular carcinoma (HCC) and premalignant lesions by hepatocyte phase Gd-EOB-DTPA magnetic resonance imaging (MRI) for hypovascular hepatocytic nodules

Only resected specimens: 30		Pathological findings	
		e-HCC	DN or RN
Signal intensity in hepato-biliary phase with Primovist	Low—slightly low (24)	23	1
	Iso—high (6)	1	5

AccuracyL 93% (23+5/30). DN, dysplastic nodule; e-HCC, early hepatocellular carcinoma; RN, regenerative nodule.

but the JIS score is most useful to predict the prognosis of many small liver cancers.

Attention needs to be paid to the fact that the BCLC stage corresponds to the Japanese treatment algorithm, but is not a prognostic prediction staging system. For countries incapable of detecting HCC early or in developing countries with insufficient screening systems and diagnostic instruments, the CLIP score may provide good stratification as a prognostic prediction system. In the future, the JIS score may be used worldwide when surveillance systems for early detection of HCC become more common.

For practical purposes, the following conditions are essential for comprehensive analysis or staging of all cases of liver cancer: the system should: (i) be simple; (ii) have no missing data; (iii) be able to be used by anyone anywhere; (iv) be easy to memorize; and (v) be superior for stratifying early, intermediate, advanced, and terminal cases. Considering these conditions, the JIS score or bm-JIS score may be the most appropriate among current systems for the overall stratification of liver cancer cases in Japan.

Hepatic arterial infusion chemotherapy for advanced HCC

Until sorafenib was introduced, there was no effective anticancer drug for advanced liver cancer. 'Far advanced liver cancer represents stage IVa liver cancer accompanied by vascular invasion and stage IVb liver cancer accompanied by distant metastasis, for which low-dose fluorouracil platinum (FP) (5FU and cisplatinum)[45] therapy, and hepatic arterial infusion of 5FU in combination with IFN treatment[46] have been established as an effective treatment option in Japan. In fact, response rate (complete response + partial response [CR+PR]) reaches to 46% according to the Nationwide Survey by LCSGJ[6] (Fig. 10). In addition, it is well established that overall survival of the responder is superior to

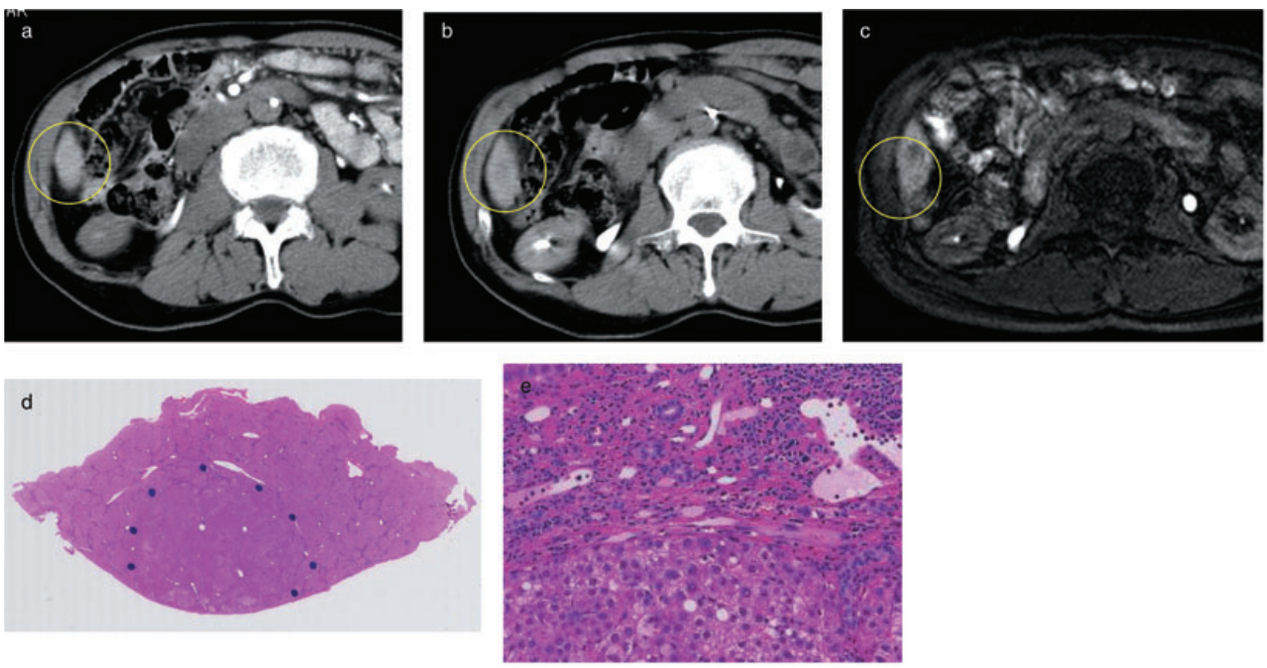


Figure 6 Early hepatocellular carcinoma (HCC), which was confirmed by Gd-EOB-DTPA magnetic resonance imaging (MRI). (a) Computed tomography hepatic arteriography (CTHA) does not show any hypervascularity. (b) CT during arterial portgraphy (CTAP) shows slight low dense mass on Segment 6. (c) Gd-EOB-DTPA magnetic resonance imaging (MRI) shows low intense mass at the hepatocyte phase, strongly suggestive of early HCC. (d) Pathological findings of resected specimen clearly shows vaguely nodular type HCC, suggesting early HCC. (e) Microscopical findings clearly show well-differentiated HCC with stromal invasion, which is a strong diagnostic clue of early HCC.

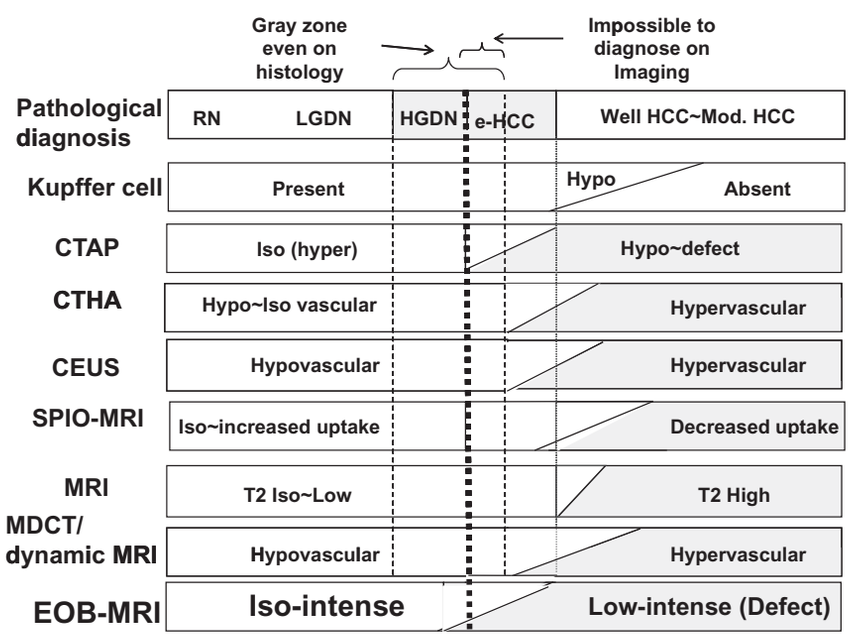


Figure 7 Gd-EOB-DTPA magnetic resonance imaging (MRI) is the most sensitive technique in the detection of initial phenotypic change of human hepatocarcinogenesis among various pre-existing imaging modalities. CEUS, contrast-enhanced ultrasound; CTAP, CT during arterial portgraphy; CTHA, computed tomography hepatic arteriography; EOB, EthOxyBenzl; HGDN, high grade dysplastic nodule; LGDN, low grade dysplastic nodule; MDCT, multidetector row CT; MRI, magnetic resonance imaging; SPIO, super paramagnetic iron oxide.

that of non-responders or best supportive care groups. However, intra-arterial infusion is complex because establishment of a reservoir port for arterial infusion is necessary; therefore, this technique is not performed in Western counties.

Recently, maintenance of the blood IFN level using pegylated IFN (PEG-IFN), and its efficacy in combination with an oral 5FU prodrug, S-1, (PEG-IFN + S1 combination therapy),[47] have been demonstrated to some extent. Further investigation, including a

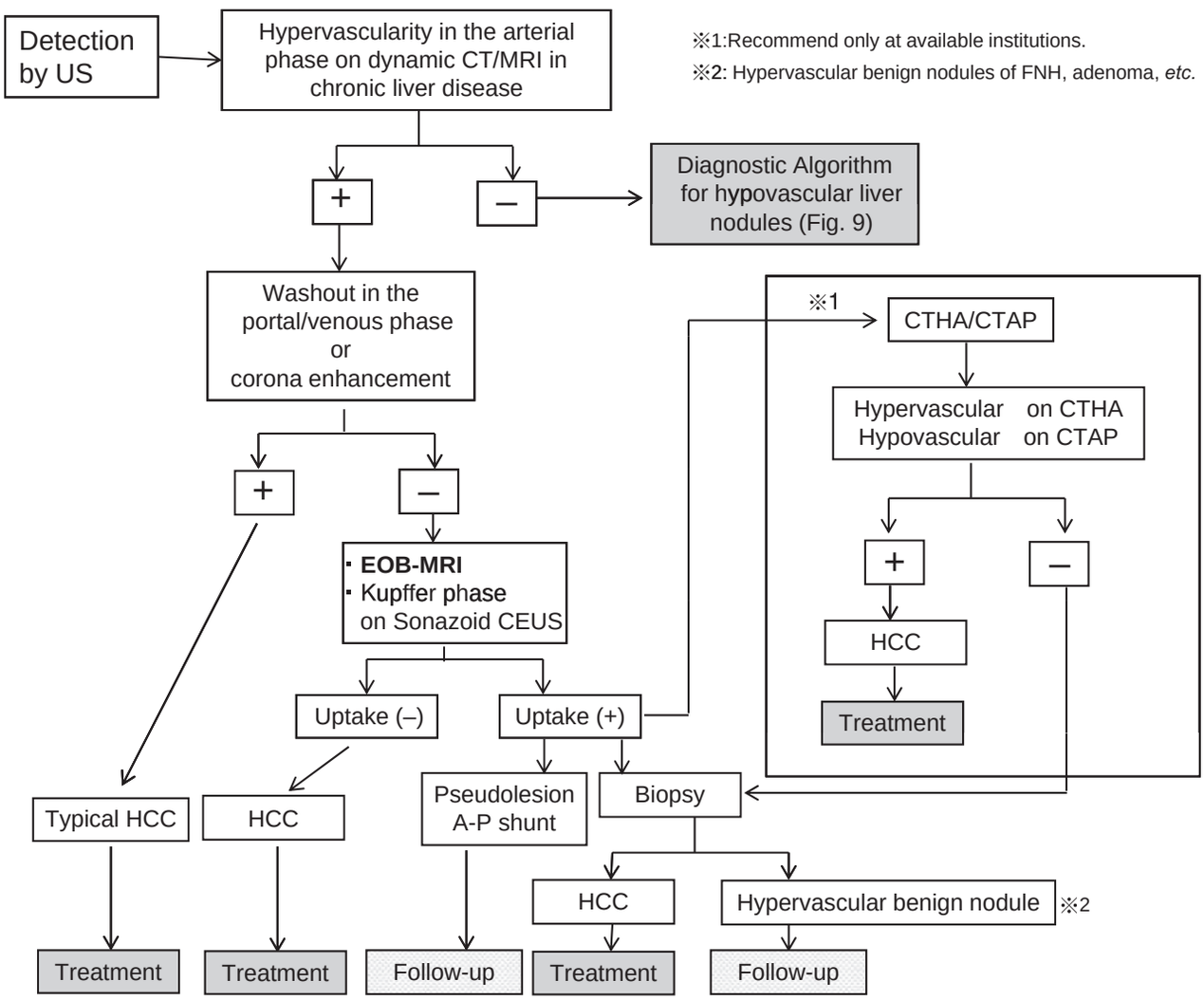


Figure 8 Diagnostic and treatment algorithm for hypervascular liver nodules according to clinical practice manual recommended by Japan Society of Hepatology (partially modified and cited from Narita et al. 2009[30]). CEUS, contrast-enhanced ultrasound; CT, computed tomography; CTAP, CT during arterial portgraphy; CTHA, computed tomography hepatic arteriography; EOB, EthOxyBenzl; FNH, focal nodular hyperplasia; HCC, hepatocellular carcinoma; MRI, magnetic resonance imaging; US, ultrasound.

prospective randomized study, is necessary. Moreover, hepatic intra-arterial infusion chemotherapy is not recommended in the AASLD guidelines.[5] Although the response rate is high, efficacy, especially survival benefit of intra-arterial infusion chemotherapy and that using an intractable delivery port system should be confirmed by further randomized studies.

New treatment option: Molecular targeted agent, Sorafenib

Molecular-targeted drugs are agents that exploit genetic differences between cancer and normal cells and specifically inhibit molecules involved in cancer growth and metastasis. The earliest successful agents have been Imatinib, Trastuzumab, and Gefitinib, all breakthrough agents developed from basic studies on tyrosine kinase or serine-threonine-mediated intracellular signal transduction.

Although HCC is the 3rd greatest cause of cancer death worldwide, the molecular mechanism(s) of its growth and progression have not been fully clarified. It is a hypervascular tumor, similar to renal cell carcinoma, but until recently, the efficacy of angiogenesis inhibitors alone has been limited. Sorafenib is a multikinase inhibitor that clearly prolongs the overall survival in patients with advanced HCC by 44%;[48] it has been approved for advanced HCC in Western countries since 2007, and is regarded as standard of care treatment option for advanced HCC with vascular invasion or extrahepatic metastases.

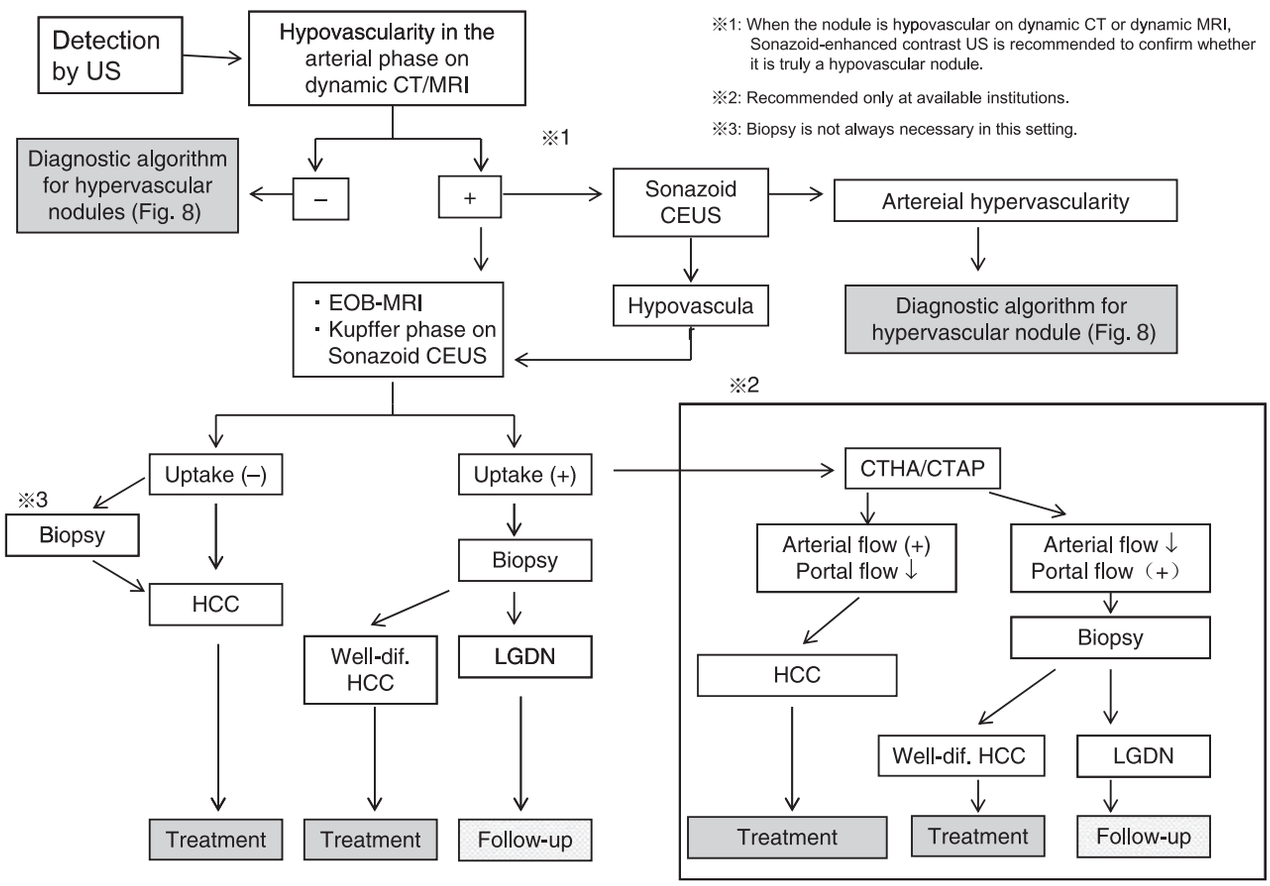


Figure 9 Diagnostic treatment algorithm for hypovascular liver nodules according to Japan Society of Hepatology (cited from Kudo et al. 2007[50]). CEUS, contrast-enhanced ultrasound; CT, computed tomography; CTAP, CT during arterial portgraphy; CTHA, computed tomography hepatic arteriography; EOB, EthOxyBenzl; FNH, focal nodular hyperplasia; HCC, hepatocellular carcinoma; LGDN, low grade dysplastic nodule; MRI, magnetic resonance imaging; US, ultrasound.

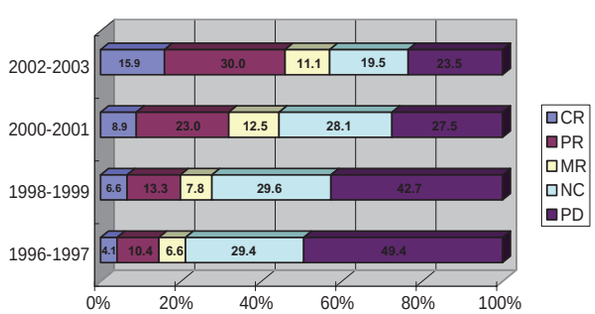


Figure 10 Response rate of hepatic arterial infusion chemotherapy (HAIC) from 1996 to 2003 reported by Nation-wide survey of Liver Cancer Study Group of Japan. Response rate during 2002–2003 reached 45.9%, which is very high. CR, complete response; MR, minor response; NC, no change; PD, progressive disease; PR, partial response. HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

Sorafenib, developed by Bayer HealthCare (Germany), is a low-molecular-weight compound discovered by screening inhibitors of Raf kinase, an important molecule in the mitogen activating protein (MAP) kinase cascade located downstream of growth factor receptors. Sorafenib exhibits strong inhibitory activity for not only wild type c-Raf, but also for V600E mutant b-Raf and other receptor tyrosine kinases involved in angiogenesis and cell growth, such as vascular endothelial growth factor receptor-2 (VEGFR-2), VEGFR-3, platelet-derived growth factor receptor (PDGFR), Fms-related tyrosine kinase-3 (Flt-3), and c-Kit.

The phase III study for HCC (SHARP trial)[48] was performed as a randomized double-blind placebo-controlled multicenter study initiated in March 2005. The subjects had advanced HCC at ECOG PS 0–2 with Child-Pugh A liver function and no previous systemic chemotherapy. There were two study groups, Sorafenib (400 mg b.i.d.) and placebo treatment, and the primary end point was overall survival (OS). Secondary endpoints were time to progression (TTP).

Six hundred and two patients met the inclusion criteria, and 299 and 303 were randomly allocated to the Sorafenib and placebo

groups, respectively. On interim analysis, the median OS was 10.7 months in the Sorafenib group and 7.9 months in the placebo group, showing 44% improvement (hazard ratio: 0.69, *P*-value = 0.0006). TTP was 5.5 months in the Sorafenib group and 2.8 months in the placebo group, showing 73% prolongation (hazard ratio: 0.587, *P*-value = 0.000007). Grade 3 and 4 adverse events for which a causal relationship with Sorafenib could not be ruled out were diarrhea and skin reaction.

In August 2007, it was reported that Sorafenib also prolonged overall and progression-free survival in a phase III study for HCC performed in the Asia Pacific region, involving 226 Chinese, Korean, and Taiwanese patients. Data demonstrated similar efficacy and safety of Sorafenib on HCC as in the SHARP study.[49] In Japan, a phase I study has been completed, and a phase III study in HCC patients following TACE is currently underway. In addition, a phase III trial for HCC of acyclic Retionid, a vitamin A analog, after resection or RFA is also underway in Japan.

A global phase III trial of Sorafenib as adjuvant therapy after surgery or ablation is now ongoing (STORM trial) and a global phase II trial of Sorafenib as a maintenance therapy with a combination of TACE is also ongoing (SPACE trial). A phase I/II trial of a combination therapy of Sorafenib with hepatic arterial infusion chemotherapy (HAIC) is also ongoing in Japan (SILIUS trial). These results are awaited to confirm its usefulness in the daily clinical practice.

Treatment algorithm for HCC and impact of molecular targeted agents

Evidence-based treatment algorithm for HCC in Japan

Treatment algorithm in the west

The treatment algorithms in Europe and North America were published as the European Association For the Study of the Liver (EASL) consensus in 2001,[35] and then as the AASLD Clinical Practice Guidelines in Hepatology in 2005.[5] Both were prepared based on BCLC staging. The BCLC staging classification consists of stages 0 to D. Palliative treatment only is specified for stage D, while stage 0 is defined as a very-early stage, specifying 2 cm or smaller solitary liver cancers with carcinoma *in situ*, which corresponds to early HCC in Japan. These are solitary, and resection is desirable when portal pressure and bilirubin levels are normal. When portal hypertension is present, other potentially curative treatments, such as liver transplantation and local treatment, are recommended. For solitary or $\leq$ 3 HCC, $\leq$ 3 cm lesions with mild portal hypertension, liver transplantation or local ablation is recommended. These are very strict criteria, and only stages 0 and A are indicated for radical treatments, that is, resection, local ablation, and liver transplantation. The intermediate stage (Stage B) specifies multinodular lesions, and the advanced stage (Stage C) specifies cases with vessel invasion or extrahepatic spread. For Stage B patients, TACE is recommended and for Stage C patients Sorafenib is recommended as a standard of care treatment.

A consensus-based treatment algorithm for HCC proposed by the Japan Society of Hepatology

A Japanese expert panel established a consensus-based treatment algorithm based on therapeutic policies widely used in Japan.[50] Since Sorafenib is proved as a standard of care treatment for advanced HCC with major vascular invasion or extrahepatic spread,[50] a modified version of this consensus-based algorithm has been proposed.[51]

The original algorithm first divides cases based on the presence or absence of extrahepatic lesions, liver function, vascular invasion, number of tumors, and tumor size. It also divides treatment options into curative treatments (resection or local ablation), TACE, arterial infusion chemotherapy, liver transplantation, and palliative treatment. The algorithm essentially follows the evidence-based treatment algorithm,[3] but treatments widely performed in Japan were included by consensus, even though evidence is not always present.

Resection or local ablation is performed for three or fewer nodules of $\leq$ 3 cm with no extrahepatic lesion, good liver function, and no vascular invasion. In this group, local ablation or resection is potentially curative and a good prognosis can be expected. Although the number of nodules is three or fewer, when the tumor exceeds 3 cm, resection or TACE is selected. Additional local ablation following transarterial treatment (Lipiodol TACE or HAIC) may increase curability. IFN therapy after curative therapy has proved to be useful for improving patient survival;[52] therefore, it is recommended to treat patients with HCV who can tolerate IFN therapy. In the future, Sorafenib may become a first choice of treatment for adjuvant therapy if positive results are obtained by ongoing global clinical trial (STORM trial) (Fig. 11).

For patients with four or more lesions, TACE or HAIC is recommended. Local ablation in combination with TACE or HAIC may be more beneficial for $\leq$ 5–6 lesions. Sorafenib may be useful as a maintenance therapy between several procedures of TACE in order to reduce the numbers of TACE, thus avoiding the impaired liver function caused by repeated TACE. As a result, it may be beneficial to improve patient survival, but there is not yet solid evidence to support this concept. The positive results of several clinical trials (SPACE trial, TACTIS trial, Brisk-TA trial) (Fig. 11) in this setting awaited before this strategy is introduced in the clinical settings.

For patients with an extrahepatic lesions and good liver functional reserve, Sorafenib is currently the standard of care.

Establishment of an original Japanese treatment algorithm was necessary because the situation in Japan, including the availability of transplantation, is different from that in Western counties. The algorithm established by the Japan Society of Hepatology is not necessarily based on scientific evidence; indeed, consensus-based algorithm was combined with an evidence-based algorithm and opinions of JSH experts. Since it is also difficult to state whether the European or North American algorithm is strictly based on evidence, the JSH consensus-based treatment algorithm may be valid; thus, a treatment algorithm based on large scale of specialists' consensus and treatment strategy performed in real practice in Japan is important. However, this algorithm should be carefully revised through prospective trials for issues lacking evidence.

Ongoing clinical trials with molecular targeted agents

In addition to STORM, SPACE trials and TACTICS trials using Sorafenib in combinations with TACE (see earlier), the SILIUS

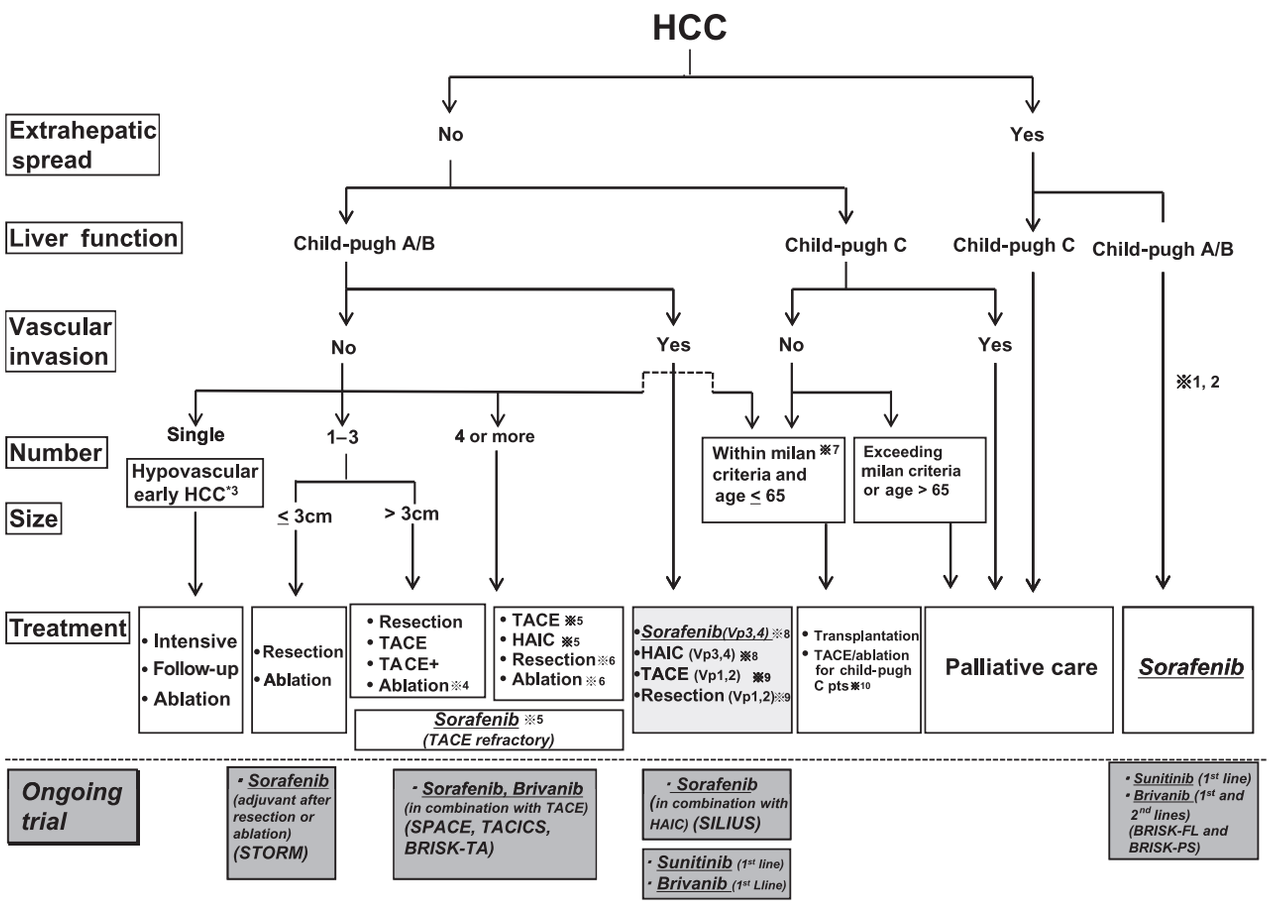


Figure 11 Consensus-based treatment algorithm for hepatocellular carcinoma (HCC) proposed by the Japan Society of Hepatology modified and updated in 2009 from its original version in 2007. Sorafenib is a standard of care for advanced HCC with extrahepatic spread and/or vascular invasion in major branches. Ongoing clinical trials include Sorafenib treatment after resection or ablation (STORM trial), combination therapy of transcatheter arterial chemoembolization (TACE) + Sorafenib (SPACE trial, TACTICS trial) and TACE + Brivanib (BRISK-TA), combination therapy of Sorafenib + Hepatic arterial infusion chemotherapy (HAIC) (SILIUS trial), and finally head-to-head trial between Sorafenib and Sunitinib/Brivanib for advanced HCC. ※1: Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not regarded as a prognostic factor. ※2: Sorafenib is the first choice of treatment in this setting as a standard of care. ※3: Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (i) when the nodule is diagnosed pathologically as early HCC; (ii) when the nodules show decreased uptake on Gd-EOB-MRI; or (iii) when the nodules show decreased portal flow by computed tomography during arterial portgraphy (CTAP), since these nodules are known to frequently progress to the typical advanced HCC. ※4: Even for HCC nodules exceeding 3 cm in diameter, combination therapy of TACE and ablation is frequently performed when resection is not indicated. ※5: TACE is the first choice of treatment in this setting. HAIC using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose fluorouracil platinum (FP) (5FU+CDDP) or intra-arterial 5FU infusion combined with systemic interferon therapy. Sorafenib is also a treatment of choice for TACE refractory patients with Child Pugh A liver function. ※6: Resection is sometimes performed even when number of nodules exceeds four. Furthermore, ablation is sometimes performed in combination with TACE. ※7: Milan criteria: Tumor size ≤ 3 cm and tumor numbers ≤ 3; or solitary tumor ≤ 5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for relatively younger patients with frequently or early recurring HCC after curative treatments. ※8: HAIC or Sorafenib is recommended for HCC patients with Vp3 (portal invasion at the 1st portal branch) or Vp4 (portal invasion at the main portal branch). ※9: Resection and TACE is frequently performed when portal invasion is minimal, such as Vp1(portal invasion at the 3rd or more peripheral portal branch) or Vp2 (portal invasion at the 2nd portal branch). ※10: Local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (< 3.0 mg/dL). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue. Even in Child-Pugh A/B patients, transplantation is sometimes performed for relatively younger patients with frequently or early recurring HCC after curative treatments.

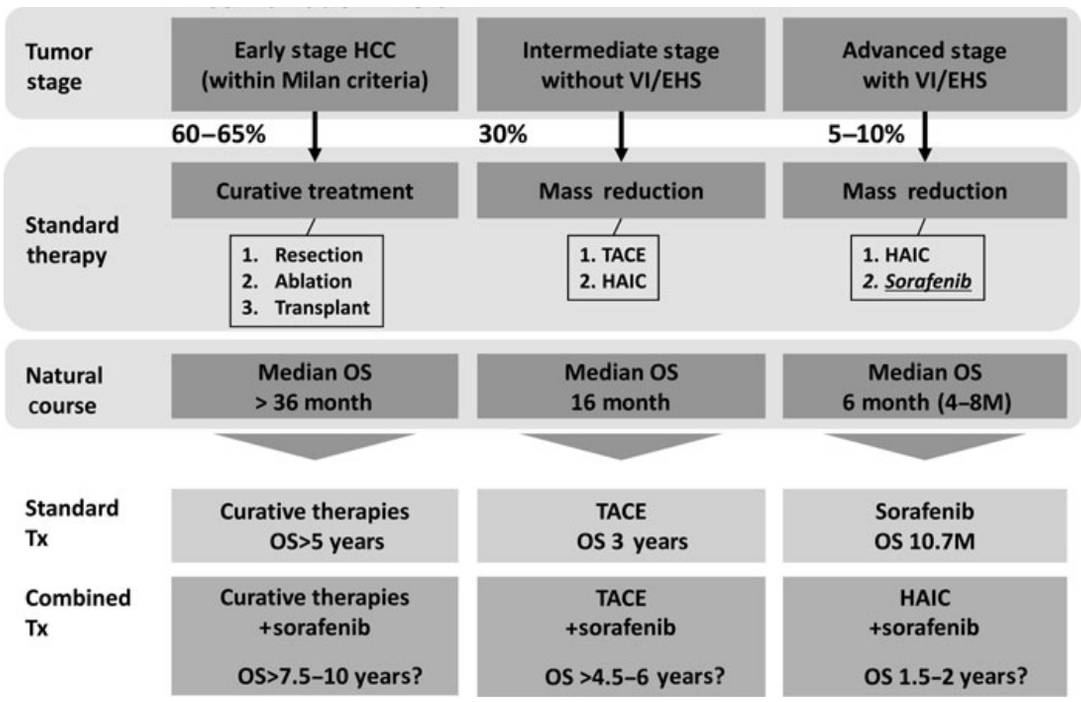


Figure 12 Outcome of standard treatment modality and expected future outcome by combination therapy with Sorafenib or other molecular targeted agents (MTAs). Prolonged life expectancy was calculated as 1.5 to 2.0 times better than the placebo arm by calculating hazard ratio (0.52) and mean survival time (MST) (14.5 *vs.* 10.2 months) by SHARP Subanalysis study, presented at the American Society of Clinical Oncology Meeting in 2008. For early stage hepatocellular carcinoma (HCC) without vascular invasion (VI) and/or extrahepatic spread (EHS), outcome is expected to be prolonged from MST of 5.0 years to 7.5 to 10.0 years by adjuvant use of Sorafenib after resection or ablation. For intermediate stage HCC without VI or EHS, outcome is expected to be prolonged from 3.0 years to 4.5–6.0 years when combination therapy with transcatheter arterial chemoembolization (TACE) is performed. Similarly, for advanced stage HCC with VI and/or EHS, outcome is expected to be prolonged from 10 months to 1.5–2.0 years when hepatic arterial infusion chemotherapy is combined with Sorafenib. HAIC, hepatic arterial infusion chemotherapy.

trial to compare Sorafenib in combination with HAIC is under investigation in Japan. Furthermore, head-to-head trials of Sunitinib versus Sorafenib and Brivanib versus Sorafenib (BRISK-FL trial) for advanced HCC are ongoing globally. Finally, second line trials of Brivanib for Sorafenib failure have been initiated as a global clinical trial (BRISK-PS trial). In addition, Brivanib in combination with TACE (BRISK-TA trial) is also ongoing. The results of all of these trials are eagerly awaited for their hope to provide better outcomes at different stages of HCC (Fig. 11). If positive results are obtained in these trails, the life expectancy at each stage could be much prolonged, at least as calculated theoretically by using hazard ratios incorporated from the SHARP trial. Subanalysis data presented at the ASCO 2008 clearly showed that in HCC patients without vascular invasion or extrahepatic spread hazard ratio of the prolongation of life expectancy is 0.52 and median survival time (MST) is 1.5 times better than placebo arms. If it can be incorporated in earlier stage HCC patients, Sorafenib will prolong the life expectancy approximately 1.5–2.0 times compared with the standard of care group in early and intermediate stage patients (Fig. 12). This could be translated that Sorafenib use in earlier stage in combination with standard of care treatment (resection, ablation, or TACE) will prolong HCC patients' life expectancy (1.5–5.0 years) (Fig. 12).

Conclusion

In this review, recent progress of the management of HCC, including issues from surveillance to molecular-targeted therapy for HCC, has been reviewed. It is strongly expected that this article will enhance the most up-to-date knowledge on HCC for the readers of the *Journal of Gastroenterology and Hepatology*.

References

1 El-Serag HB. Epidemiology of hepatocellular carcinoma in USA. *Hepatol. Res.* 2007; **37**: S88–94.
2 Fattovich G, Stroffolini T, Zagni I, Donato F. Hepatocellular carcinoma in cirrhosis: incidence and risk factors. *Gastroenterology.* 2004; **127** (5 Suppl. 1): S35–50.
3 Makuuchi M, Kokudo N, Arii S *et al.* Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol. Res.* 2008; **38**: 37–51.
4 Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology* 2007; **72**: S2–15.
5 Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208–36.
6 Ikai I, Arii S, Okazaki M *et al.* Report of the 17th nationwide

follow-up survey of primary liver cancer in Japan. *Hepatol. Res.* 2007; **37**: 676–91.
7 Adams PC, Arthur MJ, Boyer TD *et al.* Screening in liver disease: report of an AASLD clinical workshop. *Hepatology* 2004; **39**: 1204–12.
8 Gebo KA, Chander G, Jenckes MW *et al.* Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: a systematic review. *Hepatology* 2002; **36** (5 Suppl. 1): S84–92.
9 Okuda K, Tanaka M, Kanazawa N *et al.* Evaluation of curability and prediction of prognosis after surgical treatment for hepatocellular carcinoma by lens culinaris agglutinin-reactive alpha-fetoprotein. *Int. J. Oncol.* 1999; **14**: 265–71.
10 Koike Y, Shiratori Y, Sato S *et al.* Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. *Cancer* 2001; **91**: 561–9.
11 Sherman M, Klein A. AASLD single-topic research conference on hepatocellular carcinoma: Conference proceedings. *Hepatology* 2004; **40**: 1465–73.
12 Capurro M, Wanless IR, Sherman M *et al.* Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; **125**: 89–97.
13 Miura N, Maeda Y, Kanbe T *et al.* Serum human telomerase reverse transcriptase messenger RNA as a novel tumor marker for hepatocellular carcinoma. *Clin. Cancer Res.* 2005; **11**: 3205–9.
14 Wen YL, Kudo M, Zheng RQ *et al.* Characterization of hepatic tumors: value of contrast-enhanced coded phase-inversion harmonic angio. *AJR Am. J. Roentgenol.* 2004; **182**: 1019–26.
15 Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K. Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual-display mode. *Radiology* 2001; **220**: 349–56.
16 Inoue T, Kudo M, Watai R *et al.* Differential diagnosis of nodular lesions in cirrhotic liver by post-vascular phase contrast-enhanced US with Levovist: comparison with superparamagnetic iron oxide magnetic resonance images. *J. Gastroenterol.* 2005; **40**: 1139–47.
17 Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K. Contrast-enhanced subtraction harmonic sonography for evaluating treatment response in patients with hepatocellular carcinoma. *AJR Am. J. Roentgenol.* 2001; **176**: 661–6.
18 Minami Y, Kudo M, Kawasaki T *et al.* Transcatheter arterial chemoembolization of hepatocellular carcinoma: usefulness of coded phase-inversion harmonic sonography. *AJR Am. J. Roentgenol.* 2003; **180**: 703–8.
19 Ding H, Kudo M, Onda H *et al.* Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. *Radiology* 2001; **221**: 721–30.
20 Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H. Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. *AJR Am. J. Roentgenol.* 2004; **183**: 153–6.
21 Minami Y, Kudo M, Chung H *et al.* Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR Am. J. Roentgenol.* 2007; **188**: 489–94.
22 Wen YL, Kudo M, Zheng RQ *et al.* Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. *AJR Am. J. Roentgenol.* 2003; **181**: 57–63.
23 Inoue T, Kudo M, Hatanaka K *et al.* Imaging of hepatocellular carcinoma: Qualitative and quantitative analysis of postvascular phase contrast-enhanced ultrasonography with Sonazoid. *Oncology.* 2008; **75** (Suppl. 1): 48–54.
24 Kudo M, Hatanaka K, Maekawa K. Defect reperfusion imaging, a newly developed novel technology using Sonazoid in the treatment of hepatocellular carcinoma. *J. Med. Ultrasound.* 2008; **16**: 169–75.
25 Kudo M, Hatanaka K, Chung H, Minami Y, Maekawa K. A proposal of novel treatment-assist technique for hepatocellular carcinoma in the Sonazoid-enhanced ultrasonography: value of defect re-perfusion imaging. *Kanzo.* 2007; **48**: 299–301. (In Japanese.)
26 Kudo M, Hatanaka K, Maekawa K. Sonazoid-enhanced ultrasound in the diagnosis and treatment of hepatic tumors. *J. Med. Ultrasound.* 2008; **16**: 130–9.
27 Hatanaka K, Kudo M, Minami Y, Maekawa K. Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. *Oncology* 2008; **75** (Suppl. 1): 42–7.
28 Xia Y, Kudo M, Minami Y *et al.* Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: The usefulness of Sonazoid-enhanced harmonic sonography. *Oncology.* 2008; **75** (Suppl. 1): 99–105.
29 van Montfoort JE, Stieger B, Meijer DK, Weinmann HJ, Meier PJ, Fattinger KE. Hepatic uptake of the magnetic resonance imaging contrast agent gadoxetate by the organic anion transporting polypeptide Oatp1. *J. Pharmacol. Exp. Ther.* 1999; **290**: 153–7.
30 Narita M, Hatano E, Arizono S *et al.* Expression of OATP1B3 determines uptake of Gd-EOB-DTPA in hepatocellular carcinoma. *J. Gastroenterol.* 2009; **44**: 793–8.
31 Pascolo L, Petrovic S, Cupelli F *et al.* Abc protein transport of MRI contrast agents in canalicular rat liver plasma vesicles and yeast vacuoles. *Biochem. Biophys. Res. Commun.* 2001; **282**: 60–6.
32 Kanematsu M, Kondo H, Goshima S, Tsuge Y, Watanabe H. Magnetic resonance imaging of hepatocellular carcinoma. *Oncology* 2008; **75** (Suppl. 1): 65–71.
33 Kim M, Choi J, Chung Y, Choi S. Magnetic resonance imaging of hepatocellular carcinoma using contrast media. *Oncology.* 2008; **75** (Suppl. 1): 72–82.
34 Kojiro M, Wanless I, Alves V. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009; **49**: 658–64.
35 Bruix J, Sherman M, Llovet JM *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J. Hepatol.* 2001; **35**: 421–30.
36 Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin. Liver. Dis.* 1999; **19**: 329–38.
37 The Cancer of the Liver Italian Program (CLIP) Investigators. Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. *Hepatology* 2000; **31**: 810–45.
38 Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J. Gastroenterol.* 2003; **38**: 207–15.
39 Kudo M, Chung H, Haji S *et al.* Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40**: 1396–405.
40 Tateishi R, Yoshida H, Shiina S *et al.* Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. *Gut* 2005; **54**: 419–25.
41 Liver Cancer Study Group of Japan. *General Rules for the Clinical and Pathological Study of Primary Liver Cancer*. English ed 2. Tokyo: Kanehara, 2003.
42 Ikai I, Takayasu K, Omata M *et al.* A modified Japan Integrated

Stage score for prognostic assessment in patients with hepatocellular carcinoma. *J. Gastroenterol.* 2006; **41**: 884–92.
43 Toyoda H, Kumada T, Osaki Y *et al.* Staging hepatocellular carcinoma by a novel scoring system (BALAD score) based on serum markers. *Clin. Gastroenterol. Hepatol.* 2006; **4**: 1528–36.
44 Kitai S, Kudo M, Minami Y *et al.* Validation of a new prognostic staging system for hepatocellular carcinoma: A comprison of the biomarker-combined Japan integrated staging score, the conventional Japan integrated staging score and the BALAD score. *Oncology.* 2008; **75** (Suppl. 1): 83–90.
45 Ando E, Tanaka M, Yamashita F *et al.* Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. *Cancer* 2002; **95**: 588–95.
46 Sakon M, Nagano H, Dono K *et al.* Combined intraarterial 5-fluorouracil and subcutaneous interferon-alpha therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. *Cancer* 2002; **94**: 435–42.
47 Ueshima K, Kudo M, Nagai T *et al.* Combination therapy with S-1 and pegylated interferon alpha for advanced hepatocellular carcinoma. *Oncology.* 2008; **75** (Suppl. 1): 106–13.
48 Llovet JM, Ricci S, Mazzaferro V *et al.* Sorafenib in advanced hepatocellular carcinoma. *N. Engl. J. Med.* 2008; **359**: 378–90.
49 Cheng AL, Kang YK, Chen Z *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol.* 2009; **10**: 25–34.
50 Kudo M, Okanoue T. Japan Society of Hepatology: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatolgy. *Oncology* 2007; **72**: S2–15.
51 Kudo M. Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. *Oncology* 2008; **75**: S1–12.
52 Kudo M. Impact of interferon therapy after curative treatment of hepatocellular carcinoma. *Oncology* 2008; **75** (Suppl. 1): 30–41.

World Journal of Radiology

Online Submissions: http://www.wjgnet.com/1949-8470office
wjr@wjgnet.com
doi:10.4329/wjr.v2.i4.122

World J Radiol 2010 April 28; 2(4): 122-134
ISSN 1949-8470 (online)

GUIDELINES FOR CLINICAL PRACTICE

Diagnosis of pancreatic tumors by endoscopic ultrasonography

Hiroki Sakamoto, Masayuki Kitano, Ken Kamata, Muhammad El-Masry, Masatoshi Kudo

Hiroki Sakamoto, Masayuki Kitano, Ken Kamata, Masatoshi Kudo, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, 589-8511, Japan
Muhammad El-Masry, Hepatogastroenterology and Endoscopy Unit, Department of Internal Medicine, Assiut University Hospitals, Assiut 71515, Egypt
Author contributions: Sakamoto H and Kitano M both contributed equally to writing this manuscript; El-Masry M searched the literature; Kudo M revised the manuscript.
Supported by The Japan Society for Promotion of Science, Research and Development Committee Program of The Japan Society of Ultrasonics in Medicine; Japan Research Foundation for Clinical Pharmacology; Japanese Foundation for Research and Promotion of Endoscopy
Correspondence to: Masayuki Kitano, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama, 589-8511, Japan. m-kitano@med.kindai.ac.jp
Telephone: +81-72-3660221 Fax: +81-72-3672880
Received: March 8, 2010 Revised: March 29, 2010
Accepted: April 12, 2010
Published online: April 28, 2010

Abstract

Pancreatic tumors are highly diverse, as they can be solid or cystic, and benign or malignant. Since their imaging features overlap considerably, it is often difficult to characterize these tumors. In addition, small pancreatic tumors, especially those less than 2 cm in diameter, are difficult to detect and diagnose. For characterizing pancreatic tumors and detecting small pancreatic tumors, endoscopic ultrasonography (EUS) is the most sensitive of the imaging procedures currently available. This technique also provides good results in terms of the preoperative staging of pancreatic tumors. EUS-guided fine needle aspiration (EUS-FNA) has also proved to be a safe and useful method for tissue sampling of pancreatic tumors. Despite these advantages, however, it is still difficult to differentiate between benign and malignant, solid or cystic pancreatic tumors, malignant neoplasms, and chronic pancreatitis using EUS, even when EUS-FNA is performed. Recently, contrast-enhanced EUS with Doppler mode (CE-EUS) employing ultrasound contrast agents, which indicate vascularization in pancreatic lesions, has been found to be useful in the differential diagnosis of pancreatic tumors, especially small pancreatic tumors. However, Doppler ultrasonography with contrast-enhancement has several limitations, including blooming artifacts, poor spatial resolution, and low sensitivity to slow flow. Consequently, an echoendoscope was developed recently that has a broad-band transducer and an imaging mode that was designed specifically for contrast-enhanced harmonic EUS (CEH-EUS) with a second-generation ultrasound contrast agent. The CEH-EUS technique is expected to improve the differential diagnosis of pancreatic disease in the future. This review describes the EUS appearances of common solid and cystic pancreatic masses, the diagnostic accuracy of EUS-FNA, and the relative efficacies and advantages of CE-EUS and CEH-EUS along with their relative advantages and their complementary roles in clinical practice.

Key words: Contrast-enhanced endoscopic ultrasonography; Endoscopic ultrasonography; EUS-guided fine needle aspiration; Pancreas; Sonazoid

Peer reviewers: Adnan Kabaalioglu, MD, Professor, Akdeniz University Hospital, 07059, Antalya, Turkey; Wellington P Martins, PhD, Departamento de Ginecologia e, Obstetrícia da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo, Avenida dos Bandeirantes 3900, 8º andar, Ribeirão Preto, São Paulo 14049-900, Brazil; Kenneth Coenegrachts, MD, PhD, Department of Radiology, AZ St.-Jan AV, Ruddershove 10, B-8000 Bruges, Belgium; Ragab Hani Donkol, Professor, Radiology Department, Aseer Central Hospital, 34 Abha, Saudi Arabia

Sakamoto H, Kitano M, Kamata K, El-Masry M, Kudo M. Diagnosis of pancreatic tumors by endoscopic ultrasonography.

World J Radiol 2010; 2(4): 122-134 Available from: URL: http://www.wjgnet.com/1949-8470/full/v2/i4/122.htm DOI: http://dx.doi.org/10.4329/wjr.v2.i4.122

INTRODUCTION

Although the morphology of pancreatic tumors is highly diverse, these tumors can be classified broadly into solid and cystic tumors. Solid pancreatic masses may be due to the inflammation associated with chronic pancreatitis or they may be caused by a malignancy[1,2]. Ductal pancreatic adenocarcinoma is the most common malignant pancreatic neoplasm as it accounts for more than 95% of all malignant solid pancreatic tumors[3]. Only a minority of pancreatic tumors are neuroendocrine tumors. Other pancreatic tumors such as squamous cell carcinomas and primary pancreatic lymphomas are even rarer. Cystic tumors comprise 10%-15% of all cystic masses and 1%-5% of all pancreatic malignancies[4]. The imaging features of benign and malignant cystic lesions overlap considerably. Moreover, solid pancreatic tumors with cystic degeneration can mimic primary cystic tumors. Thus, it is often difficult to differentiate benign lesions from malignant lesions, and solid tumors from cystic pancreatic tumors. Compared to other imaging techniques, endoscopic ultrasonography (EUS) has been shown to be more accurate in terms of local staging and predicting vascular invasion and tumor resectability, particularly with tumors less than 2 cm in diameter[5-7]. Furthermore, EUS permits a pancreatic mass to be aspirated and/or biopsied during an examination, which allows a histological diagnosis to be made and benign masses to be differentiated from malignant masses.

EUS has also been adapted to employ an ultrasound (US) contrast agent. This technique is termed contrast-enhanced EUS (CE-EUS), and it has been used to assess the microvascular structures of pancreatic tumors. However, because this technique is associated with several imaging limitations, contrast-enhanced harmonic EUS (CEH-EUS) was developed recently. This technique employs an echoendoscope with a broad-band transducer and an imaging mode that was designed specifically for CEH-EUS with a second generation US contrast agent. All of these non-invasive methods have improved the discrimination between malignant and benign masses and the differential diagnosis of the pancreatic masses. In this article, the EUS imaging findings of the common pancreatic solid and cystic masses are reviewed. In addition, the diagnostic accuracy of EUS-guided fine needle aspiration (EUS-FNA) is examined. Finally, the efficacies and relative advantages of CE-EUS, CEH-EUS, and other diagnostic EUS adapted procedures and their complementary role in clinical practice are discussed.

ENDOSCOPIC ULTRASONOGRAPHY

EUS was developed in the 1980s to overcome problems associated with the transabdominal US imaging of the pancreas caused by intervening gas, bone, and fat. Since the EUS high-frequency transducers can be positioned *via* the stomach and duodenum in direct proximity to the pancreas, this technique yields detailed high-resolution images of the pancreas that far surpass those achieved by computed tomography (CT) or magnetic resonance imaging (MRI). The high resolution of these images permits the detection of lesions as small as 2-3 mm in diameter and their relationship with adjacent blood vessels such as the portal vein and mesenteric vasculature to be characterized. As a result, EUS is more accurate than other imaging techniques in terms of local staging and predicting vascular invasion and tumor resectability, particularly with tumors less than 2 cm in diameter[5-7]. EUS is also useful for locating occult pancreatic tumors in patients who have liver metastases and an unknown primary tumor. For example, when EUS was applied to 33 patients whose CT images only revealed metastatic tumors derived from an unknown primary tumor, primary pancreatic tumors were detected in 17 patients[8]. The identification of these primary pancreatic tumors meant that these patients could be treated with pancreas-specific chemotherapy, which improved their outcome.

SOLID PANCREATIC LESIONS

Solid pancreatic masses include benign masses, namely focal chronic pancreatitis, and malignancies, namely ductal adenocarcinomas, neuroendocrine tumors, lymphomas, and metastases.

Focal chronic pancreatitis

Regardless of whether CT, MRI, or even EUS is used, it is very difficult to reliably distinguish between chronic pancreatitis masses, namely masses that are due to advanced inflammation or fibrosis, and malignant tumors. To diagnose chronic pancreatitis, nine EUS criteria are currently accepted. Four are parenchymal criteria: hyperechogenic foci, hyperechogenic strands, pseudocysts, and lobularity. Five are ductal criteria: dilated main pancreatic ducts (MPDs), visible side branches, and hyperechogenic walls of the MPD[9-11]. When these 4-5 diagnostic criteria are used, the diagnostic sensitivity of EUS ranges between 84% and 100%, while its specificity ranges between 60% and 95%[12-16]. In addition, Rösch *et al*[17] and Glasbrenner *et al*[18] independently proposed EUS criteria that are suggestive of an inflammatory mass, namely inhomogeneous echo pattern, calcification, peripancreatic echo-rich stranding, and cysts. Their EUS criteria of malignant masses included: signs of invasion of adjacent organs, enlargement of adjacent lymph nodes, and masses with irregular outer margins (Figure 1). While these criteria markedly improved the diagnostic specificity of EUS, the sensitivity of the technique remained rather low, which means that the B-mode images of EUS are still insufficient for discriminating between chronic pancreatitis and malignant tumors.

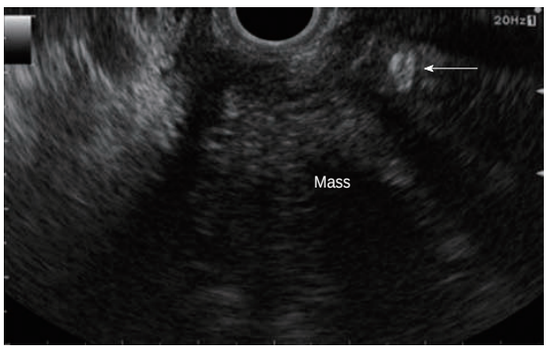


Figure 1 Focal chronic pancreatitis. Endoscopic ultrasonography (EUS) shows a mass with an irregular, inhomogeneous echo pattern, and calcification (arrow) at the head of the pancreas.

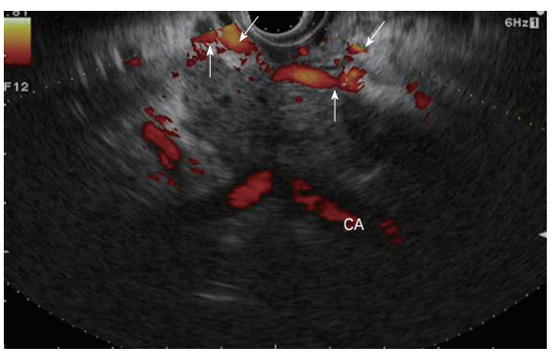


Figure 2 Pancreatic adenocarcinoma. EUS shows a heterogeneous hypoechoic mass with irregular margins at the body of the pancreas, infiltrating the celiac artery, and development of collateral vessels around the tumor (arrows). CA: Celiac artery.

Pancreatic adenocarcinoma

Pancreatic adenocarcinomas typically have the EUS appearance of a heterogeneous hypoechoic mass with irregular margins (Figure 2). However, relying on these morphological features alone only yields a diagnostic specificity of 53% since these features can also be seen in focal pancreatitis, neuroendocrine tumors, and metastases[19]. However, with a sensitivity of 89%-100%, EUS has been remarkably successful in the early detection of small adenocarcinomas[20-22]. In our institute, helical CT and EUS can detect pancreatic carcinomas 2 cm or less in diameter with a sensitivity of 50% and 94.4%, respectively. Thus EUS is significantly more sensitive than helical CT for detecting small pancreatic tumors[23].

Compared to other imaging techniques, EUS also facilitates more accurate staging, which improves the management of pancreatic cancer. Indeed, it has been suggested that EUS is most useful for assessing peripancreatic vascular and lymph node involvement. Many large series have found that when EUS is used for staging, the T stage accuracy ranges between 78%-91% and the nodal (N) stage accuracy ranges between 41%-86%[24-28]. In general, the T stage accuracy based on EUS findings

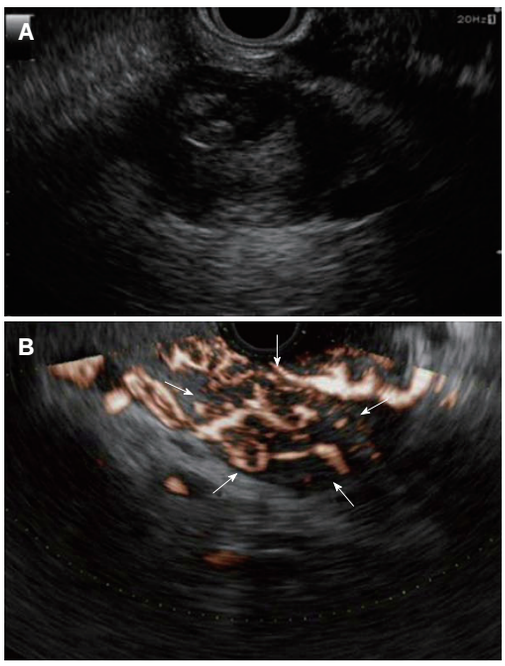


Figure 3 Neuroendocrine tumor. A: EUS shows a heterogeneous appearance; cystic, with a solid component or pure fluid 31 mm in diameter; B: EUS using Doppler mode shows a hypervascular mass at the tail of the pancreas (arrows).

is highest for patients with smaller tumors, whereas helical CT is more accurate in staging larger tumors[26-29]. When all four features that are suggestive of malignant lymph nodes, namely round shape, well-delimitated, size > 1 cm, and hypoechogenity, are present the chance of malignancy is 80%-100%[30].

Another benefit of EUS with regard to pancreatic tumors is that it can show the invasion of the great peripancreatic vessels with an accuracy of 67%-93%[17,31,32]. The splenic vein, portal vein and proximal superior mesenteric artery are easier to visualize on EUS than the other major peripancreatic vessels[33,34]. The vascular invasion criteria are as follows: irregularity of the interface with the vessels, intravascular tumor growth, and nonvisualization of the vessel, with collateral circulation growth. EUS can detect vascular invasion with a sensitivity and specificity of 42%-91% and 89%-100%, respectively[17,31,32]. While the accuracy can be rather low, this is because the staging accuracy of EUS can be influenced by several factors, including the experience of the endosonographer, the presence of imaging artifacts, and the endosonographer's knowledge of the results of previous imaging tests.

Neuroendocrine tumors

On EUS, neuroendocrine tumors usually appear as a hypoechogenic well-delimited lesion with intense vascularization; moreover, 60%-75% of all neuroendocrine tumors are less than 1.5 cm in diameter[35,36]. Lesions greater than 3 cm are likely to have an increased potential for malignancy and a heterogeneous appearance, namely cys-

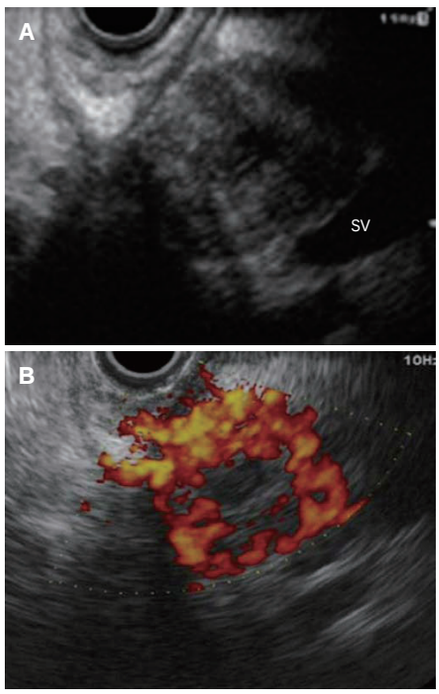


Figure 4 Metastatic pancreatic cancer from renal cell carcinoma. A: EUS shows a heterogeneous hypoechoic mass with a central necrotic area at the head of the pancreas; B: Contrast-enhanced Doppler EUS shows a hypervascular mass. SV: Splenic vein.

tic, with a solid component or pure fluid[37] (Figure 3A). The accuracy and specificity with which EUS can localize neuroendocrine tumors are 93% and 95%, respectively[38]. Since typical neuroendocrine tumors are known to be hypervascular tumors, EUS employing a Doppler mode is useful for observing the vascularity of identified neuroendocrine tumors (Figure 3B).

Primary pancreatic lymphoma

Primary pancreatic lymphoma is rare, comprising 1.3%-1.5% of all malignant pancreatic tumors. It is characterized by non-specific symptoms, laboratory tests and imaging results. Consequently, it can be very difficult to differentiate pancreatic lymphoma from pancreatic cancer on the basis of clinical and imaging data alone[39,40]. One report has described the EUS appearance of a pancreatic lymphoma as a bulky localized tumor in the pancreas without significant dilation of the MPD. Furthermore, if enlarged lymph nodes are encountered below the level of the renal veins, pancreatic lymphoma may be suspected. These EUS appearances may be useful for distinguishing between pancreatic lymphoma and other malignant pancreatic masses[41].

Metastatic pancreatic cancer

While primary pancreatic adenocarcinoma is the most common malignant tumor of the pancreas, a recent study showed that 3% of all pancreatic resections performed for malignant disease are due to pancreatic metastases of renal cell carcinomas[42]. Most pancreatic metastases develop from primary kidney, lung, breast, colon, or skin tumors[43] (Figure 4A and B). Confirming the metastatic nature of a pancreatic tumor is not an easy task, even for pathologists. However, metastatic tumors are more likely to have well-defined borders than primary pancreatic cancers[44].

CYSTIC PANCREATIC LESIONS

Cystic neoplasms of the pancreas often pose a diagnostic dilemma. They can be essentially classified according to malignant potential into mucinous and non-mucinous lesions with significant differences in the natural history and survival between the two groups. Mucinous tumors have recently been classified into mucinous cystic neoplasms (MCN) and intraductal papillary mucinous neoplasms (IPMN). Non-mucinous cysts include neoplastic cysts [serous cyst adenomas (SCAs) and solid pseudopapillary tumors], inflammatory cysts (pseudocysts), and epithelial cysts (adult polycystic disease and cystic fibrosis). Mucinous lesions are premalignant or malignant tumors, and surgical resection is generally recommended on operative candidates. Of the non-mucinous lesions, SCAs, whose potential for malignancy is low, and pseudocysts, which are always benign, are generally only resected if they are causing symptoms or complications[45-47].

The morphological features of cystic pancreatic lesions that can be determined by EUS include the presence of a wall, septa, solid component, the number and size of cysts, and the dilatation and thickening of the MPD. The presence of intracystic mucin or floating debris, pancreatic duct dilation, echogenic ductal wall thickening, and focal cyst wall nodularity or thickening are distinctly usual and suggestive of a mucinous tumor. These EUS features are thus useful for the differential diagnosis of cystic pancreatic lesions[48-53], although the accuracy with which they can be used to diagnose malignant cystic pancreatic tumors is rather low (51%-82%). Their usefulness is particularly limited in the case of large lesions (> 5-6 cm) that escape the focal field of the transducer[18,54-56].

Pseudocysts

The diagnosis of pseudocysts is generally not a clinical dilemma if there is a history of pancreatitis. However, cysts occurring in the setting of pancreatitis are not always pseudocysts; IPMN, for example may present with pancreatitis. Mature pseudocysts often have a thick wall surrounding a round collection of fluid, whereas early pseudocysts have a thin wall containing a collection of complex fluids[48] (Figure 5). To differentiate pseudocysts from cystic malignancies, it is useful to know that internal cyst debris and pancreatic parenchymal changes are observed more frequently in pseudocysts, and that mural nodules and septa are present more frequently in cystic

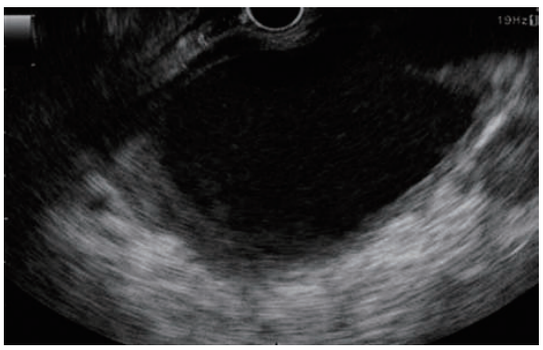


Figure 5 Pseudocyst. EUS shows a cystic lesion with a thick wall surrounding a round fluid collection at the body of the pancreas.

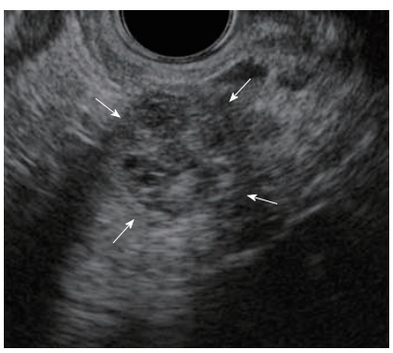


Figure 6 Serous cyst adenoma. EUS shows a mass with a "honeycomb appearance" at the body of the pancreas 13 mm in diameter (arrows).

malignancies[57]. However, several studies have concluded that when used in isolation, morphological features cannot reliably differentiate between malignancies and cystic lesions including pseudocysts[58,59].

SCAs

SCAs occur predominantly in young females. Although several reports have found that 50% to 70% are located in the pancreatic body or tail, other studies have found them more commonly in the head or neck region (63%). Although there are case reports of the malignant transformation of SCAs, they are largely benign cystic lesions and as such are often managed non-surgically[59,60]. SCAs usually appear as focal, well-demarcated lesions that contain multiple, and small (less than 1-2 cm in diameter) fluid-filled microcysts. The microcysts are separated by dense fibrous septa, producing a honeycomb appearance (Figure 6). Central fibrosis or calcification may be seen, particularly in large lesions, and can result in sunburst calcification. While this is a pathognomonic feature, it is present in only about 10% of patients with SCAs. A less common macrocystic variant contains larger (greater than 2 cm) cysts. They are typically microcystic. A solid variant contains numerous tiny cysts, each 1-2 mm in diameter, and appears as a homogeneous hypoechoic mass that can be mistaken for a ductal carcinoma.

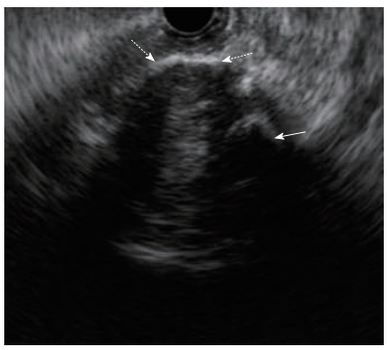


Figure 7 Solid pseudopapillary tumor. EUS shows a tumor in part of the calcified wall (dashed arrows) with acoustic shadow and inner calcifications (arrow) at the body of the pancreas 12 mm in diameter.

Solid pseudopapillary tumors

These tumors have a fairly well-defined behavior and malignant risk and are often managed surgically. In these cases, EUS plays a limited role because of the large size of the lesions and the resulting limitation of the examination field. However, typical EUS images of these tumors reveal well-delimited tumors with inner cystic formations and calcification (Figure 7). The atypical pure fluid forms are difficult to differentiate from the MCNs.

IPMN

IPMNs are more common in the elderly and are located more frequently in the head of the pancreas. IPMNs are characterized by the papillary proliferation of the ductal epithelium that is responsible for mucus production, which leads to the dilatation of the excretory pancreatic ducts. In a minority of cases, an endoscopic diagnosis of an IPMN can be established if a papulous papilla with mucin extrusion, also sometimes referred to as a "fish-eye" ampulla, is seen[61] (Figure 8A). These lesions can progress from hyperplasia to dysplasia, then to carcinoma *in situ*, and finally to invasive carcinoma. Macroscopically, IPMN is characterized by the mucinous dilatation of the pancreatic ducts, with involvement of either the MPD alone (main duct type), the side branch ducts alone (side branch type), or both (combined type)[62-64] (Figure 8B-D). Although communication with the MPD is a feature of side branch type IPMN and helps to exclude MCN, the absence of communication does not exclude IPMN because the mucus can block the flow of contrast into the abnormal side branch. EUS can: (1) visualize the communication between the MPD and a dilated side pancreatic duct; (2) help to make a differential diagnosis between an intraductal mucus deposit (as filaments or hyperechogenic round structures surrounded by a hyperechogenic ring) and a hypoechogenic intraductal polypoid lesion; and (3) visualize the thickening of the pancreatic duct wall or mural nodes. The diagnostic accuracy of EUS for IPMN is 92%, which is higher than that provided by US (82%) or endoscopic retrograde cholangiopancreatography (89%). Although not specific, an underlying malignancy

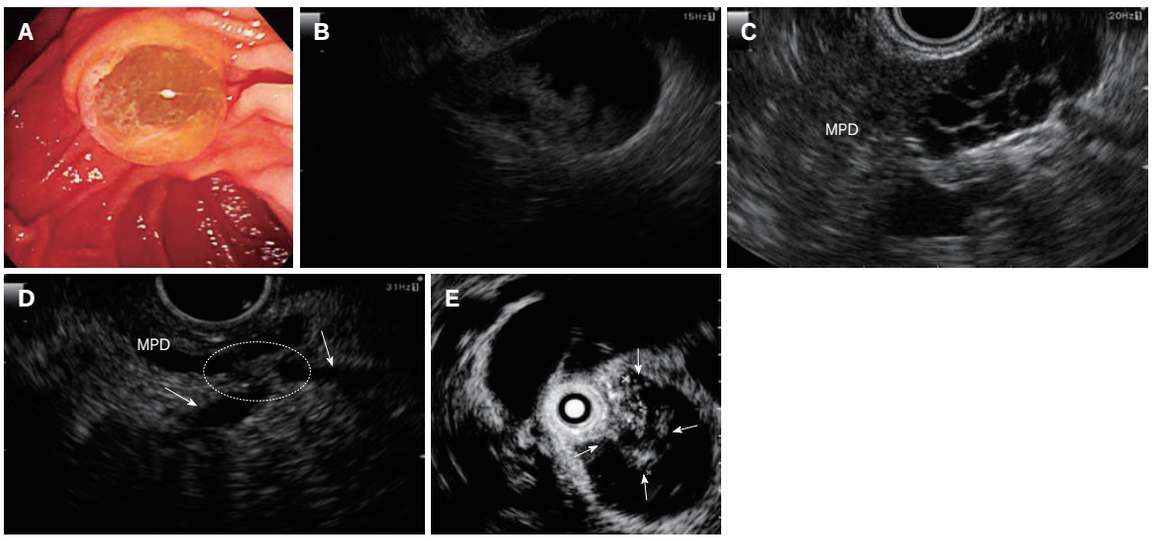


Figure 8 Intraductal papillary mucinous neoplasms (IPMN). A: An endoscopic diagnosis of an IPMN can be established if the "fish-eye" ampulla is visualized in minority cases; B: IPMN of main duct type. EUS shows a mural nodule within by the mucinous dilatation of the pancreatic ducts, with involvement of the main duct at the tail of the pancreas; C: IPMN of side branch type. EUS shows a multiple dilatation of the side branch at the neck of the pancreas; D: IPMN of the combined type. EUS show a mural nodule stretching (circle) over the main pancreatic duct and side branches (arrows) at the body of the pancreas. E: IPMN of main duct type. Intraductal ultrasonography (IDUS) can identify tumor nodule development into the main pancreatic duct (arrows). MPD: Main pancreatic duct.

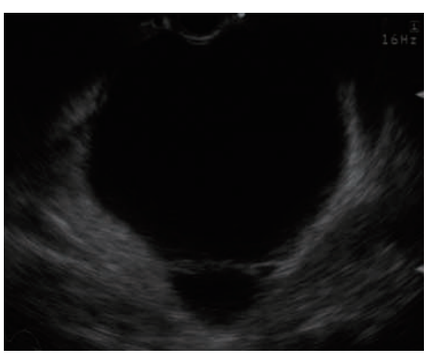


Figure 9 Mucinous cystic neoplasms (MCN). EUS shows a separated macrocyst 40 mm in diameter.

is suggested by an MPD diameter greater than 10 mm, branch-duct type IPMNs that have a cystic lesion diameter greater than 40 mm and a thick, irregular septum, and the presence of mural nodules that exceed 10 mm in diameter[49].

In cases where the pancreatic duct is sufficiently dilated, intraductal ultrasonography (IDUS) that utilizes a thin caliber (approximately 2 mm in diameter) ultrasonic probe with high-frequency ultrasound (12-30 MHz) can be useful. This technique results in images that have a high spatial resolution and can be used to determine the extent of a tumor along the MPD or the progression of a tumor from a branch duct into the MPD. Thus, it provides critical information for surgical candidates with IPMN. It can also detect flat lesions that are less than 500 μm in height[65], but the depth of image penetration is limited (Figure 8E).

MCN

MCNs are more common in middle-aged women and are located more frequently in the body and tail of the pancreas. Although MCNs are typically macrocystic tumors that are > 2 cm in diameter, there are also small MCNS that are only a few, millimeters in diameter (Figure 9). Peripheral calcifications are found in 15% of patients but can also occur in other cystic lesions, as well as in mural nodes or vegetations[66]. Pancreatic duct communication is seldom seen because MCNs originate within the peripheral ductal system. Angiography, although rarely performed on these lesions, shows that most MCNs are hypervascular. Evidence of malignancy includes the presence of cyst wall irregularity and thickening, intracystic solid regions, or an adjacent solid mass. The presence of "ovarian type stroma" is strongly suggestive of an MCN lesion, although MCNs with "non-ovarian type stroma" have also been reported[67,68].

EUS-FNA

EUS-FNA has proved to be a safe and useful method for tissue sampling of pancreatic masses. The safety of EUS-FNA for evaluating pancreatic lesions is now well established[69-71]. Several studies have reported that the rate of complications, which include pancreatitis, infection, and bleeding, is 0%-2%[69,72,73]. In addition, a multicenter study evaluating the safety of EUS-FNA of solid pancreatic masses found that, 14 of 4958 patients developed pancreatitis[69]. The accuracy of EUS-FNA for the diagnosis of pancreatic carcinoma and neuroendocrine tumors is reported to be 80%-95%[72-75] and 46%-83%[75,76], respectively. The low accuracy for endo-

crine tumors may be because inadequate hemorrhagic samples are often obtained: this reflects the vascular nature of these tumors. In terms of the diagnostic sensitivity of EUS-FNA, a study of 282 patients with pancreatic solid tumors with and without chronic pancreatitis found that the diagnostic sensitivity of EUS-FNA was significantly lower for chronic pancreatitis cases (73.9% *vs* 91.3%, $P = 0.02$)[36]. Another study of 69 patients with chronic pancreatitis showed that compared to EUS alone, EUS-FNA of the patients' masses improved the sensitivity, specificity and overall accuracy with which inflammatory conditions could be differentiated from pancreatic adenocarcinomas (63.6% *vs* 72.7%, 75.9% *vs* 100%, 73.9% *vs* 95.7%, respectively)[77]. However, the relatively poor sensitivity of EUS-FNA means that even this technique is insufficient for distinguishing between inflammatory and malignant masses. If the EUS-FNA data are suggestive of pancreatitis but other diagnostic modalities, including EUS, point to pancreatic cancer, close follow-up tests must be performed.

EUS-FNA of a cystic lesion may improve the accuracy of EUS since it permits the cystic fluid to be analyzed and a cytological diagnosis to be made. The cytological analyses include specific testing for the presence of columnar epithelial cells that stain for mucin (which is suggestive of MCNs or IPMNs), or cuboidal epithelial cells that stain for glycogen (which is suggestive of SCAs). In relation to this, a recent cooperative, multicenter trial in the United States studied 112 patients with cystic lesions of the pancreas who first underwent EUS-FNA and then surgical resection of their masses (which provided a histological diagnosis)[54]. The accuracy with which EUS, cystic fluid cytology, and staining of the cyst fluid for tumor markers such as carcinoembryonic antigen (CEA) provided the correct diagnosis was assessed. Of the 112 patients, 68, 7, 25, 5 and 5 were found to have mucinous, serous, inflammatory, endocrine, and other cystic lesions, respectively. Immunostaining for CEA differentiated between mucinous and non-mucinous cystic lesions with significantly greater accuracy (79%) than EUS morphology (51%) or cytology (59%). The investigators concluded that cystic lesions should be aspirated and that the fluid should be analyzed for CEA to differentiate between mucinous and non-mucinous lesions. In contrast, another study found that cystic fluid aspiration and CEA analysis did not improve diagnoses made on the basis of EUS[78]. In this study, 34 patients with a cystic lesion underwent EUS-FNA followed by resection of the lesion. The abilities of EUS, cytology, and cystic fluid analysis to provide a diagnosis were compared. Histological analysis revealed that the lesions were benign (simple cysts, pseudocysts, or SCAs) or malignant/potentially malignant (MCAs, IPMNs, cystic islet cell tumors, or cystic adenocarcinomas). The diagnostic sensitivities of EUS, cytology and CEA were 91%, 27%, and 28%, respectively ($P = 0.01$), their specificities were 60%, 100%, and 25%, respectively, and their accuracies were 82%, 55%, and 27%, respectively. If EUS was combined with cytopathology and CEA, its diagnostic accuracy did not improve further. It was concluded that cystic fluid cytology and CEA analysis does not improve the diagnostic ability of EUS.

Table 1 Pancreatic cyst fluid levels of amylase and tumor markers

	Serous cystadenoma	Mucinous cystic neoplasm	IPMN	Pseudocyst
Amylase	Low	Low	High	High
CEA	Low	High	High	Low
CA 72-4	Low	High	High	Low
CA 19-9	Variable	Variable	Variable	High
CA 125	Low	Variable	Low	Low

IPMN: Intraductal papillary mucinous neoplasia; CEA: Carcinoembryonic antigen; CA: Carbohydrate antigen.

Tumor markers other than CEA have also been used to analyze pancreatic cystic fluids sampled by EUS-FNA. These include CA19-9, CA125, and CA 72-4. The largest study to date that has examined the ability of multiple tumor markers in cystic fluid to detect benign and malignant mucinous cystic lesions in pancreatic cystic lesions found that mucinous cystic tumors had significant CA 72-4 levels and that this marker could detect mucinous or malignant cysts with a specificity and sensitivity of 95% and 80%, respectively[79].

Fluid obtained during FNA of pancreatic cysts could be sent for biochemical and cytological analysis, and tumor marker levels, which often determines the cyst type and the presence of malignancy[80-84]. A combined analysis of 11 studies[85,86] found that cytology from cyst fluid was diagnostic in 38% to 48% of cystic pancreatic neoplasms, and the Cooperative Pancreatic Cyst Study[84] determined the diagnostic accuracy to be 59% in this setting. When tumor markers, amylase testing and mucin staining are combined with cytological testing, the diagnostic accuracy increases to 80% or 90%[80-84] (Table 1). High levels of cyst fluid amylase are more often found in cysts that communicate with pancreatic ducts (pseudocysts and IPMN); a cyst fluid amylase level greater than 5000 U/L has a sensitivity and specificity of 61% and 58%, respectively, for distinguishing pseudocysts from other cystic neoplasms[86,87].

With regard to the complications associated with EUS-FNA of pancreatic cystic lesions, it has been reported that in 81 patients subjected to EUS-FNA, one developed an infected cystadenoma[88]. This patient did not receive prophylactic antibiotics before the procedure. The current standard of care for patients undergoing FNA of a pancreatic cystic lesion includes routine administration of antibiotics.

CONTRAST-ENHANCED EUS

While EUS is a diagnostic method that can detect small pancreatic lesions with high sensitivity, it remains difficult to differentially diagnose pancreatic lesions, especially malignant neoplasms in patients with chronic pancre-

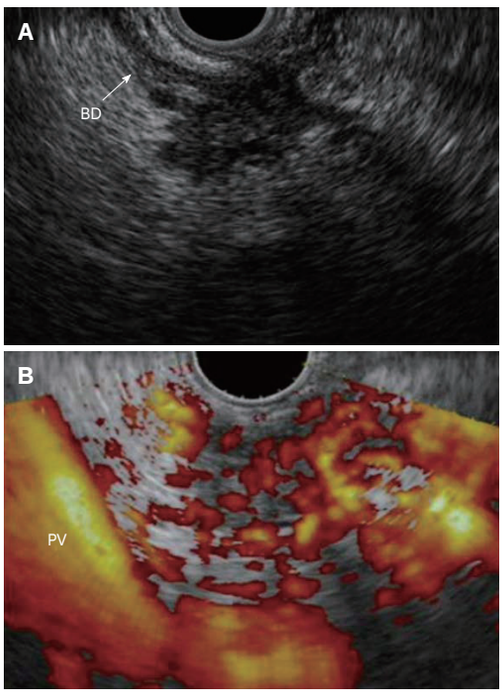


Figure 10 Focal chronic pancreatitis. A: EUS shows a mass with an irregular and inhomogeneous echo pattern at the head of the pancreas; B: Contrast-enhanced power Doppler EUS shows an isovascular nodule compared with the surrounding pancreatic tissue. BD: Bile duct; PV: Portal vein.

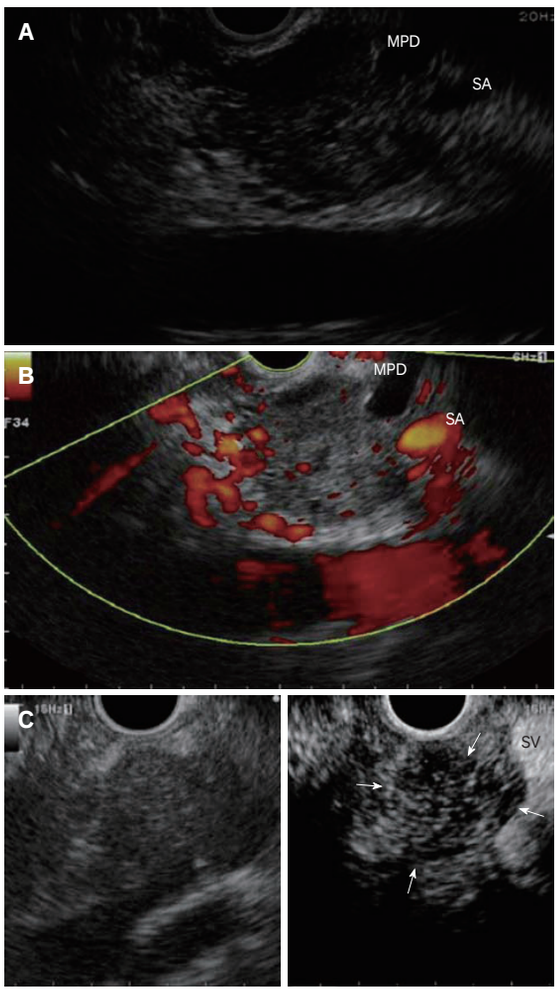


Figure 11 Pancreatic adenocarcinoma. A: EUS shows a heterogeneous hypoechoic mass with irregular margins at the body of the pancreas and tail side main pancreatic duct enlarged due to the infiltrating mass; B: Contrast-enhanced power Doppler EUS shows a hypovascular nodule compared with the surrounding pancreatic tissue; C: Contrast-enhanced harmonic EUS showing a clear margin and hypovascular nodule compared with surrounding pancreatic tissue (arrows) without blooming artifact such as that found with Doppler imaging. Left: B-mode imaging; Right: Contrast imaging. MPD: Main pancreatic duct; SA: Splenic artery.

atitis[89]. The introduction of EUS-FNA has made this task easier, however, there are cases where the diagnosis is still difficult using EUS-FNA. These include cases where the EUS-FNA aspirant contains insufficient tumor material because the pancreatic tumor is small, and cases with severe chronic pancreatitis that make it difficult to see the borders of the lesion, thereby hampering the accurate insertion of the needle. Moreover, there are cases where a non-invasive diagnostic technique is needed because the patient is using anticoagulants. For these reasons, CE-EUS was developed.

Contrast-enhanced techniques provide information on vascularity and blood flow in normal and pathological tissues. CE-US has played an important role in clinical practice by aiding the differential diagnosis of diseases in a wide array of organs, including the liver, gallbladder, bile duct, pancreas, kidney, thyroid, and prostate. It has also helped to guide interventional procedures and to evaluate treatment responses after local therapies and chemotherapy[90-95].

Several studies that assessed the utility of CE-EUS for diagnosing pancreatic tumors were reported recently[23,96-100]. One of these was our study comparing the ability of power Doppler EUS (PD-EUS), CE-EUS with power Doppler mode using first generation US contrast agent (Levovist), and contrast-enhanced helical CT (CE-CT) to diagnose small pancreatic tumors[23]. PD-EUS and CE-EUS allowed the pancreatic tumors to be classified according to their density of vessels relative to the vascularity of the surrounding pancreatic tissue, namely as, hypovascular, isovascular, and hypervascular (Figures 10 and 11): For small pancreatic tumors that were $\leqslant$ 2 cm, the sensitivity with which PD-EUS, CE-EUS and CE-CT differentiated ductal carcinoma from other tumors was 50%, 83.3% and 11%, respectively. Thus, CE-EUS was significantly more sensitive than PD-EUS and CE-CT, which suggests that CE-EUS is particularly useful for differentially diagnosing, pancreatic tumors, especially small pancreatic tumors. However, such Doppler ultrasonography with contrast enhancement has several limitations, including blooming artifacts, poor spatial resolution, and low sensitivity to slow flow[96-99]. Indeed, in our study, these limitations

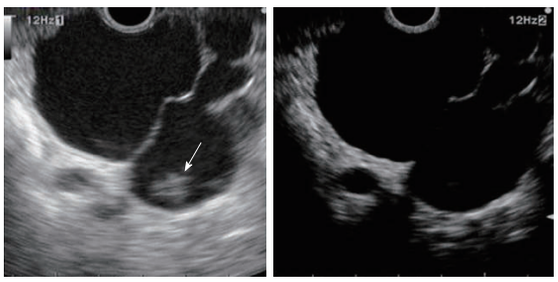


Figure 12 IPMN of side branch type. Left (B-mode image): The nodule (arrow) in dilatation of the side branch cannot be distinguished between sediment and tumor by B-EUS; Right (contrast image): Contrast-enhanced harmonic EUS reveals that this nodule is sediment.

prevented vascularity from benign evaluated in 7.8% of all patients, of whom 22.2% had carcinomas that were ⩽ 2 cm in diameter.

Hocke *et al*[100] evaluated the ability of CE-EUS with power Doppler mode using SonoVue, a second generation US contrast agent, to differentiate inflammation from pancreatic carcinoma on the basis of the perfusion characteristics of the microvessels. For this study, chronic pancreatitis without neoplasm was defined as the lack of detectable vascularization or the regular appearance of vessels both before and after the injection of SonoVue, and the detection of both arterial and venous vessels in the contrast-enhanced phase. Malignancy was defined as the lack of detectable vascularization before the injection of SonoVue, the irregular appearance of arterial vessels after the injection of SonoVue, and the absence of venous vessels in the lesion. In patients with chronic pancreatitis, combined conventional B-mode and power Doppler EUS diagnosed pancreatic cancer with a sensitivity and specificity of 73.2% and 83.3%, respectively, whereas CE-EUS with power Doppler had a sensitivity and specificity of 91.1% and 93.3%, respectively. Thus, CE-EUS is highly useful for the differential diagnosis of pancreatic cancer.

CONTRAST-ENHANCED HARMONIC EUS

Kitano *et al*[101] recently developed an echoendoscope with a broad-band transducer and an imaging mode specifically for CEH-EUS. This technology can detect signals from microbubbles in vessels with a very slow flow without Doppler-related artifacts and can be used to characterize tumor vascularity in the pancreas (Figure 11C). Second-generation US contrast agents such as SonoVue and Sonazoid, harmonic signals at low acoustic powers and thus are suitable for EUS imaging at low acoustic powers[102,103]. CEH-EUS successfully creates novel perfusion images and the vascular structures of pancreatic lesions (Figure 12). This CEH-EUS mediated evaluation of the microvasculature of pancreas lesions is expected to improve the differential diagnosis of pancreatic disease in the near future.

OTHER DIAGNOSTIC EUS ADAPTED PROCEDURES

IDUS

The list of indications of EUS is growing, which has forced gastroenterologists to think outside the lumen. Technological advances in EUS imaging has led to the development of IDUS mini propes for the evaluation of the pancreatobiliary tree and periductal structures. In the evaluation of patients with pancreatic duct stenosis, IDUS can be used to distinguish malignant strictures, allow for the early detection of small pancreatic adenocarcinomas, assist in local staging and to determine resectability[104,105]. IDUS may also be useful for the localization of pancreatic neuroendocrine tumors not visualized by other imaging modalities[104-106]. In the evaluation of IPMN, IDUS is used to determine malignant disease and disease extent before surgery. IDUS and pancreatoscopy had a reported combined sensitivity, specificity and accuracy of 91%, 82% and 88%, respectively[107].

EUS-elastography

EUS-elastography can assess tissue hardness by measuring its elasticity which might provide clinical utility in the diagnosis of pancreatic disorders. Tissue elasticity studies can provide information on both its pattern and distribution. EUS-elastography has introduced a new form of pathologic analysis, that is, tissue elasticity. This parameter appears to correlate with the malignant potential of the lesions. Importantly, the image of EUS elastography indicates the relative value in a region of interest (ROI), so the same lesion might display different colors in a different ROI. This is a limitation of EUS-elastography. The other is the distribution of tissue elasticity. With the prototype image analysis software, we can now capture and analyze features of real-time tissue elastography by using computer software. Theoretically, this will limit interpretation bias and provide a measure of pattern distribution that is constant and independent, regardless of ROIs[108]. More studies and greater experience are needed before it has a place in our diagnostic armamentarium.

Tridimensional-EUS

Tridimensional (3D)-EUS certainly facilitates anatomical interpretation of the images in the pancreatobiliary area, as well as vascular landmarks used for staging and assessment of resectability. The method might be feasible for the assessment of venous invasion and venous compression in focal pancreatic masses, in both chronic pancreatitis and pancreatic cancer[109]. The acquisition of 3D volume allows a retrospective assessment and slicing of the reconstructed cube, with accurate depiction of focal masses, even if missed on the initial real-time evaluation. However, further progress of the technology is still necessary.

CONCLUSION

EUS is established as a most accurate method for stag-

ing malignancies of the pancreas, particularly small pancreatic lesions. EUS-FNA also allows safe tissue sampling of pancreatic tumors. EUS and EUS-FNA are now indispensable for the management of pancreatic tumors. In addition, we have recently been able to use various new EUS adapted technologies such as CE-EUS and CEH-EUS in clinical practice, which are helpful in the differential diagnosis of pancreatic tumors, especially small pancreatic tumors. Further improvements in EUS technology are expected to provide more useful modalities for the detection and diagnosis of pancreatic tumors.

REFERENCES

1 **Kamisawa T**, Egawa N, Nakajima H, Tsuruta K, Okamoto A, Kamata N. Clinical difficulties in the differentiation of autoimmune pancreatitis and pancreatic carcinoma. *Am J Gastroenterol* 2003; **98**: 2694-2699
2 **Yadav D**, Notahara K, Smyrk TC, Clain JE, Pearson RK, Farnell MB, Chari ST. Idiopathic tumefactive chronic pancreatitis: clinical profile, histology, and natural history after resection. *Clin Gastroenterol Hepatol* 2003; **1**: 129-135
3 **Kalra MK**, Maher MM, Mueller PR, Saini S. State-of-the-art imaging of pancreatic neoplasms. *Br J Radiol* 2003; **76**: 857-865
4 **Visser BC**, Muthusamay VR, Mulvihill SJ, Coakley F. Diagnostic imaging of cystic pancreatic neoplasms. *Surg Oncol* 2004; **13**: 27-39
5 **Buscail L**, Pagès P, Berthélemy P, Fourtanier G, Frexinos J, Escourrou J. Role of EUS in the management of pancreatic and ampullary carcinoma: a prospective study assessing resectability and prognosis. *Gastrointest Endosc* 1999; **50**: 34-40
6 **Hunt GC**, Faigel DO. Assessment of EUS for diagnosing, staging, and determining resectability of pancreatic cancer: a review. *Gastrointest Endosc* 2002; **55**: 232-237
7 **Mertz HR**, Sechopoulos P, Delbeke D, Leach SD. EUS, PET, and CT scanning for evaluation of pancreatic adenocarcinoma. *Gastrointest Endosc* 2000; **52**: 367-371
8 **tenBerge J**, Hoffman BJ, Hawes RH, Van Enckevort C, Giovannini M, Erickson RA, Catalano MF, Fogel R, Mallery S, Faigel DO, Ferrari AP, Waxman I, Palazzo L, Ben-Menachem T, Jowell PS, McGrath KM, Kowalski TE, Nguyen CC, Wassef WY, Yamao K, Chak A, Greenwald BD, Woodward TA, Vilmann P, Sabbagh L, Wallace MB. EUS-guided fine needle aspiration of the liver: indications, yield, and safety based on an international survey of 167 cases. *Gastrointest Endosc* 2002; **55**: 859-862
9 **Sahai AV**, Zimmerman M, Aabakken L, Tarnasky PR, Cunningham JT, van Velse A, Hawes RH, Hoffman BJ. Prospective assessment of the ability of endoscopic ultrasound to diagnose, exclude, or establish the severity of chronic pancreatitis found by endoscopic retrograde cholangiopancreatography. *Gastrointest Endosc* 1998; **48**: 18-25
10 **Sahai AV**. EUS and chronic pancreatitis. *Gastrointest Endosc* 2002; **56**: S76-S81
11 Reproduction of minimal standard terminology in Gastrointestinal Endosonography. *Dig Endosc* 1998; **10**: 158-185
12 **Wiersema MJ**, Hawes RH, Lehman GA, Kochman ML, Sherman S, Kopecky KK. Prospective evaluation of endoscopic ultrasonography and endoscopic retrograde cholangiopancreatography in patients with chronic abdominal pain of suspected pancreatic origin. *Endoscopy* 1993; **25**: 555-564
13 **Wallace MB**, Hawes RH, Durkalski V, Chak A, Mallery S, Catalano MF, Wiersema MJ, Bhutani MS, Ciaccia D, Kochman ML, Gress FG, Van Velse A, Hoffman BJ. The reliability of EUS for the diagnosis of chronic pancreatitis: interobserver agreement among experienced endosonographers. *Gastrointest Endosc* 2001; **53**: 294-299
14 **Buscail L**, Escourrou J, Moreau J, Delvaux M, Louvel D, Lapeyre F, Tregant P, Frexinos J. Endoscopic ultrasonography in chronic pancreatitis: a comparative prospective study with conventional ultrasonography, computed tomography, and ERCP. *Pancreas* 1995; **10**: 251-257
15 **Catalano MF**, Lahoti S, Geenen JE, Hogan WJ. Prospective evaluation of endoscopic ultrasonography, endoscopic retrograde pancreatography, and secretin test in the diagnosis of chronic pancreatitis. *Gastrointest Endosc* 1998; **48**: 11-17
16 **Hollerbach S**, Klamann A, Topalidis T, Schmiegel WH. Endoscopic ultrasonography (EUS) and fine-needle aspiration (FNA) cytology for diagnosis of chronic pancreatitis. *Endoscopy* 2001; **33**: 824-831
17 **Rösch T**, Lorenz R, Braig C, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Endoscopic ultrasound in pancreatic tumor diagnosis. *Gastrointest Endosc* 1991; **37**: 347-352
18 **Glasbrenner B**, Schwarz M, Pauls S, Preclik G, Beger HG, Adler G. Prospective comparison of endoscopic ultrasound and endoscopic retrograde cholangiopancreatography in the preoperative assessment of masses in the pancreatic head. *Dig Surg* 2000; **17**: 468-474
19 **Brand B**, Pfaff T, Binmoeller KF, Sriram PV, Fritscher-Ravens A, Knöfel WT, Jäckle S, Soehendra N. Endoscopic ultrasound for differential diagnosis of focal pancreatic lesions, confirmed by surgery. *Scand J Gastroenterol* 2000; **35**: 1221-1228
20 **Bhutani MS**, Gress FG, Giovannini M, Erickson RA, Catalano MF, Chak A, Deprez PH, Faigel DO, Nguyen CC. The No Endosonographic Detection of Tumor (NEST) Study: a case series of pancreatic cancers missed on endoscopic ultrasonography. *Endoscopy* 2004; **36**: 385-389
21 **DeWitt J**, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004; **141**: 753-763
22 **Maguchi H**, Takahashi K, Osanai M, Katanuma A. Small pancreatic lesions: is there need for EUS-FNA preoperatively? What to do with the incidental lesions? *Endoscopy* 2006; **38** Suppl 1: S53-S56
23 **Sakamoto H**, Kitano M, Suetomi Y, Maekawa K, Takeyama Y, Kudo M. Utility of contrast-enhanced endoscopic ultrasonography for diagnosis of small pancreatic carcinomas. *Ultrasound Med Biol* 2008; **34**: 525-532
24 **Gress FG**, Hawes RH, Savides TJ, Ikenberry SO, Cummings O, Kopecky K, Sherman S, Wiersema M, Lehman GA. Role of EUS in the preoperative staging of pancreatic cancer: a large single-center experience. *Gastrointest Endosc* 1999; **50**: 786-791
25 **Palazzo L**, Roseau G, Gayet B, Vilgrain V, Belghiti J, Fékéte F, Paolaggi JA. Endoscopic ultrasonography in the diagnosis and staging of pancreatic adenocarcinoma. Results of a prospective study with comparison to ultrasonography and CT scan. *Endoscopy* 1993; **25**: 143-150
26 **Yasuda K**, Mukai H, Nakajima M, Kawai K. Staging of pancreatic carcinoma by endoscopic ultrasonography. *Endoscopy* 1993; **25**: 151-155
27 **DeWitt J**, Devereaux B, Chriswell M, McGreevy K, Howard T, Imperiale TF, Ciaccia D, Lane KA, Maglinte D, Kopecky K, LeBlanc J, McHenry L, Madura J, Aisen A, Cramer H, Cummings O, Sherman S. Comparison of endoscopic ultrasonography and multidetector computed tomography for detecting and staging pancreatic cancer. *Ann Intern Med* 2004; **141**: 753-763
28 **Legmann P**, Vignaux O, Dousset B, Baraza AJ, Palazzo L, Dumontier I, Coste J, Louvel A, Roseau G, Couturier D,

Bonnin A. Pancreatic tumors: comparison of dual-phase helical CT and endoscopic sonography. *AJR Am J Roentgenol* 1998; **170**: 1315-1322
29 **Nakaizumi A**, Uehara H, Iishi H, Tatsuta M, Kitamura T, Kuroda C, Ohigashi H, Ishikawa O, Okuda S. Endoscopic ultrasonography in diagnosis and staging of pancreatic cancer. *Dig Dis Sci* 1995; **40**: 696-700
30 **Bhutani MS**, Hawes RH, Hoffman BJ. A comparison of the accuracy of echo features during endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration for diagnosis of malignant lymph node invasion. *Gastrointest Endosc* 1997; **45**: 474-479
31 **Soriano A**, Castells A, Ayuso C, Ayuso JR, de Caralt MT, Ginès MA, Real MI, Gilabert R, Quintó L, Trilla A, Feu F, Montanyà X, Fernández-Cruz L, Navarro S. Preoperative staging and tumor resectability assessment of pancreatic cancer: prospective study comparing endoscopic ultrasonography, helical computed tomography, magnetic resonance imaging, and angiography. *Am J Gastroenterol* 2004; **99**: 492-501
32 **Ramsay D**, Marshall M, Song S, Zimmerman M, Edmunds S, Yusoff I, Cullingford G, Fletcher D, Mendelson R. Identification and staging of pancreatic tumours using computed tomography, endoscopic ultrasound and mangafodipir trisodium-enhanced magnetic resonance imaging. *Australas Radiol* 2004; **48**: 154-161
33 **Rösch T**, Dittler HJ, Strobel K, Meining A, Schusdziarra V, Lorenz R, Allescher HD, Kassem AM, Gerhardt P, Siewert JR, Höfler H, Classen M. Endoscopic ultrasound criteria for vascular invasion in the staging of cancer of the head of the pancreas: a blind reevaluation of videotapes. *Gastrointest Endosc* 2000; **52**: 469-477
34 **Brugge WR**, Lee MJ, Kelsey PB, Schapiro RH, Warshaw AL. The use of EUS to diagnose malignant portal venous system invasion by pancreatic cancer. *Gastrointest Endosc* 1996; **43**: 561-567
35 **Zimmer T**, Scherübl H, Faiss S, Stölzel U, Riecken EO, Wiedenmann B. Endoscopic ultrasonography of neuroendocrine tumours. *Digestion* 2000; **62** Suppl 1: 45-50
36 **King CM**, Reznek RH, Dacie JE, Wass JA. Imaging islet cell tumours. *Clin Radiol* 1994; **49**: 295-303
37 **McLean AM**, Fairclough PD. Endoscopic ultrasound in the localisation of pancreatic islet cell tumours. *Best Pract Res Clin Endocrinol Metab* 2005; **19**: 177-193
38 **Anderson MA**, Carpenter S, Thompson NW, Nostrant TT, Elta GH, Scheiman JM. Endoscopic ultrasound is highly accurate and directs management in patients with neuroendocrine tumors of the pancreas. *Am J Gastroenterol* 2000; **95**: 2271-2277
39 **Reed K**, Vose PC, Jarstfer BS. Pancreatic cancer: 30 year review (1947 to 1977). *Am J Surg* 1979; **138**: 929-933
40 **Volmar KE**, Routbort MJ, Jones CK, Xie HB. Primary pancreatic lymphoma evaluated by fine-needle aspiration: findings in 14 cases. *Am J Clin Pathol* 2004; **121**: 898-903
41 **Merkle EM**, Bender GN, Brambs HJ. Imaging findings in pancreatic lymphoma: differential aspects. *AJR Am J Roentgenol* 2000; **174**: 671-675
42 **Faure JP**, Tuech JJ, Richer JP, Pessaux P, Arnaud JP, Carretier M. Pancreatic metastasis of renal cell carcinoma: presentation, treatment and survival. *J Urol* 2001; **165**: 20-22
43 **Matsukuma S**, Suda K, Abe H, Ogata S, Wada R. Metastatic cancer involving pancreatic duct epithelium and its mimicry of primary pancreatic cancer. *Histopathology* 1997; **30**: 208-213
44 **DeWitt J**, Jowell P, Leblanc J, McHenry L, McGreevy K, Cramer H, Volmar K, Sherman S, Gress F. EUS-guided FNA of pancreatic metastases: a multicenter experience. *Gastrointest Endosc* 2005; **61**: 689-696
45 **Sarr MG**, Carpenter HA, Prabhakar LP, Orchard TF, Hughes S, van Heerden JA, DiMagno EP. Clinical and pathologic correlation of 84 mucinous cystic neoplasms of the pancreas: can one reliably differentiate benign from malignant (or pre-malignant) neoplasms? *Ann Surg* 2000; **231**: 205-212
46 **Wilentz RE**, Albores-Saavedra J, Zahurak M, Talamini MA, Yeo CJ, Cameron JL, Hruban RH. Pathologic examination accurately predicts prognosis in mucinous cystic neoplasms of the pancreas. *Am J Surg Pathol* 1999; **23**: 1320-1327
47 **Siech M**, Tripp K, Schmidt-Rohlfing B, Mattfeldt T, Widmaier U, Gansauge F, Görich J, Beger HG. Cystic tumours of the pancreas: diagnostic accuracy, pathologic observations and surgical consequences. *Langenbecks Arch Surg* 1998; **383**: 56-61
48 **Song MH**, Lee SK, Kim MH, Lee HJ, Kim KP, Kim HJ, Lee SS, Seo DW, Min YI. EUS in the evaluation of pancreatic cystic lesions. *Gastrointest Endosc* 2003; **57**: 891-896
49 **Johnson CD**, Stephens DH, Charboneau JW, Carpenter HA, Welch TJ. Cystic pancreatic tumors: CT and sonographic assessment. *AJR Am J Roentgenol* 1988; **151**: 1133-1138
50 **Ariyama J**, Suyama M, Satoh K, Wakabayashi K. Endoscopic ultrasound and intraductal ultrasound in the diagnosis of small pancreatic tumors. *Abdom Imaging* 1998; **23**: 380-386
51 **Gress F**, Gottlieb K, Cummings O, Sherman S, Lehman G. Endoscopic ultrasound characteristics of mucinous cystic neoplasms of the pancreas. *Am J Gastroenterol* 2000; **95**: 961-965
52 **Torresan F**, Casadei R, Solmi L, Marrano D, Gandolfi L. The role of ultrasound in the differential diagnosis of serous and mucinous cystic tumours of the pancreas. *Eur J Gastroenterol Hepatol* 1997; **9**: 169-172
53 **Brugge WR**. The role of EUS in the diagnosis of cystic lesions of the pancreas. *Gastrointest Endosc* 2000; **52**: S18-S22
54 **Brugge WR**, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology* 2004; **126**: 1330-1336
55 **Frossard JL**, Amouyal P, Amouyal G, Palazzo L, Amaris J, Soldan M, Giostra E, Spahr L, Hadengue A, Fabre M. Performance of endosonography-guided fine needle aspiration and biopsy in the diagnosis of pancreatic cystic lesions. *Am J Gastroenterol* 2003; **98**: 1516-1524
56 **Sedlack R**, Affi A, Vazquez-Sequeiros E, Norton ID, Clain JE, Wiersema MJ. Utility of EUS in the evaluation of cystic pancreatic lesions. *Gastrointest Endosc* 2002; **56**: 543-547
57 **Ahmad NA**, Kochman ML, Lewis JD, Ginsberg GG. Can EUS alone differentiate between malignant and benign cystic lesions of the pancreas? *Am J Gastroenterol* 2001; **96**: 3295-3300
58 **Gerke H**, Jaffe TA, Mitchell RM, Byrne MF, Stiffler HL, Branch MS, Baillie J, Jowell PS. Endoscopic ultrasound and computer tomography are inaccurate methods of classifying cystic pancreatic lesions. *Dig Liver Dis* 2006; **38**: 39-44
59 **Compton CC**. Serous cystic tumors of the pancreas. *Semin Diagn Pathol* 2000; **17**: 43-55
60 **Warshaw AL**, Rutledge PL. Cystic tumors mistaken for pancreatic pseudocysts. *Ann Surg* 1987; **205**: 393-398
61 **Azar C**, Van de Stadt J, Rickaert F, Devière M, Baize M, Klöppel G, Gelin M, Cremer M. Intraductal papillary mucinous tumours of the pancreas. Clinical and therapeutic issues in 32 patients. *Gut* 1996; **39**: 457-464
62 **Hruban RH**, Takaori K, Klimstra DS, Adsay NV, Albores-Saavedra J, Biankin AV, Biankin SA, Compton C, Fukushima N, Furukawa T, Goggins M, Kato Y, Klöppel G, Longnecker DS, Lüttges J, Maitra A, Offerhaus GJ, Shimizu M, Yonezawa S. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol* 2004; **28**: 977-987
63 **Seicean A**, Tantau M, Badea R, Spârchez Z. The applicability of radial endoscopic ultrasonography in pancreatic diseases. *J Gastrointestin Liver Dis* 2007; **16**: 77-83
64 **Sakamoto H**, Kitano M, Komaki T, Imai H, Kamata K, Kimura M, Takeyama Y, Kudo M. Small invasive ductal

carcinoma of the pancreas distinct from branch duct intraductal papillary mucinous neoplasm. *World J Gastroenterol* 2009; **15**: 5489-5492
65 **Hara T**, Yamaguchi T, Ishihara T, Tsuyuguchi T, Kondo F, Kato K, Asano T, Saisho H. Diagnosis and patient management of intraductal papillary-mucinous tumor of the pancreas by using peroral pancreatoscopy and intraductal ultrasonography. *Gastroenterology* 2002; **122**: 34-43
66 **Koito K**, Namieno T, Nagakawa T, Shyonai T, Hirokawa N, Morita K. Solitary cystic tumor of the pancreas: EUS-pathologic correlation. *Gastrointest Endosc* 1997; **45**: 268-276
67 **Compagno J**, Oertel JE. Mucinous cystic neoplasms of the pancreas with overt and latent malignancy (cystadenocarcinoma and cystadenoma). A clinicopathologic study of 41 cases. *Am J Clin Pathol* 1978; **69**: 573-580
68 **Tanaka M**, Chari S, Adsay V, Fernandez-del Castillo C, Falconi M, Shimizu M, Yamaguchi K, Yamao K, Matsuno S. International consensus guidelines for management of intraductal papillary mucinous neoplasms and mucinous cystic neoplasms of the pancreas. *Pancreatology* 2006; **6**: 17-32
69 **Eloubeidi MA**, Gress FG, Savides TJ, Wiersema MJ, Kochman ML, Ahmad NA, Ginsberg GG, Erickson RA, Dewitt J, Van Dam J, Nickl NJ, Levy MJ, Clain JE, Chak A, Sivak MV Jr, Wong R, Isenberg G, Scheiman JM, Bounds B, Kimmey MB, Saunders MD, Chang KJ, Sharma A, Nguyen P, Lee JG, Edmundowicz SA, Early D, Azar R, Etemad B, Chen YK, Waxman I, Shami V, Catalano MF, Wilcox CM. Acute pancreatitis after EUS-guided FNA of solid pancreatic masses: a pooled analysis from EUS centers in the United States. *Gastrointest Endosc* 2004; **60**: 385-389
70 **Sakamoto H**, Kitano M, Komaki T, Noda K, Chikugo T, Dote K, Takeyama Y, Das K, Yamao K, Kudo M. Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. *J Gastroenterol Hepatol* 2009; **24**: 384-390
71 **Sakamoto H**, Kitano M, Dote K, Tchikugo T, Takeyama Y, Kudo M. In situ carcinoma of pancreas diagnosed by EUS-FNA. *Endoscopy* 2008; **40** Suppl 2: E15-E16
72 **Harewood GC**, Wiersema MJ. Endosonography-guided fine needle aspiration biopsy in the evaluation of pancreatic masses. *Am J Gastroenterol* 2002; **97**: 1386-1391
73 **Raut CP**, Grau AM, Staerkel GA, Kaw M, Tamm EP, Wolff RA, Vauthey JN, Lee JE, Pisters PW, Evans DB. Diagnostic accuracy of endoscopic ultrasound-guided fine-needle aspiration in patients with presumed pancreatic cancer. *J Gastrointest Surg* 2003; **7**: 118-126; discussion 127-128
74 **Agarwal B**, Abu-Hamda E, Molke KL, Correa AM, Ho L. Endoscopic ultrasound-guided fine needle aspiration and multidetector spiral CT in the diagnosis of pancreatic cancer. *Am J Gastroenterol* 2004; **99**: 844-850
75 **Voss M**, Hammel P, Molas G, Palazzo L, Dancour A, O'Toole D, Terris B, Degott C, Bernades P, Ruszniewski P. Value of endoscopic ultrasound guided fine needle aspiration biopsy in the diagnosis of solid pancreatic masses. *Gut* 2000; **46**: 244-249
76 **Ardengh JC**, de Paulo GA, Ferrari AP. EUS-guided FNA in the diagnosis of pancreatic neuroendocrine tumors before surgery. *Gastrointest Endosc* 2004; **60**: 378-384
77 **Ardengh JC**, Lopes CV, Campos AD, Pereira de Lima LF, Venco F, Módena JL. Endoscopic ultrasound and fine needle aspiration in chronic pancreatitis: differential diagnosis between pseudotumoral masses and pancreatic cancer. *JOP* 2007; **8**: 413-421
78 **Sedlack R**, Affi A, Vazquez-Sequeiros E, Norton ID, Clain JE, Wiersema MJ. Utility of EUS in the evaluation of cystic pancreatic lesions. *Gastrointest Endosc* 2002; **56**: 543-547
79 **Sperti C**, Pasquali C, Guolo P, Polverosi R, Liessi G, Pedrazzoli S. Serum tumor markers and cyst fluid analysis are useful for the diagnosis of pancreatic cystic tumors. *Cancer* 1996; **78**: 237-243
80 **Carlson SK**, Johnson CD, Brandt KR, Batts KP, Salomao DR. Pancreatic cystic neoplasms: the role and sensitivity of needle aspiration and biopsy. *Abdom Imaging* 1998; **23**: 387-393
81 **Nguyen GK**, Suen KC, Villanueva RR. Needle aspiration cytology of pancreatic cystic lesions. *Diagn Cytopathol* 1997; **17**: 177-182
82 **Sperti C**, Pasquali C, Guolo P, Polverosi R, Liessi G, Pedrazzoli S. Serum tumor markers and cyst fluid analysis are useful for the diagnosis of pancreatic cystic tumors. *Cancer* 1996; **78**: 237-243
83 **Hammel P**. Role of tumor markers in the diagnosis of cystic and intraductal neoplasms. *Gastrointest Endosc Clin N Am* 2002; **12**: 791-801
84 **Brugge WR**, Lewandrowski K, Lee-Lewandrowski E, Centeno BA, Szydlo T, Regan S, del Castillo CF, Warshaw AL. Diagnosis of pancreatic cystic neoplasms: a report of the cooperative pancreatic cyst study. *Gastroenterology* 2004; **126**: 1330-1336
85 **van der Waaij LA**, van Dullemen HM, Porte RJ. Cyst fluid analysis in the differential diagnosis of pancreatic cystic lesions: a pooled analysis. *Gastrointest Endosc* 2005; **62**: 383-389
86 **Frossard JL**, Amouyal P, Amouyal G, Palazzo L, Amaris J, Soldan M, Giostra E, Spahr L, Hadengue A, Fabre M. Performance of endosonography-guided fine needle aspiration and biopsy in the diagnosis of pancreatic cystic lesions. *Am J Gastroenterol* 2003; **98**: 1516-1524
87 **Sand JA**, Hyoty MK, Mattila J, Dagorn JC, Nordback IH. Clinical assessment compared with cyst fluid analysis in the differential diagnosis of cystic lesions in the pancreas. *Surgery* 1996; **119**: 275-280
88 **Varadarajulu S**, Eloubeidi MA. The role of endoscopic ultrasonography in the evaluation of pancreatico-biliary cancer. *Gastrointest Endosc Clin N Am* 2005; **15**: 497-511, viii-ix
89 **Fujita N**, Noda Y, Kobayashi G, Kimura K, Ito K. Endoscopic approach to early diagnosis of pancreatic cancer. *Pancreas* 2004; **28**: 279-281
90 **Minami Y**, Kudo M. Contrast-enhanced harmonic ultrasound imaging in ablation therapy for primary hepatocellular carcinoma. *World J Radiol* 2009; **1**: 86-91
91 **Xu HX**. Contrast-enhanced ultrasound: The evolving applications. *World J Radiol* 2009; **1**: 15-24
92 **Minami Y**, Kudo M, Chung H, Kawasaki T, Yagyu Y, Shimono T, Shiozaki H. Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR Am J Roentgenol* 2007; **188**: 489-494
93 **Minami Y**, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H. Percutaneous radiofrequency ablation guided by contrast-enhanced harmonic sonography with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. *AJR Am J Roentgenol* 2004; **182**: 1224-1226
94 **Inoue T**, Kitano M, Kudo M, Sakamoto H, Kawasaki T, Yasuda C, Maekawa K. Diagnosis of gallbladder diseases by contrast-enhanced phase-inversion harmonic ultrasonography. *Ultrasound Med Biol* 2007; **33**: 353-361
95 **Kitano M**, Kudo M, Maekawa K, Suetomi Y, Sakamoto H, Fukuta N, Nakaoka R, Kawasaki T. Dynamic imaging of pancreatic diseases by contrast enhanced coded phase inversion harmonic ultrasonography. *Gut* 2004; **53**: 854-859
96 **Becker D**, Strobel D, Bernatik T, Hahn EG. Echo-enhanced color- and power-Doppler EUS for the discrimination between focal pancreatitis and pancreatic carcinoma. *Gastrointest Endosc* 2001; **53**: 784-789
97 **Ding H**, Kudo M, Onda H, Suetomi Y, Minami Y, Chung H, Kawasaki T, Maekawa K. Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. *Radiology* 2001; **221**: 721-730
98 **Rickes S**, Mönkemüller K, Malfertheiner P. Echo-enhanced

ultrasound with pulse inversion imaging: A new imaging modality for the differentiation of cystic pancreatic tumours. *World J Gastroenterol* 2006; **12**: 2205-2208

99 **Wen YL**, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. *AJR Am J Roentgenol* 2003; **181**: 57-63

100 **Hocke M**, Schulze E, Gottschalk P, Topalidis T, Dietrich CF. Contrast-enhanced endoscopic ultrasound in discrimination between focal pancreatitis and pancreatic cancer. *World J Gastroenterol* 2006; **12**: 246-250

101 **Kitano M**, Sakamoto H, Matsui U, Ito Y, Maekawa K, von Schrenck T, Kudo M. A novel perfusion imaging technique of the pancreas: contrast-enhanced harmonic EUS (with video). *Gastrointest Endosc* 2008; **67**: 141-150

102 **Gorce JM**, Arditi M, Schneider M. Influence of bubble size distribution on the echogenicity of ultrasound contrast agents: a study of SonoVue. *Invest Radiol* 2000; **35**: 661-671

103 **Rickes S**, Uhle C, Kahl S, Kolfenbach S, Monkemuller K, Effenberger O, Malfertheiner P. Echo enhanced ultrasound: a new valid initial imaging approach for severe acute pancreatitis. *Gut* 2006; **55**: 74-78

104 **Furukawa T**, Oohashi K, Yamao K, Naitoh Y, Hirooka Y, Taki T, Itoh A, Hayakawa S, Watanabe Y, Goto H, Hayakawa T. Intraductal ultrasonography of the pancreas: development and clinical potential. *Endoscopy* 1997; **29**: 561-569

105 **Furukawa T**, Tsukamoto Y, Naitoh Y, Hirooka Y, Hayakawa T. Differential diagnosis between benign and malignant localized stenosis of the main pancreatic duct by intraductal ultrasound of the pancreas. *Am J Gastroenterol* 1994; **89**: 2038-2041

106 **Menzel J**, Poremba C, Dietl KH, Domschke W. Preoperative diagnosis of bile duct strictures--comparison of intraductal ultrasonography with conventional endosonography. *Scand J Gastroenterol* 2000; **35**: 77-82

107 **Hara T**, Yamaguchi T, Ishihara T, Tsuyuguchi T, Kondo F, Kato K, Asano T, Saisho H. Diagnosis and patient management of intraductal papillary-mucinous tumor of the pancreas by using peroral pancreatoscopy and intraductal ultrasonography. *Gastroenterology* 2002; **122**: 34-43

108 **Giovannini M**. Contrast-enhanced endoscopic ultrasound and elastosonoendoscopy. *Best Pract Res Clin Gastroenterol* 2009; **23**: 767-779

109 **Saftoiu A**, Gheonea DI. Tridimensional (3D) endoscopic ultrasound - a pictorial review. *J Gastrointestin Liver Dis* 2009; **18**: 501-505

S- Editor Cheng JX **L- Editor** Webster JR **E- Editor** Zheng XM

Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

CLINICAL STUDIES

Radiofrequency ablation guided by contrast harmonic sonography using perfluorocarbon microbubbles (Sonazoid) for hepatic malignancies: an initial experience

Yasunori Minami, Masatoshi Kudo, Kinuyo Hatanaka, Satoshi Kitai, Tatsuo Inoue, Satoru Hagiwara, Hobyung Chung and Kazuomi Ueshima

Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kinki University School of Medicine, Ohno-Higashi Osaka-Sayama, Japan

Keywords
contrast harmonic sonography – hepatocellular carcinoma – liver metastasis – perfluorocarbon microbubbles (Sonazoid) – radiofrequency ablation

Abbreviations
HCC, hepatocellular carcinoma; RF ablation, radiofrequency ablation; TACE, transcatheter arterial chemoembolization

Correspondence
Masatoshi Kudo, Department of Internal Medicine, Division of Gastroenterology and Hepatology, Kinki University School of Medicine, 377-2 Ohno-Higashi Osaka-Sayama, 589-8511, Japan
Tel: +81 72 366 0221 (ext. 3525)
Fax: +81 72 367 2880
e-mail: m-kudo@med.kindai.ac.jp

Received 19 November 2009
Accepted 2 February 2010

DOI:10.1111/j.1478-3231.2010.02226.x

Abstract

Aim: Conventional contrast harmonic sonography has the technical problem of a short enhancement time during targeting of hepatic malignancies for radiofrequency (RF) ablation. This study investigated the effectiveness of contrast harmonic sonographic guidance using perfluorocarbon microbubbles (Sonazoid) during RF ablation of hepatic malignancies. *Materials and Methods:* Nodules were detected on contrast-enhanced computed tomography, but could not be resolved clearly by B-mode sonography. Sixty-six patients (51 men, 15 women; mean age, 65.8 years) with 108 hepatic malignancies were enrolled. Fifty-one patients with hepatocellular carcinoma and 15 patients with liver metastases were treated by RF ablation guided by contrast harmonic sonography using perfluorocarbon microbubbles for a target lesion identified as a defect image after the administration of contrast medium. *Results:* The maximal diameters of all tumours ranged from 0.7 to 3.5 cm (mean $\pm$ SD, 1.7 cm $\pm$ 0.9) on sonography. Complete tumour necrosis was achieved by a single session of RF ablation in 62 (94%) of the 66 patients, while two sessions were required for the remaining four (6%) patients. The average number of treatment sessions was 1.1 $\pm$ 0.3. In the post-vascular phase, 105 (97%) of a total of 108 malignant hepatic tumours were depicted as a defect with a margin. Clinical courses have been satisfactory without any signs of local tumour progression during 1–12 months of follow-up (mean, 4.3 months). *Conclusion:* Using perfluorocarbon microbubbles, contrast harmonic sonographic-guided RF ablation is an efficient approach for guiding further ablation of hepatic malignancies that are not clearly demarcated by B-mode sonography.

Radiofrequency (RF) ablation is widely performed as a percutaneous local treatment under real-time sonographic guidance. However, several hepatic malignancies cannot be detected clearly by B-mode sonography (1–7). Contrast harmonic sonographic imaging with an intravenous contrast agent has been demonstrated to depict tumour vascularity sensitively and accurately (8–13). Recently, contrast harmonic sonography has been improved by the development of second-generation contrast agents such as sulphur hexafluoride microbubbles (SonoVue), perflutren lipid microbubbles (Definity), and perflutren protein microbubbles (Optison). These microbubbles provide stable nonlinear oscillation in a low-power acoustic field because of the hard shell of these bubbles, producing great detail in the harmonic signals in real time (14–18). However, arterial tumour vascularity can only be seen for about 1 min during the early vascular phase. As a result, contrast harmonic sonographic-guided RF ablation using these second-generation contrast agents represents a technical problem because of the short imaging time available for targeting the enhancement of hepatic malignancies and inserting the RF electrode.

Perfluorocarbon microbubbles (Sonazoid) also belong to the second generation of contrast agents for sonography (19, 20). Unlike other second-generation contrast agents, perfluorocarbon microbubbles are phagocytosed by Kupffer cells. Therefore, these microbubbles accumulate in the liver parenchyma over time. This contrast agent can provide a detailed insight not only into the perfusion features of the microvascular bed of the liver parenchyma and tumour in the vascular phase but also liver parenchymal imaging in the post-vascular phase (21–23). Because hepatic malignancies do not contain

Kupffer cells, contrast harmonic sonography can easily distinguish these lesions as defects over time, even when the lesions are undetectable on B-mode sonography.

In this study, we evaluated the usefulness of contrast harmonic sonographic guidance using perfluorocarbon microbubbles during RF ablation of hepatic malignancies that were poorly depicted by B-mode sonography.

Materials and methods

Patient selection and eligibility

The Ethics Committee of our institution approved the study protocol. Written informed consent was obtained from each patient at the time of enrolment.

Between March 2007 and March 2008, 66 patients (51 men, 15 women; age range, 32–88 years; mean age ± SD, 65.8 years ± 11.7) with 108 hepatic malignancies were retrospectively analysed in this study (Table 1). Nodules were detected as tumour enhancement on contrast-enhanced computed tomography (CT), but could not be visualized clearly by conventional B-mode sonography. Primary malignancies included hepatocellular carcinoma (HCC) ($n = 51$). Secondary hepatic malignancies included patients with colorectal cancer ($n = 8$), gastric cancer ($n = 4$), pancreatic cancer ($n = 2$) and cervical cancer ($n = 1$). Twenty-one patients with hepatic malignancies (HCC, $n = 17$; metastasis, $n = 4$) had not been treated previously for these hepatic lesions. Twenty-eight patients with hepatic malignancies (HCC, $n = 22$; metastasis, $n = 6$) had been treated previously by RF ablation at other sites in the liver. The remaining 12 HCC patients and five liver metastasis patients had shown local tumour progression after various therapies [percutaneous RF ablation, $n = 9$; transcatheter arterial chemoembolization (TACE), $n = 8$]. Before RF ablation, all patients with liver metastasis had undergone systemic chemotherapy after surgical resection of the primary tumour. Forty patients with HCC had cirrhosis classified as Child–Pugh class A, while the remaining 11 showed Child–Pugh class B cirrhosis. The maximal diameters of all tumours ranged from 0.7 to 3.5 cm (mean ± SD, 1.7 cm ± 0.9) on contrast harmonic sonography. The mean maximum diameter was 1.5 cm ± 0.7 for HCCs and 2.0 cm ± 1.1 for metastases. The distance from the skin to the deepest edge of the tumour on sonography ranged from 3 to 12 cm (mean ± SD, 6.1 cm ± 2.1).

Table 1. Baseline clinical characteristics of the patients

Number of patients (HCC/liver metastasis)	66 (51/15)
Number of lesions (HCC/liver metastasis)	108 (68/44)
Age (years)	
Mean ± SD (range)	65.8 ± 11.7 (32–88)
Sex	
Male/female	51/15
Origin of liver metastasis	
Colorectal cancer	8
Gastric cancer	4
Pancreatic cancer	2
Cervical cancer	1
Diameter of the entire hepatic malignancies (cm)	
Mean ± SD (range)	1.7 ± 0.9 (0.7–3.5)
Previous treatments	
In-patients with HCC	
None/RFA/TACE/RFA+TACE	17/24/8/2
In-patients with liver metastasis	
SR+SC: SR+SC+RFA	4/11

Data are presented as mean ± standard deviation unless otherwise indicated.

HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; SC, systemic chemotherapy; SR, surgical resection; TACE, transcatheter arterial chemoembolization.

Hepatocellular carcinomas were diagnosed based on three-phase contrast-enhanced CT findings such as positive enhancement in the arterial phase and washout in the equilibrium phase in patients with chronic liver disease. Liver metastases were diagnosed by ring enhancement on contrast-enhanced CT in patients with past cancer illness. All patients met the following criteria for treatment with RF ablation: presence of viable hepatic malignancies with a maximum diameter not greater than 3.5 cm, percutaneous accessibility of the tumours, absence of portal tumour thrombus and extrahepatic metastasis, prothrombin time ratio $> 50\%$, total bilirubin < 3.0 mg/dl and platelet count $> 50\,000/\mu l$.

Equipment

B-mode sonographic scans were obtained using a LOGIQ 7 (GE Medical Systems, Milwaukee, WI, USA) or an EUB 8500 unit (HITACHI Medico, Tokyo, Japan). The convex-arrayed transducer of LOGIQ 7 was used at a frequency of 4 or 6.5 MHz. The acoustic power of contrast harmonic sonography was set at the default setting with a mechanical index of 0.2. A single focus point was set at a depth of 10 cm. The convex-arrayed transducer of the EUB 8500 was used at a frequency of 3.5 MHz. The acoustic power of contrast harmonic sonography was set at the default setting with a mechanical index of 0.2–0.3. A single focus point was set at the deepest point of the monitor.

The sonographic contrast agent was perfluorocarbon microbubbles (Sonazoid; Daiichi-Sankyo, Tokyo, Japan) with a median diameter of 2–3 μm (19, 20). This contrast agent was reconstituted for injection with 2 ml sterile water for injection. The anticipated clinical dose for imaging of liver lesions is 0.010 ml encapsulated gas per kilogram of body weight.

Patients were treated by RF ablation (Cooled-tip RF ablation system; Radionics, Burlington, MA, USA). Twenty centimetres long, 17 G, monopolar internally cooled electrodes with 3- or 2-cm-long exposed metallic tips (Radionics) were used to deliver RF energy. A 200 W, 480 kHz monopolar RF generator regulated by impedance (CC-1, Radionics) was used as the energy source.

A multidetecter CT (LightSpeed VCT, GE Medical Systems, Milwaukee, WI, USA) was used for diagnosis. Triple-phase contrast-enhanced CT scans were performed with a 5.0-mm slice thickness at 30, 60 and 180 s after initiating the injection of contrast media to obtain hepatic arterial, portal venous and equilibrium phase images respectively. A total of 100 ml of nonionic contrast material containing 300 mg of iodine per millilitre (Iomeprol, Eisai Co., Tokyo, Japan) was injected intravenously at a rate of 3 ml/s using an automatic power injector.

Sonazoid-enhanced harmonic sonographic-guided radiofrequency ablation procedure

All nodules were treated by percutaneous RF ablation under local anaesthesia (lidocaine 1%). Some patients were sedated but conscious following an intravenous injection of 25 mg of hydroxyzine and 15 mg of pentazocine just before this treatment if necessary. Nodules > 2 cm in diameter were treated using an electrode with a 3 cm tip, and nodules < 2 cm were treated using an electrode with a 2 cm tip.

The contrast harmonic imaging mode was adjusted after viewing the plane containing the tumour on B-mode sonography. Real-time images in the optimal scanning plane were displayed by slightly changing the scanning slice showing the nodule. Vascular findings are shown in the vascular phase (from 10 s to the last 5–7 min after injection of the contrast agent), and liver parenchymal findings are shown in the post-vascular phase (from about 10 min after injection of the contrast agent) because the contrast agent was incorporated into Kupffer cells or liver sinusoids (23, 24). Therefore, hepatic malignancies were visualized by enhancement of intratumoral vessels at the beginning and by defects in the liver parenchyma during the post-vascular phase. This defect representing the lesion could be used as a target for insertion of a single RF electrode (Fig. 1). In patients previously treated with ablation of hepatic nodules, we re-injected a new dose of perfluorocarbon microbubbles 'in order to confirm tumour vascularity' before electrode insertion because both ablated lesions and local tumour progression are shown as defects (25, 26). After the RF electrode penetrated the hepatic malignancy, each ablation was performed for >8 min with at

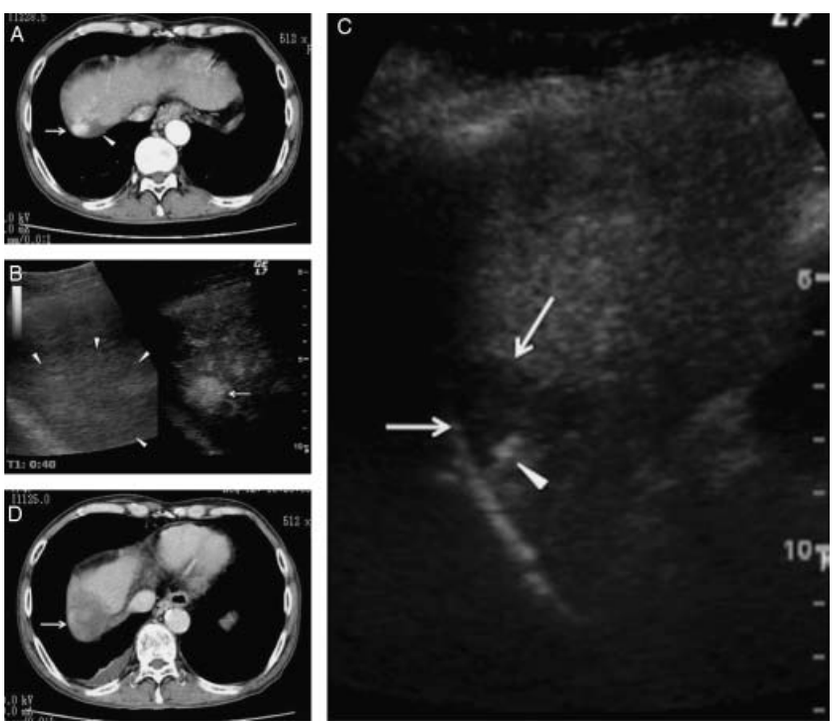


Fig. 1. A 70-year-old man with 2.0 cm local tumour progression of hepatocellular carcinoma (HCC) after percutaneous radiofrequency (RF) ablation about 1 year ago. (A) Early-phase dynamic computed tomography (CT) scan shows local tumour progression of HCC as an enhanced lesion (arrow) in segment 7 of the liver. Surrounding area that was previously treated is not enhanced (arrowhead). (B) Right: Contrast harmonic sonography shows enhancement of viable HCC focus (arrow) in the early vascular phase after administration of perfluorocarbon microbubbles. Left: A high echoic area (arrowheads) contains both a viable HCC lesion and a necrotic ablation area on B-mode sonography. (C) Contrast harmonic sonography shows the defect (arrows) imaging in the post-vascular phase and RF electrode (arrowhead) needle inserted. The RF electrode was placed through the right side of the tumour, and then the left side of the tumour was ablated after the second penetration of the RF electrode after the first ablation. (D) Early-phase dynamic CT scan obtained three days after RF ablation therapy shows that the tumour and the surrounding area (arrow) are not enhanced.

least 60 W at the beginning. However, RF electrode insertion was performed under guidance by contrast harmonic sonography based on CT information for hepatic malignancies that did not show tumour vessels or defects.

All RF ablations were performed percutaneously by one of four experienced hepatologists (M. K., H. C., Y. M., T. H.) with 10, 9, 9 and 7 years of experience, respectively, in performing sonography-guided interventional procedures including RF ablation.

Assessment of technical effectiveness and follow-up

A few days after treatment, the technical effectiveness of ablation was assessed based on contrast-enhanced CT scan findings. A tumour was considered to have been successfully ablated when there were no longer any enhanced regions either within the entire tumour during the arterial phase and at least a 0.5 cm margin of apparently normal hepatic tissue surrounding the tumour during the portal phase. Part of the tumour was diagnosed as remaining viable when images of the ablated area showed nodular peripheral enhancement (27). The residual portion was treated with additional RF ablation the following week.

Results

Among the 51 patients with 68 HCCs, seven nodules showed indistinct margins on B-mode sonography, but 61 nodules could not be accurately detected because of poor echoic signals because of large regenerated nodules in the cirrhotic liver ($n = 44$) or signals that could not distinguish between viable nodules and previously treated lesions ($n = 17$). Among 15 patients with 40 liver metastases, 14 nodules showed indistinct margins on B-mode sonography and 26 nodules could not be accurately detected because of previous treatment. In the post-vascular phase, 93 (86%) of the 108 malignant hepatic tumours were depicted as a defect with a clear margin, 12 nodules (11%) were depicted as a defect with an unclear margin and three nodules (3%) could not be detected as a defect. In 20 patients (18 HCC; two liver metastases), perfluorocarbon microbubbles were administered again to confirm tumour vessels entering the defects in the post-vascular phase. Overall enhancement of the defect was shown in 16 nodules (15 HCC; one liver metastasis), and partial enhancement was shown in four nodules (three HCC; one liver metastasis). Eventually, all tumours could be detected as defects and/or intratumoral enhancement on contrast harmonic sonography.

Technical effectiveness of ablation was achieved in a single session in 62 (94%) of 66 patients, and two sessions were required for four patients (6%). The average number of treatment sessions was 1.1 ± 0.3. Three patients with HCC and one with liver metastases received incomplete treatment at the first session. These four nodules showed an unclear defect or no defect during the post-vascular phase. For one patient with HCC and one with liver metastasis, a second treatment session was necessary because of insufficient ablative margins after the first session. These two tumours were located deep in segments 7 or 8 of the liver. For the two remaining HCC patients, a second session was needed because a viable area remained in part of the nodule after the first session. These two residual HCCs included one that had shown local tumour progression after percutaneous RF ablation in segment 6 behind the costal bone, while the other lesion was surrounded by cirrhosis and located deep within the liver. However, completion of treatment was achieved after the second session in both of these patients.

There were no serious side effects or procedure-related complications (e.g. haemorrhage, infection, needle tract seeding, hepatic failure or death). In this study, pleural effusion ($n = 1$) with mild dyspnoea occurred and was resolved by drainage. Grade one to two pain on the Common Toxicity Criteria of the National Cancer Institute was the most common side effect in 17 patients. Asymptomatic ascites ($n = 1$) occurred and then resolved spontaneously. All of these symptoms were controlled; the procedure was not discontinued in any of the cases.

Follow-up time ranged from 1 to 12 months (mean $\pm$ SD, 4.3 ± 3.1 months). During the follow-up period, none of the patients showed local tumour progression. However, five patients with HCCs and five patients with liver metastases demonstrated distant metastases in the liver. Subsequently, nine patients underwent additional RF ablation and the other underwent TACE.

Discussion

The incorporation of perfluorocarbon microbubbles into Kupffer cells and sinusoids is very helpful for differential diagnosis, and for the detection and localization of hepatic malignancies shown as defect imaging (28, 29). In this study, defect imaging in the post-vascular phase was obtained in 105 (97%) of 108 hepatic malignancies (65 of 68 HCC nodules and all 40 hepatic metastatic nodules). With perfluorocarbon microbubbles, parenchymal imaging could be performed repeatedly at a low mechanical index level. Especially in patients with liver metastases, the clear contrast between tumours and liver parenchyma could be caused by a sufficiency of Kupffer cells in the liver parenchyma. In HCC patients with Child A cirrhosis, the contrast was also clear; however, the defects were not demonstrated well in three HCC patients with Child B cirrhosis. This might be because of the decreased number of Kupffer cells and/or the poor function of phagocyte in patients with cirrhosis.

SonoVue was first launched in October 2001 and is now available in all European countries. SonoVue microbubbles are filled with sulphur hexafluoride (14). Sonazoid was first launched in January 2007 and is currently available only in Japan, as its use was suspended in the

US and Europe. Sonazoid consists of perfluorocarbon microbubbles that are stabilized with a surfactant (19, 20). Both SonoVue and Sonazoid are classified as second-generation contrast agents for sonography, and are strongly echogenic in a wide range of frequencies and acoustic pressures owing to the high flexibility of their shell. However, 99% of Sonazoid microbubbles are phagocytosed by Kupffer cells in the liver, whereas only 7.3% of SonoVue microbubbles are phagocytosed in this manner (23). Sonazoid microbubbles are taken up immediately after an intravenous injection and exist as microbubbles for 30 min within Kupffer cells, and hepatic parenchymal imaging reflects the distribution and function of Kupffer cells. Therefore, contrast harmonic sonography with Sonazoid can show a unique 'post-vascular image' in addition to a 'vascular image' (21–25).

In 20 patients (18 HCC; two liver metastases), these hepatic malignancies did not demonstrate intratumoral vessel images clearly after the first injection of perfluorocarbon microbubbles in the post-vascular phase. These included four patients with tumours that showed unclear defects or no defects in the post-vascular phase. In this study, all nodules were confirmed tumour enhancement on contrast-enhanced CT. By re-injection of the contrast medium in these patients, overall enhancement of the defect was seen in 16 (80%) nodules (15 HCC; one liver metastasis), and partial enhancement in four (20%) nodules (three HCC; one liver metastasis). If intratumoral vessel images could not be obtained clearly at the first injection, intratumoral perfusion into the defects could be observed after a second injection. This defect re-injection method facilitated an improvement of the visibility of these hepatic nodules on sonography. Moreover, re-injection of perfluorocarbon microbubbles might contribute to time shortening of a treatment session. Arterial finding of hepatic malignancies could be obtained by the defect re-injection method (24). Thus, a scanning programme in the early vascular phase after the first injection can be omitted in the diagnosis process.

Despite difficulties such as hepatic malignancies that could not be clearly demonstrated on B-mode sonography, contrast harmonic sonography guidance allowed satisfactory results to be achieved. Technically successful ablation was achieved in the first session in 62 (94%) of 66 patients. A complete treatment response was achieved after an average of 1.1 treatment sessions. In addition to improvement of visualization, these results might have been achieved because we had adequate time to perform RF ablation with careful targeting of the defects. During the early vascular phase, a very high skill level is required because the procedure time is too short to search for the enhanced hepatic malignancies and insert the RF electrode. For example, after a single intravenous administration of sulphur hexafluoride microbubbles (SonoVue; Bracco SpA, Milan, Italy) in human volunteers, the blood distribution $t_{1/2}$ was about 1 min and the elimination $t_{1/2}$ was approximately 6 min (30). Contrast-enhanced sonography with sulphur hexafluoride microbubbles could show vascular imaging for a longer duration than air-filled microbubbles (Levovist); nevertheless, contrast harmonic sonography with sulphur hexafluoride microbubbles for guidance of RF ablation might hasten the insertion of the RF electrode during the early vascular phase. Thus, the ability to find defect images in the post-vascular phase is one of the merits of perfluorocarbon microbubbles use.

In three patients with HCC and one with liver metastases, tumour ablation was incomplete after the first session. Despite secondary administration of perfluorocarbon microbubbles, only partial reperfusion imaging of these defects was achieved. Deep tumour location, location behind the costal bone or insufficient enhancement, that is, poor visibility of nodules would likely make a second treatment session necessary. Even if acoustic power of sonography was at a low level, the second-generation microbubbles became more broken as the sonography exposure increased in the field of the tumour (31). Enhancement of the liver parenchyma becomes weaker by prolonged sonographic exposure, and then the defects cannot be demonstrated clearly in the post-vascular phase. Therefore, it might be important to avoid an excessively long observation of the tumour on contrast harmonic sonography in RF ablation.

The principal limitation of this study was the retrospective and noncomparative design, which inherently decreases the statistical strength. Another limitation is the preliminary nature of this study with the relatively small number of patients and short follow-up time. Further prospective studies of this technique with a larger number of patients are warranted. The outcomes in this study can be attributed to the combined the effect of harmonic sonography and the contrast agent, perfluorocarbon microbubbles.

In conclusion, perfluorocarbon microbubbles could facilitate contrast harmonic sonography guidance of RF ablation by extending the time limitation, simplifying the procedure and improving detectability. RF ablation guided by perfluorocarbon microbubble-enhanced sonography could become an easier and more efficient approach to treating hepatic malignancies that are not clearly depicted on B-mode sonography.

Acknowledgement

Yasunori Minami, Kinuyo Hatanaka, Tatsuo Inoue, Satoru Hagiwara, Hobyung Chung and Kazuomi Ueshima analysed the data; Yasunori Minami and Masatoshi Kudo wrote the paper.

References

1. Rossi S, Di Stasi M, Buscarini E, *et al.* Percutaneous radiofrequency interstitial thermal ablation in the treatment of small hepatocellular carcinoma. *Cancer J Sci Am* 1995; **1**: 73–81.

2. Lencioni R, Cioni D, Crocetti L, *et al.* Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961–7.
3. Shiina S. Image-guided percutaneous ablation therapies for hepatocellular carcinoma. *J Gastroenterol* 2009; **44**: S122–31.
4. Minami Y, Kudo M, Chung H, *et al.* Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR* 2007; **188**: 489–94.
5. Takayasu K, Muramatsu Y, Asai S, Muramatsu Y, Kobayashi T. CT fluoroscopy-assisted needle puncture and ethanol injection for hepatocellular carcinoma: a preliminary study. *AJR* 1999; **173**: 1219–24.
6. Brennan DD, Appelbaum L, Raptopolous V, Kruskal JB, Goldberg SN. CT artifact introduced by radiofrequency ablation. *AJR* 2006; **186**: S284–6.
7. Maeda T, Hong J, Konishi K, *et al.* Tumor ablation therapy of liver cancers with an open magnetic resonance imaging-based navigation system. *Surg Endosc* 2009; **23**: 1048–53.
8. Fujimoto M, Moriyasu F, Nishikawa K, Nada T, Okuma M. Color Doppler sonography of hepatic tumors with a galactose-based contrast agent: correlation with angiographic findings. *AJR* 1994; **163**: 1099–104.
9. Quaia E, D'Onfrio M, Cabassa P, *et al.* Diagnostic value of hepatocellular nodule vascularity after microbubble injection for characterizing malignancy in patients with cirrhosis. *AJR* 2007; **189**: 1474–83.
10. Jang HJ, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007; **244**: 898–906.
11. Chami L, Lassau N, Malka D, *et al.* Benefits of contrast-enhanced sonography for the detection of liver lesions: comparison with histologic findings. *AJR* 2008; **190**: 683–90.
12. Cioni D, Lencioni R, Bartolozzi C. Therapeutic effect of transcatheter arterial chemoembolization on hepatocellular carcinoma: evaluation with contrast-enhanced harmonic power Doppler ultrasound. *Eur Radiol* 2000; **10**: 1570–5.
13. Meloni MF, Goldberg SN, Livraghi T, *et al.* Hepatocellular carcinoma treated with radiofrequency ablation: comparison of pulse inversion contrast-enhanced harmonic sonography, contrast-enhanced Power Doppler sonography, and Helical CT. *AJR* 2001; **177**: 375–80.
14. Quaia E, Calliada F, Bertolotto M, *et al.* Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence. *Radiology* 2004; **232**: 420–30.
15. Jang HJ, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007; **244**: 898–906.
16. Wang Z, Tang J, An L, *et al.* Contrast-enhanced ultrasonography for assessment of tumor vascularity in hepatocellular carcinoma. *J Ultrasound Med* 2007; **26**: 757–62.
17. Leen E, Angerson WJ, Yarmenitis S, *et al.* Multi-centre clinical study evaluating the efficacy of SonoVue (BR1), a new ultrasound contrast agent in Doppler investigation of focal hepatic lesions. *Eur J Radiol* 2002; **41**: 200–6.
18. Kono Y, Lucidarme O, Choi SH, *et al.* Contrast-enhanced ultrasound as a predictor of treatment efficacy within 2 weeks after transarterial chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2007; **18**: 57–65.
19. Ramnarine KV, Kyriakopoulou K, Gordon P, *et al.* Improved characterisation of focal liver tumours: dynamic power Doppler imaging using NC100100 echo-enhancer. *Eur J Ultrasound* 2000; **11**: 95–104.
20. Korenaga K, Korenaga M, Furukawa M, Yamasaki T, Sakaida I. Usefulness of Sonazoid contrast-enhanced ultrasonography for hepatocellular carcinoma: comparison with pathological diagnosis and superparamagnetic iron oxide magnetic resonance images. *J Gastroenterol* 2009; **44**: 733–41.
21. Kudo M. New sonographic techniques for the diagnosis and treatment of hepatocellular carcinoma. *Hepatol Res* 2007; **37**: S193–9.
22. Hatanaka K, Kudo M, Minami Y, *et al.* Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. *Intervirology* 2008; **51**: S61–9.
23. Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H. Phagocytosis of ultrasound contrast microbubbles by Kupffer cells. *Ultrasound Med Biol* 2007; **33**: 318–25.
24. Hatanaka K, Kudo M, Minami Y, Maekawa K. Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. *Oncology* 2008; **75**: S42–7.
25. Numata K, Morimoto M, Ogura T, *et al.* Ablation therapy guided by contrast-enhanced sonography with Sonazoid for hepatocellular carcinoma lesions not detected by conventional sonography. *J Med Ultrasound* 2008; **27**: 395–406.
26. Maruyama H, Takahashi M, Ishibashi H, *et al.* Ultrasound-guided treatments under low acoustic power contrast harmonic imaging for hepatocellular carcinomas undetected by B-mode ultrasonography. *Liver Int* 2009; **29**: 708–14.
27. Lim HK, Choi D, Lee WJ, *et al.* Hepatocellular carcinoma treated with percutaneous radio-frequency ablation: evaluation with follow-up multiphase helical CT. *Radiology* 2001; **221**: 447–54.
28. Ramnarine KV, Kyriakopoulou K, Gordon P, *et al.* Improved characterisation of focal liver tumours: dynamic power Doppler imaging using NC100100 echo-enhancer. *Eur J Ultrasound* 2000; **11**: 95–104.
29. Tanimoto A, Yuasa Y, Shinmoto H, *et al.* Superparamagnetic iron oxide-mediated hepatic signal intensity change in patients with and without cirrhosis: pulse sequence effects and Kupffer cell function. *Radiology* 2002; **222**: 661–6.
30. Schneider M. Characteristics of SonoVuetrade mark. *Echocarciography* 1999; **16**: 743–6.
31. Krasoviski B, Kimmel E. Stability of an encapsulated bubbles shell. *Ultrasonics* 2006; **44**: 216–20.

Hepatol Int (2010) 4:439–474
DOI 10.1007/s12072-010-9165-7

GUIDELINES

Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma

Masao Omata · Laurentius A. Lesmana · Ryosuke Tateishi · Pei-Jer Chen · Shi-Ming Lin · Haruhiko Yoshida · Masatoshi Kudo · Jeong Min Lee · Byung Ihn Choi · Ronnie T. P. Poon · Shuichiro Shiina · Ann Lii Cheng · Ji-Dong Jia · Shuntaro Obi · Kwang Hyub Han · Wasim Jafri · Pierce Chow · Seng Gee Lim · Yogesh K. Chawla · Unggul Budihusodo · Rino A. Gani · C. Rinaldi Lesmana · Terawan Agus Putranto · Yun Fan Liaw · Shiv Kumar Sarin

Received: 11 February 2009 / Accepted: 9 December 2009 / Published online: 18 March 2010

Abstract
Introduction The Asian Pacific Association for the Study of the Liver (APASL) convened an international working party on the management of hepatocellular carcinoma (HCC) in December 2008 to develop consensus recommendations.
Methods The working party consisted of expert hepatologist, hepatobiliary surgeon, radiologist, and oncologist from Asian-Pacific region, who were requested to make drafts prior to the consensus meeting held at Bali, Indonesia on 4 December 2008. The quality of existing evidence and strength of recommendations were ranked from 1 (highest) to 5 (lowest) and from A (strongest) to D (weakest), respectively, according to the Oxford system of evidence-based approach for developing the consensus statements.

M. Omata (✉) · R. Tateishi · H. Yoshida · S. Shiina
Department of Gastroenterology, Graduate School of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: omata-2im@h.u-tokyo.ac.jp; hepint_omata@yahoo.co.jp

L. A. Lesmana · U. Budihusodo · C. R. Lesmana
Department of Internal Medicine, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

P.-J. Chen
Department of Internal Medicine, College of Medicine, National Taiwan University, Taipei, Taiwan

S.-M. Lin · Y. F. Liaw
Liver Research Unit, Chang Gung Memorial Hospital, Taipei, Taiwan

M. Kudo
Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, Osaka-Sayama, Japan

J. M. Lee · B. I. Choi
Abdominal Radiology Section, Department of Radiology, Seoul National University Hospital, Seoul, Korea

R. T. P. Poon
Department of Surgery, Queen Mary Hospital, The University of Hong Kong, Hong Kong, China

A. L. Cheng
Department of Oncology, College of Medicine, National Taiwan University, Taipei, Taiwan

J.-D. Jia
Liver Research Center, Beijing Friendship Hospital, Capital Medical University, 100050 Beijing, China

S. Obi
Division of Hepatology, Kyoundo Hospital, Tokyo, Japan

K. H. Han
Department of Internal Medicine, Institute of Gastroenterology, Yonsei University College of Medicine, Seoul, Korea

W. Jafri
Department of Medicine, The Aga Khan University Hospital, Karachi, Pakistan

P. Chow
Department of General Surgery, Singapore General Hospital, Singapore, Singapore

S. G. Lim
Department of Gastroenterology and Hepatology, National University Hospital, Singapore, Singapore

Y. K. Chawla
Departments of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

R. A. Gani
Hepatology Division, Internal Medicine Department, RSUPN Cipto Mangunkusumo, Jakarta, Indonesia

T. A. Putranto
Department of Radiology, Central Army Hospital, Jakarta, Indonesia

Results Participants of the consensus meeting assessed the quality of cited studies and assigned grades to the recommendation statements. Finalized recommendations were presented at the fourth APASL single topic conference on viral-related HCC at Bali, Indonesia and approved by the participants of the conference.

Keywords Hepatocellular carcinoma · Consensus statements · Recommendations · Epidemiology · Diagnosis · Treatment algorithm

Abbreviations

AASLD	American Association for Study of Liver Diseases
AFP	α-Fetoprotein
AFP-L3	Lens culinaris agglutinin-reactive fraction of AFP
APASL	Asian Pacific Association for the Study of the Liver
ECOG	Eastern Cooperative Oncology Group
CI	Confidence interval
CEUS	Contrast-enhanced US
CSF-1	Colony-stimulating factor 1
CTAP	CT during arterial portography
CTHA	CT hepatic arteriography
DCP	Des-γ-carboxyprothrombin
DN	Dysplastic nodule
EASL	European Association for the study of the liver
FNH	Focal nodular hyperplasia
Gd-EOB-DTPA	Gadolinium-ethoxybenzyl-diethylenetriaminepentaacetic acid
GPC3	Glypican-3
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGDN	High-grade dysplastic nodules
HH	Hereditary hemochromatosis
IFN	Interferon
LAM	Lamivudine
LGDN	Low-grade dysplastic nodules
LR+	Positive likelihood ratio
MDCT	Multidetector-row CT
MTT	Molecular targeted therapy
NASH	Nonalcoholic steatohepatitis
PDGFR	Platelet-derived growth factor receptors
PIVKA-II	Prothrombin induced by vitamin K absence-II
RCT	Randomized controlled trial
RD	Risk difference
SPIO	Superparamagnetic iron oxide
TACE	Transarterial chemoembolization
US	Ultrasonography
VEGFR	Vascular endothelial growth factor receptors

S. K. Sarin
Department of Gastroenterology, G. B. Pant Hospital, University of Delhi, New Delhi, India

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of death from cancer. Approximately three-fourth of cases occur in Asian countries because of a high prevalence of chronic infection with HBV. HCC is undoubtedly a great health threat in Asian region.

The Asian Pacific Association for the Study of the Liver (APASL) convened an international working party on the management of HCC in December 2008 to develop consensus recommendations. The working party consisted of expert hepatologists, hepatobiliary surgeons, radiologists, and oncologists from Asian-Pacific region, who were requested to make drafts prior to the consensus meeting, held at Bali, Indonesia, on 4 December 2008. The consensus statements consisted of recommendations and scientific comments based on comprehensive review of the literature on each topic. The quality of existing evidence and strength of recommendations were ranked from 1 (highest) to 5 (lowest) and from A (strongest) to D (weakest), respectively, according to the Oxford system of evidence-based approach for developing the consensus statements [1]. Participants of the consensus meeting assessed the quality of cited studies and assigned grades to the recommendation statements. Finalized recommendations were presented at the fourth APASL single topic conference on viral-related HCC at Bali, Indonesia, and approved by the participants of the conference.

Epidemiology and risk factors

Recommendations
Patients with cirrhosis due to HBV or HCV are at the highest risk for HCC (2a).
The incidence of HCC was significantly higher in those who were HBeAg positive or have HBV DNA with high loads ($>10^4$ copies/mL) and older than 40 years (2a).
Coinfection with HBV and HCV may have synergistic effect on the development of HCC (2b).
Male sex, aging, and familial history are independent risk factors for HCC (2a).
Chronic and heavy alcohol intake, high body mass index (BMI > 25) and diabetes mellitus leading to liver disease increases the risk for HCC (2b).

Geographical distribution

The prevalence of HCC worldwide parallels that of viral hepatitis, and the majority of cases are associated with HBV and HCV. Chronic HBV infection is a leading cause of HCC in most African and Asian countries except Japan. HCV predominantly contributes to HCC in some southern European countries (e.g., Italy and Spain) and Japan.

HCC has large variation in incidence according to geographic locations [2]. High-incidence regions include sub-Saharan Africa, East Asia, and South-East Asia (i.e., China, Hong Kong, Taiwan, Korea, and Japan). The distribution of HCV-related HCC also differs among ethnic groups within the same country and among regions within the same country. In contrast, HBV-related HCC is evenly distributed, except in high aflatoxin exposure areas.

Hepatitis B infection

Chronic infection with HBV is the strongest risk factor for HCC in Asian countries. A landmark study by Beasley et al. [3] indicated that the relative risk of HCC in these HBsAg carriers was 223 times that of the normal population. Tsukuma et al. [4] also reported that the relative risk of HBsAg was 6.9 among 917 Japanese patients with cirrhosis or chronic hepatitis.

Some authors indicated that active viral replication of HBV increases the risk of HCC in subjects with chronic HBV infection [5–9]. Yang et al. [6] reported that the incidence of HCC was significantly higher in those who were HBsAg and HBeAg positive than in those who were HBsAg positive only. Recently, this was confirmed by showing a correlation between baseline HBV DNA levels in asymptomatic adult HBsAg carriers and the risk of HCC [10–13].

Studies have now shown that HBV genotype correlates with the risk for HCC and that genotype C carries two- to threefold higher risk than genotype B in developing HCC [10, 14–19]. Other HBV variants, such as precore, basal core, and pre-S deletion mutants, may also influence the development of HCC in carriers [15, 19–25].

The impact of genetic background of patients with chronic viral hepatitis, especially those with a family history of HCC, may need further investigation.

Hepatitis C infection

Chronic HCV infection is also strongly associated with HCC [4, 26–29]. The increased incidence of HCC in the developed world is likely to be a direct result of the HCV epidemic occurring some 20–30 years ago in the target population.

There is no clear evidence of the association between HCV genotype and HCC [30–34]. The significance of HCV viral titers in determining HCC risk needs further investigation.

HBV and HCV coinfection, HIV coinfection with HBV or HCV

A few studies have supported the synergistic effect of HBV–HCV coinfection in the development of HCC [35–40], although the mechanism of this synergy is still unknown.

HIV coinfection in HBV or HCV patients has increased in Asia. The liver disease progresses faster in patients with HIV coinfection [41, 42].

Cirrhosis

Cirrhosis is present in the majority of patients with HCC, especially in those with HCV infection [28]. It is unclear whether cirrhosis itself is biologically important in the hepatocarcinogenesis, or whether clonal expansion/tumor development and fibrogenesis take place concurrently.

Male sex

Males are more likely to develop HCC than females. Male-to-female ratios are around 3 in high-risk countries [43], and they tend to be higher in patients with HBV than in those with HCV [44–46].

Age

Age-specific incidence rates are strongly affected by the etiology of the background liver disease. Old age is an independent risk factor for HCC, especially in areas where HCV infection is endemic [2, 47]. On the other hand, the incidence rates increase after 20 years of age in countries where HBV-related carcinogenesis is dominant.

Tobacco and alcohol intake

It is still controversial whether cigarette smoking is a risk factor for HCC [37, 48, 49]. Many authors now support the supposition that heavy alcohol intake is strongly associated with HCC [37, 49–51]. Alcohol also increases the risk for HCC in chronic hepatitis B and C [52].

Aflatoxin

Aflatoxin exposure has been associated with HCC [53–57]. Aflatoxin is produced from fungi, which is a common contaminant in the food items such as corn, peanuts, and soy beans in areas such as Qidong, The People's Republic of China. Chen et al. [54] conducted a community-based

cohort study including 6,487 residents of the Penghu Islets in Taiwan and reported that patients with aflatoxin exposure had a high risk for HCC with an odds ratio of 5.5 as compared with those without aflatoxin exposure. It has also been shown that a synergistic effect exists between chronic HBV infection and aflatoxin exposure for hepatocarcinogenesis [53, 55, 56].

Metabolic factors

Recently, it has been shown that both obesity and diabetes are independent risk factors for HCC, depending on HBV and HCV infection status [58]. As both obesity and diabetes have rapidly increased in Asia, their contributions to HCC should be closely watched.

Family history

Family history of HCC is associated with a moderately increased risk of HCC [59–61]. In a cohort study, HBV carriers with a family history of HCC had a multivariate-adjusted rate ratio for HCC of 2.41 compared with HBV carriers without a family history of HCC. Risk of HCC increased as the number of affected relatives increased. For carriers with two or more affected relatives, the ratio increased to 5.55 [95% confidence interval (CI) 2.02–15.26] [61]. This factor needs to be incorporated into risk evaluation.

Hemochromatosis

Patients affected with hereditary hemochromatosis (HH), a genetic disease of iron overload, were found to lead to cirrhosis and eventually an increased risk of HCC [62–64].

Prevention

Prevention of HBV-related HCC

Recommendations
Universal hepatitis B vaccination should be implemented in the countries where HBV infection is endemic or hyperendemic (2a, A).
Interferon (IFN) therapy in adult with active hepatitis may be effective in reducing the incidence of HBV-related HCC (2b, B).
Maintained HBV suppression by oral antiviral agent(s) can reduce the risk of HCC (1b, A).

HBV vaccination (primary prevention of HCC)

About 350 million people are chronic carriers of HBV worldwide. The infection can cause acute and chronic liver diseases including cirrhosis and HCC globally. The efficacy of universal immunization has been shown in different countries to strikingly reduce the prevalence of HBV carrier in children. A nationwide vaccination program against HBV launched in Taiwan [65, 66] has drastically reduced the HBsAg carrier rate in the younger population [67]. More important, follow-up results from the Taiwan vaccination programs have shown a significant reduction in the incidence of HCC in children. The average annual incidence of HCC in children 6–14 years of age declined from 0.70/100,000 children between 1981 and 1986 to 0.57 between 1986 and 1990, and further to 0.36/100,000 between 1990 and 1994 ($P < 0.01$) [68]. An 80–85% decrease of HCC in the Taiwanese adults 3–4 decades later is anticipated. The decrease of HCC after the implementation of universal vaccination against HBV not only represents a practical approach to primary prevention of a human cancer by vaccination for the first time in history but also firmly establishes HBV as the cause of HCC in human beings [69]. These data prove that preventing HBV infection leads to a reduction in HBV-related morbidity and mortality and justify advocacy for universal hepatitis B vaccination programs worldwide.

Interferon therapy (secondary prevention of HCC)

It is evident that IFN therapy reduces the risk of HCC in chronic hepatitis B with/without cirrhosis. In HBeAg-positive patients with chronic hepatitis B, several long-term follow-up studies following 4–6 months of conventional IFN therapy have shown that sustained seroclearance of HBeAg was associated with a significant increase in survival and decreased liver decompensation, especially in patients with preexisting cirrhosis [70–75]. Among these studies, there was one randomized controlled trial (RCT) that involved 101 Taiwanese men with chronic hepatitis B, 67 of whom received IFN therapy and 34 of whom received placebo [75]. During 1.1–11.5 years of follow-up after completion of therapy, the incidence of HCC in untreated patients was higher than that in IFN-treated patients (12 vs. 1.5%, $P = 0.043$). The cumulative incidence of HCC was also higher in untreated patients than in treated patients ($P = 0.013$). However, the beneficial effect of HCC prevention was not observed in another nonrandomized study comparing 208 Chinese patients with chronic hepatitis B who were treated with IFN against 203 untreated patients [76]. These contradictory results were due to nonrandomization, patients of younger age (median 27 vs. 32 years), patients with low or normal alanine aminotransferase (ALT) (median 46 vs. 163 U/L), and associated low response rates (22 vs. 34% at 24 months, 45 vs. 82% at 132 months) in the Hong Kong study [76] compared with the Taiwan study [71]. The beneficial effect of HCC reduction was also supported by another study of

165 HBeAg-positive patients who were treated with IFN-alfa, as reported by van Zonneveld et al. [74]. On multivariate time-dependent analysis, adjusting for baseline factors that included cirrhosis, responders were found to have a significantly lower risk of HCC than nonresponders ($P = 0.027$). Because the long-term benefit of IFN therapy occurs only in patients with HBeAg loss, the actual benefit is difficult to prove when the HBeAg loss rate in untreated patients is not high enough, especially if the sample size is not big [71, 74]. Addressing these problems, a recent study comparing 233 IFN-treated patients with 233 matched, untreated controlled patients (matched for age, sex, baseline ALT, HBV DNA, and follow-up period) by Lin et al. showed a long-term significant benefit in preventing HCC development (2.7 vs. 12.5%, $P = 0.011$) [77]. This study had the superiority of including more patients with appropriate disease characteristics (active hepatitis), well-matched parameters, and a longer follow-up.

A meta-analysis of 11 randomized studies comparing IFN-treated versus untreated patients with HBV-related cirrhosis showed that IFN seemingly decreased the rate of HCC [92]. The pooled estimate of the HCC preventive effect of treatment was significantly in favor of patients undergoing IFN therapy [risk difference −4.1, 95% CI −0.8 to −7, $P < 0.013$]. However, these trials showed significant inconsistency if assessment did not take ethnicity of patients into account (European vs. Oriental studies). Consistent results were only observed when assessing data pooled from European reports, which did not show a preventive effect of HCC with treatment. Meta-analysis of longitudinal studies with prolonged follow-up showed no differences in the rate of HCC development between treated patients (1.9%, 95% CI 0.8–3.0) and controls (3.16%, 95% CI 1.8–4.5) [78].

In HBeAg-negative patients, Papatheodoridis et al. [72] studied a cohort of 209 IFN treated and 195 untreated patients and showed that the rate of HCC development was significantly reduced in IFN responders than in IFN non-responders (1.8 vs. 10.5%, $P = 0.027$), or in untreated patients (7.7%, $P = 0.048$). Another study by Lampertico et al. [73] in 101 HBeAg-negative patients showed no difference in HCC development in responders and nonresponders. The low response rate or relatively small number of patients may be one of the reasons for failure to show significant long-term benefits of IFN therapy in HBeAg-negative patients.

Lamivudine (secondary prevention of HCC)

Lamivudine (LAM) produces marked viral suppression, reduction of hepatic necroinflammatory activity, and histologic improvement of liver fibrosis [79], as well as improved liver function even in patients with decompensation [80]. However, it is still undetermined whether LAM or other oral antiviral drugs can suppress HBV-related hepatocarcinogenesis. To date, only one RCT suggests that LAM treatment of chronic hepatitis B and advanced liver disease does reduce the incidence of HCC, but with marginal significance (hazard ratio 0.49, 95% CI 0.25–0.99, $P = 0.047$) [81]. A multicenter retrospective study of 2,795 patients (657 treated with LAM, 2,138 not treated with LAM) was reported from Japan [82]. Of these, a controlled study including 377 LAM-treated patients and 377 untreated patients were selected on the basis of the propensity score. The mean follow-up period was 2.7 years in LAM-treated group and 5.3 years in the control group. In the LAM group, HCC occurred in four patients with an annual incidence rate of 0.4% per patient per year, whereas in the control group HCC occurred in 50 patients (13.3%) at a rate of 2.5% per patient per year. The cumulative HCC incidence was significantly lower in LAM group ($P < 0.001$). These findings suggest that LAM effectively reduces the incidence of HCC in patients with chronic hepatitis B. Another study including 59 patients of HBeAg-positive or HBeAg-negative cirrhosis treated with long-term LAM (median 44 months, range 15–78 months) showed that the cumulative event-free (decompensation or HCC) survival rate is significantly higher ($P = 0.001$) in patients with maintained virologic suppression than in those who did not have a complete virologic response or suffered a breakthrough [83]. On the basis of these studies, LAM was effective in HCC prevention in patients with chronic hepatitis B. Since drug resistance after long-term LAM therapy is likely to reverse or halt clinical benefit, long-term effects of HCC prevention after longer therapy with other antiviral agents with fewer drug resistance rates need to be studied.

Prevention of HCV-related HCC

Recommendations

- The control of transfusion-related, iatrogenic, and illicit drug use-related viral transmission is of paramount importance (2a, A).
- Efficient screening for HCV infection would find patients who require treatment (2b, B).
- Interferon therapy is indicated in acute hepatitis C to prevent chronicity (1b, A)
- Sustained virologic response to an IFN-based therapy reduces the risk of HCV-related HCC in patients with compensated chronic hepatitis C (1a, A).

Prevention of viral transmission

It is well known that HCV infection may be transmitted, though not commonly, by mother to neonate or by sexual transmission. In Egypt, intravenous tartar emetic injection

to prevent schistosomiasis is reported to cause an endemic of HCV infection in the country [79]. In United States, the peak of HCV viral spread coincided with the peak of injecting drug abuse from 1960s to 1980s [80]. In Japan, the peak of viral spread in 1950s and 1960s accompanied the peak of paid donors' blood transfusion, which might be contaminated with HCV because of the prior amphetamine abuse and needle sharing [81]. In many countries, new acquisition of HCV infection is decreasing due to growing concern about blood-transmitted infections, especially HIV, and this trend should be further encouraged considering the absence of effective vaccination against either HCV or HIV.

Screening for HCV infection

Patients infected with HCV usually remain asymptomatic until they develop decompensation of cirrhosis or advanced HCC, when antiviral treatments are hardly effective. The Ministry of Health, Welfare, and Labor in Japan started a national screening program in 2002 for HCV (and HBV) infection among people older than 40 years, in view of the high prevalence of HCV infection in this age group. By the end of 2006, 9 million people had been screened, among whom 110,000 patients were detected to have HCV infection and 110,000 patients with HBV infection [82]. The cost-effectiveness of such programs depends on the prevalence of viral infection among the target population.

Treatment of acute hepatitis C

Although HCV is not as infectious as HBV or HIV, chronicity is established in 70–80% of patients who have acute HCV infection. After exposure to HCV, such as needlestick injury, serum HCV should be monitored. The incidence of acute hepatitis C is reported to be 1.8% after injury with an HCV-contaminated needle. IFN therapy is to be considered to prevent chronicity once acute HCV infection is confirmed. [83, 84]

Treatment of chronic hepatitis C

Nishiguchi et al. [85] showed in an RCT that IFN therapy reduced the incidence of HCC in HCV-positive patients with compensated cirrhosis. The preventive effect was stronger in patients who showed sustained virologic response than in patients who failed to attain the response [86]. Several nonrandomized cohort studies showed similar effects on the reduction of HCC development [87–89]. One nonrandomized study detected no significant difference in HCC occurrence, but the low response rate and relatively small sample size may have been responsible for these results [90]. Several meta-analyses on randomized and nonrandomized studies on IFN therapy for patients with compensated cirrhosis concluded that the incidence of HCC was significantly reduced with therapy [91, 92].

The effect of IFN therapy on HCC incidence in noncirrhotic patients has been evaluated in nonrandomized studies. Although some studies failed to detect significant risk reduction in treated patients, all studies agree that the risk is reduced in patients who show sustained virologic response or persistent normalization of serum ALT levels [88, 93–95]. Since the incidence of HCC among noncirrhotic patients is not high, a large-sized sample and/or a long-term observation would be required to detect the effect of antiviral therapy on HCC prevention. The fact that IFN therapy improves liver histology in sustained virologic responders may also contribute to prevention of HCC [96]. Although documentation is poor, a combination with ribavirin is likely to produce a stronger effect on HCC prevention among overall treated patients [97]. In most studies, a smaller risk reduction was found in transient responders, i.e., those who showed a temporary response during IFN administration, whereas no effects were detected in nonresponders. Since treatment has a possible effect on HCC prevention even in transient responders, long-term maintenance IFN administration may be beneficial to patients with refractory chronic hepatitis C. Several nonrandomized studies reported reduction in HCC incidence with such treatments [98, 99]. However, a large-scale RCT performed in the United States revealed no reduction in HCC even with 3.5 years of peginterferon maintenance therapy [100]. The reasons for this difference are yet to be elucidated.

Viral-unrelated prevention of HCC

Recommendations
Prevention of HCC by elimination of aflatoxin contamination is advised (2a, B).
Prevention of HCC in patients with nonalcoholic steatohepatitis (NASH) is primarily through lifestyle modification with diet and exercise (2, B).

Aflatoxin

Aflatoxins are one of the most potent hepatocarcinogens and are easily acquired by human through exposure to mycotoxins. The incidence of HCC may be reduced by eliminating aflatoxin through proper food storage [78, 101]. The steady decrease in HCC incidence in affluent regions such as Singapore and Shanghai may be, in part, due to the decrease in aflatoxin contamination in the food as a result of economic development [102].

Chen et al. [54] elucidated in a community-based cohort study in Taiwan that a synergistic effect on HCC existed

between HBsAg carrier status and aflatoxin exposure. Another case–control study conducted in Sudan assessed the population-attributable risk of aflatoxin and HBV infection, jointly and separately, with respect to HCC. It demonstrated that reduction of aflatoxin contamination of foods and HBV vaccination may be useful public health strategies in HCC prevention [103].

Coffee

Coffee has a favorable effect on liver function and liver diseases, particularly in high-risk individuals, making it a substance of interest for the prevention of HCC [104–113].

Two meta-analyses on the relationship between coffee and HCC conducted by Bravi et al. [114] and Larsson et al. [115] provided substantial evidence that there is an inverse relation between coffee and HCC. The findings from these meta-analyses indicate a reduced risk of liver cancer, among both individuals with and without a history of liver disease. Although impressive reviews are available, it is still too early in making direct recommendations regarding coffee intake.

Vitamin K_2

Vitamin K_2 inhibits the growth of various neoplastic cells, including hepatoma cells, by causing cell-cycle arrest and apoptosis through different proposed mechanisms [116–122].

An RCT involving the use of vitamin K_2 in the prevention of HCC in women with HBV- or HCV-related cirrhosis proved that there could be a possible role for this as primary preventive agent [122]. The safety, relatively low cost, and ease of use make vitamin K_2 a suitable candidate for clinical trials that assess the value of combination of chemoprevention or chemotherapy in at-risk patients or in patients with a confirmed diagnosis of HCC [116, 122–125].

Although short-term effects seem appealing, additional multicenter randomized controlled studies are needed to look into long-term effects of vitamin K_2.

Tobacco and alcohol intake

It is still controversial whether cigarette smoking is a risk factor for HCC [48, 49]. Many authors support the fact that heavy alcohol intake is strongly associated with HCC [49–51]. Alcohol also increases the risk for HCC in patients with chronic hepatitis B and C [102]. Therefore, abstinence of heavy alcohol drinking is probably beneficial in reducing the risk of HCC.

NASH and HCC

Nonalcoholic steatohepatitis has been reported to affect 2–3% of the world's population, making it probably the most common liver disorder today [126]. Of these patients with NASH, 23% progress to liver cirrhosis in 10–15 years [127]. It has been observed that at the time of diagnosis, advanced fibrosis is already found in 30–40% of NASH patients, and 10–15% already have established cirrhosis. Since NASH may progress to cirrhosis (NASH being responsible for 70% of cryptogenic cirrhosis) [128], HCC development may be a part of the natural history of this disease [129]. A recent study by Chen et al. [58], which enrolled 23,820 residents in Taiwan with a 14-year follow-up, showed that extreme obesity (BMI $\geq$ 30 kg/m^2) was independently associated with a fourfold risk of HCC in anti-HCV-positive subjects and a twofold risk of HCC in those without HBV or HCV after controlling for other metabolic components. Diabetes was associated with HCC in HBsAg-positive, anti-HCV-positive, or both HBsAg- and anti-HCV-negative subjects, with the highest risk in those with HCV infection [RR (multivariate-adjusted relative risk) 3.52, 95% CI 1.29–9.24] and lowest in HBV carriers (RR 2.27, 95% CI 1.10–4.66). The study also found more than 100-fold increased risk of HCC in HBV or HCV carriers with both diabetes and obesity, indicating synergistic effects of metabolic factors and hepatitis [58].

Patients who have NASH-related cirrhosis carry a substantial risk for early development of HCC and a poor prognosis because of the limited therapeutic options due to relevant comorbidity. This raises the issue of careful screening and surveillance for HCC in NASH patients who have advanced liver disease. Control of risk factors such as type II diabetes, obesity, and dyslipidemia is recommended as the first and most important approach in managing people with NAFLD and NASH and preventing development of cirrhosis and HCC [130].

Lifestyle measures such as dietary modifications based on the metabolic profile (obesity, type II diabetes, hyperlipidemia, and hypertension) and increasing physical activity in the form of aerobic exercise should be encouraged in all patients with NAFLD. There is currently a level II evidence to support the beneficial role of dietary restriction (mainly aimed at improving insulin sensitivity) and exercise in the management of NAFLD [131].

Since NAFLD and NASH are closely associated with insulin resistance, pharmacologic treatment has been targeted on insulin-sensitizing drugs. Several studies on the use of insulin-sensitizing drugs have been done. Chavez-Tapia et al. [132] conducted a systematic review of nine studies on the use of either metformin or thiazolidinediones and indicated that these drugs improve insulin resistance and liver function.

Hemochromatosis and HCC

Hepatocellular carcinoma is long known to be associated with HH [63]. The risk for the development of HCC in patients with HH was estimated to be more than 200-fold increase in early publications [62, 133]. A subsequent Danish study also showed a 93-fold increase of HCC in HH [134]. However, the true incidence of HCC in HH may be achieved from population-based studies. Two such studies from the United States and Sweden showed a strong association of HCC and HH [64, 135]. In addition to HH, the hepatic iron overload owing to other causes, such as homozygous beta thalassemia [136] and the dietary form observed in South African blacks [137], is also associated with an increased risk of HCC. There is also evidence that marked iron overload in the setting of end-stage liver disease is also associated with HCC. However, the current data are inconclusive on the relation between mild or moderate iron overload associated with hepatitis C or alcoholic liver disease [138]. Because iron depletion by phlebotomy is safe and effective, it appears prudent to screen patients with chronic liver disease for iron overload and to institute iron depletion if iron overload is identified.

Surveillance and diagnosis

Surveillance

Recommendations
Surveillance for HCC in high-risk populations is recommended (2a, B).
Surveillance for HCC should be performed by ultrasonography (US) and α-fetoprotein (AFP) every 6 months (2a, B).

Rationale for surveillance

As described above, high-risk populations (e.g., cirrhosis with HBV or HCV infection) with HCC have been clearly identified by many epidemiological studies. However, the effectiveness of surveillance programs has still to be demonstrated through prospective RCTs, comparing the survival of participants with or without surveillance, though they may be susceptible to lead-time bias. To date, there is only one study that has proved the benefit of surveillance [139]. Zhang et al. [139] recruited 18,186 patients with chronic hepatitis due to HBV in China. The study revealed that surveillance with biannual AFP measurement and US reduced the mortality from HCC by 37% in spite of the fact that the compliance of scheduled tests was only 58.2%. It is desirable that this result should be validated in patients with other etiologies (e.g., chronic infection with HCV). However, it is highly unlikely that any such randomized study could be undertaken now because the surveillance of patients with cirrhosis is widely accepted and recruiting patients to a nonscreening arm of such a study would be almost impossible.

Who should be screened?

The efficacy of surveillance unambiguously depends on the incidence of HCC in the target population. However, because the risk of HCC in patients with chronic liver disease increases continuously with the number of risk factors, defining the population who should be screened is rather difficult. In addition, threshold for cost-effectiveness of surveillance program differs according to the economic situation of each country. Therefore, we recommend cirrhotic patients with HBV and HCV as candidates for surveillance at the present moment.

Recently, a study to better define the risk of chronic viral hepatitis by considering all important clinical and virologic features is ongoing. The results may be validated in the future [140].

What modality should be used?

Diagnostic tests universally available to date are imaging modalities including US, CT, and MRI, and a tumor marker such as AFP. AFP is the most widely studied screening test for HCC [141–143]. However, it is known that a significant proportion of small HCCs (e.g., $\leq$3 cm) do not secrete AFP to achieve a diagnostic level [142]. Furthermore, the level of AFP is elevated in patients with both HCC and chronic liver disease; thus, there is wide overlapping between the two groups [144, 145]. Most studies adopt a cutoff value of 20 ng/mL for AFP, with a sensitivity ranging from 49 to 71% and specificity from 49 to 86% in HCCs smaller than 5 cm [146–154]. Limitations in the sensitivity and specificity of AFP in surveillance of high-risk populations have led to the use of US as an additional method for the detection of HCC [142, 155–157].

Sensitivity of US is 78–90%, with 93% specificity [142, 148, 157]. In some countries such as Japan, concomitant measurement of des-γ-carboxyprothrombin (DCP) and lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) reportedly increases the detectability of small HCC [146, 147, 149–151, 153, 154]. The use of CT or MRI with contrast media can attain a higher diagnostic accuracy than US, but their use is costly.

Optimal interval for screening

The optimal interval of diagnostic tests in a surveillance program should be assessed from the view of cost-effectiveness because it is clear that more frequent tests can

detect HCC nodules of smaller size. Many studies have adopted an interval of 6 months between periodic diagnostic tests [155–157], although there are no randomized studies that have determined the optimal interval.

Thus, we propose periodic US and AFP measurements every 6 months as a minimum requirement. More frequent examinations, including new tumor markers such as DCP or AFP-L3 and CT/MRI, should be considered according to the medical circumstances of each country.

Tumor markers

Recommendations
α-Fetoprotein alone is not recommended for the diagnosis of HCC (1b, A).
Cutoff value of AFP should be set at 200 ng/mL for diagnosis (1b, A).
Simultaneous measurement of AFP and DCP provides higher sensitivity without decreasing specificity (1b, A).

Tumor makers are used in the diagnosis, prognosis, and evaluation of HCC. When a tumor marker is evaluated as a diagnostic test, its accuracy should be evaluated in terms of sensitivity, specificity, LR+, and LR+ [158]. Generally, the serum level of a tumor marker increases with the tumor size. Therefore, the range of tumor sizes should be considered in the evaluation of studies. A systematic review of studies published between 1982 and 2002 to evaluate the diagnostic accuracy of tumor markers for HCC is already available [159]. For the development of APASL consensus statement for HCC, we performed additional systematic review of studies published from 2003 to August 2008. Summary of recent studies that met the inclusion criteria is shown in Table 1 [160–169]. The results of the studies that evaluated AFP, DCP, and AFP-L3 were grossly compatible with the previous review.

α-Fetoprotein

α-Fetoprotein has served as a diagnostic test for HCC since the 1970s, when most patients with HCC were diagnosed at an advanced stage and with clinical symptoms [170]. A level of 500 ng/mL was considered diagnostic then. However, the usefulness of AFP as a diagnostic test in small HCCs is limited. According to this systematic review, the sensitivity, specificity, and LR+ of AFP in diagnosing HCC smaller than 5 cm in diameter ranged from 0.49 to 0.71, 0.49 to 0.86, and 1.28 to 4.03, respectively, with a cutoff value of 20 ng/mL and 0.04 to 0.31, 0.76 to 1.0, and 1.13 to 54.25, respectively, with a cutoff value of 200 ng/mL [159]. In a meta-analysis, AFP with a cutoff value of 200 ng/mL showed a better combined LR+ than with that of 20 ng/mL (5.85 vs. 2.45). The cutoff value of AFP should be set at 200 ng/mL instead of 20 ng/mL in the diagnosis of HCC.

Des-γ-carboxyprothrombin

Des-γ-carboxyprothrombin, also known as prothrombin induced by vitamin K absence-II, is an abnormal prothrombin protein that is increased in the serum of HCC patients. Since the report by Liebman et al. [171], DCP has been recognized as not only a highly specific marker for HCC but also a predictor of prognosis of HCC patients [172, 173]. According to the systematic review, the sensitivity, specificity, and LR+ of DCP in HCC smaller than 5 cm in diameter ranged from 0.14 to 0.54, 0.95 to 0.99, and 6.86 to 29.7, respectively, with a cutoff value of 40 mAU/mL and 0.07 to 0.56, 0.72 to 1.0, and 3.56 to 13.0, respectively, with a cutoff value of 100 mAU/mL [159]. In the meta-analysis, DCP with a cutoff value of 40 mAU/mL showed a better combined LR+ than with that of 100 mAU/mL (12.60 vs. 4.91).

Lens culinaris agglutinin-reactive fraction of AFP

AFP-L3 is a fucosylated variant of AFP that reacts with lens culinaris agglutinin A and can differentiate an increase in AFP due to HCC from that in patients with benign liver disease [174–176]. According to the systematic review, the sensitivity, specificity, and LR+ of AFP-L3 in HCC smaller than 5 cm in diameter ranged from 0.22 to 0.33, 0.93 to 0.94, and 4.63 to 30.8, respectively, with a cutoff value of 10% and 0.21 to 0.49, 0.94 to 1.0, and 8.06 to 45.1, respectively, with a cutoff value of 15% [159]. In the meta-analysis, AFP-L3 with a cutoff value of 15% earns better combined LR+ than with a cutoff value of 10% (13.1 vs. 4.89).

Glypican-3

GPC3 is a heparan sulfate proteoglycan anchored to the plasma membrane. It has been reported that GPC3 messenger RNA levels are increased in HCC [177, 178]. To date, a lot of studies reported the usefulness of GPC3 in the differential diagnosis of HCC. However, the vast majority of reports were based on the immunohistochemical studies. Capurro et al. [164] reported sensitivity of 0.53 and specificity of 0.95 with a cutoff value of 117 ng/mL on a study of serum samples from 53 healthy individuals and 71 patients with hepatitis or HCC. More evidence is needed to recommend GPC3 in daily practice.

Combination of tumor markers

Simultaneous measurement of tumor markers improves sensitivity without decreasing specificity when they have a

Table 1 Summary of studies on tumor markers for HCC published since 2003

Reference	Diagnostic test	Study design	Country	Patients with HCC				Control		
				n	Etiology	Characteristics of HCC	Modalities of diagnosis	*n*	Etiology	Characteristics of patients
Marrero et al. [160]	AFP, DCP	CC	USA	55	4% with HBV 46% with HCV 13% with ALT	NR	100% by pathology	152	7% with HBV 50% with HCV	32% with NL 34% with CH 35% with LC
Cui et al. [161]	AFP, DCP, GGTII	CC	China	120	81% with HBV 0% with HCV	26%, ≤3 cm	74% by pathology 26% by imaging	90	92% with HBV 1% with HCV	100% with LC
Wang et al. [162]	AFP, DCP	CC	China	61	46% with HBV 39% with HCV	38%, ≤2 cm 26%, 2–3 cm 36%, >3 cm	77% by pathology 23% by imaging	66	53% with HBV 42% with HCV	49% with CH 51% with LC
Sterling et al. [163]	AFP. AFP-L3	CC, CO	USA	74	100% with HCV	28%, <2 cm 68%, ≤5 cm	92% by imaging	298	100% with HCV	100% with LC
Capurro et al. [164]	AFP, GPC3	CC	Canada	34	NR	NR	NR	91	NR	58% with NL 20% with CH 22% with LC
Hippo et al. [165]	AFP, GPC3	CC	Japan	69	NR	NR	62% by pathology 38% by imaging	134	NR	28% with LC 72% with NL
Nguyen et al. [166]	AFP	CC	USA	163	100% with HCV	50%, ≤3.5 cm	53% by pathology 47% by imaging	149	100% with HCV	100% with LC
Soresi et al. [167]	AFP	CC	Italy	197	8% with HBV 75% with HCV	NR	NR	272	8% with HBV 77% with HCV	100% with LC
Arrieta et al. [168]	AFP	CC	Mexico	193	7% with HBV 30% with HCV	NR	100% by pathology	74	0% with HBV 45% with HCV	100% with LC
Paul et al. [169]	AFP	CC	India	101	NR	31%, ≤5 cm	NR	194	NR	100% with LC

AFP α-fetoprotein, *AFP-L3* lens culinaris agglutinin-reactive fraction of AFP, *CC* case–control study, *CH* chronic hepatitis, *Co* cohort study, *DCP* des-γ-carboxy prothrombin, *GGTII* hepatoma-specific band of serum γ-glutamyl transferase, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *LC* liver cirrhosis, *NL* normal liver, *NR* not reported

weak association. Sensitivity, specificity, and LR+ of AFP and DCP in small HCCs were 0.48, 0.99, and 48 with a cutoff value of 200 ng/mL for AFP and 40 mAU/mL for DCP [179].

Ultrasonography

Recommendations
Ultrasonography is a screening test and not a diagnostic test for confirmation (2b, B).
Contrast-enhanced US (CEUS) is as sensitive as dynamic CT or dynamic MRI in the diagnosis of HCC (2b, B).

The evaluation of intranodular hemodynamics is important for the diagnosis of hepatic malignancies because the pathologic findings of hepatic malignancies are closely related to intranodular hemodynamics. B-mode US is useful for the screening of liver diseases but cannot demonstrate tumor vascularity. Color Doppler imaging reveals the arterial pulsating flows, such as a basket pattern flow and a "spot" pattern flow, for hepatic tumor differentiation [180, 181]. However, color Doppler US does not detect pulsatile flow in some HCCs. The reasons for this are as follows: first, color Doppler US cannot detect flows that are perpendicular to the sound field [182]. Second, the technique uses an estimate of the mean Doppler frequency shift at a particular position. On the contrary, power Doppler imaging measures the Doppler energy, which is based on the integrated power of the Doppler signal instead of its mean Doppler frequency shift. Some studies reported that power Doppler sonography was more sensitive for the depiction of blood vessels than color Doppler imaging [182, 183]. These techniques are noninvasive and inexpensive; however, they have some limitations including a low sensitivity of detecting the microflow in the nodules.

Efforts have been made to improve both sonography equipment and contrast agents to detect flow in tumors with more sensitivity [184, 185]. Sonography with an intra-arterial CO_2 microbubble contrast agent enables the detection of intratumoral hemodynamics. The differential diagnosis of hepatic tumors has become possible with contrast-enhanced, harmonic US based on tumor vascularity [186]. CEUS using Levovist bubbles involves the use of a nonlinear backscatter property of the resonant microbubbles produced by an intravenously administered contrast agent; it allows microflow imaging of nodules and eliminates clutter signals. However, Levovist bubbles easily collapse by ultrasound wave emission because of its fragile property. Therefore, Levovist-enhanced harmonic US images are basically obtained intermittently, and real-time images can be obtained within a short period of time at an early vascular phase and Kupffer imaging in the postvascular phase by a single sweep scan of the liver.

With the development of second-generation contrast media such as SonoVue or Sonazoid, which are made of a hard shell containing bubbles, contrast-enhanced, harmonic US has entered a new era. SonoVue and Sonazoid produce stable, nonlinear oscillations in the low-power acoustic field (i.e., low mechanical index) and supply great details of the second harmonic signals in real time. These contrast agents provide detailed perfusion features of the microvascular bed of the liver parenchyma and tumor during the vascular phase. Moreover, Kupffer imaging in the postvascular phase, which is stable for at least 3 h after injection and tolerable for multiple scanning, can be obtained in the low-power acoustic field because Sonazoid microbubbles are phagocytosed by Kupffer cells [187].

D'Onofrio et al. [188] reported that SonoVue-enhanced US detected hepatic malignancy as defects in the sinusoidal phase, with a sensitivity of 85%, specificity of 88%, positive predictive value of 92%, and negative predictive value of 77%. In our study, Sonazoid-enhanced harmonic US detected hepatic malignancy with a sensitivity of 95% (208/219), specificity of 93.3% (28/30), positive predictive value of 99% (208/210), and negative predictive value of 97.4% (38/39). These favorable results can be attributed to the characteristic features of Kupffer imaging.

Hatanaka et al. [189] reported that intranodular vascularity was detected in 99.4% of HCCs on contrast-enhanced, harmonic US. In the remaining 0.6% of HCCs, no blood signal was detected. In contrast, 98.9% of HCCs showed hyper- or isoperfusion on dynamic CT. Most of the HCCs showed HCC perfusion patterns on contrast-enhanced, harmonic US. The sensitivity and specificity of the HCC pattern were 96.6 and 94.4%, respectively. The positive and negative predictive values of this pattern were 97.7 and 91.9%, respectively.

SonoVue- or Sonazoid-enhanced harmonic US is a promising technique for the noninvasive characterization of hepatic tumors on the basis of the presence/absence of the characteristic features of each tumor type.

CT, MRI, and other imaging modalities

Recommendations
Dynamic CT or dynamic MRI is recommended as a first-line diagnostic tool for HCC when a screening test result is abnormal (1a, A).
Hallmark of HCC during CT scan or MRI is the presence of arterial enhancement, followed by washout of the tumor in the portal-venous and/or delayed phases (1b, A).

Detection and characterization of focal lesions in the liver are critical for screening patients with chronic liver disease. US is the most widely used modality for HCC screening and surveillance, largely due to its relatively low

costs and ready accessibility [190]. US as a screening test in HBsAg carriers showed a sensitivity of 71% and a specificity of 93%, but its positive predictive value is only 14% [191]. Some reports suggest the use of new techniques such as CT or MRI as promising alternative surveillance tools [192, 193]. However, CT and MRI are not appropriate surveillance tests because they are too expensive, invasive (radiation with CT or intravenous injection), and have limited availability in community setting [194]. Additional use of dynamic CT or dynamic MRI is recommended in patients undergoing HCC screening while awaiting liver transplantation because it may be associated with the greatest gain in life expectancy [195–197].

Once a screening test result is abnormal or there is a clinical suspicion of HCC, imaging is very important for the diagnosis and staging of this tumor. The most reliable diagnostic tests are triple-phase, helical CT and triple-phase, dynamic, contrast-enhanced MRI, whereas hepatic angiography or angioassisted CT [CT hepatic arteriography (CTHA) and CT during arterial portography (CTAP)] has fallen out of favor in most practice settings except in Japan [198, 199]. The evaluation of blood supply in a hepatocellular nodule is extremely important to characterize the lesion because there are sequential changes in the supplying vessels and hemodynamic state during hepatocarcinogenesis [200]. Studies based on the findings at CTAP and CTHA with pathologic correlation have shown that as the grade of malignancy within the nodules evolves, there is gradual reduction of the normal hepatic arterial and portal venous supply to the nodule followed by an increase in the abnormal arterial supply via newly formed abnormal arteries (neoangiogenesis) [201]. The hallmark of HCC during CT scan or MRI is the presence of arterial enhancement followed by washout of the tumor in the portal-venous and/or delayed phases [202]. The presence of arterial enhancement followed by washout has a sensitivity and specificity of 90 and 95%, respectively. However, 71% of patients with HCC will have arterial enhancement and washout on more than one test, whereas the rest do not have these features and, therefore, will require liver biopsy for the diagnosis of HCC [202].

A study of systematic review on the accuracies of US, spiral CT, and MRI in diagnosing HCC in patients with chronic liver disease revealed that the pooled estimates of the 14 US studies showed a sensitivity of 60% and specificity of 97%; for the ten CT studies, sensitivity was 68% and specificity 93%; and for the nine MRI studies, sensitivity was 81% and specificity 85% [203]. The operative characteristics of CT are comparable, whereas MRI is more sensitive. The performance of CT and MRI is affected by the size of the lesions [204, 205]. Although CT and MRI are reported to have a sensitivity of 60–94.4% and 58.5–93%, respectively, in tumors larger than 1 cm, their sensitivities for detecting tumors smaller than 1 cm are reduced by 33–45 and 33–67%, respectively [204, 206–208]. Furthermore, small, arterially enhancing nodules are common in the cirrhotic liver, and majority of these nodules are benign [209–211]. Therefore, the most important issue remains the identification of small tumors because curative treatments can be optimally applied to improve outcome [212, 213]. If left alone, these tumors can grow aggressively and invasion can occur before tumors reach the 2-cm cutoff size for small HCC [202]. Thus, every attempt, including imaging follow-up or biopsy, should be made to characterize these nodules [205].

More recently, contrast agents other than gadolinium-based contrast media have been used for imaging HCC. Superparamagnetic iron oxide (SPIO) particles used alone [214] or in conjunction with gadolinium-based contrast agents [215–217] have been shown to be highly sensitive for the detection of HCC, particularly for small tumors. The reported sensitivity of double-contrast MRI (SPIO and gadolinium) for the detection of HCC measuring 1– 2 cm in diameter is 92% [215, 216]. Several studies demonstrated that SPIO-enhanced MRI is useful in differentiating small HCCs from small, arterially enhancing pseudolesion [214, 218]. When considering only studies with whole-liver explant, the highest performance was achieved using double-contrast liver MRI with both gadolinium and SPIO, with sensitivity ranging from 78 to 80%, compared with multidetector-row CT (MDCT) with 65–79%, SPIO-enhanced MRI with 66–82% and dynamic MRI with 55–95% [204]. A more recent study of MRI with explant pathologic correlation demonstrated that gadobenate dimeglumine, which is a hepatobiliary agent, enhanced MRI has a sensitivity of 80–85% and a positive predictive value of 65–66% in the detection of HCC but is of limited value for detecting and characterizing lesions smaller than 1 cm [219].

Hypovascular nodules associated with liver cirrhosis include low- or high-grade dysplastic nodules (HGDN), early HCCs, and well-differentiated HCCs [201, 220–222].There are significant overlaps in enhancement patterns on dynamic CT or dynamic MRI and in signal intensity on T2-weighted images [200, 201, 205]. Indeed, the noninvasive diagnostic criteria based on arterial hypervascularization in contrast-enhanced imaging techniques, published by the European Association for the study of the liver (EASL), are satisfied in only 61% of small nodules in cirrhosis [223]. Furthermore, imaging of 1- to 2-cm nodules would miss the diagnosis of HCC in up to 38% of cases. More recently, when hypovascular nodules are detected by MDCT and dynamic MRI, the guidelines published by the Japan Society of Hepatology recommend the use of Sonazoid-enhanced US and SPIO-enhanced MRI [224]. When uptake by Kupffer cells is

reduced in the Kupffer phase of SPIO-enhanced MRI, malignancy should be highly suspected [214, 225, 226].

Other imaging modalities

The less invasive imaging studies including dynamic CT, MRI, and CEUS have replaced conventional angiography for the diagnosis of HCC, except during chemoembolization of tumors or embolization for ruptured HCC. CTHA and CTAP have been used for preoperative evaluation of HCC, although they are uncommonly used except in Japan [227–229]. However, the benefit of CTHA and CTAP compared with MRI for the diagnosis of HCC is not yet clear because it is more invasive than MRI and does not appear to be more accurate than MRI [230]. The role of positron emission tomography (PET) in the diagnostic and staging evaluation of HCC still remains uncertain. Several studies have suggested a role for [^{18}F]fluorodeoxyglucose (FDG)-PET scanning for the detection of primary HCCs, tumor staging, assessing response to therapy, and for predicting prognosis [231–233]. HCCs accumulate FDG to varying degrees (only 55–65% of tumors give a positive result by PET scanning), limiting the sensitivity of PET for primary tumors [234, 235]. However, FDG-PET seems to be a useful imaging modality for identifying extrahepatic metastases, although sensitivity is limited for lesions 1 cm or smaller [231, 236].

Diagnostic algorithm

Recommendations
Typical HCC can be diagnosed by imaging regardless of the size if a typical vascular pattern, i.e., arterial enhancement with portal-venous washout, is obtained on dynamic CT, dynamic MRI, or CEUS (2b, B).
Nodular lesions show an atypical imaging pattern, such as iso- or hypovascular in the arterial phase or arterial hypervascularity alone without portal-venous washout, should undergo further examinations (2b, B).

Diagnostic algorithm of hypervascular HCC

Many institutions use US for screening tumors and MDCT or dynamic MRI for subsequent examinations. When a lesion is intensely enhanced in the early arterial phase and becomes low attenuation in the equilibrium phase, it may not be problematic to diagnose the lesion as HCC, but ruling out benign hypervascular lesions, such as focal nodular hyperplasia (FNH), and arterioportal (A-P) shunt is necessary for which uptake by Kupffer cells is best detected by SPIO-enhanced MRI or Sonazoid/Levovist-enhanced US. When high SPIO-enhanced MRI signals or a defect in the Kupffer phase of Sonazoid/Levovist-enhanced US is confirmed, the lesion is diagnosed as HCC.

When a lesion shows low attenuation in the equilibrium phase, although not intensely enhanced in the early arterial phase on MDCT, it is sometimes possible that it is a hypervascular HCC if a more sensitive tool can be used; thus, Sonazoid/Levovist-enhanced US is necessary.

Gadolinium-ethoxybenzyl-diethylenetriaminepentaacetic acid MRI is a choice of test that is useful to differentiate HCC (even early HCC) from DN. For hypervascular nodules, it is necessary to rule out pseudo tumors, such as A-P shunt, and benign hypervascular lesions (FNH, adenoma, or angiomyolipoma), which usually require a biopsy. It has been reported that SPIO-enhanced MRI or CEUS may omit procedures such as CTHA, CTAP, and the most sensitive tools in diagnosing HCC and biopsy because their diagnostic ability for HCC is equivalent to CTHA/CTAP [237] (Fig. 1).

Diagnostic algorithm of hypovascular HCC

Among nodular lesions associated with liver cirrhosis, various nodules, such as low-grade dysplastic nodules (LGDN), which are considered to be precancerous lesions, HGDN, early HCC, and nodule-in-nodule liver cancer, are included as hypovascular nodules [220, 221, 238].

The most sensitive modality capable of objectively depicting the early carcinogenesis process among currently available imaging systems is (1) CTAP, followed by (2) CTHA [239, 240], (3) CEUS [241–243], and (4) SPIO-enhanced MRI [225, 244]. Portal blood flow may be maintained in some cases of DN and early HCC but reduced in other nodules, although the pathology remains because of early HCC, in which arterial blood flow has not yet increased. CTAP may detect the earliest initial change of HCC. The second earliest initial carcinogenic change is detected by CTHA or CEUS as an increase in intranodular arterial blood flow. However, both CTHA and CTAP are commonly performed in some countries only. In majority of Asia-Pacific region, CTHA and CTAP are not common diagnostic tests. Hypervascular lesions depicted as nodule-in-nodule or as entire hypervascular nodules can be interpreted as advanced cancer, although they are small.

MDCT and dynamic MRI are sensitive for the detection of arterial blood flow but are incapable of detecting arterial vascularity in some nodules depending on the acquisition timing, tumor location, and liver function; although the lesions are hypervascular on CEUS. Nodules intensely enhanced on MDCT and dynamic MRI can be assumed to already exhibit high intensity on T2-weighted MRI.

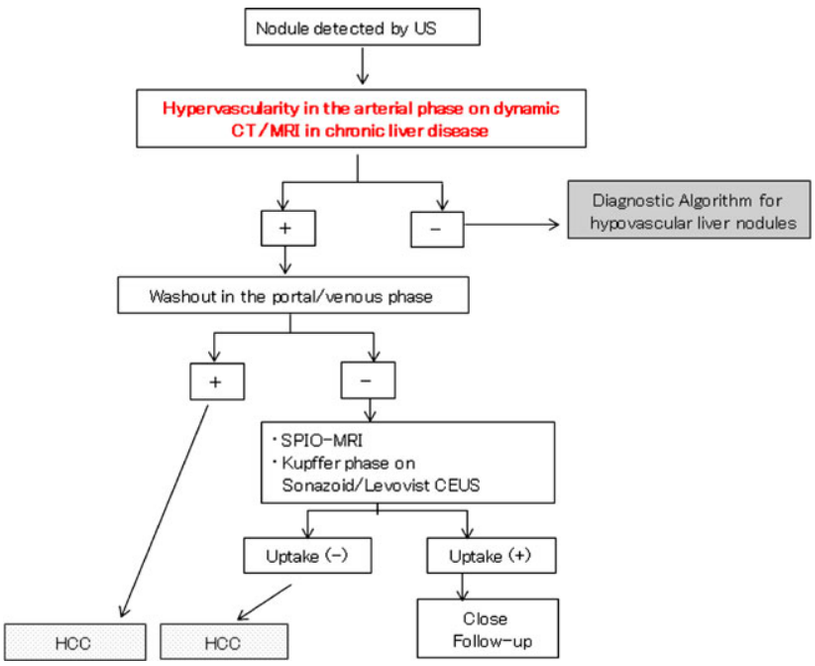


Fig. 1 Diagnostic algorithm of hypervascular HCC

On the basis of this finding, lesions detected as hypovascular nodules by MDCT and dynamic MRI should be subjected to Sonazoid- or Levovist-enhanced US (CEUS) and/or SPIO-enhanced MRI in the diagnostic algorithm for nodules. CEUS is more sensitive for detecting arterial vascularity of target nodules than dynamic CT or dynamic MRI [189, 243]. Thus, hypovascular nodules on dynamic CT may be diagnosed by CEUS. When uptake by Kupffer cells is reduced in the Kupffer phase of SPIO-enhanced MRI and CEUS, malignancy should be highly suspected. Although uptake is noted on SPIO-enhanced MRI, arterial blood flow may be increased in some cases on CEUS. When CTHA/CTAP is not available, such nodules should be closely followed up.

When Sonazoid or Levovist is used for CEUS, its combination with MDCT increases the accuracy of detecting intranodular arterial vascularity compared with that by a single method. Addition of the postvascular phase (Kupffer phase) allows an assumption of the degree of malignancy based on Kupffer function [189, 225, 244].

On the basis of this finding, when uptake is reduced in the Kupffer phase of SPIO-enhanced MRI or Kupffer phase of CEUS in nodules not depicted as hypervascular lesions by MDCT or dynamic MRI, the nodules should basically be regarded as HCC.

When uptake is noted on SPIO-enhanced MRI, close follow-up should be performed. When SPIO-enhanced MRI detects uptake and CEUS detects a malignant finding, i.e., increased arterial blood flow, the lesion should be regarded as malignant (Fig. 2).

Treatment

Liver resection and transplantation

Recommendations

Liver resection is a first-line curative treatment of solitary or multifocal HCC confined to the liver, anatomically respectable, and with satisfactory liver function reserve (2b, B)

Liver transplantation for HCC provides the best curative treatment of solitary HCC 5 or less cm or 3 or less tumor nodules, each 3 or less cm (Milan criteria) associated with Child-Pugh (C-P) class C cirrhosis (2b, B).

Bridge therapy using local ablation or chemoembolization may reduce dropout rate with long waiting time of more than 6 months, but there is no proven benefit in long-term survival or downstaging to allow expanded indication (2b, B).

Liver resection

Hepatic resection has been the mainstay of curative treatment of HCC. Like surgical treatment of other cancers, surgical resection has never been compared with conservative or drug treatment in the management of HCC, but the survival data of resection from cohort studies are so compelling that it is unethical nowadays to consider such a trial. However, there is still some controversy regarding the indications for resection of HCC. HCC with diameter of less than 5 cm is regarded by some as the best candidate for resection because of increased risk of additional nodules or vascular invasion and consequently incomplete resection

with larger HCCs [245, 246]. However, it has been shown that patients with a large solitary HCC are suitable for successful resection and reasonable long-term survival results can be achieved [247, 248]. The presence of multiple tumor nodules or vascular invasion in major intrahepatic venous branches may be associated with worse prognosis; however, surgical resection is still considered the best treatment in terms of long-term survival [249, 250]. Bilobar HCC was considered a contraindication for resection, but recent studies suggest that patients with a predominant mass in one lobe and one or two small tumor nodules in the other lobe may benefit from combined resection of the predominant tumor and ablation for the contralateral nodules [251, 252]. The presence of distant metastasis, main portal vein thrombosis, or inferior vena cava thrombosis is a definite contraindication for resection.

Hepatic resection for HCC is associated with a hospital mortality rate of less than 5% in major centers; however, the complication rate remains high, around 30–40% in large series [253–255]. Serious complications such as liver failure, postoperative bleeding, and bile leak occur in less than 5% of patients after hepatectomy nowadays [253–255]. However, less severe complications such as postoperative ascites, wound infection, and pneumonia remain common. Recently, laparoscopic liver resection has become popular, especially for minor resections or resection of the left lateral segment, and may reduce morbidity of liver resection [256]. However, thus far, no randomized trial comparing open and laparoscopic liver resection has been reported. The 5-year survival after resection of HCC is 35–50% in recent large cohort studies [257–259]. The long-term survival after hepatic resection depends on tumor characteristics. For small HCCs less than 5 cm in diameter, the 5-year survival rate is about 70% [260, 261]. However, recurrence occurs in 50–80% of patients at 5 years after resection, which is the main and long-term cause of deaths [262]. Despite several individual small trials that have demonstrated potential benefit of some adjuvant therapies, evidence from such trials is weak and there is no well-proven effective adjuvant treatment to prevent recurrence so far [263]. Aggressive management of tumor recurrence by repeat resection, ablation, or transarterial chemoembolization (TACE) is currently the most practical way to prolong patient survival [263, 264].

Liver transplantation

Orthotopic liver transplantation is theoretically the best curative treatment of HCC patients because it involves the widest possible resection margins for cancer, removes the remnant liver at risk of malignant change, and restores hepatic function. The results of transplantation for advanced HCC have been disappointing, with a 5-year survival rate of around 20%, due to a high incidence of recurrent tumors presumably from circulating tumor cells associated with large HCCs [265]. In contrast, liver transplantation is a particularly effective treatment of patients with early HCC but advanced C-P class B or C cirrhosis when other effective treatments cannot be offered. It is now well accepted that C-P class C cirrhotic patients with solitary HCC of less than 5 cm or fewer than 3 tumor nodules each of size less than 3 cm and without radiological evidence of venous invasion or distant metastasis should be treated by transplantation [266]. These criteria, called Milan criteria, are the most widely used criteria for the inclusion of HCC patients for liver transplantation on the basis of which the 4-year survival rate of up to 75% could be achieved, with a recurrence rate lower than 15%. Although there have never been any randomized studies comparing liver transplantation to conservative management or other treatments, liver transplantation has been well accepted as treatment of choice in small HCCs associated with severe cirrhosis on the basis of the favorable survival observed in cohort studies. Recently, Yao et al. [267] suggested an expanded

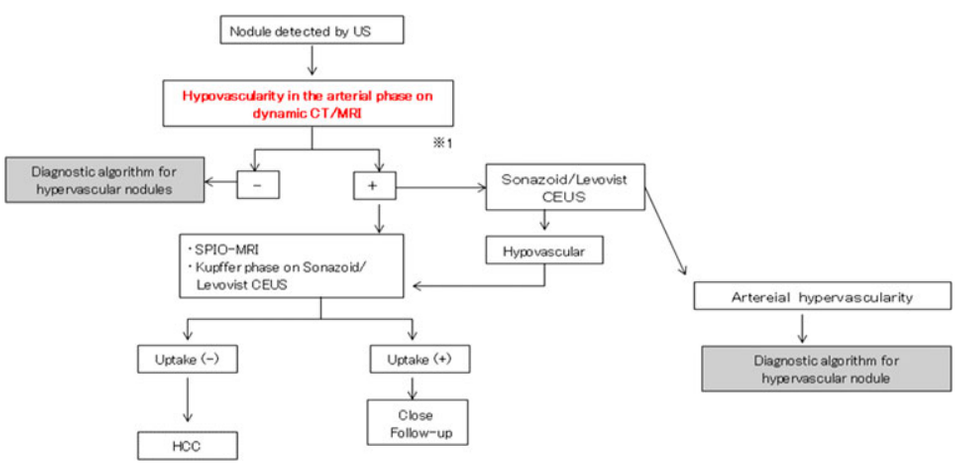


Fig. 2 Diagnostic algorithm of hypovascular HCC

criterion of solitary tumor 6.5 or less cm or three or fewer nodules with the largest lesion of 4.5 or less cm and total tumor diameter of 8 or less cm for liver transplantation. Their study showed that the long-term survival after transplantation for such patients were similar to that of liver transplantation for HCCs within the Milan criteria. Although the expanded criteria have been supported by some other studies [268], there are inadequate data in the literature to validate the long-term survival results using expanded criteria. Furthermore, it has to be noted that Yao's criteria were based on pathologic examination of explants rather than preoperative radiological imaging, which often underestimates the size of the tumor compared with measurement of tumor size in the explants. Currently, most centers worldwide still adopt Milan criteria in selection of patients for liver transplantation.

With the improvement in surgical techniques and better immunosuppressants to reduce the risk of graft rejection, the hospital mortality rate is less than 5% in major centers and the 5-year survival rate is about 60–75% [269–273]. Tumor recurrence after transplantation is lower than after resection for small HCC, and the 5-year disease-free survival rate is about 60–70%. The most important adverse prognostic factors of liver transplantation for HCC are the presence of microscopic venous invasion and histopathologic grading [272, 273]. Although the incidence of tumor recurrence is much lower after liver transplantation compared with partial hepatic resection, tumor recurrence is an important cause of long-term mortality after liver transplantation. Currently, there is no proven effective adjuvant therapy to reduce the risk of tumor recurrence.

The overall survival benefit of liver transplantation has been limited by the long waiting time for liver grafts for HCC patients. An intention-to-treat analysis has revealed a decrease in survival from 84 to 54% when the mean waiting time increased from 62 to 162 days [270]. Bridge treatments, including resection, percutaneous ablation, and TACE are commonly adopted while patients are on the waiting list to prevent tumor progression. However, the evidence for benefit of such bridge therapies is limited to retrospective case series, and it seems that bridge therapies are more likely to offer a benefit in patients with waiting time for grafts of more than 6 months [274]. Recently, live donor liver transplantation has emerged as a solution to shortage of liver grafts and is theoretically a more preferred choice for HCC patients because the waiting time is significantly reduced. However, the potential risk of donor hepatectomy (0.3–0.5% mortality) and relatively higher recipient complication (20–40%) need to be considered in offering such treatment [275]. Furthermore, live donor liver grafts are often small for size and the subsequent acute-phase injury, regeneration, and angiogenesis might increase the chance of tumor recurrence [276]. Whether this has any clinical implication on the long-term survival of patients with live donor liver transplantation remains unclear.

Whether patients with C-P class A cirrhosis with preserved liver function and a small HCC of less than 5 cm in diameter should be treated with transplantation or resection is a controversial issue. Some authors recommended liver transplantation for small HCC even in C-P class A cirrhosis patients because of the superior, disease-free survival results after transplantation, whereas others argue that hepatic resection should be the first-line therapy for such patients because of the similar overall survival results of the two treatments and the shortage of organ donors [277]. Practically, it is difficult to perform a randomized trial comparing the two approaches, and the applicability of liver transplantation depends on local graft availability in different institutions. In centers where graft shortage is a severe problem, resection as first-line treatment followed by salvage transplantation for recurrent tumors or liver failure may be a reasonable strategy [278, 279].

Ablation

Recommendations
Local ablation is an acceptable alternative to resection for small HCC (<3 cm) in C-P class A cirrhosis (2b, B).
Local ablation is a first-line treatment of unresectable, small HCC with 3 or fewer nodules in C-P class A or B cirrhosis (2b, B).

Image-guided percutaneous ablation therapies, such as percutaneous ethanol injection [280–282], microwave coagulation [283], and radiofrequency ablation (RFA) [284–286] have been widely performed on patients with small HCC, generally for those with Child A or B cirrhosis with three or fewer tumors each 3 cm or less in diameter. They are potentially curative, minimally invasive, and easily repeatable for recurrence. Percutaneous ethanol injection was first reported in the early 1980s [280–282]. Survival rates of patients treated with percutaneous ethanol injection have been reported to be 38–60% at 5 years [287–290]. Local tumor progression rates after percutaneous ethanol injection have been reported to be 6–31% depending on the size of tumor [288, 289, 291, 292]. Percutaneous ethanol injection has been considered a safe procedure, with mortality and morbidity rates of 0–3.2% and 0–0.4%, respectively [289, 291, 293]. Percutaneous microwave coagulation, in which the cancer tissue is ablated by dielectric heat produced by microwave energy emitted from the inserted 16-gauge, bipolar-type electrode, was introduced into clinical practice in the 1990s and reported to improve local tumor control [283].

Since the introduction of RFA in the 1990s [284, 285], there has been a drastic shift from ethanol injection and

microwave coagulation to RFA [286]. RCTs proved that RFA is superior to ethanol injection in the treatment of small HCCs in terms of treatment response, recurrence, and overall survival [294–297], while some investigators reported that RFA had higher complication rates [295, 297]. An RCT demonstrated that the number of treatment sessions was fewer with RFA than with microwave coagulation [298], although the rates of complete therapeutic effect, major complications, and local tumor progression were not statistically different between the two therapies. In RFA, survival rates have been reported to be 39.9–68.5% at 5 years [299–304] and local tumor progression rates to be 2.4–16.9% [299–301, 304]. Mortality and morbidity rates of RFA have been reported to be 0.9–7.9% and 0–1.5%, respectively [300–305].

Various clinical studies, involving combination of transcatheter arterial chemoembolization followed by RFA [306] or hepatic arterial balloon occlusion during RFA [307], have been attempted to increase the ablated volume of RFA by reducing the cooling effect of the blood supply. Although the extension of necrotic area was achieved, it still remains unsettled whether these trials actually improve the prognosis or not.

There have been two RCTs to compare percutaneous ablation therapies with surgical resection. One study showed no statistical significant difference for recurrence and survival between percutaneous ethanol injection and resection [308]. Another trial showed that overall survival and disease-free survival rates were not statistically different between RFA and resection, but complications were more frequent and severe after surgery [309]. No RCTs have demonstrated that surgical resection is superior to percutaneous ablation. In nonrandomized comparative studies, hepatectomy was better than percutaneous ablation in one study [213] whereas others reported no significant difference between the two therapies [310–312]. Thus, it is difficult to conclude that surgical resection is the treatment of choice for resectable HCC.

Transarterial chemoembolization

Recommendations

TACE is recommended as a first-line treatment for patients with unresectable, large/multifocal HCCs who do not have vascular invasion or extrahepatic spread (1b, A).

Selective TACE can be performed in early-stage patients in whom RFA is difficult to be performed because of tumor location or medical comorbidities (3, C).

Although the normal liver receives a dual blood supply from the hepatic artery and the portal vein, advanced HCC is supplied almost exclusively by the hepatic artery [313]. Hepatocarcinogenesis is a multistep process involving parenchymal arterialization, sinusoidal capillarization, and development of neoangiogenesis, causing gradual change in portal to arterial blood supply [314]. The blood supply of HCCs varies according to their developmental stage and growth pattern. Although well-differentiated or early HCC is supplied by the portal vein and the hepatic artery, encapsulated nodular HCC is totally supplied by the hepatic artery [315]. This specific arterial vascular profile provides the rationale for therapeutic local chemotherapy and hepatic artery occlusion of HCCs by TACE [316]. TACE exploits the preferential hepatic arterial supply of HCC for targeted delivery of chemotherapeutic agents, usually mixed with lipiodol followed by embolization or reduction in arterial flow using various types of particles, while sparing the surrounding liver parenchyma [317]. This combination of highly concentrated chemotherapy and arterial embolization may induce highly concentrated chemotherapy and ischemic damage on the tumor, which is likely to be synergistic in producing tumor necrosis [318].This reduction in arterial inflow causes not only ischemic necrosis within the tumors, which may increase tumor kill, but also significantly increases in tumor drug concentrations [319].

To date, multiple variations of TACE protocols remain in use throughout the world. Such variations revolve around the number and type of chemotherapeutic agents used, type of embolic materials, reliance on lipiodol, selectivity of catheter positioning, and the time interval between treatments [320]. However, a recent systematic review of cohort and randomized studies described the commonly used anticancer agents [321]. The most widely used single chemotherapeutic agent worldwide is doxorubicin (36%), followed by cisplatin (31%), epirubicin (12%), mitoxantrone (8%), and mitomycin C (8%). Lipiodol, an iodinated ester derived from poppy-seed oil, has been found to remain more selectively in tumor nodules for few weeks to some months when injected into the hepatic artery. It is nearly always used as a vehicle to carry and localize chemotherapeutic agents inside the tumor (tumor-seeking agents) [322]. Hepatic artery obstruction is usually achieved by Gelfoam particles, but polyvinyl alcohol, starch microspheres, metallic coils, and autologous blood clots have also been used [321]. Gelfoam powder should not be used because this may cause biliary damage [323]. In a recent study, TACE performed with drug-eluting beads loaded with doxorubicin has been shown to modify the pharmacokinetics of the injected chemotherapy, thus reducing the drug-related adverse effects while maintaining the same therapeutic efficacy as TACE [324].

The procedure requires individualized protocol according to the hepatic functional reserve and tumor extent. Every effort should be made to preserve nontumorous liver parenchyma from chemoembolization. The best way to

maximize the treatment effect and to minimize procedure-related complications is to perform selective chemoembolization of all tumor feeders [325]. The dose of lipiodol and chemotherapeutic agent depends on the size and vascularity of the tumor. The end point for the mixture administration is stasis in tumor-feeding arteries or appearance of lipiodol in portal vein branches near the tumor [321, 326, 327]. In general, the end point of the TACE procedure is the visualization of the complete blockage of the tumor-feeding branch [327]. However, there is no agreement on the degree of embolization [320]. Sometimes, the development of extrahepatic collaterals supplying liver tumors prohibits effective control of the tumor by hepatic artery chemoembolization. Therefore, it is essential to check for extrahepatic collateral arterial supply to the HCC, especially when tumor is in subcapsular location or shows exophytic tumor growth [328]. When the hepatic artery and extrahepatic collaterals supply the tumor, additional chemoembolization of the extrahepatic collaterals can be tried to increase the therapeutic efficacy of TACE [329, 330].

TACE currently is considered as the mainstay of therapy for nonsurgical HCCs that are also ineligible for percutaneous ablation [320]. In 2002, two prospectively RCTs have demonstrated a significant survival benefit from TACE in selected HCC patients with preserved liver function and adequate performance status [331, 332]. A subsequent meta-analysis confirmed these findings [333]. On the basis of the results of these studies, the guidelines published by the EASL [334] and the American Association for Study of Liver Diseases [335] recommend TACE as a first-line, noncurative therapy for nonsurgical patients with large/multifocal HCC who do not have vascular invasion or extrahepatic spread (level I). In addition, according to the guidelines published by the Japan Society of Hepatology [224], hepatectomy or TACE is recommended if there are two or three tumors of less than 3-cm diameter, and TACE or hepatic arterial infusion chemotherapy is recommended if there are more than four tumors. In addition, TACE can be performed in patients at the early stage in whom RFA cannot be performed because of tumor location (proximity to a gallbladder, biliary tree, or blood vessel) or medical comorbidities [198]. TACE is also the first-line therapy for downstaging tumors that exceed the criteria for transplantation [336–338]. Exclusion criteria in most trials are as follows: advanced liver disease (C-P class C), presence of vascular invasion or portal vein occlusion due to liver tumor, portosystemic shunt, hepatofugal blood flow, extrahepatic metastases, any contraindication to an arterial procedure (impaired clotting tests and renal failure), WHO performance stage 3 or 4, and end-stage tumorous disease (Okuda III) [339]. As the benefits of TACE procedure should not be offset by treatment-induced liver failure, patients who have liver decompensation should be excluded. A European study revealed that only 12 of the 903 patients evaluated for HCC were suitable for TACE [332].

The main complication of TACE is the so-called postembolization syndrome. The postembolization syndrome is characterized by nausea, vomiting, abdominal pain, and fever, occurring in more than 50% of patients after the procedure [335]. Although postembolization syndrome is a self-limited condition, it is an important complication of TACE that prolongs hospitalization. The incidence of major complications has been reported to be less than 5%, including hepatic insufficiency, liver abscess, parenchymal infarction, intrahepatic aneurysm, pulmonary embolism, ischemic cholecystitis or gallbladder infarction, bone marrow depression, liver rupture, and gastric or duodenal ulceration [340]. Important predisposing factors are major portal vein obstruction, compromised hepatic functional reserve, biliary obstruction, previous biliary surgery, excessive amount of iodized oil, and nonselective embolization [341]. TACE does not induce significant liver dysfunction in patients with C-P class A or B cirrhosis despite embolization of relative proximal hepatic arteries [342]. Treatment-related mortality is less than 5% [198].

Several RCTs have focused on the impact of TACE for palliation of unresectable HCC. In two RCTs and one systematic review with meta-analysis, TACE was found to improve survival compared with supportive care in patients with unresectable HCC [331–333]. Untreated patients at an intermediate stage present a median survival of 16 months. Chemoembolization increases the median survival of these patients to 19–20 months according to RCTs and meta-analysis of pooled data, and is considered the standard of care [333, 343]. In the two RCTs, 1-, 2-, and 3-year survival rates both for Asian patients and for European patients were 57 versus 96%, 31 versus 77%, and 26 versus 47%, respectively [331, 332, 339].

TACE induces extensive tumor necrosis in more than 50% of the patients [333]. According to conventional WHO criteria, the reported rate of objective responses ranges between 16 and 60%, there being no difference between TACE and transarterial embolization [316, 333]. Less than 2% of treated patients achieve a complete response [333]. However, subsegmental TACE may increase percentage of complete necrosis compared with TACE through lobar branches of hepatic artery [326, 327]. Although there are many reports suggesting satisfactory survival rates at institutions where TACE is performed on follow-up when tumor growth is detected or the tumor marker levels increase, no RCT have compared repeated TACE at regular, short intervals of 2–3 months, with TACE repeated only when tumor growth is detected [316]. Only one retrospective study demonstrated that the group receiving regular TACE at intervals of 2 months, for at least three times, showed more common complications and

lower cumulative survival rates than group that received a TACE repeat only when tumor growth was detected.

Systemic therapy

Recommendations
Sorafenib is recommended for the treatment of advanced stage patients (portal vein invasion or extrahepatic spread) who are not suitable for locoregional therapy and who have C-P class A liver function (1b, A).
Sorafenib may be used with caution in patients with C-P class B liver function (C).
Cytotoxic drugs are not routinely recommended but may be considered in highly selected patients whose general and hepatic conditions are adequate (3, C).

Recent advances in elucidating the molecular mechanisms of hepatocarcinogenesis have provided opportunities to develop molecular targeted therapy (MTT) for advanced HCC [344]. Sorafenib, an oral multikinase inhibitor, has shown survival benefit in two randomized, placebo-controlled trials [345, 346]. Several agents targeting tumor angiogenesis have also shown antitumor activity in patients with advanced HCC. Selected clinical trials of MTT for advanced HCC are summarized in Table 2.

Sorafenib

Sorafenib inhibits the kinase activity of both wild-type B-raf ($IC_{50} = 6$ nM) and mutant Raf^{V600E} ($IC_{50} = 38$ nM). In addition, sorafenib inhibits vascular endothelial growth factor receptors (VEGFR), platelet-derived growth factor receptors (PDGFR), c-kit, Flt-3, and RET ($IC_{50} < 100$ nM) [347]. Therefore, both antiproliferative and antiangiogenic mechanisms may account for the antitumor effects of sorafenib.

Two randomized, placebo-controlled trials of sorafenib for the treatment of advanced HCC have been reported [345, 346]. The first trial (SHARP trial) was conducted primarily in Europe and the United States with the primary end point of overall survival. The second trial was designed originally as a bridging study to evaluate the overall efficacy and safety of sorafenib in the Asia-Pacific population. Both trials recruited HCC patients whose tumors were not eligible for or had progressed after surgery or locoregional therapy, and patients with C-P class A liver function and Eastern Cooperative Oncology Group (ECOG) performance score was 2 or less. The treatment regimen was the same (sorafenib 400 mg twice daily). Both trials were stopped early because per-protocol interim analysis indicated significant survival benefit of sorafenib over placebo.

Patients in the Asia-Pacific trial were younger, had more symptomatic disease (ECOG score = 1 or 2), and extrahepatic metastases. Despite these differences in the baseline prognostic features, the overall treatment efficacy of sorafenib was similar between these two trials. The hazard ratios of overall survival and time to progression were 0.69 and 0.58 in the SHARP trial and 0.68 and 0.57 in the Asia-Pacific trial. Exploratory subgroup analyses of the two trials indicated that sorafenib treatment prolonged survival regardless of patients' age, performance status, and tumor burden (vascular invasion or extrahepatic spread). Time to symptomatic progression was not significantly different between patients who received sorafenib and patients who received placebo in either trial. Sorafenib is generally well tolerated. The most common drug-related adverse events included diarrhea, fatigue, hand-foot skin reaction, and rash/desquamation. These events occurred in 20–40% of patients, most of which were grade 1 or 2. The most common causes of treatment interruption or dose reduction were hand-foot skin reaction, rash, and diarrhea.

The efficacy and safety issues in patients with C-P class B cirrhosis need further clarification. A pharmacokinetic study suggested that patients with elevated bilirubin levels had lower tolerance to sorafenib treatment [348]. In the phase II trial of sorafenib for HCC, stable disease for 4 or more months was noted in 49% of patients with C-P class A cirrhosis ($n = 98$) and 26% of patients with C-P class B cirrhosis ($n = 38$). Patients with C-P class B cirrhosis had higher rate of elevated bilirubin (18 vs. 40%), encephalopathy (2 vs. 11%), and worsening ascites (11 vs. 18%) than patients with C-P class A cirrhosis, despite a similar incidence of all other adverse events and serious adverse events between these two groups of patients [349]. In the phase III SHARP trial, the incidence of serious hepatobiliary events was similar between the sorafenib group (11%) and the placebo group (9%). There are no clinical data for patients with C-P class C cirrhosis.

Antiangiogenic MTT

Hepatocellular carcinoma is typically a hypervascular tumor. Many antiangiogenic MTT have been tested for the treatment of HCC. The monoclonal anti-VEGF antibody bevacizumab has been tested at a dosing schedule of 5 or 10 mg/kg every 14 days in patients with advanced HCC [350]. The objective response rate was 13% (1 complete and 5 partial response in 46 patients). The median overall survival and progression-free survival were 12.4 and 6.9 months, respectively. The results suggest that bevacizumab may have a role in the treatment of patients with advanced HCC. The most common grade 3 or 4 toxicities included hypertension (15%), bleeding (11%), and thrombosis (6%). Careful evaluation of bleeding risk, such as esophageal and gastric varices, is recommended before the use of bevacizumab or similar agents.

Sunitinib is a multitarget tyrosine kinase inhibitor that inhibits tumor angiogenesis through its inhibition of

Table 2 Selected clinical trials of molecular targeted therapy for advanced HCC

	Treatment	Patient no.	Objective response	Median survival (months)				Level of evidence
				OS		TTP		
Phase III trials								
Llovet et al. [345]	Sorafenib 400 mg bid	299	RR: 2.3% (7 PR) SD: 71%	10.7	$P < 0.001$	5.5	$P < 0.001$	1b
	Placebo	303	RR: 0.7% (2 PR) SD: 67%	7.9		2.8		
Cheng et al. [346]	Sorafenib 400 mg bid	150	RR: 2.7% (4 PR) SD: 55%	6.5	$P = 0.014$	2.8	$P < 0.001$	1b
	Placebo	76	RR: 1.32% (1 PR) SD: 29%	4.2		1.4		
Phase II trials								
Siegel et al. [350]	Bevacizumab 5–10 mg/kg every 2 weeks	46	RR: 13% (1 CR and 5 PR) SD: 65% (progression free at 6 months)	12.4		6.9 (PFS)		4
Zhu et al. [352]	Sunitinib 37.5 mg qd for 4 weeks, followed by 2-week rest	34	RR: 2.9% (1 PR) SD: 47%	9.9		4.0		4
Faivre et al. [353]	Sunitinib 50 mg qd for 4 weeks, followed by 2-week rest	37	RR: 2.7% (1 PR) SD: 35.1%	10.3		4.8		4
Hsu et al. [356]	Thalidomide 100 mg bid	63	RR: 6.3% (1 CR, 3 PR in 63 evaluable patients)	4.3		NA		4
Patt et al. [357]	Thalidomide 400 mg qd	32	RR: 3.2% (1 PR in 32 evaluable patients) SD: 31%	6.8		NA		4
Philip et al. [368]	Erlotinib 150 mg qd	38	RR: 9% (3 PR in 34 evaluable patients) SD: 50%	13		3.2		4
Thomas et al. [369]	Erlotinib 150 mg qd	40	RR: 0 SD: 42.5%	10.8		6.5		4
O'Dwyer et al. [370]	Gefitinib 250 mg qd	31	RR: 3.2% (1 PR) SD: 22.6%	6.5		2.8 (PFS)		4
Zhu et al. [371]	Cetuximab 400 mg/m^2 loading, then 250 mg/m^2/week	30	RR: 0 SD: 16.7%	9.6		1.4 (PFS)		4

OS overall survival, *TTP* time to progression, *RR* response rate, *SD* stable disease, *PR* partial response, *PFS* progression-free survival

VEGFR and PDGFR activity [351]. Other targets of sunitinib include stem-cell factor receptor, colony-stimulating factor 1 (CSF-1), RET, and Flt-3. Two phase II trials of sunitinib for patients with advanced HCC reported a tumor stabilization rate of about 40% [352, 353]. Decreased tumor perfusion after sunitinib was demonstrated by dynamic computed tomography and magnetic resonance imaging, suggesting angiogenesis inhibition an important mechanism of its antitumor activity. Sunitinib at a daily dose of 50 mg was associated with a higher incidence of grade 3–5 toxicity, including ascites, edema, bleeding, and hepatic encephalopathy. At a daily dose of 37.5 mg, the most common toxicities included neutropenia, lymphopenia, thrombocytopenia, elevation of transaminases, fatigue, and skin rash. A phase III, randomized trial comparing the antitumor activity of sunitinib and sorafenib is under way.

Thalidomide showed antiangiogenic properties in the early 1990s and has been tested for the treatment of various cancers [354, 355]. Several phase II studies have explored the efficacy of thalidomide as a treatment of advanced HCC [21, 356–358]. Objective response, defined as complete and partial responses, was found in approximately 5% of the patients. In addition, about 10–30% of patients had disease stabilization for more than 2–4 months after thalidomide treatment. Disease stabilization after thalidomide treatment was associated with decreased tumor vascularity [359] and decreased blood perfusion [360], suggesting that the disease-controlling effect of thalidomide is mediated at

least, in part, by its antiangiogenic effect. The most common drug-related toxicities in all the series were somnolence, constipation, dizziness, and skin rash. These adverse effects were generally manageable.

Anti-EGFR MTT

The EGFR signaling pathway may play a role in hepatocarcinogenesis [361]. Expression of transforming growth factor-α, an EGFR ligand, can be induced by hepatitis viral proteins and may act synergistically with viral infection in hepatocarcinogenesis [362–364]. Results of EGFR expression in HCC tumor tissues, mainly by immunohistochemistry, varied in different studies [365], and activating mutation of EGFR, the major determinant of efficacy of EGFR inhibitors in lung cancer [366], was rarely found in HCC tumor tissue [367]. Both small-molecule EGFR inhibitors and monoclonal anti-EGFR antibodies have been tested in small-scale trials for the treatment of advanced HCC, and the response rates and patient survival were not consistent among the studies [368–371]. Correlation of tumor response with expression of EGFR yielded inconclusive results [368, 369]. The most common toxicities of these inhibitors were similar, including skin rash, diarrhea, and fatigue. The therapeutic potential of EGFR inhibitors remains unclear and needs more clinical data to support their role.

Cytotoxic therapy: single agent and combination

The role of conventional cytotoxic chemotherapy is limited by its myelosuppressive toxicity, which is particularly threatening in patients with cirrhosis, hypersplenism, and cytopenia. Objective tumor response rate to single-agent cytotoxic therapies is usually less than 10%, and no survival benefit has been observed [372–376]. An earlier randomized trial comparing doxorubicin, 60–75 mg/m^2 every 3 weeks, with no treatment indicated a borderline improvement in overall survival (10.6 vs. 7.5 weeks) for patients who received doxorubicin [377]. However, 25% of patients died of doxorubicin-related complications, including infection and cardiotoxicity. The antitumor activity of newer cytotoxic agents, such as gemcitabine [378, 379], oxaliplatin [380], and capecitabine [381], has been modest, with single-agent tumor response rate of 10% or less (Table 2). The most common grade 3–4 toxicity was myelosuppression, which occurred in 10–40% of the patients. Combination regimens, such as cisplatin/IFN/doxorubicin/fluorouracil (PIAF), gemcitabine/oxaliplatin (GEMOX), or capecitabine/oxaliplatin (XELOX), can increase the objective response rate to approximately 20% but at the expense of increased treatment-related toxicities [382, 383]. Therefore, cytotoxic chemotherapy can be used with caution only in selected patients with advanced HCC.

Future directions

Combination therapy with MTT has been continually investigated. A randomized phase II trial of sorafenib plus doxorubicin versus doxorubicin alone reported superior median overall survival (13.7 vs. 6.5 months) and time to progression (8.6 vs. 4.8 months) in patients receiving sorafenib plus doxorubicin versus doxorubicin alone [384]. These results should be interpreted with caution because a sorafenib-alone arm was not included and high incidence of adverse events related to doxorubicin was noted. Many small-scale trials of combining MTT with cytotoxic chemotherapy have been reported [385–389]. However, the treatment efficacy in terms of tumor response rate and patient survival were similar to those reported for the cytotoxic regimens alone (Table 3) [372, 375–382]. A second approach is to combine MTT targeting different molecular pathways. Preliminary results of a phase II trial combining bevacizumab with erlotinib showed a response rate of 20% and a median overall survival of 15.5 months [390]. However, these preliminary results must be validated by larger randomized trials.

Treatment algorithm

In general, treatment choice for a solid tumor should be decided taking into account the probability of cure and invasiveness of the treatments. Selecting treatment options for HCC is rather complicated because one should consider the background hepatic function that significantly affects the overall survival. In addition, probability of local cure is not a good surrogate for survival in HCC because intrahepatic recurrence occurs frequently even after curative resection. Therefore, a treatment algorithm should include both tumor- and hepatic reserve-related factors and should be based on results of studies that adopted survival as the primary end point. We propose a treatment algorithm for HCC as shown in Fig. 3.

Tertiary prevention

Recommendations
Interferon may be effective in reducing the recurrent HBV-related HCC after curative ablation of HCC (1b, B).
Lamivudine may be effective in reducing the recurrent HBV-related HCC after curative ablation of HCC (2c, C).
Interferon-based antiviral treatments after complete removal or ablation of HCV-related HCC may reduce HCC recurrence and improve survival (1b, B).

Tertiary prevention for HBV-related HCC

Interferon The short-term outcome of liver resection has dramatically improved over the last decade. The long-term

Table 3 Selected clinical trials of cytotoxic therapy for advanced HCC

Treatment	Patient no.	Objective response	Median survival (months)		Level of evidence
			OS	TTP	
Yeo et al. [375]					
Doxorubicin 60 mg/m^2 on day 1, every 3 weeks	94	RR: 10.5% (9 PR) SD: 39.4%	6.8	NA	1b
Doxorubicin 40 mg/m^2 on day 1, every 3 weeks Cisplatin 20 mg/m^2 Interferon α-2b 5 MU/m^2 5-FU 400 mg/m^2, days 1–4, every 3 weeks	94	RR: 20.9% (19 PR) SD: 37.2%	8.7	NA	
Gish et al. [376]					
Doxorubicin 60 mg/m^2 on day 1, every 3 weeks	222	RR: 2.7% (6 PR) SD: NA	7.4	2.3	1b
Nolatrexed 800 mg/m^2/ day, days 1–3, every 3 weeks	222	RR: 0.9% (1 PR) SD: NA	5.1	2.8	
Yang et al. [378]					
Gemcitabine 1,250 mg/m^2, days 1, 8, 15, every 4 weeks	28	RR: 17.8% (5PR) SD: 25%	4.3	2.8	4
Guan et al. [379]					
Gemcitabine 1,250 mg/m^2, days 1, 8, every 3 weeks	48	RR: 2.1% (2 PR) SD: 43.9%	3.2	1.5	4
Yen et al. [380]					
Oxaliplatin 100 mg/m^2 every 2 weeks	36	RR: 2.8% (1 PR) SD: 47%	6	2	4
Patt et al. [381]					
Capecitabine 2,000 mg/m^2/day, days 1–14, every 3 weeks	37	RR: 11% (1 CR, 3 PR) SD: 11%	10.1	NA	4

OS overall survival, *TTP* time to progression, *RR* response rate, *SD* stable disease, *PR* partial response

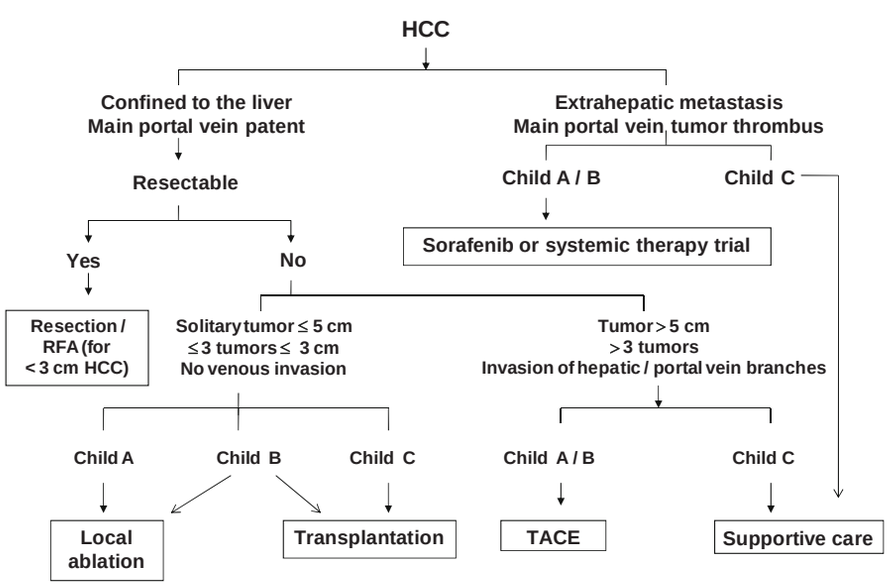


Fig. 3 Treatment algorithm of HCC

prognosis of HCC treated by hepatectomy remains a concern because of frequent development of tumor recurrence, which is the main cause of death in addition to concomitant hepatic decompensation. IFN has tumoricidal effect against a number of tumors including HCC. An RCT was performed to evaluate the safety and efficacy of adjuvant IFN

therapy after hepatic resection in a group of patients with predominantly HBV-related HCC [391]. The relative risk of death for IFN treatment was 0.42 (95% CI 0.17–1.05, $P = 0.063$). Subset analysis showed that adjuvant IFN had no survival benefit for pTNM stage I/II tumor (5-year survival 90% in both groups, $P = 0.917$) but prevented early recurrence and improved the 5-year survival of patients with stage III/IVA tumor from 24 to 68% ($P = 0.038$). HCC recurrence after locoablative treatment modalities is also common. Although candidates for medical ablation usually exhibit compensated hepatic functional status, the frequent recurrence of HCC after successful ablation contributes to short-term survival. A randomized controlled study with small sample size was conducted to evaluate the effectiveness of IFN therapy in preventing HCC recurrence after successful medical ablation therapy for primary tumors [392]. The cumulative HCC recurrence rate of the patients treated with IFN-alfa and the control group was 25 and 40% at the end of 1 year and 47 and 90% at the end of 4 years, respectively ($P = 0.0135$). Furthermore, this study also showed that the prevention of HCC recurrence using IFN-alfa was effective in HBV-related HCC [392]

Lamivudine A retrospective study was conducted to evaluate the efficacy with or without using LAM in patients following curative ablation of HBV-related HCC [393]. Cumulative recurrence rates of HCC were not significantly different between two groups ($P = 0.622$). However, median C-P score at the time of HCC recurrence was significantly different in the control group ($P = 0.005$). The cumulative survival rates of patients in the LAM group tended to be higher than those of patients in the control group ($P = 0.063$) [393]. The outcome of LAM treatment of patients with controlled HCC in terms C-P score and survival compared with a matched, LAM-untreated cohort showed no significant difference in the cumulative incidence of HCC recurrence and survival between the two groups [394]. However, there was a significant difference in the cumulative incidence of death due to liver failure ($P = 0.043$). A significant improvement in liver function was achieved by LAM treatment, even in patients with HCC. These results suggest that LAM treatment of patients with HCC may prevent death due to liver failure [394].

Recent randomized, placebo-controlled trial by Jang et al. [395] also showed that preemptive LAM therapy in patients receiving TACE significantly reduced the incidence of HBV reactivation ($P = 0.002$), overall hepatitis ($P = 0.021$), and severe hepatitis ($P = 0.035$) due to HBV reactivation after repeat TACE. However, the prevention of HCC by preemptive LAM therapy was not shown because of advanced stage of HCC in patients receiving TACE in that trial [395] Further prospective, randomized studies using a larger number of patients are required to assess its role in the tertiary prevention of HCC.

Tertiary prevention of HCV-related HCC

Hepatocellular carcinoma is characterized by very frequent recurrence even after successful initial treatments, either surgical resection or medical ablation, and the risk of recurrence remains high for many years. Recurrence is particularly frequent with HCV-related HCC, and a substantial proportion of recurrence, especially in late phase, is thought to represent de novo, or multicentric, hepatocarcinogenesis [396–398]. Therefore, it could be reasonably assumed that antiviral therapy would reduce the overall incidence of recurrence by preventing de novo carcinogenesis. Indeed, several small-sized RCTs, performed in Japan or Taiwan, showed that the incidence of recurrence was reduced in HCV-related HCC by IFN therapy subsequent to initial HCC treatment [392, 399, 400].

Other RCTs, also performed in Japan and Taiwan, failed to find a significant delay in the first recurrence with IFN therapy, but the second or third recurrence was significantly reduced especially in sustained responders and the overall survival was improved [401, 402]. Another RCT in Italy did not detect effects of IFN therapy on early recurrence but late recurrence, with more than 2 years of interval, seemed to be reduced among IFN responders [403]. These data are compatible with the hypothesis that de novo carcinogenesis was prevented by successful antiviral therapy. On the other hand, two reports on long-term observation of recurrence after IFN therapy following HCC treatment [404, 405] showed that recurrence rate in IFN-treated patients increased over time, suggesting that the growth of residual microscopic tumors had been delayed by IFN (in fact, the two presumed mechanisms are not necessarily mutually exclusive). Most of these studies used IFN monotherapy and suffered from low sustained response rates because most patients had advanced fibrosis or cirrhosis. Preventive effects of IFN on HCC recurrence are yet to be reevaluated using current more efficient protocols.

Microscopic, intrahepatic residual tumors, including intrahepatic metastases, are a possible cause of HCC recurrence. Theoretically, adjuvant chemotherapy may reduce or delay such recurrence, but few chemotherapeutic agents have been shown to be effective against HCC and not a few of them may be hepatotoxic. Hasegawa et al. [406] reported an RCT using oral administration of uracil-tegafur after curative hepatic resection but found no beneficial effects on recurrence and a possible adverse effect on overall survival. In 1966, Muto et al. [407] reported that administration of polyprenoic acid, an acyclic retinoid, reduced recurrence of HCC in an RCT. Updated, long-term

data were subsequently published [408], postulating that the eradication of premalignant or latent malignant clones is the mechanism of action. The effect is, however, yet to be confirmed in a large-scale RCT. Vitamin K_2 was reported to inhibit HCC development among female patients with cirrhosis, who had received the vitamin for the prevention of osteoporosis [122]. A small RCT suggested that vitamin K_2 was effective in suppressing HCC recurrence and may improve survival [124]. However, subsequent, large-scale RCT met with an early termination because of lacking evidence of effects.

Viral-unrelated tertiary prevention of HCC

Vitamin K_2 Apart from its use as a primary preventive agent for HCC, the use of vitamin K_2 as secondary preventive agent has also been investigated. Otsuka et al. [123] examined the biological effects of extrinsic supplementation of vitamin K_2 in HCC cells in vitro and in vivo. Administration of vitamin K_2 to nude mice inoculated with liver tumor cells reduced both tumor growth and weight loss. It was concluded that, similar to an acyclic retinoid, vitamin K_2 may be a promising therapeutic means for the management of HCC.

A pilot study by Mizuta et al. [124] on HCC patients who had undergone either percutaneous local ablation or surgery suggested that menatetrenone, a vitamin K_2 analogue, may have a suppressive effect on the recurrence of HCC and a beneficial effect on survival.

However, a later study (albeit smaller) by Hotta et al. [125] demonstrated that vitamin K_2 may not be as useful for the prevention of HCC recurrence as for primary prevention.

Sho-saiko-to Sho-saiko-to (SST or TJ9), a traditional (Chinese) herbal medicine, was demonstrated to improve liver function tests in patients with chronic active hepatitis in a multicenter, cross-over RCT by Hirayama et al. [409]. A later prospective, randomized (albeit nonblind) study by Oka et al. [410] could elucidate the use of TJ-9 in preventing the development of HCC in patients with cirrhosis, particularly in patients without HBsAg. Successive studies continued to confirm that TJ-9 could protect experimental liver injury caused by D-galactosamine and liver fibrosis by inhibition of lipid peroxide formation in liver cells [411, 412].

Juzen-taiho-to (TJ-48) A recently published study on Juzen-taiho-to, a traditional (Japanese) herbal formulation similar to Sho-saiko-to, presented new information on its anticancer effect in humans [413]. In this study, the administration of TJ-48 improved intrahepatic, recurrence-free survival after surgical treatment of HCC and its protective effects were probably due to reduction in oxidant and cytokine production by Kupffer cells.

References

1. Centre for Evidence-Based Medicine. Levels of evidence 2001 [cited 1 Dec 2008]. http://www.cebm.net/index.aspx?o=1025
2. Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB. Cancer Incidence in Five Continents, vol. VIII. Lyon: IARC Scientific; 2002. Publication No.: 155
3. Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22707 men in Taiwan. Lancet 1981;2:1129–1133
4. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993; 328:1797–1801
5. Chen CJ, Liang KY, Chang AS, Chang YC, Lu SN, Liaw YF, et al. Effects of hepatitis B virus, alcohol drinking, cigarette smoking and familial tendency on hepatocellular carcinoma. Hepatology 1991;13:398–406
6. Yang HI, Lu SN, Liaw YF, You SL, Sun CA, Wang LY, et al. Hepatitis B e antigen and the risk of hepatocellular carcinoma. N Engl J Med 2002;347:168–174
7. Tsai JF, Jeng JE, Ho MS, Chang WY, Hsieh MY, Lin ZY, et al. Additive effect modification of hepatitis B surface antigen and e antigen on the development of hepatocellular carcinoma. Br J Cancer 1996;73:1498–1502
8. Lu SN, Lin TM, Chen CJ, Chen JS, Liaw YF, Chang WY, et al. A case–control study of primary hepatocellular carcinoma in Taiwan. Cancer 1988;62:2051–2055
9. Lin TM, Chen CJ, Lu SN, Chang AS, Chang YC, Hsu ST, et al. Hepatitis B virus e antigen and primary hepatocellular carcinoma. Anticancer Res 1991;11:2063–2065
10. Yu MW, Yeh SH, Chen PJ, Liaw YF, Lin CL, Liu CJ, et al. Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. J Natl Cancer Inst 2005;97:265–272
11. Chan HL, Tse CH, Mo F, Koh J, Wong VW, Wong GL, et al. High viral load and hepatitis B virus subgenotype Ce are associated with increased risk of hepatocellular carcinoma. J Clin Oncol 2008;26:177–182
12. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65–73
13. Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. Am J Gastroenterol 2006;101:1797–1803
14. Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Chung H, et al. Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. Radiology 2001; 221:721–730
15. Yang HI, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, et al. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. J Natl Cancer Inst 2008;100:1134–1143
16. Yuen MF, Sablon E, Yuan HJ, Wong DK, Hui CK, Wong BC, et al. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology 2003;37:562–567

17. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554–559
18. Sumi H, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, et al. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology 2003;37:19–26
19. Orito E, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, et al. A case–control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology 2001;33:218–223
20. Fang ZL, Yang J, Ge X, Zhuang H, Gong J, Li R, et al. Core promoter mutations (A(1762)T and G(1764)A) and viral genotype in chronic hepatitis B and hepatocellular carcinoma in Guangxi, China. J Med Virol 2002;68:33–40
21. Chiou HE, Wang TE, Wang YY, Liu HW. Efficacy and safety of thalidomide in patients with hepatocellular carcinoma. World J Gastroenterol 2006;12:6955–6960
22. Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. Gastroenterology 2006;130:1153–1168
23. Chen CH, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, et al. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. Gastroenterology 2007;133:1466–1474
24. Chou YC, Yu MW, Wu CF, Yang SY, Lin CL, Liu CJ, et al. Temporal relationship between hepatitis B virus enhancer II/basal core promoter sequence variation and risk of hepatocellular carcinoma. Gut 2008;57:91–97
25. Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 2003;124:327–334
26. Fattovich G, Giustina G, Degos F, Tremolada F, Diodati G, Almasio P, et al. Morbidity and mortality in compensated cirrhosis type C: a retrospective follow-up study of 384 patients. Gastroenterology 1997;112:463–472
27. Tradati F, Colombo M, Mannucci PM, Rumi MG, De Fazio C, Gamba G, et al. A prospective multicenter study of hepatocellular carcinoma in Italian hemophiliacs with chronic hepatitis C. The Study Group of the Association of Italian Hemophilia Centers. Blood 1998;91:1173–1177
28. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology 1995;21:650–655
29. Sun CA, Wu DM, Lin CC, Lu SN, You SL, Wang LY, et al. Incidence and cofactors of hepatitis C virus-related hepatocellular carcinoma: a prospective study of 12,008 men in Taiwan. Am J Epidemiol 2003;157:674–682
30. Serfaty L, Aumaitre H, Chazouilleres O, Bonnand AM, Rosmorduc O, Poupon RE, et al. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. Hepatology 1998;27:1435–1440
31. Niederau C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. Hepatology 1998;28:1687–1695
32. Fattovich G, Ribero ML, Pantalena M, Diodati G, Almasio P, Nevens F, et al. Hepatitis C virus genotypes: distribution and clinical significance in patients with cirrhosis type C seen at tertiary referral centres in Europe. J Viral Hepat 2001;8:206–216
33. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. Hepatology 1997;25:754–758
34. Bruno S, Crosignani A, Maisonneuve P, Rossi S, Silini E, Mondelli MU. Hepatitis C virus genotype 1b as a major risk factor associated with hepatocellular carcinoma in patients with cirrhosis: a seventeen-year prospective cohort study. Hepatology 2007;46:1350–1356
35. Chuang WL, Chang WY, Lu SN, Su WP, Lin ZY, Chen SC, et al. The role of hepatitis B and C viruses in hepatocellular carcinoma in a hepatitis B endemic area. A case–control study. Cancer 1992;69:2052–2054
36. Chiaramonte M, Stroffolini T, Vian A, Stazi MA, Floreani A, Lorenzoni U, et al. Rate of incidence of hepatocellular carcinoma in patients with compensated viral cirrhosis. Cancer 1999;85:2132–2137
37. Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. J Gastroenterol Hepatol 1997;12:S294–S308
38. Yu MC, Tong MJ, Coursaget P, Ross RK, Govindarajan S, Henderson BE. Prevalence of hepatitis B and C viral markers in black and white patients with hepatocellular carcinoma in the United States. J Natl Cancer Inst 1990;82:1038–1041
39. Tagger A, Donato F, Ribero ML, Chiesa R, Portera G, Gelatti U, et al. Case–control study on hepatitis C virus (HCV) as a risk factor for hepatocellular carcinoma: the role of HCV genotypes and the synergism with hepatitis B virus and alcohol. Brescia HCC Study. Int J Cancer 1999;81:695–699
40. Kaklamani E, Trichopoulos D, Tzonou A, Zavitsanos X, Koumantaki Y, Hatzakis A, et al. Hepatitis B and C viruses and their interaction in the origin of hepatocellular carcinoma. JAMA 1991;265:1974–1976
41. Thio CL, Seaberg EC, Skolasky R Jr, Phair J, Visscher B, Munoz A, et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet 2002;360:1921–1926
42. Brau N, Fox RK, Xiao P, Marks K, Naqvi Z, Taylor LE, et al. Presentation and outcome of hepatocellular carcinoma in HIV-infected patients: a US–Canadian multicenter study. J Hepatol 2007;47:527–537
43. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108
44. Shiratori Y, Shiina S, Imamura M, Kato N, Kanai F, Okudaira T, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. Hepatology 1995;22:1027–1033
45. Lee CM, Lu SN, Changchien CS, Yeh CT, Hsu TT, Tang JH, et al. Age, gender, and local geographic variations of viral etiology of hepatocellular carcinoma in a hyperendemic area for hepatitis B virus infection. Cancer 1999;86:1143–1150
46. Lu SN, Su WW, Yang SS, Chang TT, Cheng KS, Wu JC, et al. Secular trends and geographic variations of hepatitis B virus and hepatitis C virus-associated hepatocellular carcinoma in Taiwan. Int J Cancer 2006;119:1946–1952
47. Yoshida H, Tateishi R, Arakawa Y, Sata M, Fujiyama S, Nishiguchi S, et al. Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. Gut 2004;53:425–430
48. Yu MC, Tong MJ, Govindarajan S, Henderson BE. Nonviral risk factors for hepatocellular carcinoma in a low-risk population, the non-Asians of Los Angeles County, California. J Natl Cancer Inst 1991;83:1820–1826
49. Tanaka K, Hirohata T, Takeshita S, Hirohata I, Koga S, Sugimachi K, et al. Hepatitis B virus, cigarette smoking and alcohol consumption in the development of hepatocellular carcinoma: a case–control study in Fukuoka, Japan. Int J Cancer 1992;51:509–514
50. Mohamed AE, Kew MC, Groeneveld HT. Alcohol consumption as a risk factor for hepatocellular carcinoma in urban southern African blacks. Int J Cancer 1992;51:537–541

51. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. Am J Epidemiol 2002;155:323–331
52. Chiesa R, Donato F, Tagger A, Favret M, Ribero ML, Nardi G, et al. Etiology of hepatocellular carcinoma in Italian patients with and without cirrhosis. Cancer Epidemiol Biomarkers Prev 2000;9:213–216
53. Wang JS, Huang T, Su J, Liang F, Wei Z, Liang Y, et al. Hepatocellular carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of China. Cancer Epidemiol Biomarkers Prev 2001;10:143–46
54. Chen CJ, Wang LY, Lu SN, Wu MH, You SL, Zhang YJ, et al. Elevated aflatoxin exposure and increased risk of hepatocellular carcinoma. Hepatology 1996;24:38–42
55. Qian GS, Ross RK, Yu MC, Yuan JM, Gao YT, Henderson BE, et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. Cancer Epidemiol Biomarkers Prev 1994;3:3–10
56. Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, et al. Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet 1992;339:943–946
57. Wang LY, Hatch M, Chen CJ, Levin B, You SL, Lu SN, et al. Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. Int J Cancer 1996;67:620–625
58. Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, et al. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology 2008;135:111–121
59. London WT, Evans AA, McGlynn K, Buetow K, An P, Gao L, et al. Viral, host and environmental risk factors for hepatocellular carcinoma: a prospective study in Haimen City, China. Intervirology 1995;38:155–161
60. Evans AA, Chen G, Ross EA, Shen FM, Lin WY, London WT. Eight-year follow-up of the 90,000-person Haimen City cohort: I. Hepatocellular carcinoma mortality, risk factors, and gender differences. Cancer Epidemiol Biomarkers Prev 2002;11:369–76
61. Yu MW, Chang HC, Liaw YF, Lin SM, Lee SD, Liu CJ, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. J Natl Cancer Inst 2000; 92:1159–1164
62. Bradbear RA, Bain C, Siskind V, Schofield FD, Webb S, Axelsen EM, et al. Cohort study of internal malignancy in genetic hemochromatosis and other chronic nonalcoholic liver diseases. J Natl Cancer Inst 1985;75:81–84
63. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer G. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. N Engl J Med 1985;313:1256–1262
64. Elmberg M, Hultcrantz R, Ekbom A, Brandt L, Olsson S, Olsson R, et al. Cancer risk in patients with hereditary hemochromatosis and in their first-degree relatives. Gastroenterology 2003;125: 1733–1741
65. Chen DS, Hsu NH, Sung JL, Hsu TC, Hsu ST, Kuo YT, et al. A mass vaccination program in Taiwan against hepatitis B virus infection in infants of hepatitis B surface antigen-carrier mothers. JAMA 1987;257:2597–2603
66. Hsu HM, Chen DS, Chuang CH, Lu JC, Jwo DM, Lee CC, et al. Efficacy of a mass hepatitis B vaccination program in Taiwan. Studies on 3464 infants of hepatitis B surface antigen-carrier mothers. JAMA 1988;260:2231–2235
67. Chen HL, Chang MH, Ni YH, Hsu HY, Lee PI, Lee CY, et al. Seroepidemiology of hepatitis B virus infection in children: ten years of mass vaccination in Taiwan. JAMA 1996;276:906–908
68. Chang MH, Shau WY, Chen CJ, Wu TC, Kong MS, Liang DC, et al. Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls. JAMA 2000;284:3040–3042
69. Kao JH, Chen DS. Recent updates in hepatitis vaccination and the prevention of hepatocellular carcinoma. Int J Cancer 2002; 97:269–271
70. Niederau C, Heintges T, Lange S, Goldmann G, Niederau CM, Mohr L, et al. Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. N Engl J Med 1996;334:1422–1427
71. Lin SM, Sheen IS, Chien RN, Chu CM, Liaw YF. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. Hepatology 1999;29:971–975
72. Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. J Hepatol 2001;34:306–313
73. Lampertico P, Del Ninno E, Vigano M, Romeo R, Donato MF, Sablon E, et al. Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. Hepatology 2003;37:756–763
74. van Zonneveld M, Honkoop P, Hansen BE, Niesters HG, Murad SD, de Man RA, et al. Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. Hepatology 2004;39:804–810
75. Lau DT, Everhart J, Kleiner DE, Park Y, Vergalla J, Schmid P, et al. Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;113:1660–1667
76. Yuen MF, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: the effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. Hepatology 2001;34:139–145
77. Lin SM, Yu ML, Lee CM, Chien RN, Sheen IS, Chu CM, et al. Interferon therapy in HBeAg positive chronic hepatitis reduces progression to cirrhosis and hepatocellular carcinoma. J Hepatol 2007;46:45–52
78. Craxi A, Camma C. Prevention of hepatocellular carcinoma. Clin Liver Dis 2005;9:329–346
79. Strickland GT. Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. Hepatology 2006;43:915–922
80. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372–1380
81. Yoshizawa H. Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62(Suppl 1):8–17
82. Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirolog 2006;49:7–17
83. Omata M, Yokosuka O, Takano S, Kato N, Hosoda K, Imazeki F, et al. Resolution of acute hepatitis C after therapy with natural beta interferon. Lancet 1991;338:914–915
84. Jaeckel E, Cornberg M, Wedemeyer H, Santantonio T, Mayer J, Zankel M, et al. Treatment of acute hepatitis C with interferon alfa-2b. N Engl J Med 2001;345:1452–1457
85. Nishiguchi S, Kuroki T, Nakatani S, Morimoto H, Takeda T, Nakajima S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. Lancet 1995;346:1051–1055
86. Nishiguchi S, Shiomi S, Nakatani S, Takeda T, Fukuda K, Tamori A, et al. Prevention of hepatocellular carcinoma in patients

with chronic active hepatitis C and cirrhosis. Lancet 2001; 357:196–197
87. Mazzella G, Accogli E, Sottili S, Festi D, Orsini M, Salzetta A, et al. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. J Hepatol 1996; 24:141–147
88. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non-cirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 1999;131:174–181
89. Shiratori Y, Ito Y, Yokosuka O, Imazeki F, Nakata R, Tanaka N, et al. Antiviral therapy for cirrhotic hepatitis C: association with reduced hepatocellular carcinoma development and improved survival. Ann Intern Med 2005;142:105–114
90. Fattovich G, Giustina G, Degos F, Diodati G, Tremolada F, Nevens F, et al. Effectiveness of interferon alfa on incidence of hepatocellular carcinoma and decompensation in cirrhosis type C. European Concerted Action on Viral Hepatitis (EUROHEP). J Hepatol 1997;27:201–205
91. Papatheodoridis GV, Papadimitropoulos VC, Hadziyannis SJ. Effect of interferon therapy on the development of hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a meta-analysis. Aliment Pharmacol Ther 2001; 15:689–698
92. Camma C, Giunta M, Andreone P, Craxi A. Interferon and prevention of hepatocellular carcinoma in viral cirrhosis: an evidence-based approach. J Hepatol 2001;34:593–602
93. Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 1998; 129:94–99
94. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999; 29:1124–1130
95. Shindo M, Ken A, Okuno T. Varying incidence of cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis C responding differently to interferon therapy. Cancer 1999; 85:1943–1950
96. Shiratori Y, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. Ann Intern Med 2000;132:517–524
97. Yu ML, Lin SM, Chuang WL, Dai CY, Wang JH, Lu SN, et al. A sustained virological response to interferon or interferon/ribavirin reduces hepatocellular carcinoma and improves survival in chronic hepatitis C: a nationwide, multicentre study in Taiwan. Antivir Ther 2006;11:985–994
98. Saito Y, Saito H, Tada S, Nakamoto N, Horikawa H, Kurita S, et al. Effect of long-term interferon therapy for refractory chronic hepatitis c: preventive effect on hepatocarcinogenesis. Hepatogastroenterology 2005;52:1491–1496
99. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Prolonged-interferon therapy reduces hepatocarcinogenesis in aged-patients with chronic hepatitis C. J Med Virol 2007; 79:1095–1102
100. Lok AS, Seeff LB, Morgan TR, Di Bisceglie AM, Sterling RK, Curto TM, et al. Incidence of hepatocellular carcinoma and associated risk factors in hepatitis c-related advanced liver disease. Gastroenterology [Epub 2008]
101. Wogan GN. Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res 1992;52:2114s–2118s
102. Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 2004;127:S72–S78
103. Omer RE, Kuijsten A, Kadaru AM, Kok FJ, Idris MO, El Khidir IM, et al. Population-attributable risk of dietary aflatoxins and hepatitis B virus infection with respect to hepatocellular carcinoma. Nutr Cancer 2004;48:15–21
104. La Vecchia C. Coffee, liver enzymes, cirrhosis and liver cancer. J Hepatol 2005;42:444–446
105. Adami HO, Hsing AW, McLaughlin JK, Trichopoulos D, Hacker D, Ekbom A, et al. Alcoholism and liver cirrhosis in the etiology of primary liver cancer. Int J Cancer 1992;51:898–902
106. Kuper H, Tzonou A, Kaklamani E, Hsieh CC, Lagiou P, Adami HO, et al. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. Int J Cancer 2000;85:498–502
107. La Vecchia C, Negri E, Cavalieri d'Oro L, Franceschi S. Liver cirrhosis and the risk of primary liver cancer. Eur J Cancer Prev 1998;7:315–320
108. Honjo S, Kono S, Coleman MP, Shinchi K, Sakurai Y, Todoroki I, et al. Coffee consumption and serum aminotransferases in middle-aged Japanese men. J Clin Epidemiol 2001;54:823–829
109. Nakanishi N, Nakamura K, Nakajima K, Suzuki K, Tatara K. Coffee consumption and decreased serum gamma-glutamyltransferase: a study of middle-aged Japanese men. Eur J Epidemiol 2000;16:419–423
110. Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. Gastroenterology 2005;128:24–32
111. Poikolainen K, Vartiainen E. Determinants of gamma-glutamyltransferase: positive interaction with alcohol and body mass index, negative association with coffee. Am J Epidemiol 1997;146:1019–1024
112. Honjo S, Kono S, Coleman MP, Shinchi K, Sakurai Y, Todoroki I, et al. Coffee drinking and serum gamma-glutamyltransferase: an extended study of Self-Defense Officials of Japan. Ann Epidemiol 1999;9:325–331
113. Tanaka K, Tokunaga S, Kono S, Tokudome S, Akamatsu T, Moriyama T, et al. Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. Int J Epidemiol 1998;27:438–443
114. Bravi F, Bosetti C, Tavani A, Bagnardi V, Gallus S, Negri E, et al. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. Hepatology 2007;46:430–435
115. Larsson SC, Wolk A. Coffee consumption and risk of liver cancer: a meta-analysis. Gastroenterology 2007;132:1740–1745
116. Tamori A, Habu D, Shiomi S, Kubo S, Nishiguchi S. Potential role of vitamin K(2) as a chemopreventive agent against hepatocellular carcinoma. Hepatol Res 2007;37(Suppl 2):S303–S307
117. Sakai I, Hashimoto S, Yoda M, Hida T, Ohsawa S, Nakajo S, et al. Novel role of vitamin K2: a potent inducer of differentiation of various human myeloid leukemia cell lines. Biochem Biophys Res Commun 1994;205:1305–1310
118. Miyakawa T, Kajiwara Y, Shirahata A, Okamoto K, Itoh H, Ohsato K. Vitamin K contents in liver tissue of hepatocellular carcinoma patients. Jpn J Cancer Res 2000;91:68–74
119. Carr BI, Wang Z, Kar S, Wang M. Prothrombin inhibits hepatocyte DNA synthesis (DNA-S) and expression of the a5 integrin gene. Proc AACR 1995;36:266
120. Kar S, Carr BI. Growth inhibition and protein tyrosine phosphorylation in MCF 7 breast cancer cells by a novel K vitamin. J Cell Physiol 2000;185:386–393
121. Varnum BC, Young C, Elliott G, Garcia A, Bartley TD, Fridell YW, et al. Axl receptor tyrosine kinase stimulated by the

vitamin K-dependent protein encoded by growth-arrest-specific gene 6. Nature 1995;373:623–626
122. Habu D, Shiomi S, Tamori A, Takeda T, Tanaka T, Kubo S, et al. Role of vitamin K2 in the development of hepatocellular carcinoma in women with viral cirrhosis of the liver. JAMA 2004;292:358–361
123. Otsuka M, Kato N, Shao RX, Hoshida Y, Ijichi H, Koike Y, et al. Vitamin K2 inhibits the growth and invasiveness of hepatocellular carcinoma cells via protein kinase A activation. Hepatology 2004;40:243–251
124. Mizuta T, Ozaki I, Eguchi Y, Yasutake T, Kawazoe S, Fujimoto K, et al. The effect of menatetrenone, a vitamin K2 analog, on disease recurrence and survival in patients with hepatocellular carcinoma after curative treatment: a pilot study. Cancer 2006; 106:867–872
125. Hotta N, Ayada M, Sato K, Ishikawa T, Okumura A, Matsumoto E, et al. Effect of vitamin K2 on the recurrence in patients with hepatocellular carcinoma. Hepatogastroenterology 2007;54:2073–2077
126. Bacon BR, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. Gastroenterology 1994;107:1103–1109
127. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116: 1413–1419
128. >Caldwell SH, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999; 29:664–669
129. Cuadrado A, Orive A, Garcia-Suarez C, Dominguez A, Fernandez-Escalante JC, Crespo J, et al. Non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma. Obes Surg 2005; 15:442–446
130. Wang RT, Koretz RL, Yee HF Jr. Is weight reduction an effective therapy for nonalcoholic fatty liver? A systematic review. Am J Med 2003;115:554–559
131. Chan HL, de Silva HJ, Leung NW, Lim SG, Farrell GC. How should we manage patients with non-alcoholic fatty liver disease in 2007? J Gastroenterol Hepatol 2007;22:801–808
132. Chavez-Tapia NC, Barrientos-Gutierrez T, Tellez-Avila FI, Sanchez-Avila F, Montano-Reyes MA, Uribe M. Insulin sensitizers in treatment of nonalcoholic fatty liver disease: systematic review. World J Gastroenterol 2006;12:7826–7831
133. Strohmeyer G, Niederau C, Stremmel W. Survival and causes of death in hemochromatosis. Observations in 163 patients. Ann N Y Acad Sci 1988;526:245–257
134. Hsing AW, McLaughlin JK, Olsen JH, Mellemkjar L, Wacholder S, Fraumeni JF Jr. Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. Int J Cancer 1995;60:160–162
135. Yang Q, McDonnell SM, Khoury MJ, Cono J, Parrish RG. Hemochromatosis-associated mortality in the United States from 1979 to 1992: an analysis of multiple-cause mortality data. Ann Intern Med 1998;129:946–953
136. Borgna-Pignatti C, Vergine G, Lombardo T, Cappellini MD, Cianciulli P, Maggio A, et al. Hepatocellular carcinoma in the thalassaemia syndromes. Br J Haematol 2004;124:114–117
137. Mandishona E, MacPhail AP, Gordeuk VR, Kedda MA, Paterson AC, Rouault TA, et al. Dietary iron overload as a risk factor for hepatocellular carcinoma in black Africans. Hepatology 1998;27:1563–1566
138. Kowdley KV. Iron, hemochromatosis, and hepatocellular carcinoma. Gastroenterology 2004;127:S79–S86
139. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol 2004;130:417–422
140. Yuen MF, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, et al. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. J Hepatol 2009;50:80–88
141. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology 2000;32:842–846
142. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. Hepatology 1995;22:432–438
143. Lok AS, Lai CL. alpha-Fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma. Hepatology 1989;9:110–115
144. Bloomer JR, Waldmann TA, McIntire KR, Klatskin G. Alpha-fetoprotein in non-neoplastic hepatic disorders. JAMA 1975; 233:38–41
145. Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. Gastroenterology 1978;74:856–858
146. Ikoma J, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita N, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. Hepatogastroenterology 2002;49:235–238
147. Kasahara A, Hayashi N, Fusamoto H, Kawada Y, Imai Y, Yamamoto H, et al. Clinical evaluation of plasma des-gamma-carboxy prothrombin as a marker protein of hepatocellular carcinoma in patients with tumors of various sizes. Dig Dis Sci 1993;38:2170–6
148. Maringhini A, Cottone M, Sciarrino E, Marceno MP, La Seta F, Fusco G, et al. Ultrasonography and alpha-fetoprotein in diagnosis of hepatocellular carcinoma in cirrhosis. Dig Dis Sci 1988; 33:47–51
149. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer 1998;82:1643–1648
150. Nomura F, Ishijima M, Horikoshi A, Nakai T, Ohnishi K. Determination of serum des-gamma-carboxy prothrombin levels in patients with small-sized hepatocellular carcinoma: comparison of the conventional enzyme immunoassay and two modified methods. Am J Gastroenterol 1996;91:1380–1383
151. Nomura F, Ishijima M, Kuwa K, Tanaka N, Nakai T, Ohnishi K. Serum des-gamma-carboxy prothrombin levels determined by a new generation of sensitive immunoassays in patients with small-sized hepatocellular carcinoma. Am J Gastroenterol 1999;94:650–654
152. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, et al. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. J Gastroenterol Hepatol 2001;16:1378–1383
153. Suehiro T, Sugimachi K, Matsumata T, Itasaka H, Taketomi A, Maeda T. Protein induced by vitamin K absence or antagonist II as a prognostic marker in hepatocellular carcinoma. Comparison with alpha-fetoprotein. Cancer 1994;73:2464–2471
154. Tanabe Y, Ohnishi K, Nomura F, Iida S. Plasma abnormal prothrombin levels in patients with small hepatocellular carcinoma. Am J Gastroenterol 1988;83:1386–1389

155. Gebo KA, Chander G, Jenckes MW, Ghanem KG, Herlong HF, Torbenson MS, et al. Screening tests for hepatocellular carcinoma in patients with chronic hepatitis C: a systematic review. Hepatology 2002;36:S84–S92
156. Cottone M, Turri M, Caltagirone M, Parisi P, Orlando A, Fiorentino G, et al. Screening for hepatocellular carcinoma in patients with Child's A cirrhosis: an 8-year prospective study by ultrasound and alpha-fetoprotein. J Hepatol 1994;21:1029–1034
157. Pateron D, Ganne N, Trinchet JC, Aurousseau MH, Mal F, Meicler C, et al. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. J Hepatol 1994;20:65–71
158. Irwig L, Tosteson AN, Gatsonis C, Lau J, Colditz G, Chalmers TC, et al. Guidelines for meta-analyses evaluating diagnostic tests. Ann Intern Med 1994;120:667–676
159. Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, Omata M. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. Hepatol Int 2008;2:17–30
160. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. Hepatology 2003;37:1114–1121
161. Cui R, He J, Zhang F, Wang B, Ding H, Shen H, et al. Diagnostic value of protein induced by vitamin K absence (PIVKAII) and hepatoma-specific band of serum gamma-glutamyl transferase (GGTII) as hepatocellular carcinoma markers complementary to alpha-fetoprotein. Br J Cancer 2003;88:1878–1882
162. Wang CS, Lin CL, Lee HC, Chen KY, Chiang MF, Chen HS, et al. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. World J Gastroenterol 2005;11:6115–6119
163. Sterling RK, Jeffers L, Gordon F, Sherman M, Venook AP, Reddy KR, et al. Clinical utility of AFP-L 3% measurement in North American patients with HCV-related cirrhosis. Am J Gastroenterol 2007;102:2196–2205
164. Capurro M, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. Gastroenterology 2003;125:89–97
165. Hippo Y, Watanabe K, Watanabe A, Midorikawa Y, Yamamoto S, Ihara S, et al. Identification of soluble NH_2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. Cancer Res 2004;64:2418–2423
166. Nguyen MH, Garcia RT, Simpson PW, Wright TL, Keeffe EB. Racial differences in effectiveness of alpha-fetoprotein for diagnosis of hepatocellular carcinoma in hepatitis C virus cirrhosis. Hepatology 2002;36:410–417
167. Soresi M, Magliarisi C, Campagna P, Leto G, Bonfissuto G, Riili A, et al. Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. Anticancer Res 2003;23:1747–1753
168. Arrieta O, Cacho B, Morales-Espinosa D, Ruelas-Villavicencio A, Flores-Estrada D, Hernandez-Pedro N. The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. BMC Cancer 2007;7:28
169. Paul SB, Gulati MS, Sreenivas V, Madan K, Gupta AK, Mukhopadhyay S. Evaluating patients with cirrhosis for hepatocellular carcinoma: value of clinical symptomatology, imaging and alpha-fetoprotein. Oncology 2007;72(Suppl 1):117–123
170. Kew MC. Alpha-fetoprotein. In Read AE, editor. Modern Trends in Gastroenterology, vol. 5. London: Butterworths; 1975. p 91
171. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med 1984;310:1427–1431
172. Koike Y, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M. Yet al. Des-gamma-carboxy prothrombin as a useful predisposing factor for the development of portal venous invasion in patients with hepatocellular carcinoma: a prospective analysis of 227 patients. Cancer 2001;91:561–569
173. Imamura H, Matsuyama Y, Miyagawa Y, Ishida K, Shimada R, Miyagawa S, et al. Prognostic significance of anatomical resection and des-gamma-carboxy prothrombin in patients with hepatocellular carcinoma. Br J Surg 1999;86:1032–1038
174. Sato Y, Nakata K, Kato Y, Shima M, Ishii N, Koji T, et al. Early recognition of hepatocellular carcinoma based on altered profiles of alpha-fetoprotein. N Engl J Med 1993;328:1802–1806
175. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. Cancer Res 1993;53:5419–5423
176. Aoyagi Y, Isemura M, Yosizawa Z, Suzuki Y, Sekine C, Ono T, et al. Fucosylation of serum alpha-fetoprotein in patients with primary hepatocellular carcinoma. Biochim Biophys Acta 1985;830:217–223
177. Hsu HC, Cheng W, Lai PL. Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. Cancer Res 1997;57:5179–5184
178. Zhu ZW, Friess H, Wang L, Abou-Shady M, Zimmermann A, Lander AD, et al. Enhanced glypican-3 expression differentiates the majority of hepatocellular carcinomas from benign hepatic disorders. Gut 2001;48:558–564
179. Sassa T, Kumada T, Nakano S, Uematsu T. Clinical utility of simultaneous measurement of serum high-sensitivity des-gamma-carboxy prothrombin and Lens culinaris agglutinin A-reactive alpha-fetoprotein in patients with small hepatocellular carcinoma. Eur J Gastroenterol Hepatol 1999;11:1387–1392
180. Tanaka S, Kitamura T, Fujita M, Nakanishi K, Okuda S. Color Doppler flow imaging of liver tumors. AJR Am J Roentgenol 1990;154:509–514
181. Tanaka S, Kitamra T, Fujita M, Kasugai H, Inoue A, Ishiguro S. Small hepatocellular carcinoma: differentiation from adenomatous hyperplastic nodule with color Doppler flow imaging. Radiology 1992;182:161–165
182. Rubin JM, Bude RO, Carson PL, Bree RL, Adler RS. Power Doppler US: a potentially useful alternative to mean frequency-based color Doppler US. Radiology 1994;190:853–856
183. Koito K, Namieno T, Morita K. Differential diagnosis of small hepatocellular carcinoma and adenomatous hyperplasia with power Doppler sonography. AJR Am J Roentgenol 1998;170:157–161
184. Fujimoto M, Moriyasu F, Nishikawa K, Nada T, Okuma M. Color Doppler sonography of hepatic tumors with a galactose-based contrast agent: correlation with angiographic findings. AJR Am J Roentgenol 1994;163:1099–1104
185. Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K. Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual-display mode. Radiology 2001;220:349–356
186. Kudo M, Tomita S, Tochio H, Mimura J, Okabe Y, Kashida H, et al. Sonography with intraarterial infusion of carbon dioxide microbubbles (sonographic angiography): value in differential diagnosis of hepatic tumors. AJR Am J Roentgenol 1992;158:65–74
187. Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H. Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. Ultrasound Med Biol 2007;33:318–325
188. D'Onofrio M, Martone E, Faccioli N, Zamboni G, Malago R, Mucelli RP. Focal liver lesions: sinusoidal phase of CEUS. Abdom Imaging 2006;31:529–536

189. Hatanaka K, Kudo M, Minami Y, Ueda T, Tatsumi C, Kitai S, et al. Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. Intervirology 2008;51(Suppl 1):61–9
190. Chalasani N, Said A, Ness R, Hoen H, Lumeng L. Screening for hepatocellular carcinoma in patients with cirrhosis in the United States: results of a national survey. Am J Gastroenterol 1999;94:2224–2229
191. Collier J, Sherman M. Screening for hepatocellular carcinoma. Hepatology 1998;27:273–278
192. Kobayashi K, Sugimoto T, Makino H, Kumagai M, Unoura M, Tanaka N, et al. Screening methods for early detection of hepatocellular carcinoma. Hepatology 1985;5:1100–1105
193. Federle MP. Use of radiologic techniques to screen for hepatocellular carcinoma. J Clin Gastroenterol 2002;35:S92–S100
194. Bruix J, Sherman M. Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236
195. Saab S, Ly D, Nieto J, Kanwal F, Lu D, Raman S, et al. Hepatocellular carcinoma screening in patients waiting for liver transplantation: a decision analytic model. Liver Transpl 2003; 9:672–681
196. Burrel M, Llovet JM, Ayuso C, Iglesias C, Sala M, Miquel R, et al. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. Hepatology 2003;38:1034–1042
197. Van Thiel DH, Yong S, Li SD, Kennedy M, Brems J. The development of de novo hepatocellular carcinoma in patients on a liver transplant list: frequency, size, and assessment of current screening methods. Liver Transpl 2004;10:631–637
198. El-Serag HB, Marrero JA, Rudolph L, Reddy KR. Diagnosis and treatment of hepatocellular carcinoma. Gastroenterology 2008;134:1752–1763
199. Arguedas MR, Chen VK, Eloubeidi MA, Fallon MB. Screening for hepatocellular carcinoma in patients with hepatitis C cirrhosis: a cost-utility analysis. Am J Gastroenterol 2003;98: 679–690
200. Kim TK, Jang HJ, Wilson SR. Imaging diagnosis of hepatocellular carcinoma with differentiation from other pathology. Clin Liver Dis 2005;9:253–279
201. Matsui O. Imaging of multistep human hepatocarcinogenesis by CT during intra-arterial contrast injection. Intervirology 2004;47:271–276
202. Marrero JA, Hussain HK, Nghiem HV, Umar R, Fontana RJ, Lok AS. Improving the prediction of hepatocellular carcinoma in cirrhotic patients with an arterially-enhancing liver mass. Liver Transpl 2005;11:281–289
203. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D, et al. Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. Am J Gastroenterol 2006; 101:513–523
204. Kim SH, Choi BI, Lee JY, Kim SJ, So YH, Eun HW. Diagnostic accuracy of multi-/single-detector row CT and contrast-enhanced MRI in the detection of hepatocellular carcinomas meeting the Milan criteria before liver transplantation. Intervirology 2008;51(Suppl 1):52–60
205. Willatt JM, Hussain HK, Adusumilli S, Marrero JA. MR Imaging of hepatocellular carcinoma in the cirrhotic liver: challenges and controversies. Radiology 2008;247:311–330
206. Ebara M, Ohto M, Watanabe Y, Kimura K, Saisho H, Tsuchiya Y, et al. Diagnosis of small hepatocellular carcinoma: correlation of MR imaging and tumor histologic studies. Radiology 1986;159:371–377
207. Rode A, Bancel B, Douek P, Chevallier M, Vilgrain V, Picaud G, et al. Small nodule detection in cirrhotic livers: evaluation with US, spiral CT, and MRI and correlation with pathologic examination of explanted liver. J Comput Assist Tomogr 2001;25:327–336
208. Krinsky GA, Lee VS, Theise ND, Weinreb JC, Rofsky NM, Diflo T, et al. Hepatocellular carcinoma and dysplastic nodules in patients with cirrhosis: prospective diagnosis with MR imaging and explantation correlation. Radiology 2001;219:445–454
209. Baron RL, Peterson MS. From the RSNA refresher courses: screening the cirrhotic liver for hepatocellular carcinoma with CT and MR imaging: opportunities and pitfalls. Radiographics 2001;21:S117–S132
210. Holland AE, Hecht EM, Hahn WY, Kim DC, Babb JS, Lee VS, et al. Importance of small (≤20-mm) enhancing lesions seen only during the hepatic arterial phase at MR imaging of the cirrhotic liver: evaluation and comparison with whole explanted liver. Radiology 2005;237:938–944
211. Brancatelli G, Baron RL, Peterson MS, Marsh W. Helical CT screening for hepatocellular carcinoma in patients with cirrhosis: frequency and causes of false-positive interpretation. AJR Am J Roentgenol 2003;180:1007–1014
212. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907–1917
213. Arii S, Yamaoka Y, Futagawa S, Inoue K, Kobayashi K, Kojiro M, et al. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. Hepatology 2000;32:1224–1229
214. Kim YK, Kwak HS, Kim CS, Chung GH, Han YM, Lee JM. Hepatocellular carcinoma in patients with chronic liver disease: comparison of SPIO-enhanced MR imaging and 16-detector row CT. Radiology 2006;238:531–541
215. Bhartia B, Ward J, Guthrie JA, Robinson PJ. Hepatocellular carcinoma in cirrhotic livers: double-contrast thin-section MR imaging with pathologic correlation of explanted tissue. AJR Am J Roentgenol 2003;180:577–584
216. Ward J, Guthrie JA, Scott DJ, Atchley J, Wilson D, Davies MH, et al. Hepatocellular carcinoma in the cirrhotic liver: double-contrast MR imaging for diagnosis. Radiology 2000;216:154–162
217. Yoo HJ, Lee JM, Lee MW, Kim SJ, Lee JY, Han JK. et al. Hepatocellular carcinoma in cirrhotic liver: double-contrast-enhanced, high-resolution 3.0T-MR imaging with pathologic correlation. Invest Radiol 2008;43:538–546
218. Mori K, Yoshioka H, Itai Y, Okamoto Y, Mori H, Takahashi N, et al. Arterioportal shunts in cirrhotic patients: evaluation of the difference between tumorous and nontumorous arterioportal shunts on MR imaging with superparamagnetic iron oxide. AJR Am J Roentgenol 2000;175:1659–1664
219. Choi SH, Lee JM, Yu NC, Suh KS, Jang JJ, Kim SH, et al. Hepatocellular carcinoma in liver transplantation candidates: detection with gadobenate dimeglumine-enhanced MRI. AJR Am J Roentgenol 2008;191:529–536
220. Sakamoto M, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. Hum Pathol 1991;22:172–178
221. Sakamoto M, Hirohashi S. Natural history and prognosis of adenomatous hyperplasia and early hepatocellular carcinoma: multi-institutional analysis of 53 nodules followed up for more than 6 months and 141 patients with single early hepatocellular carcinoma treated by surgical resection or percutaneous ethanol injection. Jpn J Clin Oncol 1998;28:604–608
222. Kojiro M. Focus on dysplastic nodules and early hepatocellular carcinoma: an Eastern point of view. Liver Transpl 2004; 10:S3–S8
223. Bolondi L, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, et al. Characterization of small nodules in cirrhosis by

assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. Hepatology 2005;42:27–34
224. Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(Suppl 1):2–15
225. Imai Y, Murakami T, Yoshida S, Nishikawa M, Ohsawa M, Tokunaga K, et al. Superparamagnetic iron oxide-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading. Hepatology 2000;32:205–212
226. Lim JH, Choi D, Cho SK, Kim SH, Lee WJ, Lim HK, et al. Conspicuity of hepatocellular nodular lesions in cirrhotic livers at ferumoxides-enhanced MR imaging: importance of Kupffer cell number. Radiology 2001;220:669–676
227. Heiken JP, Weyman PJ, Lee JK, Balfe DM, Picus D, Brunt EM, et al. Detection of focal hepatic masses: prospective evaluation with CT, delayed CT, CT during arterial portography, and MR imaging. Radiology 1989;171:47–51
228. Kanematsu M, Hoshi H, Imaeda T, Murakami T, Inaba Y, Yokoyama R, et al. Detection and characterization of hepatic tumors: value of combined helical CT hepatic arteriography and CT during arterial portography. AJR Am J Roentgenol 1997; 168:1193–1198
229. Kanematsu M, Hoshi H, Murakami T, Inaba Y, Kim T, Yamada T, et al. Detection of hepatocellular carcinoma in patients with cirrhosis: MR imaging versus angiographically assisted helical CT. AJR Am J Roentgenol 1997;169:1507–1515
230. Choi D, Kim S, Lim J, Lee W, Jang H, Lee S, et al. Preoperative detection of hepatocellular carcinoma: ferumoxides-enhanced MR imaging versus combined helical CT during arterial portography and CT hepatic arteriography. AJR Am J Roentgenol 2001;176:475–482
231. Yoon KT, Kim JK, Kim do Y, Ahn SH, Lee JD, Yun M, et al. Role of ^{18}F-fluorodeoxyglucose positron emission tomography in detecting extrahepatic metastasis in pretreatment staging of hepatocellular carcinoma. Oncology 2007;72(Suppl 1):104–110
232. Yang SH, Suh KS, Lee HW, Cho EH, Cho JY, Cho YB, et al. The role of (18)F-FDG-PET imaging for the selection of liver transplantation candidates among hepatocellular carcinoma patients. Liver Transpl 2006;12:1655–1660
233. Shiomi S, Nishiguchi S, Ishizu H, Iwata Y, Sasaki N, Tamori A, et al. Usefulness of positron emission tomography with fluorine-18-fluorodeoxyglucose for predicting outcome in patients with hepatocellular carcinoma. Am J Gastroenterol 2001;96:1877–1880
234. Khan MA, Combs CS, Brunt EM, Lowe VJ, Wolverson MK, Solomon H, et al. Positron emission tomography scanning in the evaluation of hepatocellular carcinoma. J Hepatol 2000; 32:792–797
235. Trojan J, Schroeder O, Raedle J, Baum RP, Herrmann G, Jacobi V, et al. Fluorine-18 FDG positron emission tomography for imaging of hepatocellular carcinoma. Am J Gastroenterol 1999;94:3314–3319
236. Sugiyama M, Sakahara H, Torizuka T, Kanno T, Nakamura F, Futatsubashi M, et al. ^{18}F-FDG PET in the detection of extrahepatic metastases from hepatocellular carcinoma. J Gastroenterol 2004;39:961–968
237. Tanimoto A, Wakabayashi G, Shinmoto H, Nakatsuka S, Okuda S, Kuribayashi S. Superparamagnetic iron oxide-enhanced MR imaging for focal hepatic lesions: a comparison with CT during arterioportography plus CT during hepatic arteriography. J Gastroenterol 2005;40:371–380
238. Kojiro M. Pathology of hepatocellular carcinoma. In Okuda K, Tabor E, editors. Liver Cancer. New York: Churchill Livingstone; 1997. p. 165–187
239. Tajima T, Honda H, Taguchi K, Asayama Y, Kuroiwa T, Yoshimitsu K, et al. Sequential hemodynamic change in hepatocellular carcinoma and dysplastic nodules: CT angiography and pathologic correlation. AJR Am J Roentgenol 2002; 178:885–897
240. Hayashi M, Matsui O, Ueda K, Kawamori Y, Gabata T, Kadoya M. Progression to hypervascular hepatocellular carcinoma: correlation with intranodular blood supply evaluated with CT during intraarterial injection of contrast material. Radiology 2002;225:143–149
241. Kudo M. Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. Semin Liver Dis 1999; 19:297–309
242. Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K. Contrast-enhanced subtraction harmonic sonography for evaluating treatment response in patients with hepatocellular carcinoma. AJR Am J Roentgenol 2001;176:661–666
243. Wen YL, Kudo M, Zheng RQ, Ding H, Zhou P, Minami Y, et al. Characterization of hepatic tumors: value of contrast-enhanced coded phase-inversion harmonic angio. AJR Am J Roentgenol 2004;182:1019–1026
244. Asahina Y, Izumi N, Uchihara M, Noguchi O, Ueda K, Inoue K, et al. Assessment of Kupffer cells by ferumoxides-enhanced MR imaging is beneficial for diagnosis of hepatocellular carcinoma: comparison of pathological diagnosis and perfusion patterns assessed by CT hepatic arteriography and CT arterioportography. Hepatol Res 2003;27:196–204
245. Akriviadis EA, Llovet JM, Efremidis SC, Shouval D, Canelo R, Ringe B, et al. Hepatocellular carcinoma. Br J Surg 1998;85: 1319–1331
246. Bruix J, Llovet JM. Prognostic prediction and treatment strategy in hepatocellular carcinoma. Hepatology 2002;35:519–524
247. Regimbeau JM, Farges O, Shen BY, Sauvanet A, Belghiti J. Is surgery for large hepatocellular carcinoma justified? J Hepatol 1999;31:1062–1068
248. Poon RT, Fan ST, Wong J. Selection criteria for hepatic resection in patients with large hepatocellular carcinoma larger than 10 cm in diameter. J Am Coll Surg 2002;194:592–602
249. Ng KK, Vauthey JN, Pawlik TM, Lauwers GY, Regimbeau JM, Belghiti J, et al. Is hepatic resection for large or multinodular hepatocellular carcinoma justified? Results from a multi-institutional database. Ann Surg Oncol 2005;12:364–373
250. Pawlik TM, Poon RT, Abdalla EK, Ikai I, Nagorney DM, Belghiti J, et al. Hepatectomy for hepatocellular carcinoma with major portal or hepatic vein invasion: results of a multicenter study. Surgery 2005;137:403–410
251. Liu CL, Fan ST, Lo CM, Ng IO, Poon RT, Wong J. Hepatic resection for bilobar hepatocellular carcinoma: is it justified? Arch Surg 2003;138:100–104
252. Choi D, Lim HK, Joh JW, Kim SJ, Kim MJ, Rhim H, et al. Combined hepatectomy and radiofrequency ablation for multifocal hepatocellular carcinomas: long-term follow-up results and prognostic factors. Ann Surg Oncol 2007;14:3510–3518
253. Torzilli G, Makuuchi M, Inoue K, Takayama T, Sakamoto Y, Sugawara Y, et al. No-mortality liver resection for hepatocellular carcinoma in cirrhotic and noncirrhotic patients: is there a way? A prospective analysis of our approach. Arch Surg 1999; 134:984–992
254. Poon RT, Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, et al. Improving perioperative outcome expands the role of hepatectomy in management of benign and malignant hepatobiliary diseases: analysis of 1222 consecutive patients from a prospective database. Ann Surg 2004;240:698–708 (discussion 708–710)
255. Asiyanbola B, Chang D, Gleisner AL, Nathan H, Choti MA, Schulick RD, et al. Operative mortality after hepatic resection: are literature-based rates broadly applicable? J Gastrointest Surg 2008;12:842–851

256. Polignano FM, Quyn AJ, de Figueiredo RS, Henderson NA, Kulli C, Tait IS. Laparoscopic versus open liver segmentectomy: prospective, case-matched, intention-to-treat analysis of clinical outcomes and cost effectiveness. Surg Endosc 2008;22:2564–2570
257. Grazi GL, Ercolani G, Pierangeli F, Del Gaudio M, Cescon M, Cavallari A, et al. Improved results of liver resection for hepatocellular carcinoma on cirrhosis give the procedure added value. Ann Surg 2001;234:71–78
258. Poon RT, Fan ST, Lo CM, Ng IO, Liu CL, Lam CM, et al. Improving survival results after resection of hepatocellular carcinoma: a prospective study of 377 patients over 10 years. Ann Surg 2001;234:63–70
259. Capussotti L, Muratore A, Amisano M, Polastri R, Bouzari H, Massucco P. Liver resection for hepatocellular carcinoma on cirrhosis: analysis of mortality, morbidity and survival—a European single center experience. Eur J Surg Oncol 2005; 31:986–993
260. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Difference in tumor invasiveness in cirrhotic patients with hepatocellular carcinoma fulfilling the Milan criteria treated by resection and transplantation: impact on long-term survival. Ann Surg 2007;245:51–58
261. Ishii H, Furuse J, Kinoshita T, Konishi M, Nakagohri T, Takahashi S, et al. Hepatectomy for hepatocellular carcinoma patients who meet the Milan criteria. Hepatogastroenterology 2008;55:621–626
262. Poon RT, Fan ST, Ng IO, Wong J. Significance of resection margin in hepatectomy for hepatocellular carcinoma: a critical reappraisal. Ann Surg 2000;231:544–551
263. Yamazaki S, Takayama T. Surgical treatment of hepatocellular carcinoma: evidence-based outcomes. World J Gastroenterol 2008;14:685–692
264. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Intrahepatic recurrence after curative resection of hepatocellular carcinoma: long-term results of treatment and prognostic factors. Ann Surg 1999;229:216–222
265. Penn I. Hepatic transplantation for primary and metastatic cancers of the liver. Surgery 1991;110:726–734 (discussion 734–735)
266. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, et al. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. N Engl J Med 1996;334:693–699
267. Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A, et al. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. Hepatology 2001;33:1394–1403
268. Fernandez JA, Robles R, Marin C, Sanchez-Bueno F, Ramirez P, Pons JA, et al. Can we expand the indications for liver transplantation among hepatocellular carcinoma patients with increased tumor size? Transplant Proc 2003;35:1818–1820
269. Otto G, Heuschen U, Hofmann WJ, Krumm G, Hinz U, Herfarth C. Survival and recurrence after liver transplantation versus liver resection for hepatocellular carcinoma: a retrospective analysis. Ann Surg 1998;227:424–432
270. Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. Hepatology 1999;30:1434–1440
271. Figueras J, Ibanez L, Ramos E, Jaurrieta E, Ortiz-de-Urbina J, Pardo F, et al. Selection criteria for liver transplantation in early-stage hepatocellular carcinoma with cirrhosis: results of a multicenter study. Liver Transpl 2001;7:877–883
272. Jonas S, Bechstein WO, Steinmuller T, Herrmann M, Radke C, Berg T, et al. Vascular invasion and histopathologic grading determine outcome after liver transplantation for hepatocellular carcinoma in cirrhosis. Hepatology 2001;33:1080–1086
273. Klintmalm GB. Liver transplantation for hepatocellular carcinoma: a registry report of the impact of tumor characteristics on outcome. Ann Surg 1998;228:479–490
274. Belghiti J, Carr BI, Greig PD, Lencioni R, Poon RT. Treatment before liver transplantation for HCC. Ann Surg Oncol 2008; 15:993–1000
275. Trotter JF, Wachs M, Everson GT, Kam I. Adult-to-adult transplantation of the right hepatic lobe from a living donor. N Engl J Med 2002;346:1074–1082
276. Kulik L, Abecassis M. Living donor liver transplantation for hepatocellular carcinoma. Gastroenterology 2004;127:S277–S282
277. Poon RT. Optimal initial treatment for early hepatocellular carcinoma in patients with preserved liver function: transplantation or resection? Ann Surg Oncol 2007;14:541–547
278. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. Ann Surg 2002;235: 373–382
279. Belghiti J, Cortes A, Abdalla EK, Regimbeau JM, Prakash K, Durand F, et al. Resection prior to liver transplantation for hepatocellular carcinoma. Ann Surg 2003;238:885–892 (discussion 892–893)
280. Sugiura N, Takara K, Ohto M, Okuda K, Hirokawa N. Percutaneous intratumoral injection of ethanol under ultrasound imaging for treatment of small hepatocellular carcinoma. Acta Hepatol Jpn 1983;24:920
281. Livraghi T, Festi D, Monti F, Salmi A, Vettori C. US-guided percutaneous alcohol injection of small hepatic and abdominal tumors. Radiology 1986;161:309–312
282. Shiina S, Yasuda H, Muto H, Tagawa K, Unuma T, Ibukuro K, et al. Percutaneous ethanol injection in the treatment of liver neoplasms. AJR Am J Roentgenol 1987;149:949–952
283. Seki T, Wakabayashi M, Nakagawa T, Itho T, Shiro T, Kunieda K, et al. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. Cancer 1994;74:817–825
284. Rossi S, Di Stasi M, Buscarini E, Cavanna L, Quaretti P, Squassante E, et al. Percutaneous radiofrequency interstitial thermal ablation in the treatment of small hepatocellular carcinoma. Cancer J Sci Am 1995;1:73–81
285. Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 1999;210:655–661
286. Shiina S, Teratani T, Obi S, Hamamura K, Koike Y, Omata M. Nonsurgical treatment of hepatocellular carcinoma: from percutaneous ethanol injection therapy and percutaneous microwave coagulation therapy to radiofrequency ablation. Oncology 2002;62(Suppl 1):64–68
287. Shiina S, Tagawa K, Niwa Y, Unuma T, Komatsu Y, Yoshiura K, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. AJR Am J Roentgenol 1993;160:1023–1028
288. Ebara M, Okabe S, Kita K, Sugiura N, Fukuda H, Yoshikawa M, et al. Percutaneous ethanol injection for small hepatocellular carcinoma: therapeutic efficacy based on 20-year observation. J Hepatol 2005;43:458–464
289. Sung YM, Choi D, Lim HK, Lee WJ, Kim SH, Kim MJ, et al. Long-term results of percutaneous ethanol injection for the treatment of hepatocellular carcinoma in Korea. Korean J Radiol 2006;7:187–192
290. Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, et al. Report of the 17th nationwide follow-up survey of primary liver cancer in Japan. Hepatol Res 2007;37:676–691

291. Livraghi T, Giorgio A, Marin G, Salmi A, de Sio I, Bolondi L, et al. Hepatocellular carcinoma and cirrhosis in 746 patients: long-term results of percutaneous ethanol injection. Radiology 1995;197:101–108
292. Ishii H, Okada S, Nose H, Okusaka T, Yoshimori M, Takayama T, et al. Local recurrence of hepatocellular carcinoma after percutaneous ethanol injection. Cancer 1996;77:1792–1796
293. Di Stasi M, Buscarini L, Livraghi T, Giorgio A, Salmi A, De Sio I, et al. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma. A multicenter survey of evaluation practices and complication rates. Scand J Gastroenterol 1997; 32:1168–1173
294. Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235–240
295. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma ≤4 cm. Gastroenterology 2004;127: 1714–1723
296. Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, et al. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122–130
297. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. Gut 2005;54:1151–1156
298. Shibata T, Iimuro Y, Yamamoto Y, Maetani Y, Ametani F, Itoh K, et al. Small hepatocellular carcinoma: comparison of radiofrequency ablation and percutaneous microwave coagulation therapy. Radiology 2002;223:331–337
299. Machi J, Bueno RS, Wong LL. Long-term follow-up outcome of patients undergoing radiofrequency ablation for unresectable hepatocellular carcinoma. World J Surg 2005;29:1364–1373
300. Lencioni R, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, et al. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. Radiology 2005;234:961–967
301. Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, et al. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103:1201–1209
302. Cabassa P, Donato F, Simeone F, Grazioli L, Romanini L. Radiofrequency ablation of hepatocellular carcinoma: long-term experience with expandable needle electrodes. AJR Am J Roentgenol 2006;186:S316–S321
303. Livraghi T, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, et al. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: is resection still the treatment of choice? Hepatology 2008;47:82–89
304. Yan K, Chen MH, Yang W, Wang YB, Gao W, Hao CY, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term outcome and prognostic factors. Eur J Radiol 2008;67:336–347
305. Kasugai H, Osaki Y, Oka H, Kudo M, Seki T. Severe complications of radiofrequency ablation therapy for hepatocellular carcinoma: an analysis of 3,891 ablations in 2,614 patients. Oncology 2007;72(Suppl 1):72–75
306. Cheng BQ, Jia CQ, Liu CT, Fan W, Wang QL, Zhang ZL, et al. Chemoembolization combined with radiofrequency ablation for patients with hepatocellular carcinoma larger than 3 cm: a randomized controlled trial. JAMA 2008;299:1669–1677
307. Kobayashi M, Ikeda K, Kawamura Y, Hosaka T, Sezaki H, Yatsuji H, et al. Randomized controlled trial for the efficacy of hepatic arterial occlusion during radiofrequency ablation for small hepatocellular carcinoma—direct ablative effects and a long-term outcome. Liver Int 2007;27:353–359
308. Huang GT, Lee PH, Tsang YM, Lai MY, Yang PM, Hu RH, et al. Percutaneous ethanol injection versus surgical resection for the treatment of small hepatocellular carcinoma: a prospective study. Ann Surg 2005;242:36–42
309. Chen MS, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, et al. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. Ann Surg 2006;243:321–328
310. Castells A, Bruix J, Bru C, Fuster J, Vilana R, Navasa M, et al. Treatment of small hepatocellular carcinoma in cirrhotic patients: a cohort study comparing surgical resection and percutaneous ethanol injection. Hepatology 1993;18:1121–1126
311. Livraghi T, Bolondi L, Buscarini L, Cottone M, Mazziotti A, Morabito A, et al. No treatment, resection and ethanol injection in hepatocellular carcinoma: a retrospective analysis of survival in 391 patients with cirrhosis. Italian Cooperative HCC Study Group. J Hepatol 1995;22:522–526
312. Ryu M, Shimamura Y, Kinoshita T, Konishi M, Kawano N, Iwasaki M, et al. Therapeutic results of resection, transcatheter arterial embolization and percutaneous transhepatic ethanol injection in 3225 patients with hepatocellular carcinoma: a retrospective multicenter study. Jpn J Clin Oncol 1997;27:251–257
313. Nakashima T, Kojiro M. Pathologic characteristics of hepatocellular carcinoma. Semin Liver Dis 1986;6:259–266
314. Park YN, Yang CP, Fernandez GJ, Cubukcu O, Thung SN, Theise ND. Neoangiogenesis and sinusoidal "capillarization" in dysplastic nodules of the liver. Am J Surg Pathol 1998;22:656–662
315. Goseki N, Nosaka T, Endo M, Koike M. Nourishment of hepatocellular carcinoma cells through the portal blood flow with and without transcatheter arterial embolization. Cancer 1995;76:736–742
316. Bruix J, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. Gastroenterology 2004;127:S179–S188
317. Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. Radiology 1983;148:397–401
318. Ramsey DE, Kernagis LY, Soulen MC, Geschwind JF. Chemoembolization of hepatocellular carcinoma. J Vasc Interv Radiol 2002;13:S211–S221
319. Kruskal JB, Hlatky L, Hahnfeldt P, Teramoto K, Stokes KR, Clouse ME. In vivo and in vitro analysis of the effectiveness of doxorubicin combined with temporary arterial occlusion in liver tumors. J Vasc Interv Radiol 1993;4:741–747
320. Liapi E, Geschwind JF. Transcatheter and ablative therapeutic approaches for solid malignancies. J Clin Oncol 2007;25:978–986
321. Marelli L, Stigliano R, Triantos C, Senzolo M, Cholongitas E, Davies N, et al. Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6–25
322. Vogl TJ, Naguib NN, Nour-Eldin NE, Rao P, Emami AH, Zangos S, et al. Review on transarterial chemoembolization in hepatocellular carcinoma: palliative, combined, neoadjuvant, bridging, and symptomatic indications. Eur J Radiol 2009;72:505–516
323. Makuuchi M, Sukigara M, Mori T, Kobayashi J, Yamazaki S, Hasegawa H, et al. Bile duct necrosis: complication of transcatheter hepatic arterial embolization. Radiology 1985; 156:331–334
324. Varela M, Real MI, Burrel M, Forner A, Sala M, Brunet M, et al. Chemoembolization of hepatocellular carcinoma with drug eluting beads: efficacy and doxorubicin pharmacokinetics. J Hepatol 2007;46:474–481

325. Iwamoto S, Sanefuji H, Okuda K. Angiographic subsegmentectomy for the treatment of patients with small hepatocellular carcinoma. Cancer 2003;97:1051–1056
326. Matsui O, Kadoya M, Yoshikawa J, Gabata T, Arai K, Demachi H, et al. Small hepatocellular carcinoma: treatment with subsegmental transcatheter arterial embolization. Radiology 1993; 188:79–83
327. Miyayama S, Matsui O, Yamashiro M, Ryu Y, Kaito K, Ozaki K, et al. Ultraselective transcatheter arterial chemoembolization with a 2-F tip microcatheter for small hepatocellular carcinomas: relationship between local tumor recurrence and visualization of the portal vein with iodized oil. J Vasc Interv Radiol 2007;18:365–376
328. Nakai M, Sato M, Kawai N, Minamiguchi H, Masuda M, Tanihata H, et al. Hepatocellular carcinoma: involvement of the internal mammary artery. Radiology 2001;219:147–152
329. Chung JW, Kim HC, Yoon JH, Lee HS, Jae HJ, Lee W, et al. Transcatheter arterial chemoembolization of hepatocellular carcinoma: prevalence and causative factors of extrahepatic collateral arteries in 479 patients. Korean J Radiol 2006;7:257–266
330. Miyayama S, Matsui O, Taki K, Minami T, Ryu Y, Ito C, et al. Extrahepatic blood supply to hepatocellular carcinoma: angiographic demonstration and transcatheter arterial chemoembolization. Cardiovasc Intervent Radiol 2006;29:39–48
331. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology 2002;35:1164–1171
332. Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet 2002; 359:1734–1739
333. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology 2003;37:429–442
334. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK. et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL Conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430
335. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236
336. Yao FY, Hirose R, LaBerge JM, Davern TJ III, Bass NM, Kerlan RK Jr, et al. A prospective study on downstaging of hepatocellular carcinoma prior to liver transplantation. Liver Transpl 2005;11:1505–1514
337. Hanje AJ, Yao FY. Current approach to down-staging of hepatocellular carcinoma prior to liver transplantation. Curr Opin Organ Transplant 2008;13:234–240
338. Yao FY, Kerlan RK Jr, Hirose R, Davern TJ III, Bass NM, Feng S, et al. Excellent outcome following down-staging of hepatocellular carcinoma prior to liver transplantation: an intention-to-treat analysis. Hepatology 2008;48:819–827
339. Staunton M, Dodd JD, McCormick PA, Malone DE. Finding evidence-based answers to practical questions in radiology: which patients with inoperable hepatocellular carcinoma will survive longer after transarterial chemoembolization? Radiology 2005;237:404–413
340. Chan AO, Yuen MF, Hui CK, Tso WK, Lai CL. A prospective study regarding the complications of transcatheter intraarterial lipiodol chemoembolization in patients with hepatocellular carcinoma. Cancer 2002;94:1747–1752
341. Chung JW, Park JH, Han JK, Choi BI, Han MC, Lee HS, et al. Hepatic tumors: predisposing factors for complications of transcatheter oily chemoembolization. Radiology 1996;198: 33–40
342. Caturelli E, Siena DA, Fusilli S, Villani MR, Schiavone G, Nardella M, et al. Transcatheter arterial chemoembolization for hepatocellular carcinoma in patients with cirrhosis: evaluation of damage to nontumorous liver tissue-long-term prospective study. Radiology 2000;215:123–128
343. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. J Hepatol 2008;48(Suppl 1):S20–S37
344. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008;48:1312–1327
345. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390
346. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25–34
347. Wilhelm S, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. Nat Rev Drug Discov 2006;5:835–844
348. Miller AA, Murry DJ, Owzar K, Hollis DR, Abou-Alfa GK, Desai A, et al. Pharmacokinetic (PK) and phase I study of sorafenib (S) for solid tumors and hematologic malignancies in patients with hepatic or renal dysfunction (HD or RD): CALGB 60301. J Clin Oncol 2007;25:3538 (abstract)
349. Abou-Alfa GK, Amadori D, Santoro A, Figer JDG A, Lathia C, Voliotis D, et al. Is sorafenib (S) safe and effective in patients (pts) with hepatocellular carcinoma (HCC) and Child-Pugh B (CPB) cirrhosis? J Clin Oncol 2008;26:4518 (abstract)
350. Siegel AB, Cohen EI, Ocean A, Lehrer D, Goldenberg A, Knox JJ, et al. Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol 2008;26:2992–2998
351. Chow LQ, Eckhardt SG. Sunitinib: from rational design to clinical efficacy. J Clin Oncol 2007;25:884–896
352. Zhu AX, Sahani DV, di Tomaso E, Duda DG, Catalano OA, Ancukiewicz M, et al. Sunitinib monotherapy in patients with advanced hepatocellular carcinoma (HCC): insights from a multidisciplinary phase II study. J Clin Oncol 2008;26:4521 (abstract)
353. Faivre SJ, Raymond E, Douillard J, Boucher E, Lim HY, Kim JS, et al. Assessment of safety and drug-induced tumor necrosis with sunitinib in patients (pts) with unresectable hepatocellular carcinoma (HCC). J Clin Oncol 2007;25:3546 (abstract)
354. D'Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci USA 1994;91:4082–4085
355. Kruse FE, Joussen AM, Rohrschneider K, Becker MD, Volcker HE. Thalidomide inhibits corneal angiogenesis induced by vascular endothelial growth factor. Graefes Arch Clin Exp Ophthalmol 1998;236:461–466
356. Hsu C, Chen CN, Chen LT, Wu CY, Yang PM, Lai MY, et al. Low-dose thalidomide treatment for advanced hepatocellular carcinoma. Oncology 2003;65:242–249
357. Patt YZ, Hassan MM, Lozano RD, Nooka AK, Schnirer II, Zeldis JB, et al. Thalidomide in the treatment of patients with hepatocellular carcinoma: a phase II trial. Cancer 2005;103:749–755
358. Lin AY, Brophy N, Fisher GA, So S, Biggs C, Yock TI, et al. Phase II study of thalidomide in patients with unresectable hepatocellular carcinoma. Cancer 2005;103:119–125
359. Hsu C, Chen CN, Chen LT, Wu CY, Hsieh FJ, Cheng AL. Effect of thalidomide in hepatocellular carcinoma: assessment with

power Doppler US and analysis of circulating angiogenic factors. Radiology 2005;235:509–516
360. Wang J, Chen LT, Tsang YM, Liu TW, Shih TT. Dynamic contrast-enhanced MRI analysis of perfusion changes in advanced hepatocellular carcinoma treated with an antiangiogenic agent: a preliminary study. AJR Am J Roentgenol 2004; 183:713–719
361. Breuhahn K, Longerich T, Schirmacher P. Dysregulation of growth factor signaling in human hepatocellular carcinoma. Oncogene 2006;25:3787–3800
362. Ono M, Morisawa K, Nie J, Ota K, Taniguchi T, Saibara T, et al. Transactivation of transforming growth factor alpha gene by hepatitis B virus preS1. Cancer Res 1998;58:1813–1816
363. Sato Y, Kato J, Takimoto R, Takada K, Kawano Y, Miyanishi K, et al. Hepatitis C virus core protein promotes proliferation of human hepatoma cells through enhancement of transforming growth factor alpha expression via activation of nuclear factor-kappaB. Gut 2006;55:1801–1808
364. Jakubczak JL, Chisari FV, Merlino G. Synergy between transforming growth factor alpha and hepatitis B virus surface antigen in hepatocellular proliferation and carcinogenesis. Cancer Res 1997;57:3606–3611
365. Kiss A, Wang NJ, Xie JP, Thorgeirsson SS. Analysis of transforming growth factor (TGF)-alpha/epidermal growth factor receptor, hepatocyte growth factor/c-met, TGF-beta receptor type II, and p53 expression in human hepatocellular carcinomas. Clin Cancer Res 1997;3:1059–1066
366. Yang CH. EGFR tyrosine kinase inhibitors for the treatment of NSCLC in East Asia: present and future. Lung Cancer 2008;60(Suppl 2):S23–S30
367. Su MC, Lien HC, Jeng YM. Absence of epidermal growth factor receptor exon 18–21 mutation in hepatocellular carcinoma. Cancer Lett 2005;224:117–121
368. Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, et al. Phase II study of erlotinib (OSI-774) in patients with advanced hepatocellular cancer. J Clin Oncol 2005;23:6657–6663
369. Thomas MB, Chadha R, Glover K, Wang X, Morris J, Brown T, et al. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer 2007;110:1059–1067
370. O'Dwyer PJ, Giantonio BJ, Levy DE, Kauh JS, Fitzgerald DB, Benson AB. Gefitinib in advanced unresectable hepatocellular carcinoma: results from the Eastern Cooperative Oncology Group's Study E1203. J Clin Oncol 2006;24:4143 (abstract)
371. Zhu AX, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, et al. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer 2007;110:581–589
372. Hsu C, Cheng JC, Cheng AL. Recent advances in non-surgical treatment for advanced hepatocellular carcinoma. J Formos Med Assoc 2004;103:483–495
373. Zhu AX. Systemic therapy of advanced hepatocellular carcinoma: how hopeful should we be? Oncologist 2006;11:790–800
374. Lopez PM, Villanueva A, Llovet JM. Systematic review: evidence-based management of hepatocellular carcinoma—an updated analysis of randomized controlled trials. Aliment Pharmacol Ther 2006;23:1535–1547
375. Yeo W, Mok TS, Zee B, Leung TW, Lai PB, Lau WY, et al. A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. J Natl Cancer Inst 2005;97:1532–1538
376. Gish RG, Porta C, Lazar L, Ruff P, Feld R, Croitoru A, et al. Phase III randomized controlled trial comparing the survival of patients with unresectable hepatocellular carcinoma treated with nolatrexed or doxorubicin. J Clin Oncol 2007;25:3069–3075
377. Lai CL, Wu PC, Chan GC, Lok AS, Lin HJ. Doxorubicin versus no antitumor therapy in inoperable hepatocellular carcinoma. A prospective randomized trial. Cancer 1988;62:479–483
378. Yang TS, Lin YC, Chen JS, Wang HM, Wang CH. Phase II study of gemcitabine in patients with advanced hepatocellular carcinoma. Cancer 2000;89:750–756
379. Guan Z, Wang Y, Maoleekoonpairoj S, Chen Z, Kim WS, Ratanatharathorn V, et al. Prospective randomised phase II study of gemcitabine at standard or fixed dose rate schedule in unresectable hepatocellular carcinoma. Br J Cancer 2003;89:1865–1869
380. Yen Y, Lim DW, Chung V, Morgan RJ, Leong LA, Shibata SI, et al. Phase II study of oxaliplatin in patients with unresectable, metastatic, or recurrent hepatocellular cancer: a California Cancer Consortium Trial. Am J Clin Oncol 2008;31:317–322
381. Patt YZ, Hassan MM, Aguayo A, Nooka AK, Lozano RD, Curley SA, et al. Oral capecitabine for the treatment of hepatocellular carcinoma, cholangiocarcinoma, and gallbladder carcinoma. Cancer 2004;101:578–586
382. Louafi S, Boige V, Ducreux M, Bonyhay L, Mansourbakht T, de Baere T, et al. Gemcitabine plus oxaliplatin (GEMOX) in patients with advanced hepatocellular carcinoma (HCC): results of a phase II study. Cancer 2007;109:1384–1390
383. Boige V, Raoul JL, Pignon JP, Bouche O, Blanc JF, Dahan L, et al. Multicentre phase II trial of capecitabine plus oxaliplatin (XELOX) in patients with advanced hepatocellular carcinoma: FFCD 03–03 trial. Br J Cancer 2007;97:862–867
384. Abou-Alfa GK, Johnson P, Knox J, Lacava J, Leung T, Mori A, et al. Preliminary results from a phase II, randomized, double-blind study of sorafenib plus doxorubicin versus placebo plus doxorubicin in patients with advanced hepatocellular carcinoma. In Proceedings of the 14th European cancer conference of the European Cancer Organisation (ECCO). Barcelona: ECCO; 2007. pp 23–27 (abstract no.: 3500)
385. Zhu AX, Blaszkowsky LS, Ryan DP, Clark JW, Muzikansky A, Horgan K, et al. Phase II study of gemcitabine and oxaliplatin in combination with bevacizumab in patients with advanced hepatocellular carcinoma. J Clin Oncol 2006;24:1898–1903
386. Sun W, Haller DG, Mykulowycz K, Rosen M, Soulen M, Capparo M, et al. Combination of capecitabine, oxaliplatin with bevacizumab in treatment of advanced hepatocellular carcinoma (HCC): a phase II study. J Clin Oncol 2007;25:4547 (abstract)
387. Asnacios A, Fartoux L, Romano O, Tesmoingt C, Louafi SS, Mansoubakht T, et al. Gemcitabine plus oxaliplatin (GEMOX) combined with cetuximab in patients with progressive advanced stage hepatocellular carcinoma: results of a multicenter phase 2 study. Cancer 2008;112:2733–2739
388. O'Neil BH, Bernard SA, Goldberg RM, Moore DT, Garcia R, Marroquin C, et al. Phase II study of oxaliplatin, capecitabine, and cetuximab in advanced hepatocellular carcinoma. J Clin Oncol 2008;26(Suppl):4604 (abstract)
389. Hsu CH, Yang T, Hsu C, Toh H, Epstein R, Hsiao L, et al. Phase II study of bevacizumab (A) plus capecitabine (X) in patients (pts) with advanced/metastatic hepatocellular carcinoma (HCC): final report. J Clin Oncol 2008;26(Suppl):4603 (abstr)
390. Thomas MB, Chadha R, Iwasaki M, Glover K, Abbruzzese JL. The combination of bevacizumab (B) and erlotinib (E) shows significant biological activity in patients with advanced hepatocellular carcinoma (HCC). J Clin Oncol 2007;25(Suppl):4567 (abstract)
391. Lo CM, Liu CL, Chan SC, Lam CM, Poon RT, Ng IO, et al. A randomized, controlled trial of postoperative adjuvant interferon therapy after resection of hepatocellular carcinoma. Ann Surg 2007;245:831–842
392. Lin SM, Lin CJ, Hsu CW, Tai DI, Sheen IS, Lin DY, et al. Prospective randomized controlled study of interferon-alpha in

preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. Cancer 2004;100:376–382
393. Kuzuya T, Katano Y, Kumada T, Toyoda H, Nakano I, Hirooka Y, et al. Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol Hepatol 2007;22:1929–1935
394. Piao CY, Fujioka S, Iwasaki Y, Fujio K, Kaneyoshi T, Araki Y, et al. Lamivudine treatment in patients with HBV-related hepatocellular carcinoma—using an untreated, matched control cohort. Acta Med Okayama 2005;59:217–224
395. Jang JW, Choi JY, Bae SH, Yoon SK, Chang UI, Kim CW, et al. A randomized controlled study of preemptive lamivudine in patients receiving transarterial chemo-lipiodolization. Hepatology 2006; 43:233–240
396. Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. Hepatology 1997;25:87–92
397. Poon RT, Fan ST, Ng IO, Lo CM, Liu CL, Wong J. Different risk factors and prognosis for early and late intrahepatic recurrence after resection of hepatocellular carcinoma. Cancer 2000;89:500–507
398. Sakon M, Umeshita K, Nagano H, Eguchi H, Kishimoto S, Miyamoto A, et al. Clinical significance of hepatic resection in hepatocellular carcinoma: analysis by disease-free survival curves. Arch Surg 2000;135:1456–1459
399. Ikeda K, Arase Y, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, et al. Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor—a prospective randomized study of hepatitis C virus-related liver cancer. Hepatology 2000;32:228–232
400. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Yamazaki O, et al. Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. Ann Intern Med 2001;134:963–967
401. Shiratori Y, Shiina S, Teratani T, Imamura M, Obi S, Sato S, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. Ann Intern Med 2003;138:299–306
402. Hung CH, Lee CM, Wang JH, Tung HD, Chen CH, Lu SN. Antiviral therapy after non-surgical tumor ablation in patients with hepatocellular carcinoma associated with hepatitis C virus. J Gastroenterol Hepatol 2005;20:1553–1559
403. Mazzaferro V, Romito R, Schiavo M, Mariani L, Camerini T, Bhoori S, et al. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. Hepatology 2006;44:1543–1554
404. Sakaguchi Y, Kudo M, Fukunaga T, Minami Y, Chung H, Kawasaki T. Low-dose, long-term, intermittent interferon-alpha-2b therapy after radical treatment by radiofrequency ablation delays clinical recurrence in patients with hepatitis C virus-related hepatocellular carcinoma. Intervirology 2005;48:64–70
405. Nishiguchi S, Tamori A, Kubo S. Effect of long-term postoperative interferon therapy on intrahepatic recurrence and survival rate after resection of hepatitis C virus-related hepatocellular carcinoma. Intervirology 2005;48:71–75
406. Hasegawa K, Takayama T, Ijichi M, Matsuyama Y, Imamura H, Sano K, et al. Uracil-tegafur as an adjuvant for hepatocellular carcinoma: a randomized trial. Hepatology 2006;44:891–895
407. Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, et al. Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. N Engl J Med 1996;334:1561–1567
408. Takai K, Okuno M, Yasuda I, Matsushima-Nishiwaki R, Uematsu T, Tsurumi H, et al. Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. Updated analysis of the long-term follow-up data. Intervirology 2005;48:39–45
409. Hirayama C, Okumura M, Tanikawa K, Yano M, Mizuta M, Ogawa N. A multicenter randomized controlled clinical trial of Shosaiko-to in chronic active hepatitis. Gastroenterol Jpn 1989; 24:715–719
410. Oka H, Yamamoto S, Kuroki T, Harihara S, Marumo T, Kim SR, et al. Prospective study of chemoprevention of hepatocellular carcinoma with Sho-saiko-to (TJ-9). Cancer 1995;76:743–749
411. Shimizu I, Ma YR, Mizobuchi Y, Liu F, Miura T, Nakai Y, et al. Effects of Sho-saiko-to, a Japanese herbal medicine, on hepatic fibrosis in rats. Hepatology 1999;29:149–160
412. Shiota G, Maeta Y, Mukoyama T, Yanagidani A, Udagawa A, Oyama K, et al. Effects of Sho-Saiko-to on hepatocarcinogenesis and 8-hydroxy-2′-deoxyguanosine formation. Hepatology 2002;35:1125–1133
413. Tsuchiya M, Kono H, Matsuda M, Fujii H, Rusyn I. Protective effect of Juzen-taiho-to on hepatocarcinogenesis is mediated through the inhibition of Kupffer cell-induced oxidative stress. Int J Cancer 2008;123:2503–2511

Miura *et al. BMC Gastroenterology* 2010, **10**:46
http://www.biomedcentral.com/1471-230X/10/46

BMC Gastroenterology

RESEARCH ARTICLE Open Access

A novel biomarker TERTmRNA is applicable for early detection of hepatoma

Norimasa Miura*[1], Yukio Osaki[2], Miki Nagashima[3], Michimori Kohno[4], Kensho Yorozu[5], Kohei Shomori[6], Takamasa Kanbe[7], Kenji Oyama[5], Yukihiro Kishimoto[7], Shigeo Maruyama[5], Eijiro Noma[8], Yutaka Horie[5], Masatoshi Kudo[3], Seigo Sakaguchi[8], Yasuaki Hirooka[9], Hisao Ito[6], Hironaka Kawasaki[7], Junichi Hasegawa[1] and Goshi Shiota[10]

Abstract

Backgrounds: We previously reported a highly sensitive method for serum human telomerase reverse transcriptase (hTERT) mRNA for hepatocellular carcinoma (HCC). α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP) are good markers for HCC. In this study, we verified the significance of hTERTmRNA in a large scale multi-centered trial, collating quantified values with clinical course.

Methods: In 638 subjects including 303 patients with HCC, 89 with chronic hepatitis (CH), 45 with liver cirrhosis (LC) and 201 healthy individuals, we quantified serum hTERTmRNA using the real-time RT-PCR. We examined its sensitivity and specificity in HCC diagnosis, clinical significance, ROC curve analysis in comparison with other tumor markers, and its correlations with the clinical parameters using Pearson relative test and multivariate analyses. Furthermore, we performed a prospective and comparative study to observe the change of biomarkers, including hTERTmRNA in HCC patients receiving anti-cancer therapies.

Results: hTERTmRNA was demonstrated to be independently correlated with clinical parameters; tumor size and tumor differentiation ($P < 0.001$, each). The sensitivity/specificity of hTERTmRNA in HCC diagnosis showed 90.2%/85.4% for hTERT. hTERTmRNA proved to be superior to AFP, AFP-L3, and DCP in the diagnosis and underwent an indisputable change in response to therapy. The detection rate of small HCC by hTERTmRNA was superior to the other markers.

Conclusions: hTERTmRNA is superior to conventional tumor markers in the diagnosis and recurrence of HCC at an early stage.

Background

Since the discovery of circulating nucleic acids (CNAs) in plasma in 1948, many diagnostic applications have emerged. Recently, CNAs instead of a protein has appeared on this scene of practical diagnostic assay, suggesting that cell-free CNAs in the plasma/serum of cancer patients have characteristics of tumor-derived nucleic acids. In addition to DNA-derived from tumor cells [1-4], a recent development in this new field is the finding of tumor-related RNA in the plasma/serum of cancer patients [5]. These features include tyrosine kinase mRNA [6], telomerase components [7,8], the mRNAs that are encoded by different tumor-related genes [9-13], and viral mRNA [14]. In one study, two telomerase markers in breast cancer yielded 44% of positive rates [7]. Nevertheless, telomerase RNA seems to be a promising marker by the reason that it can be found even in the serum of patients with small, undifferentiated breast cancers without any metastatic lesions. Dasi et al. showed that circulating telomerase RNA is a sensitive marker, using real-time reverse transcription-PCR (RT-PCR) [8].

The telomerase catalytic subunit (hTERT) exerts important cellular functions, including telomere homeostasis, genetic stability, cell survival and perhaps differentiation [15-20]. hTERTmRNA in serum was detected in

* Correspondence: mnmiura@hotmail.com
[1] Division of Pharmacotherapeutics, Department of Pathophysiological and Therapeutic Science, Faculty of Medicine, Tottori University, 86 Nishicho, Yonago, Tottori 683-8503, Japan
Full list of author information is available at the end of the article

breast cancer but not in benign diseases, suggesting that hTERT is available for cancer diagnosis [4].

HCC ranks high among the most common and fatal malignancies associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) infection [5]. Although HCC patients receive possible medical treatments such as transcatheter arterial chemoembolization/embolization (TACE/TAE), radiofrequency ablation (RFA), and surgery for primary tumors, intrahepatic and extrahepatic recurrence frequently limit patient's survival [6]. Although the modalities such as ultrasonography (US) and conventional tumor markers such as α-fetoprotein-L3 (AFP-L3) and DCP are widely used and important for HCC detection in clinical scenes [7], they still do not provide an entirely satisfactory solution to detect HCC at the early stage. Since HCC has been recently classified as a complex disease with a wide range of risk factors and many cellular signaling pathways have been reported to be involved in hepatocarcinogenesis, a novel biomarker for HCC is required [21]. We previously reported that measurement of serum hTERTmRNA by real-time RT-PCR method was sensitive in detection of tumor-derived hTERTmRNA even in the HCC patients whose AFP levels were low [9], and was also useful even for other malignancies such as non-small cell lung cancer, ovarian cancer, and gastric cancer [22-24]. In this large-scale study that includes follow-up cases, we focused on HCC of all malignancies and assessed the clinical significance of hTERTmRNA measurement in HCC diagnosis and monitored the clinical course.

Methods

Patients and Sample Collection

Four hundred-thirty seven consecutive patients (303 patients with HCC, 89 with CH, and 45 with LC), who were admitted at Tottori University related Hospitals, Osaka Red Cross Hospital, and Fukuoka University Chikushi Hospital between November, 2002 and December, 2006, were enrolled in this study. All the HCC patients had LC or CH as the underlying liver disease. The mean ages of patients with HCC, LC, and CH were 65, 66, and 61 years, respectively. One hundred-sixty seven patients were infected with HCV, 97 with HBV, 24 with both viruses and 15 with no viral markers. The patients were diagnosed by blood chemistry, US, computed tomography (CT), AFP and/or biopsy under US. The clinicopathological findings (age, gender, etiology, underlying liver disease (adjacent lesion), Pugh score, Child classification, total bilirubin (TB), albumin (Alb), alanine aminotransferase (ALT), AFP, AFP-L3, DCP, HCV titer, HCV subtype, tumor number, tumor size, differentiation degree of tumor, and presence of metastasis) were evaluated (Figure 1). HCC was diagnosed according to the AASLD guidelines and the differentiation of HCC was diagnosed by liver biopsy. Two hundred one healthy individuals including 144 females (from 24-87 years old: mean age 57 years) served as controls. Informed consent was obtained from each patient and the study protocols followed the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the human research committee of Tottori University. The therapies for HCC include TAE, transcatheter arterial infusion (TAI), percutaneus ethanol injection therapy (PEIT), and RFA. Regarding follow-up patients, blood samples were taken basically every two months.

RNA extraction and Real-time quantitative RT-PCR

Harvesting serum samples were performed as previously described [9]. RNA was extracted with DNase treatment from serum as reported previously [4,9]. The quantitative RT-PCR was performed as described previously [5,10]. (a) for hTERT. The RT-PCR condition was an initial incubation at 50 for 30 min followed by a 12-min incubation at 95, then 50 cycles at 95 (0 s), 55 (10 s), and 72 (15 s), and a 20 second melting at 40°C. The dynamic ranges of real-time PCR analysis for hTERTmRNA were more than approximately 5 copies in this assay and we were able to exclude the possibility of false negativity in serum samples from patients with CH, LC and controls. The PCR yielded products of 143 bp for hTERT (data not shown). The RT-PCR assay was repeated twice and the quantification was confirmed by using LightCycler (Roche, Basel, Switzerland) with reproducibility.

hTERTmRNA during the treatment and detection of small HCC

We examined the therapeutic effectiveness of hTERTmRNA during the clinical course. Serum hTERTmRNA was measured before and 7 days after TAE in 16 HCC patients. In comparison with AFPmRNA, the half-life of hTERTmRNA was examined. By monitoring gene expression in serum up to 6 months after the beginning of therapy such as TAE, TAI, RFA, PEIT, surgical treatment, the effect of therapies were estimated in 20 patients. Furthermore, we examined hTERTmRNA expression and level of other conventional tumor markers after they were categorized by the tumor size (less than 10 mm, 11-20 mm, 21-30 mm, more than 30 mm).

Immunohistochemistry

For immunohistochemical analysis, of 303 patients, 50 HCC patients (24 patients with HCV, 9 with HBV, 10 with both viruses, and 7 with unknown etiology; 5 patients with well-, 3 with well~moderately-, 32 with moderately-, 3 with moderately~poorly-, and 7 with poorly-differentiated HCC) with 35 positive and 15 negative conventional tumor markers, who underwent surgical treatment, were chosen. The immunohistochemical procedures were done as reported previously [25]. The sections were incu-

Clinical parameters		No. of HCC patient 303	hTERT mRNA *p*	AFP *p*	AFP-L3 *p*	DCP *p*
Age mean:65 years old (range:22 to 101)			NS	NS	NS	NS
Gender	M	196	NS	NS	NS	NS
	F	107				
Etiology	HBV	97	NS	NS	NS	NS
	HCV	167				
	HBV+HCV	24				
	NBNC	15				
Background lesion	CH	113	NS	NS	NS	NS
	LC	190				
Numbers of tumors	1	199	NS	NS	0.003	0.029
	2	55				
	>3	49				
Tumor size (cm)	< 1	25	<0.001	0.008	NS	NS
	1~2	79				
	2~3	100				
	> 3	99				
Differentiation of tumor			<0.001	0.019	0.001	NS
(Edmondson grade)	I	24				
	II	43				
	III	33				
	IV	1				
	unknown	101				

hTERT mRNA was independently correlated with tumor size eand differentiation degree. NS: not significant.

Figure 1 Multivariate analysis of tumor markers with clinical parameters in patients with HCC.

bated with the following monoclonal antibodies: anti-hTERT (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-Ki67 (Santa Cruz Biotechnology), anti-TUNEL (Sigma Chemical, MO, USA), HBsAg (Sigma Chemical), and HCV core antibody (Sigma Chemical). Expression degree was confirmed and estimated of hTERT, Ki-67, and TUNEL by the percentage of positively-stained cell number [26-28].

Statistical analysis

Multivariate analysis was performed using SPSS 13.0 (SPSS Corp., Tokyo, Japan). Stratified categories in each clinical parameter were evaluated by One Way ANOVA and multivariate analysis using a logistic regression analysis model. To assess the accuracy of the diagnostic tests, the matched data sets (chronic liver diseases patients and HCC patients) regarding AFP, AFP-L3, DCP, and hTERTmRNA were analyzed by using receiver operator characteristic (ROC) curve analysis. The correlation of hTERTmRNA between HCC tissue and serum was analyzed using both Paired t test and Spearman's test. The detection rates of HCC in comparison with tumor size were evaluated by Friedman test.

Results

RNA extraction and Real-time quantitative RT-PCR

In each quantitative assay, a strong linear relation was demonstrated between copy number and PCR cycles using RNA controls for concentration ($r^2 > 0.99$; data not shown). hTERTmRNA expression showed stepwise up-regulation with disease progression and the quantification was significantly higher in HCC than in LC, CH and healthy individuals ($P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively, Figure 2A). ROC curve analyses showed that the sensitivity/specificity of hTERTmRNA for HCC were 90.2%/85.4% (Figure 2B). Optimal cut-off values for hTERTmRNA expressions were predicted as 9,332 copies/0.2 ml by stressing the higher specificity. Forty six (15%) of HCC patients, whose AFP, AFP-L3, and DCP were within normal limits, had 4.23 ± 0.32 logarithmic values of hTERTmRNA, and 20 patients of 46 patients were positive for this assay.

Multivariate analysis showed that hTERTmRNA was associated with tumor size and differentiation degree of tumor ($P < 0.001$, each, Figure 1 &3). However, hTERTmRNA was not associated with age, gender, etiology, background lesion or number of tumor. On the other hand, AFP was related to tumor size and differentiation

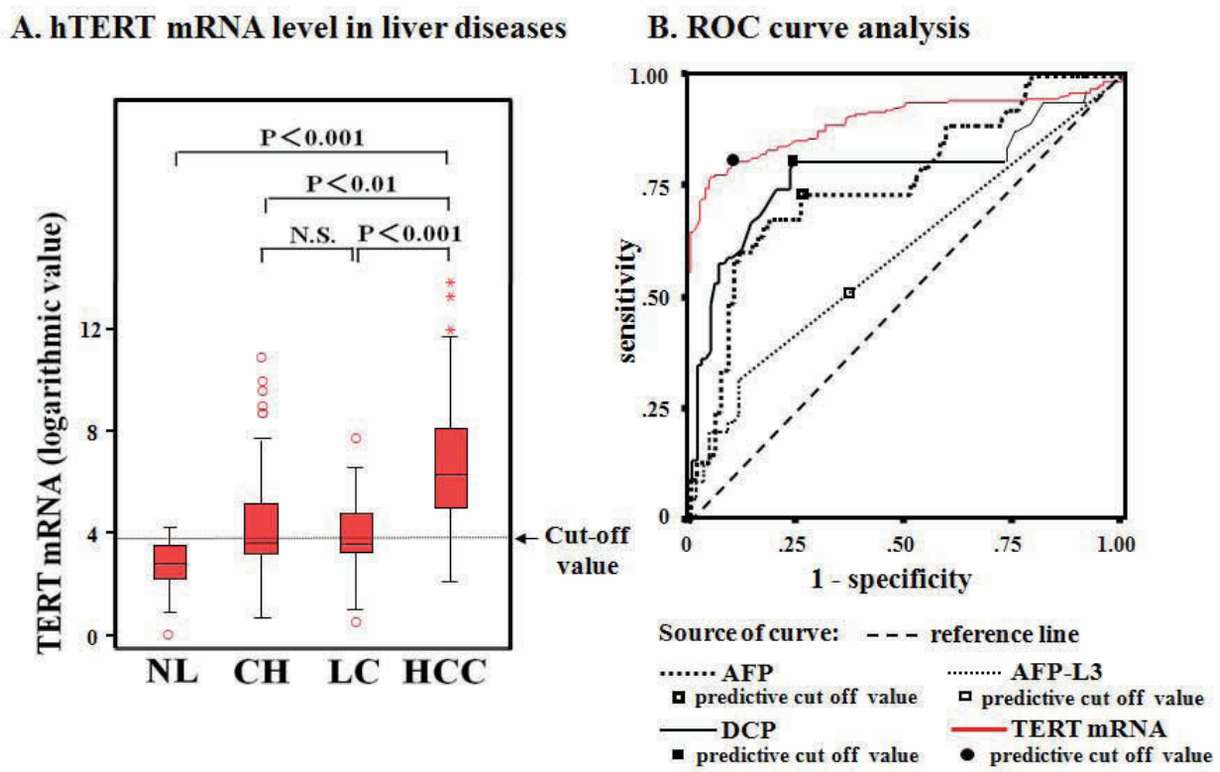


Figure 2 Serum hTERTmRNA and AFP mRNA expression in patients with HCC, LC, and CH, and in healthy individuals and receiver operator characteristics (ROC) curve analyses of hTERTmRNA, AFP, AFP-L3, and DCP. A. hTERTmRNA level in liver diseases. Serum hTERTmRNA levels and AFPmRNA level in patients with HCC, LC, CH, and healthy individuals by real-time RT-PCR were shown. The 95% confidence interval in each group is shown beside the dots. Significant differences between 4 groups are shown in the upper part of the figure. NL, individual with normal liver; CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma. Optimal cut-off values for hTERTmRNA expressions were predicted as 9,332 copies/0.2 ml. B. ROC curve analysis of hTERTmRNA, AFP, AFP-L3, and DCP was obtained by importing quantified raw data into SPSS II software. The data were analyzed by Paired t test (P = 0.01) and non-parametric Spearman's test (P = 0.017). AUC of each biomarker in ROC curve analysis is shown. Receiver operator characteristic (ROC) curve analysis of hTERTmRNA, AFP, AFP-L3, and DCP was obtained by importing quantified raw data into SPSS II. The solid line, bold dotted line, dotted line, and bold solid line correspond to DCP, AFP, AFP-L3 and hTERTmRNA, respectively.

(P = 0.008 and P = 0.0199), AFP-L3 was related to number of tumor and differentiation degree (P = 0.003 and P = 0.001), and DCP was associated with only number of tumor (P = 0.029). By Pearson relative test, serum hTERTmRNA significantly associated with tumor size and number of tumors (P < 0.033 and P < 0.003, respectively, Table 1). Importantly, hTERTmRNA was related only to DCP (P = 0.03).

ROC curve analyses showed that the sensitivity/specificity of hTERTmRNA for HCC were 90.2%/85.4% (Table 2). The sensitivity/specificity of AFP, AFP-L3, and DCP were 76.6/66.2, 60.5/88.7, and 83.4/80.3, respectively. Thus, hTERTmRNA was superior to other markers especially in sensitivity. The positive predictive value (PPV)/negative predictive value (NPV) of hTERTmRNA were 83.0/85.9. On the other hand, the PPV/NPV for AFP, AFP-L3, and DCP were 74.6/67.7, 59.6/92.2, and 78.4/73.5, respectively. Consequently, hTERTmRNA was superior to other markers in the diagnosis of HCC. Combinations of hTERTmRNA with AFP level improved the sensitivity/specificity up to 96.0%/87.2%. ROC curve analysis categorized by viruses was examined and sensitivity/specificity in HBV-infected cases was similar to that of HCV-infected cases (additional file 1). hTERT and other markers in LC was not statistically and significantly different in comparison with that in CH.

Estimation of therapeutic effect and the possibility of early HCC detection of hTERTmRNA in comparison with other biomarkers

To examine the significance of hTERTmRNA before and after TAE, serum hTERTmRNA was measured before and 7 days after TAE in 16 HCC patients (Figure 4A). As a result, hTERTmRNA significantly decreased after TAE (P = 0.018), suggesting that changes in hTERTmRNA are indicative of therapeutic effects on HCC. Comparing the follow-up data of hTERTmRNA and AFP (Figure 4B, C),

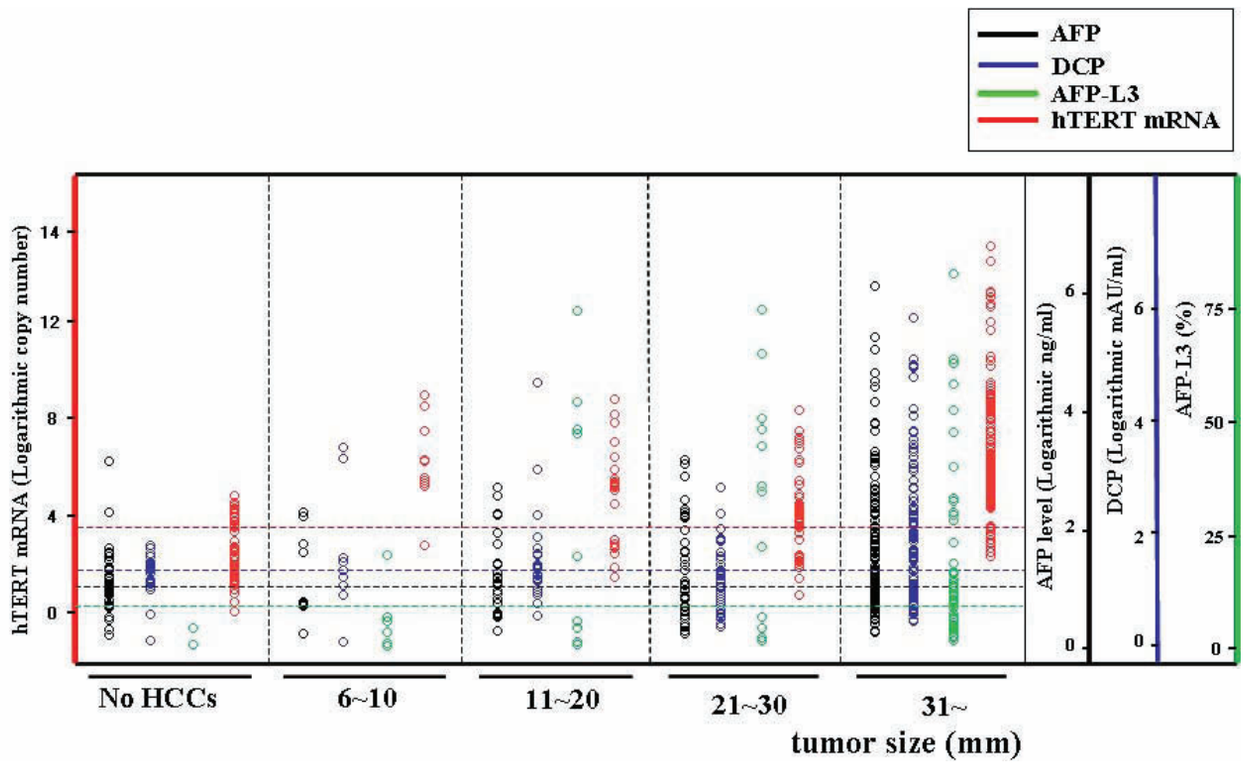


Figure 3 Levels of hTERTmRNA in regard to tumor size (6-10 mm, 11-20 mm, 21-30 mm, and over 31 mm).

the half-life of hTERTmRNA was shorter than that of AFP.

To clarify the significance of hTERTmRNA in monitoring the effect of therapies in comparison with other biomarkers, two representative cases were depicted in Figure 5. The quantification of hTERTmRNA was performed before, 2 and 5 months after RFA in a 73-year-old male patient whose HCC was a single 21 mm-sized (Figure 5A). hTERTmRNA changed similar to AFP, AFP-L3, and DCP, suggesting that hTERTmRNA is useful for monitoring the clinical course of HCC. In a 78-year-old female patient whose HCC was a single 38 mm-sized, a surgical operation was performed (Figure 5B). The values of AFP, DCP, and hTERTmRNA were measured before, 2 and 7 months after the operation. The operation was performed successfully in this patient, however recurrence was found by dynamic CT at 7 months after the operation. Although neither AFP nor DCP detected the recurrence, only hTERTmRNA did. In all the cases that hTERT detected recurrence in the earlier stage, no other imaging modality could detect it at the same time, but when we could find HCC in images such as US, CT, or MR, other markers began to arise.

Finally, we examined the relationship between the positive rates of biomarkers and tumor size. Positive rate of hTERTmRNA was higher than that of the other markers in each category of tumor size; 6-10 mm, 11-20 mm, 21-30 mm, over 31 mm by Friedman test (P = 0.017) (Figure 3). However, the positivity of hTERTmRNA expression tended to reduce slightly in tumors with diameters that exceeded than 51 mm (5.2 ± 1.9 for 56 patients with 31-50 mm of HCC, 5.0 ± 1.8 for 43 patients with HCC over 51 mm; mean ± S.D.) (additional file 2). Dot blot regarding the correlation of hTERT mRNA quantification with tumor differentiation is shown in additional file 3. In a 6 mm HCC case, no marker other than hTERTmRNA was elevated and only abdominal US caught the evidence of HCC (Figure 6(a) A, B).

Immunohistochemistry

Immunohistochemical analysis showed that Ki-67 positivity was observed in the nuclei of cancer cells (Figure 6(b) A). hTERT was observed in both the nuclei and cytoplasm of cancer cells (Figure 6(b) B). Some TUNEL-positive cells were present in cancerous lesions, however the prevalence was low (Figure 6(b) C). hTERT expression was significantly associated with the labeling index of Ki-67 (P = 0.023). When the labeling indices of Ki-67, hTERT and TUNEL were compared with the differentiation degree of HCC, both hTERT and Ki-67 were higher in poorly differentiated HCC than in well and moderately differentiated HCC (Figure 6(b) D).

Table 1: The sensitivity/specificity of each tumor marker for hepatocellular carcinoma and a statistic evaluation of hTERTmRNA level to clinical parameter were shown.

clinical parameter	average ± S.E.	Pearson test P value	Multivariate analysis P value
tumor size (mm)	21.2 ± 0.1	0.033	<0.001
(range: 6-90)			
tumor number	1.8 ± 0.1	0.003	N.S.
tumor differentiation		N.S.	<0.001
AFP (ng/ml)	6146 ± 4554	N.S.	N.S.
(n = 353)			
AFP-L3 (%)	6.7 ± 1.0	N.S.	N.S.
(n = 213)			
DCP (mAU/ml)	18780 ± 1044	0.03	N.S.
	n = 346)		

Discussion

Since HCC has been recently classified as a complex disease with a wide range of risk factors and many cellular signaling pathways have been reported to be involved in hepatocarcinogenesis, a novel biomarker for HCC is required [21]. Since an epoch-making assay to detect telomerase activity was established [11], telomerase has been examined in many kinds of cancers, precancerous lesions and normal tissues using the telomeric repeat amplification protocol and investigated the correlation with telomere length [29,30]. Notwithstanding that telomerase was definitely an unprecedented candidate tumor marker due to its specificity to cancer, it has clinically remained inapplicable because telomerase expres-

Table 2: The sensitivity/specificity of each tumor marker for HCC was depicted.

	Sensitivity	Specificity	OR	PPV/NPV	Cut-off point
hTERTmRNA	90.2	85.4	19.0	83.0/85.9	3. 97 (logarithmic copy number)
AFP	76.6	66.2	11.1	74.6/67.7	<10 (ng/ml)
AFP-L3	60.5	88.7	2.2	59.6/92.2	<10 (%)
DCP	83.4	80.3	7.6	78.4/73.5	<40 (mAU/ml)

The sensitivity/specificity values are 90.2%/85.4% for hTERTmRNA, 76.6%/66.2% for AFP, 60.5%/88.7% for AFP-L3, and 83.4%/80.3% for DCP. Regarding a diagnostic assessment in sensitivity and specificity, hTERTmRNA is identified as the most excellent tumor marker. OR: odds ratio, PPV: positive predictive value (%), NPV: negative predictive value (%).

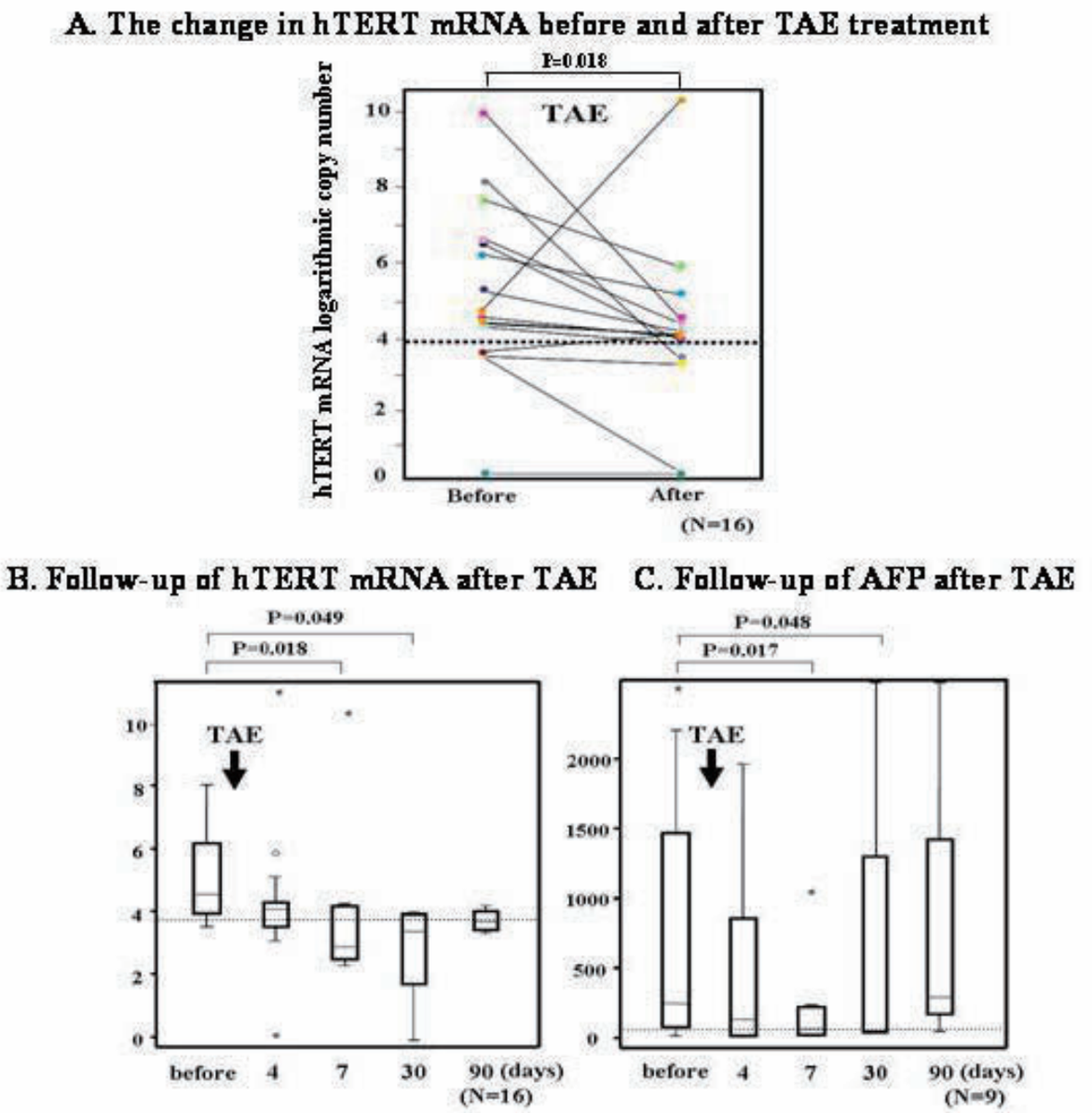


Figure 4 The change of hTERTmRNA before and 7days after TAE. A. Follow-up of serum hTERTmRNA before, 4, 7, 30 and 90 days after TAE. B. Follow-up of serum AFP before, 4, 7, 30 and 90 days after TAE.

sion has not been detected stably in body fluid [12]. In serum, the hTERTmRNA derived from cancer cells seemed to be undetectable because it becomes instable by RNase in blood. Since RNAs in serum are unexpectedly stable within 24 hrs after drawing blood due to particle-associated complex in structure [13,14], it has been suggested that they can be generally detected even in RNase-rich blood. Actually, hTERTmRNA can be detected in serum from breast cancer patients and its maximum sensitivity and specificity are at most 40% and 100%, respectively [4]. The sensitivity in patients with HCC rose to 89.7% in the semi-quantitative assay, and thus compared favorably with the previous findings in which the sensitivity and specificity of AFPmRNA were 69% and 50% for HCC, respectively [31]. Besides, with respect to HCC detection, AFPmRNA was superior to AFP level used routinely in clinic [32]. Recently, in the present study, we reported the sensitivity to detect the nucleotides in blood in the process of RNA extraction, including centrifugation steps less than 1500 × g to remove cellular proteins in serum and a primer set that can detect hTERTmRNA more efficiently than primers in the previous reports (data not shown). We previously reported that hTERT expression was very faint in the

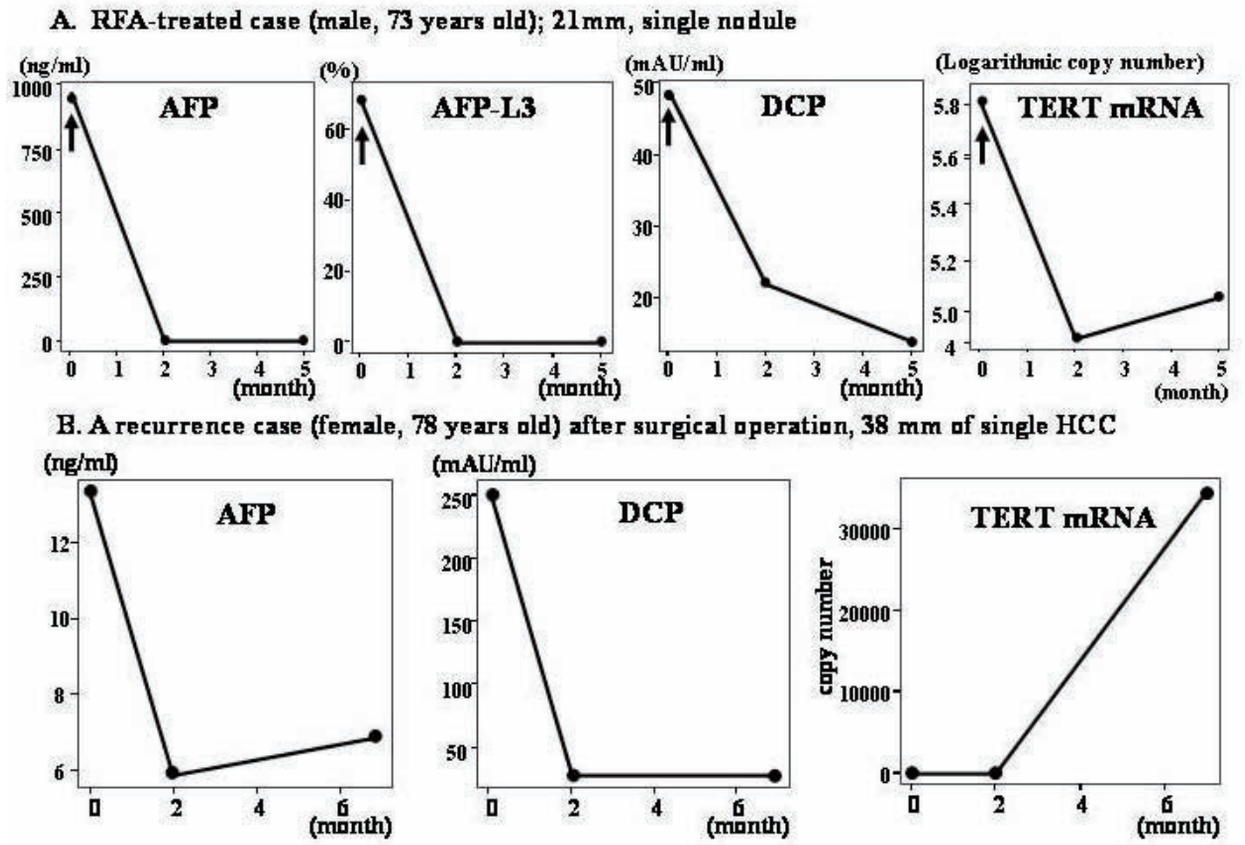


Figure 5 Conventional tumor markers and hTERTmRNA detection during clinical course with therapeutic modalities. A. Changes of AFP, AFP-L3, DCP and hTERTmRNA in RFA-treated case (male, 73 years old), of which HCC was 21 mm-sized and single. B.Changes of AFP, DCP and hTERTmRNA in surgical operation-treated case (female, 78 years old), of which HCC was 38 mm-sized and single.

serum from normal individuals indicating that lymphocytes and circulating normal cells express very low levels of hTERTmRNA [9]. Because hTERTmRNA in lymphocytes is very low, elevated hTERTmRNA levels in serum may mean that hTERTmRNA is derived from cancer cells. Since we could detect negligible amounts of lymphocyte markers after three steps of centrifugation of blood samples, the RNA extraction procedure seemed to remove lymphocytes effectively. In addition, normal or damaged hepatocytes express negligible amounts of hTERT [33,34]. Furthermore, we previously showed the significant correlation of hTERTmRNA expression between tumor tissue and serum [32]. These data suggest that hTERTmRNA detected in serum is derived from tumor cells.

Previously, we reported that qualitative analysis of serum hTERTmRNA was superior to AFP for the purpose of the early detection of HCC, because hTERTmRNA was detectable in HCC patients with normal AFP levels [9]. AFP is being widely used as a reliable marker of HCC not in earlier stage but in the advanced stage [35]. However, in this study, neither AFP was able to distinguish HCC from non-cancerous liver diseases, nor hTERTmRNA was correlated with AFP level ($P = 0.201$), suggesting that quantitative analysis of serum hTERTmRNA was much more sensitive for HCC diagnosis even in the early stage. Because the induction of the abdominal (enhanced-)US, CT, and MRI into the clinical scene enabled us to detect smaller-sized HCC [36], the sensitivity of AFP in the early detection of HCC became less than 70%. Unlike AFP level, AFPmRNA was significantly correlated with hTERTmRNA ($P < 0.001$) and more sensitive than AFP. In the present study, we measured AFP-L3, since AFP-L3 has been reported to be a more HCC-specific marker than AFP [37]. Indeed, the level of AFP-L3 correlated significantly with differentiation and number of HCC although that of AFP was correlated with tumor size and differentiation.

In the present study, of 303 HCC patients, 24 patients were negative below the calculated cut-off value (9,332; 3.97 as logarithmic number) for serum hTERTmRNA. Although the reason why hTERTmRNA was negative in

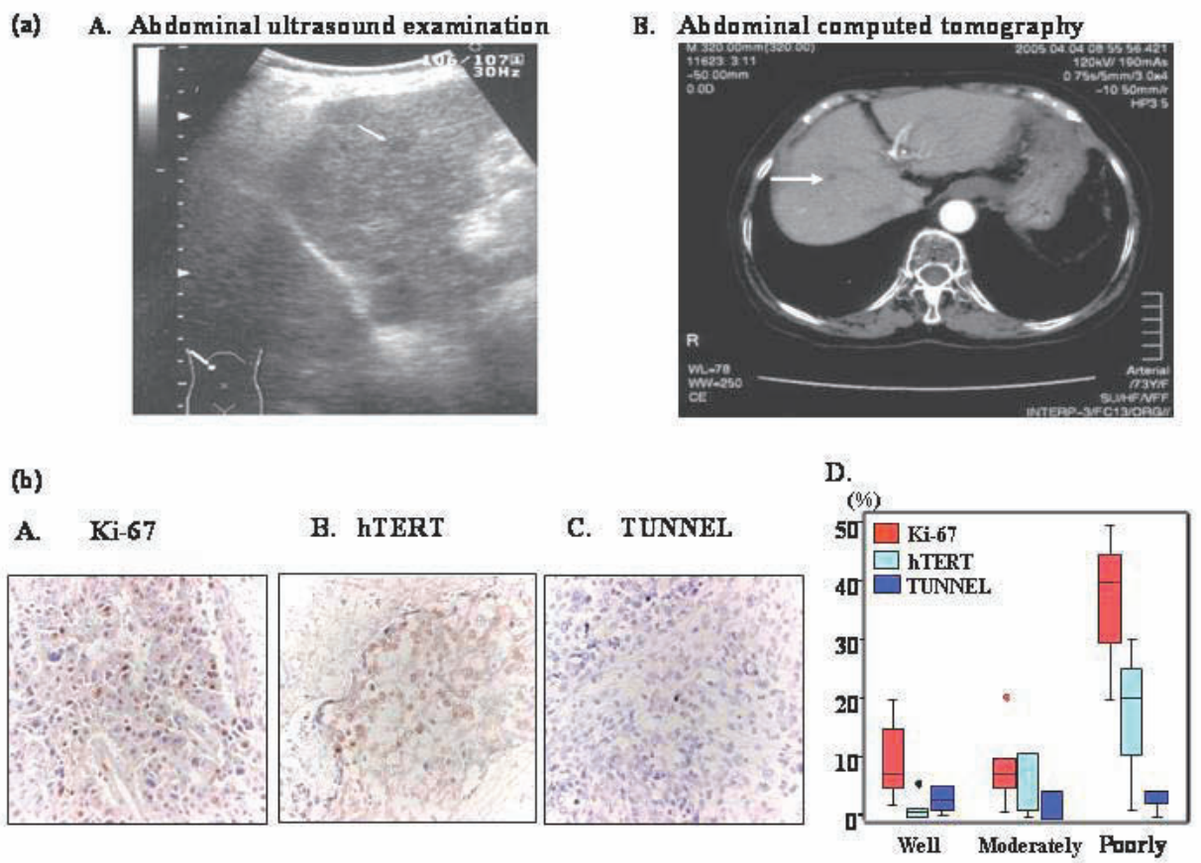


Figure 6 Early detection of small HCC by circulating hTERT mRNA and immunohistochemical analysis of HCC tissues. (a) Imaging diagnosis regarding case with 6 mm HCC. A. Ultrasonography and B. computed tomography of the smallest HCC detected by hTERTmRNA. The diameter of HCC was 6 mm. Left, ultrasonography; right, CT (b) Immunohistochemical analysis of HCC tissues. A. Ki-67 staining (×400), B. hTERT staining (×400), C. TUNEL (×400), D. Labeling indices of Ki-67, hTERT and TUNEL in regard to differential degree of HCC.

these patients is not clear, eleven of 24 hTERTmRNA-negative HCC patients had decompensated liver cirrhosis as the underlying disease. It has been reported that decompensated liver cirrhosis had higher levels of serum TGF-β that promotes apoptosis of immortalized hepatocytes and, in these cases, elevated TGF-β may stimulate apoptosis, resulting in reduction of hTERTmRNA [34,38,39]. hTERT-negative cases had no other common characteristics with age, gender, etiology, child classification etc. than tumor size, ALT, and surrounding lesion. In 23 cases (95.8%), ALT was within 1.5 fold normal limits. In 17 cases (70.8%), surrounding lesion was LC including decompensated situation. Tumor size in 12 cases (50%) was over 30 mm, reflecting on the biological features of cancer itself, as referred in Norton-Simon models regard tumor growth [40]. AFP and DCP were positive in 16 (66.7%) and 11 (45.8%) cases, respectively, suggesting that combinative use of these markers contributes to improve the diagnostic specificity.

Thus, hTERTmRNA is not only improved in both sensitivity and specificity but has a close correlation with tumor size and number in an early stage of HCC. Since HCC repeatedly recurs polyclonally after any treatment as a biological characteristic, the measurement of serum hTERTmRNA makes it possible to recognize recurrence or therapeutic effect in details as well as the usefulness for one-point diagnosis. In this respect, we have to undergo follow-up study after the treatment of HCC [24]. hTERTmRNA expression was closely associated with well to moderate differentiation degree of HCC and was enhanced with the proliferation. We should clarify that serum hTERTmRNA can be detected by what alterations of other molecules during the cancer progression [41-43]. In lower differentiated HCC, tumor cells are proliferating and hTERTmRNA has a tendency to correlate with the differentiation degree and an apoptotic event never reflect on the serum detection of cancer cell-derived mRNAs (Figure 6). Nakashio et al. previously reported the significant correlation of HCC differentiation with

telomerase expression [44]. The results in the present study confirmed their findings. hTERTmRNA showed more sensitivity and specificity compared with AFP-mRNA in HCC patients. However, in liver diseases other than HCC, hTERTmRNA was not correlated with AFP-mRNA. The higher specificity of hTERTmRNA in HCC may be related to fact that AFPmRNA is produced in HCC cells and injured hepatocytes and hTERT is produced mainly in HCC cells. Furthermore, we could detect serum hTERTmRNA expression even in HCC patients with less than 10 mm moderate-differentiated tumor, indicating that hTERT are upregulated during rapid proliferation of tumor at the early phase of oncogenesis, de-differentiation.

Waguri et al. proved that there exist circulating cancer cells derived from original HCC tissues in blood and they can detect hTERTmRNA in blood [45]. The present study suggests that quantification of hTERTmRNAs in serum has diagnostic implications for HCC. Unless apoptosis of cancer cells contributes to the early detection of HCC using serum mRNA, the essence may be immunoreactions [46]. The development of micro vessels may be also involved in the step [47]. We will evaluate the correlation of prognosis with hTERTmRNA and the availability of hTERTmRNA in other cancers by comparison of hTERT-mRNA with other tumor markers [48], and will study its usefulness for inflammatory diseases in which cellular reactions are active [49]. This method depends on RNA stability in each process of RNA purification, storage, and quantification. In the light of its superior positivity to other markers, the assay will be applied for clinical use in the strict condition because it is required to keep the serum RNA as it is in blood and avoid the degradation of RNA quality. Now we are improving RNA stability and PCR condition to better cost/benefit of this assay. In the future, another large-scale study will be required to confirm our results for monitoring HCC and the feasibility for its detection even on a primary care level.

Conclusions

In sum, our results support the suggestion that quantification of circulating hTERTmRNA expression is clinically useful for the early detection of HCC. Furthermore, hTERTmRNA is superior to conventional tumor markers in the diagnosis and recurrence of HCC at the early stage.

Additional material

Additional file 1 TIF ROC curve analysis and AUC in measurement categorized by viruses. ROC curve analysis and AUC in measurement categorized by viruses are demonstrated. Sensitivity/specificity of hTERTmRNA expression in HBV-infected cases is similar to that in HCV-infected cases.

Additional file 2 MS word Positivity of each marker for HCC. Positivity of each marker for HCC was shown, categorized by tumor size.

Additional file 3 TIF Dot blot regarding the correlation of hTERT-mRNA quantification with tumor differentiation. Serum hTERTmRNA quantification in HCC patients (n = 101) diagnosed by liver biopsy was shown, categorized by tumor differentiation. The quantification in serum of HCC patients with well-/moderately-/poorly-/un-differentiation was 4.4 ± 1.4/5.4 ± 2.0/6.3 ± 3.3/5.9 ± 1.8 (mean ± SD).

Abbreviations

(h)TERT: (human) telomerase reverse transcriptase protein; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; HBV: hepatitis B virus; LC: liver cirrhosis; CH: chronic hepatitis; AFP: α-fetoprotein; DCP: des-γ-carboxy prothrombin; ALT: alanine aminotransferase; Alb: albumin; CNA: Circulating nucleic acids.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YO analyzed biomedical data and provided blood sample as main researcher in Osaka Red Cross Hospital. MN analyzed biomedical data in Kinki University. MK analyzed biomedical data and provided blood sample as main researcher in Kinki University. KY analyzed biomedical data and provided blood sample in San-in Labor Welfare Hospital. TK analyzed clinical data and the practical analysis in San-in Labor Welfare Hospital. KO analyzed HCC imaging data and the analysis in Saiseikai Gotsu General Hospital. YK was in charge for case study in Saiseikai Gotsu General Hospital. SM analyzed biomedical data and provided blood sample as main researcher in Saiseikai Gotsu General Hospital EN was in charge for case study and biomedical analysis in Saiseikai Gotsu General Hospital. YH analyzed clinical and biomedical data as main researcher in Saiseikai Gotsu General Hospital comprehensively. MK analyzed biomedical data and provided blood sample as main researcher in Matsue City Hospital. SS analyzed biomedical data and provided blood sample as main researcher in Fukuoka University Chikushi Hospital. YH performed biomedical and clinical analysis in surgical case in Tottori University. HK analyzed biomedical data and provided blood sample as chief researcher in San-in Labor Welfare Hospital. JH provided the environment to analyze the data comprehensively. All authors read and approved the final manuscript.

Acknowledgements

This study was supported by a Grant-in-Aid (18390208) for scientific research from the Ministry of Education, Science, and Culture and the Foundation for the Promotion of Cancer Research in Japan. All the PCR primers were designed by INTEC Web and Genome Informatics, Corporation (Tokyo, Japan).

Author Details

[1]Division of Pharmacotherapeutics, Department of Pathophysiological and Therapeutic Science, Faculty of Medicine, Tottori University, 86 Nishicho, Yonago, Tottori 683-8503, Japan, [2]Department of Gastroenterology, Osaka Red Cross Hospital, 5-30 Fudegasaki-cho, Tennouji-ku, Osaka, Osaka 543-8555, Japan, [3]Department of gastroenterology, Kinki University, 3-4-1 Kowakae, Higashi-Osaka, Osaka 577-8502, Japan, [4]Department of Gastroenterology, Matsue City Hospital, 32-1 Noshira-cho, Matsue, Shimane 690-8509, Japan, [5]Department of Internal Medicine, Shimaneken Saiseikai Gotsu General Hospital, 1551 Gotsu-cho, Gotsu, Shimane 695-8505, Japan, [6]Division of Organ Pathology, Faculty of Medicine, Tottori University, Nishicho 86, Yonago, 683-8503, Japan, [7]Internal Medicine, San-in Labor Welfare Hospital, 1-8-1 Kaikeshinden, Yonago, Tottori 683-0002, Japan, [8]Department of Gastroenterology, Fukuoka University Chikushi Hospital, 1-1-1 Zokumyoin, Chikusino, Fukuoka 818-8502, Japan, [9]Department of Pathobiological Science and Technology, School of Health Science, Faculty of Medicine, Tottori University, 86 Nishicho, Yonago, Tottori 683-8503, Japan and [10]Division of Molecular and Genetic Medicine, Department of Genetic Medicine and Regenerative Therapeutics, Tottori University School of Medicine, 86 Nishicho, Yonago, Tottori 683-8503, Japan

Received: 23 July 2009 Accepted: 18 May 2010
Published: 18 May 2010

References

1. Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, Ratliff RL, Wu JR: **A highly conserved repetitive DNA sequence,**

(TTAGGG)n, present at the telomeres of human chromosomes. *Proc Natl Acad Sci USA* 1988, **85**:6622-6626.
2. Paradis V, Dargère D, Laurendeau I, Benoît G, Vidaud M, Jardin A, Bedossa P: **Expression of the RNA component of human telomerase (hTR) in prostate cancer, prostatic intraepithelial neoplasia, and normal prostate tissue.** *J Pathol* 1999, **189**:213-218.
3. Kopreski MS, Benko FA, Kwak LW, Gocke CD: **Detection of tumor messenger RNA in the serum of patients with malignant melanoma.** *Clin Cancer Res* 1999, **5**:1961-1965.
4. Chen XQ, Bonnefoi H, Pelte MF, Lyautey J, Lederrey C, Movarekhi S, Schaeffer P, Mulcahy HE, Meyer P, Stroun M, Anker P: **Telomerase RNA as a detection marker in the serum of breast cancer patients.** *Clin Cancer Res* 2000, **6**:3823-3826.
5. El-Serag HB, Mason AC: **Rising incidence of hepatocellular carcinoma in the United States.** *N Engl J Med* 1999, **340**:745-750.
6. Shirabe K, Takenaka K, Taketomi A, Kawahara N, Yamamoto K, Shimada M, Sugimachi K: **Postoperative hepatitis status as a significant risk factor for recurrence in cirrhotic patients with small hepatocellular carcinoma.** *Cancer* 1996, **15**:1050-1055.
7. Dohmen K, Shirahama M, Onohara S, Miyamoto Y, Torii Y, Irie K, Ishibashi H: **Differences in survival based on the type of follow-up for the detection of hepatocellular carcinoma: an analysis of 547 patients.** *Hepatol Res* 2000, **18**:110-121.
8. Miura N, Horikawa I, Nishimoto A, Ohmura H, Ito H, Hirohashi S, Shay JW, Oshimura M: **Progressive telomere shortening and telomerase reactivation during hepatocellular carcinogenesis.** *Cancer Genet Cytogenet* 1997, **93**:56-62.
9. Miura N, Shiota G, Nakagawa T, Maeda Y, Sano A, Marumoto A, Kishimoto Y, Murawaki Y, Hasegawa J: **Sensitive detection of hTERTmRNA in the serum of patients with hepatocellular carcinoma.** *Oncology* 2003, **64**:430-434.
10. Mitas M, Mikhitarian K, Walters C, Baron PL, Elliott BM, Brothers TE, Robison JG, Metcalf JS, Palesch YY, Zhang Z, *et al.*: **Quantitative real-time RT-PCR detection of breast cancer micrometastasis using a multigene marker panel.** *Int J Cancer* 2001, **93**:162-171.
11. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW: **Specific association of human telomerase activity with immortal cells and cancer.** *Science* 1994, **266**:2011-2015.
12. Tatsuma T, Goto S, Kitano S, Lin YC, Lee CM, Chen CL: **Telomerase activity in peripheral blood for diagnosis of hepatoma.** *J Gastroenterol Hepatol* 2000, **15**:1064-1070.
13. Ng EK, Tsui NB, Lam NY, Chiu RW, Yu SC, Wong SC, Lo ES, Rainer TH, Johnson PJ, Lo YM: **Presence of filterable and non filterable mRNA in the plasma of cancer patients and healthy individuals.** *Clin Chem* 2002, **48**:1212-1217.
14. Tsui NB, Ng EK, Lo YM: **Stability of Endogeneous and added RNA in blood specimens, serum, and plasma.** *Clin Chem* 2002, **48**:1647-1653.
15. Nørgaard R, Kassem M, Rattan SI: **Heat shock-induced enhancement of osteoblastic differentiation of hTERT-immortalized mesenchymal stem cells.** *Ann N Y Acad Sci* 2006, **1067**:443-447.
16. Zhang X, Soda Y, Takahashi K, Bai Y, Mitsuru A, Igura K, Satoh H, Yamaguchi S, Tani K, Tojo A, *et al.*: **Successful immortalization of mesenchymal progenitor cells derived from human placenta and the differentiation abilities of immortalized cells.** *Biochem Biophys Res Commun* 2006, **29**:853-859.
17. Gabet AS, Accardi R, Bellopede A, Popp S, Boukamp P, Sylla BS, Londoño-Vallejo JA, Tommasino M: **Impairment of the telomere/telomerase system and genomic instability are associated with keratinocyte immortalization induced by the skin human papillomavirus type 38.** *FASEB J* 2008, **22**:622-632.
18. Gandellini P, Folini M, Bandiera R, De Cesare M, Binda M, Veronese S, Daidone MG, Zunino F, Zaffaroni N: **Down-regulation of human telomerase reverse transcriptase through specific activation of RNAi pathway quickly results in cancer cell growth impairment.** *Biochem Pharmacol* 2007, **73**:1703-1714.
19. Burnworth B, Arendt S, Muffler S, Steinkraus V, Bröcker EB, Birek C, Hartschuh W, Jauch A, Boukamp P: **The multi-step process of human skin carcinogenesis: a role for p53, cyclin D1, hTERT, p16, and TSP-1.** *Eur J Cell Biol* 2007, **86**:763-780.
20. Xu L, Blackburn EH: **Human cancer cells harbor T-stumps, a distinct class of extremely short telomeres.** *Mol Cell* 2007, **26**:315-327.
21. Aravalli RN, Steer CJ, Cressman ENK: **Molecular mechanisms of hepatocellular carcinoma.** *Hepatology* 2008, **48**:2047-2063.
22. Miura N, Nakamura H, Sato R, Tsukamoto T, Harada T, Takahashi S, Adachi Y, Shomori K, Sano A, Kishimoto Y, *et al.*: **Clinical usefulness of serum telomerase reverse transcriptase (hTERT) mRNA and epidermal growth factor receptor (EGFR) mRNA as a novel tumor marker for lung cancer.** *Cancer Sci* 2006, **97**:1366-1373.
23. Miura N, Kanamori Y, Takahashi M, Sato R, Tsukamoto T, Takahashi S, Harada T, Sano A, Shomori K, Harada T, *et al.*: **A diagnostic evaluation of serum human telomerase reverse transcriptase mRNA as a novel tumor marker for gynecologic malignancies.** *Oncol Rep* 2007, **17**:541-548.
24. Tani N, Ichikawa D, Ikoma D, Tomita H, Sai S, Ikoma H, Fujiwara H, Kikuchi S, Okamoto K, Ochiai T, *et al.*: **Circulating cell-free mRNA in plasma as a tumor marker for patients with primary and recurrent gastric cancer.** *Anticancer Res* 2007, **27**:1207-1212.
25. Shomori K, Sakatani T, Goto A, Matsuura T, Kiyonari H, Ito H: **Thymidine phosphorylase expression in human colorectal mucosa, adenoma and carcinoma: role of p53 expression.** *Pathol Int* 1999, **49**:491-499.
26. Yeh TS, Chen TC, Chen MF: **Dedifferentiation of human hepatocellular carcinoma up-regulates telomerase and Ki-67 expression.** *Arch Surg* 2000, **135**:1334-1339.
27. Tahara H, Yasui W, Tahara E, Fujimoto J, Ito K, Tamai K, Nakayama J, Ishikawa F, Tahara E, Ide T: **Immuno-histochemical detection of human telomerase catalytic component, hTERT, in human colorectal tumor and non-tumor tissue sections.** *Oncogene* 1999, **18**:1561-1567.
28. Takeba Y, Sekine S, Kumai T, Matsumoto N, Nakaya S, Tsuzuki Y, Yanagida Y, Nakano H, Asakura T, Ohtsubo T, *et al.*: **Irinotecan-induced apoptosis is inhibited by increased P-glycoprotein expression and decreased p53 in human hepatocellular carcinoma cells.** *Biol Pharm Bull* 2007, **30**:1400-1406.
29. Nakashio R, Kitamoto M, Tahara H, Nakanishi T, Ide T, Kajiyama G: **Significance of telomerase activity in the diagnosis of small differentiated hepatocellular carcinoma.** *Int J Cancer* 1997, **22**:141-147.
30. Plentz RR, Park YN, Lechel A, Kim H, Nellessen F, Langkopf BH, Wilkens L, Destro A, Fiamengo B, Manns MP, *et al.*: **Telomere shortening and inactivation of cell cycle checkpoints characterize human hepatocarcinogenesis.** *Hepatology* 2007, **45**:968-976.
31. Wong Ih-N, Leung T, Ho S, Lau WY, Chan M, Johnson PJ: **Semiquantification of circulating hepatocellular carcinoma cells by reverse transcriptase polymerase chain reaction.** *Br J Cancer* 1997, **76**:628-633.
32. Miura N, Maeda Y, Kanbe T, Yazama H, Takeda Y, Sato R, Tsukamoto T, Sato E, Marumoto A, Harada T, *et al.*: **Serum human telomerase reverse transcriptase messenger RNA as a novel tumor marker for hepatocellular carcinoma.** *Clin Cancer Res* 2005, **1**:3205-3209.
33. Onishi T, Nouso K, Higashi T, Toshikuni N, Nakatsukasa H, Kobayashi Y, Uemura M, Yumoto E, Fujiwara K, Sato S, *et al.*: **Cellular distribution of telomerase reverse transcriptase in human hepatocellular carcinoma.** *J Gastroenterol Hepatol* 2003, **18**:1168-1174.
34. Wege H, Chui MS, Le HT, Strom SC, Zern MA: **In vitro expansion of human hepatocytes is restricted by telomere-dependent replicative aging.** *Cell Transplant* 2003, **12**:897-906.
35. Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC: **High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: significance of hepatitis virus infection, age, p53 and beta-catenin mutations.** *Int J Cancer* 2004, **112**:44-50.
36. Leoni S, Piscaglia F, Righini R, Bolondi L: **Management of small hepatocellular carcinoma.** *Acta Gastroenterol Belg* 2006, **69**:230-235.
37. Okuda H, Nakanishi T, Takatsu K, Saito A, Hayashi N, Yamamoto M, Takasaki K, Nakano M: **Clinicopathologic features of patients with hepatocellular carcinoma seropositive for a-fetoprotein-L3 and seronegative for des-g-carboxyprothrombin in comparison with those seropositive for des-g-carboxy prothrombin alone.** *J Gastroenterol Hepatol* 2002, **17**:772-778.
38. Cavin LG, Romieu-Mourez R, Panta GR, Sun J, Factor VM, Thorgeirsson SS, Sonenshein GE, Arsura M: **Inhibition of CK2 activity by TGF-beta 1 promotes IkappaB-alpha protein stabilization and apoptosis of immortalized hepatocytes.** *Hepatology* 2003, **38**:1540-1551.
39. Prade-Houdellier N, Frébet E, Demur C, Gautier EF, Delhommeau F, Bennaceur-Griscelli AL, Gaudin C, Martinel V, Laurent G, Mansat-De Mas V, *et al.*: **Human telomerase is regulated by erythropoietin and**

transforming growth factor-beta in human erythroid progenitor cells. *Leukemia* 2007, **21**:2304-2310.
40. Heitjan DF: **Generalized Norton-Simon models of tumour growth.** *Stat Med* 1991, **10**:1075-1088.
41. Yu GR, Kim SH, Park SH, Cui XD, Xu DY, Yu HC, Cho BH, Yeom YI, Kim SS, Kim SB, *et al.*: **Identification of molecular markers for the oncogenic differentiation of hepatocellular carcinoma.** *Exp Mol Med* 2007, **31**:641-652.
42. Swisher JF, Khatri U, Feldman GM: **Annexin A2 is a soluble mediator of macrophage activation.** *J Leukoc Biol* 2007, **82**:1174-1184.
43. Lin SY, Elledge SJ: **Multiple tumor suppressor pathways negatively regulate telomerase.** *Cell* 2003, **27**:881-889.
44. Takahashi S, Kitamoto M, Takaishi H, Aikata H, Kawakami Y, Nakanishi T, Shimamoto F, Tahara E, Tahara H, Ide T, *et al.*: **Expression of telomerase component genes in hepatocellular carcinoma.** *Eur J Cancer* 2000, **36**:496-502.
45. Waguri N, Suda T, Nomoto M, Kawai H, Mita Y, Kuroiwa T, Igarashi M, Kobayashi M, Fukuhara Y, Aoyagi Y: **Sensitive and specific detection of circulating cancer cells in patients with hepatocellular carcinoma; detection of human telomerase reverse transcriptase messenger RNA after immunomagnetic separation.** *Clin Cancer Res* 2003, **9**:3004-3011.
46. Mizukoshi E, Nakamoto Y, Marukawa Y, Arai K, Yamashita T, Tsuji H, Kuzushima K, Takiguchi M, Kaneko S: **Cytotoxic T cell responses to human telomerase reverse transcriptase in patients with hepatocellular carcinoma.** *Hepatology* 2006, **43**:1284-1294.
47. Piao YF, He M, Shi Y, Tang TY: **Relationship between microvessel density and telomerase activity in hepatocellular carcinoma.** *World J Gastroenterol* 2004, **15**:2147-2149.
48. Fujita Y, Fujikane T, Fujiuchi S, Nishigaki Y, Yamazaki Y, Nagase A, Shimizu T, Ohsaki Y, Kikuchi K: **The diagnostic and prognostic relevance of human telomerase reverse transcriptase mRNA expression detected in situ in patients with non small cell lung carcinoma.** *Cancer* 2003, **98**:1008-1013.
49. Miura N, Kabashima H, Shimizu M, Sato R, Tsukamoto T, Harada T, Takahashi S, Endo R, Nakayama N, Takikawa Y, *et al.*: **Clinical impact of serum transforming growth factor-alpha mRNA as a predictive biomarker for the prognosis of fulminant hepatitis.** *Hepatol Int* 2008, **2**:213-221.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-230X/10/46/prepub

doi: 10.1186/1471-230X-10-46
Cite this article as: Miura *et al.*, A novel biomarker TERTmRNA is applicable for early detection of hepatoma *BMC Gastroenterology* 2010, **10**:46

ORIGINAL PAPER

THE INTERNATIONAL JOURNAL OF CLINICAL PRACTICE

Design and rationale for the non-interventional Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib (GIDEON) study

R. Lencioni,[1] J. Marrero,[2] A. Venook,[3] S.-L. Ye,[4] M. Kudo[5]

[1]Division of Diagnostic Imaging and Intervention, Department of Liver Transplantation, Hepatology and Infectious Diseases, Pisa University School of Medicine, Pisa, Italy
[2]Multidisciplinary Liver Tumor Clinic, University of Michigan, Ann Arbor, MI, USA
[3]University of California, San Francisco, CA, USA
[4]Liver Cancer Institute, Zhongshan Hospital, Fudan University, Shanghai, China
[5]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Correspondence to:
Professor Riccardo Lencioni, Division of Diagnostic Imaging and Intervention, Department of Liver Transplantation, Hepatology and Infectious Diseases, Pisa University School of Medicine, Cisanello Hospital, Building No. 30C, Suite 197, Via Paradisa 2, IT-56124 Pisa, Italy
Tel.: + 39 050 997 321
Fax: + 39 050 997 320
Email: lencioni@med.unipi.it

Disclosures
JM has received consulting and research grant from Bayer Healthcare/Onyx; AV has received research funding from Bayer Healthcare/Onyx; MK has received lecture fee from Bayer Healthcare/Onyx.

SUMMARY

Background: Hepatocellular carcinoma (HCC) is a complicated condition influenced by multiple confounding factors, making optimum patient management extremely challenging. Ethnicity, stage at diagnosis, comorbidities and tumour morphology affect outcomes and vary from region to region, and there is no common language to assess patient prognosis and make treatment recommendations. Despite recent efforts to reduce the incidence of HCC, most patients present with unresectable disease. Non-surgical treatments include ablation, transarterial chemoembolisation and the multikinase inhibitor, sorafenib, but their effects in all patient subgroups are not known and further information is needed to optimise the use of these treatments. **Aims:** The Global Investigation of Therapeutic DEcisions in Hepatocellular Carcinoma and Of its Treatment with SorafeNib (GIDEON) study (ClinicalTrials.gov identifier NCT00812175; http://clinicaltrials.gov/) is an ongoing global, prospective, non-interventional study of patients with unresectable HCC who are eligible for systemic therapy and for whom the decision has been taken to treat with sorafenib under real-life practice conditions. The aim of this study is to evaluate the safety and efficacy of sorafenib in different subgroups, especially Child-Pugh B where data are limited. **Discussion:** This study will recruit 3000 patients from > 40 countries and follow them for approximately 5 years to compile a large and robust database of information that will be used to analyse local, regional and global differences in baseline characteristics, disease aetiology, treatment practice patterns and treatment outcomes, with a view to improve the knowledge base used to guide physician treatment decisions and to improve patient outcomes.

What's known
- HCC is a complex disease influenced by multiple confounding factors that vary from region to region, making optimum patient management extremely complex.
- Sorafenib is an oral multikinase inhibitor with proven efficacy in patients with unresectable HCC, but data in Child-Pugh B are limited.
- There is a need to fully evaluate existing treatments in all patient subgroups to optimise their use.

What's new
- GIDEON will generate data from 3000 patients to evaluate the effects of sorafenib in different patient subgroups, and the resulting large database will be used to analyse local, regional and global differences that influence patient prognosis and management, with a view to refine HCC staging and evaluation and better inform treatment decisions

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, but because of the poor prognosis associated with this disease, it is the third most common cause of cancer-related death (1). Over 80% of patients with HCC are in developing countries, with particularly high incidence rates in sub-Saharan Africa and Southeast Asia (1). There is a low incidence of HCC in developed countries such as the USA, Australia and the UK, but these rates are rising (2).

Risk factors for the development of HCC have been well documented and include the presence of cirrhosis, infection with hepatitis B and C viruses, heavy alcohol intake, diabetes and obesity (1,2). Although surveillance and vaccination programmes have reduced the incidence of HCC in certain populations (3,4), the majority of patients still present with unresectable disease and are unsuitable for surgery. Current treatments for unresectable disease include loco-regional interventions and systemic therapies, although further data on all treatments are required to fully understand their potential, e.g. in patient groups not included in clinical trials.

Non-surgical loco-regional treatment options include ablation therapy and transarterial chemoembolisation (TACE). Ablation therapy is associated with a 5-year survival rate of 40–70%, with best responses seen among patients with single tumours

© 2010 Blackwell Publishing Ltd *Int J Clin Pract*, July 2010, **64**, 8, 1034–1041
doi: 10.1111/j.1742-1241.2010.02414.x

and preserved liver function (5). However, TACE is recommended for patients with large/multifocal tumours with no vascular invasion or extra-hepatic spread and is associated with objective response rates of 16–60% (3). Although survival benefits have been reported in only two randomised controlled trials (RCTs) (6,7), a robust meta analysis showed that the treatment with TACE was associated with significant improvements in 2-year survival vs. control (8). However, TACE treatment has a number of important limitations. Residual tumour growth following treatment means that treatment repetition is necessary. Additionally, many of the clinical studies investigating TACE have used a wide range of treatment strategies, including different types of embolic particle, chemotherapy, emulsifying agent and numbers of treatment sessions. For this reason, there is no clear evidence to support an optimum treatment strategy (9). Also, TACE therapy is only possible in patients where the arterial blood supply to the tumour can be isolated, and is not recommended in patients with portal vein thrombosis, those with Child-Pugh C liver function or those with a total serum bilirubin level > 3 mg/ml, as all of these factors have been identified as predictors of poor prognosis in patients treated with TACE (9). Finally, treatment with TACE is associated with considerable side effects; the most commonly reported being postembolisation syndrome that occurs in $> 50\%$ of treated patients (3). Other less frequent but more serious complications include hepatic abscess and cholecystitis. Further research is therefore needed to optimise TACE treatment strategy and ensure treatment efforts are directed at patients who will most likely benefit.

Systemic therapies investigated for unresectable HCC have included single-agent and combination chemotherapy regimens, but their efficacy has been disappointing and their use is no longer recommended (9). More recently, the Sorafenib HCC Assessment Randomized Protocol trial, a multicentre, Phase III, double-blind, placebo-controlled trial of 602 Western patients with unresectable HCC, showed that treatment with the oral multikinase inhibitor, sorafenib (Nexavar®; Onyx Pharmaceuticals, Inc., Emeryville, CA, USA; Bayer HealthCare Pharmaceuticals, Inc., Wayne, NJ, USA; Bayer Schering Pharma AG, Berlin, Germany), was associated with a significant improvement in survival compared with placebo [median overall survival (OS) of 10.7 months vs. 7.9 months for sorafenib and placebo respectively, $p < 0.001$] (10). As a result, sorafenib is the first systemic anticancer therapy indicated for treating these patients (9). Similar benefits (median OS of 6.5 months vs. 4.2 months for sorafenib and placebo respectively, $p = 0.014$) were reported in a Phase III, randomised, double-blind, placebo-controlled trial of 226 patients with unresectable HCC from the Asia-Pacific region, thus confirming the efficacy of sorafenib in a broad geographic patient population (11). However, all patients included in these two large RCTs had preserved liver function (Child-Pugh A), and our knowledge regarding the efficacy of sorafenib in patients with hepatic impairment is limited to small subgroups of patients from Phase I and II studies (12,13). Further studies to evaluate the efficacy of sorafenib among all patient groups are therefore needed.

Current treatment guidelines for unresectable disease are therefore based on the best available evidence, including non-randomised trials, case studies and expert opinion; however, significant data gaps exist. Further evidence is needed to fully evaluate current treatment options and optimise their use to improve patient outcomes.

Against this background, the Global Investigation of Therapeutic DEcisions in Hepatocellular Carcinoma and Of its Treatment with SorafeNib (GIDEON) study is an ongoing global, non-interventional study (NIS) of patients with unresectable HCC who are to receive sorafenib as part of their standard clinical care. The study should produce the largest, most robust database of information on factors influencing treatment and outcome of patients with HCC. This manuscript will include details of the GIDEON aims and objectives, study design, target recruitment and timeline. It will also describe the planned analyses and discuss how it is hoped that findings from this study will allow us to gain a detailed understanding of the factors that influence the prognosis and management of these patients, and how this in turn will help us to refine HCC staging and evaluation, better inform treatment choices and ultimately improve outcomes for patients with HCC.

The GIDEON study

Aims, objectives and rationale for GIDEON

Non-interventional studies, or observational studies, are postauthorisation safety studies (PASS) that are usually conducted to gain further information about a licensed product. Observational studies are characterised by the fact that assignment to a particular therapy strategy is not mandated by a study protocol but reflects the participating physician's current practice. The physician alone decides which treatment, if any, is appropriate. In NIS, the decision to include a patient in a study is separate from the treatment decision. Furthermore, no additional diagnostic or monitoring interventions are mandated for the patient as a result of inclusion in an NIS. NIS serve a wide range of purposes but are of particular value

in providing information in wider populations or subgroups not covered in RCTs (14). NIS also enable information to be gathered on other parameters not usually assessed in the clinical trial setting, such as patient acceptance and compliance, physician adherence to information and directions for use and prescription behaviour. The importance of NIS in the literature is increasingly recognised, and guidelines were developed to improve the analysis and reporting of observational studies (15). NIS provide opportunities to enhance the evidence base for established drugs and therapies and increase understanding of the impact of treatments in real-world practice (14).

GIDEON is a global, prospective NIS of patients with unresectable HCC who are candidates for systemic therapy and for whom the decision has been taken to treat with sorafenib. It was initiated to fulfil the postapproval commitment to organisations such as the European Medicines Agency to gather data on the safety and efficacy of sorafenib in patients with Child-Pugh B liver function. Additional goals are to compile a large and robust database of HCC treatment patterns and outcomes among patients with unresectable disease who are candidates for systemic therapy, to answer clinically relevant questions and gain a better understanding of the safety of sorafenib with loco-regional therapies, given either concomitantly or sequentially. Data will also be gathered in the USA and possibly other regions on the characteristics, disease course and treatment outcomes of patients with newly diagnosed HCC or recurring disease after curative treatments, who are not candidates for systemic therapy with sorafenib.

Based on these goals, the primary objective of GIDEON is to evaluate the safety of sorafenib in patients with unresectable HCC in real-life practice conditions. Secondary objectives are to: evaluate the efficacy [OS, progression-free survival (PFS), time to progression (TTP), response rate and stable disease rate] of sorafenib in these patients; determine the duration of therapy according to various patient characteristics; evaluate methods of patient evaluation, diagnosis and follow-up; assess comorbidities and their influence on treatment and outcome in real-life practice rather than a controlled clinical trial setting and evaluate the practice patterns of the physicians involved in the care of these patients.

This study was conducted according to established regulations and recommendations relating to the conduct of NIS; volume 9A of the Rules Governing Medicinal Products in the European Union (16). When required, documented approval from the appropriate ethics committee(s)/institutional review board was obtained for all participating centres prior to the study, according to Good Clinical Practice and local laws, regulations and organisations.

Establishing a global NIS

GIDEON is a Phase IV, international, prospective, open-label, multicentre, non-interventional PASS of patients with unresectable HCC receiving sorafenib under real-life conditions. Approximately, 3000 eligible patients will be recruited by participating physicians from > 40 countries across Europe, Latin America and the Asia-Pacific region and from the USA (Figure 1) and will be observed from the start

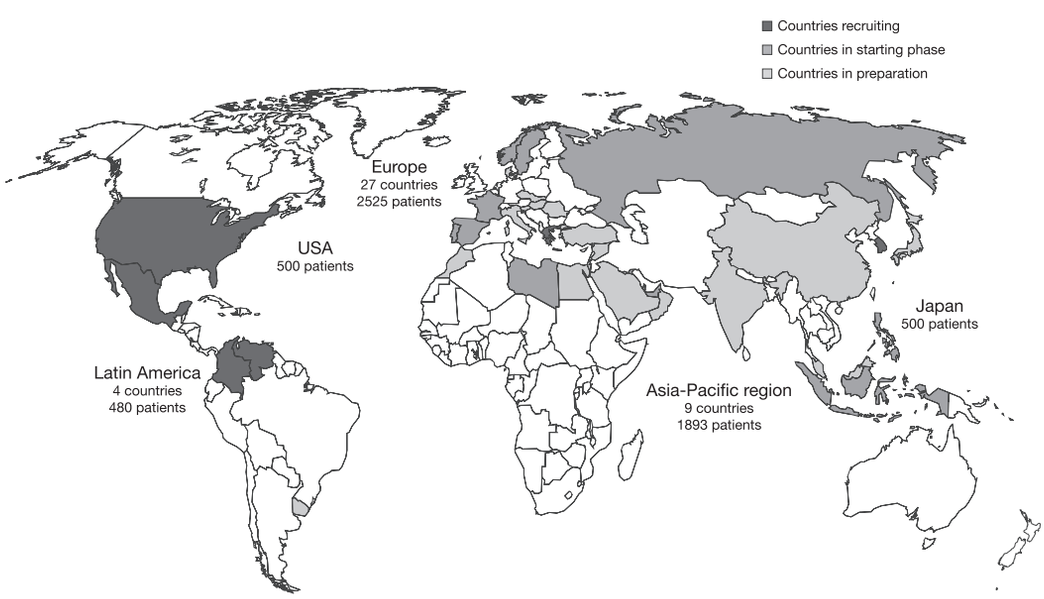


Figure 1 The global reach of GIDEON

of sorafenib therapy to patient withdrawal, loss to follow-up, death or final visit.

An overview of the GIDEON timeline and planned analyses is shown in Figure 2. The first patient's first visit was recorded in January 2009 and the last patient's first visit is anticipated to occur in quarter four of 2012. Interim analyses for safety will be conducted after 500 and 1500 patients have been recruited and followed for 4 months, with the final analysis anticipated in quarter two or three of 2014. The study will end 12 months after enrolment of the 3000th eligible patient, irrespective of whether the final patient dies or not, is lost to follow-up or survives.

Patients to be included in GIDEON

Patients with histologically or cytologically documented or radiographically diagnosed unresectable HCC who are candidates for systemic therapy, and for whom a decision has been made to treat with sorafenib, are eligible for inclusion in GIDEON if they have a life expectancy of > 8 weeks and have provided signed informed consent. Patient exclusion criteria are based on the approved local product information for sorafenib.

Data collection

All data will be collected using case report forms (CRFs). These will be available as paper and electronic versions, with participating countries and their sites able to choose the format of preference. Data will be collected from all enrolled patients at study entry and start of sorafenib, then at intervals normally used by the prescribing physician (estimation: ≥ 6 to ≤ 12 weeks), or until patient death, withdrawal or loss to follow-up, or if significant changes in a patient's disease are observed. All data will be verified through spot site monitoring, which will take place at up to 10% of all sites involved in the study. An overview of the visit schedule and data collected at each visit is shown in Figure 3. Study end-points are summarised in Figure 4. All adverse events (AEs) will be graded according to the National Cancer Institute Common Terminology Criteria version 3.0 (National Cancer Institute, Bethesda, MD, USA), and their likely relationship to sorafenib therapy will be documented. Tumour assessments will be made by computed tomography or other equivalent radiographical method and will be evaluated using the Response Evaluation Criteria in Solid Tumors.

The population of patients who entered this study and received at least one dose of sorafenib will be valid for intent-to-treat safety and efficacy analysis. However, patients who received sorafenib in the past will be excluded from efficacy analysis. All baseline demographic data will be summarised for the intent-to-treat population. AEs and other safety parameters, including blood pressure, Child-Pugh grade and Eastern Cooperative Oncology Group performance status (ECOG PS), will be summarised using the safety population.

Planned subgroup analyses conducted globally, regionally and by country will include: the impact of baseline characteristics on safety, particularly Child-Pugh B; the relationship between baseline characteristics and efficacy; the duration of sorafenib therapy and reasons for discontinuation; the effect of other treatments for HCC on outcome and the impact of different practice patterns on outcome. In addition, subgroup analyses for specific regions may be conducted, such as: an evaluation of common treatments for HCC in Asia; referral and diagnostic patterns in Europe; duration of treatment, tolerability and compliance in Latin America and patient selection for loco-regional therapy in Japan. However, all subgroup analyses performed will depend on the actual data collected.

Statistical considerations for GIDEON

An overall sample size of 3000 patients with unresectable HCC treated with sorafenib is expected to be

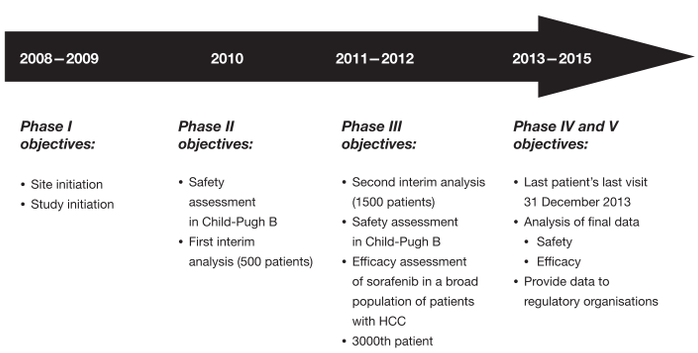
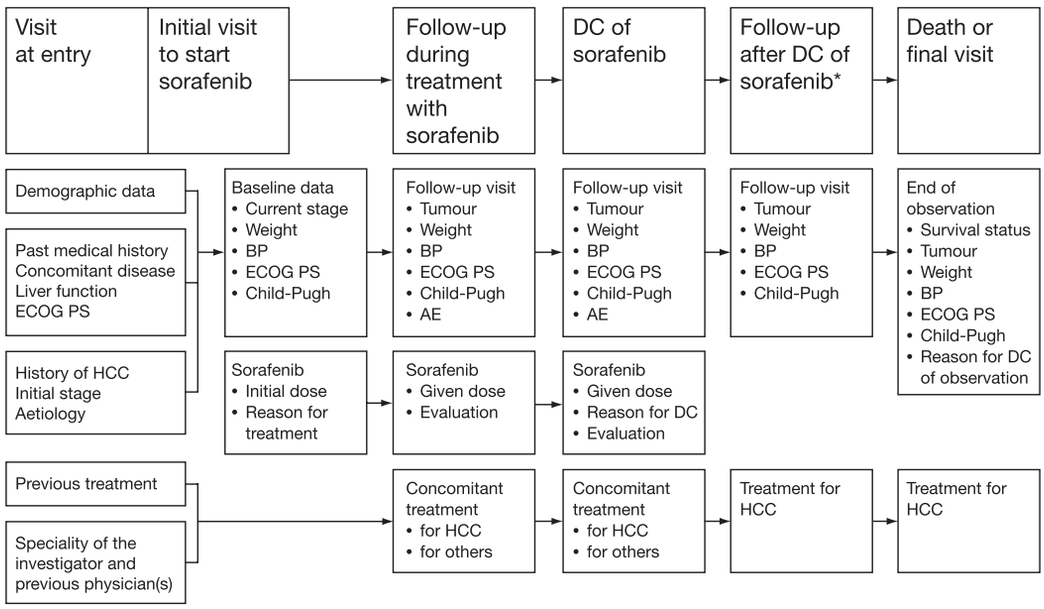
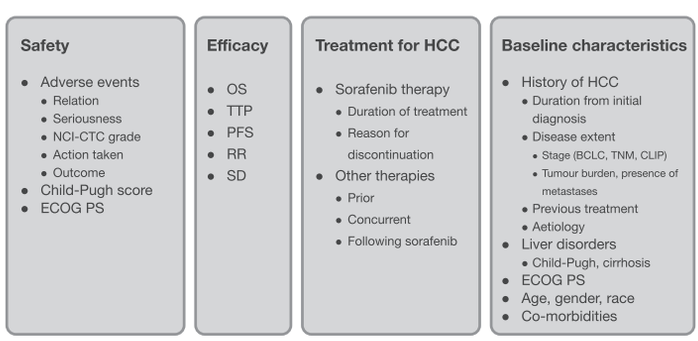


Figure 2 An overview of the GIDEON timeline and planned analyses



*Applicable if patient discontinues therapy, is alive and not lost to follow-up
AE, adverse event; BP, blood pressure; DC, discontinuation;
ECOG PS, Eastern Cooperative Oncology Group performance status; HCC, hepatocellular carcinoma

Figure 3 GIDEON patient assessment schedule



BCLC, Barcelona Clinic Liver Cancer; CLIP, Cancer of the Liver Italian Program; ECOG PS, Eastern Cooperative Oncology Group performance status; HCC, hepatocellular carcinoma; NCI-CTC, National Cancer Institute-Common Toxicity Criteria; OS, overall survival; PFS, progression-free survival; RR, response rate; SD, stable disease; TNM, tumour node metastases; TTP, time to progression

Figure 4 Overview of the GIDEON safety, efficacy, treatment and baseline patient assessments and end-points

sufficient to allow for evaluation of safety of the overall population as well as specific subgroups. With this sample size, there would be an 84% chance of observing an AE with a true incidence of 1% in at least 25 patients.

All baseline, safety and efficacy data were analysed using descriptive statistics. Kaplan–Meier estimates were calculated for the OS, PFS and TTP efficacy end-points. At the time of the analyses, any patient alive or lost to follow-up, without disease progression or death or without documented radiological progression will be censored at the last date of evaluation for OS, PFS and TTP analyses, respectively. Exploratory subgroup analyses of efficacy and safety data may also be performed, stratified by prognostic/predictive baseline factors such as stage, Child-Pugh score, ECOG PS, region and age, as appropriate. Data regarding administration of sorafenib such as duration, given dose, continuation or discontinuation of therapy and dose modification of sorafenib therapy, including reason(s) for discontinuation, will be summarised in a descriptive manner. Treatments for HCC other than sorafenib before, during and after therapy with sorafenib will be summarised descriptively as per available data. The sample size was calculated to collect data to allow for sufficient

evaluation of safety monitoring of all treated patients. Interim analyses are planned during the study primarily for summarising and monitoring safety data, and will be conducted after 500 patients and 1500 patients are enrolled and have been treated on study for at least 4 months.

Discussion

What will GIDEON achieve?

GIDEON is a global PASS initiated to collect more information on the safety and efficacy of sorafenib in patients with unresectable HCC. This is the largest prospective HCC NIS in the world, which will enrol 3000 patients from > 40 countries globally. The compilation of a database of this size in HCC has not previously been undertaken; as no other global registries exist in this area, it is anticipated that the data collected here will be an important contribution to our knowledge and will help to answer important and clinically relevant questions relating to the natural disease course of HCC and liver dysfunction, long-term efficacy and safety of sorafenib therapy, physicians' practice patterns and patients' perspectives.

The number of factors influencing HCC and its disease course make it extremely difficult to accurately assess patient prognosis and make optimum treatment recommendations. The geographical variation of these factors has also prevented the establishment of a universal system to assess all patients. Findings from GIDEON could help to establish a globally applicable staging classification, which could facilitate the accurate and consistent assessment of all patients and help to provide a common language for the broad HCC multidisciplinary team on which to base treatment recommendations. In addition, as GIDEON will collect data regarding the differences in physician treatment practice patterns and outcomes, it may be possible to analyse these data with a view to optimise the role of all members of this large multidisciplinary team and streamline patient care.

Although potentially curative therapy via surgical resection or transplant is possible for some patients with HCC, there is a lack of cadaveric transplants available, and the majority of patients are unsuitable for surgery at presentation. In addition, the recurrence rate of HCC after curative treatment is high and the long-term curable rate is low (17). Thus, non-surgical treatments play a central role in the management of these patients. However, there is still a relative shortage of RCTs to fully evaluate these treatments in all patient subgroups, and more work is needed to fully understand the benefits of each of these treatments and to establish their place in the HCC treatment armamentarium. One of the main focuses of GIDEON is to gain further information on the optimum duration of sorafenib therapy as well as the safety and efficacy of sorafenib in different subgroups of patients, especially patients who are generally excluded from RCTs to minimise errors or confounding factors in the study, i.e. patients who have moderate liver dysfunction (Child-Pugh B) where data are currently limited. However, information is also being gathered regarding the safety and outcomes following other treatments before, during and after sorafenib therapy; thus, it is anticipated that the information gathered in this study will help to improve our understanding of the risks and benefits associated with each of these treatment approaches, which could help us to establish an optimum treatment algorithm for these patients.

In addition to GIDEON, a large clinical trial programme for sorafenib is ongoing that should help us to fully establish its optimum place in therapy. One area of interest is the role of sorafenib as adjuvant therapy to improve survival of patients with HCC. To date, treatments such as radiotherapy and chemotherapy and their combination have been used to reduce tumour size and improve patients' quality of life (18). TACE has recently been shown to be the only palliative treatment that can benefit HCC patients ineligible for curative treatments because of advanced tumour stage or poor hepatic functional reserve; however, the survival gain appears marginal (19) and other effective treatments are urgently needed. Against this background, a large Phase III, randomised, double-blind, placebo-controlled trial of adjuvant sorafenib following either surgical resection or local ablation is currently in progress (STORM study) (20). The primary end-point is recurrence-free survival, with secondary end-points including time to recurrence and OS. Estimated accrual to this trial is 1100 patients and data are due to be reported in 2014.

Another area of interest is the efficacy of sorafenib in combination with, and subsequent to, TACE therapy. A large randomised Phase II trial has been initiated to evaluate the role of sorafenib in combination with TACE in the treatment of patients with intermediate disease (SPACE study) (21). Estimated enrolment to this trial is 350 patients, and final results are expected in late 2010. In addition to this, a Phase III, double-blind, randomised, placebo-controlled trial of sorafenib following TACE in Japanese patients with unresectable, advanced disease is ongoing (Japan post-TACE study) (22). The target recruitment of 414 patients has already been reached and final results are anticipated in early 2010.

Finally, the effects of sorafenib in combination with other targeted agents in HCC are also of

interest, and a large Phase III, randomised, double-blind trial evaluating the efficacy, safety and health-related quality of life of sorafenib plus erlotinib vs. sorafenib plus placebo for the first-line treatment of advanced HCC is in progress (SEARCH study) (23). The target recruitment for this study is 700 patients and the estimated final data collection date is July 2011.

These ongoing studies form a comprehensive and well-integrated clinical trial programme, which will provide data from several points in the treatment pathway. GIDEON is also a key component of this programme, as it will provide data from the entire unresectable patient population treated under real-life conditions, not in a non-restrictive setting and within the approved indication, and will enable the collection of data from populations not commonly included in RCTs, such as those with Child-Pugh B liver function. Thus, in addition to the ongoing interventional studies, the information collected in GIDEON will significantly contribute to the current body of evidence, which helps inform treatment decisions.

What are the limitations of GIDEON?

The information gathered in GIDEON will form a large and robust database that will be analysed to improve our understanding of the global, regional and local differences in patient demographics, disease course and treatment outcomes, with a view to improve our knowledge base and improve patient outcomes. However, as this is an observational NIS, it is associated with a number of limitations. The lack of randomisation to specific treatment arms and the lack of a placebo-control arm will limit any robust evaluation of the efficacy of any of the treatments received by these patients during the course of the study. Comparisons between sorafenib-treated and untreated patients in the USA will be limited because of the small sample size of patients in the USA who will not receive sorafenib. The value of some subgroup analyses may also be limited by small patient numbers, although these analyses may still be hypothesis-generating and could help direct future research. However, given these limitations, it will be important to consider findings from GIDEON together with emerging data from large RCTs, to fully evaluate the safety and efficacy of these treatments and draw any definitive conclusions.

Another possible limitation in GIDEON is that all data will be collected via the completion and submission of CRFs, which could delay data collection and evaluation. However, electronic versions of the CRF have been compiled with a view to facilitate this process and to reduce any lengthy delays in completing and analysing the data collected.

As with any observational study, a number of biases may also exist. The lack of blinding of either the treating physician or the patient to study treatment may introduce a bias in reporting treatment outcomes. It is also possible that physicians engaged in GIDEON may be more likely to choose sorafenib therapy for a larger proportion of their patients than would be representative of normal treatment practice patterns in that area, although all patients receiving sorafenib therapy must be eligible according to the locally approved product information for sorafenib. Finally, while study procedures regarding data collection and verification are in place for GIDEON, it should be noted that in an NIS, potential exists for less robust data than might be expected from an RCT.

Conclusions

GIDEON is the largest global, prospective, open-label NIS ever conducted among patients with unresectable HCC. The study was initiated to further evaluate the safety and efficacy of sorafenib in different patient subgroups, including Child-Pugh B. The collection of information regarding patient baseline demographics, disease aetiology, treatments and outcomes from approximately 3000 patients worldwide, over a period of approximately 5 years, will also enable the compilation of a large and robust database that will be used to analyse local, regional and global differences, with a view to answer some important questions and fill significant data gaps. It is therefore anticipated that findings from GIDEON, together with data from large RCTs, will help improve the knowledge base used to guide physician treatment decisions and may enable physicians to make better informed treatment choices and ultimately improve patient outcomes.

Acknowledgements

The authors take full responsibility for the scope, direction and content of the manuscript and have approved the submitted manuscript. They would like to thank Karen Brayshaw, PhD, at Complete HealthVizion for her assistance in the preparation and revision of the draft manuscript, based on detailed discussion and feedback from all the authors. Editorial assistance was supported by a grant from Bayer HealthCare Pharmaceuticals.

Research funding

The GIDEON study is funded by Bayer HealthCare Pharmaceuticals and Onyx Pharmaceuticals.

Author contributions

All authors are members of the Global Steering Committee for the GIDEON study and have been involved in the discussion and modification of the GIDEON protocol. All authors provided critical review of the manuscript and approved the final version for publication.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74–108.

2 El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557–76.

3 Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208–36.

4 Chang MH, You SL, Chen CJ et al. Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: a 20-year follow-up study. *J Natl Cancer Inst* 2009; **101**: 1348–55.

5 Lopez PM, Villanueva A, Llovet JM. Systematic review: evidence-based management of hepatocellular carcinoma–an updated analysis of randomized controlled trials. *Aliment Pharmacol Ther* 2006; **23**: 1535–47.

6 Lo CM, Ngan H, Tso WK et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164–71.

7 Llovet JM, Real MI, Montañá X et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734–9.

8 Llovet JM, Bruix J, for the Barcelona-Clinic Liver Cancer Group. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003; **37**: 429–42.

9 National Comprehensive Cancer Network. *NCCN Guidelines in Oncology 2009*. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp (accessed April 2010).

10 Llovet JM, Ricci S, Mazzaferro V et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378–90.

11 Cheng AL, Kang YK, Chen Z et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25–34.

12 Abou-Alfa GK, Schwartz L, Ricci S et al. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293–300.

13 Furuse J, Ishii H, Nakachi K et al. Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. *Cancer Sci* 2008; **99**: 159–65.

14 Ligthelm RJ, Borzi V, Gumprecht J et al. Importance of observational studies in clinical practice. *Clin Ther* 2007; **29 Spec No**: 1284–92.

15 Vandenbroucke JP, von Elm E, Altman DG et al. Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. *Ann Intern Med* 2007; **147**: W163–94.

16 European Medicines Agency. *Volume 9A of the Rules Governing Medicinal Products in the European Union*. http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-9/pdf/vol9a_09-2008.pdf (accessed April 2010).

17 Spangenberg HC, Thimme R, Blum HE. Evolving therapies in the treatment of hepatocellular carcinoma. *Biologics* 2008; **2**: 453–62.

18 Saito H, Masuda T, Tada S et al. Hepatocellular carcinoma in Keio affiliated hospitals–diagnosis, treatment, and prognosis of this disease. *Keio J Med* 2009; **58**: 161–75.

19 Shin SW. The current practice of transarterial chemoembolization for the treatment of hepatocellular carcinoma. *Korean J Radiol* 2009; **10**: 425–34.

20 NCT00692770. *Sorafenib as Adjuvant Treatment in the Prevention of Recurrence of Hepatocellular Carcinoma (STORM)*. http://clinicaltrials.gov/ct2/show/NCT00692770?term=nct00692770&rank=1 (accessed April 2010).

21 NCT00855218. *A Phase II Randomized, Double-blind, Placebo-controlled Study of Sorafenib or Placebo in Combination With Transarterial Chemoembolization (TACE) Performed With DC Bead and Doxorubicin for Intermediate Stage Hepatocellular Carcinoma (HCC)*. http://clinicaltrials.gov/ct2/show/NCT00855218?term=nct00855218&rank=1 (accessed April 2010).

22 NCT00494299. *Phase III Study of BAY 43-9006 in Japanese Patients With Advanced Hepatocellular Carcinoma*. http://clinicaltrials.gov/ct2/show/NCT00494299?term=nct00494299&rank=1 (accessed April 2010).

23 NCT00901901. *Nexavar-Tarceva Combination Therapy for First Line Treatment of Patients Diagnosed With Hepatocellular Carcinoma (SEARCH)*. http://clinicaltrials.gov/ct2/show/NCT00901901?term=nct00901901&rank=1 (accessed April 2010).

Paper received January 2010, accepted March 2010

Int J Clin Oncol (2010) 15:242–255
DOI 10.1007/s10147-010-0089-y

REVIEW ARTICLE

Current status of molecularly targeted therapy for hepatocellular carcinoma: clinical practice

Masatoshi Kudo

Received: 15 April 2010 / Published online: 28 May 2010

Abstract In recent years, molecular-targeted agents have been used clinically to treat various malignant tumors. In May 2009, sorafenib (Nexavar®) was approved in Japan for "unresectable hepatocellular carcinoma (HCC)", and was the first molecular-targeted agent for use in liver cancer. To date, sorafenib is the only molecular-targeted agent whose survival benefit has been demonstrated in two global phase III randomized controlled trials, and it has now been approved worldwide. Phase III clinical trials are now underway to compare other molecular-targeted agents with sorafenib as first-line treatment agents, and to evaluate other multi-kinase inhibitors of the vascular endothelial growth factor and platelet-derived growth factor receptors, as well as drugs targeting the epidermal growth factor receptor, insulin-like growth factor receptor, and mammalian target of rapamycin, in addition to other molecules targeting other components of the signal transduction pathways. This review outlines the main pathways involved in the development and progression of HCC and the agents that target these pathways.

Keywords Hepatocellular carcinoma · Molecular-targeted agent · Sorafenib · Sunitinib · Brivanib · Complete remission

M. Kudo (✉)
Department of Gastroenterology and Hepatology,
Kinki University School of Medicine, 377-2,
Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan
e-mail: m-kudo@med.kindai.ac.jp

Introduction

Advances in molecular cell biology over the last decade have clarified the mechanisms involved in cancer growth, invasion, and metastasis, and enabled the development of molecular-targeted agents, best represented by trastuzumab for breast cancer, imatinib and rituximab for hematopoietic tumors, and gefitinib and erlotinib for lung cancer. These molecular-targeted agents are broadly classified into two categories: drugs targeting cancer cell-specific molecules and nonspecific molecular-targeted drugs for molecular biological abnormalities induced in the host stroma or blood vessels by the presence of cancer. Examples of the former approach include: trastuzumab, which targets HER2, the expression of which is a poor prognostic factor for breast cancer; rituximab, which is used to treat B cell lymphoma, and targets CD20 expressed on normal and neoplastic mature B cells; and imatinib, which binds to the ATP-binding site of Bcr-abl, a protein that causes chronic myelogenous leukemia. However, no critical target molecules responsible for treatment response have been identified in hepatocellular carcinoma (HCC).

In recent years, clinical trials have been conducted for many agents that act on growth factor receptors (for example epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor (VEGFR)) and intracellular signaling pathways. In addition, multi-kinase inhibitors, including sorafenib, have emerged and have been evaluated. Clinical trials are now ongoing to compare drugs with the same mechanism of action and to test the combined efficacy and relative merits of these drugs with existing drugs for many cancers. Because the main treatment option for metastatic, advanced stage cancers, for example breast and colorectal cancer, is systemic chemotherapy, clinical trials are ongoing to investigate how to

combine molecular-targeted agents with standard therapies based on the results of long-term, large-scale clinical trials, and to identify which molecular-targeted agents should be used as initial or second-line therapy. However, for HCC, background liver damage limits the indication for systemic chemotherapy and no anti-cancer drugs were found to be effective in a large-scale randomized controlled trial (RCT). However, now that the usefulness of sorafenib has been demonstrated in clinical trials, the development of drugs that are effective for poor-prognosis advanced HCC with distant metastasis and vascular invasion is eagerly awaited.

Signaling pathways and molecular-targeted agents in HCC

As in other cancers, the molecular mechanisms involved in the development and progression of HCC are complex. It has been shown that, after HBV/HCV infection and alcohol or aflatoxin B1 exposure, genetic and epigenetic changes occur, including oncogene activation and tumor-suppressor gene inactivation, because of an inflammation-induced increase in hepatocyte turnover and oxidative stress-induced DNA damage. Through apoptosis and cell proliferation, these changes lead to the multistep development and progression of a hyperplastic to dysplastic nodule, early HCC, and advanced HCC. A number of studies have reported changes in gene expression, chromosomal amplification, mutations, deletions and copy number alterations (gain/loss), somatic mutations, CpG hypermethylation, DNA hypomethylation, and molecular abnormalities, which can constitute therapeutic targets [1–5].

The binding of growth factors to their receptor proteins activates protein-phosphorylating enzymes, thus activating a cascade of proliferative signaling pathways to transmit proliferative signals into the nucleus. Growth factors, for example epidermal growth factor (EGF), transforming growth factor (TGF)-α/β, insulin-like growth factor (IGF), and vascular endothelial growth factor (VEGF), also function in liver regeneration after injury, whereas fibroblast growth factor (FGF) and the platelet-derived growth factor (PDGF) family are involved in liver fibrosis and HCC growth [6–8]. The receptors for these growth factors are broadly classified into G-protein-coupled receptors and protein kinases. On ligand binding, these receptors activate their downstream intracellular molecules in a cascade fashion. Many of the growth factor receptors and oncogenes have tyrosine kinase activity, and the tyrosine kinases are classified into transmembrane receptor tyrosine kinases such as the EGFR and VEGFR, and cytoplasmic non-receptor tyrosine kinases such as Abl and Src. On the other hand, Raf, MAP kinase/ERK kinase (MEK), and mammalian target of rapamycin (mTOR) are serine/threonine kinases.

In general, the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/Akt/mTOR, c-MET, IGF, Wnt-β-catenin, and hedgehog signaling pathways, and the VEGFR and PDGFR signaling cascades show altered activity in HCC, and agents targeting these pathways are under development (Fig. 1, Table 1) [9]. Many molecular-targeted agents are now under development; the target signaling pathways and growth factors are outlined below.

MAPK pathway (RAS/RAF/MEK/ERK)

The MAPK intracellular signaling pathway, which is mainly involved in cell growth and survival, and regulates cell differentiation, is upregulated in cancer cells. Therefore, this pathway has been extensively studied as a therapeutic target. The MAPK pathway is a common downstream pathway for the EGFR, PDGFR, and VEGFR, and is universally used for signal transduction downstream of cytokine receptors, integrin complexes, and G-protein receptors to Ras. The MAPK pathway also plays an important role in HCC in that its activation is reportedly involved in HCC growth and survival [5]. The downstream extracellular signaling-regulated kinase (ERK) is activated by two upstream protein kinases, which are coupled to growth factor receptors by Ras proteins. Ras, which is activated by ligand binding, activates Raf serine/threonine kinases and MEK (MAP kinase/ERK kinase), whereas MEK phosphorylates and activates ERK, which phosphorylates proteins involved in cell growth, apoptosis resistance, extracellular matrix production, and angiogenesis [10–13].

Raf and Ras inhibitors

Raf and Ras are proto-oncogenes. In particular, K-Ras mutations are commonly observed in many cancers, including pancreatic and colorectal cancers. One study reported that 30% of HCCs have Ras mutations [14]. To our knowledge, no agents targeting Ras are planned to enter clinical trials in the near future. However, because the binding of Ras protein to the cell membrane and its functional activation require farnesylation, several farnesyltransferase inhibitors are being tested for Ras-related tumors. In addition, vaccine therapy for mutant Ras proteins is currently being tested for solid cancers, including HCC.

The Raf family consists of three isoforms, A-Raf, B-Raf, and C-Raf/Raf-1. Genetic abnormalities, for example point mutations and gene rearrangements, have been reported in various cancers [15]; however, in HCC, *ras/raf* mutations

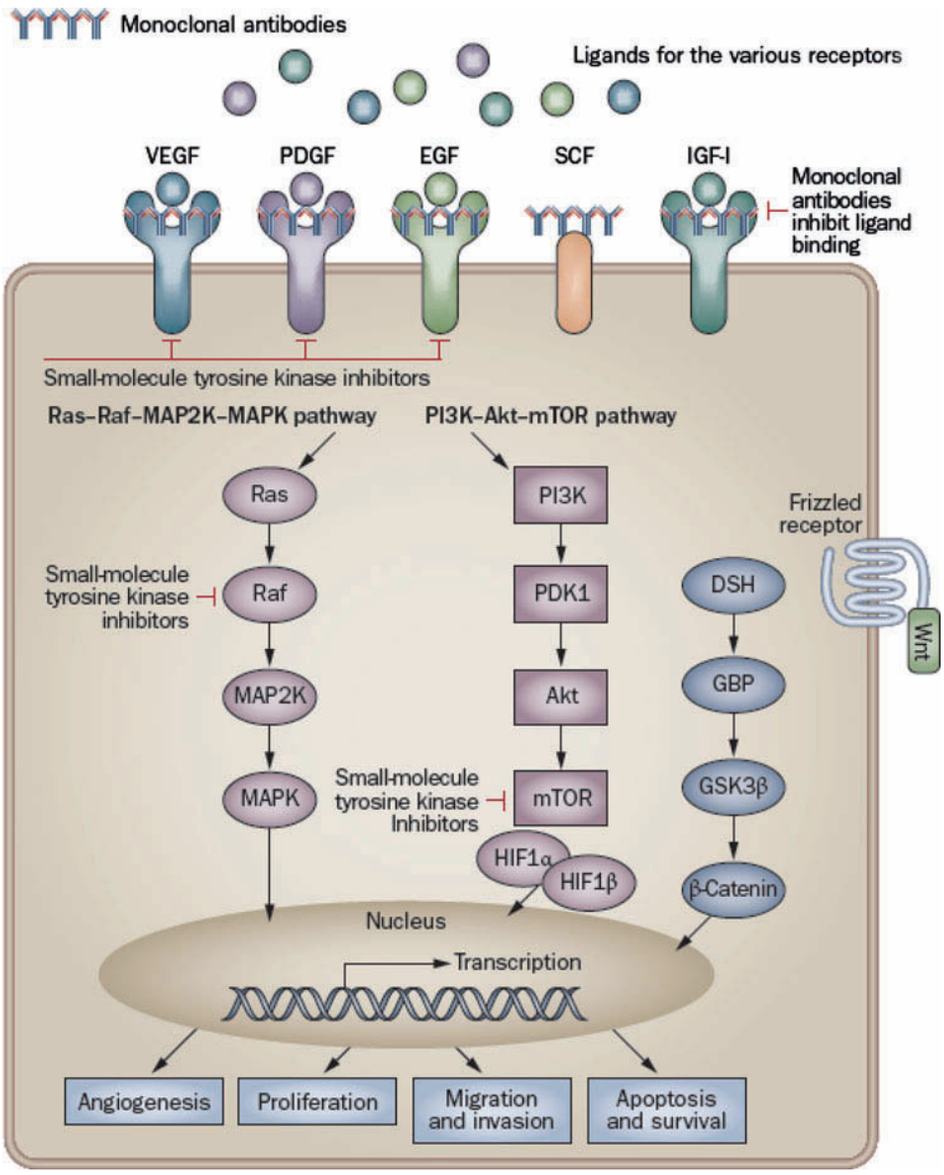


Fig. 1 Signaling pathways and the site of action of molecular-targeted agents (modified from Villanueva A et al. [3] and Llovet and Bruix [5])

are rare, and no *k-ras* or *b-raf* mutations have been detected [16]. On the other hand, wild-type Raf-1 was reported to be hyperactivated in many cancers, including HCC [17–19]. Sorafenib inhibits Raf, and has multiple characteristics in that it has strong inhibitory activity against Raf-1 (C-Raf) kinase, B-Raf (wild-type B-Raf and mutant V600E B-Raf) serine/threonine kinase, the pro-angiogenic receptor tyrosine kinases VEGFR, PDGFR, and FGFR1, and tyrosine kinases such as c-kit, Flt-3, and RET, which are involved in tumor progression and overall prognosis [20].

MEK

The MEK family consists of MEK1 and MEK2 proteins, which specifically phosphorylate tyrosine and threonine residues, and phosphorylates downstream Erk1 and Erk2 [21].

In an immunohistochemical study, MEK1/2 overexpression, ERK1/2 overexpression, and ERK1/2 phosphorylation were observed in 100% (46/46), 91% (42/46), and 69% (32/46) of HCCs, respectively, and the in-vitro treatment of HepG2 and Hep3B cells with MEK1/2 inhibitors inhibited cell growth and upregulated apoptosis [22].

The MEK inhibitors CI-1040, PD0325901, AZD6244, and RDEA119/BAY869766 have been tested in several cancers including solid tumors such as HCC. A phase II study of AZD6244 (selumetinib, ARRY-142866) and a phase I/II study of RDEA119/BAY869766 in combination with sorafenib are being conducted.

Table 1 Molecular-targeted agents being tested in HCC

Agent	Antiangiogenic targets					Antiproliferative targets				Antiepigenetic targets				Developmental status	Company
	VEGF	FGF	VEGFR	PDGFR	FGFR	EGFR	Raf	MEK	mTOR	RAR	RXR	HDAC	Heparanase		
Sorafenib[a] (Nexavar)			●	●			●							Approved	Bayer
Sunitinib[a] (Sutent)			●	●										Phase III stopped	Pfizer
NIK-333 (Acyclic Retinoid)										●	●			Phase II/III complete	Kowa
Brivanib			●		●									Phase III ongoing	Bristol-Myers Squibb
TSU-68			●	●	●									Phase II complete	Taiho
TAC-101										●				Phase II stopped	Taiho
Erlotinib (Tarceva)						●								Phase II complete	Roche
Bevacizumab (Avastin)	●													Phase II ongoing	GenentechA
AZD2171 (Cediranib)			●											Phase II recruiting	AstraZeneca
Gefitinib (Iressa)						●								Phase II complete	AstraZeneca
Lapatinib						●								Phase II ongoing	GlaxoSmithKline
Thalidomide	●													Phase II ongoing	TTY BioPharm
Linifanib			●	●										Phase III initiated Abott	
AZD6244								●						Phase II ongoing	AstraZeneca
PI-88	●	●											●	Phase II complete	Progen
Cecuximab						●								Phase II complete	Merck
RAD001									●					Phase III initiated	Novartis
PXD101 (Belinostat)												●		Phase I/II ongoing	Curagen

Sources: Trial Trove, ClinicalTrials.Gov (NCI), Evaluate Pharma, IMS Knowledge Link, Esplcom, IDdB3, BioPharm Insight, MedTrack

[a] Sorafenib and sunitinib also have antiproliferative affects through multi-tyrosine kinase inhibition

PI3K/Akt/mTOR pathway

The PI3K/Akt/mTOR pathway also plays an important role in cell growth, survival regulation, metabolism, and anti-apoptosis. The membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP_2) is phosphorylated by phosphatidylinositol 3-kinase (PI3K) into phosphatidylinositol 3,4,5-triphosphate (PIP_3), which binds to and activates the serine/threonine kinase Akt. The tumor-suppressor gene product PTEN (phosphatase and tensin homolog deleted on chromosome) is antagonistic to PI3K activity. PTEN is a lipid phosphatase that dephosphorylates inositol phosphates such as PIP_3. The inactivation of PTEN through gene deletion increases PIP_3 levels, and activates Akt, which inhibits apoptosis, leading to the development of tumors. The serine/threonine kinase mTOR is an important mediator in the PI3K/Akt pathway that binds intracellularly to a protein called raptor or rictor, and exists as two different complexes, complex 1 and 2 (mTORC1 and mTORC2). mTORC2 (mTOR-rictor) activates Akt whereas mTORC1 (mTOR-raptor) is activated downstream of Akt; thus, both molecules regulate protein synthesis [23].

A study of 528 HCC samples showed that expression of pAkt, PTEN, p27, and S6 ribosomal protein (pS6) was a poor prognostic factor for survival [24]. A tissue microarray analysis of HCC samples revealed that the loss of PTEN and overexpression of pAkt and p-mTOR were correlated with tumor grade, intrahepatic metastasis, vascular invasion, TNM stage, Ki-67 labeling index, and matrix metalloproteinase (MMP)-2 and (MMP)-9 upregulation, Meanwhile, PTEN mRNA expression in the cancerous tissue was downregulated, compared with that in the non-cancerous tissue. The levels of PTEN, MMP-2, and MMP-9 mRNA expression were correlated with tumor stage and metastasis, and the levels of PTEN and MMP-9 mRNA expression were inversely correlated [25]. In an extensive analysis of 314 HCC samples in terms of mutation analysis, DNA copy number changes, mRNA levels, and immunostaining, Villanueva et al. found that activation of the IGF pathway, upregulation of EGF, dysregulation of PTEN, and aberrant mTOR signaling were present in half of the samples, and that inhibiting mTOR activity with everolimus was effective in improved survival and suppression of recurrence [26].

The PI3K inhibitor RG7321 and the Akt inhibitor perifosine target the PI3K/Akt/mTOR pathway and are in early stages of clinical development, whereas the mTOR inhibitors everolimus (RAD001), sirolimus (Rapamune), and temsirolimus (CCI-779) are at more advanced stages of development. Everolimus is used to treat sorafenib-intolerant patients or for patients showing disease progression after sorafenib administration. A phase III study to compare everolimus and a placebo (EVOLVE-1: Advanced Hepatocellular Carcinoma after Disease Progression or Intolerance to Sorafenib *Ev*er*O*limus for *Li*v*E*r cancer *E*valuation) and a phase I/randomized phase II study (sorafenib + everolimus vs. sorafenib alone) to test the efficacy and tolerance of sorafenib in combination with everolimus are underway. Because mTOR inhibitors have cytostatic and antiangiogenic effects, they are expected to be effective in combination with other angiogenesis inhibitors such as bevacizumab, and may be appropriate for administration after transarterial chemoembolization (TACE). Furthermore, because the mTOR pathway is stimulated by factors such as EGFR, PDGFR, and TGFα, and is closely related to other signaling pathways including the Ras/Raf/MEK/ERK pathway, they are likely to show promising efficacy when used in combination with other growth factor inhibitors [27].

VEGF/VEGFR, PDGFR, FGFR

Angiogenesis is an important event not only for HCC but also for cancer growth and metastasis, and occurs because of complex alterations involving promoting factors such as VEGF, angiopoietin, and FGF, inhibitory factors including thrombospondin (TSP) and angiostatin and the surrounding tissue. The VEGF family consists of VEGF-A, B, C, D, and E, and placental growth factor (PIGF). The VEGFR family comprises VEGFR-1 (flt-1), VEGFR-2 (flk-1/KDR), and VEGFR-3 (flt-4). VEGF-A binds to VEGFR-1 and VEGFR-2 and is involved in angiogenesis and the maintenance of mature blood vessels, whereas VEGF-C and VEGF-D mainly bind to VEGFR-3, are involved in lymphangiogenesis [28, 29]. VEGF isoforms such as $VFGF_{121}$ and $VEGF_{165}$ have been identified, and isoform subtypes also exist, for example EGF_{166}b. Thus, it is clear that these growth factors do not exhibit angiogenesis-promoting effects alone, and they have attracted attention as new therapeutic targets [30].

HCC typically exhibits active angiogenesis. During the progression from early to well, and to moderately differentiated HCC, angiogenesis increases and cancer cells acquire the ability to invade vessels and metastasize. Scientific and clinical studies have revealed that, during the progression from hepatitis to cirrhosis, angiogenesis and disruption of the vascular architecture are linked to the progression of HCC, and contribute to increased hepatic vascular resistance and portal hypertension, and decreased hepatocyte perfusion [31]. In addition, a meta-analysis has demonstrated that VEGF expression is a prognostic factor in HCC [32].

Phase II studies have been started to test the usefulness of bevacizumab (Avastin®), which directly targets VEGF, in TACE-treated HCC, and the use of bevacizumab in

combination with erlotinib (Tarceva®), an EGFR tyrosine kinase inhibitor.

Sunitinib (Sutent®) is a multi-kinase inhibitor that inhibits tyrosine kinases such as VEGFR-1, 2, 3, PDGFR-α, β, and c-Kit. A phase II study of sunitinib in 37 advanced HCC patients showed that the median progression-free survival (PFS) and median overall survival (OS) were 3.7 and 8 months, respectively. In that study, adverse events included grade 3/4 thrombocytopenia in 37.8% of patients, neutropenia in 24.3%, asthenia in 13.5%, and hand–foot syndrome in 10.8% [33]. Because sunitinib has a lower IC_{50} for each target than sorafenib, it is expected to have greater antitumor activity. However, this factor may be responsible for the higher incidence of adverse events with sunitinib. The main evaluation item in the above phase II trial was the response rate, which did not reach the expected value, leading to the conclusion that it was a negative study [34]. In that study sunitinib was administered at 50 mg/day for 4 weeks followed by 2 weeks of rest per cycle [33], whereas Zhu et al. [34] used a dosing schedule of 37.5 mg/day for 4 weeks followed by 2 weeks of rest per cycle, and reported that the median PFS and OS were 3.9 and 9.8 months, respectively. An ongoing global cooperative phase III controlled clinical trial to compare sorafenib and sunitinib head-to-head and to seek approval for first-line indications for advanced HCC adopted a sunitinib dosing schedule of 37.5 mg/day. However, in a "Reflection and Reaction" regarding these trial results, Forner et al. cast doubt on whether the drugs at this dose could maintain tolerance and ensure efficacy [35]. Because recruitment is progressing well, the results are expected to be available soon.

Brivanib is a kinase inhibitor that selectively inhibits VEGFR-1, 2, and 3, and FGFR-1, 2, and 3. As for sunitinib, an international global phase III clinical trial to compare brivanib and sorafenib head-to-head and to seek approval for first-line therapy for advanced HCC has already been started, and the results are eagerly awaited. Japanese centers are participating in this clinical trial. Because brivanib targets FGF and VEGF, and is associated with relatively mild adverse effects, a second-line study of brivanib in sorafenib-ineffective and sorafenib-intolerant patients and a trial to evaluate the use of brivanib in combination with TACE are underway. Depending on the results of these trials, indications for use in HCC may be obtained; therefore, positive results are eagerly anticipated. The results have been reported for a phase II study of brivanib in 55 patients (cohort A) who had not received systemic therapy for curatively unresectable HCC and 46 patients (cohort B) previously treated with angiogenesis inhibitors such as sorafenib or thalidomide [36]. The median TTP and OS were 2.8 and 10 months, respectively, in cohort A versus 1.4 and 9.8 months, respectively, in cohort B. Adverse events included fatigue (51.5%), diarrhea (41.6%), hypertension (42.6%), anorexia (41.6%), and nausea/vomiting (40.6/30.7%). Thus, these results demonstrated the efficacy of brivanib as a second-line treatment. The results of three phase III clinical trials, BRISK-PS (sorafenib failure or sorafenib-intolerant patients; brivanib + best supportive care (BSC) vs. placebo + BSC), BRISK-FL (advanced HCC; brivanib vs. sorafenib), and BRISK-TA (patients with unresectable HCC, brivanib vs. placebo as post-TACE adjuvant therapy) are awaited. Japanese centers participated in all three trials.

In a Japanese phase I/II trial of TSU-68, an oral molecular inhibitor of VEGFR, PDGFR, and FGFR, to test its safety and efficacy in 35 HCC patients, the response rate was 5.6% (CR, PR, SD, PD, and NE in 1, 2, 15, 16, and 1 patients, respectively), and the disease control rate was 51.4% [37].

In addition, several phase I/II trials are being conducted to assess kinase inhibitors such as linifanib (ABT-869) and cediranib (AZD2171), which inhibit VEGFR, PDGFR, CSF-1R (cFms), Kit, and Flt3. Furthermore, axitinib, which is currently being tested in renal cell carcinoma, has also attracted attention as a promising agent for treatment of HCC because of its efficacy and mild side effects.

EGF/EGFR

EGFR is a member of the human epidermal growth factor receptor (HER) family that includes EGFR (erbB1), HER2/neu (erbB3), and HER4 (erb4). All members of this family, except HER3, have an intracellular tyrosine kinase domain, and the binding of a ligand to its extracellular domain triggers signal transduction through the above-described MAPK and PI3K/Akt/mTOR pathways. Thus, these receptors are involved in cell growth, differentiation, survival and adhesion [38]. EGFR overexpression has been reported in many cancers, and in HCC. For example, Buckley et al. reported that EGFR, detected by immunohistochemical analysis, was overexpressed in 50 (66%) of 76 HCCs, and that fluorescence in-situ hybridization (FISH) showed extra EGFR gene copies in 17 (45%) of 38 HCCs [39].

EGFR-targeting drugs, which include anti-EGFR antibodies, such as cetuximab and panitumumab, and small-molecule inhibitors of EGFR tyrosine kinases such as gefitinib, etc., have been used widely for treatment of several cancers other than HCC. Unfortunately, except for phase II trial data, there are few clinical data on the efficacy of these drugs for the treatment of HCC.

Similar to gefitinib (Iressa®), erlotinib (Tarceva®) is an oral EGFR tyrosine kinase inhibitor. Philip et al. and Thomas et al. have reported the results of phase II studies of erlotinib in HCC [40, 41]; the median OSs in their

studies were 13 and 10.7 months, respectively. A phase III clinical study (SEARCH study: *S*orafenib and *E*rlotinib, a R*a*ndomized T*r*ial Protoc*o*l for the Treatment of Patients with *H*epatocellular Carcinoma) of sorafenib in combination with erlotinib versus sorafenib plus placebo is ongoing. Because erlotinib is associated with a high incidence of skin rash, dry skin, and gastrointestinal toxicity, for example diarrhea, the results of the SEARCH study should be evaluated to assess whether this combination therapy can be used in clinical settings. Thomas et al. conducted a phase II clinical study of erlotinib in combination with bevacizumab in 40 advanced HCC patients, and reported promising results; the median PFS and OS were 9 and 15.7 months, respectively. However, they noted frequent treatment-related grade 3/4 toxicities, including fatigue (20%), hypertension (15%), gastrointestinal bleeding (12.5%), wound infection (5%), diarrhea (10%), elevated transaminase levels (10%), and thrombocytopenia (10%) [42], which necessitates further evaluation of drug tolerance. Although a clinical study of erlotinib in combination with bevacizumab (OPTIMOX-3 study) was also conducted in colorectal cancer patients, no tolerance was observed, which led to a change in the protocol [43, 44].

After the introduction of a number of molecular-targeted drugs, strategies for the inhibition of similar or different signaling pathways (vertical or horizontal inhibition) with several drugs have been proposed. However, the combined use of molecular-targeted agents has remained largely unsuccessful, including panitumumab in combination with bevacizumab for treatment of colorectal cancer [45]. Similarly, results for sorafenib in combination with bevacizumab (vertical inhibition) have been reported [46]. Although some therapeutic response was obtained, the combination therapy resulted in greater toxicity [46], suggesting the need for detailed evaluation of the dosing regimen.

Lapatinib (Tykerb®) is a dual inhibitor of EGFR and HER-2/neu, and inhibits tumor growth by downregulating MAPK, AKT, and p70S6 kinase [47]. In Japan, lapatinib is indicated for treatment of breast cancer. In a phase II clinical trial of lapatinib in 26 patients with unresectable advanced HCC, the median PFS and OS were 1.9 and 12.6 months, respectively, and adverse events included diarrhea (73%), nausea (54%), and skin rash (42%) [48].

Cetuximab (Erbitux®) is a human/mouse chimeric monoclonal antibody consisting of the variable region of a mouse anti-human EGFR monoclonal antibody and the human IgG1 constant region. Cetuximab inhibits the binding of endogenous EGFR ligands, for example EGF and TGFα, to EGFR. In a phase II clinical trial of cetuximab in 30 patients with unresectable or metastatic HCC, the median PFS and OS were 1.4 and 9.6 months, respectively, and treatment-related toxicities included grade 3 hypomagnesemia (3.3%) and grade 1/2 acne-like rash (83.3%), which was observed for the duration of anti-EGFR therapy in that study [49].

The EGFR is a very interesting therapeutic target. As described above, use of erlotinib in combination with sorafenib is still in the research stage. However, on the basis of results from phase II studies, the efficacy of cetuximab or lapatinib as monotherapy seems to be limited, and the results of further studies evaluating their efficacy in sorafenib-refractory or intolerant patients are awaited with interest.

HGF/c-Met pathway

Because the hepatocyte growth factor (HGF)/Met pathway is involved in tumor growth, invasion, and angiogenesis in a wide range of neoplasms, HGF and Met have recently attracted attention as therapeutic targets. HGF is a heterodimer consisting of α and β chains bound together by a disulfate bond. The α chain contains four kringle domains, and the β chain contains a serine protease-like domain. Met is a receptor tyrosine kinase for the HGF ligand, and contains a semaphorin-like domain. HGF or Met overexpression and Met gene mutations and duplications have been reported in various cancers, and abnormalities due to HGF/Met pathway activation have also been noted [50]. These abnormalities activate the downstream signaling cascade, leading to epithelial-mesenchymal transition and increased proliferative, migratory, invasive and metastatic potentials of cancer cells [50].

HGF/c-MET-targeted drugs, including kinase inhibitors, HGF inhibitors, and decoy c-Met receptor molecules, are being developed. Of particular interest is ARQ-197, a c-Met receptor tyrosine kinase inhibitor which is a non-ATP-competitive molecule that binds near the ATP-binding site. A randomized phase II study of ARQ-197 versus placebo is ongoing in patients with unresectable HCC after systemic therapy failure.

IGF/IGFR

The IGF/IGFR system is involved in cell growth and the chemotherapeutic response. The ligands IGF-I and II bind to their receptors IGF-1R and IGF-2R, and are involved in DNA synthesis and cell growth. Abnormalities in IGF and IGF-1R or their overexpression have been reported in various cancers, including HCC. Their associations with disease stage, metastasis, and survival [51] and the functions of IGF and IGFR in HCC [52] have been reported.

IGF-targeting drugs are currently being developed, and mainly include anti-IGF-1R antibodies, for example BIIB022, AVE1642, and cixutumumab (IMC-A12). A phase II study of cixutumumab, a phase Ib/II study of

Table 2 Results of the Asia–Pacific and SHARP studies

End point	Asia–Pacific		SHARP	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
OS	0.68 (0.50–0.93)	0.014	0.69 (0.55–0.87)	<0.001
TTSP	0.90 (0.67–1.22)	0.498	1.08 (0.88–1.31)	0.768
TTP	0.57 (0.42–0.79)	<0.001	0.58 (0.45–0.74)	<0.001
PFS	0.62 (0.46–0.82)	<0.001	0.65 (0.52–0.79)	<0.001

sorafenib versus sorafenib plus BIIB022, and phase I/II studies of AVE1642 as monotherapy or in combination with sorafenib or erlotinib are ongoing.

Sorafenib: trial results and clinical experience

Clinical results for sorafenib in HCC

As described above, sorafenib is a multi-kinase inhibitor of tumor growth and angiogenesis, and has a strong inhibitory effect on C-Raf and B-Raf serine/threonine kinases (comprising the Raf/MEK/ERK pathway), VEGFR and PDGFR tyrosine kinases, and FLT-3 and c-kit [20]. To date, sorafenib is the only molecular-targeted agent approved for treatment of HCC, on the basis of the results of two large-scale clinical trials, namely the SHARP (Sorafenib HCC assessment Randomized Protocol) study [53] and the Asia–Pacific study [54]. The median OSs for the sorafenib group in the SHARP and Asia–Pacific studies were 10.7 months (vs. 7.9 months for the placebo group, $P < 0.001$; HR: 0.69) and 6.5 months (vs. 4.2 months for the placebo group, $P = 0.014$; HR: 0.68), respectively, indicating that sorafenib prolongs survival by approximately 50% (Table 2). These data should compel HCC specialists to challenge their preconception that systemic anticancer drug therapy is not effective for HCC.

Current status regarding the use of sorafenib in Japan

Sorafenib was approved in Japan in May, 2009. A survey has confirmed that, at the time of writing (March, 2010), over 3,700 patients have been prescribed sorafenib. Across several centers, 15 Japanese patients have achieved CR, which was not observed in the SHARP or Asia–Pacific trials. This suggests that some Japanese patients may be very sensitive to sorafenib [55]. The reason for this, and predictive biomarkers, are now actively under investigation.

On the other hand, it has been reported that hand–foot syndrome occurs early after sorafenib administration [56] more often than was noted in the SHARP and Asia–Pacific studies, and the drug is often discontinued because of the adverse effects in many patients [56]. As demonstrated in the SHARP and Asia–Pacific studies, sorafenib is only used to achieve stable disease; it is, therefore, important to improve drug efficacy by extending the period of administration for as long as possible. Therefore, it is no exaggeration to say that, in the case of sorafenib, the "successful management of side effects" is equal to "successful treatment." According to "post-TACE phase III clinical study [56]" performed in Japan and Korea, it is strongly speculated that physicians who are unaccustomed to prescribing molecular-targeted agents and who fail to see marked efficacy, as induced by conventional chemotherapeutic agents, often do not understand the properties of this drug, and they (and the patients) do not fully comprehend therapeutic efficacy. Moreover, they feel too anxious about side effects that have not been encountered before. These circumstances may result in treatment discontinuation in many patients. Clearly, greater awareness among physicians for therapeutic efficacy and approaches to manage adverse effects is needed to improve treatment outcomes.

Experience of sorafenib use at our institute

Since the approval of sorafenib on May 20, 2009, we have treated 90 patients with sorafenib, and few have discontinued therapy because of adverse effects or patient refusal to continue. Of these 90 patients, two achieved CR [55]. These two CR patients, in whom pulmonary and adrenal metastases and intrahepatic lesions all disappeared, survived free of recurrence for more than 2 years and 1 year, respectively, at the time of writing (March, 2010), i.e., they are still alive at present. In other patients who apparently achieved SD, the tumor marker levels reached a plateau after sorafenib administration, when their levels were rising rapidly before sorafenib administration. Even if hepatic lesions do not show a clear tendency to undergo necrosis or regression on CT images, three tumor markers (AFP, PIVKA-II, and AFP-L3) are widely considered to serve as surrogate markers. In fact, there are very few data on serum tumor markers, except for AFP, outside Japan. Nevertheless, Japanese researchers have demonstrated the value of changes in these markers and the antitumor efficacy of sorafenib [55].

Interestingly, it has previously been demonstrated that the levels of PIVKA-II or DCP tend to be increased by

inducing hypoxia [57]. Therefore, PIVKA-II or DCP may be a good predictive marker for evaluating the hypoxic response to antiangiogenic therapy for HCC.

Only 17 of the 90 patients showed PD on computed tomography (CT) images although follow-up period is still short (less than 10 months). However, because the speed with which the patient develops progressive disease may slow down due to tumor growth inhibition, it is very difficult to determine when to discontinue treatment because of tumor refraction. Important issues for future studies include:

1 identification of biomarkers that can be used to predict therapeutic responses, including CR or PR, in patient groups;
2 evaluation of the role of tumor markers in the determination of therapeutic responses;
3 establishing response evaluation criteria that can determine the therapeutic responses to molecular-targeted agents; and
4 development of effective second-line therapies after sorafenib failure (Figs. 2, 3).

In the treatment algorithm (Figs. 2, 3) approved by the Consensus Meeting of the 2009 Annual Meeting of the Japan Society of Hepatology (Congress chair: Professor Masatoshi Kudo), sorafenib is indicated for treatment of patients with Child-Pugh A HCC with extrahepatic metastasis, vascular invasion, or refractoriness to TACE or arterial infusion chemotherapy.

In addition to the pharmaceutical-sponsored clinical trials of sunitinib and brivanib as first and second-line therapy in sorafenib-refractory patients, investigator-initiated trials (IIT) of sorafenib in combination with hepatic arterial infusion chemotherapy (SILIUS trial), pharmaceutical and IIT trials of sorafenib in combination with TACE (SPACE, TACICS and BRISK-TA trials), and a trial to test the inhibitory effect of sorafenib on tumor recurrence after curative treatment (STORM trial) are ongoing, and the results of these trials are eagerly awaited (Figs. 2, 3). The working hypotheses in these studies can be deduced by extrapolating the MST and hazard ratios in overall survival (OS) calculated in a subanalysis of the SHARP study (Table 3). The results obtained suggest that starting treatment with molecular-targeted drugs at an earlier tumor stage in combination with standard treatment options such as resection, ablation, TACE, or hepatic arterial infusion chemotherapy can improve the prognosis of HCC. Thus, sorafenib has the potential to induce a change of emphasis in the treatment of HCC. For example, in a subanalysis of the SHARP trial, the hazard ratios for OS and MST ratio in intermediate stage HCC without vascular invasion or extrahepatic spread were 0.52 and 1.50, respectively (Table 4). This suggests that survival of early stage HCC and intermediate stage HCC may be prolonged from 5 years to 7.5–10 years by using sorafenib in an adjuvant setting after curative treatment, and from 3 years to 4.5–6 years by using sorafenib in combination with TACE (Fig. 4).

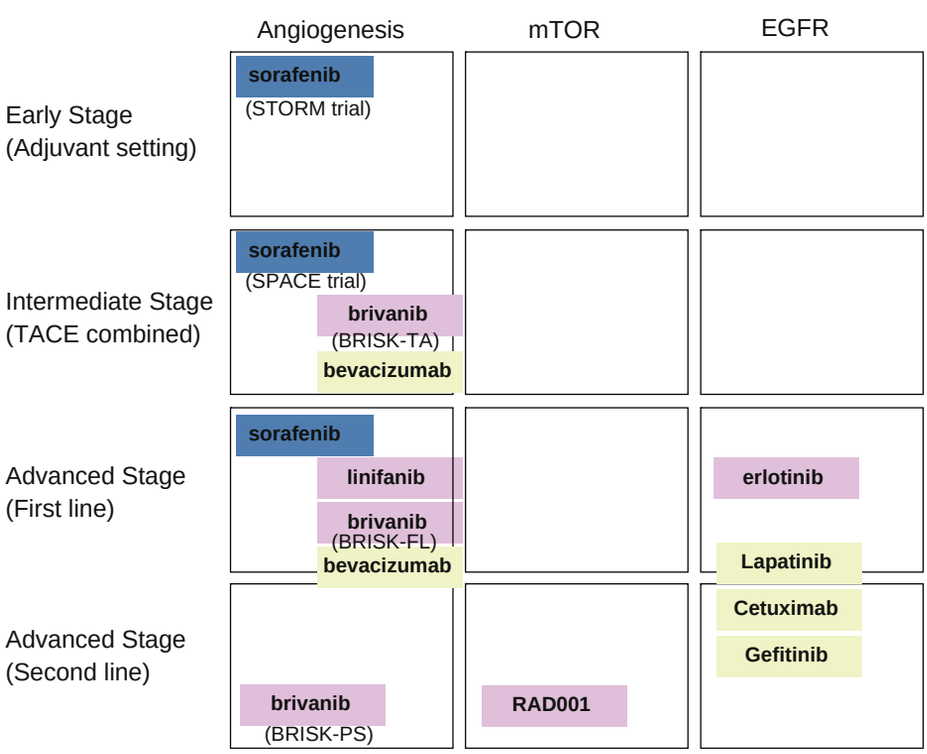


Fig. 2 Molecular targeted agents: ongoing trials in each stage of HCC

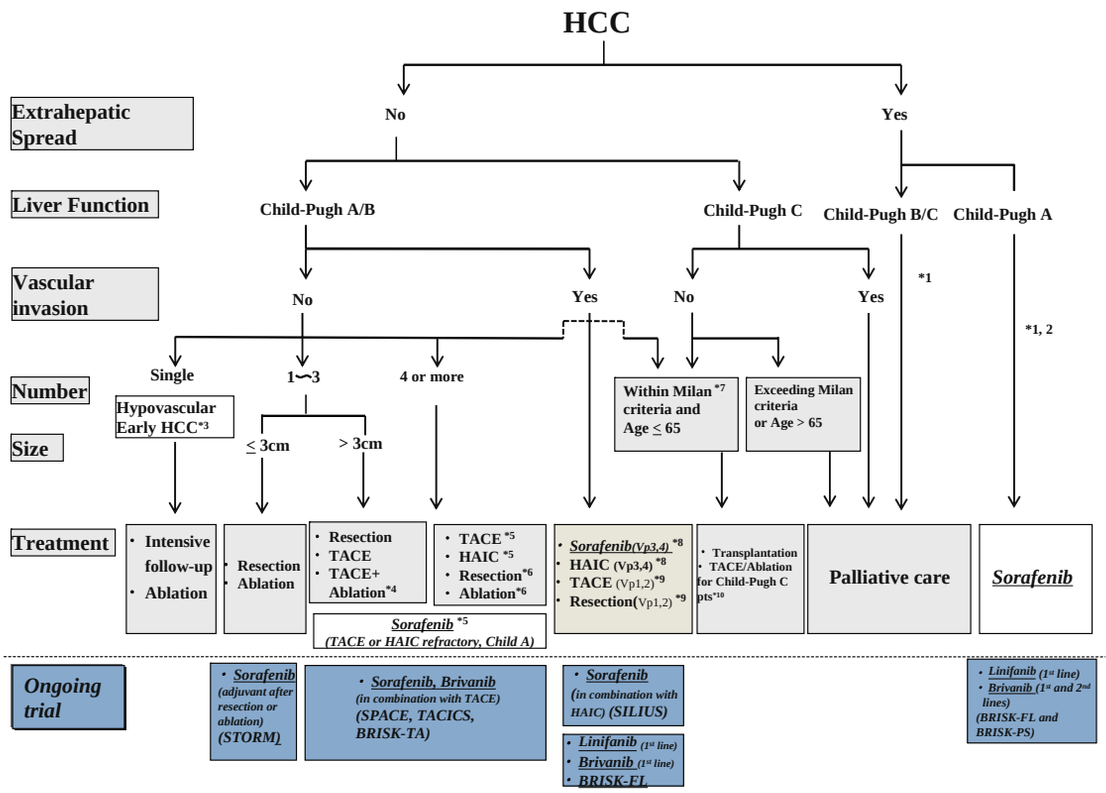


Fig. 3 Consensus-based treatment algorithm for HCC proposed by the Japan Society of Hepatology (JSH) revised in 2010. *1 Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not considered as a prognostic factor in Child-Pugh class A/B patients, *2 sorafenib is the first choice of treatment in this setting as a standard of care, *3 intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early HCC; (2) when the nodules show decreased uptake on Gd-EOB-MRI; or (3) when the nodules show decreased portal flow by CTAP, since these nodules frequently progress to advanced HCC, *4 even for HCC nodules exceeding 3 cm in diameter, transcatheter arterial chemoembolization (TACE) in combination with ablation is frequently performed when resection is not indicated, *5 TACE is the first choice of treatment in this setting. Hepatic arterial infusion chemotherapy (HAIC) using an implanted port is also recommended for TACE-refractory patients. The regimen for this treatment is usually low-dose FP (5FU+CDDP) or intra-arterial 5FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE- or HAIC-refractory patients with Child-Pugh class A liver function, *6 resection is sometimes performed when more than four nodules are detected. Ablation is sometimes performed in combination with TACE, *7 Milan criteria: tumor size ≤3 cm and tumor number ≤3, or solitary tumor ≤5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for patients with frequently recurring HCC, *8 sorafenib and HAIC are recommended for HCC patients with major portal invasion such as Vp3 (portal invasion in the first portal branch) or Vp4 (portal invasion in the main portal branch), *9 resection and TACE are frequently performed when portal invasion is minor, such as Vp1 (portal invasion in the third or more peripheral portal branch) or Vp2 (portal invasion in the second portal branch), *10 local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated, when there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (<3.0 mg/dl). However, it is regarded as an experimental treatment because there is no evidence of a survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue

Table 3 Subanalysis data of the SHARP study

		Advanced HCC with vascular invasion and extrahepatic spread	Advanced HCC without vascular invasion or extrahepatic spread
Hazard ratio		0.77 (95% CI: 0.60–0.99)	0.52 (95% CI: 0.32–0.85)
Median OS (MST)	Sorafenib	8.9 M ($n = 209$) (95% CI: 7.6–10.3 M)	14.5 M ($n = 90$) (95% CI: 14.0 M–N/E)
	Placebo	6.7 M ($n = 212$) (95% CI: 5.2–8.0 M)	10.2 M ($n = 91$) (95% CI: 8.6–15.5 M)

M, month

Sherman M et al. ASCO 2008

Table 4 Results of studies of molecular-targeted agents for HCC

Agent	Type	Target	Number of patients	RR (%)	PFS (month)	TTP (month)	OS (month)	Reference
Phase III								
Sorafenib	s.m.	C-Raf, B-Raf,	602 (299[a])	2	–	5.5	10.7	Llovet [5, 53]
		PDGFR, VEGFR	271 (150[a])	3.3	–	2.8	6.5	Cheng [54]
Phase II								
Sorafenib	s.m.	C-Raf, B-Raf, PDGFR, VEGFR	137	2.2	–	5.5	9.2	Abou-Alfa [58]
Sunitinib	s.m.	VEGFR, PDGFR,	37	2.7	3.7	5.3	8	Faivre [33]
		SCFR, FLT3	34	2.9	3.9	4.1	9.8	Zhu [34]
Brivanib	s.m.	VEGFR, FGFR	55	n.r.	–	2.8	10	Raoul [36]
Linifanib	s.m.	VEGFR, PDGFR	44	6.8	–	5.7	9.3	Toh [59]
Bevacizumab	MoAb	VEGF	46	13	6.9	–	12.4	Siegel [60]
Erlotinib	s.m.	EGFR	38	9	–	3.2	13	Philip [40]
			40	0	–	–	10.7	Thomas [41]
Gefitinib	s.m.	EGFR	31	3.2	2.8	–	6.5	O'Dwyer [61]
Lapatinib	s.m.	EGFR	40	5	2.3	–	6.2	Ramanathan [62]
			26	0	1.9	–	12.6	Bekaii-Saab [48]
Cetuximab	MoAb	EGFR	30	0	1.4	–	9.6	Zhu [49]

[a] Sorafenib arm

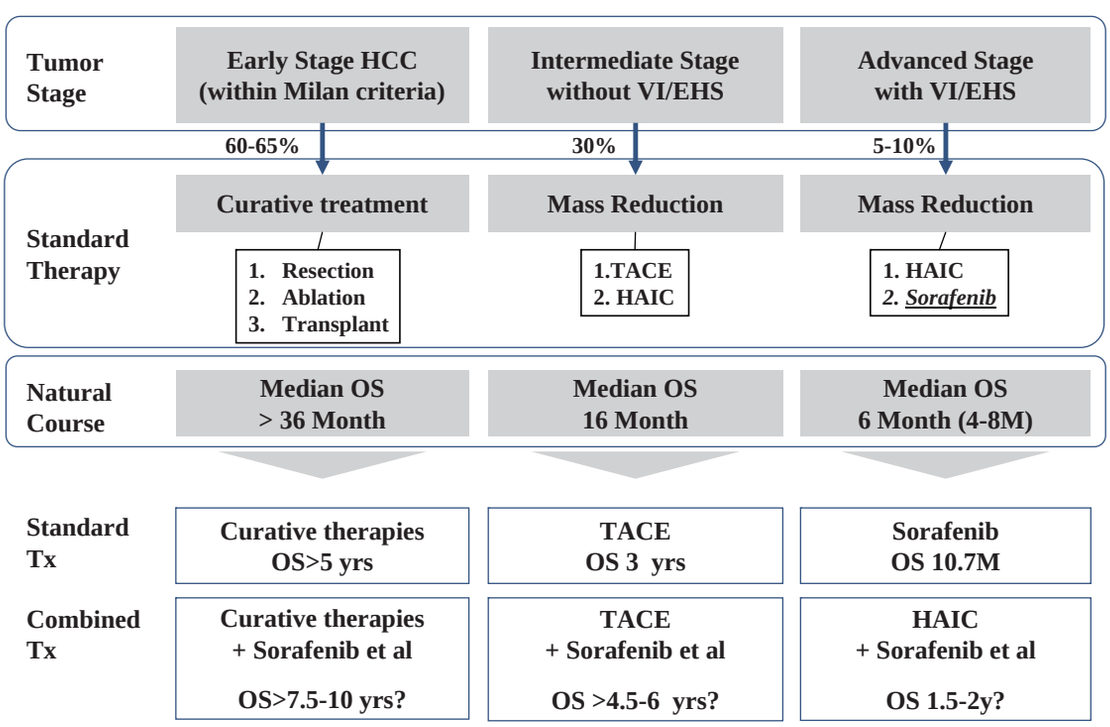


Fig. 4 Outcomes of standard treatment modalities and expected effects of combination therapy with molecular-targeted agents

Summary and future prospects

The results of clinical trials [33, 34, 36, 40, 41, 49, 58–62] of the molecular-targeted agents described above are summarized in Table 4. Angiogenesis-inhibiting drugs, particularly sorafenib, have been evaluated for HCC, and drugs targeting EGFR and mTOR are being developed. The results (numerical values) of phase II clinical trials show no marked differences in the therapeutic efficacy evaluated by time to progression (TTP) or progression-free survival

(RFS). However, results from phase II studies may be subject to patient selection bias and cannot be compared with results from other trials. Thus, when determining the therapeutic efficacy of drugs, we should review the efficacy of the respective drugs, and consider where the theoretical target molecules are present and what combinations of drugs have a theoretical rationale, and thus evaluate options for monotherapy and combination therapy based on the efficacy and safety data obtained from phase III clinical trials.

Molecular-targeted agents that have been introduced into clinical use in recent years are approved for treatment of specific cancer and are then frequently used to treat other cancers. Although not discussed here, studies to identify predictors of efficacy (i.e., biomarkers) for angiogenesis inhibitors and EGFR tyrosine kinase inhibitors, and factors involved in drug resistance, are making steady progress, and the associated therapeutic strategies are undergoing major changes. Therefore, even in the treatment of HCC, it is necessary for HCC specialists to expand their knowledge of and techniques for applying existing treatment modalities (resection, ablation, TACE, arterial infusion chemotherapy) to physically remove, destroy, or necrotize the tumor, and to better understand clinical oncology, particularly the role and mechanisms of action of molecular-targeted agents. We are entering an era in which physicians treating HCC should pay close attention to the development of therapeutic agents not only for HCC but also for other cancers, and be aware of the use of molecular-targeted agents for treating cancers in clinical and basic research settings, and understand approaches to limit or control adverse effects associated with these drugs.

Although sorafenib was recently approved, many issues remain to be addressed, including:

1 how to determine and define refractoriness; and
2 whether to continue TACE or hepatic arterial infusion chemotherapy (a de facto standard in Japan) in patients with TACE-refractory tumors or portal tumor thrombi before starting sorafenib therapy.

For oncology, in particular, the Pharmaceuticals and Medical Devices Agency (PMDA) in Japan has approved several drugs based on results from global clinical trials and on Japanese phase I study data alone. We strongly recommend that, on the basis of the molecular-targeted agents currently under development, clinical studies (including IITs) should be conducted aggressively, and therapeutic strategies should be devised to resolve the limitations of currently used therapeutic approaches and to improve therapeutic outcomes.

The introduction of sorafenib to treat HCC in 2007 in Western countries and in 2009 in Japan was undoubtedly the beginning of a change of emphasis, representing a significant breakthrough for HCC treatment not previously experienced for this unique tumor.

Conflict of interest statement M. Kudo has received honoraria for the lecture from Bayer HealthCare, Pfizer, and Bristol-Meyers.

References

1. Farazi PA, DePinho RA (2006) Hepatocellular carcinoma pathogenesis: from genes to environment. Nat Rev Cancer 6:674–687
2. Minguez B, Tovar V, Chiang D et al (2009) Pathogenesis of hepatocellular carcinoma and molecular therapies. Curr Opin Gastroenterol 25:186–194
3. Villanueva A, Newell P, Chiang DY et al (2007) Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis 27:55–76
4. Laurent-Puig P, Zucman-Rossi J (2006) Genetics of hepatocellular tumors. Oncogene 25:3778–3786
5. Llovet JM, Bruix J (2008) Molecular targeted therapies in hepatocellular carcinoma. Hepatology 48:1312–1327
6. Hopfner M, Shuppan D, Scherubl H (2008) Growth factor receptors and related signaling pathways as target for novel treatment strategies of hepatocellular cancer. World J Gastroenterol 14:1–14
7. Campbell JS, Hughes SD, Gilbertson DG et al (2005) Platelet-derived growth factor C induces liver fibrosis, steatosis, and hepatocellular carcinoma. Proc Natl Acad Sci 102:3389–3394
8. Ogasawara S, Yano H, Iemura A et al (1996) Expression of basic fibroblast growth factor and its receptors and their relationship to proliferation of human hepatocellular carcinoma cell lines. Hepatology 24:198–205
9. Kudo M (2008) Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. Oncology 75:S1–S12
10. Roberts PJ, Der CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26:3291–3310
11. Schmidt CM, McKillop IH, Cahill PA et al (1997) Increased MAPK expression and activity in primary human hepatocellular carcinoma. Biochem Biophys Res Commun 236:54–58
12. Huynh H, Nguen TTT, Chow KHP et al (2003) Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterology 3:19–30
13. Calvisi DF, Ladu S, Gorden A et al (2006) Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology 130:1117–1128
14. Bos JL (1989) ras Oncogenes in human cancer: review. Cancer Res 49:4682–4689
15. Beeram M, Patnaik A, Rowinsky EK (2005) Raf: a strategic target for therapeutic development against cancer. J Clin Oncol 23:6771–6790
16. Tannapfel A, Sommerer F, Benicke M et al (2003) Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. Gut 52:706–712
17. Jenke HS, Deml E, Oesterle D (1994) C-raf expression in early ratliver tumorigenesis after promotion with polychlorinated biphenyls or Phenobarbital. Xenobiotica 24:569–580
18. Beer DG, Neveu MJ, Paul DL et al (1998) Expression of the c-raf protooncogene, gamma-glutamyltranspeptidase, and gap junction protein in rat liver neoplasms. Cancer Res 48:1610–1617
19. Hwang YH, Choi JY, Kim S et al (2004) Over-expression *c-raf*-1 proto-oncogene in liver cirrhosis and hepatocellular carcinoma. Hepatol Res 29:113–121

20. Wilhelm SM, Adnane L, Newell P et al (2008) Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 7:3129–3140
21. Ohren JF, Chen H, Pavlovsky A et al (2004) Structures of human MAP kinase kinase 1(MEK1) and MEK2 describe novel non competitive kinase inhibition. Nat Struct Mol Biol 11:1192–1197
22. Huynh H, Nguyen TTT, Chow KHP et al (2003) Over-expression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: its role in tumor progression and apoptosis. BMC Gastroenterol 3:19–40
23. Engelma J (2009) Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer 9:550–562
24. Zhou L, Huang Y, Li J et al (2009) The mTOR pathway is associated with the poor prognosis of human hepatocellular carcinoma. Med Oncol (Epub ahead of print)
25. Chen J, Wang Q, Fu X et al (2009) Involvement of PI3K/PTEN/AKT/mTOR pathway in invasion and metastasis in hepatocellular carcinoma: association with MMP-9. Hepatol Res 39:177–186
26. Villanueva A, Chiang DY, Newell P et al (2008) Pivotal role of mTOR signaling in hepatocellular carcinoma. Gastroenterology 135:1972–1983
27. Treiber G (2009) mTOR inhibitors for hepatocellular carcinoma: a forward-moving target. Expert Rev Anticancer Ther 9:247–261
28. Ferrara N, Davis-Smyth T (1997) The biology of vascular endothelial growth factor. Endocr Rev 18:4–25
29. Griffioen AW, Molema G (2000) Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. Pharmacol Rev 52:237–268
30. Harper SJ, Bates DO (2008) VEGF-A splicing: the key to anti-angiogenic therapeutics? Nat Rev Cancer 8:880–887
31. Fernandez M, Semela D, Bruix J et al (2009) Angiogenesis in liver disease. J Hepatol 50:604–620
32. Schoenleber SJ, Kurtzl DM, Talwalkar JA et al (2009) Prognostic role of vascular endothelial growth factor in hepatocellular carcinoma: systematic review and meta-analysis. Br J Cancer 100:1385–1392
33. Faivre S, Raymond E, Boucher E et al (2009) Safety and efficacy of Sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicenter, phase II study. Lancet Oncol 10:794–800
34. Zhu AX, Sahani DV, Duda DG et al (2009) Efficacy, safety, and potential biomarker of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. J Clin Oncol 27:3027–3035
35. Forner A, Llovet JM, Bruix J (2009) Sunitinib and the benefits of a negative study. Lancet Oncol 10:743–744
36. Raoul JL, Flinn RS, Kang YK et al (2009) An open-label phase II study of first- and second-line treatment with Brivanib in patients with hepatocellular carcinoma (HCC). J Clin Oncol 27:15S Suppl; Abstr 4577
37. Kanai F, Yoshida H, Tateishi R et al (2008) Final results of a phase I/II trial of the oral anti-angiogenesis inhibitor TSU-68 in patients with advanced hepatocellular carcinoma. J Clin Oncol 26 (abstract 4589)
38. Ciardiello F, Tortora G (2008) EGFR antagonists in cancer treatment. N Engl J Med 358:1160–1174
39. Buckley AF, Burgart LJ, Sahai V et al (2008) Epidermal growth factor receptor expression and gene copy number in conventional hepatocellular carcinoma. Am J Clin Pathol 129:245–251
40. Philip PA, Mahoney MR, Allmer C et al (2005) Phase II study of erlotinib (OSI-774) in patients with advanced hepatocellular cancer. J Clin Oncol 23:6657–6663
41. Thomas MB, Chadhal R, Glover K et al (2007) Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer 110:1059–1066
42. Thomas MB, Morris JS, Chadha R et al (2009) Phase II trial of the combination of bevacizumab and Erlotinib in patients who have advanced hepatocellular carcinoma. J Clin Oncol 27:843–850
43. Modified folfox7/bevacizumab or modified Xelox/bevacizumab with or without erlotinib in first-line metastatic colorectal cancer (MCRC): results of the feasibility phase of the DREAM-OPTIMOX3 study (GERCOR). J Clinical Oncol, 2007 ASCO Annual Meeting Proceedings Part I, vol 25, No. 18S: 4097
44. Tournigand B, Samson W, Scheithauer C et al (2009) mFOLFOX-bevacizumab or XELOX-bevacizumab then bevacizumab alone or with erlotinib in first-line treatment of patients with metastatic colorectal cancer (mCRC): interim safety analysis of DREAM study. J Clin Oncol 27:15S, ASCO Annual Meeting Abstract No:4077C
45. Hecht JR, Mitchell E, Chidiac T et al (2008) A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol 27:672–680
46. Azad NS, Posadas EM, Kwitkowski VE et al (2008) Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. J Clin Oncol 26:3709–3714
47. Burris HA III, Hurwitz HI, Dees EC et al (2005) Phase I safety, pharmacokinetics, and clinical activity of Lapatinib (GW572016), a reversible dual inhibitor of epidermal growth factor receptor tyrosine kinases, in heavily pretreated patients with metastatic carcinomas. J Clin Oncol 23:5305–5313
48. Bekaii-Saab T, Markowitz J, Prescott N et al (2009) A multi-institutional phase II study of the efficacy and tolerability of Lapatinib in patients with advanced hepatocellular carcinomas. Clin Cancer Res 15:5895–5901
49. Zhu AX, Stuart K, Blaszkowsky LS et al (2007) Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer 110:581–589
50. Comoglio PM, Giordano S, Trusolino L (2008) Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Dis 7:501–516
51. Scharf JG, Braulke T (2003) The role of the IGF axis in hepatocarcinogenesis. Horm Metab Res 35:685–693
52. Chen YW, Boyartchuk V, Lewis BC (2009) Differential roles of insulin-like growth factor receptor–and insulin receptor–mediated signaling in the phenotypes of hepatocellular carcinoma cells. Neoplasia 11:835–845
53. Llovet JM, Ricci S, Mazzaferro V et al (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359:378–390
54. Cheng AL, Kang YK, Chen Z et al (2009) Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 10:25–34
55. Kudo M (2010) Positioning of a molecular-targeted agent, sorafenib, in the treatment algorithm for hepatocellular carcinoma in Japan, including its impact on complete remission. Oncology (in press)
56. Okita K, Imanaka K, Chiba N et al (2010) Phase III study of sorafenib in patients in Japan and Korea with advanced hepatocellular carcinoma (HCC) treated after transarterial chemoembolization. ASCO Gastrointestinal Cancers Symposium Proceedings 2010: 89 (LBA128)
57. Murata K, Suzuki H, Okano H et al (2010) Hypoxia-induced des-γ-carboxy prothrombin production in hepatocellular carcinoma. Int J Clin Oncol 36:161–170
58. Abou-Alfa GK, Schwartz L, Ricci S et al (2006) Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol 24:4293–4300
59. Toh H, Chen PJ, Carr BI et al (2009) A phase II study of ABT-869 in hepatocellular carcinoma (HCC) : Interim analysis. J Clin Oncol 27:15s (Suppl; Abstr 4581)

60. Siegel AB, Cohen EI, Ocean A et al (2008) Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol 26:2992–2998
61. O'Dwyer PJ, Giantonio BJ, Levy DE et al (2006) Gefitinib in advanced unresectable hepatocellular carcinoma: Results from the Eastern Cooperative Oncology Group's Study E1203. J Clin Oncol 24:18S (Suppl, Abstr 4143)
62. Ramanathan RK, Belani CP, Singh DA et al (2009) A phase II study of Lapatinib in patients with advanced biliary tree and hepatocellular cancer. Cancer Chemother Pharmacol 64:777–783

Introduction

Oncology

Oncology 2010;78(suppl 1):1–6
DOI: 10.1159/000315222

Published online: July 8, 2010

Management of Hepatocellular Carcinoma: From Prevention to Molecular Targeted Therapy

Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Key Words
Hepatocellular carcinoma, prevention · Molecular targeted therapy · Sorafenib · Treatment algorithm

Abstract
Hepatocellular carcinoma is a malignant tumor responsible for approximately 600,000–700,000 deaths worldwide, and is becoming more prevalent not only in South-East Asia and Africa, but also in Western countries; therefore, interest in hepatocellular carcinoma has mounted in recent years in the West, where little or no interest was evident 10–20 years ago.

Introduction

The 3rd International Kobe Liver Symposium on Hepatocellular Carcinoma (HCC) was held on June 6–7, 2009, in conjunction with the 45th Annual Meeting of the Japan Society of Hepatology on June 4–5 (Congress President: Prof. Masatoshi Kudo). To this symposium, a total of 20 oversea guests, all globally recognized HCC specialists, were invited (table 1). Numerous topics were presented followed by extensive discussions with Japanese HCC specialists.

This supplement issue focuses on these topics, from the prevention to molecular targeted therapy. I firmly believe that readers will gain a deeper insight into the latest progress and updated diagnosis and treatment of HCC.

Table 1. Invited overseas speakers at the 3rd IKLS 2009 in Kobe

Luigi Bolondi (Italy)	Joong-Won Park (Korea)
Jordi Bruix (Spain)	Ronnie T. Poon (Hong Kong)
Pei-Jer Chen (Taiwan)	Tania Roskams (Belgium)
Byung Ihn Choi (Korea)	Myron Schwartz (USA)
Michel Claudon (France)	Morris Sherman (Canada)
Kwang Hyub Han (Korea)	Mitchell L. Shiffman (USA)
Riccardo Lencioni (Italy)	Hui Chuan Sun (China)
Shi-Ming Lin (Taiwan)	S. Thorgeirsson (USA)
Joseph M. Llovet (Spain	Swan N. Thung (USA)
Jorge Marrero (USA)	J. Zucman-Rossi (France)
Vincenzo Mazzaferro (Italy)	

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0001$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

Prevention

Prevention of Hepatitis B Virus (HBV)-Related HCC

Primary Prevention of HCC by HBV Vaccination

About 350 million people are chronic carriers of the HBV worldwide. The efficacy of universal immunization has been shown in many countries, with striking reductions of the prevalence of HBV carriage in children. A nationwide vaccination program against HBV launched in Taiwan [1–3] has drastically reduced the HBsAg carrier rate in younger populations [4]. More importantly, follow-up results from the Taiwan vaccination programs have shown the incidence of HCC has been significantly reduced in children. The average annual incidence of HCC in children 6–14 years of age declined from 0.70/100,000 children between 1981 and 1986 to 0.57 between 1986 and 1990, and further to 0.36/100,000 between 1990 and 1994 ($p < 0.01$) [5].

Secondary Prevention of HCC by Interferon (IFN) Therapy

There was one randomized controlled trial [6] which involved 101 Taiwanese men with chronic hepatitis B, 67 of whom received IFN and 34 of whom received placebo. During 1.1–11.5 years after completion of therapy, the incidence of HCC in untreated patients was higher than that in IFN-treated patients (12 vs. 1.5%, $p = 0.043$). The cumulative incidence of HCC was also higher in untreated patients than treated patients ($p = 0.013$).

A meta-analysis of randomized studies comparing IFN-treated versus untreated patients with HBV-related cirrhosis showed that IFN seemingly decreased the rate of HCC [7].

Secondary Prevention of HCC by Nucleoside Analog

To date, only one randomized controlled trial suggests that lamivudine (LAM) treatment of chronic hepatitis B and advanced liver disease does reduce the incidence of HCC but with marginal significance (hazard ratio 0.49; 95% CI 0.25–0.99; $p = 0.047$) [8]. A multicenter retrospective study of 2,795 patients (657 treated with LAM, 2,138 not treated with LAM) was reported from Japan [9]. The cumulative HCC incidence was significantly lower in the LAM group ($p < 0.001$). These findings suggest that LAM effectively reduces the incidence of HCC in patients with chronic hepatitis B.

Prevention of HCV-Related HCC

Primary Prevention by Prevention of Viral Transmission

It is well known that HCV infection has become prevalent recently under artificial circumstances: mother-neonate transmission and sexual transmission of the virus are possible but not common. In many countries, new acquisition of HCV infection is decreasing due to growing concern about blood-transmitted infections, especially about HIV, and this trend should be further encouraged considering the absence of an effective vaccination for either HCV or HIV.

Secondary Prevention by Treatment of Chronic Hepatitis C

The effect of IFN therapy on HCC incidence in non-cirrhotic patients has been evaluated in non-randomized studies. All studies agree that the risk is reduced in patients who show sustained virologic response or persistent normalization of serum ALT levels [10–13]. Although documentation is rather scarce, the combination with ribavirin will produce a stronger effect on HCC prevention among overall treated patients [14].

Surveillance for Early Detection of HCC

Definition of the Population at High Risk for HCC

Liver cirrhosis induced by causes other than HBV and HCV is a risk for liver carcinogenesis. Since carcinogenesis occurs in some cases of liver cirrhosis associated with non-alcoholic steatohepatitis, alcoholic liver disease, primary biliary cirrhosis and autoimmune hepatitis, the course of the disease should be followed with close attention to carcinogenesis, particularly in viral liver cirrhosis. Alcohol increases the risk of chronic hepatitis B- and C-associated liver carcinogenesis.

Based on the above, patients with chronic hepatitis B and C and non-viral liver cirrhosis are defined as high-risk populations for HCC in both the Evidence-Based Practice Guidelines [15] proposed by the Japan Society of Hepatology and the Consensus-Based Clinical Practice Manual [16] in Japan and Practice Guidelines published by the American Association of Study of the Liver (AASLD) [17]. Patients with liver cirrhosis types B and C are defined as a super-high-risk population [15, 16].

Surveillance Protocol for Early Detection of HCC

No clear evidence is available to determine the optimal interval for periodic screening, but HCCs detected in pe-

riodic screening by AFP, a protein induced by vitamin K absence or antagonist-II (PIVKA-II), AFP lectin fraction (AFP-L3) measurement, and ultrasonography are solitary and small in many cases, as compared to those detected in symptomatic patients. Thus, the Japanese Evidence-Based Clinical Practice Guidelines [15] and Consensus-Based Clinical Practice Manual [16] propose ultrasonography and tumor marker measurements every 3–4 months in the super-high-risk population and every 6 months in high-risk populations.

Result of Early Detection of HCC in Japan

In Japan, approximately 65% of the patients are detected in an early stage, for which curative treatment intervention is possible according to the nationwide survey in 198,000 patients [18]. This can be attributed to the establishment of a nationwide surveillance system all over the Japan.

Newly Introduced Diagnostic Techniques

Contrast-Enhanced US (CEUS) with a New Contrast Agent, Sonazoid

Clinical Significance of CEUS

Sonazoid is a newly introduced second-generation ultrasound contrast agent exclusively approved in Japan in 2007. The important characteristics of Sonazoid are that it facilitates real-time imaging in blood flow images at low acoustic power and stable Kupffer phase imaging, tolerable for multiple scanning from 10 to 120 min after its injection [19], which resulted in the invention of the breakthrough method, defect reperfusion imaging. Sonazoid-enhanced US with defect reperfusion imaging is an innovative technology that will greatly change the daily practices of HCC.

Development of Defect Reperfusion Imaging (Dual-Phase Fusion Imaging)

We recently developed defect reperfusion imaging [20–22] using the properties of very stable Kupffer images and real-time fine blood flow images obtained with Sonazoid for typical HCC, which is depicted by CT but not by B-mode scanning. This method is a breakthrough for accurate localization and treatment guidance [21]. Until recently, diagnosis in dynamic studies is usually based on enhancing patterns according to a time sequence or phase; however, by introducing the novel idea of dual-phase imaging with the re-injection method, both Kupffer and arterial-phase images are obtained at the same slice of ultrasound plane, which is really an innovative technique. Namely, this method is performed as follows: re-injection of Sonazoid is performed into areas that show defects in the post-vascular phase [19–22]. The introduction of this method has led to dramatic solutions of many limitations in the diagnosis and treatment of HCC, such as detection of small HCCs [23], evaluation of treatment response [24], or needle insertion guidance [25]. The detection rate of small HCCs by Sonazoid-enhanced US is even more sensitive than that by MDCT [23].

MRI Using a New Contrast Agent, Gd-EOB-DTPA, in the Diagnosis of Early HCC

A newly introduced contrast agent, gadolinium-diethylene-triamine-pentaacetic acid (Gd-EOB-DTPA), approved in 2008 in Japan, is a hepatocyte-specific MRI contrast medium with a different mechanism, utilizing both dynamic and Kupffer cell imaging. This new contrast medium is useful to diagnose cases which would have been difficult using previous techniques such as dynamic MRI or SPIO-MRI.

In well-differentiated early HCC, some nodules may not be completely shown as a defective area on CTAP, but Gd-EOB-DTPA uptake is apparently lower than that in the surrounding normal liver parenchyma, being imaged as a low-intensity nodule. Well-differentiated early HCC having Kupffer cells with enhanced SPIO uptake and receiving portal blood flow on CTAP has been difficult to characterize by SPIO-MRI or CTAP; however, it can be imaged clearly as hypointense nodule using Gd-EOB-DTPA MRI in many early HCC cases due to differences in the biological characteristics, indicating that this contrast agent may lead to a breakthrough in the diagnosis of early HCC [26, 27], which has been clinically difficult and difficult even by pathological diagnosis when biopsy sample is used. In other words, this technique may be the most sensitive tool in the detection of the phenotypic change of early hepatocarcinogenesis, much more sensitive than CTAP, CTHA, or SPIO-MRI.

Therefore, diagnostic algorithm will be changed by introducing Gd-EOB-DTPA MRI in hyper- and hypovascular liver nodules [28, 29].

Value of an Integrated Staging System

The staging system integrating the TNM and liver damage stage is very important. Various staging systems, such as (1) Okuda stage, (2) BCLC stage [30, 31], (3) CLIP

score [32], (4) JIS score [33, 34], and (5) Tokyo score [35] have been proposed and used in different regions of the world. The JIS score, utilizing both the LCSGJ TNM [36] and Child-Pugh stages, is considered to be the most useful for integrated staging of HCC in Japan. In contrast, the CLIP score has several disadvantages: specification of the tumor-spreading degree is rough, only AFP is used as a biological malignancy marker, and stratification ability is also poor in advanced cases (many cases cluster to a score of 0–2). By contrast, the JIS score is superior for score stratification.

Hepatic Arterial Infusion Chemotherapy for Advanced HCC

No effective anticancer drug for advanced liver cancer had been demonstrated before sorafenib was introduced [37]. 'Far advanced liver cancer' represents stage IVa liver cancer accompanied by vascular invasion and stage IVb liver cancer accompanied by distant metastasis, for which low-dose FP (5-FU and cisplatinum) [38] therapy and hepatic arterial infusion of 5-FU in combination with IFN treatment [39] were established as an effective treatment option in Japan. In fact, response rate (CR + PR) reaches 45.9% according to the Nationwide Survey by LCSGJ [18]. In addition, it is well established that overall survival of the responder is definitely better than the non-responder or best supportive care group. However, the intra-arterial infusion procedure is complex because establishment of a reservoir port for arterial infusion is necessary; therefore, this technique is not performed in Western countries.

Hepatic intra-arterial infusion chemotherapy is not recommended in the AASLD guidelines [17]; therefore, although the response rate is high, the efficacy of especially survival benefit of intra-arterial infusion chemotherapy and that using an intractable delivery port system should be confirmed by further randomized studies.

New Treatment Option: Molecular Targeted Agent, Sorafenib

Sorafenib is a low-molecular-weight compound discovered by screening inhibitors of Raf kinase, an important molecule in the MAP kinase cascade located downstream of the growth factor receptor. Sorafenib exhibits strong inhibitory activity for not only c-Raf, the wild-type, and V600E mutant b-Raf, but also receptor tyrosine kinases involved in angiogenesis and cell growth, such as vascular endothelial growth factor receptor (VEGFR)-2, VEGFR-3, platelet-derived growth factor receptor, Fms-related tyrosine kinase-3 (Flt-3), and c-Kit.

The positive results of a phase III study for HCC (SHARP trial) [40] gave a strong impact on treatment strategy of HCC. This study was performed as a randomized double-blind placebo-controlled multicenter study initiated in March 2005. The subjects were advanced HCC patients at ECOG PS 0–2 with Child-Pugh A liver function without previous systemic chemotherapy. Regarding the study design, two groups, sorafenib (400 mg b.i.d.) and placebo treatment, were established and the primary endpoints were overall survival. The secondary endpoints were time to progression.

Ongoing Clinical Trials with Molecular Targeted Agents

As stated earlier, the STORM trial using sorafenib as an adjuvant setting after curative treatment such as resection or ablation is ongoing as a global trial. In addition, the SPACE trial and TACTICS trial using sorafenib in combination with TACE are ongoing in Western countries and Japan, respectively. The SILIUS trial using sorafenib in combination with hepatic arterial infusion chemotherapy is under investigation in Japan. Furthermore, head-to-head trials of sunitinib versus sorafenib and brivanib versus sorafenib for advanced HCC are ongoing globally. Finally, a second-line trial of brivanib for the sorafenib failure is also ongoing as a global clinical trial [29]. These trial results are awaited to bring better outcomes for different stages of HCC. If positive results are obtained by these clinical trials, the life expectancy in each stage is expected to be considerably prolonged if a theoretical calculation using the hazard ratio on overall survival is incorporated from the SHARP trial [29].

In Japan, although a phase III study in HCC patients following TACE was revealed to be a negative study [41], an investigator-sponsored trial of investigating efficacy and tolerability of a combination of TACE with sorafenib is underway. In addition, a phase III trial for HCC of acyclic retinoid, a vitamin A analog, after resection or RFA has been completed and will be presented at the American Society of Clinical Oncology Meeting, 2010.

A global phase III trial of sorafenib as an adjuvant therapy after surgery or ablation is now ongoing (STORM trial) and a global phase II trial of sorafenib as a maintenance therapy with a combination of TACE is also ongoing (SPACE trial). These results are awaited to

confirm its usefulness in daily clinical practice. A paradigm shift in HCC treatment may be induced if the positive results are obtained by these currently ongoing sorafenib trials.

Conclusion

Recent progress in the management of HCC, including issues from prevention to molecular targeted therapy for HCC, has been discussed at this symposium. It is strongly expected that this supplement issue will enhance the most up-to-date knowledge on HCC of the readers of *Oncology*.

Disclosure Statement

The author declares that he has no financial conflict of interest.

References

1 El-Serag HB: Epidemiology of hepatocellular carcinoma in USA. Hepatol Res 2007; 37:S88–S94.
2 Chen DS, Hsu NH, Sung JL, Hsu TC, Hsu ST, Kuo YT, Lo KJ, Shih YT: A mass vaccination program in Taiwan against hepatitis B virus infection in infants of hepatitis B surface antigen-carrier mothers. JAMA 1987;257: 2597–2603.
3 Hsu HM, Chen DS, Chuang CH, Lu JC, Jwo DM, Lee CC, Lu HC, Cheng SH, Wang YF, Wang CY, et al: Efficacy of a mass hepatitis B vaccination program in Taiwan. Studies on 3,464 infants of hepatitis B surface antigen-carrier mothers. JAMA 1988;260:2231–2235.
4 Chen HL, Chang MH, Ni YH, Hsu HY, Lee PI, Lee CY, Chen DS: Seroepidemiology of hepatitis B virus infection in children: ten years of mass vaccination in Taiwan. JAMA 1996;276:906–908.
5 Chang MH, Shau WY, Chen CJ, Wu TC, Kong MS, Liang DC, Hsu HM, Chen HL, Hsu HY, Chen DS: Hepatitis B vaccination and hepatocellular carcinoma rates in boys and girls. JAMA 2000;284:3040–3042.
6 Lau DT, Everhart J, Kleiner DE, Park Y, Vergalla J, Schmid P, Hoofnagle JH: Long-term follow-up of patients with chronic hepatitis B treated with interferon alfa. Gastroenterology 1997;113:1660–1667.
7 Yang YF, Zhao W, Zhong YD, Xia HM, Shen L, Zhang N: Interferon therapy in chronic hepatitis B reduces progression to cirrhosis and hepatocellular carcinoma: a meta-analysis. J Viral Hepat 2009;16:265–271.
8 Liaw YF, Sung JJ, Chow WC, Farrell G, Lee CZ, Yuen H, Tanwandee T, Tao QM, Shue K, Keene ON, et al: Lamivudine for patients with chronic hepatitis B and advanced liver disease. N Engl J Med 2004;351:1521–1531.
9 Matsumoto A, Tanaka E, Rokuhara A, Kiyosawa K, Kumada H, Omata M, Okita K, Hayashi N, Okanoue T, Iino S, et al: Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. Hepatol Res 2005;32:173–184.
10 Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, et al: Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 1999;131:174–181.
11 Imai Y, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, et al: Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. Ann Intern Med 1998;129:94–99.
12 Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, Tsubota A, Nakamura I, Murashima N, Kumada H, et al: Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–1130.
13 Shindo M, Ken A, Okuno T: Varying incidence of cirrhosis and hepatocellular carcinoma in patients with chronic hepatitis C responding differently to interferon therapy. Cancer 1999;85:1943–1950.
14 Yu ML, Lin SM, Chuang WL, Dai CY, Wang JH, Lu SN, Sheen IS, Chang WY, Lee CM, Liaw YF: A sustained virological response to interferon or interferon/ribavirin reduces hepatocellular carcinoma and improves survival in chronic hepatitis C: a nationwide, multicentre study in Taiwan. Antivir Ther 2006;11:985–994.
15 Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, et al: Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008; 38:37–51.
16 Kudo M, Okanoue T: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(suppl):2–15.
17 Bruix J, Sherman M: Management of hepatocellular carcinoma. Hepatology 2005;42: 1208–1236.
18 Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, et al: Report of the 17th Nationwide Follow-Up Survey of Primary Liver Cancer in Japan. Hepatol Res 2007;37: 676–691.
19 Inoue T, Kudo M, Hatanaka K, Takahashi S, Kitai S, Ueda T, Ishikawa E, Hagiwara S, Minami Y, Chung H, et al: Imaging of hepatocellular carcinoma: qualitative and quantitative analysis of postvascular phase contrast-enhanced ultrasonography with Sonazoid. Oncology 2008;75(suppl 1):48–54.
20 Kudo M, Hatanaka K, Maekawa K: Defect reperfusion imaging, a newly developed novel technology using Sonazoid in the treatment of hepatocellular carcinoma. J Med Ultrasound 2008;16:169–175.
21 Kudo M, Hatanaka K, Chung H, Minami Y, Maekawa K: A proposal of novel treatment-assist technique for hepatocellular carcinoma in the Sonazoid-enhanced ultrasonography: value of defect re-perfusion imaging (in Japanese). Kanzo 2007;48:299–301.
22 Kudo M, Hatanaka K, Maekawa K: Sonazoid-enhanced ultrasound in the diagnosis and treatment of hepatic tumors. J Med Ultrasound 2008;16:130–139.

23 Hatanaka K, Kudo M, Minami Y, Maekawa K: Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. Oncology 2008;75(suppl 1):42–47.

24 Xia Y, Kudo M, Minami Y, Hatanaka K, Ueshima K, Chung H, Hagiwara S, Inoue T, Ishikawa E, Kitai S, et al: Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: the usefulness of Sonazoid-enhanced harmonic sonography. Oncology 2008;75(suppl 1):99–105.

25 Minami Y, Kudo M: Contrast-enhanced harmonic ultrasound imaging in ablation therapy for primary hepatocelular carcinoma. World J Radiol 2009;31:86–91.

26 Kanematsu M, Kondo H, Goshima S, Tsuge Y, Watanabe H: Magnetic resonance imaging of hepatocellular carcinoma. Oncology 2008;75(suppl 1):65–71.

27 Kim M, Choi J, Chung Y, Choi S: Magnetic resonance imaging of hepatocellular carcinoma using contrast media. Oncology 2008; 75(suppl 1):72–82.

28 Kudo M: Will Gd-EOB-MRI change the diagnostic algorithm for hepatocellular carcinoma? Oncology 2010;78(suppl 1):87–93.

29 Kudo M: The 2008 Okuda Lecture: Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. J Gastroenterol Hepatol 2010;439–452.

30 Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J: Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona 2000 EASL Conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.

31 Llovet JM, Bru C, Bruix J: Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19:329–338.

32 The Cancer of the Liver Italian Program (CLIP) Investigators: Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatology 2000;31:810–845.

33 Kudo M, Chung H, Osaki Y: Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). J Gastroenterol 2003;38:207–215.

34 Kudo M, Chung H, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T: Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. Hepatology 2004;40:1396–1405.

35 Tateishi R, Yoshida H, Shiina S, Imamura H, Hasegawa K, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, et al: Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. Gut 2005;54:419–425.

36 Liver Cancer Study Group of Japan: General Rules for the Clinical and Pathological Study of Primary Liver Cancer, Engl ed 2. Tokyo, Kanehara, 2003.

37 Kitai S, Kudo M, Minami Y, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T: Validation of a new prognostic staging system for hepatocellular carcinoma: a comparison of the biomarker-combined Japan integrated staging score, the conventional Japan integrated staging score and the BALAD score. Oncology 2008;75(suppl 1): 83–90.

38 Ando E, Tanaka M, Yamashita F, Kuromatsu R, Yutani S, Fukumori K, Sumie S, Yano Y, Okuda K, Sata M: Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. Cancer 2002;95:588–595.

39 Sakon M, Nagano H, Dono K, Nakamori S, Umeshita K, Yamada A, Kawata S, Imai Y, Iijima S, Monden M: Combined intraarterial 5-fluorouracil and subcutaneous interferon-α therapy for advanced hepatocellular carcinoma with tumor thrombi in the major portal branches. Cancer 2002;94:435–442.

40 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, et al: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.

41 Okita K, Imanaka K, Chiba N, Tak W, Nakachi K, Takayama T, Suh D, Kumada H, Wada M, Kudo M: Phase III study of sorafenib in patients in Japan and Korea with advanced hepatocellular carcinoma treated after transarterial chemoembolization. ASCO Gastrointestinal Cancers Symposium Proceedings 2010, No 89 (LBA128).

Oncology

Oncology 2010;78(suppl 1):40–45
DOI: 10.1159/000315229

Published online: July 8, 2010

Newly Developed Novel Ultrasound Technique, Defect Reperfusion Ultrasound Imaging, Using Sonazoid in the Management of Hepatocellular Carcinoma

Masatoshi Kudo[a] Kinuyo Hatanaka[a] Kiyoshi Maekawa[b]

[a]Department of Gastroenterology and Hepatology, and [b]Division of Ultrasound, Kinki University School of Medicine, Osaka, Japan

Key Words

Ultrasound, contrast-enhanced harmonic · Sonazoid · Hepatic tumors · Radiofrequency ablation · Guidance, treatment-assisted · Defect reperfusion imaging · Kupffer phase imaging

Abstract

The aim of this study is to clarify the usefulness of defect reperfusion ultrasound (US) imaging using Sonazoid in the management of hepatocellular carcinoma (HCC). A total of 33 HCC nodules and 34 local recurring nodules after radiofrequency ablation (RFA), which could not be identified by B-mode US but were depicted by dynamic CT, were studied by defect reperfusion US imaging with Sonazoid. In addition, Kupffer phase Sonazoid-enhanced US in combination with defect reperfusion US imaging were used for screening HCC in 262 consecutive cirrhotic patients. As a result, 33 US undetectable HCC nodules and 34 local recurring HCC nodules were successfully confirmed by Sonazoid-enhanced US with defect reperfusion imaging. Subsequently, RFA was successfully performed in all of 67 HCC nodules with a Sonazoid-enhanced US guidance. A total of 7 small HCCs were depicted and confirmed as HCCs by Kupffer phase surveillance and defect reperfusion US imaging. In conclusion, defect reperfusion US imaging is extremely useful in the depiction and confirmation of US undetectable HCCs as well as in the surveillance of HCC in cirrhotic patients.

Introduction

Imaging techniques in the treatment of hepatocellular carcinoma (HCC) are very important. In the management of HCC, despite advances in diagnostic imaging techniques such as ultrasound (US), computed tomography (CT) and magnetic resonance imaging (MRI), many difficulties remain, such as screening, staging, evaluation of treatment response, treatment guidance, identification of local recurring nodules after treatment, and diagnosis of intrahepatic recurrence after treatment. In fact, among these problems, Levovist-enhanced US has made a certain contribution to differential diagnosis [1–3], evaluation of malignancy grade [4], evaluation of therapeutic response to transcatheter arterial chemoembolization (TACE) [5–7], and needle insertion guidance [8, 9]. However, there are still significant limitations in the evaluation of the therapeutic response to radiofrequency ablation (RFA) [10], screening or staging.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0040$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

The main characteristics of Sonazoid are that it facilitates stable Kupffer phase imaging tolerable for repeated scanning from 10 to 60 min after its injection and the acquisition of real-time blood flow images at low acoustic power. Before the introduction of Sonazoid, it was thought that Sonazoid would be more effective than Levovist in real-time vascular imaging, be easier to use, and allow a good image even using a non-high-end machine, and therefore, dependence on skills/machines would be decreased, which may facilitate the widespread use of contrast-enhanced US. In addition, although Sonazoid US provides very stable Kupffer phase images, it would be tolerable for multiple scanning. Taking this into account, it was not expected that Sonazoid would markedly change HCC practice before its clinical use. However, this preconception has changed since it was launched on January 10, 2007, in Japan.

In particular, because an epoch-making technique has been invented, we became convinced that Sonazoid US with this method is an innovative breakthrough technique that will greatly change the daily practice with regard to HCC. This epoch-making method is called double-contrast US, performed by Sonazoid reinjection into areas that show defects in the Kupffer phase [11].

The aim of this study is to evaluate the diagnostic efficacy of double-contrast US imaging in US ill-defined nodules.

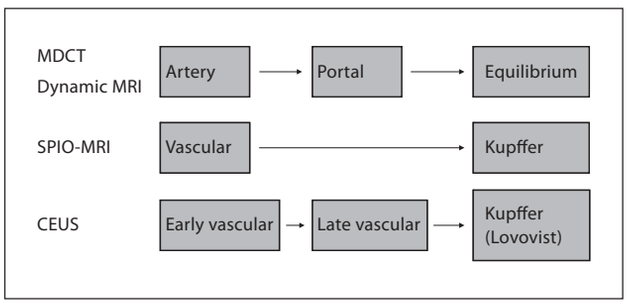


Fig. 1. Common sense of imaging/dynamic studies such as CEUS, MDCT, and MRI. Diagnostic imaging is performed based on enhancing patterns according to time sequence or phase.

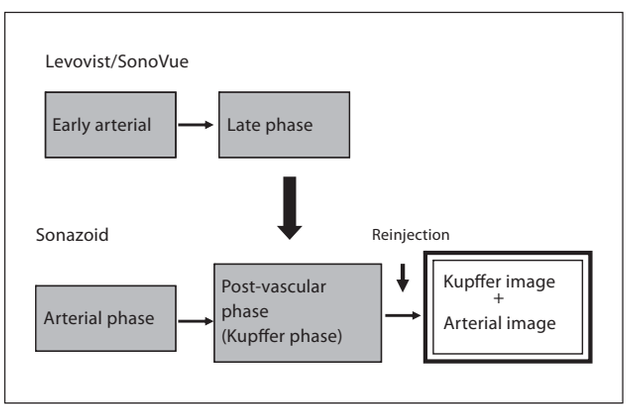


Fig. 2. Innovation: change of IDEA. An innovative technique has been developed by reinjecting Sonazoid. Dual-phase imaging that combines Kupffer and arterial phase images is obtained by this method.

Patients and Methods

US Ill-Defined Nodules

From January 2007 to May 2008, 33 of 847 consecutive patients with HCC nodules underwent defect reperfusion US study using Sonazoid. The nodules could not be visualized on B-mode US but were visualized as typical HCC on dynamic CT with the characteristic findings of arterial enhancement with venous washout.

Locally Recurring Nodules after RFA

After RFA 34 patients with locally recurring nodules that were not identified by B-mode US, but were easily identified by dynamic CT, were evaluated by defect reperfusion US imaging with Sonazoid.

Screening by Kupffer Phase of Sonazoid CEUS with Defect Reperfusion US Imaging

262 consecutive cirrhotic patients (198 cases of hepatitis C-related cirrhosis; 64 cases of hepatitis B-related cirrhosis) were screened by Kupffer phase contrast-enhanced US with Sonazoid, and defect reperfusion US study was performed in cases in which a Kupffer defect was found.

Sonazoid (0.01 ml/kg) is intravenously injected into a patient at high risk of HCC (hepatitis B and C liver cirrhosis) in the outpatient clinic. Subsequently, in the US department, technologists perform Kupffer phase imaging between 10 and 60 min after intravenous injection. When a defective area is found on Kupffer phase, reinjection of Sonazoid is performed to depict whether an arterial supply is present or not in the Kupffer defect nodule.

Methods

Double-contrast US imaging using the properties of very stable Kupffer images and real-time fine blood flow images provided by Sonazoid were performed for typical HCC, which is depicted by CT but not by B-mode scanning. Diagnosis in a dynamic study is usually based on enhancing patterns according to time sequence or phase (fig. 1). However, by changing the basic idea, combined Kupffer and arterial phase images are obtained by Sonazoid reinjection at the Kupffer phase (fig. 2). The ultrasound machine used in this study is GE LOGIQ7 (GE Healthcare, Milwaukee, Wisc., USA).

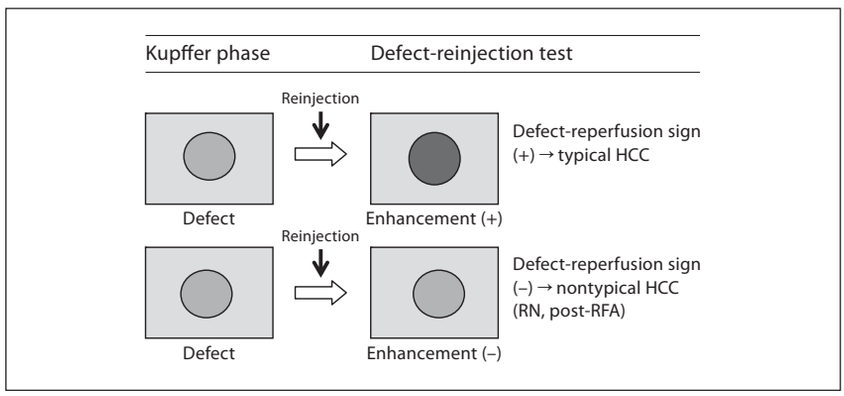


Fig. 3. Defect reperfusion imaging for B-mode ill-defined nodule. Even typical HCC nodules that are not identified by B-mode US and are shown as early enhancement with portal/venous washout can be correctly diagnosed if arterial enhancement is obtained within the Kupffer defect area (Kupffer defect with hypervascularization).

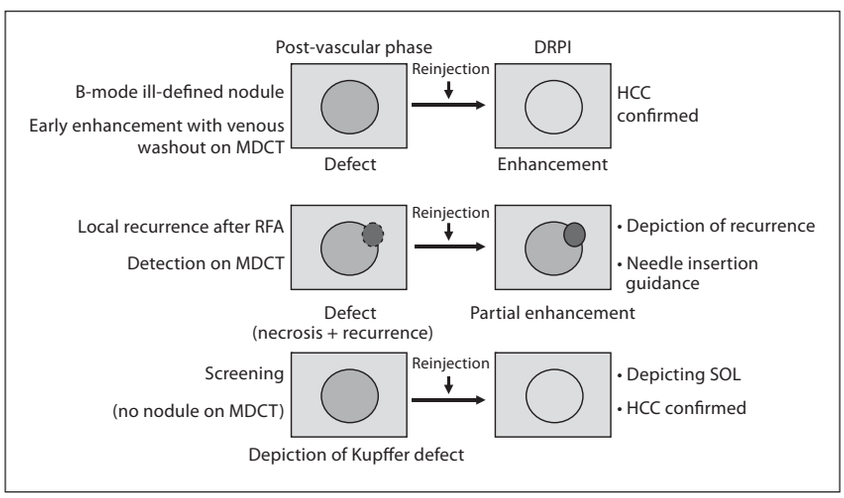


Fig. 4. Defect reperfusion US image. Defect reperfusion imaging is useful in the detection of nodules that are ill defined by B-mode US, locally recurrent nodules and nodules depicted at screening/surveillance.

After intravenous injection of Sonazoid (0.01 ml/kg), early staining is observed in the vascular phase, and the presence or absence of defects is determined by an entire liver scan in the Kupffer phase 10–60 min after injection. Subsequently, the probe is applied to the area that shows a defect in the Kupffer phase 10–60 min after intravenous injection of Sonazoid. Sonazoid (0.01 ml/kg) is additionally injected, and whether or not arterial blood flow enters the defective area is determined (defect reinjection test). When the fast wash in of arterial flow is observed in the Kupffer defect area, this is regarded as a confirmative sign of HCC (fig. 3).

Results

US Ill-Defined Nodules

For 33 HCC nodules that could not be visualized by B-mode US but were clearly demonstrated by dynamic CT, clear defects could be detected at the Kupffer phase of Sonazoid-enhanced US. Furthermore, after reinjection of Sonazoid, clear wash in and staining could be detected in the Kupffer defect area (defect reperfusion sign, positive). These patients were all candidates for RFA, and RFA under the guidance of Kupffer phase Sonazoid-enhanced US was possible in all 33 HCC cases that were not identified by B-mode US. Sensitivity was as high as 100% (fig. 3–5).

Locally Recurring Nodules after RFA

Similarly, in 34 cases of local progression nodules or recurrence at a different region after RFA, which were not identified by B-mode US, localization of the recurring portion was clearly identified in all cases (sensitivity 100%) with the defect reperfusion US technique (fig. 3–6). Subsequently, RFA was successfully performed on all 33 nodules. The remaining one HCC nodule was surgically resected because of the potential bleeding risk due to close location to the heart (fig. 6).

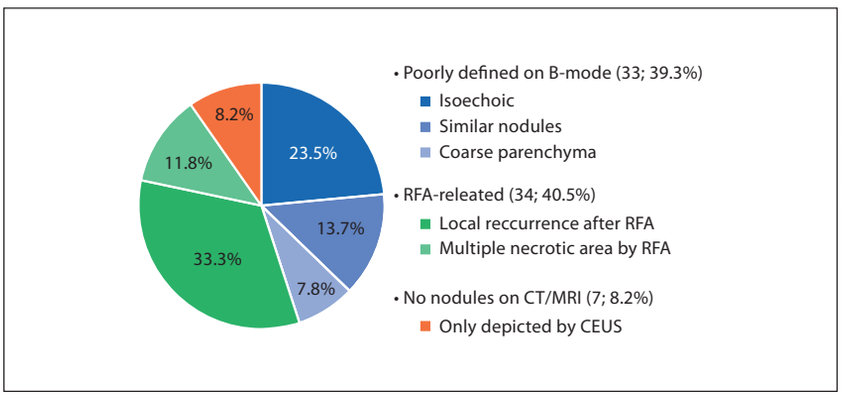
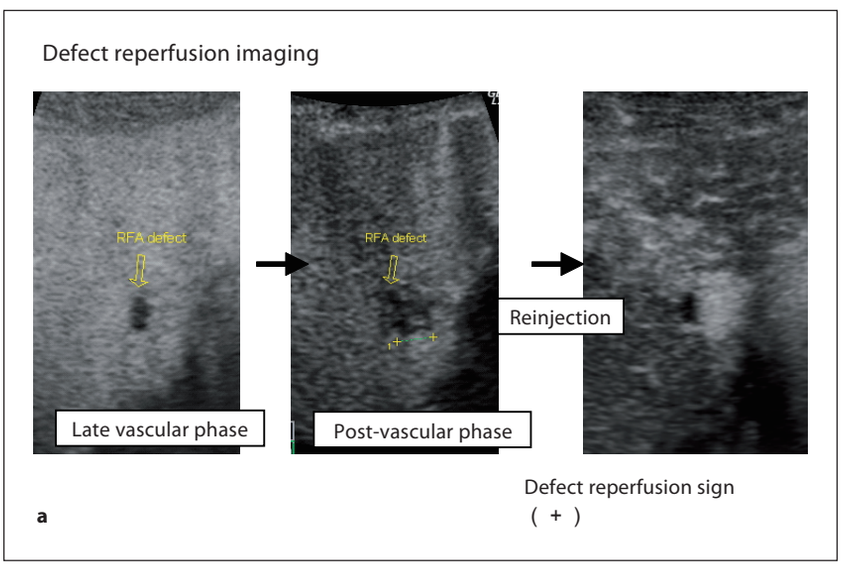


Fig. 5. Distribution of useful cases by defect reperfusion images with Sonazoid-enhanced US.



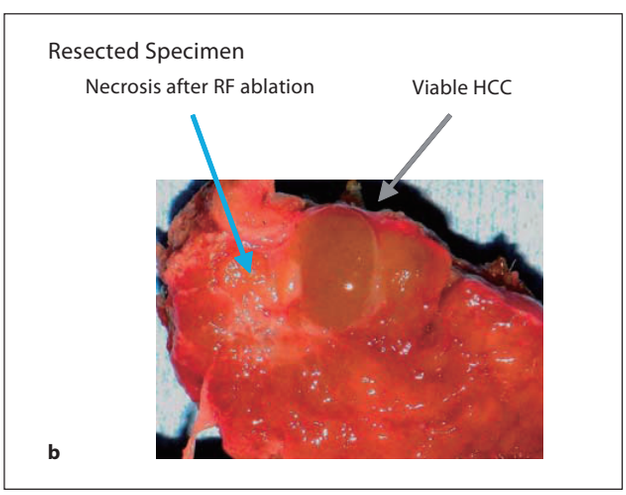


Fig. 6. Local recurring HCC after RFA. **a** The vascular phase only shows a small perfusion defect, whereas the post-vascular phase (Kupffer phase) clearly shows a larger area demonstrated as defected area, suggesting local recurrence. **b** Resected specimen clearly shows viable recurring HCC next to the necrotic area induced by previous RFA therapy.

Depiction of HCC by Kupffer Phase Surveillance and Confirmation by Reinjection

Of 262 consecutive cirrhotic patients, 9 nodules were depicted as Kupffer defect by Kupffer phase imaging and confirmed as HCC in 7 nodules by reinjection of Sonazoid (defect reperfusion US imaging). One nodule was correctly diagnosed as a hemangioma by defect reperfusion US imaging, and another nodule was confirmed as a simple cyst by defect reperfusion US imaging (fig. 3–5).

The sensitivity and specificity of double-contrast US imaging in the diagnosis of HCC were both 100%.

Discussion

It has been extremely difficult to depict small nodular lesions in the coarse liver parenchyma even though dynamic CT or dynamic MRI clearly show an arterial enhancing nodule with venous washout. In our series, 77 (9.1%) of 847 HCC nodules, which were not identified by B-mode US, have been clearly identified by defect reperfusion US imaging. If the technique is confined to Kupffer phase scanning, the false-positive rate is increased. In addition to the Kupffer defect, information on arterial vascularity, i.e. reinjection method, increases the diagnostic accuracy to 100% even in deep-seated nodules. This is really a breakthrough technique.

With this method, nodules that cannot be visualized by B-mode US are detected as defects on Kupffer images in the stable first Kupffer phase, and subsequently whether nodules with Kupffer defects have arterial blood flow is determined by the reinjection test. This method may be a breakthrough in diagnostic imaging (fig. 2) [11]. This double-contrast US technique requires no special apparatus or analysis, and is the result of a change in the way of thinking for contrast-enhanced US. For a typical CT image (so-called early enhancement with late washout nodules), defects are easily detected in the Kupffer phase, and subsequently arterial perfusion within the defect is demonstrated by the reinjection test (visualization of staining within the Kupffer defects, which is the reverse phenomenon of early enhancement with late washout; fig. 3). The introduction of this technique has allowed almost 100% accuracy of detecting lesions that are shown on CT images and are not visualized on B-mode US images.

If the reperfusion test shows no enhancement of a Kupffer defect, this defect differs from the nodule detected by CT (fig. 3). Therefore, this method could be an epoch-making treatment aid for HCC, which facilitates needle insertion guidance. In addition, this defect reperfusion imaging has many possibilities, and is applicable for screening HCC in the coarse liver parenchyma, and staging HCCs before treatment (fig. 4).

In screening, this procedure facilitates easy and capable screening because Sonazoid US can be performed in the setting of a routine examination, without taking extra time for contrast US study. In addition, operators only need to concentrate on the depiction of Kupffer defects in the Kupffer phase in contrast to routine B-mode US, in which many regenerative nodules mimic malignant ones. If defects are detected, Sonazoid reinjection leads to confirmation of HCC because of information on both Kupffer cell function and arterial blood flow in the same cross-sectional image (fig. 4, 5). This dual-phase fusion imaging allows detection and definitive diagnosis of HCC with 100% confidence. In this respect, Sonazoid also markedly improves the efficiency of HCC depiction on screening.

In the past, contrast-enhanced US has been considered only for nodules previously depicted by B-mode US and is inappropriate for screening. However, this concept has changed markedly with the introduction of double-contrast US imaging using Sonazoid.

Defect reperfusion US imaging is also useful for the localization of recurrent lesions located in a previously ablated area (identification of a locally recurring nodule by B-mode US is very difficult because of the inhomogeneous echo pattern mixed with viable lesions, ablated area, and ablated surrounding liver). In this setting, even skilled operators have great difficulty in determining the viable area on B-mode US images alone, which corresponds to the enhancing area on CT due to numerous US cross sections [12]. This problem was readily overcome by defect reperfusion US imaging with Sonazoid, which is quite revolutionary (fig. 4).

Defect reperfusion imaging was particularly useful for needle insertion guidance for the treatment of HCC. For invisible nodules on B-mode US, needle insertion has been performed under the guidance of either real-time virtual sonography [13] or Levovist- or SonoVue-enhanced US [8, 9]. However, real-time virtual sonography requires CT volume data and a special apparatus. In addition, complete concordance of synchronized images from B-mode US that correspond to the cross-sectional plane of CT volume data is sometimes difficult. Similarly, under Levovist- or SonoVue-enhanced US, puncture should be performed in a very short time in the early vascular phase [7, 8]. Since this technique requires enough experience and expertise, this method has not been widely accepted.

On the other hand, in Sonazoid-enhanced US, Kupffer defects are detected very easily, and whether blood flow is present in the defective areas can be determined by the reinjection test (defect reperfusion US imaging) in all cases. Therefore, needle insertion can be easily performed during a stable period in the Kupffer phase, and accurate needle placement followed by sufficient treatment is possible with Sonazoid-enhanced US (fig. 4).

Conclusions

Sonazoid-enhanced US is reportedly useful in the characterization of hepatic tumors when compared with MDCT or Levovist-enhanced US [14, 15]. More importantly, we are convinced that Sonazoid-enhanced US with defect reperfusion US imaging is an epoch-making approach in the diagnosis and treatment of HCC. This innovative technique has been invented based on two major favorable properties of Sonazoid: real-time blood flow images with low acoustic power, and stable Kupffer images tolerable for repeated scanning in the Kupffer phase. Therefore, this breakthrough technique will markedly change the therapeutic strategy for HCC. In addition, the most important point is that this technique is not possible with SonoVue or Definity, which have been approved in Europe and China, but is possible only with Sonazoid. In this respect, Sonazoid should be made more available worldwide.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Kudo M: Contrast Harmonic Imaging in the Diagnosis and Treatment of Hepatic Tumors. Tokyo, Springer, 2003, pp 1–253.

2 Wen YL, Kudo M, Zheng RQ, et al: Characterization of hepatic tumors: value of contrast-enhanced coded phase inversion harmonic US. AJR Am J Roentgenol 2004;182:1019–1026.

3 Ding H, Kudo M, Onda H, et al: Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual display mode. Radiology 2001;220:349–356.

4 Inoue T, Kudo M, Watai R, et al: Differential diagnosis of nodular lesions in cirrhotic liver by Kupffer phase contrast-enhanced US with Levovist: comparison with superparamagnetic iron oxide magnetic resonance images. J Gastroenterol 2005;40:1139–1147.

5 Ding H, Kudo M, Onda H, et al: Contrast-enhanced subtraction harmonic sonography for evaluating treatment response in patients with hepatocellular carcinoma. AJR Am J Roentgenol 2001;176:661–666.

6 Minami Y, Kudo M, Kawasaki T, Kitano M, Chung H, Maekawa K, Shiozaki H: Transcatheter arterial chemoembolization of hepatocellular carcinoma: usefulness of coded phase-inversion harmonic sonography. AJR Am J Roentgenol 2003;180:703–708.

7 Ding H, Kudo M, Onda H, et al: Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. Radiology 2001;221:721–730.

8 Minami Y, Kudo M, Kawasaki T, et al: Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. AJR Am J Roentgenol 2004;183:153–156.

9 Minami Y, Kudo M, Chung H, et al: Contrast harmonic sonographic-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. AJR Am J Roentgenol 2007;188:489–494.

10 Wen YL, Kudo M, Minami Y, et al: Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. AJR Am J Roentgenol 2003;181:57–63.

11 Kudo M, Hatanaka K, Chung H, et al: A proposal of novel treatment-assist technique for hepatocellular carcinoma in the Sonazoid-enhanced ultrasonography: value of defect re-perfusion imaging. Kanzo 2007;48:299–301.

12 Kudo M: Local ablation therapy for hepatocellular carcinoma: current status and future perspective. J Gastroenterol 2004;39:205–214.

13 Minami Y, Chung H, Kudo M, et al: Percutaneous radiofrequency ablation of sonography-unidentifiable liver tumors: feasibility and usefulness of a novel guiding technique with an integrated system of CT and sonographic images. Oncology 2007;72(suppl):111–116.

14 Hatanaka K, Kudo M, Minami Y, et al: Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. Oncology 2008;75(suppl 1):42–47.

15 Hatanaka K, Kudo M, Minami Y, et al: Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. Intervirology 2008;51(suppl 1):61–69.

Oncology

Oncology 2010;78(suppl 1):53–59
DOI: 10.1159/000315231

Published online: July 8, 2010

Usefulness of the Post-Vascular Phase of Contrast-Enhanced Ultrasonography with Sonazoid in the Evaluation of Gross Types of Hepatocellular Carcinoma

Kinuyo Hatanaka[a] Hobyung Chung[a] Masatoshi Kudo[a] Seiji Haji[b] Yasunori Minami[a] Kiyoshi Maekawa[c] Sousuke Hayaishi[a] Tomoyuki Nagai[a] Masahiro Takita[a] Kanae Kudo[a] Taisuke Ueda[a] Chie Tatsumi[a] Satoshi Kitai[a] Emi Ishikawa[a] Norihisa Yada[a] Tatsuo Inoue[a] Satoru Hagiwara[a] Kazuomi Ueshima[a]

[a]Division of Gastroenterology and Hepatology, Department of Internal Medicine, [b]Department of Surgery, and [c]Abdominal Ultrasound Unit, Kinki University School of Medicine, Osaka, Japan

Key Words

Hepatocellular carcinoma · Contrast-enhanced ultrasonography · Sonazoid · Macroscopic classification · Post-vascular phase · Single nodular type · Single nodular with extranodular growth type · Confluent multinodular type

Abstract

Objective: The purpose of this study was to assess the usefulness of post-vascular phase (PVP) images of contrast-enhanced ultrasonography (CE-US) in the evaluation of the gross types of hepatocellular carcinoma (HCC) that is closely related to the malignant potential of the tumor. ***Methods:*** A total of 29 patients with 40 HCCs of <5 cm in diameter, who underwent hepatic resection, were enrolled. The gross type of the tumor was evaluated using real-time scanning during the PVP of CE-US with Sonazoid prior to surgery. The tumors were classified into three types based on the macroscopic classification of the Liver Cancer Study Group of Japan: single nodular (SN) type, single nodular with extranodular growth (SNEG) type, and confluent multinodular (CMN) type. The ability of CE-US to correctly depict the gross type of HCC was evaluated. ***Results:*** 26 tumors were macroscopically diagnosed as the SN type, 11 tumors as the SNEG type, and 3 tumors as the CMN type. The sensitivity, specificity and accuracy of CE-US were 96, 80 and 90%, respectively. ***Conclusion:*** The PVP image of CE-US with Sonazoid is a useful tool in the evaluation of the gross type of HCC and is considered essential in deciding treatment strategy.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world. Although the prognosis of HCC has become more favorable as a result of regular screening of the high-risk population for HCC and recent progress in treatment methods [1–5], some patients still have a poor prognosis. Various clinicopathological features, such as portal vein invasion and satellite lesions, have been identified as the prognostic factors in patients with HCC who underwent hepatic resection [6–12].

The nodular type HCCs are classified into three gross types according to the macroscopic classification of the Liver Cancer Study Group of Japan, namely the single nodular (SN) type, the single nodular with extranodular growth (SNEG) type, and the confluent multinodular

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0053$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Division of Gastroenterology and Hepatology, Department of Internal Medicine
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

(CMN) type [13]. Among these three gross types, the SNEG type and CMN type are associated with portal vein invasion, intrahepatic metastasis and poorly differentiated histology [11, 12]. Patients with the SN type HCC showed better survival as compared with patients with SNEG type HCC [12]. Thus, it is important to assess the gross type of HCC before treatment, especially in patients with early-stage HCC who are candidates for curative treatments.

Recently, contrast-enhanced ultrasonography (CE-US) with an intravenous contrast agent has enabled the detection of intratumoral vascularity with higher sensitivity and specificity [14–16]. Levovist (Schering, Berlin, Germany) was a first-generation contrast agent. It was made from galactose-palmitic acid. CE-US using Levovist cannot only provide microflow imaging of liver tumors but can also eliminate clutter signals [16]. However, Levovist bubbles are easily collapsed by ultrasonography (US) emissions because of their fragile properties. Therefore, the images of CE-US using Levovist were obtained intermittently and real-time images were only obtained within a short period of time. Sonazoid (Daiichi Sankyo, Tokyo, Japan; GE Healthcare, Oslo, Norway), a second-generation contrast agent, has been commercially available in Japan since January 2007. This contrast agent contains perflubutane within a hard shell [17–19]. It can produce stable non-linear oscillations under low acoustic pressure with greater detail in the second harmonic signals in real time [20, 21]. The post-vascular phase (PVP) image of CE-US with Sonazoid represents the function of the reticuloendothelial system. Malignant liver tumors are usually depicted as defects through the PVP, because Kupffer cells are absent or the number of Kupffer cells has decreased in these lesions [22]. Therefore, this image can be used to estimate the gross type of HCC according to the shape of the defect.

The aim of the present study was to assess the usefulness of PVP images of CE-US using Sonazoid in evaluating the gross types of HCC.

Patients and Methods

Patients

Twenty-nine patients who had been diagnosed as having HCC and had undergone hepatic resection at the Kinki University School of Medicine were included in this study. Inclusion criteria were as follows: (1) patients with a single tumor of up to 5 cm in diameter or with less than four tumors of up to 3 cm in diameter; (2) patients without extrahepatic metastasis, and (3) patients who underwent curative hepatic resection, defined as the removal of all macroscopic residual tumors.

Macroscopic and Pathological Examination

Resected specimens were cut in the coronal plane so that the largest cross section of the tumor was exposed. An experienced surgeon (S.H.), who was blinded to the results of CE-US analysis, assessed the classification of the gross types of HCC by careful observation of the surgical specimens. The gross type was determined by the appearance of the tumor in the largest cross section, according to the macroscopic classification of the Liver Cancer Study Group of Japan. Thus, all of the tumors were classified into the following three gross types: SN type defined as those tumors showing a clear round shape; SNEG type defined as those tumors with similarities to the SN type but accompanied by extranodular growths, and the CMN type defined as those tumors forming a cluster of small and confluent nodules. For light microscopic examination, paraffin-embedded sections were stained with hematoxylin and eosin and the histological grade of the tumor, vascular invasion, intrahepatic metastasis and capsule invasion were determined.

Comparison of Clinicopathologic Features and Gross Type

Of 29 patients with HCC enrolled in this study, 21 patients had a single HCC and were divided into the SN group and non-SN group (i.e. SNEG and CMN groups) according to the gross type of the tumor. The clinicopathologic features of the two groups were compared.

Contrast-Enhanced Ultrasonography

Sonazoid was reconstituted with 2 ml sterile water for injection. The injected dose was 0.010 ml/kg body weight. Sonazoid was injected as a bolus at a rate of 1 ml/s via a 22-gauge cannula placed in the antecubital vein and flushed with 10 ml of normal saline.

The coded phase-inversion harmonic mode of GE LOGIQ 7 (GE Medical Systems, Milwaukee, Wisc., USA) with a 4-MHz convex transducer or a 6.5-MHz transducer was used in this study. The acoustic power of the CE-US was set at the default setting with a mechanical index of 0.2 and the dynamic range was fixed at 60–65 dB. A single focus point was set at 10 cm deep from the surface of the skin. Since the images of the ideal scanning plane were displayed in real time, the patients were requested to hold their breath. The images were stored as a cine clip with GE exclusive raw-data format files in the LOGIQ 7 computer.

To minimize variation between operators, the CE-US studies were performed by either one of two operators (K.H., K.M.) using the same examination protocol. Figure 1 details the protocol for CE-US with Sonazoid in our hospital. PVP images, obtained at least 10 min after the intravenous injection of Sonazoid, were used to characterize the gross type of HCC. Defect reperfusion imaging [23, 24] was also performed for all nodules to confirm the intratumoral vascularity, as described previously. All PVP images were reviewed by two physicians (H.C., T.I.). These reviewers were blinded to any other information regarding the patients, such as clinical data and the results of other imaging modalities. When there were discrepancies, the reviewers assessed the cine clips together and discussed them to reach a consensus.

Statistical Analysis

The χ^2 test and Mann-Whitney U test were used to compare the clinicopathologic features of patients. Sensitivity, specificity and accuracy were calculated to evaluate the diagnostic value of

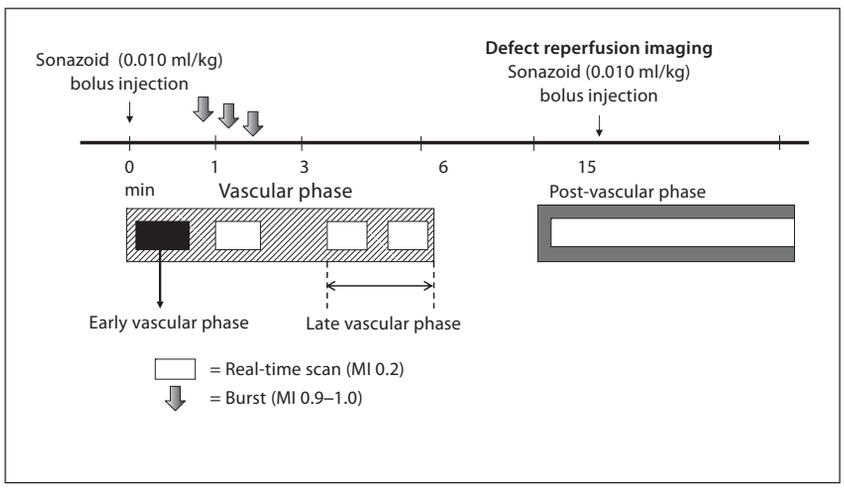


Fig. 1. Protocol for CE-US with Sonazoid. Change the US mode to coded phase inversion (CPI) mode after B-mode scanning. Bolus injection of Sonazoid (0.01 ml/kg). Intratumoral arterial enhancement in the early-vascular phase (<1 min). PVP (>10 min) image to depict perfusion defects for evaluating the gross types of HCC and for searching for other new nodules. Defect reperfusion imaging (obtained by reinjection of Sonazoid) for evaluating the intratumoral arterial vascularity especially for the nodules undetected in the early-vascular phase and for those newly detected.

CE-US in the evaluation of the gross type of HCC. A p value of <0.05 was considered statistically significant. All the statistical analyses were performed using SPSS software version 11.5 (SPSS, Inc., Chicago, Ill., USA).

Results

Characteristics of Patients

The characteristics of patients are detailed in table 1. There were 19 males (65.5%) and 10 female (34.5%) patients, with a mean age of 67.2 years, involved in the study. The underlying liver diseases, diagnosed using a combination of the serum biomarkers and the imaging findings, were as follows: HCV-related cirrhosis (n = 13); HCV-related chronic hepatitis (n = 7); HBV-related cirrhosis (n = 4); HBV-related chronic hepatitis (n = 3); HCV- and HBV-related chronic hepatitis (n = 1), and cirrhosis of unknown causes (n = 1). The mean tumor size was 2.4 cm. Macroscopically, 26 tumors (65.0%) were classified into the SN group, 11 (27.5%) tumors into the SNEG group, and 3 (7.5%) tumors into the CMN group. The surgical procedures applied were partial hepatectomy in 19 patients, subsegmentectomy in 6 patients, segmentectomy in 3 patients, and lobectomy in 1 patient.

Differences in Clinicopathologic Findings according to Gross Type

Of 21 patients who had single HCC, 13 patients were classified into the SN group, 6 patients into the SNEG group, and 2 patients into the CMN group, according to the macroscopic findings. Table 2 summarizes the inter-

Table 1. Characteristics of 29 patients with 40 HCCs

Age, years	67.2 ± 7.1 (52–78)
Male/female	19/10
HBs-Ag/HCV-Ab/HBs-Ag + HCV-Ab/NBNC	7/20/1/1
Child-Pugh grade, A/B/C	25/4/0
Platelet count, $\times 10^4/\mu l$	14.0 ± 8.8 (3.4–46.9)
ICG R15, %	16.5 ± 10.1 (1–54)
Tumor size, cm	2.4 ± 1.1 (0.8–5.0)
Tumor marker	
DCP, mAU/ml	1,232.7 ± 5,146.8 (9–27,333)
AFP, ng/ml	294.6 ± 961.4 (2–4,825)
AFP-L3, %	10.7 ± 22.6 (0–83.7)
Macroscopic classification	
SN	26
SNEG	11
CMN	3
Operative procedures	
Lobectomy	1
Segmentectomy	3
Subsegmentectomy	6
Partial hepatectomy	19

Continuous data are expressed as the mean ± SD. Data in parentheses express the range. NBNC = Patients negative for both HBs antigen and HCV antibody; ICG R15 = indocyanine green retention rate at 15 min; DCP = des-γ-carboxy prothrombin; AFP = α-fetoprotein; AFP-L3 = lens culinaris agglutinin A-reactive fraction of α-fetoprotein; SN = single nodular type; SNEG = single nodular type with extranodular growth; CMN = confluent multinodular type.

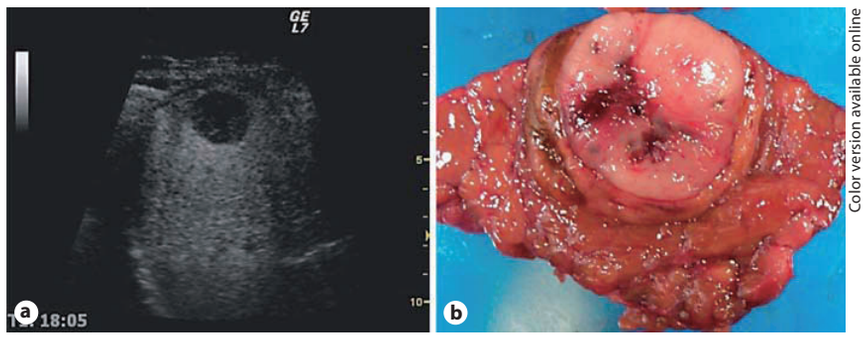


Fig. 2. SN type HCC. **a** PVP image depicting an SN type. **b** The surgical specimen was also diagnosed as an SN type.

Table 2. Comparison of clinicopathologic features between SN group and non-SN group in patients with single HCC

Variables	SN group (n = 13)	Non-SN group (n = 8)	p value
Age, years	65.8 ± 7.4	71.6 ± 4.7	0.041
Male/female	7/6	5/3	0.948
HBs-Ag-positive	4	1	0.669
HCV-Ab-positive	8	5	0.675
Child-Pugh grade (A/B)	9/4	8/0	0.128
Platelets, $\times 10^4/\mu l$	10.3 ± 4.8	16.7 ± 13.3	0.121
DCP, ≥400 mAU/ml	0	4	0.024
AFP, ≥100 ng/ml	2	2	0.978
AFP-L3, ≥20%	1	2	0.701
Tumor size, cm	2.2 ± 1.0	2.8 ± 1.2	0.093
Capsule invasion, %	7.7	50.0	0.094
Positive vascular invasion, %	0	25.0	0.068
Positive IM, %	15.4	37.5	0.471
Differentiation of HCC, % (well/moderate/poorly)	8.3/66.7/25	0/83.3/16.7	0.671

Continuous data are expressed as mean ± SD. Data in parentheses express the range. p value indicates whether any significant differences exist between the SN group and the non-SN group. DCP = Des-γ-carboxy prothrombin; AFP = α-fetoprotein; AFP-L3 = lens culinaris agglutinin A-reactive fraction of α-fetoprotein; SN = single nodular; ICG R15 = indocyanine green retention rate at 15 min.

comparison of clinicopathologic features between the SN group and the non-SN group. Patients in the non-SN group were significantly older and had significantly higher serum des-γ-carboxyl prothrombin (DCP) levels, as compared with the SN group (p = 0.041 and p = 0.024). In addition, there were tendencies for the non-SN group to have larger tumors. The capsule invasion and the vascular invasion were also more frequently observed in the non-SN group.

Table 3. Summary of the results of CE-US in **a** evaluating the gross type of HCC and **b** sensitivity, specificity and accuracy of PVP images

a		Macroscopic classification		Total
		SN type	non-SN type	
CE-US	SN type	24	3	27
	non-SN type	1	12	13
	total	25	15	40

b	Sensitivity	Specificity	Accuracy
CE-US	96.0%	80.0%	90.0%

Diagnostic Value of Contrast-Enhanced Ultrasonography

Among a total of 40 nodules, the CE-US diagnosed the gross type correctly in 36 nodules, while the remaining 4 nodules were misdiagnosed as shown in table 3a. The representative cases are shown in figures 2–4. The sensitivity, specificity and accuracy of CE-US for distinguishing between the SN type and the non-SN type were 96, 80 and 90%, respectively (table 3b). The characteristics of the 4 misdiagnosed nodules are detailed in table 4. One SN type nodule was misdiagnosed as an SNEG type on CE-US, while 3 SNEG type nodules were misdiagnosed as the SN type. It seems unlikely that there is a fixed cause for the misdiagnosis with respect to the size, location, depth from the surface of liver, histological grade and tumor markers.

Discussion

Establishment of a regular screening program for the high-risk population with HCC has increased the number of cases with small HCC suitable for curative treat-

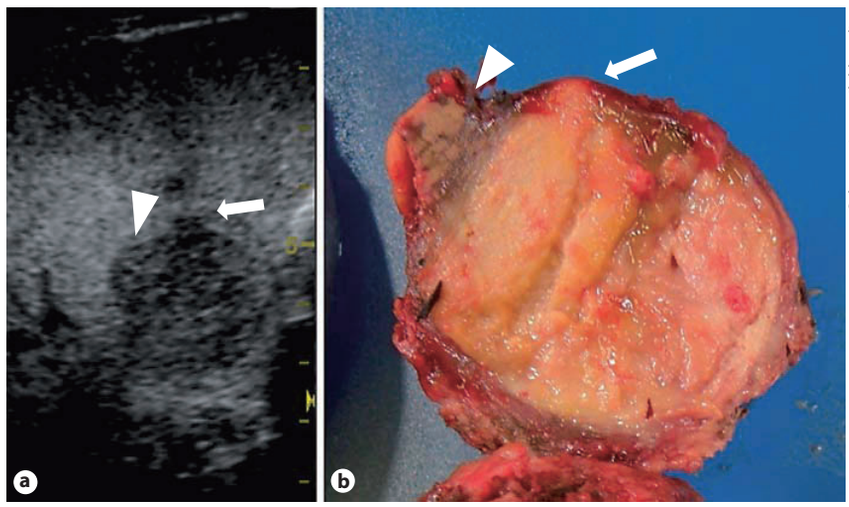


Fig. 3. SNEG type. **a** PVP image depicting SNEG. **b** The surgical specimen was also diagnosed as SNEG. Arrowheads and arrows represent portions of extranodular growth.

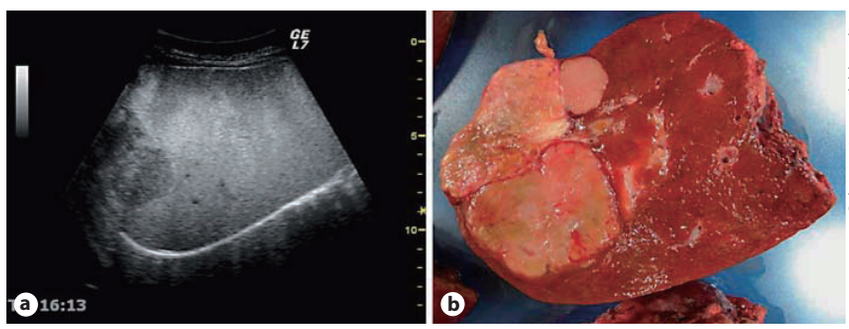


Fig. 4. CMN type. **a** PVP image depicting confluent multinodular type. **b** The surgical specimen was also diagnosed as confluent multinodular type. Usefulness of the PVP in evaluating the gross types of HCC.

Table 4. Characteristics of 4 misdiagnosed nodules

Nodule No.	Gross type (specimen)	Segment	Size cm	Diagnosis on CE-US	Depth cm	Histological grade	DCP mAU/ml	AFP ng/ml	AFP-L3 %
1	SN	S6	3.0	SNEG	9	moderate HCC	43	68	<0.5
2	SNEG	S8	1.8	SN	11	moderate HCC	26	24	<0.5
3	SNEG	S7	1.6	SN	4	moderate HCC	1,910	4,825	68.1
4	SNEG	S8	0.8	SN	4	moderate HCC			

Nodule Nos. 3 and 4 were found in a patient. CE-US = Contrast-enhanced US; DCP = des-γ-carboxy prothrombin; AFP = α-fetoprotein; AFP-L3, lens culinaris agglutinin A-reactive fraction of α-fetoprotein.

ments, such as resection and percutaneous ablation. However, the number of candidates for resection is limited, even in cases with small HCC, because of impaired liver function due to cirrhosis. Radiofrequency ablation (RFA) was introduced in the 1990s and has been widely performed for the treatment of unresectable liver tumors ever since [25, 26]. Several factors, such as larger tumor size, tumor multiplicity and elevated serum des-γ-carboxy prothrombin, were identified as a prognostic factor in patients with HCC who underwent RFA [27–29]. However, the association between the gross type of HCC and the efficacy of RFA has not yet been investigated.

Previous studies have demonstrated the association between the gross type of HCC and the prevalence of por-

tal vein invasion and intrahepatic metastasis [11–12, 30]. Portal vein invasion was found in 18.7–19.5% of SN types, while it occurred in 44.4–50.0% of SNEG types and 33.3–63.2% of CMN types. Intrahepatic metastasis was found in 4.1–30.7% of SN types, 26.7–60.0% of SNEG types and 26.3–47.6% of CMN types [11, 12, 30]. Thus, we assume that the gross type of HCC is closely correlated with the efficacy of RFA and that the assessment of the gross type of early-stage HCC before curative treatments is essential. In cases of SNEG type or CMN type HCC with preserved liver function, hepatic resection is considered to be a preferable treatment strategy to RFA.

Sonazoid-enhanced US can repeatedly demonstrate the real-time images in a low-power acoustic field because Sonazoid has hard shells. Therefore, it has become much easier to obtain the PVP image that is applicable to the evaluation of liver tumors, as well as being a treatment guide for RFA. Furthermore, Sonazoid-enhanced US demonstrated a higher sensitivity and accuracy for the detection and diagnosis of hepatic malignancies than dynamic CT [20, 21]. The advantage of US, such as higher spatial and temporal resolution, and sustainable real-time scanning with Sonazoid has increased the detection ability of CE-US. In the present study, 4 nodules were newly detected on the preoperative PVP image, all of which had not been detected by either preoperative B-mode US or CE-CT. It should be noted that supplemental partial hepatectomy was performed for each nodule and that all 4 nodules were histologically diagnosed as HCC.

The PVP image reflects the function of the reticuloendothelial system that is mainly determined by the number and function of Kupffer cells. Additionally, the number of Kupffer cells present in cancerous tissues decreases as the histological grade of the tumor worsens [22]. We previously reported the usefulness of the PVP image using Levovist to assess the histological grade of nodular lesions in cirrhotic liver [31, 32]. In the present study, all nodules were shown as clear perfusion defects on the PVP image, with the exception of 7 nodules, 5 nodules were shown as defects with unclear boundaries and 2 nodules as lower degree defects with clear boundaries. The former 5 cases were diagnosed as having severe liver cirrhosis according to the resected specimens. Therefore, deteriorated liver function, which is closely correlated with the decreased function of the reticuloendothelial system in the liver parenchyma, is considered to be mainly attributable to the unclear boundaries on the PVP image. The latter 2 cases were histologically diagnosed as well-differentiated HCC. The reticuloendothelial system function has been found to be preserved in the tissue of well-differentiated HCC [22], and this resulted in the lower degree of defects on the PVP images of these 2 nodules.

In the present study, we failed to diagnose the gross type of HCC in 4 nodules. Although the reasons attributable to this failure have hardly been determined, 1 SN type nodule (No. 1), which had a septum formation, was depicted as a nodule with an irregular margin on CE-US and was misdiagnosed as an SNEG type. Septum formation might be one of the key issues related to the correct diagnosis of the gross type of HCC using CE-US.

The results presented in our study with a relatively small number of patients are preliminary. The usefulness of CE-US in the evaluation of gross type HCC needs to be compared with other imaging modalities. Although there are some limitations in the present study mentioned above, the PVP image of CE-US using Sonazoid is a useful tool in the diagnosis of the gross type of HCC and is considered to be essential in deciding the treatment strategy for HCC.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Hasegawa K, Makuuchi M, Takayama T, Kokudo N, Arii S, Okazaki M, Okita K, Omata M, Kudo M, Kojiro M, Nakanuma Y, Takayasu K, Monden M, Matsuyama Y, Ikai I: Surgical resection vs. percutaneous ablation for hepatocellular carcinoma: a preliminary report of the Japanese nationwide survey. J Hepatol 2008;49:589–594.

2 Kudo M, Sakaguchi Y, Chung H, Hatanaka K, Hagiwara S, Ishikawa E, Takahashi S, Kitai S, Inoue T, Minami Y, Ueshima K: Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation: a matched case-control study. Oncology 2007;72(suppl):132–138.

3 O'Suilleabhain CB, Poon RTP, Yong JL, Ooi GC, Tso WK, Fan ST: Factors predictive of 5-year survival after transarterial chemoembolization for inoperable hepatocellular carcinoma. Br J Surg 2003;90:325–331.

4 Lee HS, Kim JS, Choi IJ, Chung JW, Park JH, Kim CY: The safety and efficacy of transcatheter arterial chemoembolization in the treatment of patients with hepatocellular carcinoma and main portal vein obstruction. A prospective controlled study. Cancer 1997;79:2087–2094.

5 Kudo M: Local ablation therapy for hepatocellular carcinoma: current status and future perspectives. J Gastroenterol 2004;39:205–214.

6 Nakanishi K, Sakamoto M, Yamasaki S, Toda S, Hirohashi S: Akt phosphorylation is a risk factor for early disease recurrence and poor prognosis in hepatocellular carcinoma. Cancer 2005;103:307–312.

7 Yeh CN, Chen MF, Lee WC, Jeng LB: Prognostic factors of hepatic resection for hepatocellular carcinoma with cirrhosis: univariate and multivariate analysis: J Surg Oncol 2002;81:195–202.

8 Portolani N, Coniglio A, Ghidoni S, Giovanelli M, Benetti A, Tiberio GA, Giulini SM: Early and late recurrence after liver resection for hepatocellular carcinoma: prognostic and therapeutic implications. Ann Surg 2006;243:229–235.

9 Iguchi T, Aishima S, Taketomi A, Nishihara Y, Fujita N, Sanefuji K, Maehara Y, Tsuneyoshi M: Extracapsular penetration is a new prognostic factor in human hepatocellular carcinoma. Am J Surg Pathol 2008;32:1675–1682.

10 Kondo K, Chijiiwa K, Makino I, Kai M, Maegara M, Ohuchida J, Haganuma S: Risk factors for early death after liver resection in patients with solitary hepatocellular carcinoma. J Hepatobiliary Pancreat Surg 2005; 12:399–404.

11 Shimada M, Rikimaru T, Hamatsu T, Yamashita Y, Terashi T, Taguchi K, Tanaka S, Shirabe K, Sugimachi K: The role of macroscopic classification in nodular-type hepatocellular carcinoma. Am J Surg 2001;182: 177–182.

12 Nakashima Y, Nakashima O, Tanaka M, Okuda K, Nakashima M, Kojiro M: Portal vein invasion and intrahepatic micrometastasis in small hepatocellular carcinoma by gross type. Hepatol Res 2003;26:142–147.

13 Liver Cancer Study Group of Japan: The General Rules for the Clinical and Pathological Study of Primary Liver Cancer, ed 5. Tokyo, Kanehara, 2008, pp 17.

14 Fujimoto M, Moriyasu F, Nishikawa K, Nada T, Okuma M: Color Doppler sonography of hepatic tumors with a galactose-based contrast agent: correlation with angiographic findings. AJR Am J Roentgenol 1994;163: 1099–1104.

15 Kudo M, Tomita S, Tochio H, Mimura J, Okabe Y, Kashida H, Hirasa M, Ibuki Y, Todo A: Sonography with intraarterial infusion of carbon dioxide microbubbles (sonographic angiography): value in differential diagnosis of hepatic tumors. AJR Am J Roentgenol 1992;158:65–74.

16 Kudo M: Contrast Harmonic Imaging in the Diagnosis and Treatment of Hepatic Tumors. Tokyo, Springer, 2003.

17 Sontum PC, Ostensen J, Dvrstad K, Hoff L: Acoustic properties of NC100100 and their relation with the microbubble size distribution. Invest Radiol 1999;34:268–275.

18 Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H: Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. Ultrasound Med Biol 2007;33:318–325.

19 Watanabe R, Matsumura M, Munemasa T, Fujimaki M, Suematsu M: Mechanism of hepatic parenchyma-specific contrast of microbubble-based contrast agent for ultrasonography: microscopic studies in rat liver. Invest Radiol 2007;42:643–651.

20 Hatanaka K, Kudo M, Minami Y, Ueda T, Tatsumi C, Kitai S, Takahashi S, Inoue T, Hagiwara S, Chung H, Ueshima K, Maekawa K: Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. Intervirology 2008;51: 61–69.

21 Hatanaka K, Kudo M, Minami Y, Maekawa K: Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. Oncology 2008;75(suppl):42–47.

22 Tanaka M, Nakashima O, Wada Y, Kage M, Kojiro M: Pathomorphological study of Kupffer cells in hepatocellular carcinoma and hyperplastic nodular lesions in the liver. Hepatology 1996;24:807–812.

23 Kudo M, Hatanaka K, Chunhg H, Minami Y, Maekawa K: A proposal of novel treatment-assist technique in the Sonazoid-enhanced ultrasonography: value of defect reperfusion imaging. Acta Hepatol Jap 2007;48:299–301.

24 Kudo M, Hatanaka K, Maekawa K: Defect reperfusion imaging, a newly developed novel technology using Sonazoid in treatment of hepatocellular carcinoma. J Med Ultrasound 2008;16:169–176.

25 Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS: Small hepatocellular carcinoma: treatment with radiofrequency ablation versus ethanol injection. Radiology 1999;210:655–661.

26 Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M: Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1,000 cases. Cancer 2005;103: 1201–1209.

27 Kobayashi M, Ikeda K, Kawamura Y, Yatsuji H, Hosaka T, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Saitoh S, Arase Y, Kumada H: High serum des-γ-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. Cancer 2009;115:571–580.

28 Takahashi S, Kudo M, Chung H, Inoue T, Ishikawa E, Kitai S, Tatsumi C, Ueda T, Nagai T, Minami Y, Ueshima K: PIVKA-II is the best prognostic predictor in patients with hepatocellular carcinoma after radiofrequency ablation therapy. Oncology 2008;75(suppl): 91–98.

29 Lencioni R, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C: Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. Radiology 2005;234:961–967.

30 Okusaka T, Okada S, Ueno H, Ikeda M, Shimada K, Yamamoto J, Kosuge T, Yamasaki S, Fukushima N, Sakamoto M: Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. Cancer 2002;95:1931–1937.

31 Inoue T, Kudo M, Maenishi O, Komuta M, Nakashima O, Kojiro M, Maekawa K: Value of liver parenchymal phase contrast-enhanced sonography to diagnose premalignant and borderline lesions and overt hepatocellular carcinoma. AJR Am J Roentgenol 2009;192:698–705.

32 Inoue T, Kudo M, Watai R, Zhou P, Kawasaki T, Minami Y, Chung H, Fukunaga T, Awai K, Maenishi O: Differential diagnosis of nodular lesions in cirrhotic liver by post-vascular phase contrast-enhanced US with Levovist: comparison with superparamagnetic iron oxide magnetic resonance images. J Gastroenterol 2005;40:1139–1147.

Oncology

Oncology 2010;78(suppl 1):60–67
DOI: 10.1159/000315232

Published online: July 8, 2010

Depiction of Portal Supply in Early Hepatocellular Carcinoma and Dysplastic Nodule: Value of Pure Arterial Ultrasound Imaging in Hepatocellular Carcinoma

Masatoshi Kudo[a] Kinuyo Hatanaka[a] Tatsuo Inoue[a] Kiyoshi Maekawa[b]

[a]Department of Gastroenterology and Hepatology, and [b]Division of Ultrasound, Kinki University School of Medicine, Osaka, Japan

Key Words

Hepatocellular carcinoma, dysplastic nodule · Pure arterial phase ultrasound imaging · Arterial supply · Portal supply · Early hepatocellular carcinoma · Nodule-in-nodule type early HCC · Sonazoid · Contrast-enhanced US

Abstract

Ultrasound (US) contrast agents such as SonoVue and Sonazoid are commercially available worldwide. Innovation of contrast agents and advances of new US technologies have dramatically changed both diagnostic and treatment strategies for hepatocellular carcinoma (HCC). Recently, the breakthrough technique, pure arterial phase (PAP) US imaging, which depicts only intranodular arterial supply by use of maximum intensity projection (MIP) images, was developed from advanced raw data-storing and accumulation technologies. A total of 8 dysplastic nodules (DNs), 16 early HCCs, 5 nodule-in-nodule type early HCCs and 48 overt HCCs were included in this study. All 8 DNs (100%) showed arterial hypovascularity in the PAP followed by preserved portal perfusion at the portal phase and isouptake at the Kupffer phase by Sonazoid-enhanced contrast US. A total of 12 out of 16 early HCCs (75%) showed similar patterns on vascular and Kupffer phase imaging of contrast-enhanced ultrasonography. The remaining 4 HCCs showed slightly hypervascular pattern without venous washout and slightly decreased Kupffer uptake. All 5 nodule-in-nodule type early HCCs presented partial arterial enhancement within hypovascular nodule at the PAP followed by isovascular pattern at the portal phase and partial Kupffer defect within isouptake nodules. All 48 overt HCCs showed a hypervascular pattern with Kupffer defect on contrast-enhanced ultrasonography. This technique can clearly identify whether blood supply in the tumor is of arterial or portal origin, and facilitate the noninvasive characterization of nodular lesions associated with liver cirrhosis. In conclusion, this newly developed innovative technique can depict pure portal supply in early HCC and DN, enabling differentiating premalignant lesions and early HCCs from overt HCC even though dynamic CT or MRI does not have such capabilities.

Introduction

Innovation of contrast agents and advances of new ultrasound (US) technologies have dramatically changed both diagnostic and treatment strategies for hepatocellular carcinoma (HCC) [1–9].

The second-generation US contrast agents, SonoVue or Sonazoid, are now routinely available for the diagnosis and treatment of HCC. Recently, a new US technique called pure arterial phase (PAP) US imaging (PAP-US)

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0060$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

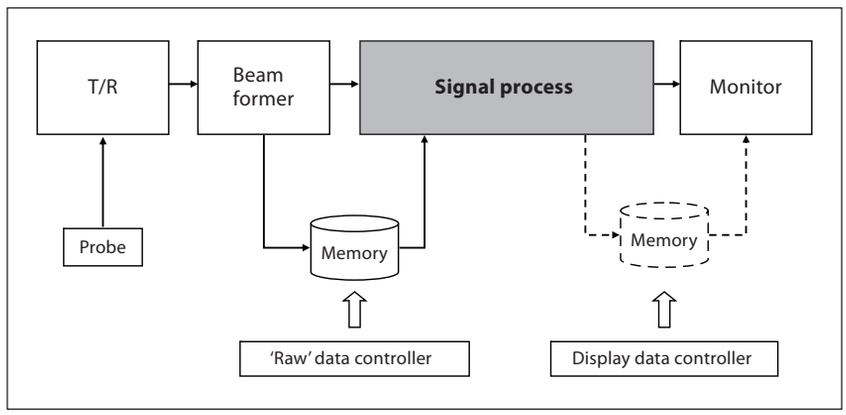


Fig. 1. This ultrasound machine (LOGIQ7) can store raw data before signal processing, unlike other ultrasound machines.

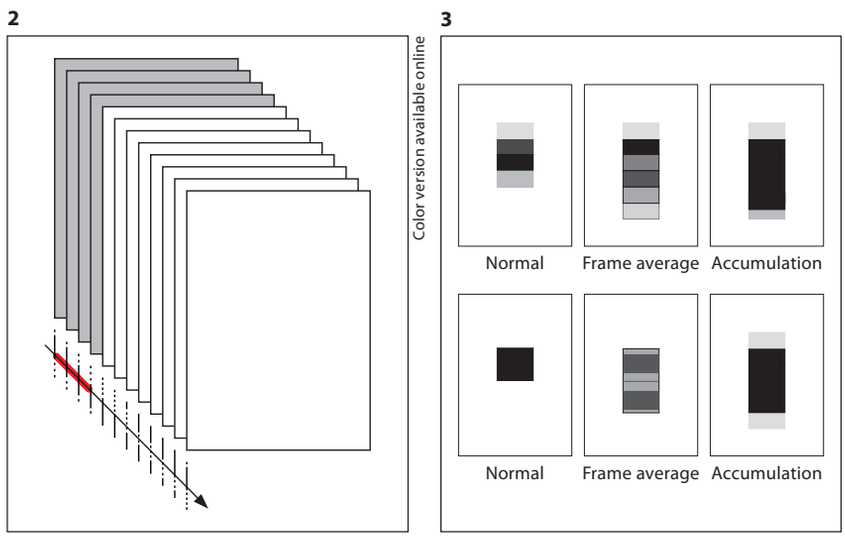


Fig. 2. Schematic representation of accumulation image. This technology displays the MIP image at given frame (N) at given time. $A_n = \max(I_n, I_{n-1}, I_{n-2} \ldots I_{n-N+1})$. Even though there is motion due to respiration, relatively good quality MIP image is obtained by this method.

Fig. 3. Difference between accumulation image and frame average. Frame average displays average intensity during a certain time; therefore, the contrast of intensity becomes weak as compared with the accumulation image, which displays maximum intensity.

has been developed in Japan. This technique clearly depicts arterial supply to the nodules at the PAP, and consequently makes it possible to evaluate whether there is a portal supply or not within the nodule at the portal phase. The purpose of this study is to clarify the sensitivity and specificity in detecting intranodular arterial and portal supply in dysplastic nodules (DNs), early HCCs, nodule-in-nodule (N-in-N) type HCCs, and overt HCCs.

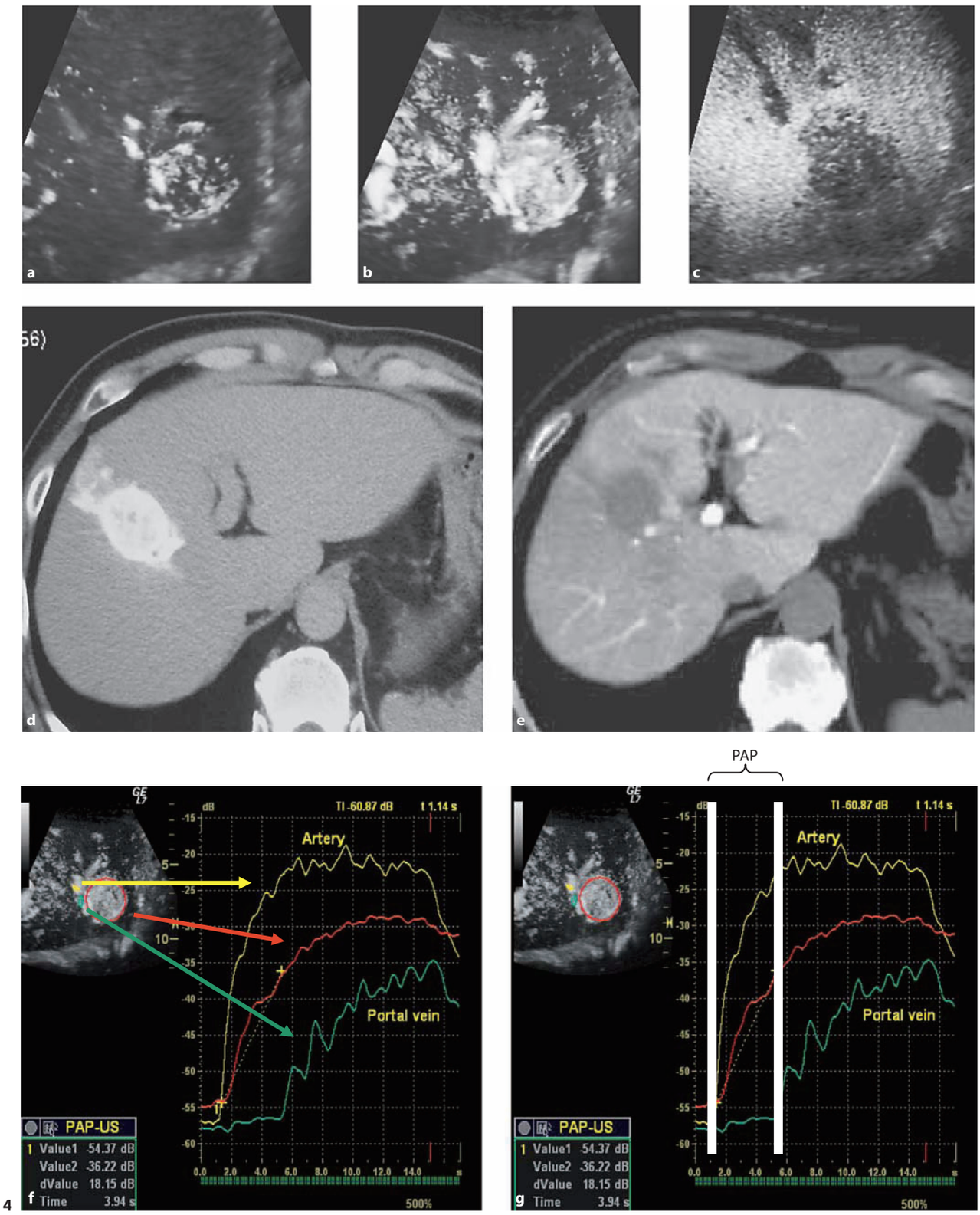
Patients and Methods

Pure Arterial Phase Ultrasound Imaging

PAP-US images can be obtained from a combination of two technologies: raw data-storing technology (fig. 1) and accumulation image technology of maximum intensity projection (MIP) images (fig. 2, 3). The US machine used in this study was GE LOGIQ 7 (GE HealthCare, Milwaukee, Wisc., USA). PAP was determined as follows: range of interest (ROI) was placed on the extranodular hepatic artery, portal vein, and tumor. Contrast agent appears in the liver around 5–10 s after intravenous injection of Sonazoid. The time difference between appearance of arterial flow and portal venous flow at the liver is a *pure arterial phase* – PAP. The time-intensity curve in the ROI placed on the liver parenchyma can reveal whether the nodule is supplied by artery or portal flow (fig. 4, 5).

Patients

A total of 73 hepatocyte nodules were included in this study. PAP-US imagings were performed in all of these 73 nodules. Eight nodules were diagnosed as dysplastic nodule (DN) by histopathology of biopsied samples. Twelve early HCCs were diagnosed as early HCC by histopathology of biopsied samples. Five nodules were diagnosed as N-in-N type early HCC by CT during hepatic arteriography (CTHA), CT during arterial portography (CTAP)


a
b
c
d
e
PAP
Artery
Portal vein
PAP-US
Value1 -54.37 dB
Value2 -36.22 dB
dValue 18.15 dB
Time 3.94 s
f
g

4

and histopathological study of biopsied specimens. Seven out of 48 overt HCCs were diagnosed as moderately differentiated HCCs by resected specimens. The remaining 41 overt HCCs were diagnosed as typical vascular pattern on dynamic CT or dynamic MRI. Typical HCC findings represent arterial enhancement with venous washout. CTHA and CTAP were performed to confirm arterial supply or portal supply in hypovascular nodules on dynamic CT in all 73 nodules.

Results

On dynamic CT, 8 DNs, 12 early HCCs, and 5 N-in-N type HCCs were demonstrated as low dense nodules (hypovascular nodules). All 8 DNs (100%) showed arterial hypovascularity in the PAP followed by preserved portal perfusion at the portal phase and isouptake at the Kupffer phase of Sonazoid-enhanced contrast US (fig. 6). A total of 12 out of 16 early HCCs (75%) showed similar patterns on vascular and Kupffer phase imaging of contrast-enhanced ultrasonography. The remaining 4 HCCs showed slight hypervascular pattern without venous washout and slight decreased Kupffer uptake. All 5 N-in-N type early HCCs presented partial arterial enhancement within the hypovascular nodule at the PAP followed by isovascular pattern at the portal phase and partial Kupffer defect within isouptake nodules (fig. 7). All 48 overt HCCs showed a hypervascular pattern with Kupffer defect on contrast-enhanced ultrasonography. This technique can clearly identify whether blood supply in the tumor is of arterial or portal origin, and facilitate the noninvasive characterization of nodular lesions associated with liver cirrhosis. CT or MRI could not demonstrate whether the nodules are supplied by the hepatic artery or portal vein. As a result, this innovative technique can help differentiate premalignant lesions and early HCCs from overt HCC (fig. 8) (table 1).

Absent arterial supply with preserved portal supply was confirmed in 100% (8/8) of DNs and 75% (12/16) of early HCCs by CTAP. The remaining 4 (25%) early HCCs were confirmed as arterial supply by CTHA and CTAP. Partial portal supply in the outer part of the nodule with central partial arterial supply in the center of the nodule was also confirmed on CTHA and CTAP. As a result, detection of intranodular arterial and portal supply were completely compatible between PAP US and CTHA/CTAP (table 2).

Table 1. Arterial and portal supply determined by PAP-US

	DN	eHCC	N-in-N eHCC	Overt HCC
Arterial supply, %	0 (0/8)	25 (4/16)	100 (5/5)*	100 (48/48)
Portal supply, %	100 (8/8)	75 (12/16)	100 (5/5)**	0 (0/48)

DN = Dysplastic nodule; eHCC = early hepatocellular carcinoma; N-in-N eHCC = nodule-in-nodule type early HCC; PAP-US = pure arterial phase ultrasound imaging.
* Partial arterial supply in the center of the nodule.
** Partial portal supply with outer part of the nodule.

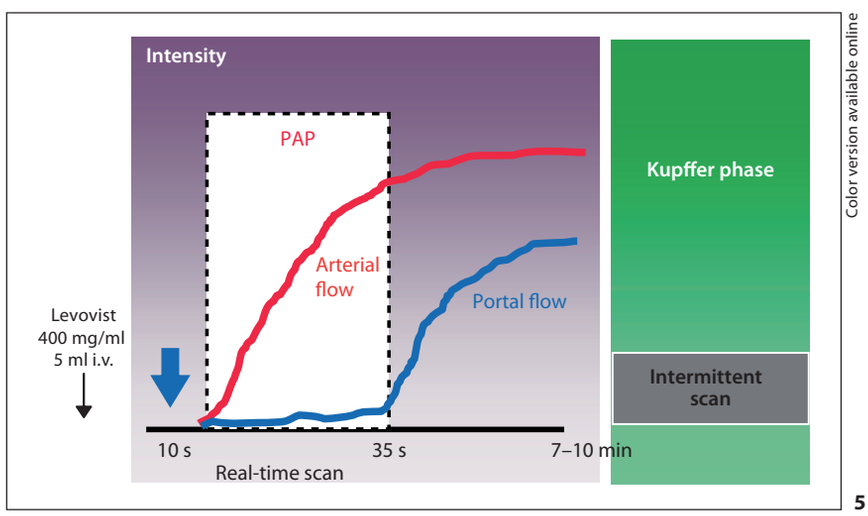


Fig. 4. Example case of PAP US imaging in overt hypervascular HCC. **a** PAP. **b** (Mixed) vascular phase. **c** Post-vascular phase. **d** CTHA. **e** CTAP. **f** Time-intensity curves on ROIs placed on the tumor (red), extranodular artery (yellow), and extranodular portal vessel (green). **g** PAP in this case was revealed to be 3.94 s. By this analysis, this nodule was revealed to be supplied only by the hepatic artery since tumor vascularity is increased during PAP (red).

Fig. 5. Phase of Sonazoid-enhanced US. The vascular phase is divided into two phases: PAP and portal phase. In addition to the vascular phase, there is a post-vascular (Kupffer) phase, 10 min after intravenous injection of Sonazoid.

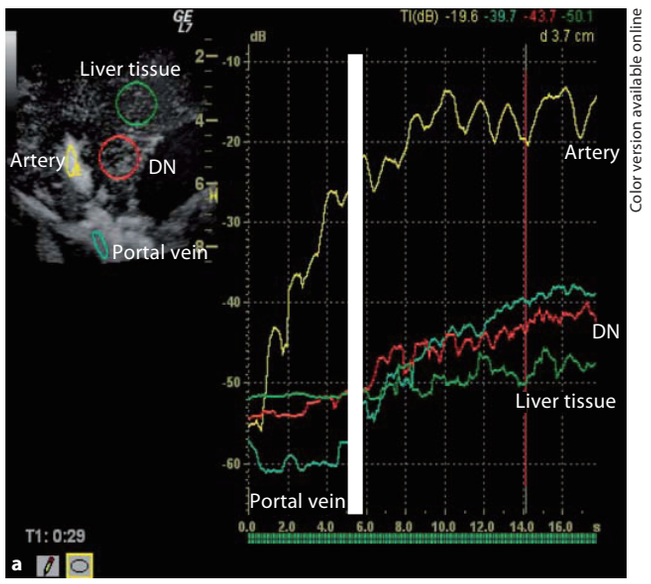
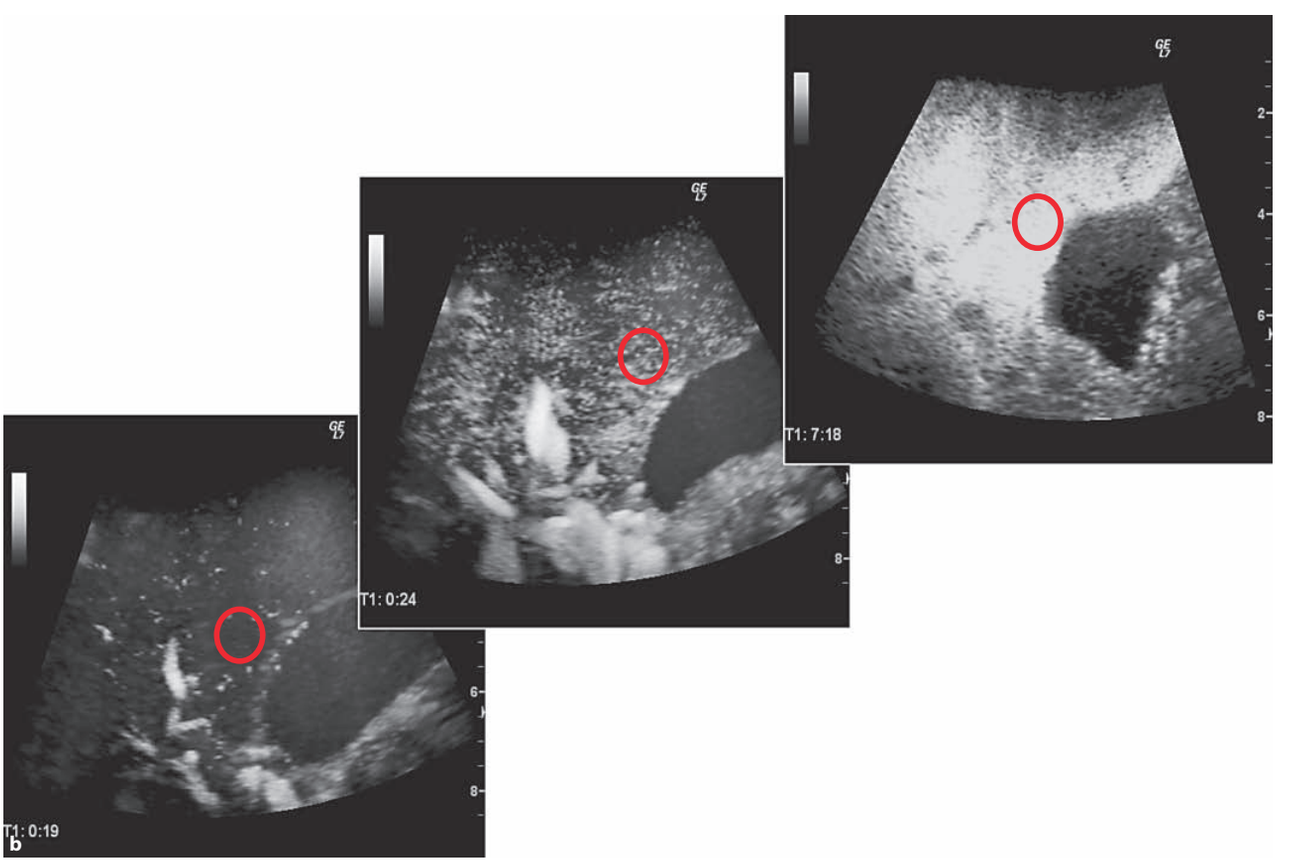


Fig. 6. a A case of DN, which was already proven to be a portal supplying nodule by CTAP. At the PAP there is no arterial supply by the analysis of time-intensity curves at the ROI placed on the artery, portal vein and nodule. **b** There is no arterial supply at the PAP (left), but preserved portal supply at the portal phase (center). Kupffer uptake is also observed within the nodule (right).



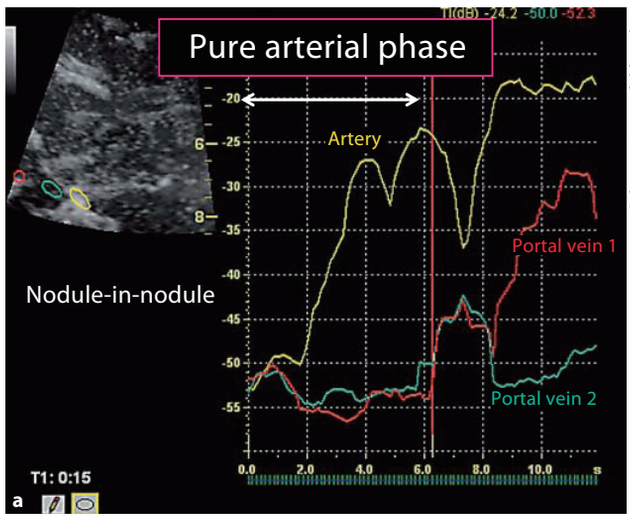
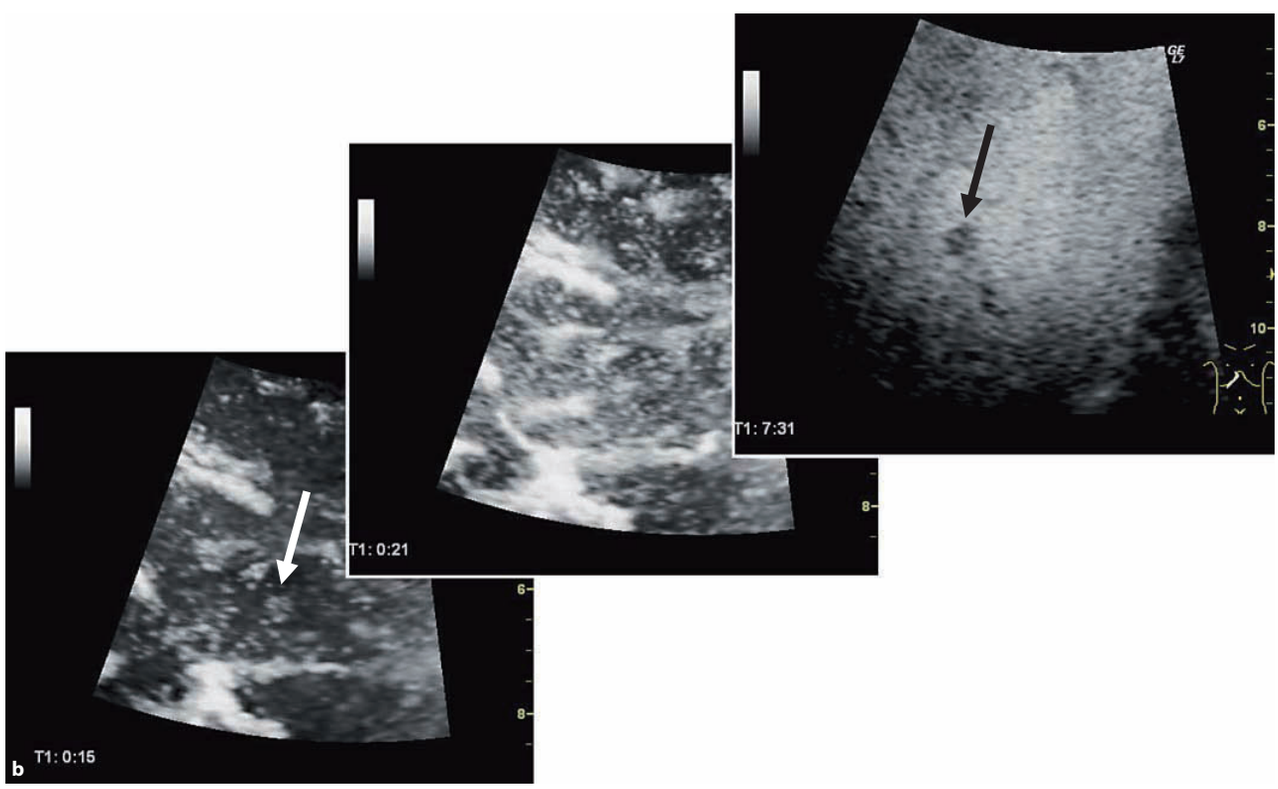


Fig. 7. PAP US image in a case of N-in-N type HCC. **a** PAP was revealed to be 6 s in this case. **b** The PAP US image clearly shows the intranodular lesion is supplied by the hepatic artery (left). Arterial vascularity (arrow) is clearly visualized by PAP-US, which corresponds to the defective area on Kupffer phase imaging (right). At the portal phase (center) the outer part of nodules can be evaluated to be supplied by portal venous flow.



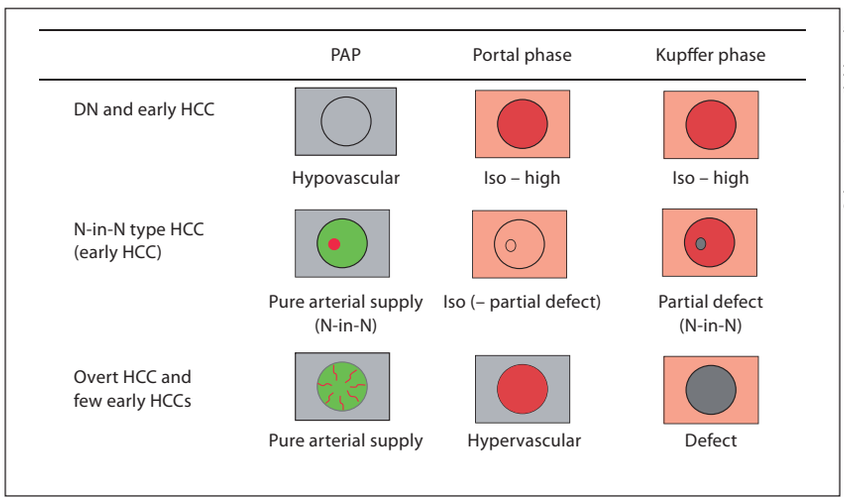


Fig. 8. Differential diagnosis of HCC from premalignant/borderline lesions with PAP US image. DNs and 75% of early HCC are demonstrated as pure portal venous supply and N-in-N type early HCCs are demonstrated as a small hypervascular lesion within the hypovascular lesion. Overt HCC is revealed to be purely supplied by the hepatic artery.

Table 2. Comparison of PAP-US and CTHA/CTAP

Diagnosis	n	PAP-US		Arterial flow on CTHA	Portal flow on CTAP
		arterial supply	portal supply		
Dysplastic nodule	8	– (100%)	+ (100%)	– (100%)	+ (100%)
Early HCC	12	+ (25%)	+ (75%)	+ (25%)	+ (75%)
N-in-N HCC	5	+ partial (100%)	+ partial (100%)	+ partial (100%)	+ partial (100%)
Overt HCC	41	+ (100%)	– (100%)	+ (100%)	– (100%)

PAP-US = Pure arterial phase ultrasound; CTHA = CT during hepatic arteriography; CTAP = CT during arterial portography.

Discussion

The results obtained by PAP-US imaging clearly showed that both DNs and eHCCs are mainly fed by portal flow and not arterial flow. Therefore, it is not possible to differentiate early HCCs from DNs. Gadolinium ethoxybenzyl (Gd-EOB) MRI is reportedly much more sensitive in the differentiation of these two pathological conditions [10]. However, at least PAP-US imaging can clearly identify the portal flow feeding nodules, such as DNs and eHCCs, facilitating the differentiation from overt HCC. This technique also provides portal flow supplying nodules, which are basically benign nature nodules [11]. These nodules develop to the overt HCCs at a very slow rate.

In addition to the arterial and portal supply to the nodule, isovascularity with low uptake of the Sonazoid at the Kupffer phases is a more important finding to differentiate early HCCs from DNs, although its sensitivity is low and its specificity high. This technique allows the depiction of intranodular portal flow in DNs and early-stage HCC noninvasively. In addition, sensitivity is as high as CTAP (table 2). Therefore, N-in-N type HCCs and hypovascular HCCs including DNs and early HCCs are correctly diagnosed by PAP-US.

Conclusion

This technique will be useful for characterization of nodular lesions associated with liver cirrhosis such as DNs, N-in-N type HCC, early HCC, and overt HCC.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Wen YL, Kudo M, Zheng RQ, Ding H, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K: Characterization of hepatic tumors: value of contrast-enhanced coded phase inversion harmonic US. AJR Am J Roentgenol 2004;182:1019–1026.

2 Wen YL, Kudo M: Detection of the intratumoral vascularity in small hepatocellular carcinoma by coded phase inversion harmonics. Intervirology 2004;47:169–178.

3 Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K: Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual display mode. Radiology 2001;220:349–356.

4 Ding H, Kudo M, Maekawa K, Suetomi Y, Minami Y, Onda H: Detection of tumor parenchymal blood flow in hepatic tumors: value of second harmonic imaging with a galactose-based contrast agent. Hepatol Res 2001; 21:242–251.

5 Kudo M: Contrast Harmonic Imaging in the Diagnosis and Treatment of Hepatic Tumors. Tokyo, Springer, 2003, pp 1–253.

6 Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H: Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. AJR Am J Roentgenol 2004;183:153–156.

7 Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Chung H, Kawasaki K, Maekawa K: Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. Radiology 2001;221:721–730.

8 Wen YL, Kudo M, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K: Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. AJR Am J Roentgenol 2003;181:57–63.

9 Ding H, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K: Contrast-enhanced subtraction harmonic sonography for evaluating treatment response in patients with hepatocellular carcinoma. AJR Am J Roentgenol 2001;176:661–666.

10 Kudo M: The 2008 Okuda Lecture: Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. J Gastroenterol Hepatol 2010;25:439–452.

11 Kudo M: Atypical large well-differentiated hepatocellular carcinoma with benign nature: a new clinical entity. Intervirology 2004;47(suppl):227–237.

Oncology

Oncology 2010;78(suppl 1):68–77
DOI: 10.1159/000315233

Published online: July 8, 2010

Contrast-Enhanced Ultrasound Techniques for Guiding and Assessing Response to Locoregional Treatments for Hepatocellular Carcinoma

Lorenzo Andreana[a, b] Masatoshi Kudo[b] Kinuyo Hatanaka[b] Hobyung Chung[b]
Yasunori Minami[b] Kiyoshi Maekawa[c] Giuseppe Ruggiero[a]

[a]Division of Internal Medicine and Hepatology, Second University of Naples Medical School, Naples, Italy;
[b]Department of Gastroenterology and Hepatology and [c]Abdominal Ultrasound Unit, Kinki University School of Medicine, Osaka, Japan

Key Words
Contrast-enhanced ultrasound · Embolization · Hepatocellular carcinoma · Percutaneous ablation

Abstract
Most hepatocellular carcinomas (HCC) are diagnosed in patients with cirrhosis and/or when tumor burden is too advanced for surgical treatment. In many of these cases the only suitable therapy is locoregional, percutaneous and/or intraarterial treatment. Moreover, the best way to guide and assess response to locoregional HCC treatment are two issues under discussion today. First-generation and subsequent second-generation microbubble contrast agents, together with contrast-enhanced ultrasound (US) imaging, have expanded the role of US techniques in HCC treatments. In this review our purpose is to illustrate the advantages, limits and potential of contrast-enhanced US application for locoregional HCC treatment.

Introduction

Hepatocellular carcinoma (HCC) is the most common liver malignancy worldwide, accounting for 75–90% of all hepatic cancers in Europe and North America [1]. Most of these primitive liver neoplasms are found in patients with cirrhosis and account for 80% of all newly diagnosed HCC [2]. It is estimated that 65% of patients with HCC will be considered for tumor-directed treatment [3]. Amongst these, due to multicentricity or advanced primitive liver disease, only 15% will be considered suitable candidates for surgery [3]. In such cases, locoregional percutaneous and intraarterial treatments have been developed for non-surgical alternatives for HCC patients. Percutanous treatment is one of the potentially curative therapies for early-stage HCC [4] and is principally carried out by inducing thermal or chemical necrosis with radiofrequency ablation (RFA) or percutaneous ethanol injection (PEI), respectively [5]. Intraarterial treatment is considered the best option for intermediate-stage HCC [4] and is performed mainly by embolizing HCC with or without contemporaneous administration of cytotoxic agents, with transarterial chemoembolization (TACE) and transarterial embolization (TAE) [6].

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0068$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

All these locoregional treatments require imaging targeting. Conventional B-mode ultrasound (cUS) is frequently employed for guiding percutaneous treatment of the liver [7, 8]. Contrast-enhanced ultrasound (CEUS) imaging, which generally improves HCC detection and may render lesions detectable, even if not previously identified with cUS, could, in many cases, be used for HCC targeting. Moreover, in several instances, CEUS imaging has been employed to help the fluoroscopic guidance of transarterial treatments as well [9].

Assessment of post-HCC treatment response is a current issue as it is of paramount importance to detect residual or recurrent viable HCC in order to schedule new treatment for patients and accurately target residual/recurrent lesions. Dynamic computed tomography (CT) 1 month after ablation is currently preferred as the standard imaging modality for assessing treatment efficacy [10]. However, CEUS represents a viable alternative with several inherent advantages: no exposure to ionizing radiation, examination can be done not only by axial planes but also by different/infinite planes, real-time scanning of the lesion during contrast phases, and, lastly, the same imaging and operator could subsequently target and treat the viable lesion. Moreover, CEUS imaging is currently performed at a lower cost when compared to dynamic cross-sectional imaging [11] and this is of particular importance in emerging economies where HCC is prevalent [1].

Targeting HCC

Ultrasound imaging is more often used as a primary guidance technique in percutaneous HCC treatment due to its facility and easy access to bedside. However, cUS is severely limited when HCC cannot be detected at baseline as well as in patients with residual liver tumors following percutaneous or intraarterial treatments. It is in such cases that contrast-enhanced imaging is best able to detect most previously undetectable and residual lesions. Upon application of CEUS, the most frequent HCC feature during the vascular phase is of an enhanced nodule (typical HCC). In the postvascular phase HCC becomes an unenhanced area surrounded by enhanced parenchyma. Even if atypical HCC remains unenhanced during the CEUS vascular phase, this defect in the postvascular phase could allow for nodule targeting.

CEUS before Harmonic Gray-Scale Imaging

Carbon dioxide CEUS (US angiography) is a rather complicated procedure, which requires hepatic artery cannulation under fluoroscopic guidance before injecting carbon dioxide directly into the HCC arterial supply. Moreover, it has a very high sensitivity and is able to detect HCC vascularization in most cases even if the lesion is only 1 cm in diameter [12]. In addition, a possible advantage of carbon dioxide CEUS is its ability to more clearly depict viable lesions with optimal time-resolution during the arterial phase. This important characteristic can improve the diagnosis of residual tumors or their local recurrence after treatment which justifies its use nowadays for selected cases [13].

The first proof of the usefulness of CEUS in guiding percutaneous treatments of HCC is found in a study on carbon dioxide CEUS published in 1992 by Imari et al. [14]. In this study of 22 patients with 31 histologically-proven HCC, carbon dioxide CEUS enabled the detection of 7 nodules that were previously undetectable at cUS. Iodized oil-ethanol was injected into 5 of these nodules under carbon dioxide CEUS guidance rending the lesions hyperechoic, and allowing further successful treatment with PEI under cUS guidance.

PEI has also been attempted under carbon dioxide CEUS guidance whereby the nodules were treated directly without injection of the iodized oil-ethanol. Takeshima et al. [15] employed this procedure for 28 early and very early HCCs that had not been detected with the cUS and had a detection rate of only 35.7% at the dynamic CT.

In a prospective study on 46 cirrhotic patients with 64 HCC, carbon dioxide CEUS permitted PEI treatment for 5 lesions not previously detected, 3 residual HCCs after primary PEI and/or TACE, 5 residual HCCs after secondary PEI treatment as well as 14 new lesions found during follow-up [16]. Recently, Miyamoto et al. [13] employed carbon dioxide CEUS to depict viable tumors in 13 out of 14 recurrent HCCs, previously assessed with dynamic CT 6 of which had been treated with RFA under carbon dioxide CEUS guidance.

In selected cases, US guidance was able to permit selective cannulation of the HCC feeding artery during intraarterial procedures. Two studies have been published on this topic which included a total of 86 patients who had received TAE guided by carbon dioxide CEUS [9, 17]. In these studies, the rationale was to use the highly sensitive US angiography when digital subtraction angiography failed to detect the tumor. Another study, utilizing microbubbles produced by mixing carbon dioxide together with human albumin or intralipid, employed carbon dioxide CEUS-guided TAE in 21 patients and PEI in 1 patient [18]. The study reported that carbon dioxide CEUS depicted nodules not previously detected in 9 of the pa-

Table 1. Microbubble contrast agents for CEUS in 51 original studies on guiding or assessing response to locoregional treatments for HCC [9, 13–27, 35–47, 49–70]

Agent	Core	Other compounds	External shell	(Routine) Use	Studies
Microbubbles which do not pass pulmonary circulation (injected into the hepatic artery)					
Carbon dioxide	CO_2	saline and/or autologous blood and/ or heparin or human albumin or intralipid		ultrasound angiography for selected cases	16%
First-generation microbubble agents					
Levovist	air		galactose and palmitic acid	abdominal, cardiologic	55%
Second-generation microbubble agents					
SonoVue	sulfur exafluoride		phospholipids	abdominal, cardiologic	12%
Optison	perfluoropropane		albumin	cardiologic	2%[1]
Imagent	perfluoroexane		phospholipids	cardiologic	2%[1]
Definity	perfluoropropane		liposomes	abdominal, cardiologic	2%
Sonazoid	perfluorobutane		phospholipids	abdominal	12%

[1] Optison and Imagent were both employed indifferently in the same study [62].

tients. With regard to the procedure itself, the author emphasized that the imaging was clearer with the intralipid and carbon dioxide mixture than that made using human albumin.

An attempt to use Doppler CEUS for guiding percutaneous ablation of HCC was reported only in one study whereby 20 patients were treated with PEI after transarterial infusion of chemotherapy for 23 HCC [19]. Each of the lesions was hypervascular at color Doppler CEUS before PEI. After treatment, both the dynamic CT and the biopsy showed the absence of enhancement for each nodule and complete necrosis, respectively.

Harmonic Gray-Scale CEUS

Microbubble disruption (first-generation microbubble agents) or simple collapsing-expanding (second-generation microbubble agents) due to US pressure result in non-linear emission of US with the generation of harmonics from the insonating frequency. The combination of transducer/receiver systems and software dedicated to harmonic imaging allows for the enhancement of signals emanating from microbubbles and reduces the signal from tissue.

First-generation microbubble agents, when injected in the peripheral vein, survive pulmonary transit reaching the liver through the hepatic artery and portal vein after an interval of 10–30 s and slightly more seconds later, respectively. These microbubbles are made of air with an external shell of albumin, galactose and/or palmitic acid, which stabilize their structure (table 1).

The enhancement of this generation of agents requires the disruption of microbubbles with a high-energy output (high mechanical index). Therefore, CEUS studies with these compounds are technically challenging because the microbubbles are disrupted as they enter the field of view, limiting their effect.

Second-generation compounds for CEUS are made up of a core of inert gasses such as esafluoride sulfur or perfluorocaburs, stabilized by an external shell of albumin or phospholipids (table 1). With respect to the first generation, these contrast agents enable strong enhancement without microbubble disruption using a contrast-specific imaging mode with a low mechanical index. The microbubbles have excellent durability, enabling continuous real-time imaging.

Since these technologies were first available, they have been used for HCC targeting during percutaneous treatments. To date, five prospective studies [20–24], two retrospective analyses [25, 26] and one prospective randomized trial [27] have been published on this theme. Four studies report on the use of Levovist [20, 21, 26, 27], three on Sonazoid [22–24] and only one on SonVue [25]. The latter study included patients with liver metastases as well [25].

Table 2. Detection rate for harmonic gray-scale CEUS associated with percutaneous treatments for HCC

Reference (first author)	Patients/ HCC, n	Size of nodule (range), cm	Local recurrent HCC, n	Percutaneously treated HCC, n	Microbubble agent	Detection rate, %
Numata, 2003 [20]	30/56	0.6–4.8	40	RFA 7/PEI 19	Levovist	93
Minami, 2004 [21]	15/16[1]	1–2.5	16	RFA 14	Levovist	81
Maruyama, 2007 [26]	40[2]/32	0.5–2	NR	RFA 5/PEI 14	Levovist	75
Numata, 2008 [22]	85/108	0.7–2.5	74	RFA 14	Sonazoid	97
Maruyama, 2009 [23]	44[2]/NR	0.5–2.4	NR	RFA 11/PEI 20	Sonazoid	96[3]
Miyamoto, 2009 [24]	42/52	0.7–3	12	RFA 50	Sonazoid	96
	12[4]/12		12	RFA 12	Sonazoid	100

NR = Not reported.
[1] Only recurrent nodules located at hepatic dome were included.
[2] Even hypervascular nodules not definitively diagnosed as HCC were included.
[3] More lesions found after CEUS with angiography.
[4] Subgroup of local recurrence patients, assessed with defect-reperfusion imaging.

Harmonic gray-scale CEUS targeting emerges from the published literature as a highly sensitive and effective image-guiding tool, proving a consistent detection rate of 75–100% [20–24, 26, 27] (table 2). The detection rate is lower than 93% in only two studies [21, 26]. In the first of these, Minami et al. [21] treated 16 recurrent HCCs with RFA located at the hepatic dome and reported on 13 of them (81%). Visualization of these difficult lesions was possible by using Levovist-enhanced harmonic CEUS together with production of artificial pleural effusion. Artificial pleural effusion with the mean infused volume of 673 ml obtained both an increase in tumor depiction and lung covering during RFA treatment. The second study is a retrospective analysis beginning with HCC surveillance for 40 patients with cirrhosis [26]. In this study, only 24 of the 32 HCCs diagnosed with a total of 47 hypervascular lesions were detected (75%). Harmonic CEUS then guided PEI or RFA for 19 of those HCC.

The only randomized controlled trial on harmonic gray-scale CEUS-guided RFA against cUS-guided RFA [27] produced interesting results. Forty patients diagnosed with 40 local HCC progressions were randomized into the two arms. The harmonic CEUS-guided arm demonstrated a significantly higher complete response rate after one RFA course (94.7 vs. 65.0%, p = 0.043). Unfortunately, follow-up results were inconsistent. During the medium time of 21.5 and 19.4 months, respectively, both arms showed two local recurrences (10.5 vs. 10%, p = 0.96). We believe that a larger trial could eventually provide more conclusive results. Moreover, a large retrospective analysis of 162 patients treated with RFA-guided harmonic gray-scale CEUS for 192 HCC and 97 liver metastases [25] resulted in recommending its routine use. In this study the impressive results of only 5.9% incomplete tumor necrosis (17 on 289 treated nodules) were obtained after the introduction of CEUS targeting compared to the previous 16.1% of cUS-guided treatments. Unfortunately, the recurrence rate in these patients was not reported.

A more specific indication may pertain to treatments guided by harmonic gray-scale CEUS. Tamai et al. [28] reported on a single case suggesting harmonic gray-scale CEUS-guided RFA as an optimal approach for treating spontaneously ruptured HCC. TAE is currently considered a first-line treatment for treatment of spontaneously ruptured HCC [29]. However, angiographic localization of the ruptured bleeding site is reportedly successful in 20% of patients at most [30]. In the above-mentioned case, harmonic CEUS with Levovist was able to accurately evidence the damaged area of the tumor. Subsequently, due to the patient's poor liver function, RFA was the preferred choice at TAE for in a single session it simultaneously contained hemostasis and complete HCC necrosis. Further studies are needed to elucidate indications for such treatment.

The postvascular/Kupffer phase, which starts 5–10 min after Sonazoid injection and lasts for more than 60 min, enables the new technique of defect-reperfusion imaging that was recently proposed by Kudo et al. [11]. With this technique the HCC nodule is evidenced as a defect during the postvascular/Kupffer phase and is enhanced during the vascular phase by reinjecting Sonazoid. Particularly in cases of previously treated HCC, this enables

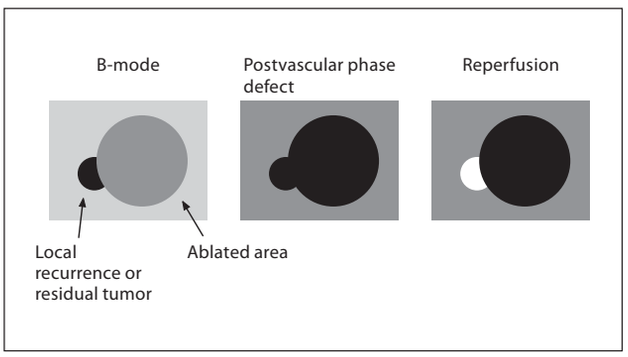


Fig. 1. Defect-reperfusion imaging proposed by Kudo et al. [11]. During reperfusion, an enhanced viable tumor is clearly contrasted by enhanced parenchyma and a defect of a previously treated area [reproduced with permission].

easy focus on the portion of residual or recurrent HCC, which is contrasted with both the normal liver and the defect area of the treated HCC (fig. 1).

Myamoto et al. [24] used this defect-reperfusion imaging together with the RFA for 12 previously treated HCC in 12 patients. Conventional gray-scale CEUS guided the RFA for 38 more untreated HCCs in 30 patients. The detection rate with this strategy was 96% for all nodules (52 HCC) and 100% for local recurrent HCC with defect-reperfusion imaging. Complete necrosis was obtained after a first RFA course for 48 of these tumors.

Assessing Treatment Response

After locoregional percutaneous or intraarterial HCC treatments, an imaging assessment may allow for further treatment of any residual or recurrent HCC. An accurate diagnosis of this residual/recurrent HCC can be pivotal for patient prognosis. Nowadays the use of the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines [31] for the assessment of tumor response is not considered the correct approach for HCC. This is because of the scarce relationship between HCC necrosis in HCC and tumor shrinkage, especially after locoregional treatments [32]. Moreover, when the tumor is not detectable at baseline by unenhanced imaging, the RECIST guidelines are technically not applicable.

For transarterial treatments which contemplate the use of lipiodol, several authors evaluated the response to treatment by observing iodized oil retention [33]. In these cases, complete dense lipiodol retention is considered synonymous with complete response. However, retained lipiodol could possibly mask enhancement of the viable tumor with dynamic CT, and could only indicate that the procedure has been optimally executed.

The European Association for the Study of the Liver (EASL) guidelines [10] indicate that the presence of viable HCC is detected by enhancement of the treated lesions whereas the non-enhanced area reflects tissue necrosis. In fact, successfully treated HCC is devoid of vascularity showing constant avascular features during follow-up if there is no recurrence. Based upon this, we believe that evaluation of tumor vascularity is the best way to assess accurate response to locoregional treatments and that only non-enhancing atypical HCC must be assessed following RECIST guidelines [34].

Since April 1998, 34 studies [35–69] that consider different CEUS techniques have been published. Of these, 23 were on harmonic gray-scale CEUS [48–70], 12 were on Doppler CEUS modalities [36–48], one study compared both approaches [48] and another reported on carbon dioxide CEUS [35] Two studies have been excluded from our discussion due to the unavailability of papers [71, 72].

CEUS before Harmonic Gray-Scale Imaging

Carbon dioxide CEUS has also been employed for the assessment of HCC after locoregional treatment. Currently, only one study exists on this theme [35]. In 66 HCCs treated with PEI, TACE (or a combination of both) and carbon dioxide, CEUS proved an excellent positive predictive value of 100% when compared with follow-up results. In the same cohort the positive predictive value for angiography was lower (87.8%).

Power and color Doppler modalities have been used with early developing microbubble agents. However, their main limitation was poor time resolution during the vascular phases (arterial phase is masked from color blooming), which was responsible for many HCCs showing up as hypovascular at the workup before treatment. For this reason, in the 12 Doppler CEUS studies considered for assessing response to locoregional treatments, several nodules had been excluded from analysis [36–47]. Out of 507 nodules from 444 patients which represent the overall considered population subject of our review, a total of 22 nodules were excluded from analysis.

Specificity of Doppler CEUS modalities in assessing treatment response shows an average value of 99.4% (range 93–100%) [36–47]. Unfortunately, its sensitivity and negative predictive value has proven to be frequently low with a reported average value of only 74.4% (range 33.3–100%) and 77.5% (range 8.3–100%), respectively

Table 3. Harmonic gray-scale CEUS in assessing response to locoregional treatments for HCC

Reference (first author)	Patients/ HCC, n	Size of nodule (range), cm	Treatment scheduled	Microbubble agent	Gold standard	Sensitivity (PPV/NPV, %)
Ding, 2001 [50]	43/50	1–10	RFA 15/TACE 7/ TACE + RFA 19/TACE + PEI 2	Levovist	CT	94.6 (100/95.1)
Ding, 2001 [49]	26/32	1–6	RFA 6/TACE 10/RFA + PEI 3/[1] TACE + RFA 3/TACE + PEI 8	Levovist	CT	93.3 (100/93.8)
Numata, 2001 [51]	29/39	2.7[2]	TACE	Levovist	CT	100 (100/100)
Meloni, 2001 [42]	35/43	1.2–5.8	RFA	Levovist	CT	83.3 (100/93.9)
Morimoto, 2003 [52]	29/49	NR	TACE	Levovist	biopsy	100 (81.3/100)
Minami, 2003 [53]	40/44	1.5–11	TACE	Levovist	CT	100 (50/100)
Wen, 2003 [54]	67/91	1–5	RFA	Levovist	CT	95.3 (100/97)
Choi, 2003 [55]	75/81	1.3–4.8	RFA	Levovist	CT	100 (100/100)
Shimizu, 2004 [56]	40/64	1–6	RFA	Levovist	CT[3]	100 (86.7/100)
Pompili, 2005 [57]	47/56	1–7.5	RFA 3/PEI 31/TACE 12/[1] RFA + PEI 3/TACE + PEI 7	SonoVue	CT	100 (88.5/100)
Morimoto, 2005 [58]	48/72	0.7–3	RFA	Levovist	biopsy or Rx	83.3 (100/98.5)
Vilana, 2006 [59]	41/41	13–41	RFA 5/PEI 36	SonoVue	CT	27.3 (75/78.3)
Kim, 2006 [60]	29/31	0.6–12	TACE	Levovist	angiography	93 (68/92)
Dill-Macky, 2006 [61]	19/22	1.5–3.7	RFA	Definity	CT or MRI	40 (66/83)
Kono, 2007 [62]	33/42	1–10	TACE	Imagent of Optison	CT	100 (94.4/100)
Lu, 2007 [63]	151/NR	NR	RFA or MWTA	SonoVue	CT or MRI	66.7[4] (98.2/66.7)
Xia, 2008 [64]	28/43	0.9–10	TACE	Sonazoid	MDCT	100 (68/100)
Ricci, 2008 [65]	100/100	2.6–4.8	RFA	SonoVue	CT	92.3 (97.4/100)
Salvaggio, 2009 [66]	139/148	NR	RFA 100/TACE 39	SonoVue	MDCT	83.3 (100/96.8)
Gallotti, 2009 [67]	69/90	0.5–4.9	RFA 34/PEI 43[5]	SonoVue	MDCT	61.3 (73.1/81.3)
Luo, 2009 [68]	63/63	10–30	RFA	Sonazoid	MDCT	100 (50/100)
Shiozawa, 2010 [69]	71/87	0.8–6.5	RFA 55/TACE 22/[1] TACE + RFA 10	Sonazoid	MDCT	81.5 (NR/NR)

MWTA = Microwave thermoablation; RX = resection; MRI = gadolinium-enhanced magnetic resonance imaging; MDCT = multidetector computed tomography; NR = not reported.
[1] Nodules. [2] Average diameter. [3] Even biopsy when CT was negative. [4] Patient-based analysis. [5] Eight patients had both treatments.

[36–47]. As such, its low negative predictive value is discouraging for routine use. In the only study comparing the efficacy of power Doppler CEUS and harmonic gray-scale CEUS, the power Doppler CEUS performed poorly (sensitivity of 33.3 vs. 83.3%) [42].

Harmonic Gray-Scale CEUS

Doppler CEUS modalities have been greatly supplanted by harmonic gray-scale CEUS which has proven to be more sensitive and less complex. Indeed, the joint guidelines of the American Association of the Study of the Liver (AASLD) and the EASL [4] and the Japanese 'evidence-based guidelines' [48] all indicate harmonic gray-scale CEUS amongst the diagnostic tools useful for HCC imaging.

Studies published on harmonic gray-scale CEUS for assessing the response to locoregional treatments encompass a total of 1,244 enrolled patients [42, 49–70]. Post-treatment assessment was made on 1,314 nodules in 16 studies following RFA [42, 49, 50, 54–57, 59, 61, 63, 65–70], in 3 studies following PEI [57, 59, 67], and in 11 studies following TACE [49–53, 57, 60, 62, 64, 66, 69]. Five of these studies also contemplated the use of combined treatments [49, 50, 57, 67, 69].

The overall detection rate for residual/local-recurrent HCC was 3.2–92.3% reporting an average sensitivity, specificity, positive predictive value and negative predictive value of 86.1% (range 27.3–100%), 90.3% (range 65–100%), 84.1% (range 50–100%) and 95.6% (range 78.3–100%), respectively [42, 49–70] (table 3). In the few studies that reported its value, the interobserver agreement was very high, with a range of 92.1–97.3% [66, 68]. In one study the κ coefficient for harmonic gray-scale CEUS was higher than that for multidetector CT (0.89 vs. 0.66) [69].

Reported assessment timing was wide ranging from a few hours to 1,882 days [42, 49–70]. However, for most studies assessment was made around 1 week after treatment [49, 51–54, 56, 60, 64], when artifacts due to procedure disappeared. Moreover, Salvaggio et al. [66] report-

ed on a subgroup analysis from his series of patients who were screened with harmonic gray-scale CEUS immediately after RFA and had a subsequent extension of the ablated area if a residual tumor was present and was showed better sensitivity in this group compared with those assessed at 30 days (100 vs. 95.6%).

In most studies dynamic CT at 30 days represented the gold standard [55, 57, 59, 62, 65–68]. However, in several instances this imaging technique proved to have a lower sensitivity when compared to harmonic gray-scale CEUS with values of 64–92.3 and 93–100%, respectively, whereby the gold standard was angiography or follow-up results [60, 65].

Xia et al. [64] comparing results 1 week after TACE of Sonazoid-enhanced CEUS with defect-reperfusion imaging and multidetector CT reported significantly higher sensitivity for the former (100 vs. 61.5%, $p < 0.01$). When analyzing studies which only included lesions of ≤5 cm in diameter, the average sensitivity for harmonic gray-scale CEUS reported lower values when compared with all lesions, whereas specificity appeared to have improved (74.9 vs. 86.1% and 96.9 vs. 90.3%) [54, 55, 58, 59, 61, 65, 67, 68]. The more constantly developed vascularity in bigger lesions may justify this evidence. The detection rate reported for residual/local-recurrent HCC with harmonic gray-scale CEUS was 3.2–45.1% after RFA [42, 54–56, 58, 61, 63, 65, 68]. Average sensitivity, specificity, positive predictive value and negative predictive value for harmonic gray-scale CEUS in assessing response to RFA were 84.5% (range 66.7–100%), 98.3% (range 94–100%), 85.2% (range 66–100%) and 96.7% (range 83–100%), respectively [42, 54–56, 58, 61, 63, 65, 68].

TACE, which is currently not a standardized procedure, had a wide recurrence rate ranging from 25.6 to 92.3% with harmonic gray-scale CEUS which reflects its current acceptance [51–53, 60, 62, 64]. Upon assessment of the response to TACE, average sensitivity, specificity, positive predictive value and negative predictive value for harmonic gray-scale CEUS were 98.8% (range 93–100%), 71.7% (range 31.6–100%), 77% (range 50–100%) and 100%, respectively [51–53, 60, 62, 64], proving that assessment for TACE with harmonic gray-scale CEUS had an average-to-lower specificity when compared to RFA (71.7 vs. 98.3%).

In the above-referenced study, Salvaggio et al. [66] in patients treated with RFA or TACE reported a slightly higher accuracy in defining the efficacy of RFA over TACE with an agreement of 97 and 92%, respectively. They attributed this result to the presence of dense lipiodol retention in 3 false negatives at the CT. In the only study that considered different percutaneous procedures analyzing harmonic gray-scale performance separately for each procedure [67], overall specificity for RFA and PEI combined was 88.1% while for RFA only it was 100%. Nonetheless, the reported sensitivity was very low (20%), perhaps because of the artifacts during assessment which were done immediately after the RFA session.

From an oncologic point of view, HCC ablation should be performed with an established safety margin. An ablated area that exceeds a tumor diameter of 0.5–1 cm appears to prevent HCC recurrence as proven in studies on RFA [73, 74]. Therefore it is reasonable to consider complete HCC response if the ablation area includes a safety margin of ≥0.5 mm.

In several cases the systematic creation of a safety margin during RFA has been reported as part of treatment strategy [54, 55, 57, 70]. Wen et al. [54] tried to assess the presence of at least 0.5 cm of safe margins by employing harmonic gray-scale CEUS, demonstrating a poor sensitivity of 10% when compared with dynamic CT as the current gold standard. Virtual ultrasonography, together with harmonic gray-scale CEUS, shows that first-time CEUS was able to detect these safety margins [70]. In this case, virtual reconstruction of images, merging data from multidetector CT before RFA and Levovist-enhanced CEUS after treatment enabled real-time detection of safety margins with a sensitivity rate of 92.3%.

Three-dimensional CEUS represents a new CEUS-associated technology which enables a detailed visualization of nodule vascularization together with high spatial resolution. The only published study on this technology utilized for post-RFA assessment showed a sensitivity and specificity of 97 and 100%, respectively [68]. We believe that this method to evaluate the treatment response shows promise, especially for the planning of further percutaneous treatment. In fact, when there is residual/recurrent HCC acquisition of three-dimensional images, subsequent reviewing from different orientations may allow for correct needle placement.

Conclusion

In current clinical practice, CEUS is becoming ever more common, involving many diagnostic and therapeutic fields which are expanding its application. At present, CEUS is part of the hepatologist's or interventional radiologist's armory for treatment of HCC in combination with locoregional procedures.

The two main chapters of this review have outlined the history of CEUS technologies as they have developed and been applied for guiding and assessing locoregional treatments of HCC. Since the first studies reporting good results with carbon dioxide and Doppler approaches have been published, the first true breakthrough has been in introducing harmonic US technology. Following this, harmonic gray-scale CEUS has been accepted as a reliable diagnostic tool for HCC [4, 48].

For HCC targeting, CEUS represents a significant improvement in all steps of tumor ablative treatment. Published literature has shown that it can greatly simplify patient management and even possibly reduce costs by lowering the number of courses of treatment. However, comparative studies need to be made.

The use of CEUS in assessing treatment is now a widely accepted alternative to dynamic cross-sectional imaging. Overall sensitivity and specificity in the detection of HCC vascularity after treatment has proven comparable to other imaging tools. In addition, CEUS can also be reliably employed in targeting treatment if percutaneous ablation is required.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Parkin DM, Bray F, Ferlay J, et al: Global cancer statistics, 2002. CA Cancer J Clin 2005; 55:74–108.

2 Llovet JM, Burroughs A, Bruix J: Hepatocellular carcinoma. Lancet 2003;362:1907–1917.

3 Pleguezuelo M, Germani G, Marelli L, et al: Evidence-based diagnosis and locoregional therapy for hepatocellular carcinoma. Expert Rev Gastroenterol Hepatol 2008;2:761–784.

4 Bruix J, Sherman M: Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005; 42:1208–1236.

5 Germani G, Pleguezuelo M, Gurusamy K, et al: Clinical outcomes of radiofrequency ablation, percutaneous alcohol and acetic acid injection for hepatocelullar carcinoma: a meta-analysis. J Hepatol 2010;52:380–388.

6 Marelli L, Stigliano R, Triantos C, et al: Transarterial therapy for hepatocellular carcinoma: which technique is more effective? A systematic review of cohort and randomized studies. Cardiovasc Intervent Radiol 2007;30:6–25.

7 Lin DY, Lin SM, Liaw YF: Non-surgical treatment of hepatocellular carcinoma. J Gastroenterol Hepatol 1997;12:S319–S328.

8 Solbiati L, Ierace T, Goldberg SN, et al: Percutaneous US-guided radiofrequency tissue ablation of liver metastases: treatment and follow-up in 16 patients. Radiology 1997;202: 195–203.

9 Hashimoto M, Watanabe O, Hirano Y, et al: Use of carbon dioxide microbubble-enhanced sonographic angiography for transcatheter arterial chemoembolization of hepatocellular carcinoma. AJR Am J Roentgenol 1997;169:1307–1310.

10 Bruix J, Sherman M, Llovet JM, et al: EASL Panel of Experts on HCC. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona 2000 EASL Conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.

11 Kudo M, Hatanaka K, Maekawa K: Defect reperfusion Imaging, a newly developed novel technology using Sonazoid in the treatment of hepatocellular carcinoma. J Med Ultrasound 2008;16:169–176.

12 Kudo M, Tomita S, Tochio H, et al: Small hepatocellular carcinoma: diagnosis with US angiography with intraarterial CO_2 microbubbles. Radiology 1992;182:155–160.

13 Miyamoto N, Hiramatsu K, Tsuchiya K, et al: Carbon dioxide microbubbles-enhanced sonographically guided radiofrequency ablation: treatment of patients with local progression of hepatocellular carcinoma. Radiat Med 2008;26:92–97.

14 Imari Y, Sakamoto S, Shiomichi S, et al: Hepatocellular carcinoma not detected with plain US: treatment with percutaneous ethanol injection under guidance with enhanced US. Radiology 1992;185:497–500.

15 Takeshima K, Kumada T, Kimura T, et al: The usefulness of percutaneous ethanol injection therapy under guidance with carbon dioxide contrast-enhanced ultrasound sonography. Nippon Rinsho 1998;56:1001–1006.

16 Numata K, Tanaka K, Kiba T, et al: Nonresectable hepatocellular carcinoma: improved percutaneous ethanol injection therapy guided by CO_2-enhanced sonography. AJR Am J Roentgenol 2001;177:789–798.

17 Miyajima Y, Sakaguchi S, Tohara K: Transcatheter arterial embolization of hepatocellular carcinoma using CO_2 US. Nippon Rinsho 1998;56:990–993.

18 Irie T, Yamada T, Ganaha F, et al: Usefulness of CO_2 US angiography in treating hepatocellular carcinoma. Nippon Igaku Hoshasen Gakkai Zasshi 1998;58:338–342.

19 Shirato K, Morimoto M, Tomita N, et al: Hepatocellular carcinoma: therapeutic experience with percutaneous ethanol injection under real-time contrast-enhanced color Doppler sonography with the contrast agent Levovist. J Ultrasound Med 2002;21:1015–1022.

20 Numata K, Isozaki T, Ozawa Y, et al: Percutaneous ablation therapy guided by contrast-enhanced sonography for patients with hepatocellular carcinoma. AJR Am J Roentgenol 2003;180:143–149.

21 Minami Y, Kudo M, Kawasaki T, et al: Percutaneous radiofrequency ablation guided by contrast-enhanced harmonic sonography with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. AJR Am J Roentgenol 2004;182:1224–1226.

22 Numata K, Morimoto M, Ogura T, et al: Ablation therapy guided by contrast-enhanced sonography with Sonazoid for hepatocellular carcinoma lesions not detected by conventional sonography. J Ultrasound Med 2008;27:395–406.

23 Maruyama H, Takahashi M, Ishibashi H, Okugawa H, Okabe S, Yoshikawa M, Yokosuka O: Ultrasound-guided treatments under low acoustic power contrast harmonic imaging for hepatocellular carcinomas undetected by B-mode ultrasonography. Liver Int 2009;29:708–714.

24 Miyamoto N, Hiramatsu K, Tsuchiya K, et al: Sonazoid-enhanced sonography for guiding radiofrequency ablation for hepatocellular carcinoma: better tumor visualization by Kupffer-phase imaging and vascular-phase imaging after reinjection. Jpn J Radiol 2009; 27:185–193.

25 Solbiati L, Ierace T, Tonolini M, et al: Guidance and control of percutaneous treatments with contrast-enhanced ultrasound. Eur Radiol 2003;13(suppl 3):N87–N90.
26 Maruyama H, Kobayashi S, Yoshizumi H, et al: Application of percutaneous ultrasound-guided treatment for ultrasonically invisible hypervascular hepatocellular carcinoma using microbubble contrast agent. Clin Radiol 2007;62:668–675.
27 Minami Y, Kudo M, Chung H, et al: Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. AJR Am J Roentgenol 2007;188:489–494.
28 Tamai H, Oka M, Maeda H, et al: Contrast harmonic sonographically guided radiofrequency ablation for spontaneous ruptured hepatocellular carcinoma. J Ultrasound Med 2005;24:1021–1026.
29 Zhu LX, Wang GS, Fan ST: Spontaneous rupture of hepatocellular carcinoma. Br J Surg 1996;83:602–607.
30 Uchida K, Nakata S, Iwase H, et al: Imaging diagnosis of ruptured site in hepatocellular carcinoma. Nippon Shokakibyo Gakkai Zasshi 1989;86:1287–1291.
31 Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–216.
32 Forner A, Ayuso C, Varela M, et al: Evaluation of tumor response after locoregional therapies in hepatocellular carcinoma: are response evaluation criteria in solid tumors reliable? Cancer 2009;115:616–623.
33 Maki S, Konno T, Maeda H: Image enhancement in computerized tomography for sensitive diagnosis of liver cancer and semiquantitation of tumor-selective drug targeting with oily contrast medium. Cancer 1985;56:751–757.
34 Llovet JM, Di Bisceglie AM, Bruix J, et al: Panel of Experts in HCC-Design Clinical Trials. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
35 Chen RC, Wang CK, Chiang LC, et al: Intraarterial carbon dioxide-enhanced ultrasonogram of hepatocellular carcinoma treated by transcatheter arterial embolization and percutaneous ethanol injection therapy. J Gastroenterol Hepatol 1998;13:41–46.
36 Bartolozzi C, Lencioni R, Ricci P, et al: Hepatocellular carcinoma treatment with percutaneous ethanol injection: evaluation with contrast-enhanced color Doppler US. Radiology 1998;209:387–393.
37 Catalano O, Cusati B, Esposito M, et al: The correlation between Doppler echography with a contrast medium and CT in the study of a hepatocarcinoma submitted to chemoembolization. Radiol Med 1998;95:608–613.
38 Catalano O, Esposito M, Lobianco R, et al: Hepatocellular carcinoma treated with chemoembolization: assessment with contrast-enhanced Doppler ultrasonography. Cardiovasc Intervent Radiol 1999;22:486–492.
39 Choi D, Lim HK, Kim SH, et al: Hepatocellular carcinoma treated with percutaneous radiofrequency ablation: usefulness of power Doppler US with a microbubble contrast agent in evaluating therapeutic response-preliminary results. Radiology 2000;217:558–563.
40 Cioni D, Lencioni R, Bartolozzi C: Therapeutic effect of transcatheter arterial chemoembolization on hepatocellular carcinoma: evaluation with contrast-enhanced harmonic power Doppler ultrasound. Eur Radiol 2000;10:1570–1575.
41 Fiore F, Vallone P, Ricchi P, et al: Levovist-enhanced Doppler sonography versus spiral computed tomography to evaluate response to percutaneous ethanol injection in hepatocellular carcinoma. J Clin Gastroenterol 2000;31:164–168.
42 Meloni MF, Goldberg SN, Livraghi T, et al: Hepatocellular carcinoma treated with radiofrequency ablation: comparison of pulse inversion contrast-enhanced harmonic sonography, contrast-enhanced power Doppler sonography, and helical CT. AJR Am J Roentgenol 2001;177:375–380.
43 Cioni D, Lencioni R, Rossi S, et al: Radiofrequency thermal ablation of hepatocellular carcinoma: using contrast-enhanced harmonic power Doppler sonography to assess treatment outcome. AJR Am J Roentgenol 2001;177:783–788.
44 Cedrone A, Pompili M, Sallustio G, et al: Comparison between color power Doppler ultrasound with echo-enhancer and spiral computed tomography in the evaluation of hepatocellular carcinoma vascularization before and after ablation procedures. Am J Gastroenterol 2001;96:1854–1859.
45 Choi D, Lim HK, Kim SH, et al: Assessment of therapeutic response in hepatocellular carcinoma treated with percutaneous radiofrequency ablation: comparison of multiphase helical computed tomography and power Doppler ultrasonography with a microbubble contrast agent. J Ultrasound Med 2002;21:391–401.
46 Vilana R, Llovet JM, Bianchi L, et al: Barcelona Clinic Liver Cancer Group: Contrast-enhanced power Doppler sonography and helical computed tomography for assessment of vascularity of small hepatocellular carcinomas before and after percutaneous ablation. J Clin Ultrasound 2003;31:119–128.
47 Vallone P, Gallipoli A, Izzo F, et al: Local ablation procedures in primary liver tumors: Levovist US versus spiral CT to evaluate therapeutic results. Anticancer Res 2003;23:5075–5079.
48 Makuuchi M, Kokudo N, Arii S, et al: Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008;38:37–51.
49 Ding H, Kudo M, Onda H, et al: Contrast-enhanced subtraction harmonic sonography for evaluating treatment response in patients with hepatocellular carcinoma. AJR Am J Roentgenol 2001;176:661–666.
50 Ding H, Kudo M, Onda H, et al: Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. Radiology 2001;221:721–730.
51 Numata K, Tanaka K, Kiba T, et al: Using contrast-enhanced sonography to assess the effectiveness of transcatheter arterial embolization for hepatocellular carcinoma. AJR Am J Roentgenol 2001;176:1199–1205.
52 Morimoto M, Shirato K, Sugimori K, et al: Contrast-enhanced harmonic gray-scale sonographic-histologic correlation of the therapeutic effects of transcatheter arterial chemoembolization in patients with hepatocellular carcinoma. AJR Am J Roentgenol 2003;181:65–69.
53 Minami Y, Kudo M, Kawasaki T, et al: Transcatheter arterial chemoembolization of hepatocellular carcinoma: usefulness of coded phase-inversion harmonic sonography. AJR Am J Roentgenol 2003;180:703–708.
54 Wen YL, Kudo M, Zheng RQ, et al: Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. AJR Am J Roentgenol 2003;181:57–63.
55 Choi D, Lim HK, Lee WJ, et al: Early assessment of the therapeutic response to radiofrequency ablation for hepatocellular carcinoma: utility of gray scale harmonic ultrasonography with a microbubble contrast agent. J Ultrasound Med 2003;22:1163–1172.
56 Shimizu M, Iijima H, Horibe T, et al: Usefulness of contrast-enhanced ultrasonography with a new contrast mode, agent detection imaging, in evaluating therapeutic response in hepatocellular carcinoma treated with radiofrequency ablation therapy. Hepatol Res 2004;29:235–242.
57 Pompili M, Riccardi L, Covino M, et al: Contrast-enhanced gray-scale harmonic ultrasound in the efficacy assessment of ablation treatments for hepatocellular carcinoma. Liver Int 2005;25:954–961.
58 Morimoto M, Nozawa A, Numata K, et al: Evaluation using contrast-enhanced harmonic gray scale sonography after radiofrequency ablation of small hepatocellular carcinoma: sonographic-histopathologic correlation. J Ultrasound Med 2005;24:273–283.
59 Vilana R, Bianchi L, Varela M, et al: BCLC Group. Is microbubble-enhanced ultrasonography sufficient for assessment of response to percutaneous treatment in patients with early hepatocellular carcinoma? Eur Radiol 2006;16:2454–2462.

60 Kim HJ, Kim TK, Kim PN, et al: Assessment of the therapeutic response of hepatocellular carcinoma treated with transcatheter arterial chemoembolization: comparison of contrast-enhanced sonography and three-phase computed tomography. J Ultrasound Med 2006;25:477–486.

61 Dill-Macky MJ, Asch M, Burns P, et al: Radiofrequency ablation of hepatocellular carcinoma: predicting success using contrast-enhanced sonography. AJR Am J Roentgenol 2006;186(suppl):S287–S295.

62 Kono Y, Lucidarme O, Choi SH, et al: Contrast-enhanced ultrasound as a predictor of treatment efficacy within 2 weeks after transarterial chemoembolization of hepatocellular carcinoma. J Vasc Interv Radiol 2007;18:57–65.

63 Lu MD, Yu XL, Li AH, et al: Comparison of contrast-enhanced ultrasound and contrast-enhanced CT or MRI in monitoring percutaneous thermal ablation procedure in patients with hepatocellular carcinoma: a multicenter study in China. Ultrasound Med Biol 2007;33:1736–1749.

64 Xia Y, Kudo M, Minami Y, et al: Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: the usefulness of Sonazoid-enhanced harmonic sonography. Oncology 2008;75(suppl 1):99–105.

65 Ricci P, Cantisani V, Drudi F, et al: Is contrast-enhanced US alternative to spiral CT in the assessment of treatment outcome of radiofrequency ablation in hepatocellular carcinoma? Ultraschall Med 2009;30:252–258.

66 Salvaggio G, Campisi A, Lo Greco V, et al: Evaluation of posttreatment response of hepatocellular carcinoma: comparison of ultrasonography with second-generation ultrasound contrast agent and multidetector CT. Abdom Imaging 2009 (in press).

67 Gallotti A, D'Onofrio M, Ruzzenente A, et al: Contrast-enhanced ultrasonography immediately after percutaneous ablation of hepatocellular carcinoma. Radiol Med 2009;114:1094–1105.

68 Luo W, Numata K, Morimoto M, et al: Role of Sonazoid-enhanced three-dimensional ultrasonography in the evaluation of percutaneous radiofrequency ablation of hepatocellular carcinoma. Eur J Radiol 2009 (in press).

69 Shiozawa K, Watanabe M, Takayama R, et al: Evaluation of local recurrence after treatment for hepatocellular carcinoma by contrast-enhanced ultrasonography using Sonazoid: comparison with dynamic computed tomography. J Clin Ultrasound 2010;38:182–189.

70 Kisaka Y, Hirooka M, Kumagi T, et al: Usefulness of contrast-enhanced ultrasonography with abdominal virtual ultrasonography in assessing therapeutic response in hepatocellular carcinoma treated with radiofrequency ablation. Liver Int 2006;26:1241–1247.

71 Hotta N, Tagaya T, Maeno T, et al: Usefulness of contrast-enhanced ultrasonography with dynamic flow imaging to evaluate therapeutic effects for hepatocellular carcinoma. Hepatogastroenterology 2003;50:1867–1871.

72 Nishiharu T, Maeda N, Hara M, et al: Usefulness of enhanced ultrasonography after administration of intravenous contrast agent in the evaluation of therapeutic effect in treatment of hepatocellular carcinoma, and efficacy of percutaneous ethanol injection therapy for residual tumor. Nippon Igaku Hoshasen Gakkai Zasshi 2001;61:790–795.

73 Ikeda K, Seki T, Umehara H, et al: Clinicopathologic study of small hepatocellular carcinoma with microscopic satellite nodules to determine the extent of tumor ablation by local therapy. Int J Oncol 2007;31:485–491.

74 Livraghi T, Meloni F, Di Stasi M, et al: Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? Hepatology 2008;47:82–89.

Oncology

Oncology 2010;78(suppl 1):87–93
DOI: 10.1159/000315235

Published online: July 8, 2010

Will Gd-EOB-MRI Change the Diagnostic Algorithm in Hepatocellular Carcinoma?

Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Key Words
Gd-EOB-DTPA · Early hepatocellular carcinoma · Dysplastic nodule · Liver tumor · Hepatocellular carcinoma

Abstract
A hepatocyte-specific contrast agent, gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA), was approved in Japan in 2008. This contrast agent enhances the blood pool and also is hepatocyte specific: it is taken up by hepatocytes and excreted into the biliary tract. Approximately 50% of the administered dose of Gd-EOB-DTPA is taken up by normal hepatocytes and subsequently excreted into the biliary tract, while the remaining 50% is excreted via the kidney. Hepatocellular uptake is considered to represent passive diffusion mediated by organic anion transporter polypeptide 1 (OATP1), which is expressed on the hepatocyte membrane. Gd-EOB-DTPA-enhanced MRI may offer a breakthrough for the diagnosis of liver tumors, particularly early hepatocellular carcinoma (HCC). The differentiation of dysplastic nodules from early HCC has remained difficult, even for pathologists specialized in liver tumors, but Gd-EOB-DTPA MRI facilitates an objective diagnosis with accuracy close to that of HCC-specialized pathologists. In conclusion, Gd-EOB-DTPA MRI facilitates the diagnosis of hypervascular advanced HCC and the differentiation of early HCC and dysplastic nodules, which used to be difficult, even with CT during arterial portography, and offers a high accuracy rate. Thus, Gd-EOB-DTPA MRI will have a significant impact on diagnostic algorithm for HCC.

Introduction

A hepatocyte-specific contrast agent, gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) (Primovist®), was approved in Japan in January 2008. This contrast agent is hepatocyte specific and enhances the blood pool: it is taken up by hepatocytes and excreted into the biliary tract. Compared with another liver-specific contrast agent, superparamagnetic iron oxide (SPIO; Resovist®), the liver parenchyma is strongly stained white in the hepatocyte phase on T_1-weighted images, 20 min after the intravenous injection of Gd-EOB-DTPA. Nodules lacking normal hepatocytes, such as hepatocellular carcinoma (HCC), are depicted as low-intensity masses. These effects occur in addition to its ability for hemodynamic evaluation. Thus, Gd-EOB-DTPA may be referred to as a white liver agent. In contrast, SPIO stains the entire liver black (black liver) on T_2-weighted images, which are inferior to T_1-weighted images in terms of spatial resolution. Thus, Gd-EOB-DTPA contrast imaging offers a diagnostic tool that is

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0087$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3149, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

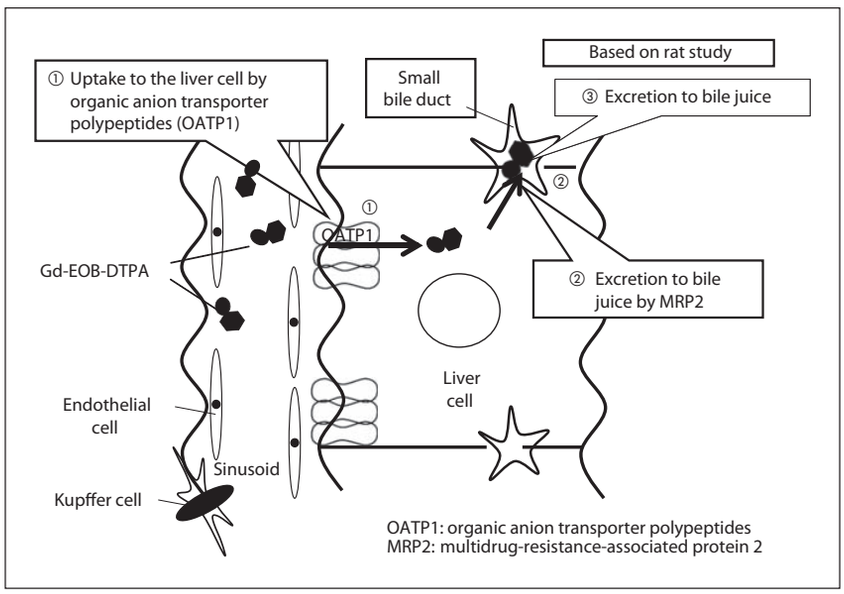


Fig. 1. Pharmacokinetics of Gd-EOB-DTPA.

simple to interpret for hepatologists and MRI-specialized radiologists, and is a breakthrough diagnostic tool in liver imaging.

Imaging Findings in HCC according to Pathological Differentiation Grade

Approximately 50% of the administered Gd-EOB-DTPA is taken up by normal hepatocytes and subsequently excreted into the biliary tract, while the remaining 50% is excreted via the kidney. Hepatocellular uptake is considered to represent passive diffusion mediated by organic anion transporter polypeptide 1 (OATP1), which is expressed on the hepatocyte membrane [1], ATP-dependent active transport by the canalicular membrane organic anion transporter (cMOAT) or multidrug resistance-associated protein 2 (MRP2) is considered to be responsible for the excretion from hepatocytes into bile ducts [2] (fig. 1). The dependence of Gd-EOB-DTPA uptake on OATP1B3 in human liver was recently reported [3], as OATP1B3 was expressed in cases with positive Gd-EOB-DTPA uptake in the hepatocyte phase during Gd-EOB-MRI, even in well- to moderately differentiated HCC. However, OATP1B3 expression was not associated with bile production ability (green hepatoma) or the pathological differentiation grade [3]. Based on these findings, the mechanism shown in table 1 is also considered to be one explanation for the relationship of expression of OATP1B3 and Gd-EOB-MRI findings. The expression of the uptake transporter OATP1B3 and the excretory transporter MRP2 remains normal in dysplastic nodules; therefore, dysplastic nodules are not shown as low-intensity lesions on the hepatocyte phase of Gd-EOB-MRI.

Table 1. Relationship between the expression of OATP1B3/MRP2 and findings in the hepatocyte phase

	Uptake transporter (OATP 1B3)	Excretory transporter (MRP2)	Hepatocyte phase imaging of EOB-MRI
Dysplastic nodule	+	+	Iso- to high intensity
	– (rare?)	+	Low intensity
Early HCC	+ (rare?)	+	Isointensity
	–	+	Low intensity
Well- to mod. diff. HCC	+ (5–10%)	+	Iso- to high intensity
		–	Iso- to high intensity
	– (90–95%)	–	Low intensity
Poorly diff. HCC	–	–	Low intensity

OATP1 = Organic anion transporter polypeptides; MRP2 = multidrug-resistance-associated protein 2.

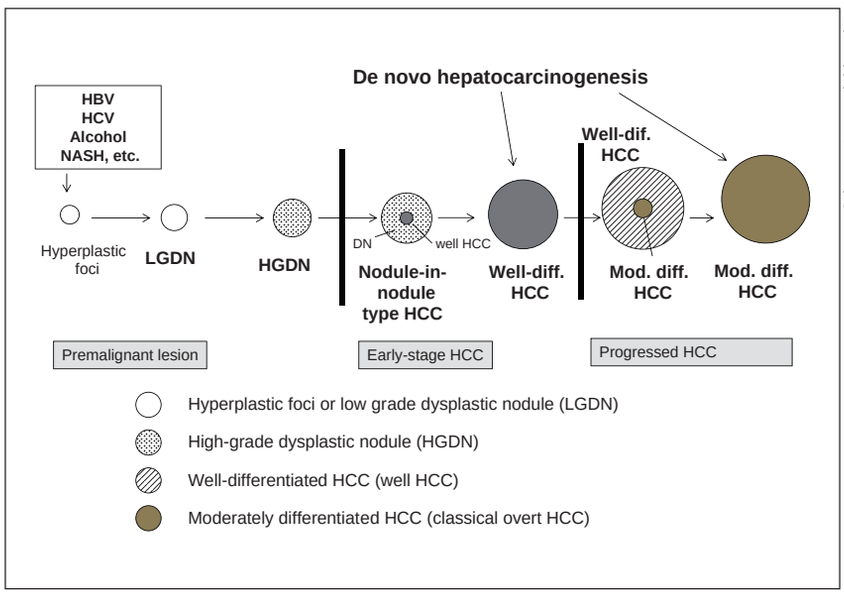


Fig. 2. Schematic representation of multistep progression of human hepatocarcinogenesis.

Table 2. Accuracy of the differentiation of early HCC and premalignant lesions by hepatocyte-phase Gd-EOB-DTPA MRI for hypovascular hepatocytic nodules [cited from 10]

Only resected specimens (n = 30)	Pathological findings	
	eHCC	DN or RN
Signal intensity in hepatobiliary phase with Primovist		
Low to slightly low (n = 24)	23	1
Iso to high (n = 6)	1	5

Accuracy: 93% (23+5/30). eHCC = Early hepatocellular carcinoma; DN = dysplastic nodule; RN = regenerative nodule.

Gd-EOB-MRI Findings in Dysplastic Nodules and HCC according to Pathological Differentiation Grade

HCC develops in a multistep fashion from dysplastic nodules and early-stage well-differentiated HCC (early HCC) (fig. 2). In early HCC with pathological HCC stromal invasion [4–9], OATP1B3 expression decreases, and EOB uptake is decreased, visualized as an area of low intensity in the hepatocyte phase of Gd-EOB-MRI. However, even in some early HCCs, the expression of OATP1B3 may be reduced or lost, similar to that of moderately to poorly differentiated HCC; therefore, these early HCCs may show hypointensity in images on the hepatocyte phase of Gd-EOB-MRI.

By contrast, the signal intensity is not reduced in the hepatocyte phase Gd-EOB-MRI in about 5% of cases of well- to moderately differentiated hypervascular HCC. These were reported to be so-called 'green hepatomas', suggesting that OATP1B3 is even expression in some cases of well- to moderately differentiated HCCs. In cases expressing OATP1B3, but with decreased MPR2 expression, Gd-EOB-DTPA may be retained in bile and hepatocytes, which may mimic 'green hepatoma'. In cases showing normal MRP2 expression, because Gd-EOB-DTPA is excreted in a similar fashion from the HCC cells, similarly to that from normal hepatocytes, equivalent signal intensity is shown. Based on these assumptions, early HCC cases with normal OATP1B3 expression may also be present at each stage of human hepatocarcinogenesis from a dysplastic nodule to early HCC and well- to moderately differentiated HCC (fig. 2) (table 2) [10].

In every-day clinical practice, some nodules detected by ultrasonography were diagnosed as well-differentiated HCC based on the histopathological diagnosis of biopsied specimens, even though the signal intensity was not reduced in the hepatocyte phase of Gd-EOB-MRI. Thus, some exceptional well-differentiated HCCs may express OATP1B3 on the hepatocyte membrane.

Another problem that should be clarified is whether all dysplastic nodules do not show low signal intensity in

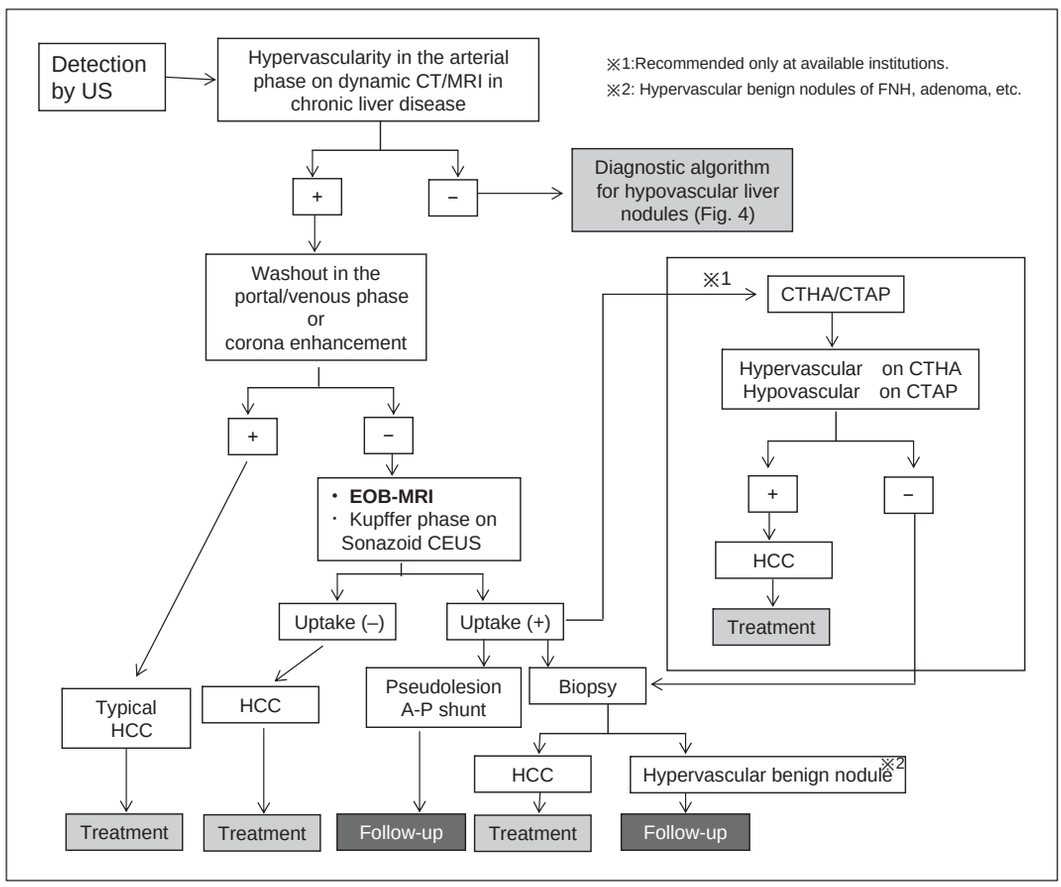


Fig. 3. Diagnostic and treatment algorithms for hypervascular liver nodules proposed by the Japan Society of Hepatology, revised in 2010.

the hepatocyte phase of Gd-EOB-MRI. Because dysplastic nodules are usually diagnosed based on the pathology of biopsied samples, there is a possibility that such nodules exist. However, in a study performed by Sano and Ichikawa's group [11] in which only resected specimens, not biopsied specimens, were investigated, all of the dysplastic nodules showed isointensity in the hepatocyte phase. Thus, OATP1B3 expression may rarely be reduced in dysplastic nodules, as determined pathologically in resected specimens by liver-specialized pathologists, as reported by the authors of a recently published consensus paper on early HCC [8]. In our experience, we have rarely encountered dysplastic nodules showing low intensity on Gd-EOB-DTPA hepatocyte phase in resected cases [10]. Pathological diagnosis by biopsy sample alone is often limited and may underestimate the pathology because it is difficult to diagnose biopsied specimens as early HCC, even by liver-specialized pathologists, because of possible sampling error problems, limitations of biopsy, or the lack of evidence for stromal invasion, even if atypical cells and atypical structures mimicking early HCC are noted [4–9]. Therefore, comparisons of pathological findings of resected specimens and Gd-EOB-DTPA MRI findings are essential.

Differentiation of Early HCC from Dysplastic Nodules by Gd-EOB-MRI

As described above, early HCC is hypovascular on dynamic imaging in most cases. Its accurate diagnosis has remained difficult even with CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP). Although slightly reduced portal blood flow on

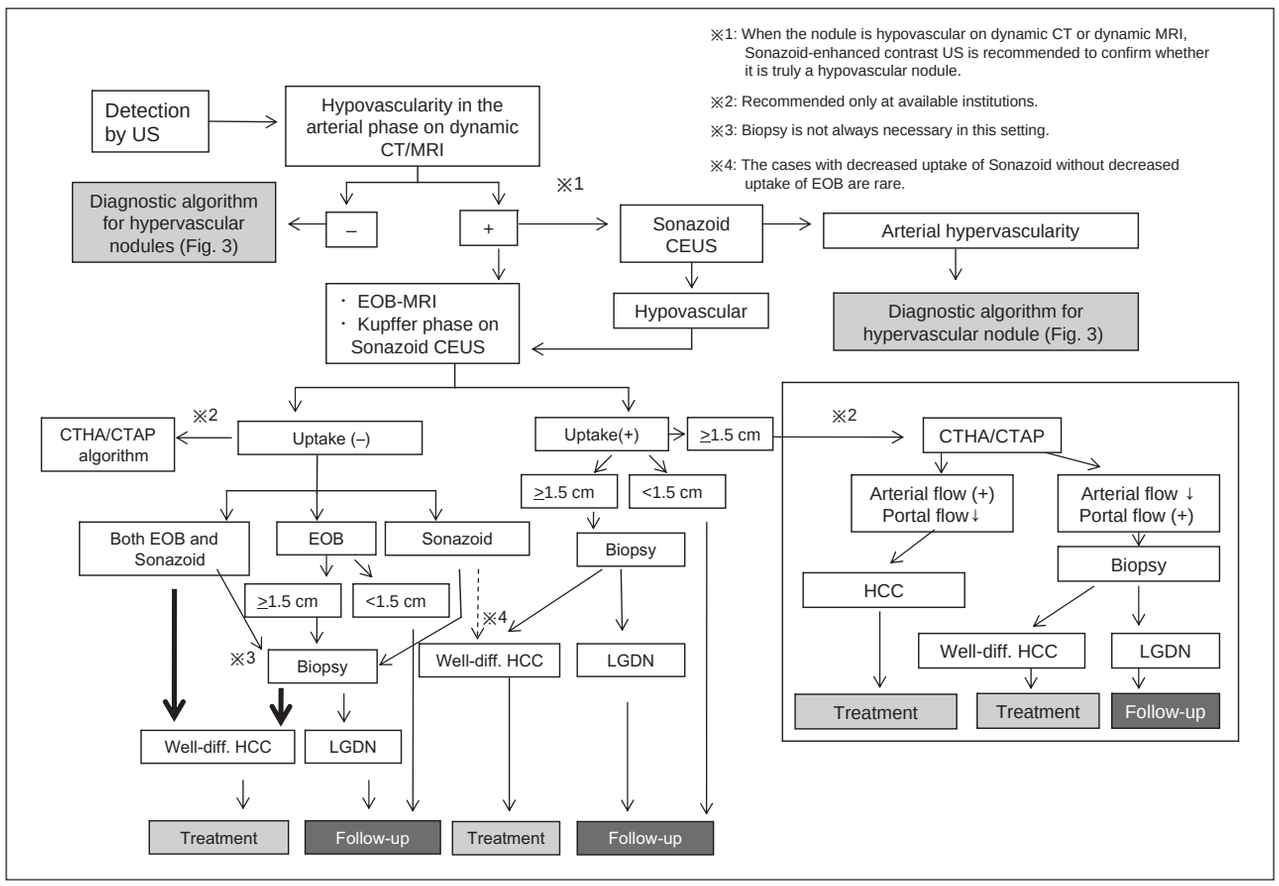


Fig. 4. Diagnostic and treatment algorithms for hypovascular liver nodules proposed by the Japan Society of Hepatology, revised in 2010.

CTAP is suggestive of early HCC, some cases show isoperfusion. The pathological diagnosis of resected specimens by liver-specialized pathologists was early HCC in most cases showing hypointensity in the hepatocyte phase of Gd-EOB-MRI, and that of most cases showing isointensity or high intensity on the hepatocyte phase was dysplastic nodules (table 2). This indicates that the functional diagnosis using Gd-EOB-DTPA MRI is more sensitive for the detection of early changes during hepatocarcinogenesis than hemodynamic imaging [12–16], SPIO-MRI [17, 18], Kupffer phase imaging of Sonazoid-enhanced ultrasound [19, 20] and evaluation of portal blood flow on CTAP, which was previously the most sensitive technique for the differentiation of early HCC and dysplastic nodules.

There are two problems remaining: (1) whether some dysplastic nodules showing low intensity in the hepatocyte phase of Gd-EOB-MRI are actually present, and (2) how frequently the nodules showing isointensity or high intensity in the hepatocyte phase of Gd-EOB-MRI include those pathologically diagnosed as early HCC. These two points should be urgently clarified by collecting cases worldwide. A hypovascular nodule showing positive uptake on SPIO-MRI and in the Kupffer phase of contrast-enhanced ultrasonography with Sonazoid can be diagnosed as a hepatocellular nodule (i.e. nodules of hepatocyte origin). It is essential to obtain biopsy specimens of such nodules showing hypointensity on the hepatocyte phase of Gd-EOB-MRI, and to compare the findings with those from follow-up studies to assess their natural courses. This approach will allow us to grade the malignant potential of nodules showing low intensity on the hepatocyte phase of Gd-EOB-MRI. This study is now being conducted by the Japan Liver Oncology Group (JLOG 0902 trial).

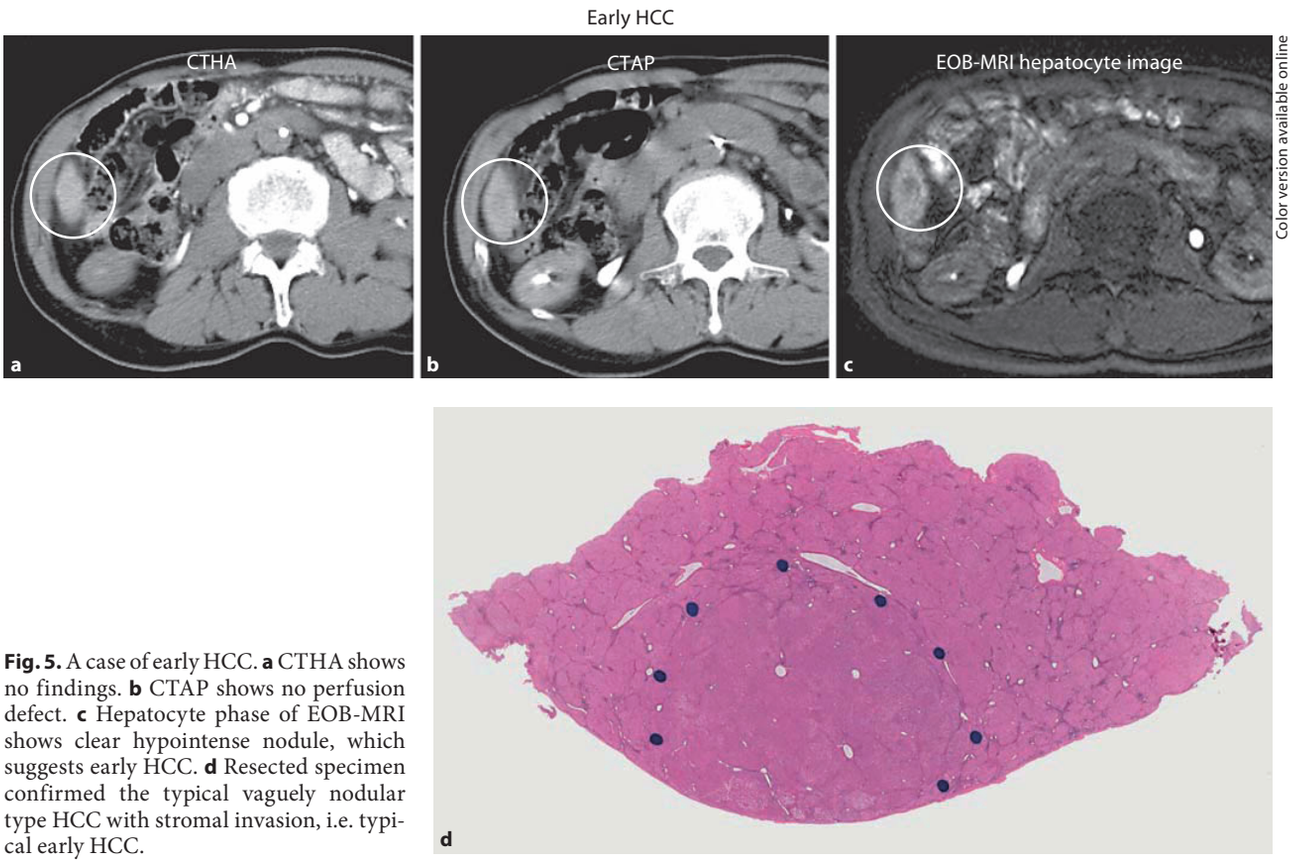


Fig. 5. A case of early HCC. **a** CTHA shows no findings. **b** CTAP shows no perfusion defect. **c** Hepatocyte phase of EOB-MRI shows clear hypointense nodule, which suggests early HCC. **d** Resected specimen confirmed the typical vaguely nodular type HCC with stromal invasion, i.e. typical early HCC.

Impact of Gd-EOB-DTPA on the Diagnostic Algorithm for Hypovascular Hepatocellular Nodules

The 2010 revisions of the diagnostic algorithms for hypovascular HCC, as presented in the consensus-based clinical practice manual edited by the Japan Society of Hepatology in 2007 [21], are shown in figures 3 and 4. For the hypervascular nodules diagnosed by dynamic CT or MRI in which no washout is observed, SPIO-MRI and Sonazoid-enhanced US was recommended as the next imaging tool in the 2007 version; however, SPIO-MRI has been almost completely replaced by the more specific and sensitive technique, Gd-EOB-MRI. CTHA and CTAP can also be omitted from the diagnostic procedure in routine clinical settings. These techniques can also be performed when deemed necessary. Similarly, SPIO-MRI and Kupffer phase imaging of Sonazoid-enhanced US were recommended for hypovascular nodules detected by dynamic CT or MRI in the first version. However, similar to that for hypervascular nodules, SPIO-MRI has been completely replaced by Gd-EOB-MRI. CTHA and CTAP can also be omitted in the routine clinical setting. However, MDCT should not be omitted because of the limited availability of MRI instruments compared with MDCT in Japan. However, the value of Gd-EOB-MRI as an examination tool will undoubtedly become more important in the future [10, 22].

As proposed by Sano and Ichikawa's group [11], to investigate the malignant potential of HCC nodules in high-risk patients, Gd-EOP-DTPA MRI should be performed at least once a year to determine the presence of hypointensity nodules in the hepatocyte phase in cases with cirrhotic liver. Furthermore, as described above, it is important to obtain biopsy samples in such nodules showing low intensity and compare them with the natural course to establish a therapeutic strategy.

It is possible to diagnose early HCC by Gd-EOP-DTPA MRI with an accuracy close to that of pathologists specialized in evaluating early HCC (fig. 5). However, whether such cases should be clinically treated at that point is unresolved. This should be clarified by comparing the pathological findings with their natural courses.

Conclusion

Gd-EOB-MRI offers a breakthrough technique for the diagnosis of liver tumors, particularly early HCC. The differentiation of dysplastic nodules from early HCC is difficult, even for pathologists specialized in liver tumors. However, Gd-EOB-MRI is an objective diagnosis method that offers accurate diagnosis similar to that of pathologists specialized in HCC. As described above, there are several problems that need to be resolved: (1) How frequently does early HCC possess normal expression levels of OATP1B3 and show isointensity or high intensity on the hepatocyte phase of Gd-EOB-MRI, and (2) whether the intensity of dysplastic nodules is not reduced. These problems should be clarified in studies of resected liver specimens in which the precise pathological examination is possible. Moreover, whether transporters other than OATP1B3 and MRP2 are responsible for Gd-EOB-DTPA uptake and excretion, and whether the expression level of MRP2 at each differentiation stage affects the image findings of Gd-EOB-MRI, remains to be solved.

In conclusion, Gd-EOB-DTPA MRI facilitates the diagnosis of hypervascular advanced HCC, and the differentiation of early HCC and dysplastic nodules with a high accuracy rate [10], which was difficult, even with CTAP. In addition, because Gd-EOB-DTPA MRI, which has been a breakthrough in hepatic tumor imaging over the last two decades, will play a major role in the staging of primary and metastatic liver cancer and the diagnosis of benign nodules, it will provide a significant impact on diagnostic imaging of the liver tumors, especially of HCCs.

Disclosure Statement

The author declares that he has no financial conflict of interest.

References

1 Van Montfoort JE, Stieger B, Meijer DK, et al: Hepatic uptake of the magnetic resonance imaging contrast agent gadoxetate by the organic anion transporting polypeptide OATP1. J Pharmacol Exp Ther 1999;290:153–157.

2 Pascolo L, Petrovic S, Cupelli F, et al: ABC protein transport of MRI contrast agents in canalicular rat liver plasma vesicles and yeast vacuoles. Biochem Biophys Res Commun 2001;282:60–66.

3 Narita M, Hatano E, Arizono S, et al: Expression of OATP1B3 determines uptake of Gb-EOB-DTPA in hepatocellular carcinoma. J Gastroenterol 2009;44:793–798.

4 Kojiro M, Nakashima O: Histopathologic evaluation of hepatocellular carcinoma with a special reference to small early stage tumor. Semin Liver Dis 1999;19:287–296.

5 Kojiro M: Diagnostic discrepancy of early hepatocellular carcinoma between Japan and West. Hepatol Res 2007;37:S249–S252.

6 Nakano M, Saito A, Yamamoto M, et al: Stromal invasion and blood vessel wall invasion in well-differentiated hepatocellular carcinoma. Liver 1997;17:41–46.

7 Park YN, Kojiro M, Di Tommaso L, et al: Ductular reaction is helpful in defining early stromal invasion, small hepatocellular carcinomas, and dysplastic nodules. Cancer 2007;109:915–923.

8 International Consensus Group for Hepatocellular Neoplasia: Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology 2009;49:658–664.

9 Desmet VJ: East-West pathology agreement on precancerous liver lesions and early hepatocellular carcinoma. Hepatology 2009;49:355–357.

10 Kudo M: The 2008 Okuda Lecture: Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. J Gastroenterol Hepatol 2010;25:439–452.

11 Sano K, Ichikawa T, Matsuda M, et al: Usefulness of Gd-EOB-DTPA-enhanced MRI for detecting early hepatocellular carcinoma (in Japanese). Hepatobiliary Pancreat Imaging 2009;11:513–517.

12 Matsui O, Kadoya M, Kameyama T, et al: Benign and malignant nodules in cirrhotic livers: distinction based on blood supply. Radiology 1991;178:493–497.

13 Hayashi M, Matsui O, Ueda K, et al: Correlation between the blood supply and grade of malignancy of hepatocellular nodules associated with liver cirrhosis: evaluation by CT during intraarterial injection of contrast medium. AJR 1999;72:969–976.

14 Ueda K, Matsui O, Kawamori Y, et al: Hypervascular hepatocellular carcinoma: evaluation of hemodynamics with dynamic CT during hepatic arteriography. Radiology 1998;206:161–166.

15 Kudo M: Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. Semin Liver Dis 1999;19:297–309.

16 Kudo M: Multistep human hepatocarcinogenesis: correlation of imaging with pathology. J Gastroenterol 2009;44:112–118.

17 Kim YK, Kwak HS, Kim CS, et al: Hepatocellular carcinoma in patients with chronic liver disease: comparison of SPIO-enhanced MR imaging and 16-detector row CT. Radiology 2006;238:531–541.

18 Imai Y, Murakami T, Yoshida S, et al: Superparamagnetic iron oxide-enhanced magnetic resonance images of hepatocellular carcinoma: correlation with histological grading. Hepatology 2000;32:205–212.

19 Kudo M, Hatanaka K, Maekawa K: Sonazoid-enhanced ultrasound in the diagnosis and treatment of hepatic tumors. J Med Ultrasound 2008;16:130–139.

20 Kudo M, Hatanaka K, Maekawa K: Defect reperfusion imaging, a newly developed novel technology using Sonazoid in the treatment of hepatocellular carcinoma. J Med Ultrasound 2008;16:169–176.

21 Kudo M, Okanoue T; Clinical Practice Manual of HCC Expert Panel: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology (JSH). Oncology 2007;72(suppl):2–15.

22 Kudo M: Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. Oncology 2008;75(suppl):1–12.

Oncology

Oncology 2010;78(suppl 1):94–101
DOI: 10.1159/000315236

Published online: July 8, 2010

Radiofrequency Ablation for Hepatocellular Carcinoma: Assistant Techniques for Difficult Cases

Tatsuo Inoue[a] Yasunori Minami[a] Hobyung Chung[a] Sousuke Hayaishi[b] Taisuke Ueda[b] Chie Tatsumi[b] Masahiro Takita[b] Satoshi Kitai[a] Kinuyo Hatanaka[a] Emi Ishikawa[b] Norihisa Yada[a] Satoru Hagiwara[a] Kazuomi Ueshima[b] Masatoshi Kudo[a]

Departments of [a]Gastroenterology and Hepatology, and [b]Internal Medicine, Kinki University School of Medicine, Osaka, Japan

Key Words
Radiofrequency ablation · Pleural effusion · Endoscopic nasobiliary drainage

Abstract
Purpose: To confirm the safety and effectiveness of techniques to assist radiofrequency ablation (RFA) for difficult cases, we retrospectively evaluated successful treatment rates, early complications and local tumor progressions. ***Patients and Methods:*** Between June 1999 and April 2009, a total of 341 patients with 535 nodules were treated as difficult cases. Artificial pleural effusion assisted ablation was performed on 64 patients with 82 nodules. Artificial ascites-assisted ablation was performed on 11 patients with 13 nodules. Cooling by endoscopic nasobiliary drainage (ENBD) tube-assisted ablation was performed on 6 patients with 8 nodules. When the tumors were not well visualized with conventional B-mode ultrasonography (US), contrast-enhanced US-assisted ablation with Levovist® or Sonazoid® or virtual CT sonography-assisted ablation was performed. Contrast-enhanced US-assisted ablation was performed on 139 patients with 224 nodules and virtual CT sonography-assisted ablation was performed on 121 patients with 209 nodules. ***Results:*** In total, complete ablation was achieved in 514 of 535 (96%) nodules in difficult cases. For RFA with artificial pleural effusion, artificial ascites and ENBD, complete response was confirmed in all cases. For contrast-enhanced US- and CT sonography-assisted ablation, complete response was 95%. Early complications were recognized in 24 cases (4.5%). All cases recovered with no invasive treatment. Local tumor recurrence was investigated in 377 nodules of 245 patients, and 69 (18%) nodules were positive. Tumor recurrences in each assisted technique were 14.7% in artificial pleural effusion cases, 7% in artificial ascites, 12.5% in ENBD tube cases, 31% in virtual CT sonography, and 8.5% in contrast-enhanced US. ***Conclusion:*** Although local tumor progression needs to be carefully monitored, assisted techniques of RFA for difficult cases are well tolerated and expand the indications of RFA.

Introduction

Hepatocellular carcinoma (HCC) is a common disease worldwide. In patients with HCC, radiofrequency ablation (RFA) has recently been established as a promising

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0094$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

and safe percutaneous technique [1–3]. RFA has steadily become first-line ablative management of small- to intermediate-sized (3 cm) HCC at many centers. It has a primary effectiveness rate of 88–99% in the management of HCC [4–8].

However, there are some limitations to RFA. The first limitation is the location of HCC. When the HCC is located directly under the diaphragm, peripheral lesions that abut organs such as the large bowel or stomach and are located no more than 5 mm from a major bile duct, RFA can damage these organs.

Resection is recommended in patients who have HCCs in such locations, if they have good liver function. However, in patients in whom liver function is poor and resection is not recommended, so far there is no curative therapy.

A second problem is that multiple sessions of RFA therapy are often needed to manage HCCs poorly defined by B-mode ultrasonography (US) [9]. Tumors located near lesions as mentioned above or tumors that are poorly defined by B-mode US are considered too difficult to treat by RFA. Thus, these cases possibly result in complications or do not allow sufficient treatment.

We performed RFA with assistant techniques for difficult cases, for example, artificial pleural effusion, artificial ascites, contrast-enhanced US with Levovist® (Schering, Berlin, Germany) or Sonazoid® (Daiichi-Sankyo, Tokyo, Japan; GE Healthcare, Milwaukee, Wisc., USA), virtual CT sonography, and cooling by endoscopic nasobiliary drainage (ENBD) tube to resolve the difficult status.

To confirm the safety and effectiveness of these assistant techniques, we retrospectively evaluated successful treatment rates, early complications and local tumor recurrence as indicators of insufficient treatment from the perspective of tumor location and status in HCC patients treated with RFA.

Patients and Methods

Between June 1999 and April 2009, 1,105 (724 primary and 381 for recurrent) HCC patients were treated using percutaneous RFA at the Department of Gastroenterology and Hepatology, Kinki University School of Medicine. Curative RFA was intended in 680 (94%) of 724 patients, and a total of 1,259 nodules were completely ablated. In 44 patients, RF ablation was intended for a total of 160 nodules to reduce tumor burden, with some nodules being left unablated in each patient because of their multiplicity. 535 nodules were in a high-risk location as defined above, and 341 patients (41%) had at least one nodule in a high-risk location (table 1). All patients met the following criteria for treatment with percutaneous RFA: percutaneous accessibility of the tumor; absence of portal venous and extrahepatic metastasis; presence of liver cirrhosis (Child-Pugh A or B); prothrombin time ratio >50%; total bilirubin concentration <3.0 mg/dl, and platelet count >50,000/μl. Patients were excluded if they had an excessive bleeding tendency (platelet count <50,000/μl or prothrombin activity <50%), or refractory ascites. The study was performed according to the guidelines of the Helsinki Declaration. Written, fully informed consent was obtained from each patient before treatment.

Table 1. Characteristics of the 341 patients with 535 nodules

Variables	
Age, years	69.4 ± 9.6
Sex, M/F	237/104
HBsAg (+)	40
HCV Ab (+)	256
Non-B/non-C	45
Size of tumor, cm	1.7 ± 1.09
Child-Pugh classification	
A	231
B	110
C	0
Number of nodules	
1	189
2	99
3	37
4	16
Curative cases	
Yes	514 (96%)
No	21 (4%)
Follow-up, days	617 (23–2,894)

Diagnosis of HCC

The diagnosis of HCC was made by the presence of typical tumor features, using dynamic CT or MRI, such as hyperattenuations in the arterial phase and hypoattenuations in the portal phase [10, 11]. All patients underwent dynamic CT or MRI 1 month before RFA.

Definition of Difficult Cases for RFA

On the basis of previous literature, we defined locations adjacent to large vessels (i.e. <5 mm from a first or second branch of the portal vein, the base of the hepatic veins and the inferior vena cava) or extrahepatic organs (i.e. lung, gallbladder, right kidney and gastrointestinal tract) as difficult cases. We also defined tumors poorly defined on B-mode US as difficult cases.

Ablation-Assisted Technique in Difficult Cases

When the targeted nodule was close to the diaphragm, we used an artificial pleural effusion method with 5% glucose to separate from the lung [12]; when it was close to the gastrointestinal tract, we infused 5% glucose as artificial ascites into the abdominal cavity to separate from the gastrointestinal tract to pre-

vent thermal injury; when the tumor edge was located no more than 5 mm from a major bile duct, as revealed by CT or US, ENBD tube was performed to cool and reduce the rates of biliary complications; when the tumors were not well visualized using conventional B-mode US, we performed contrast-enhanced US-guided ablation with Levovist® or Sonazoid® or virtual CT sonography [13].

Equipment and Techniques

We used GE LOGIQ 700 EXPERT Series, LOGIQ 7 (General Electric Medical Systems, Milwaukee, Wisc., USA) and Aplio (Toshiba Medical Systems, Toshiba, Tokyo, Japan) when we performed RFA with artificial pleural effusion, artificial ascites and contrast-enhanced US with Levovist and Sonazoid. EUB 8500 (Hitachi Medico) was used as the virtual CT sonography system with magnetic navigation.

When the target nodule was located close to the bile duct, an ENBD tube was inserted deeply into the peripheral branch of the target bile duct before RFA.

RFA Technique and Equipment

All RFAs were performed percutaneously by experienced hepatologists, each of whom had more than 6 years of experience in US-guided interventional procedures and RFA. The treatments were performed under local anesthesia and conscious sedation. Conscious sedation was induced with 5–20 mg of diazepam. Local infiltration anesthesia was induced with 5–15 ml of 1% lidocaine (Liduokayin, Yimin). The patients were conscious when the electrode was placed. Vital signs, including blood pressure, heart rate, and oxygen saturation, were continuously monitored during the procedure. Patients were treated with a cooled-tip needle RFA system (Cool-tip, Covidien), which is a 480-kHz alternative current generator that can produce a maximum power of 180 W through a 17-gauge monopolar cooled-tip needle electrode. A thermocouple embedded in the electrode ensures that the temperature at the tip of the needle is constantly monitored. The radiofrequency electrode temperature was maintained at <18°C by application of circulating chilled (0°C) saline solution to the cannula sheath [14].

We selected a single 2-cm exposed tip for nodules of <2 cm in diameter and a single 3-cm exposed tip for larger nodules.

Assessing the Effect of RFA and Follow-Up

We assessed treatment response, early complications and local tumor recurrence rates. Treatment response was assessed by dynamic CT or MRI 1–5 days after the end of treatment. Complete response was confirmed by the absence of enhanced areas. Early complication was defined as that occurring within 30 days after RFA and requiring additional invasive therapy and/or lengthened hospitalization of >1 week. Dynamic CT and US were performed every 3–4 months after RFA. Local tumor recurrence was defined as the appearance of viable tumor during follow-up that was contiguous with the zone that had been considered completely ablated.

Statistical Analysis

Cumulative incidence of local tumor recurrence was calculated using the Kaplan-Meier technique. All analyses were performed with statistics software (SPSS, version 11, SPSS) for Microsoft Windows.

Results

Successful Treatment Rates

Complete ablation was achieved in 514 of 535 (96%) nodules in difficult cases.

Complete response was confirmed in all cases in whom RFA was performed with artificial pleural effusion (fig. 1), artificial ascites (fig. 2) and cooling by ENBD tube (fig. 3). We obtained complete responses in 213 of 224 cases (95%) with contrast-enhanced US-assisted RFA (fig. 4). In 11 residual cases, one additional session of percutaneous ethanol injection (PEI) was performed for 4 nodules because the residual tumor areas were closely located adjacent to large vessels. Transcatheter arterial chemoembolization (TACE) was performed for the remaining 7 cases because we detected other multiple nodules using dynamic CT after the first RFA session in 5 cases, and we were unable to obtain complete responses in the last 2 cases using one additional RFA session. In virtual CT sonography-assisted RFA sessions (fig. 5), we obtained complete responses in 199 of 209 cases (95%). In the remaining 10 cases, hepatectomy was performed in one because of the bleeding during the RFA session, and the other 9 cases had TACE because we could not ablate the residual tumors by a further RFA session.

Early Complications

Among the 535 nodules, complications were recognized in 25 cases (4.6%). In the artificial pleural effusion cases, 5 patients (6%) presented complications: 3 patients presented with pneumothorax and 2 patients presented with injury to the diaphragm. They recovered without treatment, and there were no further complications. There were no complications in the artificial ascites cases. In the ENBD tube cases, 4 patients (67%) presented complications: 2 patients with hepatic infarction and 2 patients with dilatation of the bile duct. They recovered with antimicrobial therapy. In the contrast-enhanced US cases, 9 patients (4%) presented complications: 3 patients presented with hepatic infarction, 2 with pneumothorax, 2 with injury to the diaphragm, and 2 with dilatation of the bile duct. They recovered without treatment, and there were no further complications (table 2). In the virtual CT sonography cases, 7 patients (5.8%) presented complications: 2 with dilatation of the bile ducts, 2 with injury to the diaphragm, 1 with hemothorax, 1 with hepatic infarction, and 1 with bleeding.

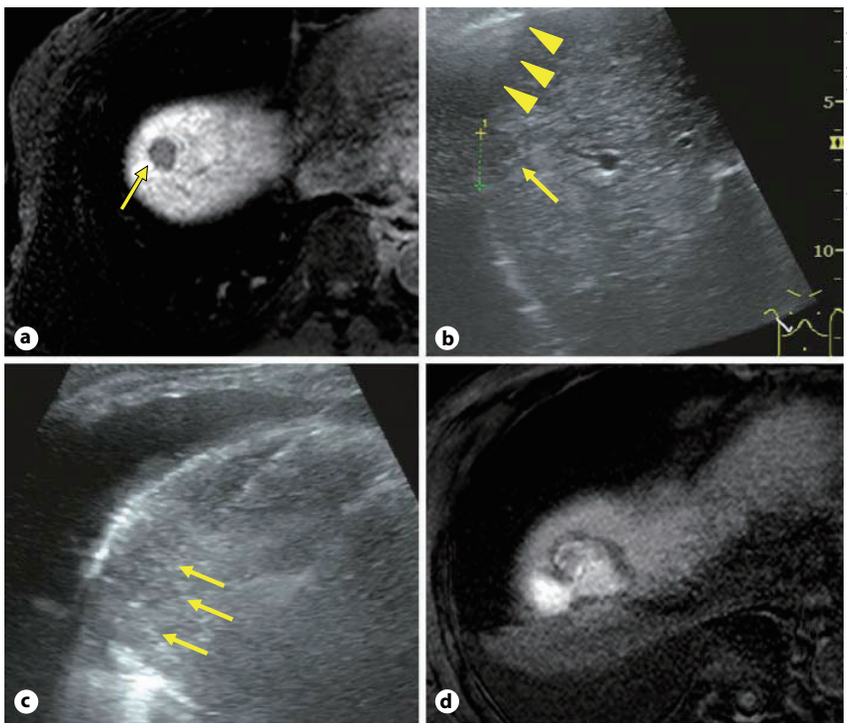


Fig. 1. Case presentation of RFA with artificial pleural effusion. **a** A 72-year-old man with 1.0-cm HCC in the right hepatic dome. Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance (MR) imaging shows tumor (arrow) as a low-intensity area. **b** On B-mode US, we could not detect the entirety of the tumor (arrow) because of the influence of lung artifacts (arrowheads). **c** After intrathoracic injection of 5% glucose solution, we could detect all parts of the tumor (arrows), located in segment VIII of the liver. **d** After the session, the nodule was completely ablated.

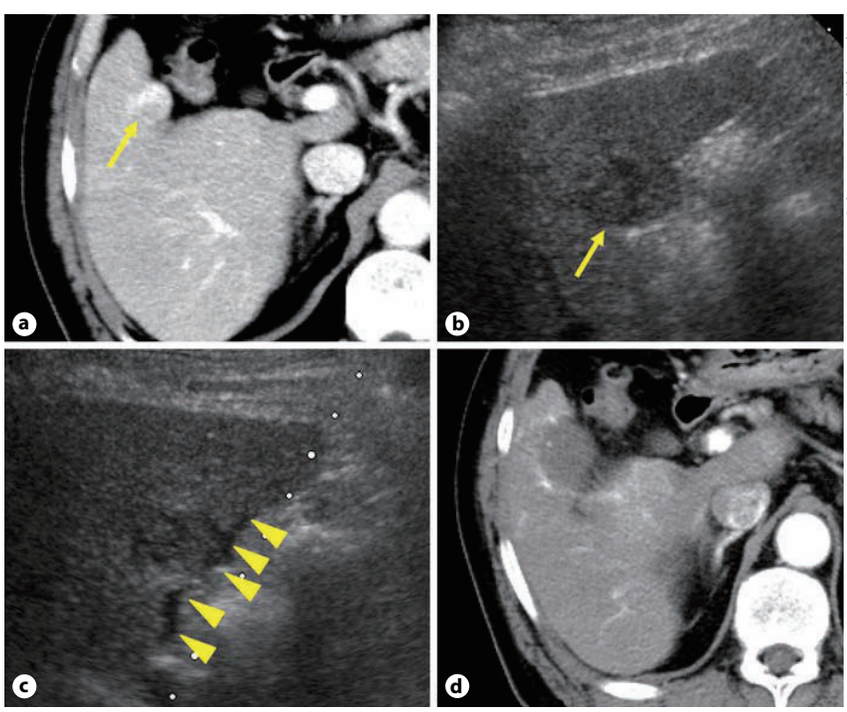


Fig. 2. Case presentation of RFA with artificial ascites. **a** A 62-year-old man with 2.0-cm HCC. Dynamic CT shows a hypervascular tumor (arrow) in segment V. **b** On B-mode US, the tumor (arrow) is seen close to the gastrointestinal tract. **c** A fluid space using 5% glucose was prepared to function as an insulator between the nodule and the gastrointestinal tract (arrowheads). **d** We achieved complete necrosis of the tumor without complications.

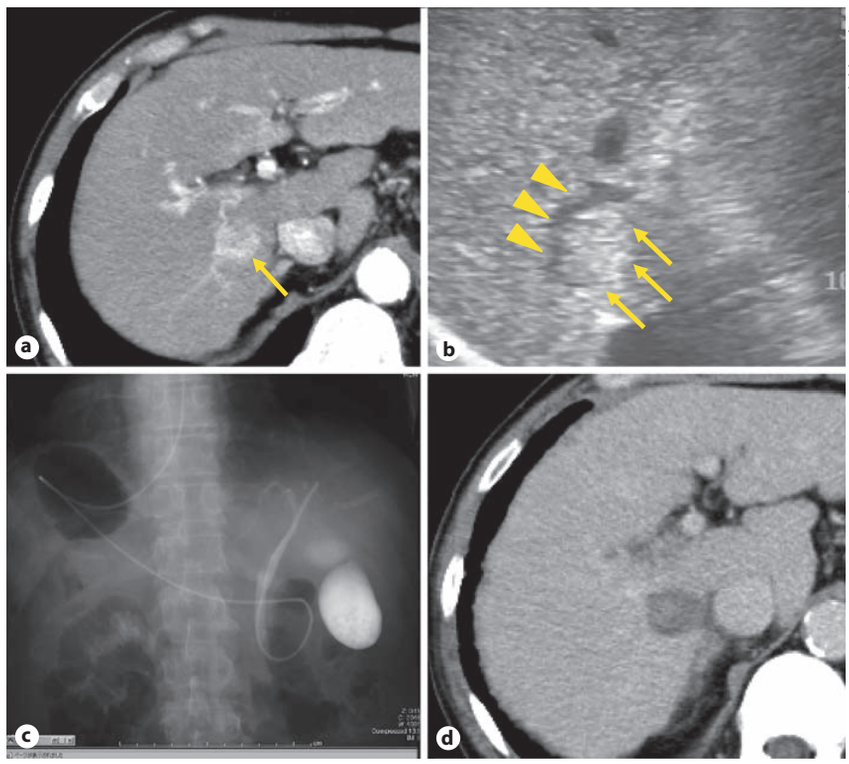


Fig. 3. Case presentation of RFA with cooling with ENBD. **a** A 70-year-old man with 2.2-cm HCC. Dynamic CT shows a hypervascular tumor (arrow) closed to the portal vein. **b** On B-mode US, the tumor (arrows) is seen close to the portal vein (arrowheads). **c** A nasobiliary drainage tube was inserted endoscopically into the right hepatic bile duct. **d** We achieved complete necrosis without biliary complications.

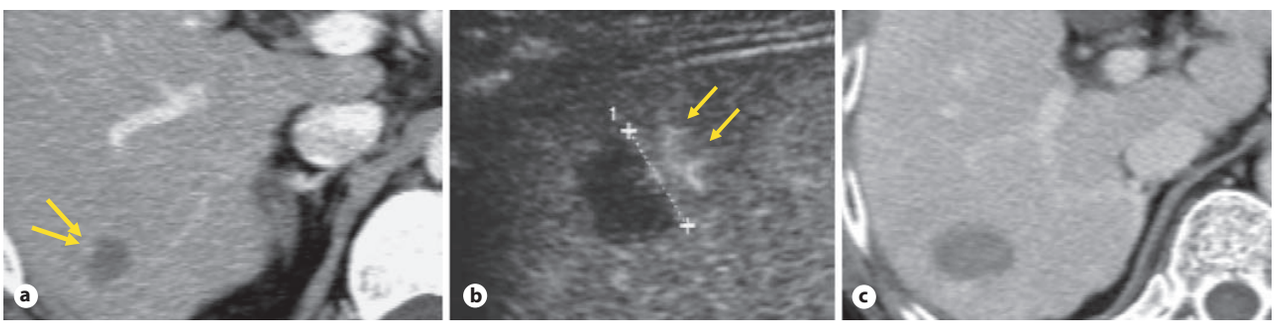


Fig. 4. Case presentation of RFA with contrast-enhanced US. **a** A 70-year-old man with recurrent HCC. Early-phase dynamic CT scan shows an outgrowth pattern of locally progressive HCC (arrows) in the right hepatic lobe. The lesion borders on an unenhanced area which was previously treated. **b** Contrast-enhanced US shows enhancement of the viable focus of the HCC (arrows). **c** After the session, the nodule was completely ablated.

Local Tumor Recurrence

Local tumor recurrence was only evaluated among the curative cases. Among the 341 patients in whom all nodules were to be ablated, 99 were lost to follow-up before the first routinely performed follow-up CT 3–4 months after RFA. Thus, local tumor recurrence was investigated in 377 nodules of 242 patients, and 69 (18%) nodules were positive. In the artificial pleural effusion cases, 9 of 61 (14.7%) nodules revealed local tumor recurrence (fig. 5); artificial ascites cases and ENBD tube cases had 1 nodule each (7 and 12.5%); virtual CT sonography cases had 45 of 144 (31%) nodules (fig. 6), and 13 of 152 (8.5%) nodules

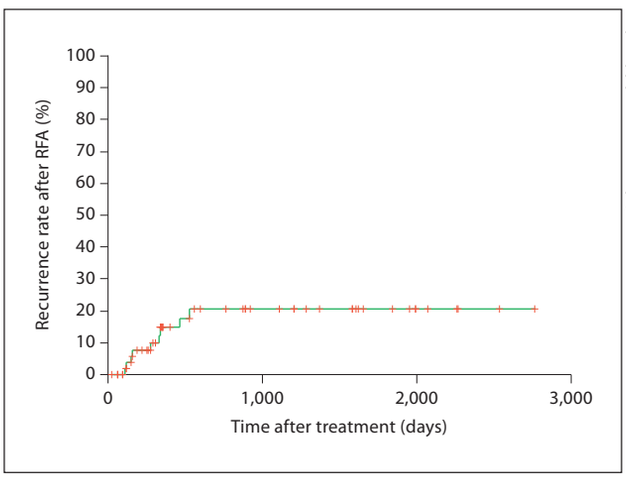


Fig. 5. Kaplan-Meier estimates of local recurrence in 61 nodules assigned to RFA with artificial pleural effusion.

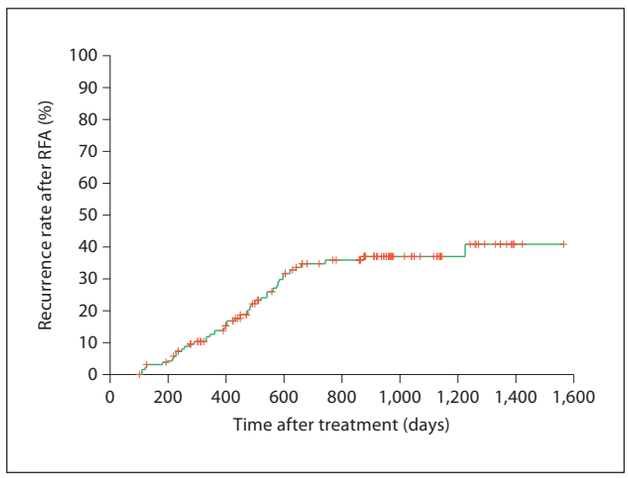


Fig. 6. Kaplan-Meier estimates of local recurrence in 144 nodules assigned to RFA with virtual CT sonography.

Table 2. Early complications and local recurrence in 341 patients

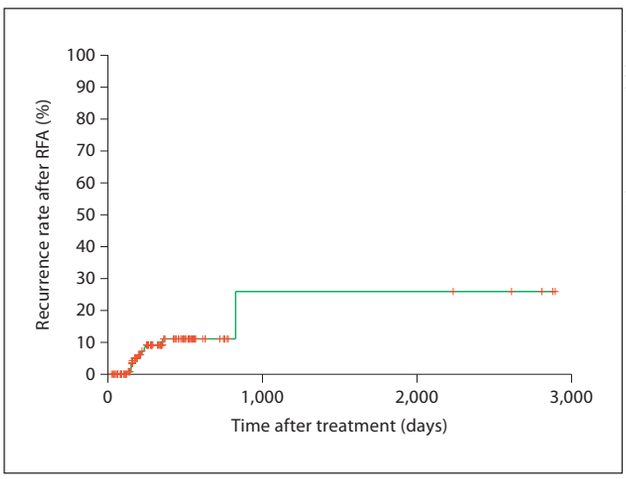
Assistant methods:	Artificial pleural effusion (64 patients with 82 nodules)	Artificial ascites (11 patients with 13 nodules)	ENBD (6 patients with 8 nodules)	CT virtual sonography (121 patients with 209 nodules)	CE-US (139 patients with 224 nodules)
Complications					
Pneumothorax	2				2
Injury of diaphragm	3			2	2
Hepatic infarction			2	1	3
Dilatation of bile duct			2	2	2
Hemothorax				1	
Bleeding				1	
Total cases	5/64 (6%)	0	4/6 (67%)	7/121 (5.8%)	9/139 (4%)

Data presented as number of cases. CE-US = Contrast-enhanced US.

(fig. 7) in contrast-enhanced US revealed local tumor recurrence. All local recurrence tumors were again treated with RFA, PEI, or TACE.

Discussion

There are some reports about the assistant methods for RFA of difficult cases. But there are no reports that mention the variety of assistant methods for RFA. In this study, complete ablation was achieved in 515 of 535 (96%) nodules in difficult cases. The treatment success rate in this study was equal to those reported by others [1, 16–20]. The overall early complication rate of our study was 4.6% [21, 22] which may be higher than those reported in other studies. We have experienced many complications especially in the ENBD tube cases (67%). Because this procedure has a potentially cooling effect on tumor cells near the cooled bile duct, it is necessary to ablate the side of the tumor located near the bile duct to reduce the re-



Color version available online

Fig. 7. Kaplan-Meier estimates of local recurrence in 152 nodules assigned to RFA with contrast-enhanced US.

sidual tumor. The effort of thorough ablation increased the total number of electrode insertions, and this may have led to an increase in complications.

Local tumor progression was 18%, which is high compared to previous reports [23, 24]. The main reason may have been the lack of adequate safety margins for the ablated area surrounding the target rumors. In the present study, we performed RFA with artificial pleural effusion or ascites for 94 nodules. In these cases located in the capsule or sub-capsule area, it is difficult to obtain a safety margin along the capsule. This is also thought for ENBD tube-assisted RFA cases because of its cooling effect as mentioned above [21]. Although RFA for difficult cases has a relatively high tumor progression rate due to the location of the tumor, we can cover and treat local tumor progression by careful follow-up.

In the present study, RFA therapy with CT sonography had the highest local tumor progression rate (31%). The main reason is that in CT sonography cases the tumor was not clearly visualized with B-mode US and the puncture area was determined by CT images. Although we easily compared the virtual CT sonographic images with B-mode US images, virtual CT sonography sometimes did not show the images coincidently with the B-mode US images, making view adjustment difficult. Imaging incompatibility might have occurred due to variation in the length of breath-holding during CT and the sonographic examinations. The difference also enhanced by an increase in distance between the magnetic sensor attached to the transducer and the magnetic generator [14]. Therefore, the needle might not reach the appropriate area, resulting in a lack of adequate safety margins and an increase in local tumor progression.

In contrast-enhanced US-assisted RFA, we first used Levovist. It has been reported [22] that contrast-enhanced harmonic US with Levovist is useful to guide percutaneous local ablation therapy for HCC poorly depicted using conventional B-mode US. However, microbubble-based contrast agents such of Levovist can easily collapse when exposed to the sonographic pulse. After the second-generation contrast medium Sonazoid was commercially used in Japan in January 2007, contrast-enhanced US has entered a new era. Sonazoid consists of shelled microbubbles and is strongly echogenic in a wide range of frequencies and acoustic pressures [25]. Furthermore, this contrast agent is stable for at least up to 3 h after injection and tolerable for multiple scannings in the low-power acoustic field [26]. When the target area of the tumor is difficult to detect at first, we can again try to observe the tumorous vascularity by re-injecting the contrast agent, while Levovist failed. Contrast harmonic US guidance with Sonazoid is an efficient approach to RFA of HCC nodules that are not clearly demarcated using B-mode US, particularly in the case of local tumor progression of HCC.

In conclusion, assistant techniques for RFA of difficult cases are well tolerated and expand the indications for RFA, especially for nodules that are poorly defined on B-mode US. However, local tumor recurrence needs to be carefully monitored.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Lin SM, Lin CJ, Lin CC, et al: Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or = 4 cm. Gastroenterology 2004;127:1714–1723.

2 Livraghi T, Goldberg SN, Lazzaroni S, et al: Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 1999;210:655–661.

3 Rossi S, Di Stasi M, Buscarini E, et al: Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. AJR Am J Roentgenol 1996;167:759–768.

4 Teratani T, Yoshida H, Shiina S, et al: Radiofrequency ablation for hepatocellular carcinoma in so-called high-risk locations. Hepatology 2006;43:1101–1108.
5 Tateishi R, Shiina S, Teratani T, et al: Percutaneous radiofrequency ablation for hepatocellular carcinoma: an analysis of 1000 cases. Cancer 2005;103:1201–1209.
6 Shiina S, Teratani T, Obi S, et al: A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005; 129:122–130.
7 Camma C, Di Marco V, Orlando A, et al: Treatment of hepatocellular carcinoma in compensated cirrhosis with radio-frequency thermal ablation (RFTA): a prospective study. J Hepatol 2005;42:535–540.
8 Lencioni RA, Allgaier HP, Cioni D, et al: Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235–240.
9 Cioni D, Lencioni R, Rossi S, et al: Radiofrequency thermal ablation of hepatocellular carcinoma: using contrast-enhanced harmonic power Doppler sonography to assess treatment outcome. AJR Am J Roentgenol 2001;177:783–788.
10 Lee HM, Lu DS, Krasny RM, et al: Hepatic lesion characterization in cirrhosis: significance of arterial hypervascularity on dual-phase helical CT. AJR Am J Roentgenol 1997; 169:125–130.
11 Mortele KJ, De Keukeleire K, Praet M, et al: Malignant focal hepatic lesions complicating underlying liver disease: dual-phase contrast-enhanced spiral CT sensitivity and specificity in orthotopic liver transplant patients. Eur Radiol 2001;11:1631–1638.
12 Koda M, Ueki M, Maeda Y, Mimura K, Okamoto K, Matsunaga Y, et al: Percutaneous sonographically guided radiofrequency ablation with artificial pleural effusion for hepatocellular carcinoma located under the diaphragm. Am J Roentgenol 2004;183:538–588.
13 Minami Y, Chung H, Kudo M, Kitai S, Takahashi S, Inoue T, Ueshima K, Shiozaki H: Radiofrequency ablation of hepatocellular carcinoma: value of virtual CT sonography with magnetic navigation. AJR Am J Roentgenol 2008;190:W335–W341.
14 Lim HK, Choi D, Lee WJ, et al: Hepatocellular carcinoma treated with percutaneous radio-frequency ablation: evaluation with follow-up multiphase helical CT. Radiology 2001;221:447–454.
15 Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS: Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. Radiology 1999;210:655–661.
16 Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Ierace T, Solbiati L, et al: Hepatocellular carcinoma: radio-frequency ablation of medium and large lesions. Radiology 2000;214: 761–768.
17 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC: Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. Gut 2005; 54:1151–1156.
18 Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, et al: A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122–130.
19 Brunello F, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, et al: Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: a randomized controlled trial. Scand J Gastroenterol 2008; 43:727–735.
20 Cho YK, Kim JK, Kim MY, Rhim H, Han JK: Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. Hepatology 2009;49:453–459.
21 Ogawa T, Kawamoto H, Kobayashi Y, Nakamura S, Miyatake H, Harada R, Tsutsumi K: Prevention of biliary complication in radiofrequency ablation for hepatocellular carcinoma – cooling effect by endoscopic nasobiliary drainage tube. Eur J Radiol 2010;73: 385–390.
22 Minami Y, Kudo M, Chung H, et al: Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. AJR Am J Roentgenol 2007;188:489–494.
23 Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M: Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. Cancer 2005;103: 1201–1209.
24 Komorizono Y, Oketani M, Sako K, Yamasaki N, Shibatou T, Maeda M, Kohara K, Shigenobu S, Ishibashi K, Arima T: Risk factors for local recurrence of small hepatocellular carcinoma tumors after a single session, single application of percutaneous radiofrequency ablation. Cancer 2003;97: 1253–1262.
25 Myreng Y, Molstad P, Ytre-Arne K, et al: Safety of the transpulmonary ultrasound contrast agent NC100100: a clinical and haemodynamic evaluation in patients with suspected or proved coronary artery disease. Heart 1999;82:333–335.
26 Yanagisawa K, Moriyasu F, Miyahara T, Yuki M, Iijima H: Phagocytosis of ultrasound contrast agent microbubbles by Kupffer cells. Ultrasound Med Biol 2007;33:318–325.

Oncology

Oncology 2010;78(suppl 1):113–124
DOI: 10.1159/000315239

Published online: July 8, 2010

Radiofrequency Ablation for Hepatocellular Carcinoma: Updated Review in 2010

Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Key Words
Hepatocellular carcinoma · Radiofrequency ablation · Treatment algorithm · RFA, complications · RFA, outcomes

Abstract
Percutaneous radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC) was introduced in Japan in 1999. It has been established as a main local treatment method worldwide including Japan. On comparing outcomes between resection and RFA, they were comparable when cases were limited to those with 3 or fewer tumors 3 cm or smaller in many reports, based on which RFA has become the main treatment for small HCCs. The 5-year survival rate following RFA was as high as 57% in patients registered in the Liver Cancer Study Group of Japan, 73% when cases were limited to liver damage A (Child-Pugh A), and 83.8 and 76.3% in liver damage A (Child-Pugh A) cases with a single 2-cm or smaller and 2- to 5-cm liver tumor, respectively, showing outcomes equivalent to those of resection. The outcomes at our facility were also favorable: the 5-year survival rates of Child-Pugh A liver function HCC cases with 3 or fewer tumors 3 cm or smaller following RFA and resection were 84 and 78%, respectively. Various complications and limitations of RFA have previously been reported, but the advances of physicians' skills and development of various techniques have reduced complications and expanded the indications for RAF. TACE-combined, artificial pleural effusion- and ascites-combined, and contrast-enhanced ultrasonography-guided RFAs are good examples. Adjuvant therapy, such as interferon and molecular targeted therapies following curative therapy, is expected to further improve survival after RFA.

Introduction

Radiofrequency ablation (RFA) for hepatocellular carcinoma (HCC) was introduced in Japan in 1999, and started to be covered by the national health insurance in April 2004. This is a breakthrough therapy for HCC. This report will outline the latest progress in HCC treatment: the indications for RFA, techniques devised to treat complications and difficult cases, and positioning in the treatment algorithm.

Indications and Contraindications for RFA

Percutaneous RFA is a treatment method causing the coagulation necrosis of liver tumors by dielectric heating with radio waves (460 ± 5 Hz) around an electrode inserted into a lesion. Since RFA is currently considered to be a superior local treatment to ethanol injection with

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0113$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3149, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

regard to the overall survival and recurrence rates worldwide, it is indicated for local treatment [1–4] as a first choice of treatment modality.

Expandable and Cool-tip types of electrode needles are available for RFA at present. An about 3-cm area can be necrotized by a single ablative treatment, and, accordingly, treatment of a 2.5-cm or smaller tumor can be completely ablated by a single RFA session. Currently, 3 or fewer tumors 3 cm or smaller are generally indicated for RFA. It is occasionally applied to tumors >3 cm, but a combination with transcatheter arterial chemoembolization (TACE) described below is desirable for hypervascular HCC >3 cm because of associated problems, such as local residual and microsatellite lesions and residual microvascular invasion.

Contraindications for RFA are jaundice, refractory ascites, a bleeding tendency, and a 50,000/mm [3] or lower platelet count. Relative contraindications include lesions close to the stomach and intestine, gallbladder, and bile duct and the heart. For these lesions, other approaches, such as laparoscopic ablation, are recommended. Patients with pacemakers are also contraindicated for RFA.

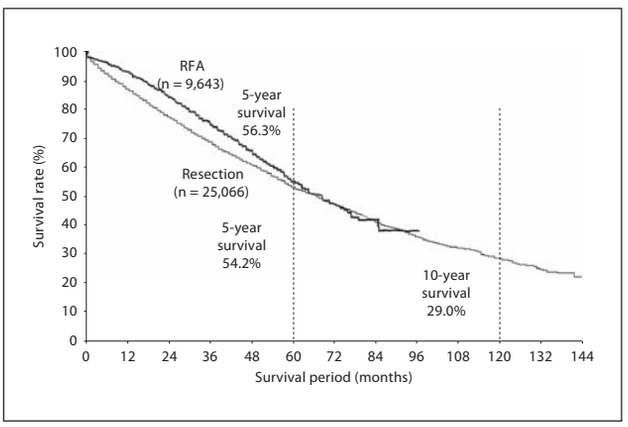


Fig. 1. Overall survival of resection and RFA (all cases) cited from the report of the 18th Nationwide Follow-Up Survey of Primary Liver Cancer performed by the LCSGJ.

Position in Treatment Algorithm in Japan, North America, and Europe

There are two established treatment algorithms in Japan: 'Evidence-based clinical guidelines for the diagnosis and treatment of HCC' published in 2005 [5] and revised in 2009 [6], and 'Consensus-based clinical practice manual for HCC' [7, 8] edited by the Japan Society of Hepatology (JSH). The former recommends: (1) hepatectomy for a single tumor regardless of the tumor size, but local treatment may be selected for a 2-cm or smaller tumor in Child-Pugh B patients, (2) hepatectomy or local treatment when the number of tumors is 2 or 3 and the tumor size is within 3 cm, and (3) liver transplantation for Child-Pugh C patients with 3 or fewer tumors 3 cm or smaller or a single tumor with a tumor size within 5 cm (Milan Criteria).

In the latter, edited by the JSH, in addition to the above indications, RFA combined with TACE is recommended for tumors >3 cm. RFA is also recommended for 4 or more nodules if applicable. Furthermore, the application of RFA may be attempted for Child-Pugh C HCC patients within the Milan Criteria when no refractory ascites is present and the bilirubin level is 3 mg/ml or lower. Local treatment is recommended as well as 'strict extensive close follow-up' for a single early-stage hypovascular HCC diagnosed by liver biopsy or imaging. Since the 'Consensus-based HCC treatment algorithm' reflects treatment actually performed in clinical practice, the indication for RFA is wider than that by the 'Evidence-based algorithm'.

In Europe and North America, the algorithm established by the American Association of the Study of the Liver Disease (AASLD) [9] recommends local treatment for 3 or fewer 3-cm or smaller early-stage HCCs and 2-cm or smaller very-early-stage HCCs with complications, such as portal hypertension. Although the typical treatment algorithms in Japan, North America, and Europe are slightly different, basically, RFA is recommended for 3 or fewer 3-cm or smaller HCCs.

Outcomes and Survival following RFA: Comparison with Those after Resection

Indications for RFA are 3 or fewer 3-cm or smaller tumors, as descried above. According to the Nationwide Follow-Up Survey of Primary Liver Cancer in Japan reported by the Liver Cancer Study Group of Japan (LCSGJ), there were no significant differences in the 5-year survival rate between RFA and resected cases in all RFA- and hepatectomy-treated cases (fig. 1), liver damage A (Child-Pugh A) patients (fig. 2), single 2-cm or smaller HCC cases (fig. 3), or single 2- to 5-cm HCC cases (fig. 4) [10]. However, Hasegawa et al. [11] recently reported that the recurrence rate was significantly higher

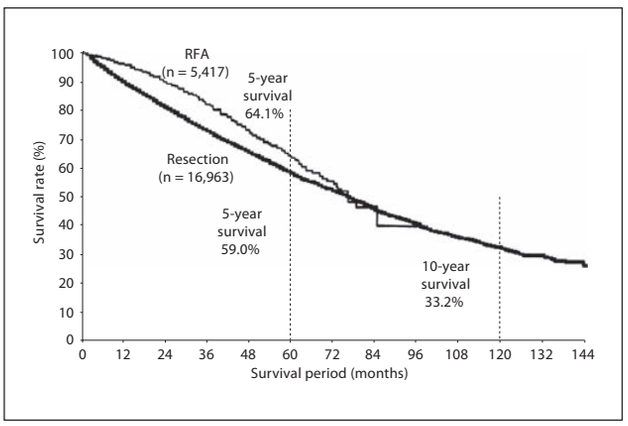


Fig. 2. Overall survival of resection and RFA (liver damage class A cases only) cited from the report of the 18th Nationwide Follow-Up Survey of Primary Liver Cancer performed by the LCSGJ.

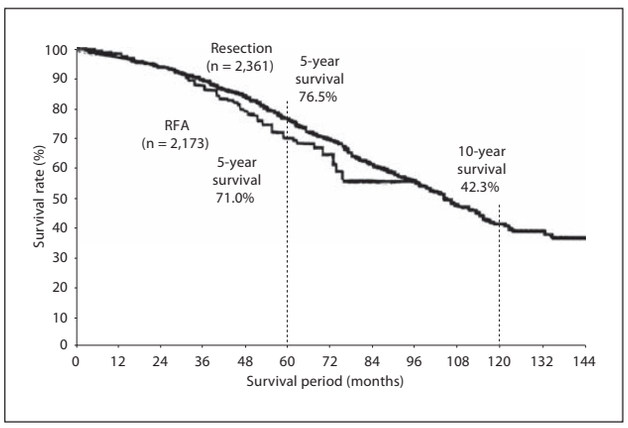


Fig. 3. Overall survival of resection and RFA (liver damage class A cases with TNM stage I: a single 2-cm or smaller tumor without vascular invasion) cited from the report of the 18th Nationwide Follow-up Survey of Primary Liver Cancer performed by the LCSGJ.

in RFA-treated cases, although no significant difference was noted in the overall survival rate. Similarly, no significant difference in the 5-year survival rate was noted between RFA and resected cases at our institution (fig. 5). Similar findings were also noted in other reports [12–18]. Based on these, resection is recommended for cases with 3 or fewer tumors 3 cm or smaller in which resection is readily applicable with regard to the liver function and localization, and only a small amount of liver tissue is sacrificed, but RFA is recommended for tumors located in a deep region, when the liver function capacity is graded as Child-Pugh B, or the tumor extends over the bilateral lobes.

In actual cases, RFA is applicable for all cases of Child-Pugh B (or liver damage class B) with 3 or fewer 3-cm or smaller HCCs, but hepatectomy is not in many cases. The applicability of resection is limited even in Child-Pugh A patients with 3 or fewer 3-cm or smaller HCCs. Cases with 3 or fewer 3-cm or smaller HCCs for which both resection and RFA may be applicable account for only 10% of all cases. Inversely, resection should be selected for some cases when complete control by RFA is impossible while the liver function is well preserved. Currently, a multicenter randomized controlled study (prospective randomized study of surgery or RFA for early HCC: SURF Trial) is underway in Japan, involving cases with 3 or fewer tumors 3 cm or smaller for which both hepatectomy and RFA are applicable, and the results are anticipated. It has been reported that protein induced by vitamin K absence II (PIVKA-II) was the most sensitive recurrence-predictive factor following curative RFA of HCC [19].

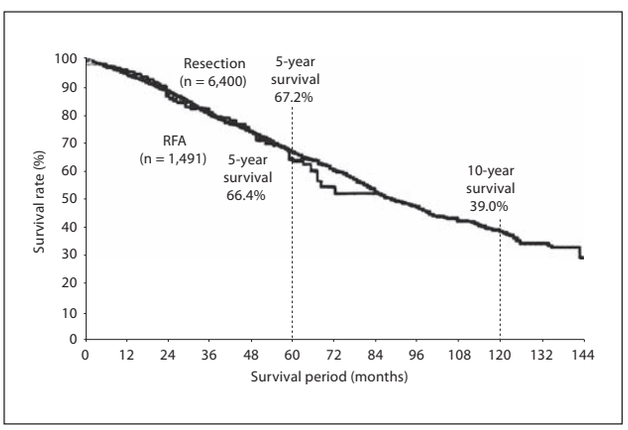


Fig. 4. Overall survival of resection and RFA (stage I: liver damage class A cases with a single 2- to 5-cm tumor) cited from the report of the 18th Nationwide Follow-Up Survey of Primary Liver Cancer performed by the LCSGJ.

Advances of Techniques for RFA Difficult Cases

The indication for RFA has been expanded by devising various techniques to previously contraindicated cases with difficulty in the application or the high-risk nature of RFA [20].

Large HCC

In cases of HCCs exceeding 3 cm in size, recurrence (often appearing as local recurrence) arises from a satel-

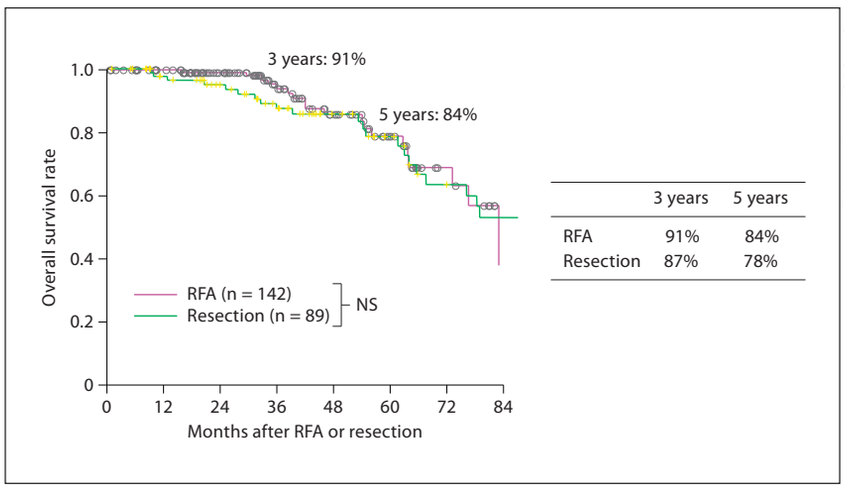


Fig. 5. Comparison between the cumulative survival rates following resection and RFA in initial development cases of 3 or fewer 3-cm or smaller HCCs treated at Kinki University Medical Center.

lite lesion around the main nodule and the proximity of the ablated area due to residual microscopic vascular invasion overlooked on imaging. For such lesions, lipiodol TACE-preceded RFA is actively performed, aiming at the treatment of satellite nodules and microscopic vascular invasion and ensuring an accurate margin by lipiodol injection [21–26]. It was clarified in the consensus meeting 'HCC Treatment' at the 45th Annual Meeting of the JSH in Kobe in 2009 (Congress Chairman: Prof. Masatoshi Kudo) [27] that about 90% of physicians performing RFA employ lipiodol TACE-preceded RFA for 3-cm or larger HCCs. Lipiodol TACE-preceded RFA is relatively curative and can be readily performed for the following reasons: (1) lipiodol regurgitates into the portal branches via the peribiliary venous plexus, causing a transient liver infarction state, which reduces the cooling effect, expanding ablative area, and results in (2) the coagulation of satellite lesions [28]. Yamakado et al. [24] reported that the survival rates of large HCC cases treated with resection and lipiodol TACE-preceded RFA were almost equivalent.

Right Subdiaphragmatic Lesion

Ultrasonographic imaging of right subdiaphragmatic lesions may be difficult and treatment was previously impossible, but these problems were overcome by RFA in the presence of 5% glucose solution or normal saline infused into the intrapleural cavity as artificial pleural effusion [25, 29–32]. By introducing this technique, our department can treat all cases with right subdiaphragmatic lesions with great success.

Cases Unclear on B-Mode Ultrasonography

Although HCCs may show typical features on contrast CT or MRI, arterial enhancement with venous washout may not be imaged on B-mode ultrasonography because of a coarse liver parenchymal echo or because the differentiation of a new recurrent lesion from necrotic nodules may be difficult on B-mode ultrasonography alone due to previously performed RFA. Such cases were previously treated with CT-guided RFA [33] and RFA under synchronized ultrasonography and CT, i.e. real-time virtual sonography (RVS)-guided RFA. By introducing these techniques, the therapeutic efficiency was drastically increased [34–37]. However, the recent advances of contrast media for ultrasonography, particularly Sonazoid, facilitated accurate pinpoint treatment guidance by Kupffer-phase and subsequent defect reperfusion imaging through reinjection [38–40]. This is really a major breakthrough [40–45].

Localization of Locally Recurrent Cancer

Recurrent lesions may not be localized by B-mode ultrasonography in some cases of local recurrence, despite local marginal recurrence being apparently present on dynamic CT. For these cases, defect reperfusion imaging with Sonazoid is extremely useful [38–40]. RVS was also previously used in these cases [34–37], but this is no longer necessary.

Tumors Located on the Intraabdominal Free Surface

Tumors present on the intraabdominal free surfaces possess the risk for complications, such as gastrointestinal and gallbladder perforations, because they are located close to the other organs, such as the intestine and gall-

bladder [46]. Since such cases have actually been reported, special caution is necessary to avoid fatal complications. Although it was previously contraindicated, artificial preparation of a space between the intestine and nodule by infusing normal saline or 5% glucose (artificial ascites method) for treatment has recently become possible [47, 48]. These techniques markedly expanded the indication for RFA. Laparoscopic resection or laparotomic RFA had to be inevitably performed in these cases before the introduction of artificial ascites, but more than 90% of cases are now treatable by the 'artificial ascites method'.

Lesions Proximal to the Hepatic Portal Glisson's Capsule

When a HCC is located near a peripheral vessel, although it sometimes injures an artery or portal vein and causes hepatic infarction, the damage is minimal. What is of concern is the risk of bile duct injury-associated complications, such as biloma or abscess. For a large tumor present in the hepatic portal region, injuring a large bile duct in the Glisson's capsular region by ensuring a safety margin is of concern, which may cause major complications. On the other hand, less strict treatment may result in local recurrence. Taking this into consideration, ethanol injection can be performed for lesions near vessels in such cases. Ethanol injection is effective to some extent, but the therapeutic effect is limited compared to RFA. For such cases, many institutions including ours employ a method in which an endoscopic nasobiliary drainage tube is inserted into the bile duct, and RFA is performed with cooling by the perfusion of ice-cold water through the tube [49, 50]. This procedure markedly reduced the probability of bile duct injury. We consider that this method should be routinely employed and investigation involving a larger number of cases is necessary because an increased risk for liver abscess has been reported [51].

Lesions in the Caudate Lobe

The risk of RFA for lesions in the caudate lobe is high because many vessels are present when adopting a right intercostal approach. Similarly, the RFA needle inevitably penetrates into the intraabdominal cavity in a left lobe approach. Treatment is possible in more than 70% of cases if the puncture pathway is carefully selected [52], but laparoscopic treatment (resection or RFA) is recommended for institutions with insufficient experience and cases to avoid a risk of hemorrhage. Laparoscopic surgery is selected for about 10% of cases with lesions in the caudate lobe at our institution.

Importance of Ensuring a Safety Margin in RFA

Concept of a Safety Margin Based on RECICL

The concept of a 'safety margin' has been considered important in RFA for HCC. In the assessment criteria of local liver cancer treatment in the 2010 revised version (RECICL: response evaluation criteria in cancer of the liver) [53], the assessment is distinctly divided into two categories based on the presence or absence of a safety margin: when the region with an intensity lower than that in the late phase on pretreatment CT is slightly wider than the lesion in terms of the entire circumference, the lesion is judged as 100% necrotized (TEIVa), while the disappearance of the tumor stain alone lacking a slightly wider unstained region is judged as TEIVb [53].

Basis for the Necessity of a Safety Margin for RFA

In simple nodule-type HCCs, intrahepatic metastatic lesions are present at a site within 2 mm from the primary lesion in 66.7% of cases and 5 mm in 11.1% [54]. Accordingly, for this type, intrahepatic metastatic lesions can be included in the treatment area by setting a safety margin of 5 mm. Similar findings have been reported in which satellite nodules were present in 19% of 194 resected cases of 3-cm or smaller tumors, and 33% of these were present within 5 mm from the main tumor [55], indicating that local recurrence develops from residual microsatellite HCC lesions overlooked on RFA due to undetectable residual cancer by imaging, and local recurrence may occur even after a sufficient margin is ensured. Recurrence appears to arise from residual cancer after RFA, while recurrence from a microsatellite or by microvascular invasion other than the main nodule may also appear as a local recurrence (late local recurrence) in some cases. Therefore, ensuring a safety margin in RFA is important for not only the simultaneous treatment of microsatellite lesions, but also to ensure an enough tumor ablation on the assumption of partial volume effect-associated limitation on evaluation of the therapeutic effect by imaging.

Does a Safety Margin Inhibit Local Recurrence?

In our study, the local recurrence rates after 4 years were 2.6 and 20.8% in cases in which a 5-mm or wider safety margin could be ensured and in cases in which the margin was narrower than 5 mm, respectively, showing a significant difference [28].

A significant difference in the local recurrence rate between cases with a tumor size of $\leq$2.3 and $>$2.3 cm has been reported, while the local recurrence rate was significantly lower in cases with a sufficient safety margin

than in those with an insufficient safety margin, regardless of the tumor size in another report [56]. Nishijima et al. [57] categorized the presence of no margin, a partially lacking margin, margin narrower than 5 mm, and complete margin wider than 5 mm as R0, R1, R2, and R3 on the assessment of the therapeutic effect of RFA, respectively, and found that significant differences were present between R0 and R1 or between R2 and R3, but not between R1 and R2, showing that ensuring a margin, even though it is narrow, reduces the local recurrence rate. This was also extracted as the sole significant independent factor on multivariate analysis. Similarly, the local recurrence rate was significantly different between cases with and those without a sufficient safety margin [58]. A sufficient margin was also the sole predictor of the local recurrence rate on multivariate analysis [58]. For tumors >2 cm, preceding TACE was extracted as a prognostic factor, in addition to a sufficient safety margin.

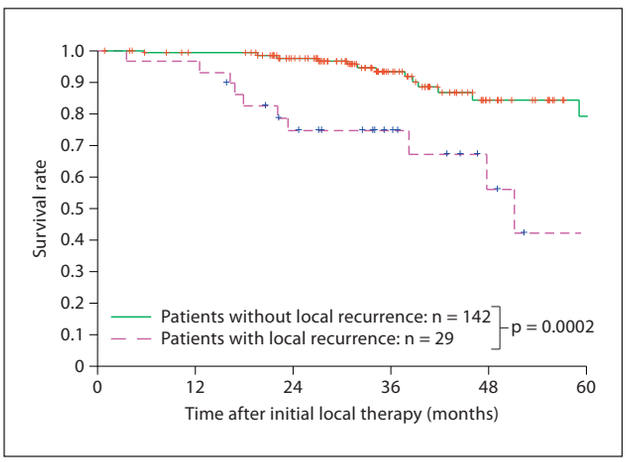


Fig. 6. Comparison of the overall survival rate between cases of Child-Pugh A liver function and HCC within the Milan Criteria with and without local recurrence. Overall survival in patients without local recurrence was significantly better than that in patients with local recurrence.

Does the Inhibition of Local Recurrence Improve Survival?

Although the Barcelona Clinic Liver Cancer (BCLC) group reported that a complete response to the first treatment was related to later survival [59], this report has problems: the initial response rate was 70%, showing a markedly lower local control rate following the initial treatment as compared with Japanese standard, and the 5-year local recurrence rate was markedly high (74%), suggesting that there is no concept of a safety margin in RFA in Western countries. The therapeutic effect is normally assessed only by CT 1–2 months after RFA in Western countries, unlike in Japan where RFA is repeated thoroughly until arterial enhancement of the tumor completely disappears and a safety margin is ensured on the first treatment [27]. Accordingly, the 5-year survival rate is nearly 50% in cases indicated for local treatment in Japan, but only 27% in their results. In any case, on the comparison of Child-Pugh A cases, the survival rate of complete responders who 'eventually' developed no local recurrence was significantly higher than of those who 'eventually' developed local recurrence, and a significant difference in the survival rate was also noted in Child-Pugh B cases. This is the first report that the presence or absence of local recurrence determines survival.

It also indicates that the survival of recurrence-free cases including recurrence in other regions (sustained complete response) is significantly better than that of cases with recurrence in local or other regions.

In a report involving 192 cases of complete ablation by RFA, there was a significant difference in survival between groups with and without local recurrence, and this recurrence pattern was also significant on multivariate analysis [60]. In another report, the survival time of patients who developed local recurrence within 1 year (early local recurrence group) was significantly shorter than that of patients who developed local recurrence after 1 year (late local recurrence group) [61]. The safety margin-ensured rate was significantly different between RFA and ethanol injection. Generally, a safety margin can be more easily ensured in RFA than in ethanol injection. Subsequently, as is widely recognized, the recurrence rate is lower in RFA, and the overall survival rate following RFA is also superior to that following ethanol injection [1, 2, 62, 63].

We also observed a significant difference in survival between groups which developed local recurrence and 'eventually' did not (fig. 6). Multivariate analysis also clarified this, showing that the presence or absence of local recurrence was a significant independent factor.

It is clear that the establishment of a safety margin inhibits local recurrence. It is also clear that survival is improved in cases in which a safety margin is 'eventually' achieved. Therefore, syllogistically, 'ensuring a safety margin on the first treatment improves survival'.

Summary of the Importance of a Safety Margin

In summary: (1) ensuring a safety margin is essential to treat microsatellite lesions and compensate for the un-

certainty of imaging evaluation; (2) local recurrence is inhibited in nodules with an 'eventually' achieved safety margin; (3) the survival of 'eventually' local recurrence-free cases is favorable compared to cases who develop local recurrence, and (4) the presence or absence of a safety margin in the first treatment is a prognostic factor.

Assessment of the Therapeutic Effect of RFA

The assessment of the therapeutic effect of RFA is very important. Basically, the local recurrence rate following a single RFA treatment depends on how strictly the therapeutic effect is assessed. In Japan, as a rule, treatment is completed when the presence of a safety margin (ablative margin) around the entire tumor is three-dimensionally confirmed [27, 53]. Accordingly, it is difficult to establish a safety margin employing the standard procedure in the absence of lipiodol in many cases when the tumor size exceeds 2 cm. Therefore, for hypervascular HCCs >2 cm, a far lower local recurrence rate can be achieved when TACE or arterial lipiodol injection is performed prior to RFA, although invasiveness slightly increases [58].

The assessment was previously performed employing CT alone, but after a second-generation contrast medium for ultrasonography (SonoVue or Sonazoid) was introduced, assessment using this medium may be optimal when the original tumor can be confirmed on B-mode ultrasonography because contrast-enhanced ultrasonography does not require pretreatment images, and the temporary and spatial resolutions are markedly high [64, 65]. This excellent method may omit the need for the therapeutic effect assessment by CT. In our study, confirmation of the margin of the original tumor on B-mode ultrasonography was optimal on the day of RFA or the following day. Thus, evaluation employing contrast-enhanced ultrasonography should be performed early after treatment [66]. A demerit of the therapeutic effect assessment immediately after treatment is the presence of RFA-induced inflammatory hyperemia, but as long as the margin of the nodule before treatment can be confirmed, the differentiation of residual tumor staining or inflammatory hyperemia can be easily distinguished. However, the margin can be identified immediately after treatment or the following day in only 50–60% of cases; therefore, dynamic CT is still necessary for the remaining approximately 40%. The use of superparamagnetic iron oxide MRI is unique, but its procedure is slightly complex [67].

Adverse Events and Complications of RFA

Literature Reports

Generally, adverse events represent unexpected results which may frequently occur, including pain, asymptomatic pleural effusion, a small volume of ascites around the liver, and blood retention. Symptoms called postablation syndrome, such as slight fever and systemic malaise, may develop after RFA. Their persistence mainly depends on the volume of necrotized tissue.

Complications are divided into serious and non-serious. When serious complications are left untreated, life is threatened, or some disease/disorder develops and requires readmission or the prolongation of hospitalization [68–70].

Mulier et al. [51] analyzed 82 reports published before the end of 2001, and identified 20 (0.5%) fatal and 327 (8.9%) moderately to severely complicated cases out of 3,670 cases: abdominal hemorrhage occurred in 1.6%, abdominal infection in 1.1%, bile duct injury in 1.0%, liver failure in 0.8%, pulmonary complications in 0.8%, burn in 0.6%, intrahepatic vascular injury in 0.6%, visceral organ disorder in 0.5%, and cardiac complications in 0.4%. They considered that mild complications may have been neglected and some complications may have been overlooked due to a short follow-up period, such as biliary stenosis and seeding, pointing out that the incidence may have been underestimated.

Livraghi et al. [71] reported 6 (0.3%) fatal cases and 50 (2.2%) and 110 (4.7%) cases of severe and mild complications, respectively, out of 2,320 cases with 3,554 lesions treated with RFA in 41 medical institutions in Italy. The mortality was due to intestinal perforation-associated multiorgan failure in 2 patients, and peritonitis-induced sepsis, mass bleeding following tumor rupture, liver failure following right intrahepatic bile duct stenosis, and sudden death 3 days after RFA in 1 patient each. Many cases of severe complications involved intraabdominal hemorrhage, seeding, intraabdominal abscess, and intestinal perforation, and the incidence of these complications was high in cases punctured many times. Although many experts think that RFA is simple, Livraghi et al. [71] pointed out that the procedure is complex, and sufficient experience is necessary to perform it safely.

Curley et al. [72] divided 608 cases of complications following RFA into those which occurred within (early phase) and after (late phase) 30 days, and found that the incidence of early-phase complications was high in cases treated with laparotomic RFA or those who had hepatic cirrhosis (43 cases, 7.1%), while the late-phase complica-

tions (15 cases, 2.4%) were bile duct injury, ascites, and liver failure. They emphasized that complications may appear several months after RFA, and HCCs located in the hepatic portal region should not be included in the indications for RFA because of a high risk of complications, such as bile duct injury.

Kasugai et al. [73] reported that 9 patients (0.3%) died and complications occurred in 207 patients (7.9%) within 3 months after 3,891 ablations in 2,614 patients in Japan. The mortality was due to acute aggravation/sarcomatous change in 3 patients, liver failure in 2 patients, and bile duct injury/biloma, ascites, gastrointestinal hemorrhage, and acute myocardial infarction in 1 patient each. When pleural effusion and ascites were excluded, as reported in other countries, complications occurred in 114 patients (4.4%) and 1 patient died within 1 month (0.04%), showing a markedly low incidence of complications and mortality. The main complications are as follows: (1) intrahepatic complications: bile duct injury/biloma, portal vein thrombus, liver abscess, acute aggravation/sarcomatous change; (2) complications of the puncture line and proximal organs: pleural effusion, ascites, abdominal wall burn, injuries of other organs (peritonitis, large intestinal perforation), hemothorax, liver capsular hematoma, pneumothorax, gastrointestinal hemorrhage, and peritoneal dissemination, and (3) systemic complications: jaundice, liver failure, and hypotension. However, the complication rate was almost 0% in about half of the departments, suggesting that the incidence of complications can be reduced by making efforts to perform RFA safely. There have been several reports on complications of tumor seeding [74–79], but it can be dealt with by devising various techniques.

Prevention of Complications

RFA has been attracting attention as a treatment method for HCC because of its relatively low invasiveness. Not only elevating the survival rate and reducing the incidence of local recurrence but also avoiding complications as much as possible are major tasks.

Indication

When Curley et al. [80] treated 110 cases of HCC complicated by hepatic cirrhosis with RFA, complications occurred in 4 of 50 Child-Pugh A cases (8%), 2 of 31 Child-Pugh B cases (6.5%), and 8 of 29 Child-Pugh C cases (27.6%), showing that the incidence of complications was high in Child-Pugh C cases. Although RFA is less invasive, sufficient caution is necessary in cases with an impaired liver function.

The risk for infection, such as liver abscess, after RFA is high in patients following biliojejunal anastomosis and in diabetic patients [71, 81]. Preventive antibiotic administration to high-risk patients has been attempted, but its scientific basis is unclear. The diagnosis of liver abscess is easy when fever, leukocytosis, and gas in the lesion are noted after RFA, but it may be asymptomatic or manifest only as malaise and a slight fever. Moreover, it may develop late after several months, to which attention should be paid.

The bile duct near the hepatic portal tract is considered protected by a cooling effect of the portal vein and hepatic artery distributing in parallel to the bile duct, but many complications in the early phase have been reported. Moreover, tumors located at a site within 1 cm of the hepatic portal tract were excluded from the indication in many reports. Some reports stated that ethanol injection should be selected, instead of RFA, for tumors in the hepatic portal tract [82].

Other reports stated that RFA is applicable for all intrahepatic regions, and that tumors adjacent to the gallbladder [82] and digestive tract [46] can be safely treated. However, once intestinal perforation occurs, it may lead to multiorgan failure. The application of RFA should be carefully decided on when the tumor is adjacent to the intestine, gallbladder, hilar bile duct, and diaphragm, and close follow-up after treatment is necessary [51, 71, 82]. When the tumor is adjacent to the intestine after surgery in the upper abdominal region, it is desirable to perform laparoscopic or laparotomic RFA, and not percutaneous treatment, or switch to ethanol injection [71].

Technical Aspect

Since the treatment method is not simple, complication-inducing elements of RFA are complex: some involve RFA instruments and electrodes, while others involve various concomitantly employed techniques and treatments. Elements of instruments and electrodes include types of generator and electrode, anesthetic and guidance methods, way of elevating the output, frequency of puncture, timing of treatment completion, and the presence or absence of coagulation of the puncture pathway.

Thermal coagulation of the puncture pathway under the liver capsule before removing the electrode is the most reliable method to prevent mass hemorrhage, blood leakage and needle tract tumor seeding, and its adoption is strongly recommended [51]. When the tumor is present near the liver surface, hemorrhage is particularly likely to cause peritoneal dissemination [79], but thermal coagulation of the puncture pathway may prevent seeding.

Since the risk of hemorrhage is always present after puncture, the vital sign should be carefully monitored for 3–4 h after surgery.

Giorgio et al. [83] performed 375 RFAs with concomitant saline infusion in 336 patients, and severe complications occurred in 3 (0.9%), including 1 fatal case.

Many studies reported that RFA may rather promote tumor advancement when a residual tumor is present due to insufficient treatment or when portal invasion is present [84–88].

Intrahepatic hematoma may develop due to injury of large vessels [81], and the avoidance of blood vessel puncture prevents its occurrence. When an expandable needle is used, it is difficult to completely avoid blood vessel puncture because the simultaneous observation of all electrodes is impossible, but this is possible when a monopolar needle electrode is used.

In a study reported by Kasugai et al. [73], technical procedures, such as laparotomic RFA, the use of artificial pleural effusion and ascites, a CT guidance, and concomitant therapy, such as TACE or PEI, were associated with a high incidence of complications. It should be kept in mind that the addition of these procedures and concomitant treatment may increase the risk of complications.

RFA is performed percutaneously in most of the cases, but there is no marked difference in the incidence of complications in laparoscopic or laparotomic RFA [51]. Thus, the differential application of these methods is desirable.

Tertial Prevention for Cases following Curative RFA

Recurrence occurs at 15–20% of the annual incidence rate even after curative treatment, such as resection or RFA, which is a characteristic of HCC. Therefore, tertial prevention, i.e. inhibition of recurrence, is the most urgent issue. The most frequently employed treatment is interferon therapy [89–91]. When the liver function damage is mild, hepatitis C should basically be treated by eradicating hepatitis C virus through pegylated interferon and ribavirin combination therapy. A marked inhibition of the recurrence rate in virus-eradicated cases has been reported. It has also been reported that even though the virus was not eliminated, long-term low-dose interferon maintenance therapy is significantly effective for inhibition of second and third recurrences, although no difference was noted for the first recurrence. As a result, because of the lowering the recurrence and the numbers of treatments such as RFA or TACE, long-term low-dose interferon maintenance therapy improves the prognosis in patients with curative RFA [89]. Therefore, at present, from an academic viewpoint, improvement of survival by recurrence-inhibitory long-term low-dose interferon therapy is highly recommended for cases following curative RFA for which conventional interferon ribavirin combination therapy is not feasible because of low platelet counts or other complications.

Difference in the Concept of RFA Therapy between Japan and Western Countries

Although RFA was originally developed in Western countries, the local control rate by RFA is in the 90% range in the literature, not reaching 100%. In contrast, the CR rate is usually 100% in Japan because treatment is repeated until the necrosis rate reaches 100% and a safety margin is ensured [27]. Even if a safety margin cannot be ensured, treatment is continued until arterial enhancement disappears in most cases, being practically 100% necrosis. This is the difference in the way of thinking about RFA between Western countries and Japan. In Western countries, physicians do not consider repeating RFA until tumor staining disappears and a safety margin is ensured. In most of the cases, patients are discharged after only a single RFA treatment without assessment of the therapeutic effect by CT, and the effect is assessed after about 1–2 months by CT. This condition may be the cause of the markedly poorer outcomes of RFA in Child-Pugh A cases with 3 or fewer 3-cm or smaller HCCs in Western countries.

TACE-combined RFA is sometimes performed in Western countries, but less frequently. In any case, RFA conducted in Western countries may be imprecise compared to that performed in Japan. The quality of RFA in Japan is the best in the world and it may be the most frequent method considering that more than 100 cases are annually treated at more than 40 institutions in Japan. In Western countries, RFA with the concomitant use of doxorubicin-eluting beads has recently been performed [92]. The results of this treatment may attract some interest.

Conclusion

The current status and outcomes of RFA for HCC in Japan and other countries were described. In addition to interferon administered to inhibit recurrence after RFA, an international cooperative clinical trial (STORM trial)

on adjuvant therapy with a molecular targeting agent, Sorafenib, is currently underway. In addition, a clinical study on the inhibition of recurrence following resection or ablation by retinoid, developed in Japan, has been performed, and its results were favorable to some extent, although it did not meet the primary endpoint. These adjuvant therapies are expected to markedly improve the outcome of RFA.

Disclosure Statement

The author declares that he has no financial conflict of interest.

References

1 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC: Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. Gut 2005; 54:1151–1156.

2 Shiina S, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, et al: A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. Gastroenterology 2005;129:122–130.

3 Orlando A, Leandro G, Olivo M, Andriulli A, Cottone M: Radiofrequency thermal ablation vs. percutaneous ethanol injection for small hepatocellular carcinoma in cirrhosis: meta-analysis of randomized controlled trials. Am J Gastroenterol 2009;104:514–524.

4 Brunello F, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, Sacchetto P, Gandini G, Rizzetto M: Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: a randomized controlled trial. Scand J Gastroenterol 2008;43:727–735.

5 Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, et al: Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008; 38:37–51.

6 The Japan Society of Hepatology: Evidence-Based Clinical Practice Guideline for Hepatocellular Carcinoma (revised version) (in Japanese). Tokyo, Kanehara, 2009.

7 The Japan Society of Hepatology: Consensus-Based Clinical Practice Manual. Tokyo, Igakushoin, 2007.

8 Kudo M, Okanoue T: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(suppl):2–15.

9 Bruix J, Sherman M: Management of hepatocellular carcinoma. Hepatology 2005;42: 1208–1236.

10 Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, et al: Report of the 17th Nationwide Follow-Up Survey of Primary Liver Cancer in Japan. Hepatol Res 2007;37: 676–691.

11 Hasegawa K, Makuuchi M, Takayama T, Kokudo N, Arii S, Okazaki M, Okita K, Omata M, Kudo M, Kojiro M, et al: Surgical resection vs. percutaneous ablation for hepatocellular carcinoma: a preliminary report of the Japanese nationwide survey. J Hepatol 2008;49:589–594.

12 Ogihara M, Wong LL, Machi J: Radiofrequency ablation versus surgical resection for single nodule hepatocellular carcinoma: long-term outcomes. HPB (Oxford) 2005;7: 214–221.

13 Lupo L, Panzera P, Giannelli G, Memeo M, Gentile A, Memeo V: Single hepatocellular carcinoma ranging from 3 to 5 cm: radiofrequency ablation or resection? HPB (Oxford) 2007;9:429–434.

14 Ueno S, Sakoda M, Kubo F, Hiwatashi K, Tateno T, Baba Y, Hasegawa S, Tsubouchi H: Surgical resection versus radiofrequency ablation for small hepatocellular carcinomas within the Milan Criteria. J Hepatobiliary Pancreat Surg 2009;16:359–366.

15 Molinari M, Helton S: Hepatic resection versus radiofrequency ablation for hepatocellular carcinoma in cirrhotic individuals not candidates for liver transplantation: a Markov model decision analysis. Am J Surg 2009; 198:396–406.

16 Livraghi T, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S: Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? Hepatology 2008;47:82–89.

17 Guglielmi A, Ruzzenente A, Valdegamberi A, Pachera S, Campagnaro T, D'Onofrio M, Martone E, Nicoli P, Iacono C: Radiofrequency ablation versus surgical resection for the treatment of hepatocellular carcinoma in cirrhosis. J Gastrointest Surg 2008;12:192–198.

18 Vivarelli M, Guglielmi A, Ruzzenente A, Cucchetti A, Bellusci R, Cordiano C, Cavallari A: Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. Ann Surg 2004;240:102–107.

19 Takahashi S, Kudo M, Chung H, Inoue T, Ishikawa E, Kitai S, Tatsumi C, Ueda T, Nagai T, Minami Y, et al: PIVKA-II is the best prognostic predictor in patients with hepatocellular carcinoma after radiofrequency ablation therapy. Oncology 2008;75(suppl 1): 91–98.

20 Chen MH, Yang W, Yan K, Hou YB, Dai Y, Gao W, Zhang H, Wu W: Radiofrequency ablation of problematically located hepatocellular carcinoma: tailored approach. Abdom Imaging 2008;33:428–436.

21 Takaki H, Yamakado K, Uraki J, Nakatsuka A, Fuke H, Yamamoto N, Shiraki K, Yamada T, Takeda K: Radiofrequency ablation combined with chemoembolization for the treatment of hepatocellular carcinomas larger than 5 cm. J Vasc Interv Radiol 2009;20:217–224.

22 Kirikoshi H, Saito S, Yoneda M, Fujita K, Mawatari H, Uchiyama T, Higurashi T, Goto A, Takahashi H, Abe Y, et al: Outcome of transarterial chemoembolization monotherapy, and in combination with percutaneous ethanol injection, or radiofrequency ablation therapy for hepatocellular carcinoma. Hepatol Res 2009;39:553–562.

23 Peng ZW, Chen MS, Liang HH, Gao HJ, Zhang YJ, Li JQ, Zhang YQ, Lau WY: A case-control study comparing percutaneous radiofrequency ablation alone or combined with transcatheter arterial chemoembolization for hepatocellular carcinoma. Eur J Surg Oncol 2010;36:257–263.

24 Yamakado K, Nakatsuka A, Takaki H, Yokoi H, Usui M, Sakurai H, Isaji S, Shiraki K, Fuke H, Uemoto S, et al: Early-stage hepatocellular carcinoma: radiofrequency ablation combined with chemoembolization versus hepatectomy. Radiology 2008;247:260–266.

25 Lee MW, Kim YJ, Park SW, Jeon HJ, Yi JG, Choe WH, Kwon SY, Lee CH: Percutaneous radiofrequency ablation of liver dome hepatocellular carcinoma invisible on ultrasonography: a new targeting strategy. Br J Radiol 2008;81:e130–e134.

26 Kitamoto M, Imagawa M, Yamada H, Watanabe C, Sumioka M, Satoh O, Shimamoto M, Kodama M, Kimura S, Kishimoto K, et al: Radiofrequency ablation in the treatment of small hepatocellular carcinomas: compari-

son of the radiofrequency effect with and without chemoembolization. AJR Am J Roentgenol 2003;181:997–1003.
27 Arii S, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, Kokudo N, Tanaka M, Takayama T, et al: Management of Hepatocellular Carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology 2009. Hepatol Res 2010, in press.
28 Kudo M: Local ablation therapy for hepatocellular carcinoma: current status and future perspectives. J Gastroenterol 2004;39:205–214.
29 Rhim H, Lim HK, Kim YS, Choi D: Percutaneous radiofrequency ablation with artificial ascites for hepatocellular carcinoma in the hepatic dome: initial experience. AJR Am J Roentgenol 2008;190:91–98.
30 Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H: Percutaneous radiofrequency ablation guided by contrast-enhanced harmonic sonography with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. AJR Am J Roentgenol 2004;182:1224–1226.
31 Wang ZY, Sun WB, Li MY, Zhang XX, Ding XM: Percutaneous extrapulmonary radiofrequency ablation for tumors in the hepatic dome. Hepatogastroenterology 2008;55:1164–1166.
32 Kondo Y, Yoshida H, Tateishi R, Shiina S, Kawabe T, Omata M: Percutaneous radiofrequency ablation of liver cancer in the hepatic dome using the intrapleural fluid infusion technique. Br J Surg 2008;95:996–1004.
33 Park BJ, Byun JH, Jin YH, Won HJ, Shin YM, Kim KW, Park SJ, Kim PN: CT-guided radiofrequency ablation for hepatocellular carcinomas that were undetectable at US: therapeutic effectiveness and safety. J Vasc Interv Radiol 2009;20:490–499.
34 Nakai M, Sato M, Sahara S, Takasaka I, Kawai N, Minamiguchi H, Tanihata H, Kimura M, Takeuchi N: Radiofrequency ablation assisted by real-time virtual sonography and CT for hepatocellular carcinoma undetectable by conventional sonography. Cardiovasc Intervent Radiol 2009;32:62–69.
35 Minami Y, Chung H, Kudo M, Kitai S, Takahashi S, Inoue T, Ueshima K, Shiozaki H: Radiofrequency ablation of hepatocellular carcinoma: value of virtual CT sonography with magnetic navigation. AJR Am J Roentgenol 2008;190:W335–W341.
36 Minami Y, Kudo M, Chung H, Inoue T, Takahashi S, Hatanaka K, Ueda T, Hagiwara H, Kitai S, Ueshima K, et al: Percutaneous radiofrequency ablation of sonographically unidentifiable liver tumors. Feasibility and usefulness of a novel guiding technique with an integrated system of computed tomography and sonographic images. Oncology 2007;72(suppl 1):111–116.
37 Kawasoe H, Eguchi Y, Mizuta T, Yasutake T, Ozaki I, Shimonishi T, Miyazaki K, Tamai T, Kato A, Kudo S, et al: Radiofrequency ablation with the real-time virtual sonography system for treating hepatocellular carcinoma difficult to detect by ultrasonography. J Clin Biochem Nutr 2007;40:66–72.
38 Kudo M, Hatanaka K, Maekawa K: Sonazoid-enhanced ultrasound in the diagnosis and treatment of hepatic tumors. J Med Ultrasound 2008;16:130–139.
39 Kudo M, Hatanaka K, Maekawa K: Defect reperfusion imaging, a newly developed novel technology using Sonazoid in the treatment of hepatocellular carcinoma. J Med Ultrasound 2008;16:169–175.
40 Kudo M, Hatanaka K, Chung H, Minami Y, Maekawa K: A proposal of novel treatment-assist technique for hepatocellular carcinoma in the Sonazoid-enhanced ultrasonography: value of defect re-perfusion imaging (in Japanese). Kanzo 2007;48:299–301
41 Minami Y, Kudo M, Chung H, Kawasaki T, Yagyu Y, Shimono T, Shiozaki H: Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. AJR Am J Roentgenol 2007;188:489–494.
42 Maruyama H, Takahashi M, Ishibashi H, Okugawa H, Okabe S, Yoshikawa M, Yokosuka O: Ultrasound-guided treatments under low acoustic power contrast harmonic imaging for hepatocellular carcinomas undetected by B-mode ultrasonography. Liver Int 2009;29:708–714.
43 Miyamoto N, Hiramatsu K, Tsuchiya K, Sato Y, Terae S, Shirato H: Sonazoid-enhanced sonography for guiding radiofrequency ablation for hepatocellular carcinoma: better tumor visualization by Kupffer-phase imaging and vascular-phase imaging after reinjection. Jpn J Radiol 2009;27:185–193.
44 Minami Y, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H: Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. AJR Am J Roentgenol 2004;183:153–156.
45 Miyamoto N, Hiramatsu K, Tsuchiya K, Sato Y: Carbon dioxide microbubbles-enhanced sonographically guided radiofrequency ablation: treatment of patients with local progression of hepatocellular carcinoma. Radiat Med 2008;26:92–97.
46 Choi D, Lim HK, Kim MJ, Kim SH, Lee WJ, Lim JH, Paik SW, Koh KC, Yoo BC: Therapeutic efficacy and safety of percutaneous radiofrequency ablation of hepatocellular carcinoma abutting the gastrointestinal tract. AJR Am J Roentgenol 2004;183:1417–1424.
47 Park SY, Tak WY, Jeon SW, Cho CM, Kweon YO, Kim SK, Choi YH: The efficacy of intraperitoneal saline infusion for percutaneous radiofrequency ablation for hepatocellular carcinoma. Eur J Radiol 2010;74:536–540.
48 Song I, Rhim H, Lim HK, Kim YS, Choi D: Percutaneous radiofrequency ablation of hepatocellular carcinoma abutting the diaphragm and gastrointestinal tracts with the use of artificial ascites: safety and technical efficacy in 143 patients. Eur Radiol 2009.
49 Lam VW, Ng KK, Chok KS, Cheung TT, Wat J, Fan ST, Poon RT: Safety and efficacy of radiofrequency ablation for periductal hepatocellular carcinoma with intraductal cooling of the central bile duct. J Am Coll Surg 2008;207:e1–e5.
50 Ohnishi T, Yasuda I, Nishigaki Y, Hayashi H, Otsuji K, Mukai T, Enya M, Omar S, Soehendra N, Tomita E, et al: Intraductal chilled saline perfusion to prevent bile duct injury during percutaneous radiofrequency ablation for hepatocellular carcinoma. J Gastroenterol Hepatol 2008;23:e410–e415.
51 Mulier S, Mulier P, Ni Y, Miao Y, Dupas B, Marchal G, De Wever I, Michel L: Complications of radiofrequency coagulation of liver tumours. Br J Surg 2002;89:1206–1222.
52 Peng ZW, Liang HH, Chen MS, Zhang YJ, Li JQ, Zhang YQ, Lau WY: Percutaneous radiofrequency ablation for the treatment of hepatocellular carcinoma in the caudate lobe. Eur J Surg Oncol 2008;34:166–172.
53 Kudo M, Kubo S, Takayasu K, Sakamoto M, Tanaka M, Ikai I, Furuse J, Nakamura K, Makuuchi M, et al: Response evaluation criteria in cancer of the liver (RECICL) proposed by the Liver Cancer Study Group of Japan (2010 Revised Version). Hepatol Res 2010, in press.
54 Nakashima Y, Nakashima O, Tanaka M, Okuda K, Nakashima M, Kojiro M: Portal vein invasion and intrahepatic micrometastasis in small hepatocellular carcinoma by gross type. Hepatol Res 2003;26:142–147.
55 Okusaka T, Okada S, Ueno H, Ikeda M, Shimada K, Yamamoto J, Kosuge T, Yamasaki S, Fukushima N, Sakamoto M: Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. Cancer 2002;95:1931–1937.
56 Zytoon AA, Ishii H, Murakami K, El-Kholy MR, Furuse J, El-Dorry A, El-Malah A: Recurrence-free survival after radiofrequency ablation of hepatocellular carcinoma. A registry report of the impact of risk factors on outcome. Jpn J Clin Oncol 2007;37:658–672.
57 Nishijima N, Osaki Y, Kita R, Eso Y, Yamanaka S, Kawakami H, et al: Proposal of the radicality grading as a criterion for therapeutic effectiveness of RFA against hepatocellular carcinoma, in relation to the local recurrence rate (in Japanese). Acta Hepatol Jpn 2008;49:192–199.

58 Takahashi S, Kudo M, Chung H, Inoue T, Ishikawa E, Kitai S, Tatsumi C, Ueda T, Minami Y, Ueshima K, et al: Initial treatment response is essential to improve survival in patients with hepatocellular carcinoma who underwent curative radiofrequency ablation therapy. Oncology 2007;72(suppl 1):98–103.

59 Sala M, Llovet JM, Vilana R, Bianchi L, Sole M, Ayuso C, Bru C, Bruix J: Initial response to percutaneous ablation predicts survival in patients with hepatocellular carcinoma. Hepatology 2004;40:1352–1360.

60 Ng KK, Poon RT, Lo CM, Yuen J, Tso WK, Fan ST: Analysis of recurrence pattern and its influence on survival outcome after radiofrequency ablation of hepatocellular carcinoma. J Gastrointest Surg 2008;12:183–191.

61 Lam VW, Ng KK, Chok KS, Cheung TT, Yuen J, Tung H, Tso WK, Fan ST, Poon RT: Risk factors and prognostic factors of local recurrence after radiofrequency ablation of hepatocellular carcinoma. J Am Coll Surg 2008;207:20–29.

62 Lencioni RA, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, et al: Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. Radiology 2003;228:235–240.

63 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC: Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or = 4 cm. Gastroenterology 2004;127:1714–1723.

64 Wen YL, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K: Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. AJR Am J Roentgenol 2003;181:57–63.

65 Bartolotta TV, Taibbi A, Midiri M, De Maria M: Hepatocellular cancer response to radiofrequency tumor ablation: contrast-enhanced ultrasound. Abdom Imaging 2008;33:501–511.

66 Zhou P, Kudo M, Minami Y, Chung H, Inoue T, Fukunaga T, Maekawa K: What is the best time to evaluate treatment response after radiofrequency ablation of hepatocellular carcinoma using contrast-enhanced sonography? Oncology 2007;72(suppl 1):92–97.

67 Mori K, Fukuda K, Asaoka H, Ueda T, Kunimatsu A, Okamoto Y, Nasu K, Fukunaga K, Morishita Y, Minami M: Radiofrequency ablation of the liver: determination of ablative margin at MR imaging with impaired clearance of ferucarbotran – feasibility study. Radiology 2009;251:557–565.

68 De Baere T, Risse O, Kuoch V, Dromain C, Sengel C, Smayra T, Gamal El Din M, Letoublon C, Elias D: Adverse events during radiofrequency treatment of 582 hepatic tumors. AJR Am J Roentgenol 2003;181:695–700.

69 Goldberg SN, Charboneau JW, Dodd GD 3rd, Dupuy DE, Gervais DA, Gillams AR, Kane RA, Lee FT Jr, Livraghi T, McGahan JP, et al: Image-guided tumor ablation: proposal for standardization of terms and reporting criteria. Radiology 2003;228:335–345.

70 Chen TM, Huang PT, Lin LF, Tung JN: Major complications of ultrasound-guided percutaneous radiofrequency ablations for liver malignancies: single center experience. J Gastroenterol Hepatol 2008;23:e445–e450.

71 Livraghi T, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN: Treatment of focal liver tumors with percutaneous radiofrequency ablation: complications encountered in a multicenter study. Radiology 2003;226:441–451.

72 Curley SA, Marra P, Beaty K, Ellis LM, Vauthey JN, Abdalla EK, Scaife C, Raut C, Wolff R, Choi H, et al: Early and late complications after radiofrequency ablation of malignant liver tumors in 608 patients. Ann Surg 2004;239:450–458.

73 Kasugai H, Osaki Y, Oka H, Kudo M, Seki T: Severe complications of radiofrequency ablation therapy for hepatocellular carcinoma: an analysis of 3,891 ablations in 2,614 patients. Oncology 2007;72(suppl 1):72–75.

74 Stigliano R, Marelli L, Yu D, Davies N, Patch D, Burroughs AK: Seeding following percutaneous diagnostic and therapeutic approaches for hepatocellular carcinoma. What is the risk and the outcome? Seeding risk for percutaneous approach of HCC. Cancer Treat Rev 2007;33:437–447.

75 Latteri F, Sandonato L, Di Marco V, Parisi P, Cabibbo G, Lombardo G, Galia M, Midiri M, Latteri MA, Craxi A: Seeding after radiofrequency ablation of hepatocellular carcinoma in patients with cirrhosis: a prospective study. Dig Liver Dis 2008;40:684–689.

76 Imamura J, Tateishi R, Shiina S, Goto E, Sato T, Ohki T, Masuzaki R, Goto T, Yoshida H, Kanai F, et al: Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. Am J Gastroenterol 2008;103:3057–3062.

77 Perkins JD: Seeding risk following percutaneous approach to hepatocellular carcinoma. Liver Transpl 2007;13:1603.

78 Kong WT, Zhang WW, Qiu YD, Zhou T, Qiu JL, Zhang W, Ding YT: Major complications after radiofrequency ablation for liver tumors: analysis of 255 patients. World J Gastroenterol 2009;15:2651–2656.

79 Llovet JM, Vilana R, Bru C, Bianchi L, Salmeron JM, Boix L, Ganau S, Sala M, Pages M, Ayuso C, et al: Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. Hepatology 2001;33:1124–1129.

80 Curley SA, Izzo F, Ellis LM, Nicolas Vauthey J, Vallone P: Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. Ann Surg 2000;232:381–391.

81 De Baere T, Roche A, Amenabar JM, Lagrange C, Ducreux M, Rougier P, Elias D, Lasser P, Patriarche C: Liver abscess formation after local treatment of liver tumors. Hepatology 1996;23:1436–1440.

82 Chopra S, Dodd GD 3rd, Chanin MP, Chintapalli KN: Radiofrequency ablation of hepatic tumors adjacent to the gallbladder: feasibility and safety. AJR Am J Roentgenol 2003;180:697–701.

83 Giorgio A, Tarantino L, de Stefano G, Coppola C, Ferraioli G: Complications after percutaneous saline-enhanced radiofrequency ablation of liver tumors: 3-year experience with 336 patients at a single center. AJR Am J Roentgenol 2005;184:207–211.

84 Seki T, Tamai T, Ikeda K, Imamura M, Nishimura A, Yamashiki N, Nakagawa T, Inoue K: Rapid progression of hepatocellular carcinoma after transcatheter arterial chemoembolization and percutaneous radiofrequency ablation in the primary tumour region. Eur J Gastroenterol Hepatol 2001;13:291–294.

85 Koda M, Maeda Y, Matsunaga Y, Mimura K, Murawaki Y, Horie Y: Hepatocellular carcinoma with sarcomatous change arising after radiofrequency ablation for well-differentiated hepatocellular carcinoma. Hepatol Res 2003;27:163–167.

86 Portolani N, Tiberio GA, Ronconi M, Coniglio A, Ghidoni S, Gaverini G, Giulini SM: Aggressive recurrence after radiofrequency ablation of liver neoplasms. Hepatogastroenterology 2003;50:2179–2184.

87 Takada Y, Kurata M, Ohkohchi N: Rapid and aggressive recurrence accompanied by portal tumor thrombus after radiofrequency ablation for hepatocellular carcinoma. Int J Clin Oncol 2003;8:332–335.

88 Ruzzenente A, Manzoni GD, Molfetta M, Pachera S, Genco B, Donataccio M, Guglielmi A: Rapid progression of hepatocellular carcinoma after radiofrequency ablation. World J Gastroenterol 2004;10:1137–1140.

89 Kudo M, Sakaguchi Y, Chung H, Hatanaka K, Hagiwara S, Ishikawa E, Takahashi S, Kitai S, Inoue T, Minami Y, et al: Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation. A matched case-control study. Oncology 2007;72(suppl):132–138.

90 Kudo M: Impact of interferon therapy after curative treatment of hepatocellular carcinoma. Oncology 2008;75(suppl 1):30–41.

91 Ishikawa T: Secondary prevention of recurrence by interferon therapy after ablation therapy for hepatocellular carcinoma in chronic hepatitis C patients. World J Gastroenterol 2008;14:6140–6144.

92 Lencioni R, Crocetti L, Petruzzi P, Vignali C, Bozzi E, Della Pina C, Bargellini I, Cioni D, Oliveri F, De Simone P, et al: Doxorubicin-eluting bead-enhanced radiofrequency ablation of hepatocellular carcinoma: a pilot clinical study. J Hepatol 2008;49:217–222.

Oncology

Oncology 2010;78(suppl 1):148–153
DOI: 10.1159/000315244

Published online: July 8, 2010

Hepatic Arterial Infusion Chemotherapy Using Low-Dose 5-Fluorouracil and Cisplatin for Advanced Hepatocellular Carcinoma

Kazuomi Ueshima Masatoshi Kudo Masahiro Takita Tomoyuki Nagai Chie Tatsumi Taisuke Ueda Satoshi Kitai Emi Ishikawa Norihisa Yada Tatsuo Inoue Satoru Hagiwara Yasunori Minami Hobyung Chung

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Key Words
Hepatocellular carcinoma · Hepatic arterial infusion chemotherapy · Cisplatin · 5-Fluorouracil · Low-dose FP

Abstract
Background: Although hepatic arterial infusion chemotherapy (HAIC) using low-dose 5-fluorouracil (5-FU) and cisplatin (low-dose FP) is commonly used for advanced hepatocellular carcinoma (HCC) with vascular invasion in Japan, few reports have investigated the efficacy and safety of this approach. We investigated the efficacy and toxicity of HAIC using low-dose FP for patients with advanced HCC as a phase II trial. ***Methods:*** Low-dose FP consisted of a continuous arterial infusion of 5-FU (250–500 mg/day, 5 days/week, for the first 2 weeks) and cisplatin (10 mg/day, 5 days/week, for the first 2 weeks). Then, 5-FU (1,000 mg/body for 5 h) and cisplatin (10 mg/body) were administered once weekly. ***Results:*** In these patients treated with low-dose FP, the response rate was 38.5%, the median time to progression was 4.1 months (95% CI 2.1–6.1 months) and the median survival time was 15.9 months (95% CI 9.8–22.0 months). The most frequent adverse events were myelosuppression such as neutropenia or thrombocytopenia. ***Conclusions:*** HAIC using low-dose FP is an effective treatment option for locally advanced HCC. However, it is not well tolerated hematologically because of potent pancytopenia and poor hepatic reserve. Therefore, this regimen should be performed carefully with regular monitoring of hematological function.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Japan and the fifth most common cancer worldwide [1–3]. It was established in Japan that surveillance of high-risk groups, such as patients with hepatitis C virus (HCV) or hepatitis B virus (HBV), can enable the detection of HCC in the early stage. However, about 10% of patients with HCC are still detected in advanced stages [4]. In the early stage, HCC is treatable by standard therapies such as hepatic resection, radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE). Interferon therapy improves the prognosis of patients who are curatively treated by hepatic resection or RFA [5–9]. However, many patients require repeated treatment, and the standard therapies may not be effective in such patients. In the intermediate stage, TACE is considered to be the standard treatment, but TACE is often not curative and is commonly repeated. Moreover, TACE is contraindicated in patients with HCC

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0148$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

that has progressed into the portal vein, particularly the main trunk. In such cases, hepatic arterial infusion chemotherapy (HAIC) using low-dose 5-fluorouracil (5-FU) and cisplatin (low-dose FP) is often selected. HAIC has been established as a treatment option in Japan. However, there is little evidence of the efficacy of this method. Therefore, we performed a phase II trial to investigate the efficacy and toxicity of low-dose FP for patients with advanced HCC.

Materials and Methods

Patients

Fifty-two patients with advanced HCC, who were admitted to the Department of Gastroenterology and Hepatology, Kinki University School of Medicine, were enrolled in the current study between April 2004 and August 2006. Advanced HCC was considered as the presence of a portal vein tumor thrombus or HCC refractory to TACE. Written informed consent was obtained from each participant after explaining the advantages and risks of HAIC using low-dose FP.

Eligibility Criteria

The eligibility criteria of this therapy were as follows: (1) advanced HCC, which was uncontrollable with standard treatment such as TACE, or HCC with vascular invasion; (2) age under 80 years; (3) Eastern Cooperative Group performance status of 0 or 1; (4) Child-Pugh grade A or B; (5) encephalopathy degree 0; (6) leukocyte count >2,000 cells/mm^3, hemoglobin level >10 g/dl, platelet count >75,000 cells/mm^3; (7) serum creatinine <1.5 mg/dl, and (8) serum total bilirubin level <3.0 mg/dl.

HAIC was performed in cases in which the intrahepatic tumor threatened the patient's life, even in the presence of extrahepatic spread.

Catheter Placement

Angiography was performed from the right femoral artery using the Seldinger technique. Arteriography of the celiac trunk and the superior mesenteric artery was performed to detect HCC and its feeding artery, and arterioportography via the superior mesenteric artery was performed to evaluate the portal vein patency. To avoid gastrointestinal mucosal injury due to anticancer agents, arteries supplying the gastrointestinal tract were embolized by the metallic coils. A 5-Fr heparin-coated catheter was placed and the tip of the catheter was located in the gastroduodenal artery and fixed by the metallic coils. The side hole of the catheter was located at the common hepatic artery (GDA-fixed method) [10]. Another tip of the catheter was connected to the injection port system and implanted in a subcutaneous pocket in the right inguinal part in front of the femoral region.

Treatment

Cisplatin and 5-FU were administered via the implanted port system. 5-FU was administered continuously using an ambulatory balloon infusion pump (LV2; Baxter, Chicago, Ill., USA) at a dose of 250–500 mg/day for 5 days/week for the first 2 weeks. Cisplatin was administered manually at the dose of 10 mg/day for 5 days/week for the first 2 weeks (fig. 1). The dose of these drugs was reduced according to hepatic reserve. To prevent emesis caused by cisplatin, 5-HT3 antagonists were given. After 2 weeks, the dose of cisplatin was changed to 10 mg/body and 5-FU was administered using an ambulatory balloon infusion pump (LV50; Baxter) at the dose of 1,000 mg/body for 5 h every week until HCC progressed (requiring a change in treatment) or an unacceptable adverse event occurred.

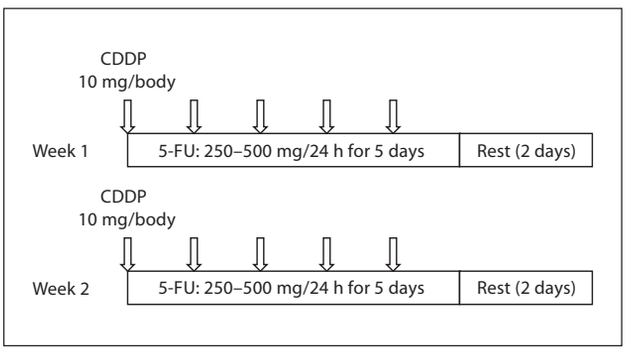


Fig. 1. Schedule of the low-dose FP during the first 2 weeks.

Evaluation and Statistical Analysis

Response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) [11] every 2 months on treatment. The overall survival time was calculated from the date of initiation of this therapy to the date of any cause or confirmed survival. The time to progression was calculated from the date of initiation of this therapy to the date of radiological progression. The overall survival and time to progression were analyzed using the Kaplan-Meier method. Statistical analysis was conducted using SPSS version 11.5.1 for Windows. Toxicity was assessed according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE) [12].

Results

Table 1 summarizes the clinical profiles of the 52 patients (46 males, 6 females) treated with low-dose FP. The mean age was 61.3 years (range 30–79; median age 63.5 years old). The numbers of patients at United International Consensus Committee (UICC) stages III, IVA and IVB were 10, 36 and 6, respectively. The number of patients at American Joint Committee of Cancer (AJCC) [13] stages II, IIIA, IIIB and IV were 9, 37, 2 and 4, respectively. Three patients were at an intermediate stage and 49 at an advanced stage based on Barcelona Clinic Liver Cancer (BCLC) [14] grading. Forty-two patients had macroscopic vascular invasion, including portal vein thrombosis. Four patients had extrahepatic spread including bone metasta-

Table 1. Patient characteristics

Age		61.3 (30–79)
Sex	male/female	46/6
Stage (UICC)	III/IVA/IVB	10/36/6
Stage (AJCC)	II/IIIA/IIIB/IV	9/37/2/4
Stage (BCLC)	B (intermediate)/ C (advanced)	3/49
MVI	yes/no	42/10
EHS	yes/no	4/48
Cause of disease	HBV/HCV/NBNC/ HBV+HCV	12/30/9/1
Child-Pugh class	A/B	38/14
ALB, g/dl	median (range)	3.5 (2.7–4.8)
BIL, mg/dl	median (range)	1.0 (0.3–2.5)
PT, %	median (range)	81.5 (48.3–120.0)

UICC = United International Consensus Committee; AJCC = American Joint Committee of Cancer; BCLC = Barcelona Clinic Liver Cancer; MVI = macroscopic vascular invasion; EHS = extrahepatic spread; NBNC = not infected with HBV and HCV.

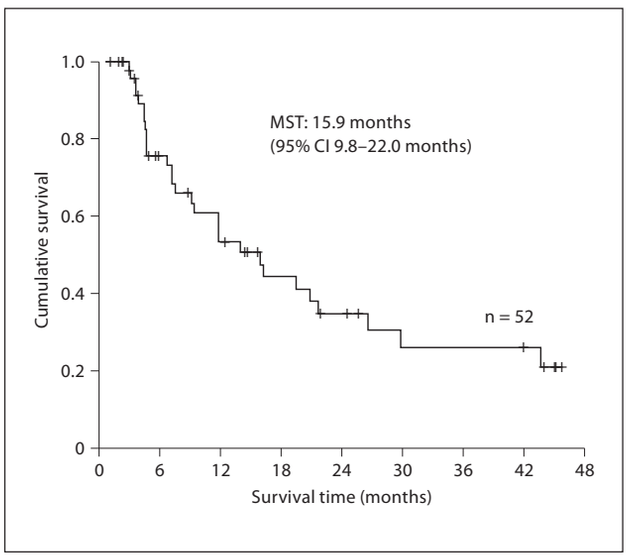


Fig. 2. Kaplan-Meier analysis of overall survival of 52 patients treated by HAIC with low-dose FP.

ses or lung metastases. Twelve patients were infected with HBV, 30 were infected with HCV, 9 were not infected with either HBV or HCV, and 1 was infected with HBV and HCV. Overall, 38 patients were at Child-Pugh grade A and 14 at Child-Pugh grade B.

Three patients (5.8%) withdrew during follow-up on their own accord and were not evaluated; therefore, tumor response could be assessed in 49 of 52 patients. In the intent-to-treat analysis (comprising all treated patients), 4 of 52 patients (7.7%) achieved complete response (CR) and 16 (30.8%) achieved partial response (PR). Stable disease (SD) was observed in 14 patients (26.9%) and progressive disease (PD) was observed in 15 patients (28.8%). Therefore, the objective response rate was 38.5%. The disease control rate (CR + PR + SD) was 65.4%.

The cumulative survival rate of the 52 patients is shown in figure 2. The 1-, 2- and 3-year cumulative survival rates were 53.3, 34.8, and 26.1%, respectively. The median survival time was 15.9 months (95% CI 9.8–22.0 months). The patients with PR or CR had a median survival of 40.7 months (95% CI 11.3–70.1), whereas the patients with SD or PD had a median survival of 6.8 months (95% CI 5.6–8.0 months). The overall survival (OS) of the patients with PR or CR was significantly longer than that of patients with SD or PD (median OS: 40.7 vs. 6.8 months; $p < 0.0001$) (fig. 3). The median time to progression was 4.1 months (95% CI 2.8–5.2 months) (fig. 4).

Adverse Effects

The treatment-related adverse effect was assessed in two categories: events potentially related to the anticancer agent and events potentially related to the implanted catheter system. The events related to the antitumor agent are summarized in table 2. Hematologic toxicities, including leukopenia, neutropenia, anemia, and thrombocytopenia, were relatively severe. Grade 3 leukocytopenia, neutropenia, anemia and thrombocytopenia were observed in 10 (19.2%), 12 (23.1%), 5 (9.6%), and 22 (42.3%) patients, respectively. Grade 4 thrombocytopenia without any associated bleeding event was observed in 2 patients (3.8%); 1 of these patients required thrombocyte transfusion. Grade 3 hyperbilirubinemia was observed in 7 patients (13.5%). These toxicities were improved by discontinuing the treatment or reducing the dose of anticancer agents. Non-hematological events were mild and well tolerated. Grade 1 anorexia, fatigue, mucositis and diarrhea were often observed. These events were managed by symptomatic therapy without discontinuing the treatment.

In terms of implanted catheter system-related complications, obstruction of the hepatic artery was observed in 1 patient. Anticoagulation therapy was performed but the obstruction did not improve. No infection of the catheter system was observed. No treatment-related deaths were observed.

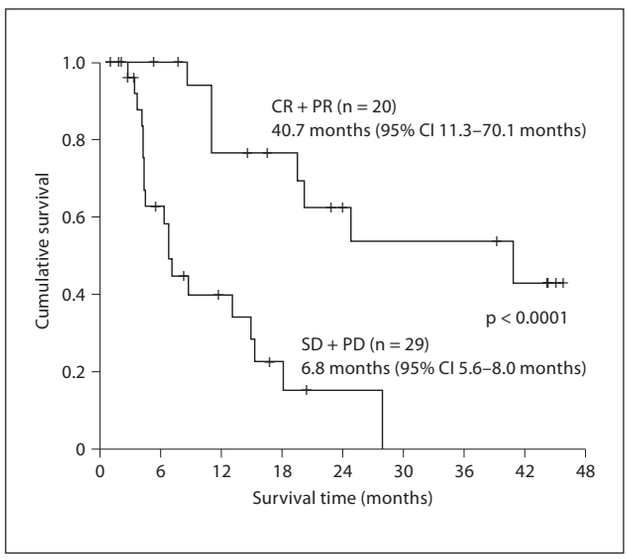


Fig. 3. The overall survival of patients with CR and PR was significantly longer than that of patients with SD and PD. The median overall survival was 40.7 months (95% CI 11.3–70.1 months) in the CR + PR group versus 6.8 months (95% CI 5.6–8.0 months) in the SD + PD group ($p < 0.0001$).

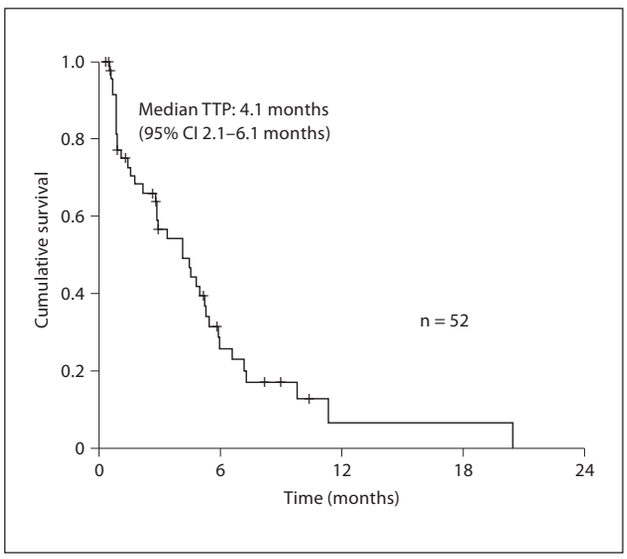


Fig. 4. Kaplan-Meier analysis of time to progression in 52 patients treated by HAIC using low-dose FP. The median time to progression was 4.1 months (95% CI 2.1–6.1).

Table 2. Adverse events

	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Leukocytes	12 (23.1)	16 (30.8)	10 (19.2)	0
Hemoglobin	30 (57.7)	10 (19.2)	5 (9.6)	0
Platelets	10 (19.2)	16 (30.8)	22 (42.3)	2 (3.8)
Neutrophil	5 (9.6)	14 (26.9)	12 (23.1)	0
Bilirubin	15 (28.8)	19 (36.5)	7 (13.5)	0
Anorexia	8 (15.4)	0	0	0
Fatigue	10 (19.2)	0	0	0
Fever	7 (13.5)	0	0	0
Mucositis	5 (9.6)	0	0	0
Diarrhea	4 (7.7)	0	0	0

Classified according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE).

Discussion

According to the Consensus-Based Treatment Algorithm for HCC of Japan Society of Hepatology [15], TACE is recommended if there are two or three tumors and their diameters exceed 3 cm, or if there are more than four tumors. However, once HCC has progressed into the portal vein, particularly the main trunk, TACE is contraindicated. Treatment with anticancer agents is necessary if the standard therapy is not indicated.

Chemotherapy as a treatment option for HCC has limitations, as follows: (1) HCC is a malignancy that is commonly less sensitive to anticancer agents, and (2) because of the pancytopenia and poor hepatic reserve caused by the underlying liver cirrhosis, it is not possible to administer adequate doses of anticancer agents to cause tumor shrinkage.

HAIC, a regional chemotherapy, offers a feasible approach to elicit a greater antitumor effect than systemic chemotherapy and can reduce toxicity against other systemic organs [16]. In addition, the HCC tends to remain in the liver, even if it advances. Accordingly, HAIC is the most suitable treatment option for locally advanced HCC.

On the other hand, there are several problems associated with HAIC, as follows: (1) skill is required to appropriately insert the catheter; (2) catheter placement is very invasive for patients; (3) infection may occur via the catheter system, and (4) the catheter system or hepatic artery may become obstructed [17]. In this study, 1 patient experienced obstruction of the hepatic artery. If injection into the port system is difficult or if the patient has any

complaint about the gastrointestinal tract, obstruction of the catheter system or hepatic artery should be considered and examined.

The pharmacokinetic rationale of HAIC using low-dose FP can be divided into two concepts. The first is the role of cisplatin as a biochemical modulator, and the second is the dose and duration of 5-FU administration.

Low-dose FP consists of a combination of low-dose cisplatin plus 5-FU, because of their synergistic effects [18, 19]. This combination is frequently used in the treatment of gastrointestinal tract malignancies. Cisplatin has a wide spectrum of antitumor effects in various malignancies. In combination with 5-FU, cisplatin plays a role as a modulator rather than an effector, and enhances the antitumor effect of 5-FU by increasing the intracellular concentration of reduced folate [20].

5-FU is also widely used to treat various malignancies. The advantage of continuous arterial infusion of 5-FU is that 5-FU acts time-dependently on tumor cells. It was reported that administration of lower doses of 5-FU for longer times was more effective in producing direct cytotoxic effects in human tumor cells than when administered at higher doses for shorter times [21]. Many investigators have reported the efficacy of this combination therapy for advanced HCC [22–25]. Okuda et al. [26] reported that the CR rate and effective response rate of HAIC using cisplatin and 5-FU were 29.0 and 71.0%, respectively. Meanwhile, Ando et al. [27] reported that the response rate of HAIC using low-dose cisplatin and 5-FU was 48.0%.

In this study, the objective response rate for low-dose FP was 38.5% and successful disease control was achieved in 65.4% of patients. This result is relatively high considering that these patients were contraindicated to standard therapies such as hepatic resection, RFA or TACE. In addition, the prognosis of the patients who achieved CR or PR was markedly improved.

Most patients with HCC have poor hepatic reserve and pancytopenia caused by underlying viral-related cirrhosis. In this study, grade 3–4 hematological toxicities were relatively common, but no subjective symptom was observed and these toxicities were improved by discontinuing the treatment. Non-hematological toxicities such as anorexia, fatigue and fever were observed but were not severe. Thus, it seems that HAIC will not deteriorate patients' quality of life.

However, HAIC has several limitations, as follows: (1) HAIC is not effective for patients with extrahepatic spread and (2) HAIC cannot be performed if the hepatic artery is obstructed. In such cases, systemic chemotherapy is required. Sorafenib, a multikinase inhibitor, was recently introduced for unresectable HCC.

Sorafenib is a low-molecular-weight compound discovered by screening inhibitors of Raf kinase. It exhibits strong inhibitory activity for tumor progression and angiogenesis [28, 29]. Positive results of a phase III study for HCC (SHARP trial) [30] has had a marked impact on the treatment strategy for HCC. Therefore, sorafenib will likely be used in various stages of HCC, and various clinical trials such as in an adjuvant or combination setting are ongoing.

It is still unclear whether HAIC using low-dose FP or systemic chemotherapy using sorafenib should be used in patients with vascular invasion, such as in the presence of a portal vein tumor thrombus, without extrahepatic spread. Our results indicate that the overall survival of patients with CR and PR was significantly longer than that of patients with SD and PD. The SHARP trial [30] revealed that sorafenib prolonged the overall survival and time to progression, but the response rate of sorafenib was extremely low (CR and PR were 0 and 2%, respectively) compared with that of HAIC using low-dose FP (CR and PR were 7.7 and 30.8%, respectively). Accordingly, we suggest that HAIC using low-dose FP might be more efficacious than systemic chemotherapy using sorafenib in this clinical setting.

HAIC using low-dose FP is an effective treatment option for locally advanced HCC and offers advantages over sorafenib, such as tumor shrinkage. However, low-dose FP may not be well tolerated hematologically because of potent pancytopenia and poor hepatic reserve. Therefore, this regimen should be performed carefully with regular monitoring of hematological function. Sorafenib in combination with HAIC using low-dose FP might provide greater clinical efficacy for advanced HCC and we have started a phase I/II study to investigate this approach.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 El-Serag HB: Epidemiology of hepatocellular carcinoma. Clin Liver Dis 2001;5:87–107.
2 El-Serag HB: Hepatocellular carcinoma: an epidemiologic view. J Clin Gastroenterol 2002;35:S72–S78.
3 Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB: Hepatitis C infection and the increasing incidence of hepatocellular carcinoma: a population-based study. Gastroenterology 2004;127:1372–1380.
4 Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, Takayasu K, Nakanuma Y, Makuuchi M, Matsuyama Y, Monden M, Kudo M: Report of the 17th Nationwide Follow-Up Survey of Primary Liver Cancer in Japan. Hepatol Res 2007;37:676–691.
5 Suou T, Mitsuda A, Koda M, Matsuda H, Maruyama S, Tanaka H, Kishimoto Y, Kohno M, Hirooka Y, Kawasaki H: Interferon-α inhibits intrahepatic recurrence in hepatocellular carcinoma with chronic hepatitis C: a pilot study. Hepatol Res 2001;20:301–311.
6 Hung CH, Lee CM, Wang JH, Tung HD, Chen CH, Lu SN: Antiviral therapy after non-surgical tumor ablation in patients with hepatocellular carcinoma associated with hepatitis C virus. J Gastroenterol Hepatol 2005;20:1553–1559.
7 Jeong S, Aikata H, Katamura Y, Azakami T, Kawaoka T, Saneto H, Uka K, Mori N, Takaki S, Kodama H, Waki K, Imamura M, Shirakawa H, Kawakami Y, Takahashi S, Chayama K: Low-dose intermittent interferon-α therapy for HCV-related liver cirrhosis after curative treatment of hepatocellular carcinoma. World J Gastroenterol 2007;13:5188–5195.
8 Kudo M, Sakaguchi Y, Chung H, Hatanaka K, Hagiwara S, Ishikawa E, Takahashi S, Kitai S, Inoue T, Minami Y, Ueshima K: Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation. A matched case-control study. Oncology 2007;72(suppl 1):132–138.
9 Someya T, Ikeda K, Saitoh S, Kobayashi M, Hosaka T, Sezaki H, Akuta N, Suzuki F, Suzuki Y, Arase Y, Kumada H: Interferon lowers tumor recurrence rate after surgical resection or ablation of hepatocellular carcinoma: a pilot study of patients with hepatitis b virus-related cirrhosis. J Gastroenterol 2006;41:1206–1213.
10 Shindoh N, Ozaki Y, Kyogoku S, Yamana D, Sumi Y, Katayama H: Stabilization of a percutaneously implanted port catheter system for hepatic artery chemotherapy infusion. Cardiovasc Intervent Radiol 1999;22:344–347.
11 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–216.
12 Japanese translation of Common Terminology Criteria for Adverse Events (CTCAE), and instructions and guidelines. Int J Clin Oncol 2004;9(suppl 3):1–82.
13 Greene FL: American Joint Committee on Cancer: AJCC Cancer Staging Atlas. New York, Springer, 2006.
14 Llovet JM, Bru C, Bruix J: Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19:329–338.
15 Kudo M, Okanoue T: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(suppl 1):2–15.
16 Collins JM: Pharmacologic rationale for regional drug delivery. J Clin Oncol 1984;2:498–504.
17 Seki H, Kimura M, Yoshimura N, Yamamoto S, Ozaki T, Sakai K: Hepatic arterial infusion chemotherapy using percutaneous catheter placement with an implantable port: assessment of factors affecting patency of the hepatic artery. Clin Radiol 1999;54:221–227.
18 Scanlon KJ, Newman EM, Lu Y, Priest DG: Biochemical basis for cisplatin and 5-fluorouracil synergism in human ovarian carcinoma cells. Proc Natl Acad Sci USA 1986;83:8923–8925.
19 Shirasaka T, Shimamoto Y, Ohshimo H, Saito H, Fukushima M: Metabolic basis of the synergistic antitumor activities of 5-fluorouracil and cisplatin in rodent tumor models in vivo. Cancer Chemother Pharmacol 1993;32:167–172.
20 Scanlon KJ, Safirstein RL, Thies H, Gross RB, Waxman S, Guttenplan JB: Inhibition of amino acid transport by *cis*-diammine-dichloroplatinum(II) derivatives in l1210 murine leukemia cells. Cancer Res 1983;43:4211–4215.
21 Calabro-Jones PM, Byfield JE, Ward JF, Sharp TR: Time-dose relationships for 5-fluorouracil cytotoxicity against human epithelial cancer cells in vitro. Cancer Res 1982;42:4413–4420.
22 Lai YC, Shih CY, Jeng CM, Yang SS, Hu JT, Sung YC, Liu HT, Hou SM, Wu CH, Chen TK: Hepatic arterial infusion chemotherapy for hepatocellular carcinoma with portal vein tumor thrombosis. World J Gastroenterol 2003;9:2666–2670.
23 Toyoda H, Nakano S, Kumada T, Takeda I, Sugiyama K, Osada T, Kiriyama S, Suga T, Takahashi M: The efficacy of continuous local arterial infusion of 5-fluorouracil and cisplatin through an implanted reservoir for severe advanced hepatocellular carcinoma. Oncology 1995;52:295–299.
24 Yamasaki T, Kimura T, Kurokawa F, Aoyama K, Ishikawa T, Tajima K, Yokoyama Y, Takami T, Omori K, Kawaguchi K, Tsuchiya M, Terai S, Sakaida I, Okita K: Prognostic factors in patients with advanced hepatocellular carcinoma receiving hepatic arterial infusion chemotherapy. J Gastroenterol 2005;40:70–78.
25 Lim TY, Cheong JY, Cho SW, Sim SJ, Kim JS, Choi SJ, Choi JW, Kwon HC, Lee KM, Kim JK, Won JH, Yoo BM, Lee KJ, Hahm KB, Kim JH: Effect of low-dose 5-fluorouracil and cisplatin intra-arterial infusion chemotherapy in advanced hepatocellular carcinoma with decompensated cirrhosis (in Korean). Korean J Hepatol 2006;12:65–73.
26 Okuda K, Tanaka M, Shibata J, Ando E, Ogata T, Kinoshita H, Eriguchi N, Aoyagi S, Tanikawa K: Hepatic arterial infusion chemotherapy with continuous low-dose administration of cisplatin and 5-fluorouracil for multiple recurrence of hepatocellular carcinoma after surgical treatment. Oncol Rep 1999;6:587–591.
27 Ando E, Tanaka M, Yamashita F, Kuromatsu R, Yutani S, Fukumori K, Sumie S, Yano Y, Okuda K, Sata M: Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. Cancer 2002;95:588–595.
28 Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA: BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–7109.
29 Chang YS, Adnane J, Trail PA, Levy J, Henderson A, Xue D, Bortolon E, Ichetovkin M, Chen C, McNabola A, Wilkie D, Carter CA, Taylor IC, Lynch M, Wilhelm S: Sorafenib (BAY 43-9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. Cancer Chemother Pharmacol 2007;59:561–574.
30 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Haussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.

Oncology

Oncology 2010;78(suppl 1):154–166
DOI: 10.1159/000315245

Published online: July 8, 2010

Positioning of a Molecular-Targeted Agent, Sorafenib, in the Treatment Algorithm for Hepatocellular Carcinoma and Implication of Many Complete Remission Cases in Japan

Masatoshi Kudo Kazuomi Ueshima

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Key Words

Hepatocellular carcinoma · Liver cancer treatment algorithm · Molecular-targeted therapy · Sorafenib · Complete remission · Tyrosine kinase inhibitor

Abstract

Sorafenib, a molecular-targeted agent that inhibits tumor cell proliferation and angiogenesis by inhibiting RAF serine-threonine kinase and VEGF, PDGF, Flt-3, c-Kit receptor tyrosine kinase, was approved in Europe and North America in 2007 and in Japan on May 20, 2009. In the 10 months since its approval, sorafenib has been prescribed for more than 3,700 patients with advanced hepatocellular carcinoma (HCC), and its efficacy has been confirmed in many cases. According to the consensus statements of the Japan Society of Hepatology in 2010, sorafenib is recommended for advanced HCC with extrahepatic spread or major vascular invasion such as invasion of the 1st branch of the portal vein or the main portal branch of the portal vein in patients with Child-Pugh A liver function. In addition to that, transcatheter arterial chemoembolization (TACE) or hepatic arterial infusion chemotherapy (HAIC) refractory HCC patients with Child-Pugh A liver function are also candidates of sorafenib monotherapy as a second-line treatment option. To date, 15 cases with complete remission (CR) to sorafenib in metastatic advanced HCC patients have been reported in Japan, an event that is rarely reported in other countries. Of the 90 cases treated by ourselves, 2 achieved CR. Factors indicating systemic cancer spread, including multiple liver lesions, lymph node metastases, adrenal metastases, lung metastases and vascular invasion, were completely absent in both cases of CR by 2 and 1 year, respectively. Similarly, three tumor markers (AFP, PIVKA-II, and AFP-L3) completely returned to normal values. Although cases of CR are rare, it seems that there might be racial differences in terms of gene mutations. Clinical trials for other molecular-targeted agents, including sunitinib, brivanib, or linifanib, are ongoing and their outcomes are eagerly awaited. According to a subanalysis of the SHARP study, it is expected that sorafenib in combination with resection, ablation, TACE or HAIC will markedly prolong the overall survival in early-, intermediate- and advanced-stage HCCs.

Introduction

Sorafenib is a multikinase inhibitor that targets tumor growth (RAF-MEK-ERK) and angiogenesis (VEGFR, PDGER) signal transduction pathways. Two global phase III trials (SHARP [1] and Asia-Pacific Study [2]) showed

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0154$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3149, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

Table 1. Comparison between the Asia-Pacific and SHARP studies

End point	Asia-Pacific		SHARP	
	hazard ratio (95% CI)	p value	hazard ratio (95% CI)	p value
OS	0.68 (0.50–0.93)	0.014	0.69 (0.55–0.87)	<0.001
TTSP	0.90 (0.67–1.22)	0.498	1.08 (0.88–1.31)	0.768
TTP	0.57 (0.42–0.79)	<0.001	0.58 (0.45–0.74)	<0.001
PFS	0.62 (0.46–0.82)	<0.001	0.65 (0.52–0.79)	<0.001

OS = Overall survival; TTSP = time to symptomatic progression; TTP = time to progression; PFS = progression-free survival.

that sorafenib prolonged the survival of patients with advanced hepatocellular carcinoma (HCC). The results of these studies were rapidly disseminated worldwide and were enthusiastically accepted by physicians specializing in liver cancer treatment. Based on the positive results of the SHARP trial [1], the EU and USA approved sorafenib for advanced HCC in October and November 2007, respectively.

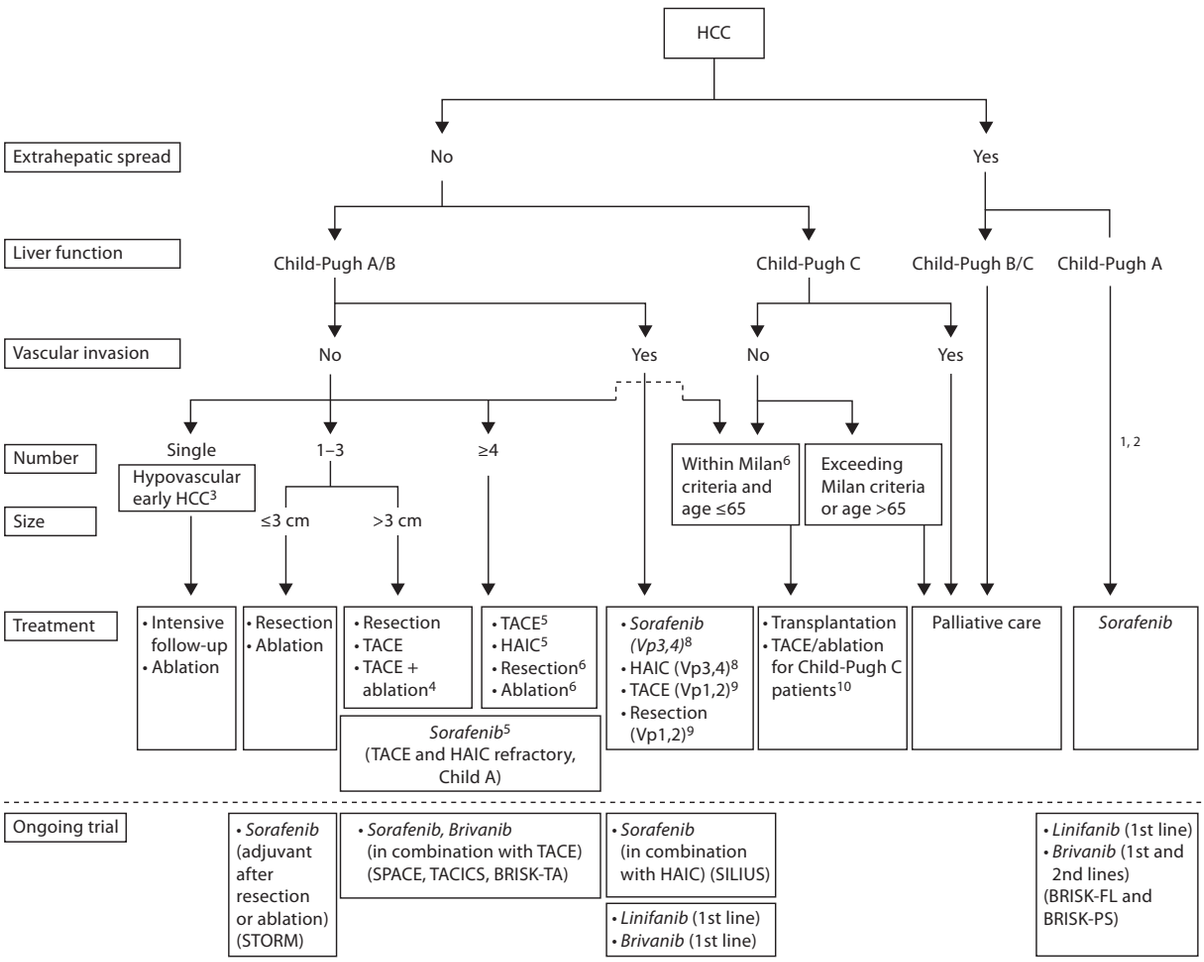
The following four factors may explain why the results of this study were well accepted worldwide, particularly in Japan. First, the study quashed the strong assumption or belief held by physicians specialized in liver cancer that systemic chemotherapy is not effective for liver cancer, unlike other cancers. Although no effective systemic chemotherapeutic drug was available before the introduction of sorafenib, very effective locoregional therapy was available for unresectable HCC, unlike other cancers, and survival for locoregional therapy was similar to that for resection. This is a major difference between liver cancer and other cancers. The commonly held view for liver cancer therapy is that 'treatment by physically destroying cancer cells' is effective and, thus, preferred over chemotherapy. Locoregional interventional treatments include transcatheter arterial chemoembolization (TACE), ethanol injection therapy, microwave coagulation therapy, and radiofrequency ablation (RFA), and physically destroying cancer cells.

In addition, HCC is often complicated by liver cirrhosis, and the accompanying pancytopenia rapidly results in excess toxicity, such as bone marrow suppression, of systemically administered cytotoxic anticancer drugs, limiting their doses and reducing their therapeutic effect. Thus, poor tolerability is a significant problem of systemic anticancer therapy and explains the poor efficacy of these drugs for HCC.

Table 2. Molecular-targeted agents for HCC under development

Agent	Anti-angiogenic targets					Antiproliferative targets				Antiepigenetic targets				Developmental status	Company
	VEGF	FGF	VEGFR	PDGFR	FGFR	EGFR	Raf	MEK	mTOR	RAR	RXR	HDAC	Heparanase		
Sorafenib[1] (Nexavar)			●	●			●							Approved	Bayer
Sunitinib[1] (Sutent)			●	●										Stopped	Pfizer
NIK-333 (Acyclic Retinoid)										●	●			Phase II/III complete	Kowa
Brivanib			●		●									Phase III ongoing	Bristol-Myers Squibb
TSU-68			●	●	●									Phase II complete	Taiho
TAC-101										●				Phase II stopped	Taiho
Erlotinib (Tarceva)						●								Phase II complete	Roche
Bevacizumab (Avastin)	●													Phase II ongoing	GenentechA
AZD2171 (Cediranib)			●											Phase II recruiting	AstraZeneca
Gefitinib (Iressa)						●								Phase II complete	AstraZeneca
Lapatinib						●								Phase II ongoing	GlaxoSmithKline
Thalidomide	●													Phase II ongoing	TTY BioPharm
ZD6474 (Zactima)			●			●								Phase II ongoing	AstraZeneca
AZD6244								●						Phase II ongoing	AstraZeneca
PI-88	●	●											●	Phase II complete	Progen
Cecuximab						●								Phase II complete	Merck
RAD001									●					Phase III initiated	Novartis
PXD101 (Belinostat)												●		Phase I/II ongoing	Curagen

[1] Sorafenib and sunitinib also have antiproliferative effects through multi-tyrosine kinase inhibition.
Sources: Trial Trove, ClinicalTrials. Gov (NCI), Evaluate Pharma, IMS Knowledge Link, Esplcom, IDdB3, BioPharm Insight, MedTrack.



1 Treatment should be performed as if extrahepatic spread were negative, when extrahepatic spread is not regarded as a prognostic factor.

2 Sorafenib is the first choice of treatment in this setting as a standard of care.

3 Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early HCC, (2) when the nodules show decreased uptake on Gd-EOB-MRI, or (3) when the nodules show decreased portal flow by CTAP, since these nodules are known to frequently progress to the typical advanced HCC.

4 Even for HCC nodules >3 cm in diameter, combination therapy of TACE and ablation is frequently performed when resection is not indicated.

5 TACE is the first choice of treatment in this setting. HAIC using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5-FU+CDDP) or intra-arterial 5-FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE refractory patients.

6 Resection is sometimes performed even when there are >4 nodules. Furthermore, ablation is sometimes performed in combination with TACE.

7 Milan criteria: tumor size ≤3 cm and tumor numbers ≤3; or a solitary tumor ≤5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients.

8 Sorafenib and HAIC are recommended for HCC patients with Vp3 (portal invasion at the first portal branch) or Vp4 (portal invasion at the main portal branch).

9 Resection and TACE is frequently performed when portal invasion is minimal such as Vp1 (portal invasion at the third or more peripheral portal branch) or Vp2 (portal invasion at the second portal branch).

10 Local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated as there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (<3.0 mg/dl). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue.

Fig. 1. Consensus-based treatment algorithm for hepatocellular carcinoma proposed by the Japan Society of Hepatology in 2010. The positioning of sorafenib and the ongoing trials on sorafenib or other molecular-targeted agents are shown.

Table 3. Molecular-targeted agents for hepatocellular carcinoma: study results

Agent	Type	Target	Number of patients	RR %	PFS months	TTP months	OS months	References
Phase III								
Sorafenib	s.m.	C-Raf, B-Raf, PDGFR, VEGFR	602 (299*)	2.0	–	5.5	10.7	Llovet et al. [1], 2008
			271 (150*)	3.3	–	2.8	6.5	Cheng et al. [2], 2009
Phase II								
Sorafenib	s.m.	C-Raf, B-Raf, PDGFR, VEGFR	137	2.2	–	5.5	9.2	Abou-Alfa [18], 2006
Sunitinib	s.m.	VEGFR, PDGFR, SCFR, FLT3	37	2.7	3.7	5.3	8.0	Faivre et al. [15], 2007
			34	2.9	3.9	4.1	9.8	Zhu et al. [16], 2009
Brivanib	s.m.	VEGFR, FGFR	55	n.r.	–	2.8	10	Raoul [19], 2009
Linifanib	s.m.	VEGFR, PDGFR	44	6.8	–	5.7	9.3	Toh [20], 2009
Bevacizumab	MoAb	VEGF	46	13	6.9	–	12.4	Siegel [21], 2008
Erlotinib	s.m.	EGFR	38	9	–	3.2	13	Philip [22], 2005
			40	0	–	–	10.7	Thomas [23], 2007
Gefitinib	s.m.	EGFR	31	3.2	2.8	–	6.5	O'Dwyer [24], 2006
Lapatinib	s.m.	EGFR	40	5	2.3	–	6.2	Ramanathan [25], 2009
			26	0	1.9	–	12.6	Bekaii-Saab [26], 2009
Cetuximab	MoAb	EGFR	30	0	1.4	–	9.6	Zhu [27], 2007

n.r. = Not reported; s.m. = small molecule; MoAb = monoclonal antibody.

Table 4. Subanalysis of the SHARP study

	Advanced HCC with vascular invasion or extrahepatic spread	Advanced HCC without vascular invasion or extrahepatic spread
Hazard ratio	0.77 (95% CI 0.60–0.99)	0.52 (95% CI 0.32–0.85)
Median overall survival (MST)		
Sorafenib, months	8.9 (n = 209; 95% CI 7.6–10.3)	14.5 (n = 90; 95% CI 14.0–N/E)
Placebo, months	6.7 (n = 212; 95% CI 5.2–8.0)	10.2 (n = 91; 95% CI 8.6–15.5)

N/E = Not evaluable. Sherman et al., ASCO 2008.

Second, although Japanese HCC specialists have been active, believing that their treatment historically leads the world in liver cancer therapy since most of the treatment options including TACE, ablation, hepatic arterial infusion chemotherapy (HAIC), and systematic hepatectomy (anatomical resection) were invented in Japan, there has been no effective treatment for advanced-stage HCC with extrahepatic spread for the reason described above. Therefore, the finding that sorafenib prolongs the survival of patients with advanced-stage HCC, particularly those with distant metastases, was unexpected and surprising.

Third, unlike the current developmental process for cytotoxic anticancer agents, sorafenib was the first drug to have been developed by identifying the target molecule through researching the molecular mechanisms involved in carcinogenesis and progression, resulting in drug development. Although it is well known that molecular-targeted agents for lung cancer (gefitinib, erlotinib), renal cancer (sorafenib, sunitinib), and colorectal cancer (bevacizumab, cetuximab) have been introduced into clinical practice, liver cancer specialists never expected that such a marked survival-prolonging effect could be achieved by a drug for HCC, which is a completely different situation from solid tumors in other organs as described above.

Fourth, the results of the SHARP and Asia-Pacific studies dispelled the common belief that the response rate is a

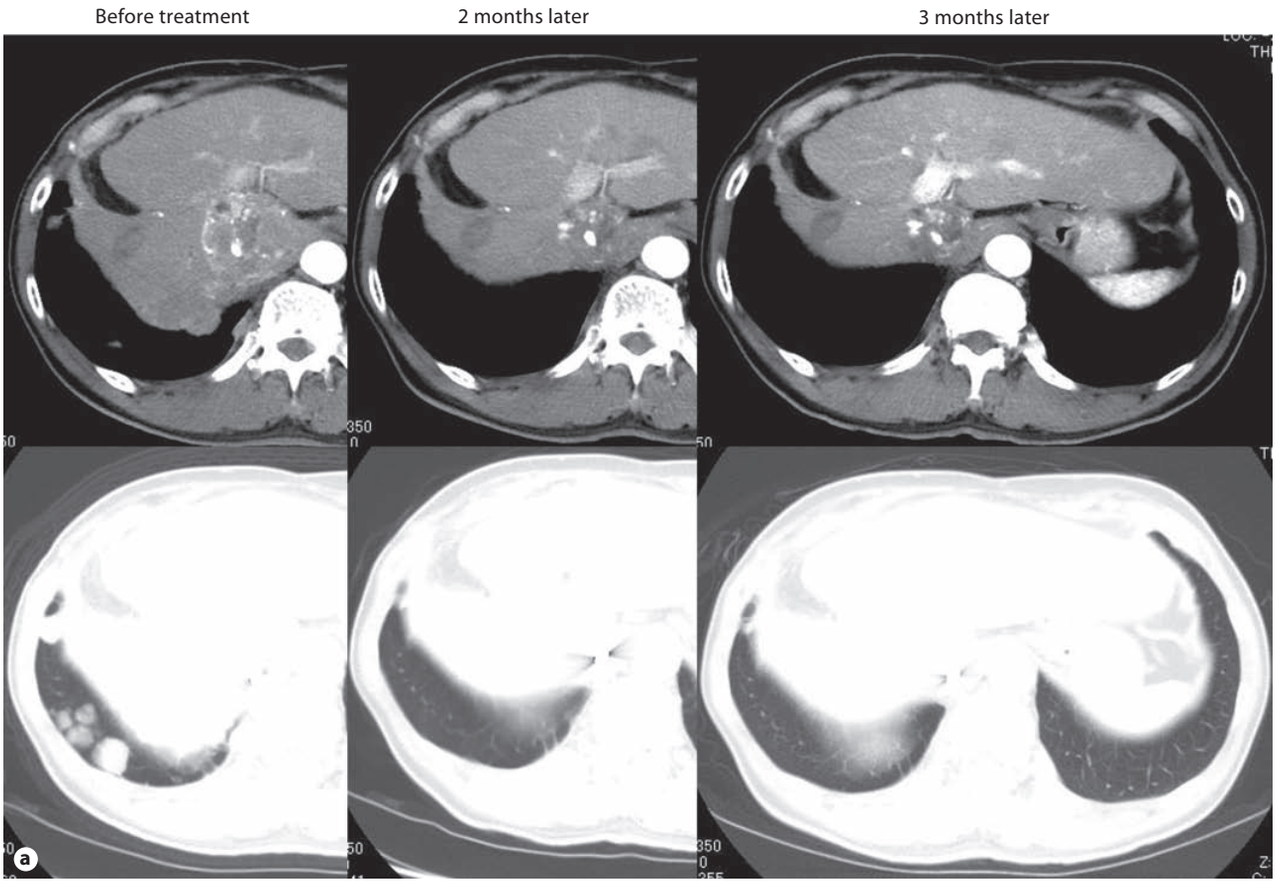


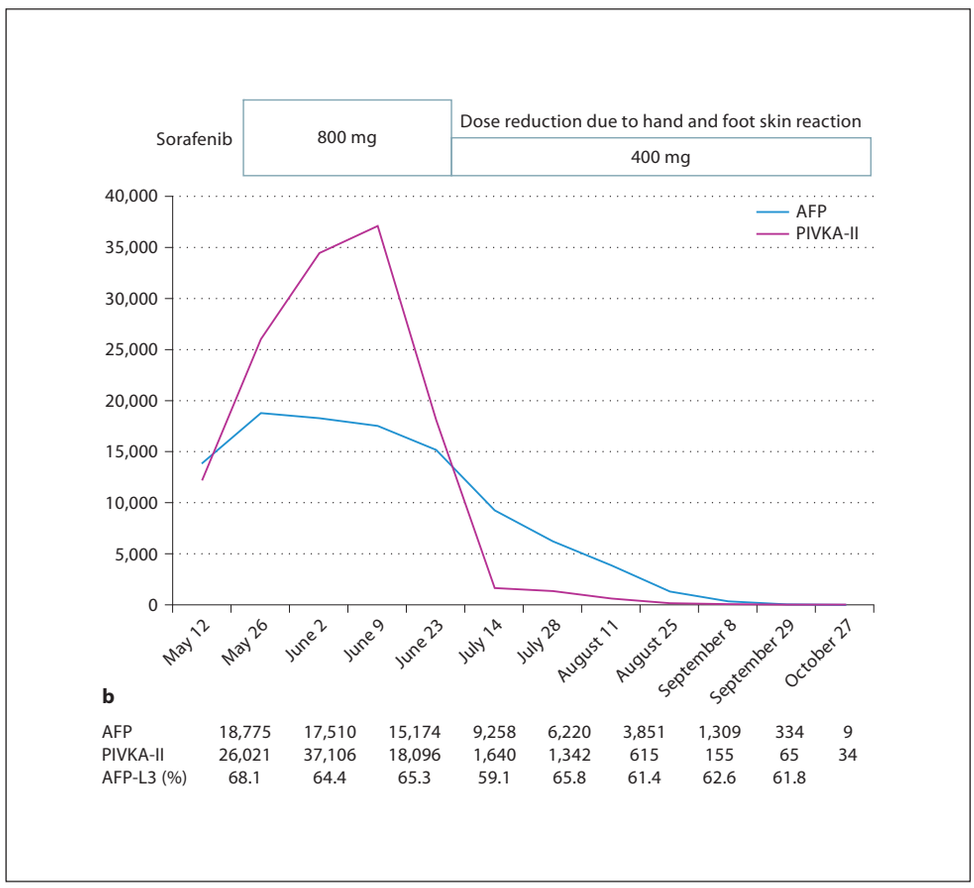
Fig. 2. Complete remission case 1. A 68-year-old male with chronic hepatitis B and stage IVB HCCs and Child-Pugh A liver function. **a** In 2004, the patient underwent surgery followed by nine sessions of TACE. In 2009, HCC invasions were found in the inferior vena cava and multiple metastases were found in the lung. Sorafenib monotherapy (800 mg) was then started. 2 months later, all tumors including a tumor in the inferior vena cava and lung disappeared completely.

surrogate of survival. Physicians treating liver cancer have endeavored to increase the local control rate, believing that the presence of a tumor response prolongs survival, while a poor response indicates treatment failure of TACE, RFA, or HAIC. However, the results of the SHARP study proposed a new concept: patients live longer on molecular-targeted therapy, even though the objective response rate is low, leading to a paradigm shift in liver cancer therapy.

In this review, we discuss the positioning of sorafenib in the treatment algorithm in Japan, complete remission (CR) cases treated with sorafenib, and the future perspectives including current ongoing clinical trials of molecular-targeted agents.

Mechanism of Action of Sorafenib and Results of Recent Studies

The mitogen-activated protein kinase (MAPK) cascade, located downstream of growth factor receptors, plays an important role in cell growth and survival. Raf protein is an important regulatory factor in this cascade, and sorafenib was discovered by screening for inhibitors of Raf protein activity [3, 4]. Sorafenib is a potent inhibitor not only for the RAF isoforms c-RAF (RAF1) and wild-type and mutant (V600E) b-RAF but also vascular endothelial growth factor receptor-2 (VEGFR-2), VEGFR-3, platelet-derived growth factor receptor



Color version available online

b

AFP	18,775	17,510	15,174	9,258	6,220	3,851	1,309	334	9
PIVKA-II	26,021	37,106	18,096	1,640	1,342	615	155	65	34
AFP-L3 (%)	68.1	64.4	65.3	59.1	65.8	61.4	62.6	61.8	

Fig. 2. Complete remission case 1. A 68-year-old male with chronic hepatitis B and stage IVB HCCs and Child-Pugh A liver function. **b** Clinical course of the tumor markers. The AFP and PIVKA-II levels and the AFP-L3 fraction markedly decreased and normalized during sorafenib treatment. The patient is now under long-term treatment with sorafenib at 400 mg/day, and the HCC has not recurred for more than 1 year.

(PDGFR) and Fms-related tyrosine kinase-3 (Flt-3), which are involved in angiogenesis and are receptor tyrosine kinases involved in cell growth. Thus, sorafenib is a multikinase inhibitor that exhibits multiple effects: it acts directly on cancer cells to inhibit their growth, and affects the surrounding vascular endothelial cells to inhibit angiogenesis [5–10].

In the SHARP [1] and Asia-Pacific [2] studies, the median overall survival with sorafenib was 10.7 months (placebo group 7.9 months; $p < 0.01$) in the SHARP study and 6.5 months in the Asia-Pacific study (placebo group 4.2 months; $p < 0.01$), showing an apparent difference between the 2 studies. However, the hazard ratios for overall survival, time to progression, and progression-free survival were similar in both studies (table 1). Overall, sorafenib appeared to prolong patients' survival (table 1). The Asia-Pacific study tended to include more patients with advanced stage cancer compared with the SHARP study. In the SHARP study, approximately 30% of the patients did not exhibit distant metastases or vascular invasion, suggesting that patients in an intermediate stage, who are usually candidates for TACE, were included in the SHARP study. Taken together, the poorer conditions of the Asian patients in the Asia-Pacific study may at least partly explain the shorter overall survival in that study.

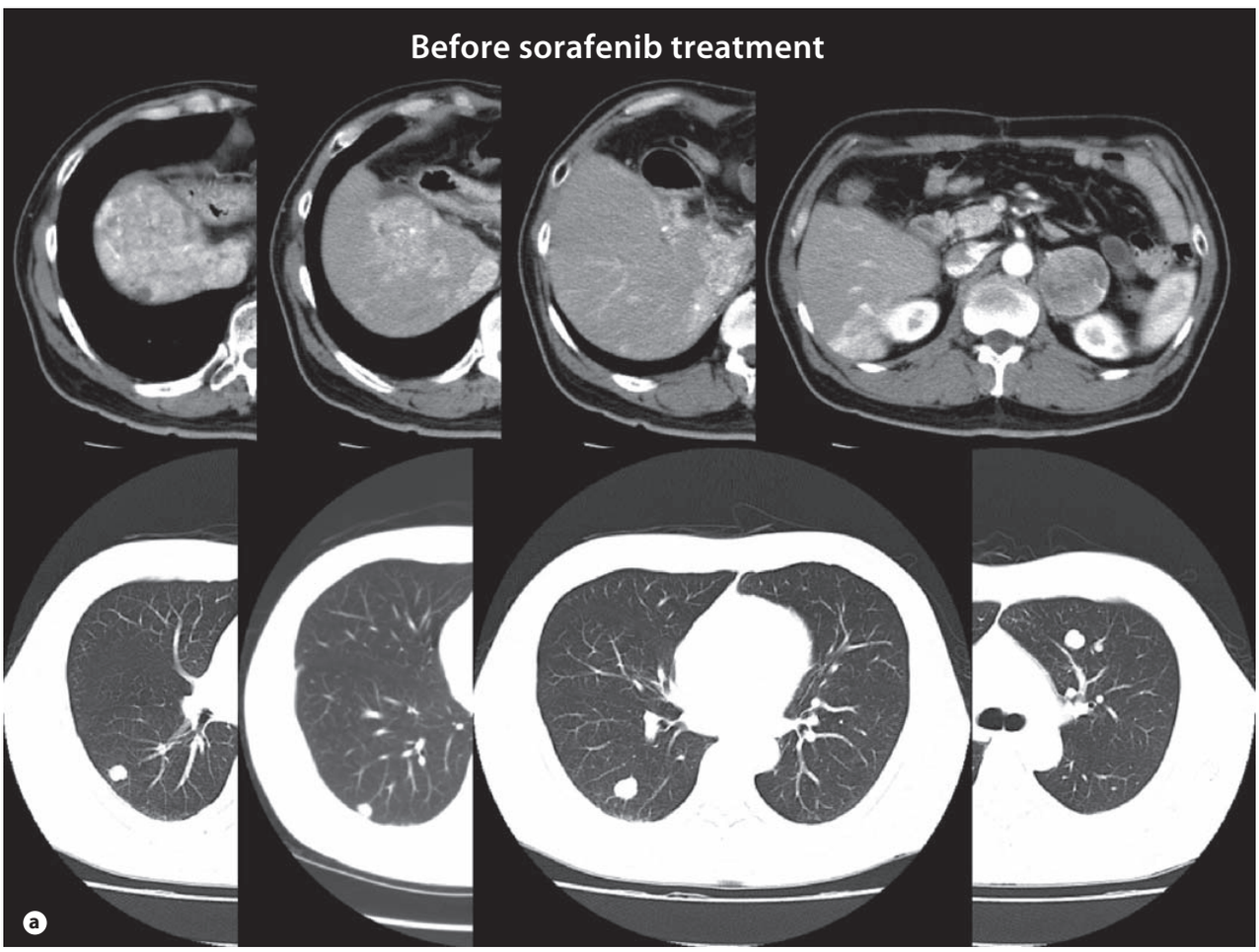


Fig. 3. Complete remission case 2. A 68-year-old male with chronic hepatitis B with stage IVB HCC and Child-Pugh A liver function. **a** The initial development of HCC was detected in February 2007, and TACE was performed. The HCC recurred in April 2007 and hepatectomy was performed, followed by intraarterial infusion chemotherapy with an implanted port, but the infusion was discontinued due to arterial obstruction. Multiple lung, lymph node, and left adrenal metastases were confirmed, and the patient was referred to our institute. At our hospital, S-1 + PEG-IFN combination therapy was performed, but the response was progressive disease (PD). Epirubicin and MMC were systemically administered, but the PD response remained. Oral administration of 800 mg sorafenib was initiated on January 5, 2008. Computed tomography (CT) before sorafenib administration shows intrahepatic multiple HCCs, portal tumor thrombus, and left adrenal, lymph node and multiple lug metastases can be seen.

Positioning of Sorafenib in the HCC Treatment Algorithm

According to the consensus statements of the Japan Society of Hepatology in 2010, sorafenib is recommended for advanced HCC with extrahepatic spread or major vascular invasion such as the 1st branch of the portal vein invasion or the main branch of the portal vein invasion in patients with Child-Pugh A liver function. In addition to that, TACE or HAIC refractory HCC patients with Child-Pugh A liver function are also candidates of sorafenib monotherapy as a second-line treatment option [11] (fig. 1).

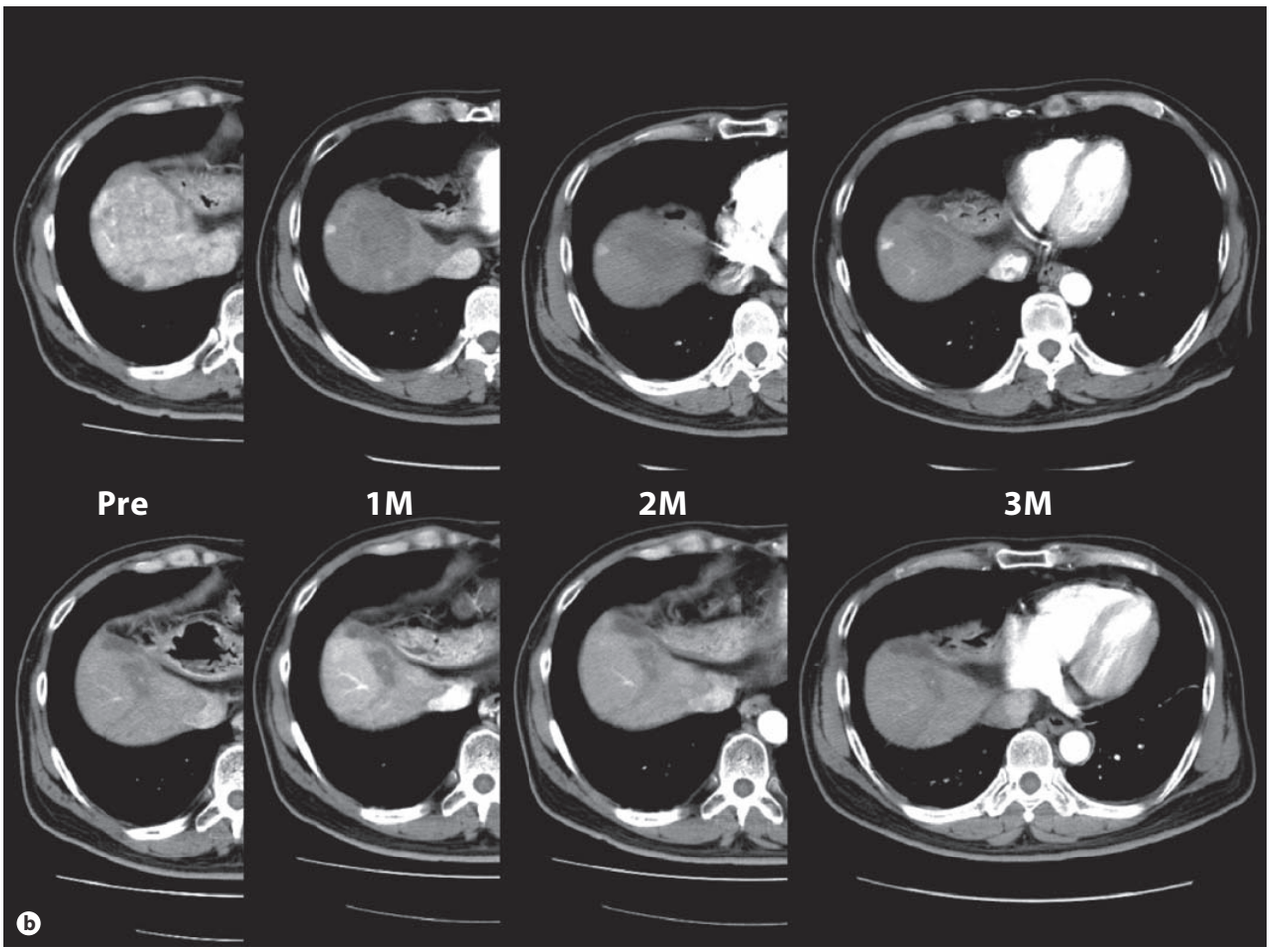


Fig. 3. Complete remission case 2. A 68-year-old male with chronic hepatitis B with stage IVB HCC and Child-Pugh A liver function. **b** One month later, all of the tumors in the lung, liver and the lymph node metastases completely disappeared except in left adrenal gland.

Clinical Experience of Complete Remission Cases with Sorafenib

Many patients with advanced-stage HCC accompanied by distant metastases that is considered untreatable at many hospitals visit our institution and are willing to have any potential treatment. Since the approval of sorafenib in Japan on May 20, 2009, it has been used to treat more than 3,700 patients with advanced HCC in Japan. Of these, 15 patients have been reported to achieve CR [12]. To date, we have treated 90 patients at our institution with sorafenib monotherapy, and 2 achieved CR (fig. 2, 3). By contrast, there have been very few reports of cases achieving CR in other countries [13, 14]. Based on these findings, there might be a racial difference concerning gene mutation that influences the response to sorafenib, differing between ethnic groups, similar to the EGFR mutation for gefitinib.

Clinical Trial Status of Molecular-Targeted Agents for HCC

The agents shown in tables 2 and 3 are currently under development. Drugs that have entered phase III clinical trials are briefly outlined here. Molecular-target-

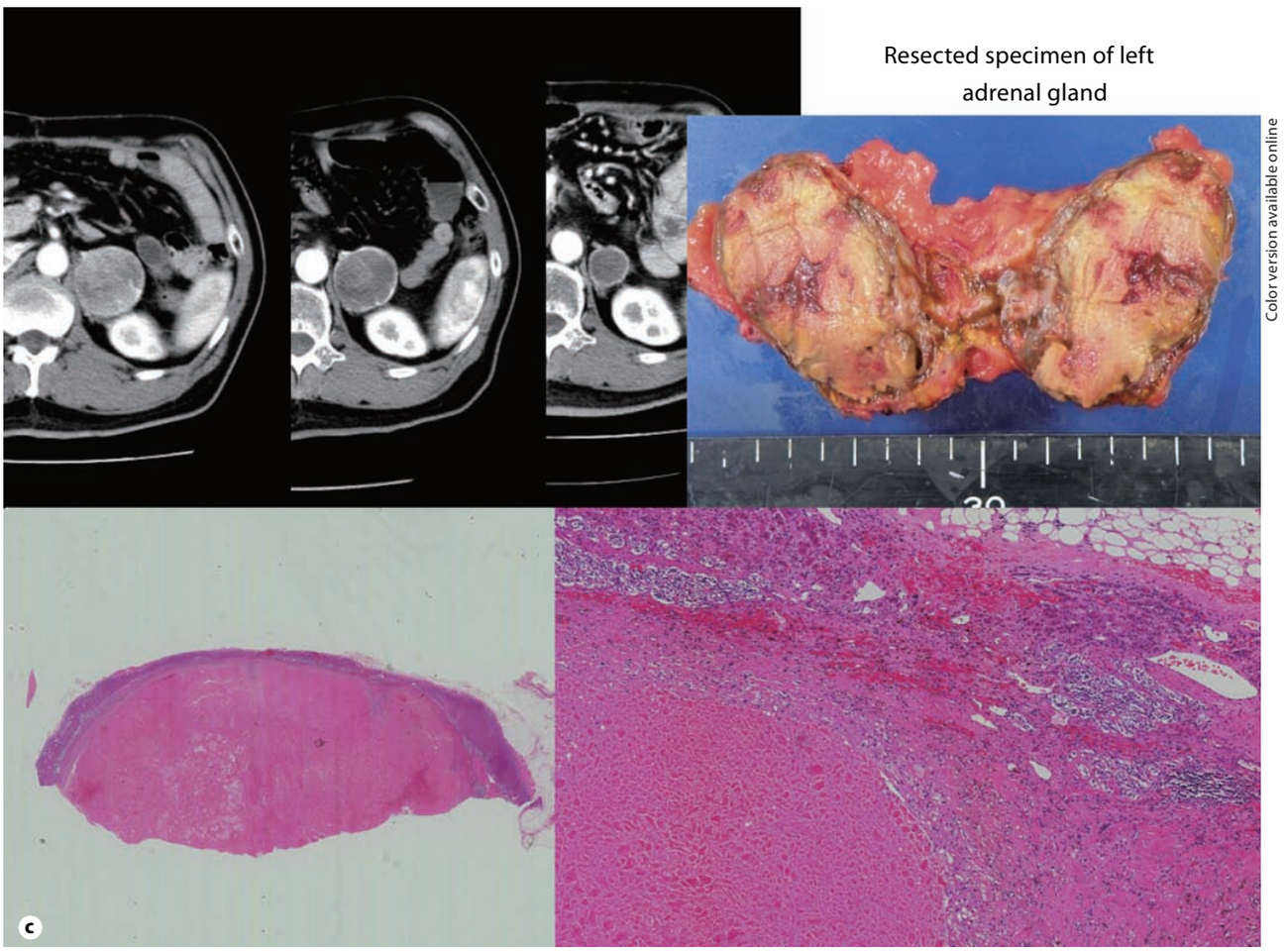


Fig. 3. Complete remission case 2. A 68-year-old male with chronic hepatitis B with stage IVB HCC and Child-Pugh A liver function. **c** Left adrenal metastasis became small during 6-month follow-up; however, there remains enhancing thin layer at the peripheral area. Therefore, the left adrenal gland was surgically resected. Pathological study of the resected specimen showed entire necrosis at the central area with normal adrenal gland at the periphery of the adrenal gland. Cancer-free status was therefore confirmed.

ing drugs for liver cancer and their target molecules are shown in figure 4 [15]. The results of clinical trials of molecular-targeted agents for HCC are summarized in table 3 [16–27].

Sunitinib (Sutent®; Pfizer)

Sunitinib is a low-molecular-weight oral tyrosine kinase inhibitor, which not only inhibits VEGFR and PDGFR but also Flt-3 and C-Kit. Compared with sorafenib, sunitinib slightly more frequently showed grade 3–4 toxicity in phase II studies [16, 17], including thrombocytopenia, neutropenia, and hemorrhage. Sunitinib also strongly inhibits angiogenesis, which is thought to be involved in its strong efficacy.

On a global basis, a head-to-head study of sunitinib versus sorafenib as a control in patients with advanced HCC has unfortunately been terminated in April 2010 because of its toxicity and insufficient efficacy based on the recommendation by an independent data monitoring committee.

Brivanib (Bristol-Myers)

Brivanib is a low-molecular-weight oral kinase inhibitor that selectively inhibits VEGFR and FGFR. In a phase

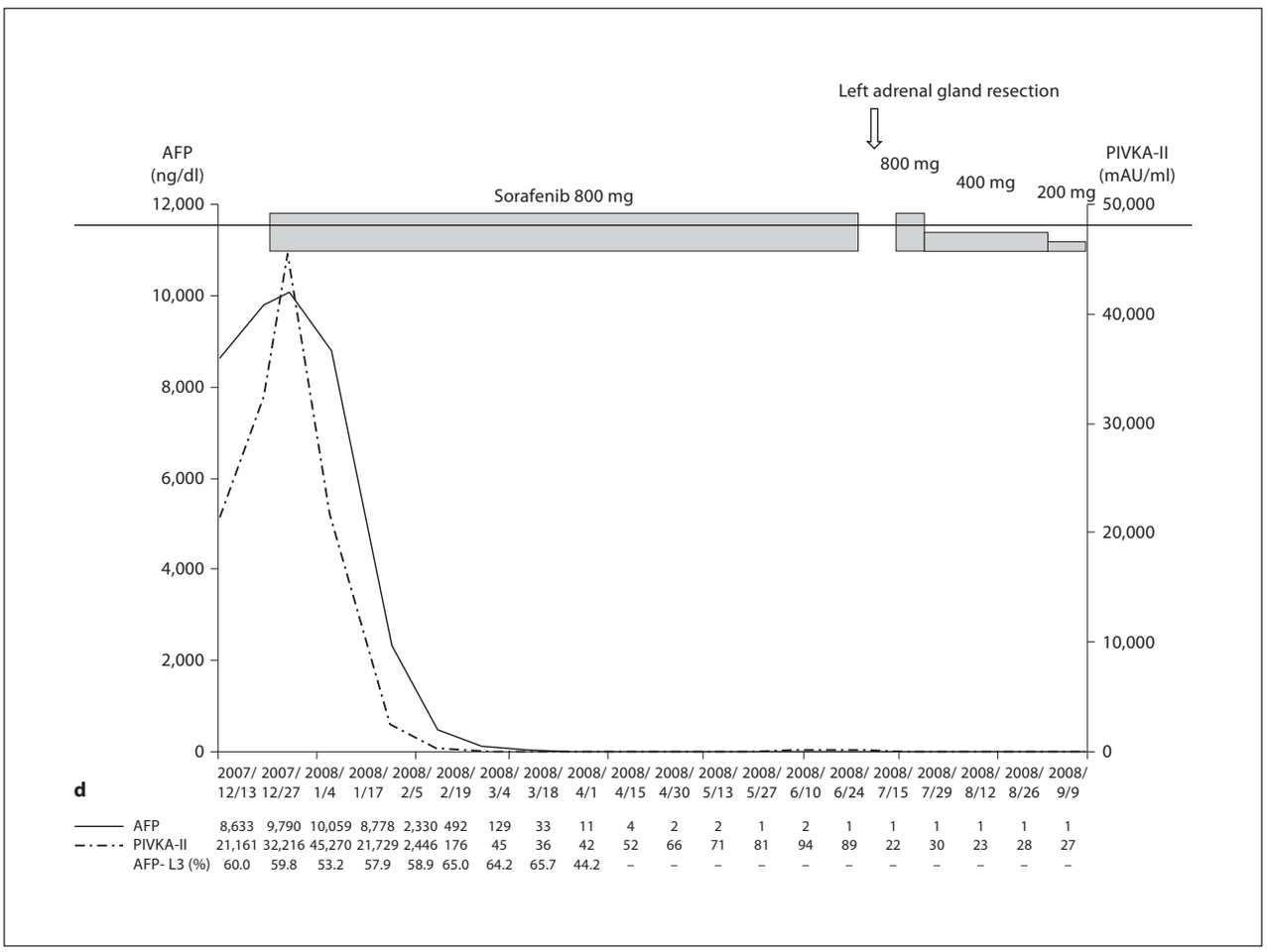


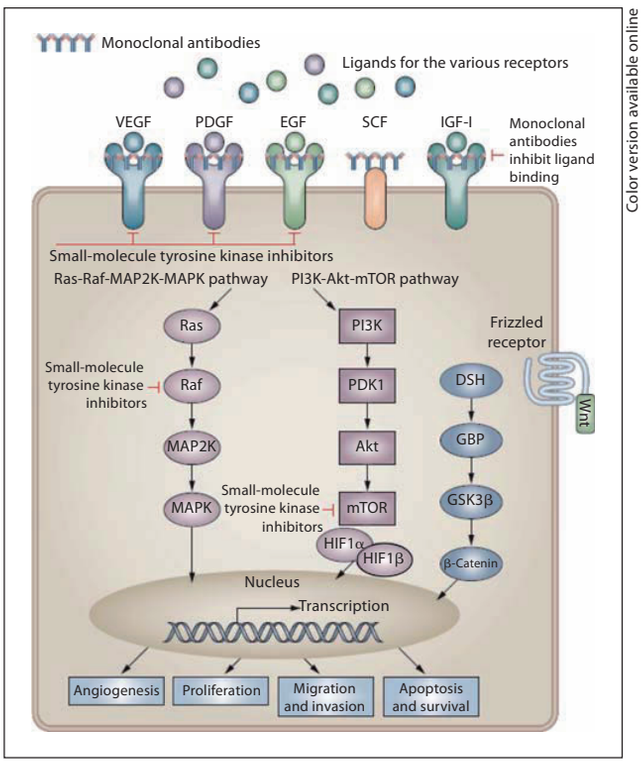
Fig. 3. Complete remission case 2. A 68-year-old male with chronic hepatitis B with stage IVB HCC and Child-Pugh A liver function. **d** For more than 1 year, there was no recurrence (sustained cancer-free status) and the patient is now under longterm treatment with sorafenib at 200 mg/day. Clinical course of the tumor markers: 5 months after sorafenib administration, the high AFP level (10,559 ng/ml) returned to normal (1 ng/ml). Similarly, the high PIVKA-II level (45,270 mAU/ml) returned to normal (27 mAU/ml) and the high AFPL3 fraction (60.0 %) returned to normal (<10%). The patient is now under long-term treatment with sorafenib at 200 mg/day.

II study of 36 Asian and 20 non-Asian patients with advanced HCC, the overall survival rate in the Asian patients was 10.0 months, showing a favorable outcome compared with that (6.2 months) achieved by sorafenib in the Asia-Pacific study. However, a simple comparison of the 2 studies is not appropriate because of differences in patient characteristics.

Three global trials of brivanib are now ongoing: one, a placebo-controlled study, is for adjuvant therapy after TACE (BRISK-TA trial); the second is a first-line clinical trial for brivanib versus sorafenib for advanced HCC (BRISK-FL trial); and the third is a second-line, placebo-controlled clinical trial in sorafenib-resistant HCC (BRISK-PS trial).

Retinoid (NIK-333; Kowa)

Retinoids represent a broad range of compounds that bind to and activate retinoic acid (RAR) and retinoid (RXR) receptors, two nuclear hormone receptors. Retinoid-333 is an acyclic retinoid that was developed in Japan. It activates transcription via RAR and RXR, and induces differentiation, and is expected to induce apoptosis



Color version available online

Fig. 4. Signaling pathways and molecular-targeted agents. Monoclonal antibodies (VEGFR: bevacizumab, EGFR: cetuximab), tyrosine kinase inhibitors (VEGFR: sorafenib and sunitinib, EGFR: erotinib, lapatinib), serine/threonine kinase inhibitors (Raf: sorafenib, mTOR: rapamycin and everolimus, PIK: KL-755). Cited from Spangenberg et al. [15]. Reproduced with permission.

of precancerous HCC cells, and inhibit carcinogenesis by inducing differentiation [28, 29]. In Japan, a phase II/III study of adjuvant therapy with retinoid after resection or RFA was recently completed and the results were presented at the annual meeting of the American Society of Clinical Oncology (ASCO) in 2010. Although the study did not reach its primary endpoint, the results in recurrence-free survival were favorable to some extent.

Other Drugs

A phase II study of TSU-68 (Tiho Pharmaceuticals) in combination with TACE was recently completed and presented at ASCO in 2010. At present, the feasibility of a phase III study is being investigated. C-met inhibitor and mTOR inhibitor (RAD001) is also entering a phase III clinical study as a second-line therapy in patients with sorafenib-intolerant or resistant cancer (table 2).

Future Perspectives of Molecular-Targeted Therapy for HCC

The SHARP study showed that sorafenib inhibits the growth and progression of HCC and inhibits angiogenesis. What do these findings mean? Even cases of liver cancer indicated for curative treatment, such as resection, RFA or TACE, show the similar phenotype of advanced cancer, including hypervascularity, vascular invasiveness, and a high recurrence rate of intrahepatic metastasis. Although the therapeutic policy varies depending on the cancer stage, all of hypervascular HCCs are included in the same category; so-called 'advanced cancer' which has a strong potential to recur at a yearly rate of 15–20%. In other words, the treatment policy is dictated by the cancer stage; however, the existence of these characteristics indicates that the cancer should be treated as an advanced cancer. Accordingly, it may be possible to extrapolated the results of the SHARP study to most HCC cases classified into various stages. Of course, this should be evaluated in prospective clinical trials and, in fact, global trials are already underway, which are expected to show that sorafenib improves prognosis in the following settings: (1) adjuvant therapy after curative treatment (STORM trial); (2) TACE combination therapy (global SPACE trial and Japanese TACTICS trial), and (3) combination therapy with HAIC (Japanese SILIUS trial). Indeed, when considering a subanalysis of the SHARP study presented at ASCO in 2008 by Sherman et al. [30], the hazard ratio for overall survival in patients without extrahepatic spread or vascular invasion was 0.52, indicating that sorafenib improved survival twofold relative to the placebo group. Furthermore, the median survival time of these patients was approximately 15 months with sorafenib compared with 10 months in the placebo group (table 4). These results indicate that when sorafenib is used in combination with TACE or adjuvant therapy after resection or ablation, overall survival should be much prolonged as presented in figure 5.

It must be noted that sorafenib is associated with some unusual adverse events that are not normally encountered with other cytotoxic chemotherapeutic agents, and include skin reactions to the hands and feet, diarrhea and hypertension. In addition, liver dysfunction, hepatic encephalopathy, acute interstitial pneumonia, or bleeding are the big issues that need to be well managed as they are life-threatening events. Hepatologists mainly prescribe this drug in Japan as opposed to other countries where oncologists prescribe sorafenib as well. To adequately prescribe and manage molecular-targeted agents, hepa-

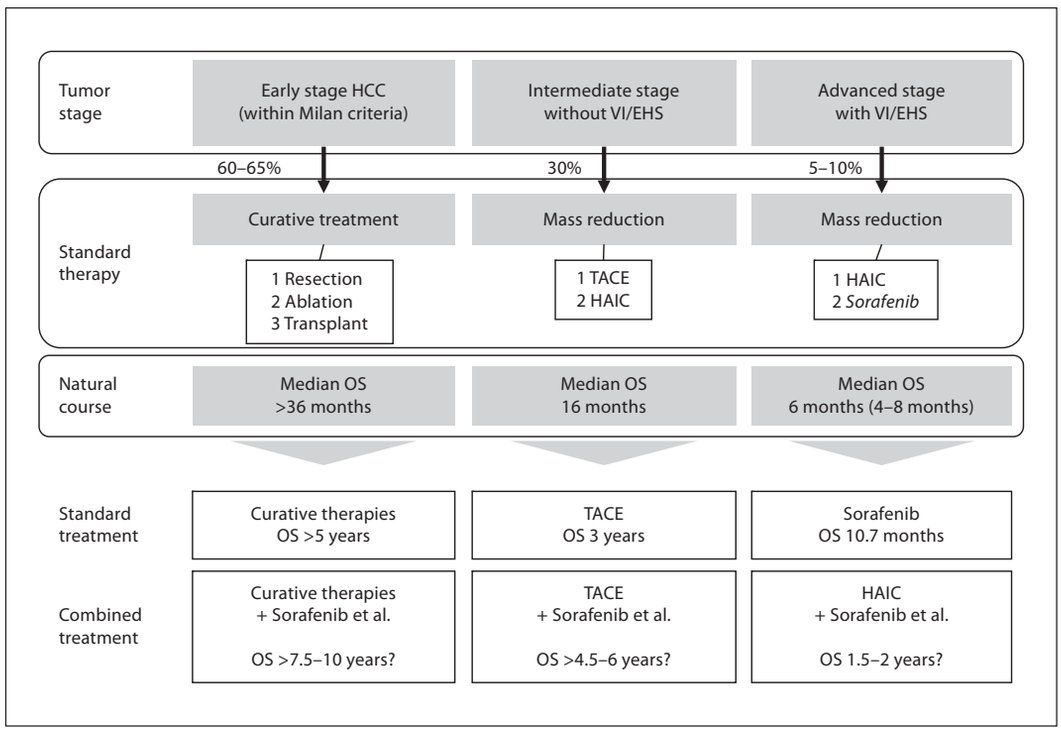


Fig. 5. Outcomes of standard treatment modalities and expected future outcomes of combination therapy with molecular-targeted agents. By combining molecular-targeted agents with resection or ablation, life expectancy is expected to be increased to 7.5–10 years. In addition, for intermediate stage HCC, the prognosis is expected to be increased to 4.5–6 years by combination with TACE. OS = Overall survival.

tologists should have a thorough knowledge of the possible adverse events and be aware of treatment options. This is important not only to avoid unnecessary adverse events, but also to maximize the efficacy of such agents by continuing drug administration for as long as possible, and thus prolonging survival.

One year has passed since sorafenib was approved in Japan on May 20, 2009. Molecular-targeted agents, such as sorafenib, may have a significant impact on the treatment of liver cancer and markedly change the algorithm originally established in 2007 for treating liver cancer in Japan, as shown in figure 5 [31, 32], and revised by the Japan Society of Hepatology in 2010 [11]. The results of the SHARP and Asia-Pacific studies and the 1-year experience in Japan with 15 CR cases among a total of more than 3,700 cases offer hope to many HCC patients, particularly those with advanced HCC with major vascular invasion or extrahepatic spread.

Disclosure Statement

The authors declare that they have no financial conflict of interest.

References

1 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, et al: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.
2 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, et al: Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009; 10:25–34.
3 Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, et al: Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:855–867.

4 Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, et al: BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 2004;64:7099–7109.
5 Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M: Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 2008;7:3129–3140.
6 Rini BI: Sorafenib. Expert Opin Pharmacother 2006;7:453–461.
7 Wu H, Huang C, Chang D: Anti-angiogenic therapeutic drugs for treatment of human cancer. J Cancer Mol 2008;4:37–45.
8 Kano MR, Komuta Y, Iwata C, Oka M, Shirai YT, Morishita Y, Ouchi Y, et al: Comparison of the effects of the kinase inhibitors imatinib, sorafenib, and transforming growth factor-beta receptor inhibitor on extravasation of nanoparticles from neovasculature. Cancer Sci 2009;100:173–180.
9 Tanaka S, Arii S: Molecularly targeted therapy for hepatocellular carcinoma. Cancer Sci 2009;100:1–8.
10 Llovet JM, Bruix J: Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008;48:1312–1327.
11 Arii S, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, et al: Management of Hepatocellular Carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology 2009. Hepatol Res 2010, in press.
12 Japan Association of Molecular Targeted Therapy: 1st Meeting Abstract Book. Osaka, Japan Association of Molecular Targeted Therapy, 2010.
13 Wang SX, Byrnes A, Verma S, Pancoast JR, Rixe O: Complete remission of unresectable hepatocellular carcinoma treated with reduced dose of sorafenib: a case report. Target Oncol 2010, Epub ahead of print.
14 So BJ, Bekaii-Saab T, Bloomston MA, Patel T: Complete clinical response of metastatic hepatocellular carcinoma to sorafenib in a patient with hemochromatosis: a case report. J Hematol Oncol 2008;1:18.
15 Spangenberg HC, Thimme R, Blum H: Targeted therapy for hepatocellular carcinoma. Nat Rev Gastroenterol Hepatol 2009;6:423–432.
16 Faivre S, Raymond E, Douillard J, et al: Assessment of safety and drug induced tumor necrosis with sunitinib in patients with unresectable hepatocellular carcinoma (HCC). J Clin Oncol 2007;25:149s.
17 Zhu A, Sahani D, Duda DG, di Tomaso E, et al: Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. J Clin Oncol 2009;27:3027–3035.
18 Abou-Alfa GK, Schwartz L, Ricci S, et al: Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol 2006;24:4293–4300.
19 Raoul JL, Flinn RS, Kang YK, et al: An open-label phase II study of first- and second-line treatment with brivanib in patients with hepatocellular carcinoma. J Clin Oncol 2009; 27:15S; Abstr 4577.
20 Toh H, Chen PJ, Carr BI, et al: A phase II study of ABT-869 in hepatocellular carcinoma: interim analysis. J Clin Oncol 2009;27: 15S; Abstr 4581.
21 Siegel AB, Cohen EI, Ocean A, et al: Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. J Clin Oncol 2008;26: 2992–2998.
22 Philip PA, Mahoney MR, Allmer C, et al: Phase II study of erlotinib (OSI-774) in patients with advanced hepatocellular cancer. J Clin Oncol 2005;23:6657–6663.
23 Thomas MB, Chadhal R, Glover K, et al: Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer 2007;110:1059–1066.
24 O'Dwyer PJ, Giantonio BJ, Levy DE, et al: Gefitinib in advanced unresectable hepatocellular carcinoma: results from the Eastern Cooperative Oncology Group's Study E1203. J Clin Oncol 2006;24:18S; Abstr 4143.
25 Ramanathan RK, Belani CP, Singh DA, et al: A phase II study of Lapatinib in patients with advanced biliary tree and hepatocellular cancer. Cancer Chemother Pharmacol 2009; 64:777–783.
26 Bekaii-Saab T, Markowitz J, Prescott N, et al: A multiinstitutional phase II study of the efficacy and tolerability of lapatinib in patients with advanced hepatocellular carcinomas. Clin Cancer Res 2009;15:5895–5901.
27 Zhu AX, Stuart K, Blaszkowsky LS, et al: Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer 2007;110:581–589.
28 Muto Y, Moriwaki H, Ninomiya M, Adachi S, Saito A, Takasaki KT, Tanaka T, et al: Prevention of second primary tumors by an acyclic retinoid, polyprenoic acid, in patients with hepatocellular carcinoma. Hepatoma Prevention Study Group. N Engl J Med 1996; 334:1561–1567.
29 Muto Y, Moriwaki H, Saito A: Prevention of second primary tumors by an acyclic retinoid in patients with hepatocellular carcinoma. N Engl J Med 1999;340:1046–1047.
30 Sherman M, Mazzaferro V, Amadori D, et al: Efficacy and safety of sorafenib in patients with advanced hepatocellular carcinoma and vascular invasion or extrahepatic spread: a subanalysis from the SHARP trial. J Clin Oncol 2008;26(suppl); Abstr 4584.
31 Kudo M, Okanoue T: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(suppl):2–15.
32 Kudo M: Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. Oncology 2008;75 (suppl):1–12.

Oncology

Oncology 2010;78(suppl 1):180–188
DOI: 10.1159/000315740

Published online: July 8, 2010

Real Practice of Hepatocellular Carcinoma in Japan: Conclusions of the Japan Society of Hepatology 2009 Kobe Congress

Masatoshi Kudo

Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

Key Words
Hepatocellular carcinoma · Japan Society of Hepatology · Consensus Meeting

Abstract
This article presents the current consensus on the management of hepatocellular carcinoma (HCC) formed at the 45th Annual Meeting of the Japan Society of Hepatology (June 4–5, 2009) and the 3rd International Kobe Liver Symposium (June 6–7, 2009) held in Kobe. Concluded important consensuses, which were well accepted by Japanese HCC specialists, are as follows. (1) Patients with type B or type C liver cirrhosis, who are an ultrahigh-risk group of liver cancer, should be screened every 3–4 months by ultrasonography and measurement of AFP and PIVKA-II. (2) Gd-EOB-MRI is useful for the diagnosis of early HCC. (3) The JIS score is more useful for the staging of liver cancer than the BCLC staging system, which is a global standard. (4) The TNM staging system by the Liver Cancer Study Group of Japan is superior to the TNM stage by the AJCC/UICC. (5) The therapeutic algorithm in the Japanese guidelines for the management of liver cancer is superior to the BCLC treatment algorithm. (6) Early stage. Liver cancers should be treated by radiofrequency ablation if they are ≤2 cm, and by surgical resection if they are Child-Pugh A solitary lesions. (7) Liver transplantation is only indicated for Child-Pugh C patients within Milan Criteria. In conclusion, these consensuses seem to well reflect the real practice pattern of the management of HCC in Japan and provide valuable information for other countries as well.

Introduction

A total of three Consensus Meetings were held during the 45th Annual Meeting of the Japan Society of Hepatology (JSH) on June 4–5, 2009, and the 3rd International Kobe Liver Symposium on Hepatocellular Carcinoma (HCC) (IKLS; June 6–7, 2009) held in succession in Kobe. The first one was the Consensus Meeting on HCC (participated in by Japanese HCC specialists only) of the Annual Meeting of the JSH, one held as part of the international symposium during the session of the Annual Meeting also participated in by foreign experts, and one during the 3rd IKLS, for which 20 foreign HCC experts and 200 Japanese HCC experts were selected from a total of 786 Council members representing the 10,737 members of the JSH who voted using answer pads after topic presentations.

The experts consisted of 68% internists or hepatologists, 25% surgeons, 3% radiologists, 2% pathologists, and

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/10/0787–0180$26.00/0

Accessible online at:
www.karger.com/ocl

Masatoshi Kudo, MD, PhD
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi, Osaka-Sayama, Osaka 589-8511 (Japan)
Tel. +81 72 366 0221, ext. 3525, Fax +81 72 367 2880, E-Mail m-kudo@med.kindai.ac.jp

2% from other fields. This report outlines the current consensus regarding the latest diagnostic and therapeutic issues for HCC in Japan by presenting excerpts of the results of these meetings.

Screening

According to Western guidelines (BCLC algorithm), the screening interval need not be changed depending on the degree of fibrosis or stage of the liver disease [1], but Japanese guidelines recommend modification of the screening intervals according to the risk of carcinogenesis [2–5]. In a questionnaire survey of 200 experts also using answer pads, a majority of experts (91%) answered that the screening interval should be changed according to the degree of fibrosis. This view, reflecting the actual contents of clinical practice in Japan, is considered reasonable. More specifically, 53% of the experts considered that patients with hepatitis B or C should be screened by ultrasonography every 6 months, with monitoring of the tumor marker levels every 3 months. However, 84% of the experts answered that patients with type B or C liver cirrhosis, who are an ultrahigh-risk group, should be screened every 3–4 months, following the Japanese algorithm. For the screening of high-risk groups, 72% simultaneously examined AFP, PIVKA-II and AFP-L3 among tumor markers, and 44% combined them with ultrasonography. These figures are considered to accurately reflect common practice in Japan (fig. 1).

ConsensusS tatement1

The surveillance interval needs to be shortened for patients at higher risk of HCC, such as hepatitis B- or C-related liver cirrhosis.

Consensus Statement 2

Surveillance should be performed using both ultrasonography and three tumor markers including AFP, PIVKA-II, and AFP-L3.

Diagnosis

HCC has usually been diagnosed by dynamic CT, but it is notable that 58% of the experts described gadolinium diethylenetriamine ethoxybenzyl-MRI (Gd-DTPA-EOB-MRI) as the primary modality, outnumbering those who answered dynamic MDCT. In addition, 91% of the experts agreed that biopsy is unnecessary when a hypervascular tumor of ≥1.5 cm shows typical features of wash-in and wash-out on imaging, and 67% stated that biopsy should be performed, in principle, for hypovascular tumors of ≤1.5 cm. These results also reflect the current Japanese standard of clinical practice for liver cancer.

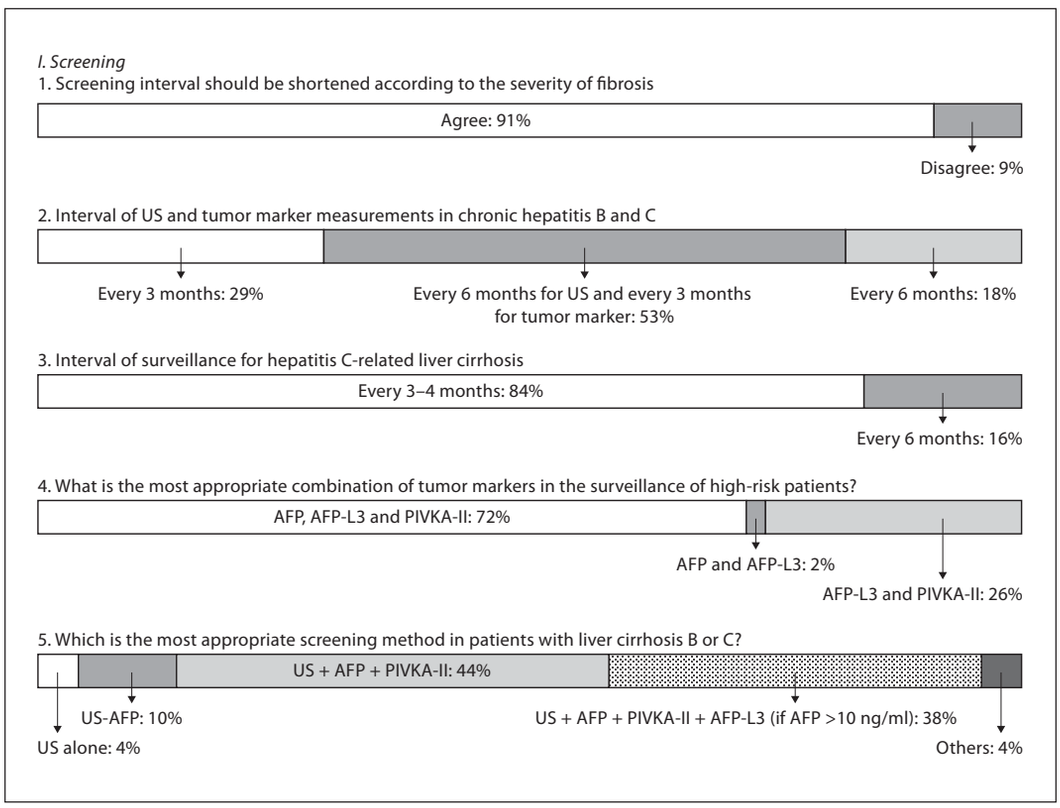
Also, Western guidelines require the agreement of two dynamic studies for tumors 1–2 cm in diameter even when they present typical images [1], but 83% of the Japanese experts considered that one imaging modality suffices for tumors of any size (even those of 1–2 cm in diameter). This is a marked difference between Japan and Western countries, and it is considered that the Japanese view is more theoretically reasonable. Concerning small nodules presenting non-typical images, a majority (55%) answered, to my surprise, that they would follow-up without biopsy. This was probably because they assumed a situation in which HCC cannot be diagnosed definitively even with the extensive use of modalities including Gd-EOB-MRI or contrast-enhanced ultrasonography, and, if so, the approach may be justified.

ConsensusS tatement3

Even though a nodule is as small as 1–2 cm in size, HCC can be correctly diagnosed by the typical imaging findings by only one dynamic imaging study.

Staging and Prognostic Stages

While the TNM staging system by the AJCC/UICC is a global standard, the TNM stage of the Liver Cancer Study Group of Japan has been used for a long time in Japan, because the cutoff size employed in the AJCC/UICC system is huge (5 cm). In Japan, many liver cancers of ≤2 cm are detected frequently due to the nationwide coverage of the screening system, and AJCC/UICC TNM staging is not adequate. Reflecting this, 97% of the participants of the International Symposium quite reasonably supported the Japanese TNM staging. Also, 65% of the experts agreed with the view that integrated staging should be employed for the staging for a predicting prognosis of liver cancer, and 69% answered that BCLC staging is inappropriate for a prognostic prediction. Indeed, the BCLC staging system is a therapeutic algorithm, and the classification of tumors and patients' conditions into early, intermediate, and advanced naturally results in the progressive exacerbation of the outcome and favorable agreement between stratification of the survival curve and the prognosis. Therefore, in a strict sense, BCLC staging is not a prognostic staging system. The view that the JIS score is appropriate as an integrated staging system for a predicting prognosis in Japan was supported by 71%.



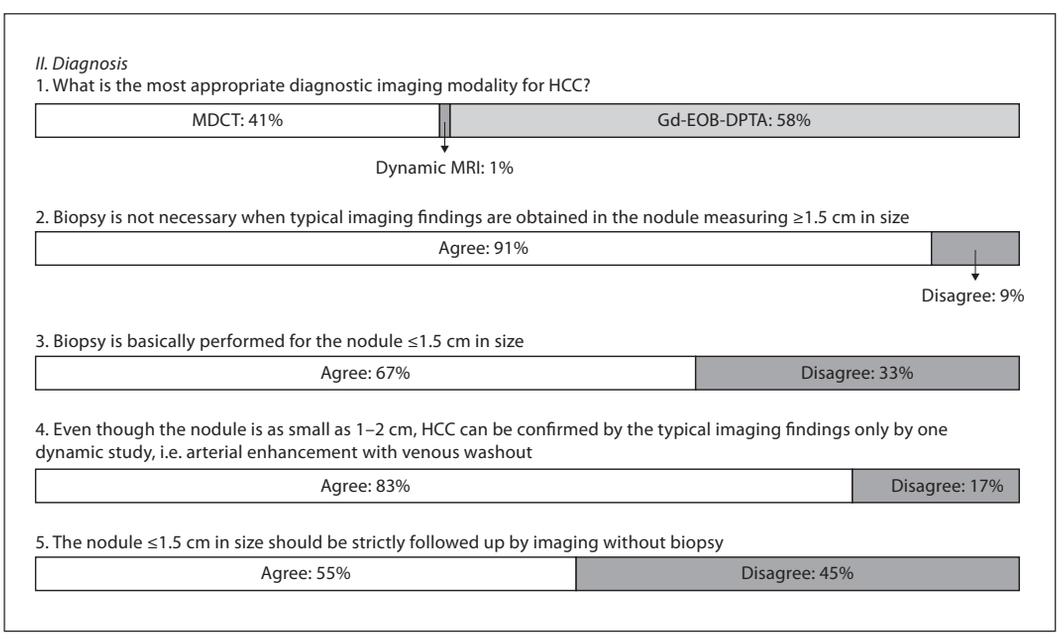


Fig. 1. Differences in many aspects of both the concept and clinical practice concerning the diagnosis and treatment of liver cancer between Japan and Western countries. AFP = Alpha-fetoprotein; PIVKA-II = protein induced by vitamin K absence or antagonist-II; AFP-L3 = AFP-L3 fraction; LCSGJ = Liver Cancer Study Group of Japan.

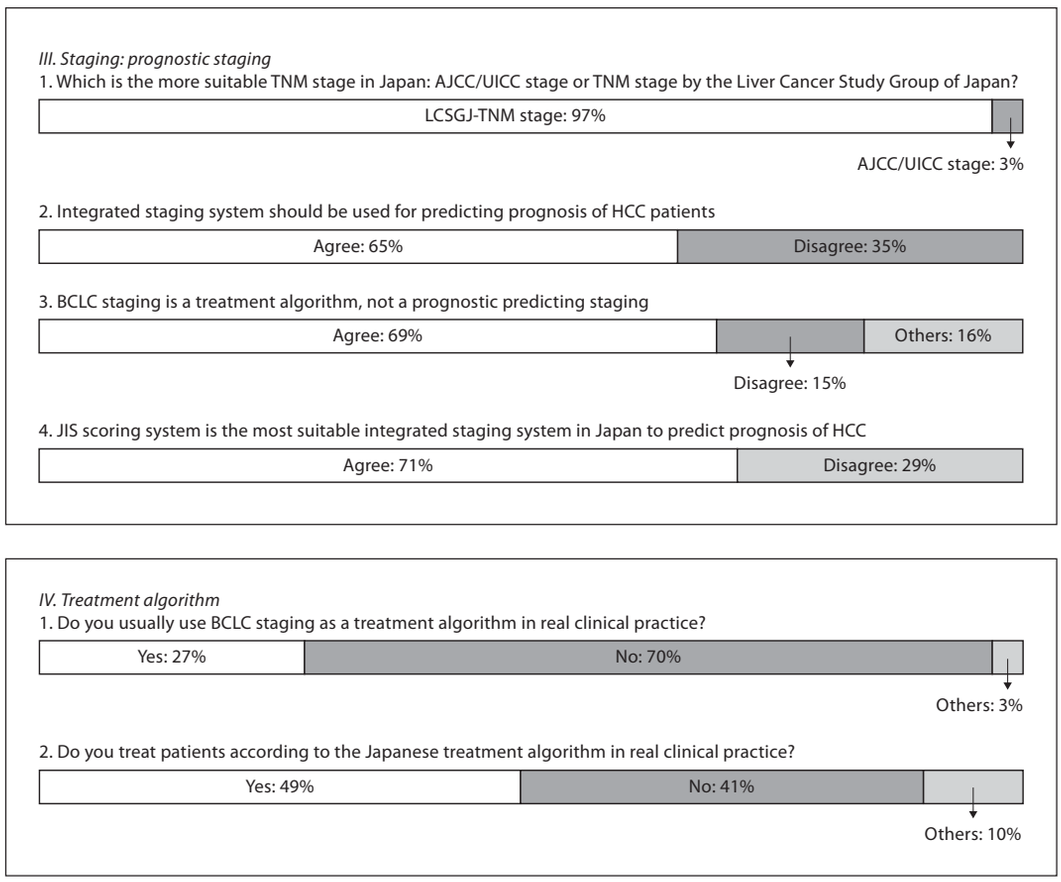


Fig. 1.

ConsensusS tatement4

TNM stage by the Liver Cancer Study Group of Japan is the more appropriate stage than the AJCC/UICC TNM stage.

ConsensusS tatement5

The JIS scoring system is the most suitable integrated staging system in Japan to predict a prognosis of HCC.

Treatment Algorithm

Concerning the treatment algorithm, 49% of the experts answered that they determined the therapeutic approach on the basis of Japanese guidelines for the management of liver cancer (fig. 1, 2) [2–4], and only 27% used the BCLC treatment algorithm. This is another marked difference in the approach to liver cancer between Western countries and Japan.

As expected, most of the experts (94%) considered that a circumferential ablative margin should be secured for ablation with the aim of the locally curative treatment of small liver cancer. This view is unique to Japanese physicians, not observable as part of the general Western practice. Also, 94% supported CT scanning at slice intervals of ≤5 mm for CT-based assessment after RFA. To my knowledge, in no country is the effect of RFA evaluated so carefully by CT, aiming at 100% necrosis and the securing of an ablative margin.

A minority (36%) answered that they would perform TACE followed by RFA for hypervascular liver cancers of ≤2 cm in diameter, but a majority (81%) answered that they would perform them, in principle, for hypervascular liver cancers of ≥3 cm, because microsatellite lesions and mircrovascular invasion are present around hypervascular liver cancers 2–3 cm in diameter, and they may lead to subsequent local recurrence even after complete necro-

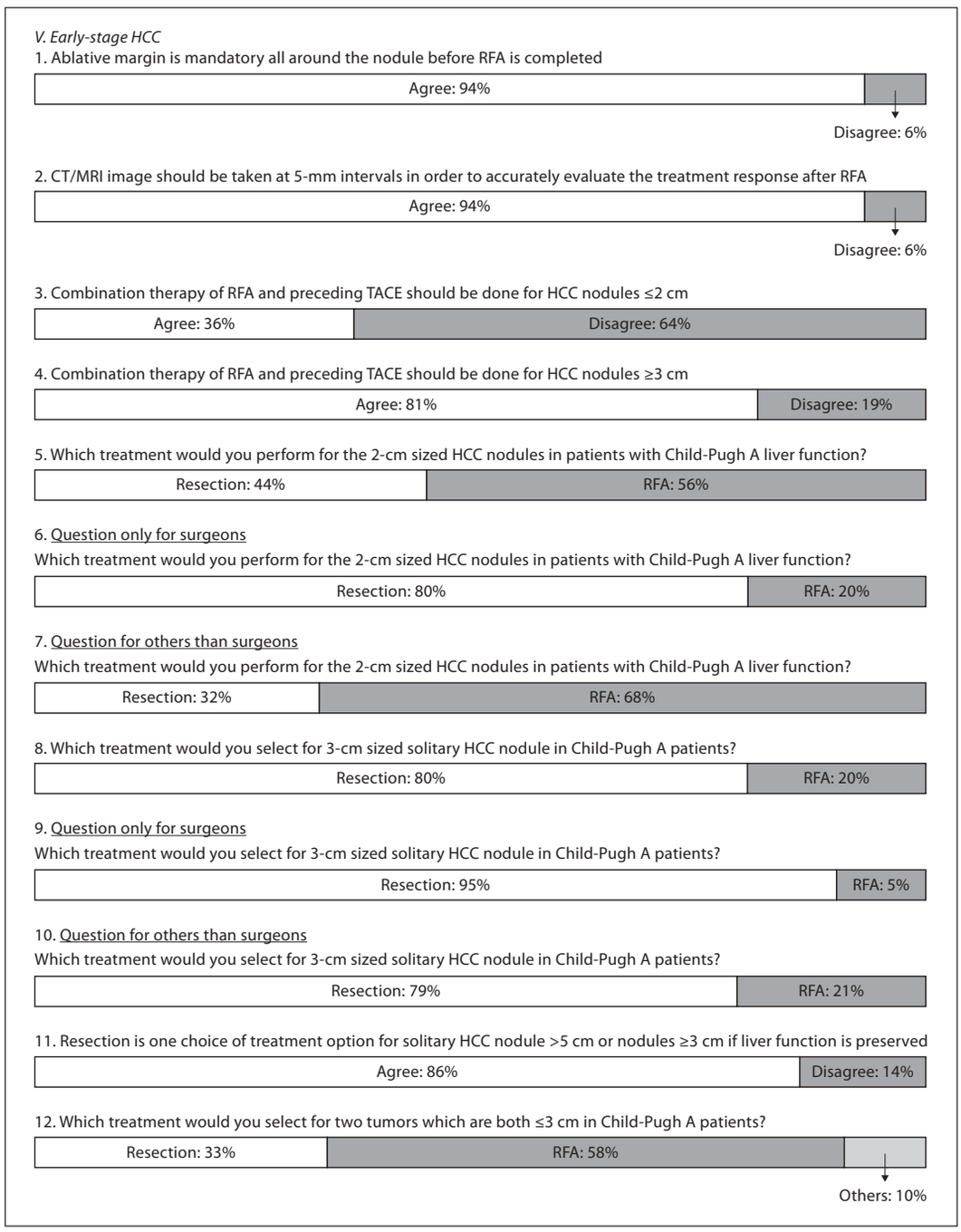

V. Early-stage HCC
1. Ablative margin is mandatory all around the nodule before RFA is completed
Agree: 94%
Disagree: 6%
2. CT/MRI image should be taken at 5-mm intervals in order to accurately evaluate the treatment response after RFA
Agree: 94%
Disagree: 6%
3. Combination therapy of RFA and preceding TACE should be done for HCC nodules ≤2 cm
Agree: 36%
Disagree: 64%
4. Combination therapy of RFA and preceding TACE should be done for HCC nodules ≥3 cm
Agree: 81%
Disagree: 19%
5. Which treatment would you perform for the 2-cm sized HCC nodules in patients with Child-Pugh A liver function?
Resection: 44%
RFA: 56%
6. Question only for surgeons
Which treatment would you perform for the 2-cm sized HCC nodules in patients with Child-Pugh A liver function?
Resection: 80%
RFA: 20%
7. Question for others than surgeons
Which treatment would you perform for the 2-cm sized HCC nodules in patients with Child-Pugh A liver function?
Resection: 32%
RFA: 68%
8. Which treatment would you select for 3-cm sized solitary HCC nodule in Child-Pugh A patients?
Resection: 80%
RFA: 20%
9. Question only for surgeons
Which treatment would you select for 3-cm sized solitary HCC nodule in Child-Pugh A patients?
Resection: 95%
RFA: 5%
10. Question for others than surgeons
Which treatment would you select for 3-cm sized solitary HCC nodule in Child-Pugh A patients?
Resection: 79%
RFA: 21%
11. Resection is one choice of treatment option for solitary HCC nodule >5 cm or nodules ≥3 cm if liver function is preserved
Agree: 86%
Disagree: 14%
12. Which treatment would you select for two tumors which are both ≤3 cm in Child-Pugh A patients?
Resection: 33%
RFA: 58%
Others: 10%
VI. Intermediate-stage HCC
1. Is TACE with lipiodol mixed with an anticancer agent such as epirubicin, doxorubicin, or CDDP a more effective treatment than TACE with doxorubicin-eluting beads (DC beads)?
Yes: 45%
No: 24%
Others: 31%

Fig. 1.

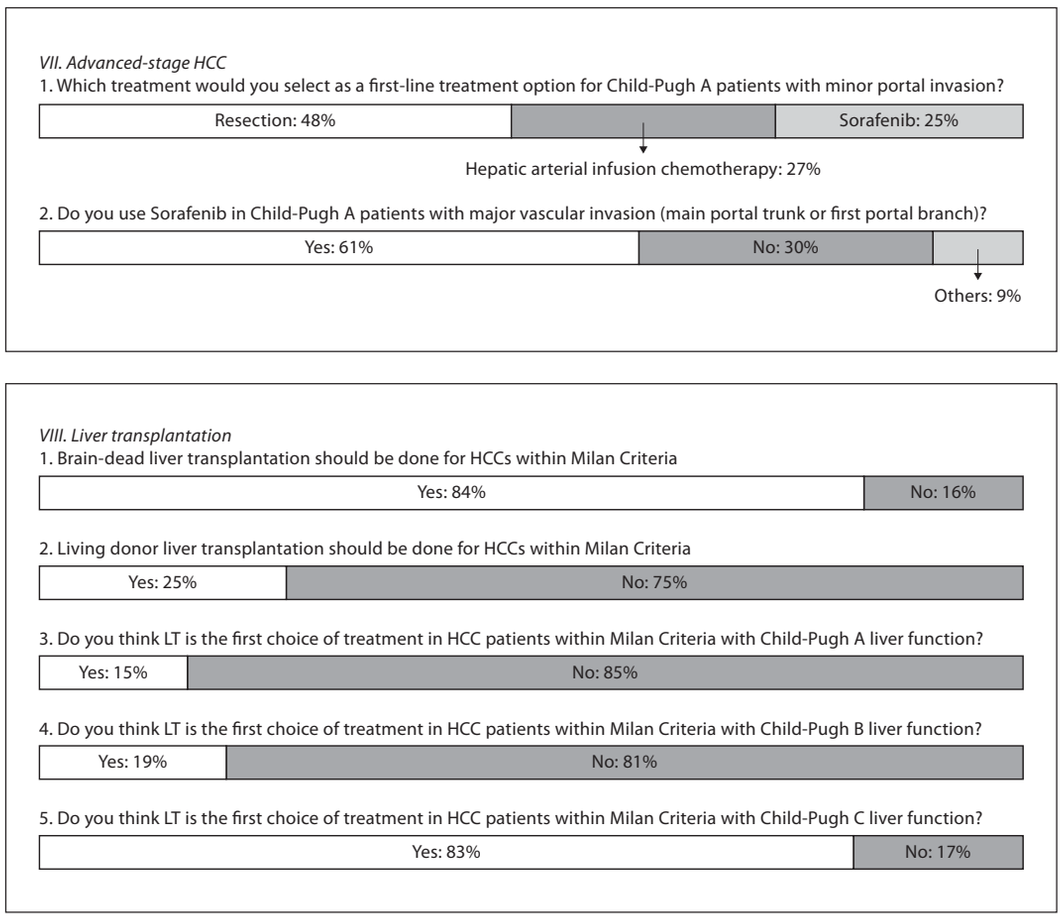


Fig. 1.

sis of the main tumor, and because the intra-arterially injected lipiodol itself is considered extremely useful for the evaluation of whether or not a ablative margin has been secured in assessing the therapeutic effect. This, being different from the Western approach, is also considered to very accurately reflect the current clinical practice in Japan. For the same reason, concerning the therapeutic approach to Child-Pugh A solitary liver cancers of 2 cm in diameter, a slight majority (56%) of all experts including internists and surgeons selected RFA rather than resection, but 80% of the surgeons and only 32% of non-surgeons selected resection. For Child-Pugh A solitary liver cancers of 3 cm in diameter, resection was supported, quite understandably, by 80% of all experts, 95% of surgeons, and 79% of non-surgeons. Thus, surgical resection should be selected if possible for hypervascular liver cancers >3 cm in diameter, because local recurrence from surrounding microsatellite lesions and microvascular invasion are observed frequently after RFA. Also, 86% of the experts agreed that resection is an alternative for liver cancers of ≥5 cm in diameter and 3 or more concurrent nodules if the liver function reserve is satisfactory. However, 58% chose RFA for 2 concurrent Child-Pugh A liver cancers of ≤3 cm in diameter, outnumbering those who chose surgical resection.

TACE using doxorubicin-eluting (DC) beads is rapidly spreading in Western countries as a treatment for moderately advanced liver cancers, but Japanese experts have slightly negative views regarding this therapy, and 45% of them answered that conventional superselective TACE also using lipiodol is a better choice. This is also understandable, because a greater effect is expected from subsegmental TACE as superselective lipiodol injection induces the reflux of lipiodol to the portal vein as well as

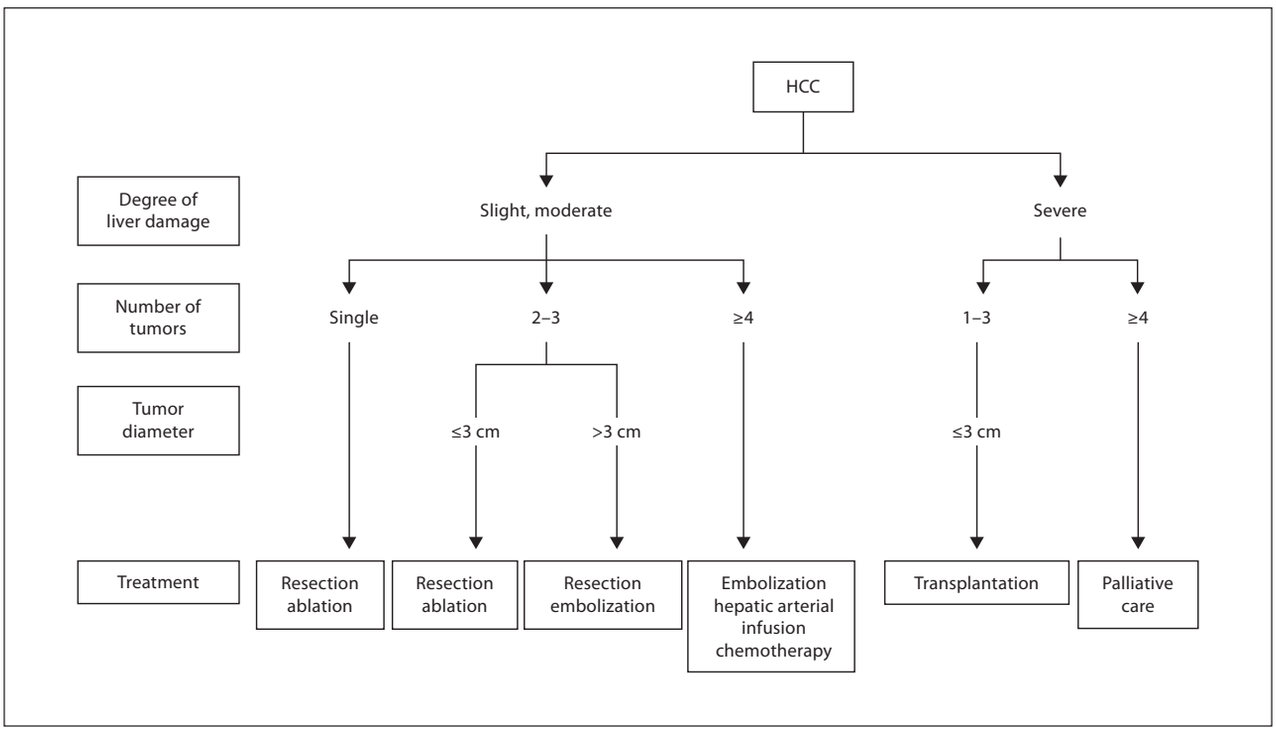


Fig. 2. Evidence-based treatment algorithm in Japan revised in 2009 [reproduced with permission].

arterial side, possibly causing temporary hepatic infarction, while this is impossible using DC beads.

Regarding advanced liver cancers, a very high percentage (48%) of the experts selected resection for Child-Pugh A patients with no distant metastasis and mild portal invasion. However, 27 and 25% selected intrahepatic arterial infusion chemotherapy and sorafenib, respectively. Actually, this selection should be made on an individual basis since resection is considered impossible in many patients. On the other hand, 61% answered that they would select sorafenib for Child-Pugh A patients with marked vascular invasion, symbolically exceeding those who supported intra-arterial infusion chemotherapy, which has been a Japanese de facto standard.

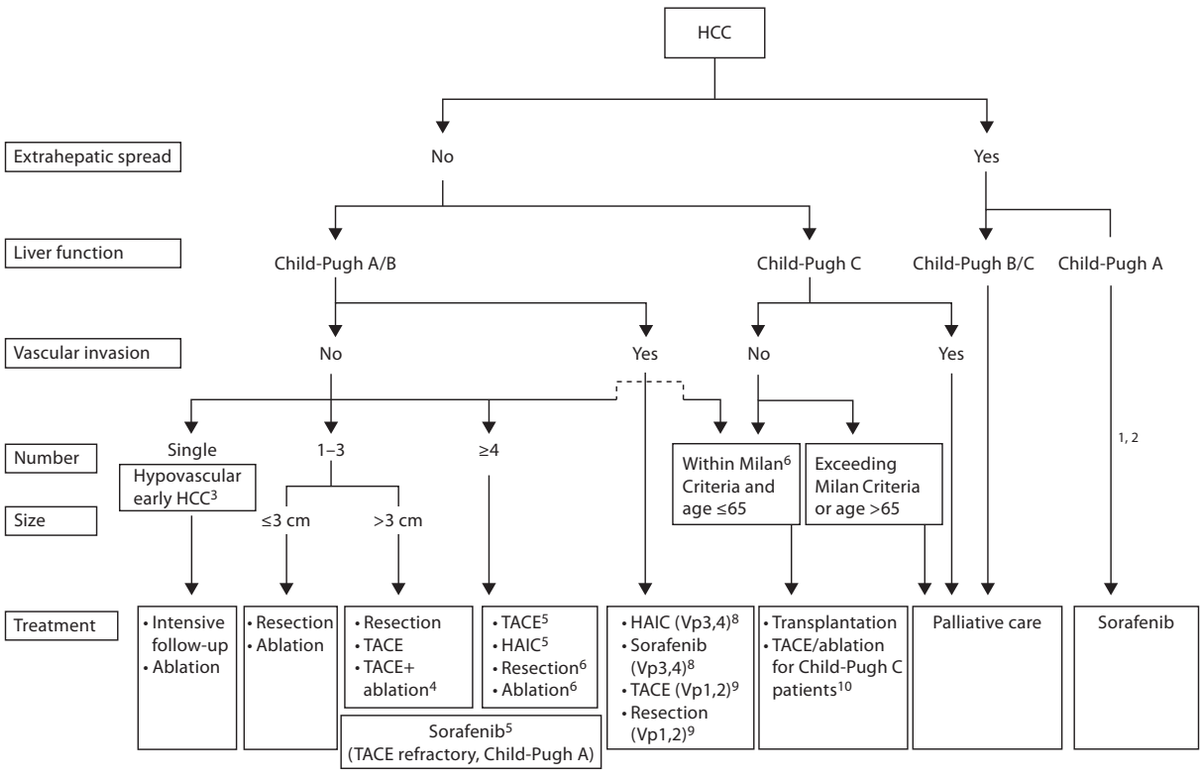
Concerning the criteria for liver transplantation, opposing results were obtained regarding liver transplantations from brain-dead and living donors. For liver transplantations from brain-dead donors, 84% of the experts answered that cancers should be confined to the Milan Criteria, but 75% considered that they need not meet the Milan Criteria for liver transplantations from living donors. These results may be reasonable as liver transplantations from living donors are mostly carried out within families, and the organs are not considered to be donated publicly. Also, the view widely shared in Japan that liver transplantation should be limited to Child-Pugh C patients and that it is not the first choice for Child-Pugh A or B patients was shown clearly in terms of numbers (fig. 1, VIII: 3–5). The answers are, again, understandable in Japan, where the skill and results of surgical resection, ablation, and TACE are all far superior to those in Western countries. Of course, the fact that liver transplantation is not a standard treatment in Japan, because there are few brain-dead liver donors, is also a major limiting factor influencing these results.

ConsensusS tatement6

Japanese HCC specialists use the Japanese treatment algorithm more often than the BCLC treatment algorithm in the real practice setting.

ConsensusS tatement7

An ablative margin is mandatory all around the nodule before RFA treatment is completed.



1 Treatment should be performed as if extrahepatic spread were negative, when extrahepatic spread is not regarded as a prognostic factor.

2 Sorafenib is the first choice of treatment in this setting as a standard of care.

3 Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (1) when the nodule is diagnosed pathologically as early HCC, (2) when the nodules show decreased uptake on Gd-EOB-MRI, or (3) when the nodules show decreased portal flow by CTAP, since these nodules are known to frequently progress to the typical advanced HCC.

4 Even for HCC nodules >3 cm in diameter, combination therapy of TACE and ablation is frequently performed when resection is not indicated.

5 TACE is the first choice of treatment in this setting. HAIC using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5-FU+CDDP) or intra-arterial 5-FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE refractory patients with Child-Pugh A liver function.

6 Resection is sometimes performed even when there are >4 nodules. Furthermore, ablation is sometimes performed in combination with TACE.

7 Milan criteria: tumor size ≤3 cm and tumor numbers ≤3; or a solitary tumor ≤5 cm. Even when liver function is good (Child-Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients.

8 Sorafenib and HAIC are recommended for HCC patients with Vp3 (portal invasion at the first portal branch) or Vp4 (portal invasion at the main portal branch).

9 Resection and TACE is frequently performed when portal invasion is minimal such as Vp1 (portal invasion at the third or more peripheral portal branch) or Vp2 (portal invasion at the second portal branch).

10 Local ablation therapy or subsegmental TACE is performed even for Child-Pugh C patients when transplantation is not indicated as there is no hepatic encephalopathy, no uncontrollable ascites, and a low bilirubin level (<3.0 mg/dl). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child-Pugh C patients. A prospective study is necessary to clarify this issue. Even in Child-Pugh A/B patients, transplantation is sometimes performed for relatively young patients with frequently or early recurring HCC after curative treatments.

Fig. 3. Consensus-based treatment algorithm for HCC proposed by the JSH 2009, revised in 2010 [reproduced with permission].

ConsensusS tatement8

Combination therapy of RFA and proceeding TACE should be done for a HCC nodule 63 cm.

ConsensusS tatement9

Resection is one choice of treatment option even for a solitary HCC nodule 15 cm, or a 63-cm nodule if liver function is preserved.

Consensus Statement 10

Liver transplantation is confined to Child-Pugh C patients with HCC meeting Milan Criteria.

In summary, the above results clearly illustrate marked differences in many aspects of both the concept and clinical practice concerning the diagnosis and treatment of liver cancer between Japan and Western countries including: (1) screening, (2) diagnosis, (3) TNM stage, (4) integrated staging system, (5) indications for surgical resection, (6) technique of RFA, (7) technical procedures with TACE, (8) combination of TACE and RFA, and (9) transplantation (see fig. 1).

Disclosure Statement

The author declares that he has no financial conflict of interest.

References

1 Bruix J, Sherman M: Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.

2 Makuuchi M, Kokudo N, Arii S, et al: Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res 2008;38:37–51.

3 Arii S, Sata M, Sakamoto M, et al: Management of hepatocellular carcinoma. Report of the Consensus Meeting at the 45th Annual Meeting of the Japan Society of Hepatology, 2009. Hepatol Res 2010 (in press).

4 Kudo M, Okanoue T: Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. Oncology 2007;72(suppl):2–15.

5 Kudo M: The 2008 Okuda Lecture. Management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. J Gastroenterol Hepatol 2010;25:439–452.

INTERNAL MEDICINE

□ CASE REPORT □

Cecal Intussusception in an Adult with Cronkhite-Canada Syndrome Relieved by Colonoscopy

Emi Ishikawa[1], Masatoshi Kudo[1], Yasunori Minami[1], Kazuomi Ueshima[1], Satoshi Kitai[1] and Kazuki Ueda[2]

Abstract

Cronkhite-Canada syndrome (CCS) is a rare, noninherited gastrointestinal polyposis syndrome associated with characteristic ectodermal abnormalities. Here, we report a case of Cronkhite-Canada syndrome with cecal intussusception relieved by colonoscopy. A 52-year-old man who was diagnosed as CCS pathologically two years previously presented abdominal pain and sub fever-up. Physical examination revealed the palpable mass sized approximate 10 cm in diameter in the upper abdominal site, in addition to the symptoms of alopecia, absent fingernails and toenails. However, abdominal wall rigidity and rebound tenderness were never expressed. Abdominal plain CT showed concentric circles from the ascending to the middle of the transverse colon, and a tumor in the lumen at the middle of the transverse colon. Colonoscopic reduction was performed first because we diagnosed it as intussusception due to CCS polyps without peritoneal irritation, and his symptoms were improved dramatically after careful reduction. Therefore, he was able to undergo the laparoscopic ascending colectomy as scheduled.

Key words: Cronkhite-Canada syndrome, intussusception, colonoscopic reduction

(Inter Med 49: 1123-1126, 2010)
(DOI: 10.2169/internalmedicine.49.2813)

Introduction

The patients with sigmoid volvulus or intussusception require an emergent surgery or colonoscopy to avoid a necrosis or perforation of digestive organs. The cause of intussusception in adulthood is often a tumor in the colon or small intestine (1). Therefore, some investigators have pointed out the risks of perforation and cancer cells seeding into the abdominal cavity in patients with advanced cancers caused by colonoscopic reduction (2-4). However, colonoscopic reduction may be able to reduce the necessity of an emergent surgery in intussusceptive patients with low malignant potentials and no peritoneal irritation sign.

Cronkhite-Canada syndrome (CCS) is a rare acquired gastrointestinal polyposis syndrome of unknown etiopathogenesis, accompanied by alopecia, custaneous hyper pigmentation, onychodystrophy, diarrhea, and dysgeusia (5). The syndrome is characterized by the presence of nonadenomatous inflammatory polyps that occur throughout the gastrointestinal tract, except for the esophagus (6-13). We report a case of Cronkhite-Canada syndrome presenting cecal intussusception relieved by colonoscopy.

Case Report

After several days of abdominal pain and low-grade fever, a 52-year-old man presented at our hospital. This patient had been seen regularly at our hospital during the past two years as an outpatient for follow-up of CCS. He had taken cox-2 inhibitor orally, but did not have a history of steroid use because of no phenomenon of protein-losing enteropathy and malnutrition. The present physical examination revealed a palpable mass approximately 10 cm in diameter in the upper abdomen, as well as symptoms of alopecia and missing finger and toenails. However, no abdominal wall rigidity or rebound tenderness was observed. Laboratory data indicated normal electrolytes and kidney and liver function. His he-

[1]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, Osaka-Sayama and [2]Department of Surgery, Kinki University School of Medicine, Osaka-Sayama
Received for publication August 24, 2009; Accepted for publication January 21, 2010
Correspondence to Dr. Masatoshi Kudo, minkun@med.kindai.ac.jp

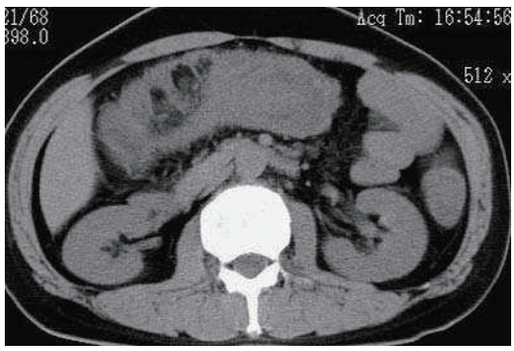


Figure 1. **Abdominal plain CT showing a pattern of concentric circles from the ascending colon to the middle of the transverse colon.**

moglobin was 12.9 g/dL, white blood cell count was 4,100/mm^3, CRP 0.063 mg/dL. Serum proteins were 6.4 g/dL and albumin was 3.7 g/dL. Serum CEA and CA19-9 were 1.1 ng/mL and 10 IU/mL, respectively. Plain abdominal CT showed a pattern of concentric circles from the ascending colon to the middle of the transverse colon, where a tumor was lodged in the lumen (Fig. 1). There were no signs of severe ischemia or necrosis at the base and the head of the intussusception.

Based on our diagnosis of intussusception due to CCS polyps, but with no peritoneal irritation, we performed colonoscopic reduction with the support of a surgeon. Colonoscopy revealed a focus of redness in the middle of transverse colon that centered on a large polyp with an irregular surface (Fig. 2a). Fluorography with meglumine sodium amidotrizoate during colonoscopy showed an obstruction that did not fill the proximal transverse colon (Fig. 2b). We took care that the scope was not pushed against consistent rigidity, and used the handling of air insufflation efficiently. Therefore, with careful handling of the colonoscope, we confirmed that the intussusceptions were relieved (Fig. 3a). Images also revealed a large and irregular polyp in the cecum (Fig. 3b), and small polyps in the colon.

The patient improved dramatically after intussusception reduction, however he underwent laparoscopic ascending colectomy as scheduled due to relapses. The main polyp in the cecum was grounded with a thick stalk that had an irregular, granulated surface. Dilative cystic ductal structures and a severe infiltration of inflammatory cells were confirmed pathologically for this polyp, but no colon cancer cells were detected. Thereafter, the patient showed no postoperative symptoms and remained in good condition for one year after surgical operation.

Discussion

CCS is characterized by the presence of diffuse gastrointestinal (GI) polyposis, dystrophic changes in the fingernails, alopecia, cutaneous hyperpigmentation, diarrhea, weight loss, abdominal pain, and other GI complications such as

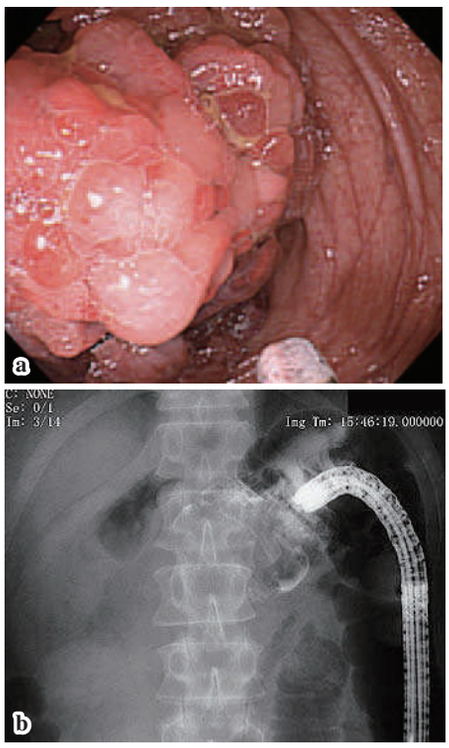


Figure 2. **Colonoscopic appearance of broad-based hamartomas typical of CCS in the middle of transverse colon (a). Fluorography with meglumine sodium amidotrizoate showed an obstruction and the subsequent extended colon (b).**

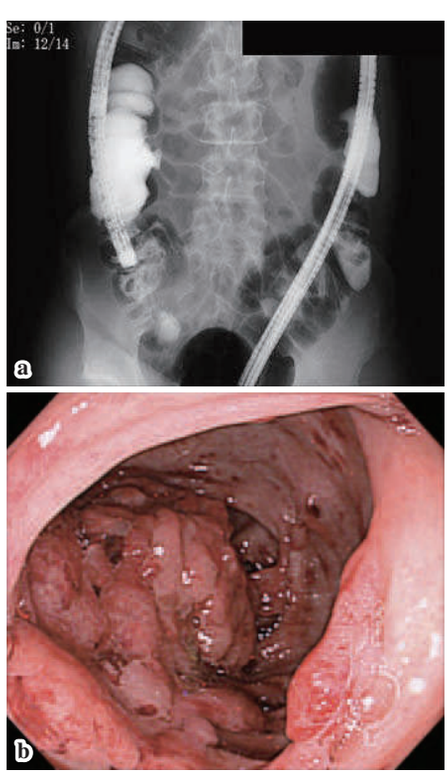


Figure 3. **Images of fluorography with meglumine sodium amidotrizoate (a) and colonoscopy (b) were obtained after the reduction of intussusception.**

protein-losing enteropathy and malnutrition (5-13). Malignant transformation of polyps is considered to be infrequent, however, there were 50 cases of CCS associated with gastrointestinal cancer reported up to 2002, including 31 cases of colon cancer and 19 cases of gastric cancer (12, 13). Therefore, surveillant upper and lower endoscopy should be performed regularly.

Intussusception itself in adults occurs relatively rarely; however, a specific lead point is identified in more than 90% of cases (14, 15). A correct and timely diagnosis is not only necessary to avoid the complications of bowel infarction and perforation secondary to high-grade obstruction but also to resect the underlying lesion that serves as a lead point. Therefore, knowledge of the imaging spectrum and the clinical features of intussusception are important because imaging plays a crucial role in the diagnosis and management of these patients. Typical intussusception is well diagnosed on CT, which shows a pathognomonic bowel-within-bowel configuration (15, 16), appears as a sausage-shaped mass when CT images is obtained parallel to its longitudinal axis of digestive tract but as a target-like mass when CT images is perpendicular to the cross sections of digestive tract (15, 16). Sonography can facilitate the diagnostic decision when the characteristic sign of a target like lesion or bull's eye lesion is shown, similar to the CT findings (15). We could diagnose the intussusception in this patient immediately by these typical findings of imaging.

Intussusceptions associated with CCS have been reported as a case report in at least four patients in Japanese language up to 2007 (17-19) in spite of no report in the English language literature. According to these literatures, intussusceptions had occurred at the ascending colon/cecum. This patient underwent laparoscopy as a therapeutic procedure, and improved. No 30-day mortality was encountered in this review. Intraoperative reduction before resection was attempted in only one patient. It was successful; nevertheless, the surgical treatment was required because of a relapse of intussusceptions.

There has been controversy associated with the option of preliminary reduction of the intussusception before resection vs. primary resection without reduction (1). The theoretic objections to reduction of grossly viable bowel with mucosal necrosis are: 1) intraluminal seeding and venous embolization of malignant cells in the region of ulcerated mucosa, 2) possible perforation during manipulation, 3) increased risk of anastomotic complications in the face of edematous and inflamed bowel (2-4). Reduction should not be attempted if there are signs of bowel ischemia or inflammation. Based on a high incidence of an underlying malignancy, which may be difficult to confirm intraoperatively, many authors recommend primary resection whenever possible. However, emergency operation was generally associated with high rates of complications and mortality (20). In patients with early stage of intussusceptions without peritoneal irritation, careful colonoscopic reduction would have less risk of tumor cell seeding, perforation, and anatomic complications; therefore, colonoscopic reduction ought to be attempted in cooperation with surgeons for the avoidance of emergency surgery.

Several reports have discussed intussusceptions associated with Peutz-Jeghers syndrome, a kind of disease of multiple polyposis (21-23). Peutz-Jeghers syndrome is characterized by hamartomas throughout the gastrointestinal tract, mucocutaneous melanotic spots and increased predisposition to malignancy. The polyp stalk of Peutz-Jeghers syndrome tends to lengthen with the growing of polyps. The bigger polyp with long stalk as the lead point of an intussusception may be outlined distal to the tapered lumen of the intussusceptum. On the other hand, polyps of CCS tend to be relatively small without a long stalk. However, in these patients, the larger size of the cecum polyp might be occur in spite of the thick stalk of the polyp. Not only polyposis but also inflammatory bowel disease could cause giant pseudopolyps. Colonic intussusception of a giant pseudopolyp was reported in a patient with inflammatory bowel disease (24, 25).

In conclusion, we described an adult with cecal intussusception due to CCS polyps that was relieved by colonoscopy. Colonoscopic reduction may reduce the necessity of an emergent surgery in intussusceptive patients with low malignant potential and no peritoneal irritation sign.

Author contributions:

Guarantors of integrity of entire study, E.I., M.K.; study concepts and design, E.I; definition of intellrctual content, E.I, S.H, M.Y; literature research, U.K, H.S; clinical study, E.I, U.K; data acquisition and analysis, E.I, H.S; manuscript preparation, E.I; manuscript editing and review, E.I, M.Y, K.M

References

1. Zubaidi A, Faisal AS, Silverman R. Adult intussusception: a retrospective review. Dis Colon Rectum **49**: 1546-1551, 2006.
2. Weibaecher D, Bolin JA, Hearn D, et al. Intussception in adults. Review of 160 cases. Am J Surg **121**: 531-535, 1971.
3. Reijnen HA, Joosten HJ, de Boer HH. Diagnosis and treatment of adult intussception. Radiology **124**: 791-792, 1977.
4. Eisen LK, Cunningham JD, Aufses AH Jr. Intussception in adults: institutional review. J Am Coll Surg **188**: 390-395, 1999.
5. Cronkhite LW Jr, Canada WJ. Generalized gastrointestinal polyposis: An unusual syndrome of polyposis, pigmentation, alopecia and onychotrophia. N Engl J Med **252**: 1011-1015, 1955.
6. Ward EM, Wolfsen HC. Review article: The non-inherited gastrointestinal polyposis syndromes. Aliment Pharmacol Ther **16**: 333-342, 2002.
7. Takakura M, Adachi H, Tsuchihashi N, et al. A case of Cronkhite-Canada syndrome markedly improved with mesalazine therapy. Dig Endosc **16**: 74-78, 2004.
8. Kubo T, Hirose S, Aoki S, et al. Canada-Cronkhite syndrome associated with systemic lupus erythematosus. Arch Intern Med **146**: 995-996, 1986.

9. Murata L, Yoshikawa L, Endo M, et al. Cronkhite-Canada syndrome: report of two cases. J Gastroenterol **35**: 706-711, 2000.
10. Takeuchi Y, Yoshikawa M, Tsukamoto N, et al. Cronkhite-Canada syndrome with colon cancer, portal thrombosis, high titer of antinuclear antibodies, and membranous glomerulonephritis. J Gastroenterol **38**: 791-795, 2003.
11. Goto A, Shimokawa K. Cronkhite-Canada syndrome associated with lesions predisposing to development of carcinoma. Cancer Ther **29**: 1767-1777, 1994.
12. Negoro K, Takahashi S, Kinouchi Y, et al. Analysis of the PTEN gene mutation in polyposis syndromes and sporadic gastrointestinal tumors in Japanese patients. Dis Colon Rectum **43**: S29-S33, 2000.
13. Yashiro M, Kobayashi H, Kubo N, Nishiguchi Y, Wakasa K, Hirakawa K. Cronkhite-Canada syndrome containing colon cancer and serrated adenoma lesions. Digestion **69**: 57-62, 2004.
14. Agha FP. Intussusception in adults. AJR **146**: 527-531, 1986.
15. Choi AH, Han JK, Kim AH, et al. Intussusception in adults: from stomach to rectum. AJR **183**: 691-698, 2004.
16. Floemer F, Bissig H, Oertli D, et al. Multislice CT in adult colocolic intussusception: case report and review of the literature. Emerg Radiol **15**: 361-366, 2008.
17. Futagami Y, Tanaka S, Haruma K, et al. A case of Cronkhite-Canada syndrome complicated with colonic invagination. Gastroenterological endoscopy **39**: 1602-1607, 1997.
18. Yamamoto T, Tanaka Y, Motizuki S, et al. A case report of intussusception due to the polyp of Cronkhite-Canada syndrome. Jap J Gastroenterol Surg **40**: 1250, 2007.
19. Morinaga N. Two cases report of intussusception with Cronkhite-Canada syndrome. J Abd Emerg Med **14**: 243, 1995.
20. Kim J, Mittal R, Konyalian V, et al. Outcome analysis of patients undergoing colorectal resection for emergent and elective indication. Am Surg **73**: 991-993, 2007.
21. Talwar N, Mohan S, Andley M, et al. Prograde and retrograde intussusception: A rarity in Peutz-Jeghers syndrome. Int Surg **91**: 265-266, 2006.
22. Akimaru K, Katoh S, Ishiguro S, et al. Resection of over 290 polyps during emergency surgery for four intussusceptions with Peutz-Jeghers syndrome: Report of a case. Surg Today **36**: 997-1002, 2006.
23. Jaremko JL, Rawat B. Colo-colonic intussusception caused by a solitary Peutz-Jeghers polyp. Br J Radiol **78**: 1047-1049, 2005.
24. Atten MJ, Attar BM, Mahkri MA, Del Pino A, Orsay CP. Giant pseudopolyps presenting as colocolic intussusception in Crohn's colitis. Am J Gastroenterol **93**: 1591-1592, 1998.
25. Maldonado TS, Firoozi B, Stone D, Hiotis K. Colocolonic intussusception of a giant pseudopolyp in a patient with ulcerative colitis: a case report and review of the literature. Inflamm Bowel Dis **10**: 41-44, 2004.

INTERNAL MEDICINE

□ CASE REPORT □

Autoimmune Thrombocytopenic Purpura during Pegylated Interferon α Treatment for Chronic Hepatitis C

Soo Ryang Kim[1], Susumu Imoto[1], Masatoshi Kudo[2], Taisuke Nakajima[1], Kenji Ando[1], Keiji Mita[1], Katsumi Fukuda[1], Hyun Soo Hong[1], Yeong Ho Lee[1], Keiichi Nakashima[1], Ikuo Shoji[3], Motoko Nagano-Fujii[3], Hak Hotta[3] and Yoshitake Hayashi[4]

Abstract

We describe a 72-year-old woman with chronic hepatitis C and autoimmune thrombocytopenic purpura (AITP) during pegylated interferon (PEG-IFN) α. Immunoglobulin G and antinuclear antibody were 2,113 mg/dL and 1,280 at the start, respectively. A liver biopsy negated autoimmune hepatitis. After a 48-week combination therapy with ribavirin, PEG-IFN α-2a was administered. At the 30th month, the platelet count was decreased to 1.1×10^4/μL. Bone marrow biopsy disclosed normocellular marrow compatible with AITP. The platelet-associated IgG (PAIgG) titer rose to 500 ng/10^7 cells. Corticosteroid therapy was successful, and the platelet count and PAIgG titer reverted to 6.4×10^4/μL and 57.3 ng/10^7 cells, respectively.

Key words: autoimmune thrombocytopenic purpura, interferon, platelet-associated immunoglobulin G, steroid therapy, chronic hepatitis C, immunological disorder

(Inter Med 49: 1119-1122, 2010)
(DOI: 10.2169/internalmedicine.49.3413)

Introduction

A number of autoimmune disorders attributed to interferon treatment such as thyroid disease and diabetes and others have been reported, among which are blood cell-related disorders including red blood cells and platelets. Here, we describe a case of autoimmune thrombocytopenic purpura (AITP) during pegylated interferon (PEG-IFN) α therapy.

Case Report

A 72-year-old woman (149 cm tall, weighing 54 kg) with chronic hepatitis C genotype 1b infection was started on PEG-IFN α-2b (80 μg/week) and ribavirin (600 mg/day) (PEG-IFN/RBV) in July 2006. Laboratory values were as follows: aspartate aminotransferase (AST) 43 (normal, 8-38) IU/L, alanine aminotransferase (ALT) 25 (4-43) IU/L, γ-glutamyl transpeptidase 47 (≤48) IU/L, bilirubin 0.6 (0.2-1.2) mg/dL, hepatitis C virus (HCV)-RNA 2,400 KIU/mL, hemoglobin 12.4 (11.3-15.2) g/dL, white blood cells count (WBC) 2,700 (3,500-9,100) /μL, platelets 12.4 (13.0-36.9)×10^4/μL, immunoglobulin (Ig) G 2,113 (870-1,700) mg/dL, IgA 331 (110-410) mg/dL, IgM 334 (46-260) mg/dL, and antinuclear antibody (ANA) 1,280 (<40). A liver biopsy showed moderate inflammation and severe fibrosis (F3, A2) according to the new classification of Desmet et al (1) without plasma cell infiltration (Figs. 1a, 1b). Autoimmune hepatitis was ruled out. During the therapy, serum HCV-RNA remained positive and liver functions such as AST and ALT did not reach normal levels. After completing the 48-week course of PEG-IFN/RBV therapy in June 2007, the patient was put on PEG-IFN α-2a (90 μg) without ribavirin; however, liver functions were not normalized. During both treatment protocols, the number of platelets remained between 4 and 14×10^4/μL.

At the 30th month (October 2008), the platelet count rap-

[1]Department of Gastroenterology, Kobe Asahi Hospital, Kobe, [2]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, [3]Division of Microbiology, Kobe University Graduate School of Medicine, Kobe and [4]Center for Infectious Diseases (CID), Kobe University Graduate School of Medicine, Kobe
Received for publication January 19, 2010; Accepted for publication February 24, 2010
Correspondence to Dr. Soo Ryang Kim, asahi-hp@arion.ocn.ne.jp

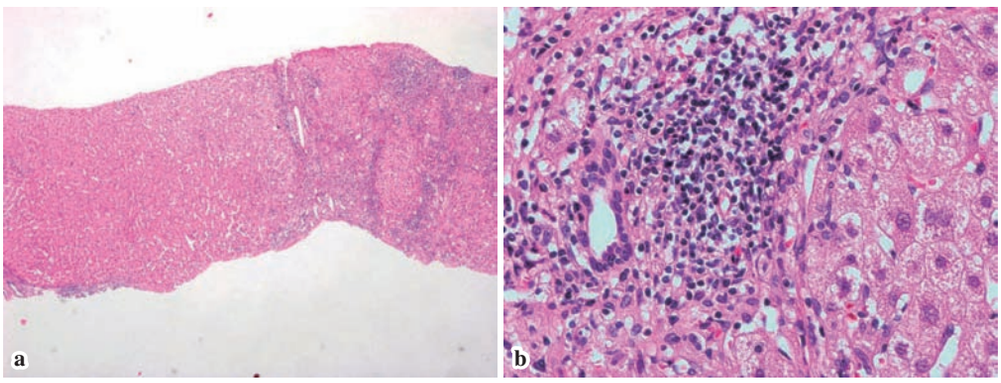


Figure 1. **Histological features of the liver: moderate inflammation and severe fibrosis (F3, A2) without plasma cell infiltration. a: Hematoxylin and Eosin staining ×40. b: Hematoxylin and Eosin staining ×400.**

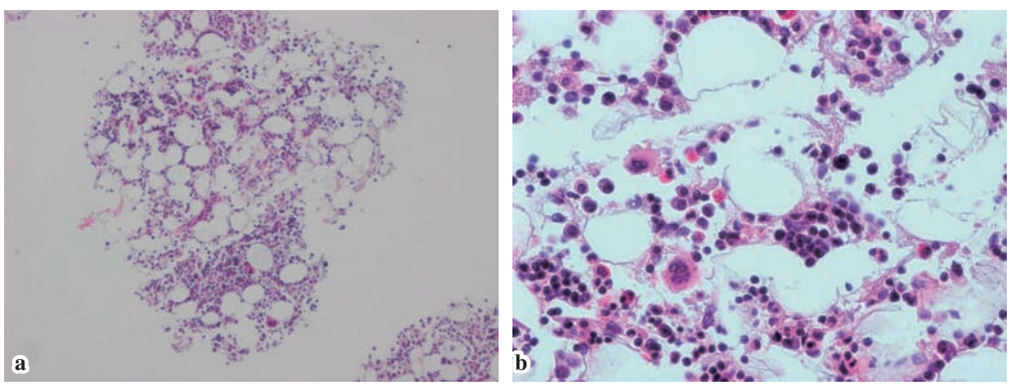


Figure 2. **Histological features of the bone marrow: normocellular marrow with myeloid: erythroid (M:E) ratio 3:1 and increased numbers of megakaryocytes (64/mm^3) compatible with a diagnosis of autoimmune thrombocytopenic purpura (AITP). a: Hematoxylin and Eosin staining ×40. b: Hematoxylin and Eosin staining ×400.**

idly declined to 1.1×10^4/μL (just 1 week before 6.2×10^4/μL), other values were WBC 1,500/μL, hemoglobin 9.0 g/dL, and hematocrit 26.8% and petechiae appeared on the patient's upper extremities. PEG-IFN α-2a was discontinued, and bone marrow biopsy showed normocellular marrow with a myeloid : erythroid (M : E) ratio of 3:1 and an increased number of megakaryocytes (64/mm^3) compatible with the diagnosis of AITP (Figs. 2a, 2b). Coagulation test results were normal, a direct Coombs' test was negative, ANA was 1,280 times (cytoplasmic×160 times), the anticardiolipin antibody was negative and cryoglobulins were positive. The platelet-associated IgG (PAIgG) level on the platelet surface had increased to 500 (normal, 9.0-25.0) ng/10^7 cells as measured by enzyme-linked immunoassay. The results of a ^{13}C-urea breath test were negative for *Helicobacter pylori* infection, obviating bacteria removal therapy; instead, corticosteroid pulse therapy was started with the intravenous administration of 1,000 mg methylprednisolone sodium succinate for three days, followed by 30 mg of oral prednisolone for 2 weeks and gradually reduced to 5 mg per day. The platelet count reverted to 6.4×10^4/μL in 14 days, and remained normal while the prednisolone dose was tapered off until the 39th month (July 2009). The PAIgG titer decreased to 57 ng/10^7 cells in response to the corticosteroid therapy (Fig. 3). HCV-RNA remained positive and liver functions did not return to normal after the withdrawal of PEG-IFN α-2a.

Discussion

Mild-to-moderate thrombocytopenia is a common adverse event of treatment with conventional interferon or with PEG-IFN α, attributed primarily to bone marrow suppression, in patients with chronic hepatitis C. Nevertheless, severe, life-threatening AITP has rarely been associated with IFN treatment (2-8). The pathogenesis of AITP is not fully understood, but IgG-type antibodies against platelet membrane glycoproteins (IIb/IIIa, Ib/IX, etc.) are known to be involved (9).

AITP, an autoimmune disorder characterized by peripheral consumption of platelets and clinical manifestations of hemorrhagic diathesis (9), is a diagnosis of exclusion and often

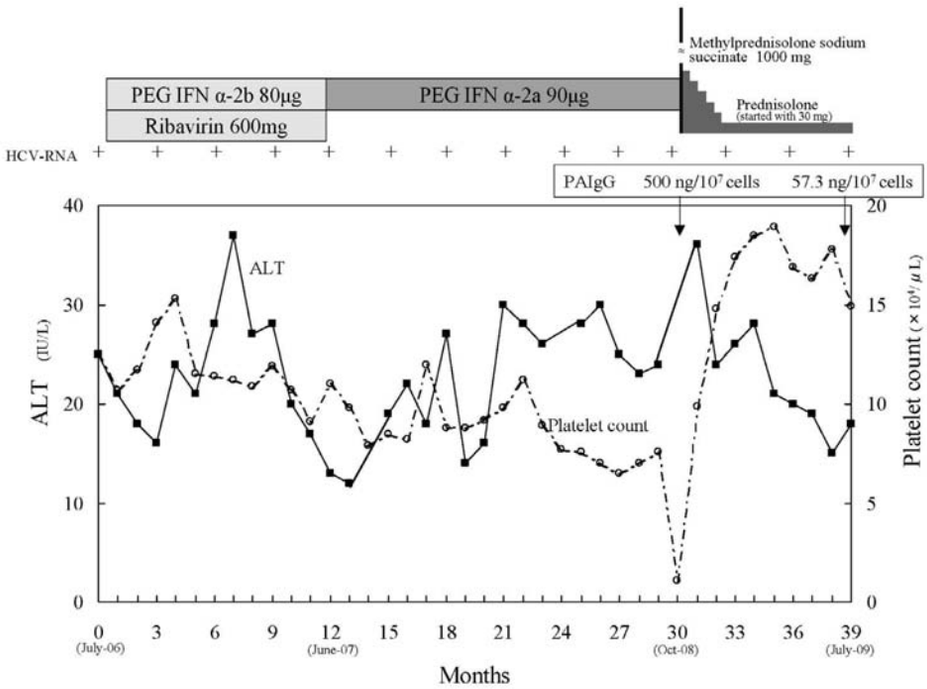


Figure 3. Clinical course of the present case.

difficult to establish. IFN-induced AITP has been reported to develop after 4 weeks to 12 months of therapy (3, 5) and even 6 months after the completion of therapy (7). AITP has been reported irrespective of the kind of IFN: IFN α-2b, PEG-IFN α-2a, and PEG-IFN with or without RBV (10).

The age of patients and baseline platelet count have varied widely, ranging from 27 (7) to 73 (6) years and from 8 (4) to 26 (5)×10^4/μL, respectively. The detection of circulating antiplatelet antibodies unbound to platelets is not sensitive enough for the diagnosis. Such autoantibodies can develop in patients immunized by pregnancy, allogenic transfusions or organ transplantation and are, thus, not specific for AITP. In contrast, direct assay of PAIgG is more useful in the diagnosis of AITP, with a sensitivity of 49-66% and a specificity of 78-92% (9). In the present case, PAIgG increased to 500 ng/10^7 cells (well above the normal range in the diagnosis of AITP) then decreased to 57.3 ng/10^7 cells (within the normal range) at the remission stage of AITP. As demonstrated in our patient, the response to steroid treatment was consistent with the diagnosis of AITP and PAIgG was also helpful in monitoring the response to corticosteroid therapy, and immunological disorders such as high γ-globulin levels of IgG and ANA positivity were found at the start of PEG-IFN/RBV therapy. Autoimmune hepatitis was ruled out by histological examination and the patient was started on PEG-IFN/RBV therapy. The clinical course was carefully monitored, focusing on the occurrence of autoimmune disease including diabetes, arthritis, sicca syndrome, vasculitis, thyroid abnormalities and others. Thirty months after the start of IFN therapy with PEG-IFN α-2b/RBV and PEG-IFN α-2a, AITP might have occurred by an autoimmunological mechanism. Clinicians should be vigilant about the occurrence of AITP during and after IFN therapy, especially in the presence of immunological disorders (11).

References

1. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Sheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. Hepatology **19**: 1513-1520, 1994.
2. Dourakis SP, Deutsch M, Hadziyannis SJ. Immune thrombocytopenia and alpha-interferon therapy. J Hepatol **25**: 972-975, 1996.
3. Sevastianos VA, Deutsch M, Dourakis SP, et al. Pegylated interferon-2b-associated autoimmune thrombocytopenia in a patient with chronic hepatitis C. Am J Gastroenterol **98**: 706-707, 2003.
4. Medeiros BC, Seligman PA, Everson GT, et al. Possible autoimmune thrombocytopenia associated with pegylated interferon-alpha 2a plus ribavirin treatment for hepatitis C. J Clin Gastroenterol **38**: 84-86, 2004.
5. Weitz IC. Treatment of immune thrombocytopenia associated with interferon therapy of hepatitis C with the anti-CD20 monoclonal antibody, rituximab. Am J Hematol **78**: 138-141, 2005.
6. Lambotte O, Gelu-Simeon M, Maigne G, et al. Pegylated interferon alpha-2a-associated life-threatening Evans' syndrome in a patient with chronic hepatitis C. J infect **51**: e113-e115, 2005.
7. Elefsiniotis IS, Pantazis KD, Fotos NV, et al. Late onset autoimmune thrombocytopenia associated with pegylated interferon-alpha-2b plus ribavirin treatment for chronic hepatitis C. J Gastroenterol Hepatol **21**: 622-623, 2006.
8. Couto CA, Faria LC, Ribeiro DD, et al. Life-threatening thrombocytopenia and nephrotic syndrome due to focal segmental glomerulosclerosis associated with pegylated interferon alpha-2b and ribavirin treatment for hepatitis C. Liver Int **26**: 1294-1297, 2006.

9. Cines DB, Blanchette VS. Immune thrombocytopaenic purpura. N Engl J Med **346**: 995-1008, 2002.
10. Enomoto M, Yamane T, Hino M, Ohnishi M, Tamori A, Kawada N. Platelet-associated IgG for the diagnosis of immune thrombocytopaenic purpura during peginterferon α and ribavirin treatment for chronic hepatitis C. Liver Int **28**: 1314-1315, 2008.
11. Lambotte O, Gelu-Simeon M, Maigne G, et al. Pegylated interferon alpha-2a-associated life-threatening Evans' syndrome in a patient with chronic hepatitis C. J Infect **51**: 113-115, 2005.

Hepatology Research 2010; **40** (Suppl. 1): 113–119 doi: 10.1111/j.1872-034X.2010.00659.x

Chapter 6: Local ablation therapy

INTRODUCTION

DURING THE PAST quarter of a century, various procedures were developed as local therapy for hepatocellular carcinoma. In 1979, Yamada *et al.* developed transcatheter arterial embolization (TAE), and this can be regarded as the first treatment approach that clarified the efficacy of local therapy for hepatocellular carcinoma.

Next, with the spread and progress of abdominal ultrasound diagnostic devices, Sugiura *et al.* created percutaneous ethanol injection therapy (PEIT) in 1983. PEIT may be the prototype of various subsequently devised local therapies that are conducted under ultrasonographic imaging. Because this procedure requires only a simple technique, and local injection needles and ethanol are inexpensive, it has quickly spread not only in Japan but also worldwide and is highly valued for its primary role in hepatocellular carcinoma treatment. Nonetheless, because PEIT is a treatment involving the infusion of a solution, "ethanol", and because ethanol does not uniformly diffuse in a tumor and pass through the septum or the capsule, problems of residual tumors and local recurrence remain.

In order to overcome these disadvantages of PEIT, treatments aimed at thermo-coagulation of tumors by emitting microwaves or radiofrequency waves from the inserted needle were developed. In 1994, Seki *et al.* presented percutaneous microwave coagulation therapy (PMCT) developed by percutaneous microwave application that had been used in the surgical field.

In 1993, Rossi *et al.* performed percutaneous radiofrequency ablation (RFA) in patients with small hepatocellular carcinoma and reported good therapeutic efficacy; treatment with radiofrequency waves for hepatocellular carcinoma quickly gained attention. In Japan, it has been conducted at many institutions since 1999. Because the range of necrosis achieved by one session is wider for RFA than for PMCT, RFA has been far more widely adopted than PMCT. In April 2004, RFA was finally covered by the National Health Insurance. Around the time when the 2005 Clinical Practice Guidelines for Hepatocellular Carcinoma were published, RCT comparing PEIT and RFA were presented in Japan and foreign countries. Their results all showed that RFA prolonged life expectancy more than PEIT. Based on such evidence, RFA has become the current standard treatment among local ablation therapies.

In this section, we organized evidence on PEIT, PMCT and RFA available as of June 2007.

SELECTION OF PUBLISHED WORK

The area of local therapy is classified into the following categories according to treatment procedures: (i) percutaneous ethanol injection therapy; (ii) microwave coagulation therapy; and (iii) radiofrequency ablation.

We created a published work list on each procedure included in MEDLINE and Japana Centra Revuo Medicina during the period from 1983 to June 2007 and extracted reports that seemed to be useful for establishment of the Guidelines. Furthermore, we read the abstracts and picked up the original articles of those that should be reviewed, and selected articles with as high an evidence level as possible. Evaluations were chosen based on article style, sample size and study design.

CQ48 In what patients should local ablation therapy be performed?

RECOMMENDATION

Good candidates for local ablation therapy are patients with liver function graded Child–Pugh class A or B, and three or fewer tumors measuring 3 cm or less in diameter. **(grade C1)**

SCIENTIFIC STATEMENT

In an analysis ($n = 12\,888$) of the follow-up survey by the Liver Cancer Study Group of Japan, the therapeutic results of hepatectomy were better than those of PEIT in Clinical Stage (CS) I (current liver damage A) patients with a solitary tumor less than 2 cm in diameter ($P = 0.01$), whereas there was no significant difference between hepatectomy and PEIT in CS II (liver damage B) or more advanced-stage patients. In contrast, the therapeutic results of hepatectomy were good in patients with a solitary tumor larger than 2 cm in diameter. In CS II (liver damage B) with a tumor larger than 2 cm in diameter, the therapeutic results of hepatectomy were also favorable (LF00178[1] level 2a).

In a retrospective study in hepatocellular carcinoma patients ($n = 3225$) involving 18 institutions in Japan, the 5-year survival rate was equivalent between hepatectomy and PEIT in CS I (liver damage A) patients with three or fewer tumors measuring 3 cm or less in diameter. In CS II (liver damage B) patients, the survival rate was higher for PEIT (LF00472[2] level 2b).

In a retrospective study in patients with a single hepatocellular carcinoma measuring 5 cm or less in diameter with cirrhosis, patients were assigned to hepatectomy ($n = 120$), PEIT ($n = 155$) or non-treatment ($n = 116$) and studied. The results showed the 3-year survival rate to be equivalent between hepatectomy and PEIT in both Child–Pugh class A and B patients (LF00600[3] level 2b).

Huang *et al.* conducted an RCT of hepatectomy and PEIT in 76 Child–Pugh class A or B hepatocellular carcinoma patients with two or fewer tumors measuring 3 cm or less in diameter and reported that there was no difference in the recurrence rate or the survival rate between the two (LF10134[4] level 1b). Nonetheless, there were only eight cancer deaths in both groups, and the follow-up period was found to be insufficient. Chen *et al.* performed an RCT of hepatectomy and RFA in 180 patients with a single tumor measuring no more than 5 cm in diameter and reported that there was no difference in the recurrence rate or the survival rate (LF10135[5] level 1b). However, 19 of 90 patients, who were assigned to RFA, withdrew consent and underwent hepatectomy; thus, the appropriateness of making this comparison was questionable.

Murakami *et al.* examined the local recurrence rate in 258 consecutive hepatocellular carcinoma patients with three or fewer tumors measuring 3 cm or less in diameter or a single tumor measuring 5 cm or less in diameter who underwent RFA or transcatheter arterial chemoembolization (TACE), and reported that RFA was significantly superior to TACE ($P = 0.013$) (LF11840[6] level 2a).

The local recurrence rate for PEIT increased when the tumor was larger than 3 cm (LF01555[7] level 2a).

COMMENTS

The conclusion as to whether local ablation therapy can be employed as the first-line treatment instead of hepatectomy in hepatocellular carcinoma treatment has not yet been reached. The analysis of the follow-up survey by the Liver Cancer Study Group of Japan has the largest sample size presented, to date, for examining this issue. However, liver function was only matched to liver damage stages, and the tumor diameter was categorized into 2 cm or less versus 2–5 cm. Consequently, hepatectomy was quite likely to be performed in hepatocellular carcinoma patients with better liver function even if the liver damage stages were comparable, or in those with larger tumors even if the category was the same; the appropriateness of comparison was thus questionable. In addition, this was a comparison between PEIT and hepatectomy; thus, had a comparison been made with RFA, which could conceivably provide a better survival rate, the result would probably have been different. Two RCT were subsequently presented, but both had problems

of study design. It may be too early to draw any firm conclusions or reach consensus on this issue.

When limiting the candidates to unresectable patients, the indications for local ablation therapy are determined in comparison with the third-line treatment, TACE. Murakami *et al.* examined the local recurrence rate in 258 consecutive hepatocellular carcinoma patients with three or fewer tumors measuring 3 cm or less in diameter or a single tumor measuring 5 cm or less in diameter who underwent RFA or TACE, and reported that RFA was significantly superior to TACE ($P = 0.013$) (LF11840[6] level 2a). There are no RCT comparing the survival rate between TACE alone and local therapy alone for tumors in this range; however, based on this evidence, we recommend local therapy for unresectable hepatocellular carcinoma measuring 3 cm or less in diameter and three or fewer lesions.

Many studies of indications for PEIT as local therapy for tumors selected candidates with three or fewer tumors measuring 3 cm or less in diameter. It has been reported that the local recurrence rate for PEIT increased when tumor diameter exceeded 3 cm. In principle, the range of ablation can be expanded for RFA, which is thermo-coagulation therapy, by increasing the number of punctures. However, increases in the range of ablation and the number of punctures are anticipated to raise the incidence of complications. Considering that the ablation range for many RFA electrodes is approximately 3 cm, we decided to also adhere to the PEIT candidate indications (three or fewer tumors measuring ≤3 cm in diameter) for those receiving RFA.

REFERENCES

1 LF00178 Arii S, Yamaoka Y, Futagawa S *et al.* Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. *Hepatology* 2000; **32** (6): 1224–9.

2 LF00472 Ryu M, Shimamura Y, Kinoshita T *et al.* Therapeutic results of resection, transcatheter arterial embolization and percutaneous transhepatic ethanol injection in 3225 patients with hepatocellular carcinoma: a retrospective multicenter study. *Jpn J Clin Oncol* 1997; **27**: 251–7.

3 LF00600 Livraghi T, Bolondi L, Buscarini L *et al.* No treatment, resection and ethanol injection in hepatocellular carcinoma: a retrospective analysis of survival in 391 patients with cirrhosis. Italian Cooperative HCC Study Group. *J Hepatol* 1995; **22**: 522–6.

4 LF10134 Huang GT, Lee PH, Tsang YM *et al.* Percutaneous ethanol injection versus surgical resection for the treatment of small hepatocellular carcinoma: a prospective study. *Ann Surg* 2005; **242** (1): 36–42.

5 LF10135 Chen MS, Li JQ, Zheng Y *et al.* A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321–8.

6 LF11840 Murakami T, Ishimaru H, Sakamoto I *et al.* Percutaneous radiofrequency ablation and transcatheter arterial chemoembolization for hypervascular hepatocellular carcinoma: rate and risk factors for local recurrence. *Cardiovasc Intervent Radiol* 2007; **30**: 696–704.

7 LF01555 Ishii H, Okada S, Nose H *et al.* Local recurrence of hepatocellular carcinoma after percutaneous ethanol injection. *Cancer* 1996; **77**: 1792–6.

CQ49 How should each local ablation therapy be chosen?

RECOMMENDATION

Radiofrequency ablation can be recommended for patients who are candidates for local ablation therapy. **(grade A)**

SCIENTIFIC STATEMENT

With RFA, local control is superior to that with PEIT and survival rate is improved.

Four RCT comparing RFA and PEIT have been published. Their outlines are summarized in Table 1. In all four articles, the local recurrence rate was significantly lower for RFA than for PEIT. In three of the four articles, the survival rate in patients treated with RFA was significantly better than in those treated with PEIT (LF10941[1] level 1b, LF10457[2] level 1b, LF11869[3] level 1b, LF10468[4] level 1b). Lencioni *et al.* randomized 102 hepatocellular carcinoma patients with a single tumor 5 cm or less in diameter or with three or fewer tumors measuring 3 cm or less in diameter into two groups and treated them with RFA or PEIT. During a mean follow-up period of approximately 22 months, the 2-year survival rate was 98% in the RFA group and 88% in the PEIT group, showing no significant difference (hazard ratio = 0.20; $P = 0.138$). However, events occurred in six patients in the two groups combined, such that the follow-up period was found to be too short.

With regard to complications, three of the four RCT revealed no significant differences. In an article comparing RFA, PEIT and percutaneous acetic acid injection (PAI), Lin *et al.* reported that hemothorax requiring drainage in two patients and gastric perforation requiring laparotomy in one patient occurred in the RFA group (LF10468[4] level 1b).

COMMENTS

There are RCT comparing PEIT, PAI, PMCT and RFA as local ablation therapies. Because PAI and PMCT are rarely conducted at present in Japan, we adopted articles comparing PEIT and RFA. Based on the RCT results, we find that RFA should be selected when both PEIT and RFA are applicable. As the issue of whether RFA more frequently causes complications than PEIT, no conclusion has been drawn in the RCT performed to date; however, the incidence of complications may be higher for RFA based on the results of non-RCT and past reports. In particular, gastrointestinal perforation is a complication specific to thermo-coagulation therapy.

Table 1 Summary of RCTs comparing local ablation therapies in hepatocellular carcinoma patients

Reference ID	Therapy (No. of patients)	Hazard ratio of local recurrence	*P* value	Hazard ratio of entire survival rate, High-dose PEIT	*P* value
LF10941[1]	RFA (52), PEIT (50)	0.17	0.02	0.20	0.138
LF10457[2]	RFA (52), PEIT (52), high-dose PEIT (53)	0.37† 0.49‡	0.012† 0.037‡	0.34† 0.39‡	0.014† 0.023‡
LF11869[3]	RFA (118), PEIT (114)	0.12	0.006	0.54	0.02
LF10468[4]	RFA (62), PEIT (62), PAI (63)	0.35† 0.41§	0.012† 0.017§	0.42† 0.45§	0.031† 0.038§

†Comparison of RFA and PEIT.
‡Comparison of RFA and high-dose PEIT.
§Comparison of RFA and PAI.

When there is a high risk of gastrointestinal perforation such as an adhesion after surgery, that is located near the digestive tract, the selection of PEIT should also be considered.

REFERENCES

1 LF10941 Lencioni RA, Allgaier HP, Cioni D *et al.* Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003; **228** (1): 235–40.

2 LF10457 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma< or =4 cm. *Gastroenterology* 2004; **127** (6): 1714–23.

3 LF11869 Shiina S, Teratani T, Obi S *et al.* A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122–30.

4 LF10468 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151–6.

CQ50 Does TACE in combination with local ablation therapy improve the prognosis of patients with hepatocellular carcinoma larger than 3 cm or four or more lesions?

RECOMMENDATION

Therapy combining TACE and PEIT in patients with hepatocellular carcinoma larger than 3 cm or four or more lesions improves the prognosis as compared with TACE alone. **(grade B)**

SCIENTIFIC STATEMENT

Tanaka *et al.* conducted a comparative study of TACE alone and TACE plus PEIT in 43 patients with a single hepatocellular carcinoma larger than 3 cm and reported prognosis to be significantly better in the TACE plus PEIT group (LF01754[1] level 2a). Bartolozzi *et al.* performed an RCT of TACE plus PEIT combination therapy versus TACE alone in patients with hepatocellular carcinoma measuring 3.1–8 cm in diameter, and reported that there was no significant difference in the survival rate, but the recurrence-free survival was better with the combination therapy. In addition, hepatic functional reserve worsened 1 year later in the TACE group after repeating the treatment for two to five courses (LF01635[2] level 1b). Becker *et al.* carried out an RCT of TACE alone and TACE plus PEIT in 52 hepatocellular carcinoma patients (tumors ≥5 cm in diameter, $n = 34$; four or more lesions, $n = 11$) and reported that there was no difference in prognosis for the entire patient population, but the prognosis was better in the TACE plus PEIT group in an analysis of just the 26 Okuda stage I patients (hazard ratio = 0.4; $P = 0.04$) (LF11055[3] level 1b).

COMMENTS

We examined whether the addition of local therapy after TACE in patients with tumors larger than 3 cm in diameter or multiple tumors, which are usually not indicated for local therapy but instead for TACE, would contribute to the improvement of prognosis. There were only reports on RCT with a small sample size or non-RCT, but all of the results showed that the prognosis was better for TACE plus PEIT. However, many issues remain unknown, for example, among tumors larger than 3 cm or four or more lesions, prolongation of survival can be obtained up to what diameter of the tumors and up to how many lesions. Also, the addition of local ablation therapy may worsen the prognosis in patients with poor liver function. Thus, the indications should be carefully considered. In terms of whether TACE in combination with RFA improves prognosis, adequate evidence is lacking at present.

REFERENCES

1 LF01754 Tanaka K, Nakamura S, Numata K *et al.* Hepatocellular carcinoma: treatment with percutaneous ethanol injection and transcatheter arterial embolization. *Radiology* 1992; **185** (2): 457–60.
2 LF01635 Bartolozzi C, Lencioni R, Caramella D *et al.* Treatment of large HCC: transcatheter arterial chemoembolization combined with percutaneous ethanol injection versus repeated transcatheter arterial chemoembolization. *Radiology* 1995; **197** (3): 812–18.
3 LF11055 Becker G, Soezgen T, Olschewski M, Laubenberger J, Blum HE, Allgaier HP. Combined TACE and PEI for palliative treatment of unresectable hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6104–9.

CQ51 Does RFA with the interruption of blood flow improve prognosis?

RECOMMENDATION

The range of necrosis increases when RFA is performed with blood flow interruption, but whether this improves the prognosis needs to be investigated in the future. **(grade C1)**

SCIENTIFIC STATEMENT

Yamasaki *et al.* compared RFA (four patients, five nodules) with hepatic arterial balloon occlusion and routine RFA (six patients, seven nodules) in patients with hepatocellular carcinoma measuring less than 4 cm in diameter and noted an increase in the volume of necrosis (major axis 38.2 ± 2.8 vs 30.0 ± 4.1 mm, $P = 0.009$, minor axis 35.0 ± 1.7 vs 27.0 ± 4.3 mm, $P = 0.006$). No serious complications occurred (LF00034[1] level 2a). Kobayashi *et al.* conducted an RCT of RFA alone and RFA with hepatic arterial balloon occlusion in 30 patients with a single hepatocellular carcinoma measuring 3 cm or less and reported that the minor axis of the ablation area was significantly larger for the RFA plus hepatic arterial balloon occlusion group than for the RFA alone group at 36 mm vs 26 mm (LF10855[2] level 1b).

COMMENTS

The aforementioned clinical studies designed to increase the range of necrosis by RFA by reducing the cooling effect of blood flow were implemented. All of the reports revealed an increase in the range of necrosis, but no conclusion was drawn on the improvement of prognosis because the follow-up period was too short.

REFERENCES

1 LF00034 Yamasaki T, Kurokawa F, Shirahashi H, Kusano N, Hironaka K, Okita K. Percutaneous radiofrequency ablation therapy with combined angiography and computed tomography assistance for patients with hepatocellular carcinoma. *Cancer* 2001; **91**: 1342–8.

2 LF10855 Kobayashi M, Ikeda K, Kawamura Y *et al.* Randomized controlled trial for the efficacy of hepatic arterial occlusion during radiofrequency ablation for small hepatocellular carcinoma – direct ablative effects and a long-term outcome. *Liver Int* 2007; **27**: 353–9.

Hepatology Research

Hepatology Research 2010; **40**: 667–685 doi: 10.1111/j.1872-034X.2010.00673.x

Special Report

Management of hepatocellular carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009)

Shigeki Arii,[1] Michio Sata,[2] Michiie Sakamoto,[3] Mitsuo Shimada,[4] Takashi Kumada,[5] Shuichiro Shiina,[6] Tatsuya Yamashita,[7] Norihiro Kokudo,[8] Masatoshi Tanaka,[9] Tadatoshi Takayama[10] and Masatoshi Kudo[11]

[1]*Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University Graduate School of Medicine,* [3]*Department of Pathology, Keio University School of Medicine,* [6]*Department of Gastroenterology, University of Tokyo, Graduate School of Medicine,* [8]*Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, University of Tokyo Graduate School of Medicine, and* [10]*Department of Digestive Surgery, Nihon University School of Medicine, Tokyo,* [2]*Department of Gastroenterology and Hepatology, Kurume University School of Medicine, and* [9]*Department of Gastroenterology, Kurume University Medical Center, Kurume,* [4]*Department of Surgery, The University of Tokushima, Tokushima,* [5]*Department of Gastroenterology, Ogaki Municipal Hospital, Ogaki,* [7]*Department of Gastroenterology, Kanazawa University, Graduate School of Medical Science, Kanazawa,* [11]*Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan*

Hepatocellular carcinoma (HCC) is responsible for approximately 600 000–700 000 deaths worldwide. It is highly prevalent in the Asia–Pacific region and Africa, and is increasing in Western countries. The evidence-based guideline for HCC in Japan was published in 2005 and revised in 2009. Apart from this guideline, a consensus-based practice manual proposed by the HCC expert panel of the Japan Society of Hepatology (JSH), which reflects widely accepted daily practice in Japan, was published in 2007. At the occasion of the 45th Annual meeting of the JSH in Kobe 4–5 June 2009, a consensus meeting of HCC was held. Consensus statements were created based on 67% agreement of 200 expert members.This article describes the up-to-date consensus statements which largely reflect the real world HCC practice in Japan. We believe readers of this article will gain the newest knowledge and deep insight on the management of HCC proposed by consensus of the HCC expert members of JSH.

Key words: hepatocellular carcinoma, Japan Society of Hepatology, staging system, surveillance, treatment algorithm, consensus-based guideline

INTRODUCTION

THE LAST EVIDENCE-BASED guideline for hepatocellular carcinoma (HCC) for Japan was published in 2005,[1] and has prevailed nationwide. This document was developed by a committee composed of 14 experts (Chairman: Professor Masatoshi Makuuchi) and was based on a critical review of 7118 English reports published between 1966 and 2002. This guideline includes 58 research questions regarding important issues for the prevention, diagnosis, surveillance and treatment of HCC. The utility of this guideline is recognized by many Japanese clinicians and has provided a great contribution to clinical practice. However, there are several issues in which solid evidence is still lacking; thus, clear recommendations for clinical practice cannot be stated. In fact, 45% of the research questions are of grade C recommendation level, representing a lack of adequate evidence. These issues are left to the clinician's discretion within the clinical setting. Furthermore, because the guidelines did not include the most up-to-date articles, no recommendation or statements were made regarding newly established evidence. In addition, the clinical practices that follow these guidelines are considered to account for 70–80% of general practice institutions.

Correspondence: Professor Masatoshi Kudo, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan. Email: m-kudo@med.kindai.ac.jp
Received 23 December 2009; revision 3 March 2010; accepted 15 March 2010.

As mentioned above, Congress President, Professor Masatoshi Kudo, at the 45th Annual Meeting of the Japan Society of Hepatology organized the Consensus Meeting of Hepatocellular Carcinoma. The program was chaired by Professors M. Sata and S. Arii and covered the updated problems and clarified some controversial issues. Eight experts were selected to contribute to the meeting and they were assigned the following topics based on their specialties. Professor M. Sakamoto presented recommendations regarding diagnostic problems for small-sized HCC from the clinicopathological point of view. Professor M. Shimada discussed the utility of clinical staging and prognosis. Dr T. Kumada reviewed the current status of diagnostic imaging and tumor markers. Dr S. Shiina discussed important issues on ablative treatment. Dr Yamashita reviewed transarterial chemoembolization and chemotherapy. Professor N. Kokudo discussed surgical treatment, including liver transplantation. Dr M. Tanaka presented a treatment algorithm from the point-of-view of hepatologists. Finally, Professor T. Takayama comprehensively discussed the appropriateness of the present treatment algorithm.

In each presentation, the speakers raised clinical questions regarding the remaining problems that needed to be clarified in the present guidelines, and the HCC specialists (a total of 200 physicians: hepatologists, 70%; surgeons, 24%; radiologists, 2%; and pathologists, 4%) answered these questions using a question and answer analyzer system. Recommendations were approved when at least 67% of the HCC experts reached agreement. For instances where agreement was between 50% and 67%, the statements were considered informative, and are cited here as "informative statements".

In this consensus paper, each presenter has provided a summary of the recommendations and consensus. It is highly expected that this Consensus Statement established by the Japan Society of Hepatology (JSH)will provide valuable insight, and will greatly contribute to the future improvement of the guidelines and appropriate clinical practices for patients with HCC worldwide.

PATHOLOGICAL ASSESSMENT

PATHOLOGICAL ASSESSMENT OF HCC is described in the General Rules for the Clinical and Pathological Study of Primary Liver Cancer.[2] It focuses on macroscopic typing and tumor grading based on tumor differentiation and reflects the aggressiveness of the tumors; differential diagnosis between multicentric development and intrahepatic metastasis of multiple tumors; and diagnosis of early HCC and precancerous lesions. Historically, careful and detailed histological evaluation of surgical specimens enabled us to understand the clinicopathological features of HCC development and extension, and to establish the above-mentioned diagnostic criteria. However, the recent increase in non-surgical treatments for HCC, such as radiofrequency ablation (RFA), is rapidly changing the role and position of pathological diagnosis. Thus, we discussed the indications for liver tumor biopsy for the diagnosis and treatment of HCC.

When we consider the indications for liver biopsy, the risk and benefit of this procedure must be considered.[3–8] The risk includes complications caused by the procedure itself, such as hemorrhage by needle insertion, and by tumor seeding. The incidence of tumor seeding has been reported in approximately 1–5% of cases. Certainly, we have to note that the incidence depends on the characteristics of the tumor such as tumor size and tumor differentiation. Liver biopsy is important in terms of tumor diagnosis, assessment of prognosis and decision making for treatment. For example, for a typical HCC larger than 2 cm in size with a typical vascular pattern on imaging, and elevated tumor markers such as α-fetoprotein (AFP) and/or des-γ-carboxy prothrombin (DCP), the benefit of performing tumor biopsy to confirm the diagnosis of HCC seems minimal. In contrast, only liver biopsy can be used to confirm the diagnosis of cancer in cases with suspected HCC or borderline lesions on clinical and imaging diagnosis. However, controversy remains because of the inconsistent treatment strategy for suspected lesions, particularly in cases with poor liver function.

Previous follow-up data of suspected HCC and borderline lesions showed that the tumors grow slowly during the precancerous or early HCC stages, but grow rapidly in some early HCC cases or in progressed HCC.[9] The transition from slow growing to rapidly growing tumors was supposed to take place once the tumor reaches approximately 1.5 cm in size. Therefore, the proposed recommendations for liver biopsy are as follows.

Recommendation 1. Liver biopsy should be discouraged in cases with a typical HCC over 1.5 cm in size, which shows typical pattern on imaging.

Recommendation 2. Liver biopsy should be considered in cases with a suspected HCC or borderline lesions/early HCC of 1.5 cm in size or less, which does not show typical pattern on imaging.

In addition to these recommendations, the requirement of liver biopsy should increase if the detection and diagnostic ability of imaging techniques increases for

smaller lesions. The emergence of new contrast agents such as gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) are expected to reveal suspected HCC nodules, including early HCC at approximately 1 cm in size. Tumor biopsy should then be performed to confirm the diagnosis of early cancer before it can progress to overt HCC. It is also expected that the increase in therapeutic options will increase the need for more detailed information of the tumor characteristics, such as tumor differentiation and immunophenotype reflecting tumor aggressiveness, which can only be determined by tumor biopsy.

PROGNOSTIC STAGING SYSTEM

IN TERMS OF estimating the prognosis of HCC, there are currently insufficient evidence-based data; therefore, no definite recommendations can be made, unlike other fields of HCC management. It is well known that the prognosis of HCC is defined by the behavior of the HCC itself, and by host factors such as hepatic functional reserve. The major questions that still need to be answered in terms of estimating the prognosis of HCC are: (i) whether an integrated staging system is necessary for the management of HCC; (ii) what is the best integrated staging system; and (iii) should the integrated staging system be included in the algorithm for HCC treatment?

Tumor staging (TNM staging)

There are two major classifications used for tumor staging of HCC. One is the tumor–node–metastasis (TNM) stage, developed by the American Joint Committee on Cancer (AJCC). This classification can also be applied to liver transplant recipients. However, the cut-off value for tumor diameter of 5 cm is too large to define small HCC, which are frequently found in Japan.

The other is the TNM stage proposed by the Liver Cancer Study Group of Japan (LCSGJ). The cut-off of 2 cm is very appropriate for patients in countries such as Japan, where small HCC are often found in an established nationwide screening system. However, in this system, the weighting of the strongest prognostic factor, vascular invasion, is equal to that of other factors used to estimate prognosis, which might not be adequate.

Staging for hepatic functional reserve

There are two major classifications for estimating liver functional reserve. One is the Child–Pugh classification, which is widely used worldwide, but is difficult to apply for decision making for hepatectomy. The other is the Liver Damage Classification scheme proposed by the LCSGJ, which is useful for hepatectomy. However, this scheme is not widely accepted because of the need to perform the indocyanine green retention at 15 min test ($ICGR_{15}$).

Integrated staging system for HCC

The combined classification of TNM stage and liver function stage, namely, an integrated staging system, is extremely important to estimate patient prognosis and guide decision making for patient management. The integrated staging system contributes to: (i) estimate patient prognosis; (ii) select the best treatment option for each patient; (iii) compare different treatment modalities; and (iv) compare treatment outcomes among different institutions.

Since the Okuda classification in 1985,[10] several integrated staging systems have been reported, including the Cancer of the Liver Italian Program (CLIP) score,[11] the Barcelona Clinic Liver Cancer (BCLC) stage[12] and the Japan Integrated Staging (JIS) score.[13] The Okuda classification scheme is simple and has been found to be suitable in the past, but does not seem to be suitable at the present time, now that relatively small HCC can be detected. The CLIP score is popular in Western countries, but its discriminating power is weak for small HCC, particularly at higher scores of 4–6, and over 50% of Japanese HCC patients are classified as score 0. The BCLC staging is thought to be useful as an integrated staging system and for guiding treatment. Therefore, it is recommended as an integrated treatment algorithm by the European Association for the Study of the Liver and the American Association for the Study of Liver Disease (AASLD). However, it is not suitable for the estimation of patient prognosis, and a large number of variables are used. In contrast, the JIS score essentially consists of the Child–Pugh score and the LCSGJ TNM stage, and is widely accepted in Japan. The discriminating power for relatively small HCC is excellent, and is particularly suitable for countries such as Japan, where many small HCC are detected.

In terms of a comparison of these integrated staging systems, Cillo *et al.*[14] reported that the BCLC was the best system among the Okuda, CLIP, BCLC and French classifications. Meanwhile, Tateishi *et al.*[15] reported that the Tokyo score was superior to BCLC staging and comparable to the CLIP score in predicting prognosis after hepatectomy and ablation. Kudo *et al.*[16] reported that the JIS score was better than the CLIP score, particularly in terms of discriminating power for each subgroup. Similarly, Chung *et al.*[17] reported that the JIS score was

the most excellent staging system among the BCLC, Tokyo and JIS staging systems. Therefore, JIS score is currently considered to be the best integrated staging system in Japan. Regarding other integrated staging systems, modified JIS score has been reported[13,18] to be useful for patients undergoing hepatectomy. Biomarker combined JIS score has also been reported to be useful in discrimination in patients with good prognosis.[19] However, the usefulness of these new staging systems will remain unclear until they are assessed in a range of patient sets with HCC.

Regarding the estimation of HCC prognosis, most hepatologists recognize the importance of an integrated staging system rather than applying the TNM stage and hepatic functional reserve scales individually. Furthermore, the JIS score is considered to be the best integrated staging system for current clinical practice. However, it is still difficult to incorporate the integrated staging systems, such as the JIS score, into algorithms for HCC treatment.

Recommendation 3. Integrated staging system should be used to assess the prognosis of patients with HCC, instead of individually applying scales for TNM stage and liver function stage.

Recommendation 4. The JIS score is the best staging system to estimate the prognosis of patients with HCC.

Informative Statement 1. Integrated staging systems, such as the JIS score, are not yet suitable for inclusion in algorithms for HCC treatment.

SURVEILLANCE AND DIAGNOSIS

Surveillance programs

IT IS WELL known that HCC mainly occurs in cases with chronic liver disease, particularly cirrhosis. Several cohort studies have shown that the surveillance of high-risk patients with hepatitis B virus (HBV)- or hepatitis C virus (HCV)-related chronic liver disease improves the rate of early detection and the rate of curative treatments.[20–27] For this reason, UK[28], European[29] and American[3] practice guidelines for HCC recommend routine surveillance of HCC among individuals with viral hepatitis or cirrhosis. Almost all gastroenterologists in Japan conduct surveillance programs using a combination of tumor markers such as AFP, the *lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3%) and DCP, and by ultrasound (US).[30] However, no consensus has been reached in terms of the optimal surveillance strategy. Thompson *et al.* calculated the number of people who need to be under surveillance to prevent either a single death from HCC or a single premature death (defined as death before the age 75 years) and showed the effectiveness of surveillance programs.[31] In the absence of surveillance, approximately 20% of the mixed etiology cohort died as a result of HCC.

Recommendation 5. Surveillance with US and three tumor markers including AFP, DCP and AFP-L3 should be performed for early detection of HCC in patients with HBV- and HCV-related chronic liver disease, particularly cirrhosis.

Tumor markers

In Japan, AFP, AFP-L3 and DCP are widely and routinely used as serological tumor markers for the surveillance, diagnosis and prognostic estimation of HCC. The Evidence-Based Clinical Practice Guidelines of HCC published in 2005[1] recommended that AFP, AFP-L3 and DCP should be measured at intervals of 3–4 months for very high-risk patients (defined as HBV- or HCV-related liver cirrhosis), and at 6-month intervals for high-risk patients (defined as HBV- or HCV-related chronic liver disease or other causes of liver cirrhosis).[32] Although AFP is the most widely used tumor marker for HCC, the levels of AFP are also increased in patients with liver diseases other than HCC, including viral hepatitis, with a prevalence of 10–42%.[33–35] In contrast, AFP-L3 and DCP are very specific for HCC, compared with AFP alone. The combination assay for AFP, AFP-L3 and DCP should be performed for the early detection of HCC.[36,37] The specificity and sensitivity of the combination assay of AFP and DCP were 83% and 84%, respectively, to detect small HCC of less than 3 cm in diameter.[38] The specificity and sensitivity of the combination assay of DCP and AFP-L3 were 41.7–66.7% and 89.5–89.8%, respectively, to detect small HCC of less than 3 cm in diameter.[39,40]

Recommendation 6. Periodical measurement of more than two kinds of tumor markers (particularly AFP and DCP) is recommended for the early detection of HCC in high-risk and very high-risk patients.

Recommendation 7. The surveillance interval needs to be shorter in very high-risk patients than in high-risk patients.

Imaging modalities

Periodic follow-up of chronic liver disease by US, multidetector row computed tomography (MDCT) and magnetic resonance imaging (MRI) allows relatively

easy detection of small HCC.[41–43] However, it is sometimes difficult to characterize small hepatic nodular lesions detected by these imaging modalities. Definitive diagnosis requires invasive methods such as US-guided liver biopsy. Hemodynamic evaluation of the nodule is also important to assess the biological behavior of HCC. The recent advances in MRI and computed tomography (CT) procedures, such as CT during hepatic arteriography (CTHA) and CT during arterial portography (CTAP), have enabled the detailed hemodynamic evaluation of small hepatic nodules.

Recently, liver-specific contrast agents such as superparamagnetic iron oxide particles (SPIO), which are taken up by Kupffer cells, and Gd-EOB-DTPA, which is taken up by hepatocytes, are frequently used in MRI for early diagnosis of HCC. Gd-EOB-DTPA is a superb agent because it provides dynamic and liver-specific MR images.[44–46] This contrast agent is highly liver specific; approximately 50% of the injected dose is taken up by functioning hepatocytes and is excreted in bile, compared with just 3–5% for gadobenate dimeglumine.[46] Early studies comparing Gd-EOB-DTPA-enhanced dynamic MRI with dynamic MDCT showed that Gd-EOB-DTPA-enhanced MRI is significantly more accurate, sensitive and specific than dynamic MDCT for the diagnosis of HCC in patients with cirrhosis.[47,48] In addition, Gd-EOB-DTPA-enhanced MRI has a high detection rate for early stage HCC nodules that are not enhanced in dynamic studies. However, although the differentiation of early HCC from dysplastic nodule by hepatobiliary phase images of Gd-EOB-DTPA MRI is promising, more data are still needed.

Informative statement 2. Gd-EOB-DTPA-enhanced MRI provides dynamic and hepatocyte-specific images and is more accurate than dynamic MDCT or SPIO-MRI for the detection and characterization of small HCC, including early HCC.

ABLATION THERAPIES

IMAGE-GUIDED PERCUTANEOUS ablation therapies have long played important roles in the treatment of HCC. Percutaneous ethanol injection has been used for unresectable, small HCC since the early 1980s[49–51] and offers us the potential to treat HCC using non-surgical means. Percutaneous microwave coagulation therapy became popular in Japan in the late 1990s.[52] However, since the introduction of radiofrequency ablation (RFA) into clinical practice around 1999, there has been a dramatic shift from ethanol injection or microwave coagulation to RFA.[53] RFA for HCC has been covered by public health insurance since April 2004 in Japan. Although more than 1700 institutions have experienced RFA in Japan, RFA is estimated to be performed routinely in approximately 1000 institutions throughout Japan at the present.

Radiofrequency ablation often seems to be performed with less than adequate treatment planning or preparation compared with surgical resection. RFA appears to be a very simple procedure. Thus, some physicians may perform RFA without adequate training or experience. In addition, RFA does not require expensive equipment. Thus, several hospitals have introduced RFA into clinical practice without high-performance US and CT.

However, RFA is indicated for malignant tumors and inadequate outcome should be avoided. Thus, only physicians with sufficient experience and appropriate skill should perform the procedure. Furthermore, only well-equipped hospitals should perform RFA because the outcomes of RFA are strongly influenced by the performance of the CT and US equipment available at each institution. It is crucial to offer consistent outcomes for RFA at all institutions and for all operators.

More importantly, before commencing RFA, the tumors should be evaluated by US, contrast-enhanced CT or MRI to determine tumor size, shape, number, presence or absence of extracapsular invasion, presence or absence of satellite lesions, location relative to Glisson's capsule or other critical structures, and to determine the optimal route to approach the tumor.

Within 1–3 days after RFA, contrast-enhanced CT or MRI is essential to objectively assess the treatment response. If the tumor is completely ablated with a sufficient safety margin, the treatment may be considered complete. However, if there is any residual cancer tissue or an insufficient safety margin, RFA should be repeated until complete tumor destruction with a sufficient ablative margin is achieved. The following recommendation was supported by 94% of the experts.

Recommendation 8. Imaging should be performed within 1–3 days after RFA to evaluate treatment response. It is essential that RFA is repeated until entire tumor destruction with a sufficient ablative margin is achieved.

For accurate tumor evaluation, CT and MRI performed before and after RFA should be done using a thin slice interval. The following recommendation was agreed by 94% of the experts.

Recommendation 9. CT and MRI before and after RFA should be done using a slice thickness and interval of 5 mm or less; slice thickness and interval of 10 mm or more is not adequate.

A histopathological study has revealed that, in cases with incomplete necrosis, viable cancer tissue remains around the main tumor, in portions isolated by the septa, or along the edge of the tumor after ablation therapies.[54] There may also be extranodular growth, satellite nodules or portal vein invasion, which cannot be detected by imaging modalities.[55,56] The incidence of satellite nodules and portal vein invasion is associated with the gross appearance of the main tumor. The single nodular type with extranodular growth and the confluent multinodular type both show satellite lesions more frequently than early HCC (vaguely nodular-type HCC showing preservation of the preexisting liver structure) and the single nodular type. Thus, it is important to determine the gross appearance of the tumor by imaging. It is also essential to ablate beyond the tumor border to achieve complete tumor necrosis and prevent local tumor progression (ablative margin or safety margin). Sonazoid-enhanced US in the Kupffer phase is useful to determine the gross tumor appearance.[57] The width of the safety margin should be modified based on the gross appearance of the tumor, the number of tumors, the initial tumor or recurrent tumor, the duration of time between the previous treatment and recurrence in recurrent cases, tumor location (particularly in relation to the Glisson's capsule), liver function, comorbid conditions and the patient's age.

Furthermore, the accuracy of contrast-enhanced CT or MRI for evaluating the extent of necrosis is limited because of the partial volume effect.[58] The following recommendation was agreed by 94% of the experts.

Recommendation 10. A safety margin completely surrounding the lesion should be achieved in cases in which RFA is performed as a locally curative treatment (level 6, grade A).

Ablation therapies, including RFA, are widely accepted as the preferred treatment for unresectable small HCC. On the other hand, it has been strongly debated whether ablation therapies can provide a treatment option for resectable HCC since the introduction of ethanol injection. Although the number of patients treated by RFA has steadily increased, the Clinical Practice Guidelines for Hepatocellular Carcinoma in Japan recommends surgery rather than ablation.[1] Their scientific statement recommends the following: "(i) if only one tumor is present, liver resection is recommended irrespective of the diameter of the tumor. Ablation therapy may also be selected if the severity of liver damage is class B and the diameter of the tumor is no more than 2 cm; (ii) if two to three tumors with diameters of no more than 3 cm are present, liver resection or local ablation therapy is recommended". This scientific statement is based on a cohort study of patients at clinical stage I (fair liver function), with a solitary tumor of less than 2 cm in diameter, patients across all clinical stages with a solitary tumor greater than 2 cm, and patients of clinical stage II (moderately impaired liver function) with two tumors greater than 2 cm. In that cohort, those who underwent hepatic resection showed higher survival rates than those who received non-surgical interventions.[59]

However, those findings were not based on randomized controlled trials (RCT) and the different survival rates may be subject to bias arising from the background characteristics of the patients. Of note, the hepatic resection group was younger than the ethanol injection group. Furthermore, even among patients at clinical stage I, most patients with normal liver or chronic hepatitis seemed to undergo resection while many with cirrhosis seemed to receive ethanol injection. This might reduce the recurrence rate because of multicentric carcinogenesis and less frequent development of liver failure in the resection group. Moreover, the trend that patients with severe comorbid conditions, such as cardiopulmonary diseases and others, received ethanol injection rather than resection might explain some of the disparity in survival. By contrast, in one RCT the recurrence and survival rates were comparable between surgical resection and ethanol injection.[60] In addition, other non-randomized trials have reported similar or better overall survival after ethanol injection than after resection.[61–63]

In addition, the findings described above only compared resection with ethanol injection. For example, our RCT showed that RFA had higher survival and lower recurrence rates than ethanol injection while the adverse events were similar between the two therapies.[64] Similarly, other RCT have shown that RFA is superior to ethanol injection in terms of treatment outcomes for HCC.[65–67] Another RCT has shown that there was no difference between resection and RFA in terms of overall and disease-free survival, while post-treatment complications occurred more frequently and were more severe after surgery.[68]

Hence, it is inappropriate to generalize the findings for ethanol injection to other percutaneous local ablation therapies such as RFA, and it should not be concluded that hepatectomy is recommended over percutaneous local ablation.

Further trials are needed to determine whether RFA can become a preferred treatment for "resectable HCC". In such trials, the primary end-point should be overall

survival.[69] The AASLD practice guideline clearly states the following: "although a treatment might be less active against the tumor than another treatment and thus result in a higher recurrence rate after initial treatment, the overall survival might not differ or may even be better".[3]

Recurrence-free survival can be misleading and should not be considered as a surrogate end-point for overall survival. In HCC, unlike other solid tumors, recurrence can still be treated, and the first recurrence does not cause death in most cases. Furthermore, surgery theoretically offers better disease-free survival than RFA because it removes larger liver tissue. However, the better curability associated with hepatectomy could be cancelled out by the surgical invasion and the potential deterioration in liver function. The following recommendation was agreed by 84% of the experts.

Recommendation 11. Overall survival should be the end-point to compare results between ablation and hepatectomy.

SURGICAL TREATMENT: RESECTION AND TRANSPLANTATION

A NATIONWIDE SURVEY by the Japanese Liver Transplantation Society found that a total of 4725 cases of living-donor liver transplantations (LDLT) were reported in Japan as of the end of 2007 since its initiation in 1989. By contrast, during the same period, only 46 cases of deceased-donor liver transplantation (DDLT) were documented. At the end of 2006, 778 patients with HCC had undergone an LDLT in Japan.[70] Because of the severe shortage of brain-dead donors and the extremely long waiting time for such organs, DDLT is not a realistic treatment option for HCC patients in Japan.

Algorithm for the treatment of patients with HCC in Japan

Figure 1 shows the treatment algorithm presented in the Japanese evidence-based guideline for the diagnosis and treatment of HCC.[1] Liver transplantation is recommended for HCC patients with liver damage C (similar to Child–Pugh C), but only when the patients meet the Milan criteria proposed by Mazzaferro.[71] In the revised version of the guidelines published at the end of 2009, an age limit of 65 years was added to the criteria for liver transplantation.

Can the indications for liver transplantation be expanded beyond the Milan criteria?

Until the mid-1990s, HCC was considered a contraindication for liver transplantation because of the extremely poor outcome of early series.[72,73] This pessimistic view was reversed by Mazzaferro *et al*. who conducted a prospective cohort study to identify subgroups of HCC patients who may benefit from DDLT. They presented clear eligibility criteria for transplantation, as follows: the presence of a solitary tumor of 5 cm or less in diameter and no more than three tumor nodules, each 3 cm or less in diameter, in patients with multiple tumors, and the absence of vascular invasion or extrahepatic disease. In their series, the overall and recurrence-free survival rates

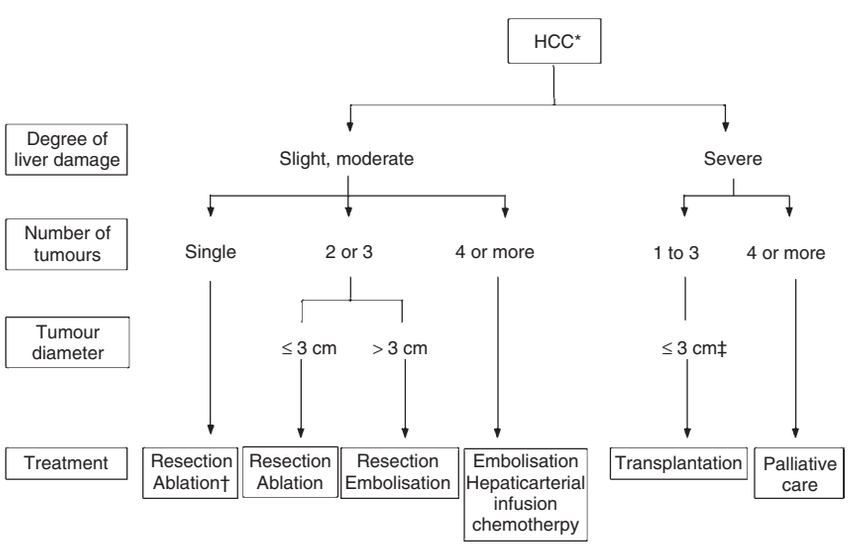


Figure 1 Japanese evidence-based treatment algorithm. HCC, hepatocellular carcinoma.

at 4 years for 35 patients who met the above criteria were as high as 85% and 92%, respectively. These criteria were named the "Milan criteria" and became the gold standard for patient selection for liver transplantation. The Milan criteria were also validated for LDLT using data from a nationwide survey in Japan.[74] Since 2004, LDLT for HCC has been covered by social medical insurance in Japan when the preoperative imaging studies indicate that the patient's condition meets the Milan criteria.

The Milan criteria have encouraged transplant surgeons to increase the number of liver transplantations performed in HCC patients, and the United Network for Organ Sharing (UNOS) has incorporated the Milan criteria as conditions for listing HCC patients. During the extensive application of liver transplantation for HCC, transplant surgeons have noticed that the outcomes of some patients who slightly exceeded the Milan criteria were also favorable. To expand the indications for liver transplantation, several groups from different countries have challenged these restrictive criteria (Table 1).[75–79] Yao *et al.* at the University of California at San Francisco (UCSF) proposed criteria consisting of a single tumor of less than 6.5 cm in diameter or two lesions of less than 4.5 cm in diameter, with a total tumor diameter of less than 8 cm; these criteria are known as the "UCSF criteria".[76] The utility of the UCSF criteria was subsequently confirmed by the University of California at Los Angeles.[80]

Regarding the indications for LDLT in HCC patients, several proposals from Asian centers have extended the eligibility criteria (Table 1). For example, a group at the University of Tokyo proposed the "5-5 rule", which allows up to five nodules with a maximum diameter of 5 cm.[77] The 3-year recurrence-free rate of 72 patients who met the Tokyo 5-5 rule was as high as 94%, which was comparable with that of patients within the Milan criteria. A group at the University of Kyoto subsequently proposed a further expansion of the criteria, increasing the upper limit of the number of tumors to 10.[79]

Because LDLT is not governed by an organ-sharing system, some authors have argued that the indications for LDLT in patients with HCC could be further extended. One might say that "If the patient (recipient) and his/her family (donor) strongly wish to undergo LDLT even in cases of very advanced HCC with full knowledge of potential for poor outcomes, there is no reason for transplant surgeons to reject their wish. The family members may accept the poor outcome after LDLT without doing any harm to the community." However, we should always remember that, while LDLT does not require a donor from the community, it does require extensive medical resources, including a large workload for surgeons and other hospital staff members, medical supplies, drugs and blood products. Furthermore, the premature death of the recipient is well known to cause severe emotional trauma to the living donors and their family members.

Based on an answer-pad vote at the consensus meeting of 45th JSH congress, 84% of the experts supported keeping the Milan criteria for DDLT, but only 25% supported keeping these criteria for LDLT. Although any expansion of the criteria should be modest, no consensus exists as to the extent to which the criteria can be extended.

Recommendation 12. For DDLT, the HCC status of the recipients should meet the Milan criteria.

Recommendation 13. For LDLT, the HCC status of the recipients does not need to be within the Milan criteria.

Which is better, liver resection or transplantation, for HCC patients who are eligible for either treatment?

Because liver transplantation replaces the whole liver, removing the highly carcinogenic background and the cirrhotic liver can avoid multicentric or de novo cancer recurrence.[80] In contrast, liver resection is associated with a very high risk of tumor recurrence. Even after curative liver resection in patients with good liver function, the 5-year recurrence rate is as high as 70–79%.[80] Roughly half of these recurrences are multicentric or de novo recurrences. For this reason, liver transplantation

Table 1 Summary of proposed criteria for indication of liver transplantation for HCC

Criteria	Conditions	References
Milan criteria	Up to 5 cm for single nodule or up to 3 nodules with a maximum diameter of 3 cm	[70]
UCSF criteria	Up to 6.5 cm for single nodule or up to 3 nodules with a maximum diameter of 4.5 cm	[76]
Tokyo 5-5 rule	Up to 5 nodules with a maximum diameter of 5 cm	[77]
Asan criteria	Up to 6 nodules with a maximum diameter of 5 cm	[78]
Kyoto criteria	Up to 10 nodules with a maximum diameter of 5 cm and PIVKA-II <400 mAU/mL	[79]
Up-to-seven criteria	Up to seven as the sum of the size of the largest tumor [in cm] and the number of tumors	[75]

may be recommended for HCC patients with good liver function who are also eligible for liver resection, as in Western countries.

Another issue is the operative risk of the two treatments. In Japan, the operative mortality rates for LDLT and liver resection are estimated to be 4–10% and 0.8–1.2%, respectively. This striking difference in operative mortality rates might preclude LDLT for patients with good liver function.

Using two databases at the National Cancer Center Hospital in Japan and the University of Pittsburgh Medical Center in the USA, Yamamoto *et al*. compared the long-term outcome of liver resection and transplantation in cirrhotic patients with HCC.[81] The overall survival of Child–Pugh A patients who underwent liver resection was similar to that of the patients without vascular invasion or lymph node metastases who underwent transplantation (most cases with Child-Pugh C). The recurrence rate was significantly lower in the transplantation group. For cases in which either treatment can be performed, the outcome of liver transplantation might be better than that of hepatic resection, particularly in cases with only a few small lesions.[81,82] In cases with large lesions, superior outcomes are achieved with hepatectomy. Because some patients may withdraw from treatment during the pre-transplantation period,[83] the outcomes with resection are better than those for liver transplantation based on intention-to-treat analysis of patients who meet the criteria for resection.

The evidence-based guideline[1] recommends the following: considering the occurrence of dropouts during the pre-transplantation period, the outcome of resection is better than that of liver transplantation among patients who meet the criteria for resection (grade B).

According to a question and answer-analyzer vote at this consensus meeting, 83% of the HCC experts selected LDLT for Child–Pugh C patients meeting the Milan criteria, whereas only 15–19% of the audience selected LDLT for Child–Pugh A or B patients.

Recommendation 14. LDLT should not be recommended for HCC patients with Child–Pugh A or B liver function.

PALLIATIVE TREATMENTS: TRANSARTERIAL CHEMOEMBOLIZATION AND CHEMOTHERAPY

PALLIATIVE TREATMENTS FOR HCC include transarterial chemoembolization (TACE), hepatic arterial infusion chemotherapy (HAIC) and systemic chemotherapy.

Transarterial embolization/TACE

Transcatheter arterial embolization (TAE)/TACE is one of the treatment options to treat hypervascular HCC. The theoretical basis of embolization is to induce ischemic tumor necrosis by acute arterial occlusion in hypervascular classical HCC. Embolization may be done alone (TAE) or in combination (TACE) with antineoplastic agents such as doxorubicin, epirubicin or cisplatin and a contrast agent, lipiodol. TACE is more effective and, thus, more widely used than embolization alone.

The technique for TACE is well established. The subsegmental artery or a peripheral artery near the target tumor is selected by a micro-catheter technique, followed by selective injection of antineoplastic agents mixed with lipiodol (lipiodol emulsion). The artery is then selectively obstructed with gelatin sponge particles. For bi-lobular multiple HCC with moderately impaired hepatic function (Child–Pugh B), TACE might need to be performed twice with an interval of several weeks to avoid hepatic decompensation.

The survival benefit of TAE/TACE was controversial until the publication of two RCT in 2002, which showed that TACE improved the survival of selected patients (Child–Pugh A with no vascular invasion) compared with conservative treatment.[84,85] A subsequent meta-analysis of seven RCT comparing TAE/TACE as a primary treatment for HCC in comparison with conservative management and/or suboptimal therapies showed a significant improvement in the 2-year survival, favoring TAE/TACE (odds ratio [OR] = 0.53; 95% confidence interval [CI] = 0.32–0.89, $P = 0.017$).[86,87]

According to the Nationwide Follow-up Survey of Primary Liver Cancer in Japan, one-third of all patients with primary HCC were treated by TAE/TACE (Fig. 2). Thus, TAE/TACE, hepatic resection and local ablation therapy are commonly used in Japan. TAE/TACE is the most widely used treatment for unresectable HCC.

In two Japanese treatment guidelines for HCC, evidence-based[1,30,88] and consensus-based guidelines,[89] TACE is recommended for patients with the severity of the liver damage categorized into A or B, in whom there are two or three tumors with a diameter greater than 3 cm, or four or more tumors.

In early stages of HCC, TACE is not indicated as first-line treatment because the outcome review of the Nationwide Follow-up Survey by the LCSGJ reported worse results for TACE than surgery or percutaneous ablation. This survey revealed that the 5-year survival rates for resection, ablation and TACE were 59.2%,

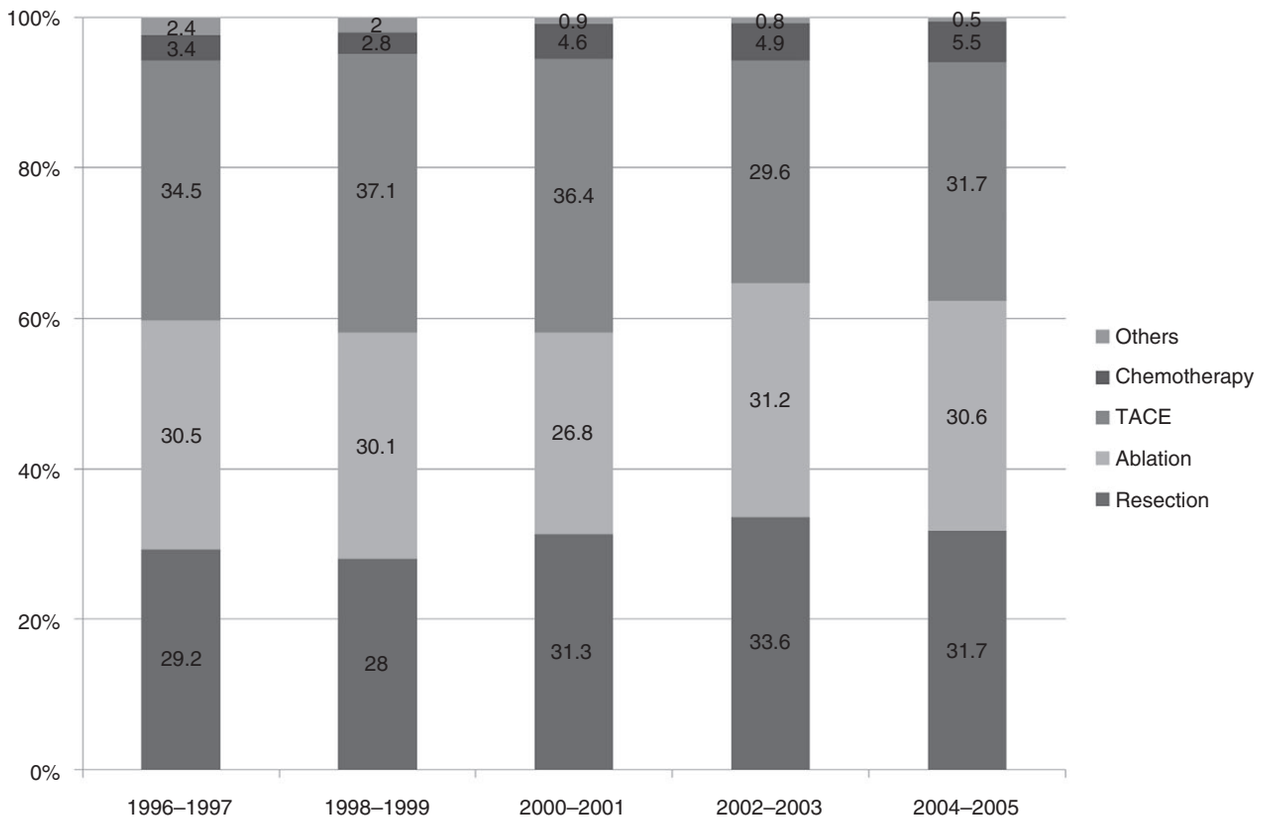


Figure 2 Change of treatment method for hepatocellular carcinoma in Japan. TACE, transcatheter arterial chemoembolization.

48.4% and 29.7%, respectively, for single tumors, and 46.4%, 37.3% and 23.0%, respectively, for two tumors.[90]

In contrast, in a large prospective cohort study of 8510 patients who received TACE for unresectable HCC, according to the LCSGJ, the median survival was 34 months with 1-, 2-, 3-, 5- and 7-year survival rates of 82%, 63%, 47%, 26% and 16%, respectively.[91] In patients with early stage HCC, single tumors of 2 cm or more and preserved liver function (clinical stage I and liver damage A according to the LCSGJ),[92] the median survival was 62 months with 1-, 2-, 3-, 5- and 7-year survival rates of 98%, 92%, 73%, 52% and 38%, respectively.[91] These results for TACE with early stage HCC seem comparable with those for surgery or ablation. Thus, although curative therapies are highly recommended for patients with early stage HCC, TACE can be applied in these patients contraindicated for curative therapies.

Transcatheter arterial chemoembolization can be used in combination with percutaneous ablation, including RFA. A meta-analysis of four RCT comparing combination therapy (TACE plus percutaneous ethanol injection [PE]) or RFA) versus monotherapy (TACE alone, PEI or RFA alone) showed a significant decrease in mortality favoring combination therapy versus monotherapy in patients with small (<3 cm) or large (>3 cm) HCC (OR = 0.534; 95% CI = 0.288–0.990; $P = 0.046$).[93]

In RFA treatment, as the tumor size increases, the therapeutic response decreases because of the limited volume of coagulation necrosis induced by the electrode. Blood flow also promotes heat loss to result in insufficient necrosis; therefore, reducing blood flow during RFA increases the ablation volume. Therefore, it seems to be reasonable to perform RFA after reducing blood flow by preceding RFA with TACE. Several cohort studies have shown that performing TACE before RFA is feasible and safe, and offers a useful treatment in compensated cirrhosis (Child–Pugh A or B) with relatively small HCC nodules (20–50 mm).[94–97] RFA in combination with preceding TACE is already recommended in the consensus-based treatment algorithm proposed by the JSH[89].

In the current consensus meeting, for hypervascular HCC of 2 cm in size, 51% of the experts used TACE

before RFA treatment. By contrast, for hypervascular HCC of 3 cm in size, 81% of the experts performed TACE before RFA. This is theoretically reasonable because the possibility of incomplete ablation is greater for tumors of 2–3 cm in size, compared with tumors of less than 2 cm in size, based on the limited volume possible with a single ablation procedure. Additionally, the accumulation of lipiodol in the tumor should facilitate the decision on whether additional RFA treatment is required following the response evaluation by dynamic CT scan. However, the survival benefit of TACE in combination with RFA should be verified by well-designed RCT.

Transcatheter arterial chemoembolization is performed in various stages in the clinical management of HCC, not only for the initially detected HCC, but also for recurrent HCC. TACE has been shown to be valuable for improving the overall survival of HCC patients, although it is difficult to assess its clinical efficacy as second- or third-line therapy.

Informative Statement 3. TACE performed before RFA is favorable for the curative treatment of hypervascular HCC of 2–3 cm in size.

Recommendation 15. TACE performed before RFA is recommended for curative treatment of hypervascular HCC larger than 3 cm in size.

Chemotherapy

Chemotherapy for HCC is divided into two types according to the route of administration; the first is systemic chemotherapy and the second is hepatic arterial infusion chemotherapy (HAIC). Systemic chemotherapy can also divided into two types: intravenous and oral chemotherapy.

According to the Nationwide Follow-up Survey of Primary Liver Cancer by the LCSGJ, chemotherapy is used in 3.4–5.5% of primary HCC patients (Fig. 2). HAIC is theoretically more favorable for HCC than systemic chemotherapy because hepatic arterial infusion of anticancer drugs enables the delivery of high doses of drugs directly to the hypervascular HCC. In addition, HAIC provides a lower systemic level of the drugs than systemic administration, because the first-pass effect in the liver, and thus reduces toxicity and side-effects. Because of these advantages, HAIC is frequently used in Japan for intrahepatic advanced HCC with portal vein tumor thrombosis and/or intrahepatic multiple HCC. A recent report from the Japanese Nationwide Survey revealed that almost 90% of the chemotherapeutic regimens for HCC are done by hepatic arterial infusion. Thus, HAIC has become widely used in Japan, despite there being no solid evidence for a survival benefit of HAIC compared with systemic chemotherapy or best supportive care (Fig. 3).

Recommendation 16. HAIC is recommended for advanced HCC with major portal vein tumor thrombi with preserved liver function.

Various anticancer drugs and treatment regimens are used for HAIC in Japan. Two regimens in particular are widely used for HAIC. The first is interferon (IFN) in combination with 5-fluorouracil (5-FU); the second is low-dose cisplatin (CDDP) in combination with 5-FU. For IFN plus 5-FU, the response rate was reported to be 52.6%, with 16.4% achieving complete response (CR) and 36.2% achieving partial response (PR) among 116 patients with tumor thrombosis of the major portal vein or first branches of the portal vein. The survival rates at 6, 12 and 24 months were 53%, 34% and 18%, respectively, with a median survival of 6.9 months, compared with survival rates of 40%, 15% and 5%, respectively, in the historical control group.[98] The survival was significantly different between the two groups ($P < 0.01$). For low-dose CDDP plus 5-FU, the response rate was 48%, including 8% with CR and 40% with PR among 48 patients with portal vein tumor thrombosis. The 1-, 2-, 3- and 5-year cumulative survival rates were 45%, 31%, 25% and 11%, respectively, with a median survival of 10.2 months.[99]

In a review of previously reported small-size phase II studies of HAIC for advanced HCC,[10,17,98–108] the response rate varied from 14% to 71%. The mean survival duration also varied from 2.6 months to 32.4 months. However, few reports have compared systemic chemotherapy or HAIC using cytotoxic agents with placebo or best supportive care (Table 2).

The results of a randomized placebo-controlled double-blind phase III study with the multikinase inhibitor sorafenib were recently reported, representing a breakthrough in the chemotherapy for advanced HCC. Sorafenib is an oral drug that inhibits the platelet-derived growth factor (PDGF)-R, vascular endothelial growth factor (VEGF)-R, c-Kit-R and raf signaling pathways in tumor cells and in surrounding endothelial cells. In that study, 602 patients with advanced HCC, who were not indicated for other loco-regional treatments such as hepatic resection, who had not received prior systemic treatment and who had good liver functional reserve (Child–Pugh A) were randomized to sorafenib (400 mg b.i.d.) or placebo. Sorafenib was well tolerated and yielded a statistically significant improvement (44%) in overall survival. The median survival increased from 7.9 to 10.7 months (hazard ratio, 0.69;

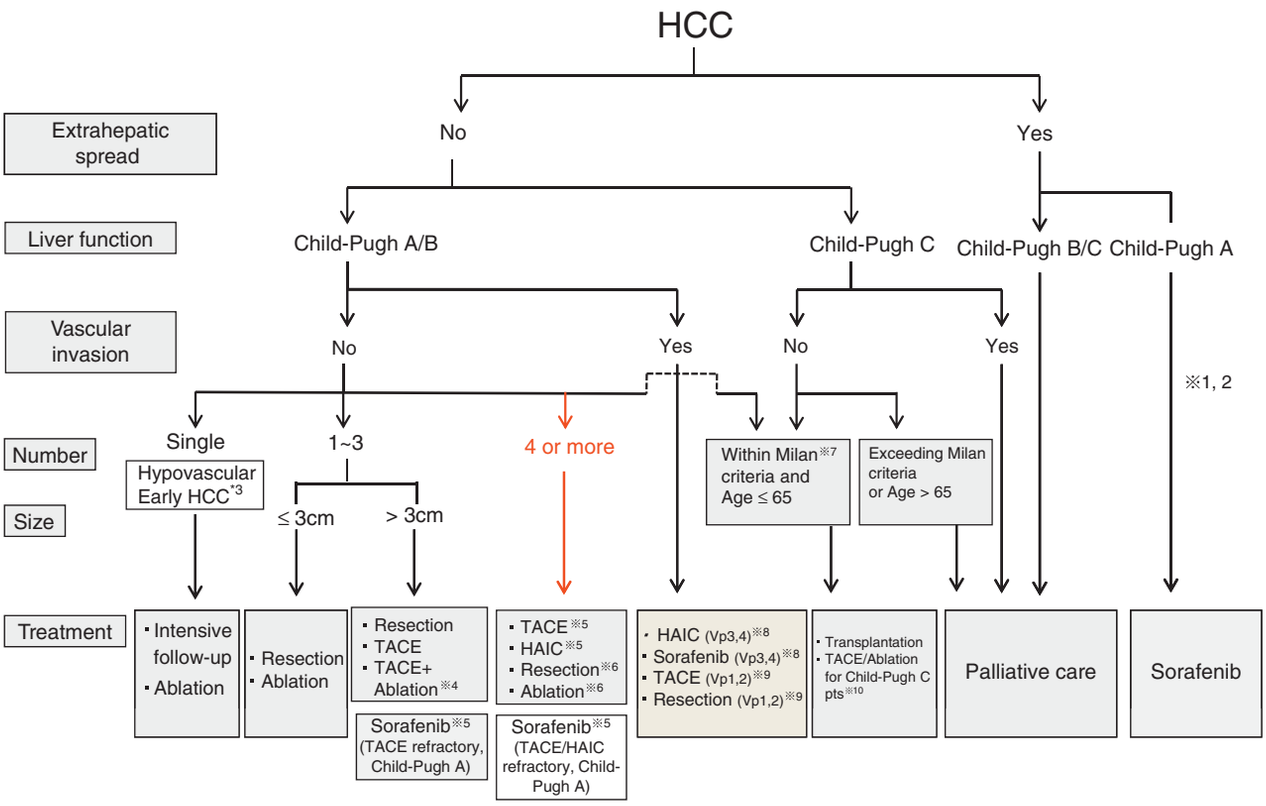


Figure 3 Consensus-based treatment algorithm for hepatocellular carcinoma proposed by the Japan Society of Hepatology (JSH) revised in 2010. (1) Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not regarded as a prognostic factor. (2) Sorafenib is the first choice of treatment in this setting as a standard of care. (3) Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following cases: (i) when the nodule is diagnosed pathologically as early hepatocellular carcinoma (HCC); (ii) when the nodules show decreased uptake on gadolinium ethoxybenzyl magnetic resonance imaging (Gd-EOB-MRI); or (iii) when the nodules show decreased portal flow by computed tomography during arterial portography (CTAP), because these nodules are known to frequently progress to the typical advanced HCC. (4) Even for HCC nodules exceeding 3 cm in diameter, combination therapy of transcatheter arterial chemoembolization (TACE) and ablation is frequently performed when resection is not indicated. (5) TACE is the first choice of treatment in this setting. Hepatic arterial infusion chemotherapy (HAIC) using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5-fluorouracil [5-FU] + cisplatin [CDDP]) or intra-arterial 5-FU in fusion combined with systemic interferon therapy. Sorafenib is also a treatment of choice for TACE/HAIC refractory patients with Child–Pugh A liver function. (6) Resection is sometimes performed even when numbers of nodules are over 4. Furthermore, ablation is sometimes performed in combination with TACE. (7) Milan criteria: tumor size ≤ 3 cm and tumor numbers ≤ 3; or solitary tumor ≤ 5 cm. Even when liver function is good (Child–Pugh A/B), transplantation is sometimes considered for relatively younger patients with frequently or early recurring HCC after curative treatments. (8) HAIC or sorafenib is recommended for HCC patients with Vp3 (portal invasion at the 1st portal branch) or Vp4 (portal invasion at the main portal branch). Sorafenib is only recommended for HCC patients with Child–Pugh A liver function. (9) Resection and TACE is frequently performed when portal invasion is minimal such as Vp1 (portal invasion at the 3rd or more peripheral portal branch) or Vp2 (portal invasion at the 2nd portal branch). (10) Local ablation therapy or subsegmental TACE is performed even for Child–Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites and a low bilirubin level (<3.0 mg/dL). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child–Pugh C patients. A prospective study is necessary to clarify this issue. Even in Child–Pugh A/B patients, transplantation is sometimes performed for relatively younger patients with frequently or early recurring HCC after curative treatments.

Table 2 Response rates and survival periods in studies of intrahepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma

	Drugs	No. of Patients	Response rate (CR + PR, %)	Median survival time (months)	References
Single	Doxorubicin (IHAC)	72	60	7.0	Tzoracoleftherakis *et al.*[102]
	Doxorubicin (systemic)		44.1	6.5	
	CDDP	67	37	10.7	Court *et al.*[103]
Multiple	CDDP, 5-FU (low FP)	52	71	ND	Okuda *et al.*[104]
	CDDP, 5-FU (low FP)	48	48	10.2	Ando *et al.*[99]
	CDDP, 5-FU (low FP)	37	56.3	32.4	Sumie *et al.*[101]
	CDDP, 5-FU (low FP)	38	47	6.2	Tanioka *et al.*[105]
	CDDP, 5-FU	41	22	12.0	Park *et al.*[106]
	CDDP, Mitomycin C, 5-FU, LV	53	28.3	13.2	Lin *et al.*[100]
	IFN, CDDP	68	33	4.4	Chung *et al.*[107]
	CDDP		14	2.6	
	BSC			1.2	
	IFN, CDDP, 5-FU, MTX, LV	34	45	ND	Kaneko *et al.*[108]
	IFN, 5-FU	116	52	6.9	Obi *et al.*[98]

IHAC, intrahepatic arterial chemotherapy; CDDP, cisplatin; 5-FU, 5-fluorouracil; low FP, 5-fluorouracil + cisplatin. BSC, best support care; IFN, interferon; LV, leucovorin; MTX, methotrexate.

95% CI = 0.55–0.87). Side-effects included hand–foot skin reaction, diarrhea and fatigue, but sorafenib was not found to be toxic to the liver.[109] Similar findings were reported in a subsequent Asia–Pacific RCT.[110]

Based on the results of these RCT, sorafenib has become the first-line therapy for advanced HCC worldwide. Some Japanese experts for HCC are claiming low response rates, although the survival was significantly prolonged compared with placebo. This phenomenon could be explained by a longer period with stable disease with sorafenib than with placebo, or the necrotic change in the tumor is present without size reduction.

In Japan, sorafenib was approved for the treatment of HCC on 20 May 2009. In the consensus meeting held in June, 35% of the Japanese experts agreed that sorafenib should be selected as the first-line therapy for advanced HCC considered unsuitable for resection, RFA or TACE. A further 36% of the experts were undecided because they did not have enough experience with using sorafenib.

Informative Statement 4. Sorafenib is the first-line therapy for advanced HCC with major vascular invasion and/or extrahepatic spread and good liver function. However, further studies are needed to compare the overall efficacy of HAIC and sorafenib.

TREATMENT ALGORITHM

TO TREAT HCC, the most appropriate therapeutic option needs to be selected among the available treatment modalities, including resection, percutaneous ablation, TACE and transplantation, but few evidence-based guidelines have been developed to aid decision-making.[1,28,29,88,89,111] Recently, two treatment algorithms for HCC have been proposed in the Japanese guidelines. The profile of these algorithms is briefly described here, in addition to the results of two questions and answers at the JSH Consensus Meeting for HCC at Kobe.

Evidence-based treatment algorithm

The Clinical Practice Guidelines for HCC was established in 2005 based on evidence-based methodology, and covers six topics including prevention, diagnosis, surgery, chemotherapy, TACE and percutaneous ablation. To develop these guidelines, a systematic review of the English medical published work was performed and a total of 7118 articles on HCC were identified, mainly from MEDLINE (1966–2002), of which 334 were selected based on the evidence level to form 58 pairs of clinical questions and recommendations.[1,88] For convenience in clinical use, two algorithms were created for the surveillance and treatment of HCC. A full English version was uploaded to the website of the JSH (www.jsh.or.jp/) in 2006.

The treatment algorithm for HCC was made on the basis of three independent factors: degree of liver damage, tumor number and tumor size. For the resulting six patients' subgroups, the first- and second-line therapies were recommended as objectively as possible (Fig. 1). The degree of liver damage is a modified system based on the Child–Pugh classification: "encephalopathy" was replaced by $ICGR_{15}$, to provide an accurate evaluation of liver functional reserve, particularly in surgical candidates.

Patients with mild (class A) or moderate (class B) liver damage are subject to the following recommendations: (i) in patients with a single tumor, liver resection is recommended, irrespective of the tumor size (percutaneous ablation may be performed if liver damage is of class B and the tumor is no more than 2 cm in size); (ii) for patients with two or three tumors smaller than 3 cm, resection or ablation are recommended; (iii) for patients with two or three tumors larger than 3 cm, resection or TACE are recommended; and (iv) for patients with more than four tumors, TACE or HAIC is recommended. The recommendations for patients with severe (class C) liver damage are as follows: (v) in patients with tumor(s) meeting the Milan criteria, liver transplantation is recommended; and (vi) for patients with more than four tumors, palliative treatment is recommended. For patients with extrahepatic metastasis, chemotherapy may be performed.

The rationale for selecting resection or ablation in patients with class A or B liver damage is based on the outcome of the largest multicenter study involving 12 888 patients in Japan.[59] The recommendation for TACE is based on the findings of two RCT showing a significant improvement in the survival of patients with multiple tumors and class A or B liver damage.[84,85] The indication for liver transplantation is derived from a prospective cohort study using the Milan criteria,[71] and a nationwide survey of Japan justifying the criteria in living donor transplantation.[74]

Consensus-based treatment algorithm

An expert panel of the JSH established a consensus-based treatment algorithm based on the therapeutic policies that are widely used in Japan.[89,111] This algorithm categories the patients on five clinical variables (extrahepatic spread, liver function, vascular invasion, tumor number and tumor size), and it divides the treatment options into resection, ablation, TACE, HAIC, liver

transplantation and palliative treatment (Fig. 3).[89,111] Because of the recent introduction of sorafenib in Japan, this consensus-based treatment algorithm was further revised and approved by the experts at the consensus meeting.[111,112]

Essentially, the consensus-based algorithm follows the evidence-based algorithm, but the treatments widely used in Japan were included by consensus, even though the evidence may be weak. The major differences in the consensus-based algorithm include: (i) ablation is sometimes performed in patients with a single, hypovascular early HCC; (ii) sorafenib is recommended for use in Child–Pugh A patients with vascular invasion, TACE failure or extrahepatic spread of HCC;[109,112] and (iii) liver transplantation is recommended, even for Child–Pugh A/B patients, if the Milan criteria are met.

The consensus-based algorithm based on the consensus of a large number of specialists, and a treatment strategy for management of HCC in Japan is important, and should be revised based on prospective trials for aspects of the algorithm lacking sufficient evidence.[111,112]

Informative statement 5. RFA might be recommended as a first-line treatment option in patients with a single, hypervascular HCC of less than 2 cm in size and with preserved liver function (Child–Pugh A or Liver Damage Class A). However, there was a discrepancy between surgeons and non-surgeons for this statement. This statement is strongly supported by non-surgeons (68%), whereas 80% of the surgeons favor resection rather than RFA. Recommendation 17. Resection should be considered as the first-line treatment option for patients with a single, hypervascular HCC of 3 cm or more in size and with preserved liver function (Child–Pugh A or Liver Damage Class A).

The revised version of the consensus-based treatment algorithm for HCC proposed by the JSH (Fig. 3) should aid decision-making at every stage in clinical practice. By sharing the information contained within the treatment algorithm chart, the physicians can offer recommended treatment options to the patient who can then choose one based on their preference (Fig. 3).

CONCLUSIONS

THIS CONSENSUS STATEMENT is a conclusion of the consensus meeting of HCC, which was held at the 45th JSH meeting, Kobe, Japan on 4–5 June 2009 (Congress President: Professor Masatoshi Kudo). This manuscript and recommendations largely reflect the daily practice in the real world carried out throughout Japan. The biggest difference of Japan's HCC practice from Western countries are pathological assessment issue, prognostic staging system, surveillance and diagnostic strategy, treatment strategy including role of HAIC, and method of RFA procedure, and treatment algorithm shown in Figure 3.

We believe every reader of this manuscript will well understand the real Japanese HCC practice much better than the other already published arterial articles. It is needless to say that consensus statements like this article should be regularly revised every 3–4 years because solid evidence or new diagnostic and treatment tool/drug or concept will be published and then established in clinical practice every year.

REFERENCES

1 Makuuchi M, Kokudo N, Arii S *et al*. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008; **38** (1)**:** 37–51.
2 Liver Cancer Study Group of Japan. *General Rules for the Clinical and Pathological Study of Primary Liver Cancer*. English 2nd edn. Tokyo: Kanehara: 2003.
3 Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42** (5)**:** 1208–36.
4 Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. *Hepatology* 2009; **49** (3)**:** 1017–44.
5 Liu YW, Chen CL, Chen YS, Wang CC, Wang SH, Lin CC. Needle tract implantation of hepatocellular carcinoma after fine needle biopsy. *Dig Dis Sci* 2007; **52** (1)**:** 228–31.
6 Chang S, Kim SH, Lim HK, Lee WJ, Choi D, Lim JH. Needle tract implantation after sonographically guided percutaneous biopsy of hepatocellular carcinoma: evaluation of doubling time, frequency, and features on CT. *AJR Am J Roentgenol* 2005; **185:** 400–5.
7 Durand F, Regimbeau JM, Belghiti J *et al*. Assessment of the benefits and risks of percutaneous biopsy before surgical resection of hepatocellular carcinoma. *J Hepatol* 2001; **35:** 254–8.
8 Takamori R, Wong LL, Dang C, Wong L. Needle-tract implantation from hepatocellular cancer: is needle biopsy of the liver always necessary? *Liver Transpl* 2000; **6:** 67–72.
9 Sakamoto M, Hirohashi S. Natural history and prognosis of adenomatous hyperplasia and early hepatocellular carcinoma: multi-institutional analysis of 53 nodules followed up for more than 6 months and 141 patients with single early hepatocellular carcinoma treated by surgical resection or percutaneous ethanol injection. *Jpn J Clin Oncol* 1998; **28:** 604–8.
10 Okuda K, Ohtsuki T, Obata H *et al*. Natural history of hepatocellular carcinoma and prognosis in relation to

treatment. Study of 850 patients. *Cancer* 1985; **56** (4): 918–28.
11 The Cancer of the Liver Italian Program (CLIP) investigators. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients. *Hepatology* 1998; **28** (3): 751–5.
12 Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362** (9399): 1907–17.
13 Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J Gastroenterol* 2003; **38:** 207–15.
14 Cillo U, Bassanello M, Vitale A *et al*. The critical issue of hepatocellular carcinoma prognostic classification: which is the best tool available? *J Hepatol* 2004; **40:** 124–31.
15 Tateishi R, Yoshida H, Shiina S *et al*. Proposal of a new prognostic model for hepatocellular carcinoma: an analysis of 403 patients. *Gut* 2005; **54:** 419–25.
16 Kudo M, Chung H, Haji S *et al*. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40** (6): 1396–405.
17 Chung H, Kudo M, Takahashi S *et al*. Comparison of three current staging systems for hepatocellular carcinoma: Japan integrated staging score, new Barcelona Clinic Liver Cancer staging classification, and Tokyo score. *J Gastroenterol Hepatol* 2008; **23:** 445–52.
18 Ikai I, Takayasu K, Omata M *et al*. A modified Japan Integrated Stage score for prognostic assessment in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41:** 884–92.
19 Kitai S, Kudo M, Minami Y *et al*. Validation of a new prognostic staging system for hepatocellular carcinoma: a comprison of the biomarker-combined Japan integrated staging score, the conventional Japan integrated staging score and the BALAD score. *Oncology* 2008; **75** (Suppl 1): 83–90.
20 Oka H, Kurioka N, Kim K *et al*. Prospective study of early detection of hepatocellular carcinoma in patients with cirrhosis. *Hepatology* 1990; **12** (4 Pt 1): 680–7.
21 Colombo M, de Franchis R, Del Ninno E *et al*. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med* 1991; **325:** 675–80.
22 Pateron D, Ganne N, Trinchet JC *et al*. Prospective study of screening for hepatocellular carcinoma in Caucasian patients with cirrhosis. *J Hepatol* 1994; **20:** 65–71.
23 Zoli M, Magalotti D, Bianchi G, Gueli C, Marchesini G, Pisi E. Efficacy of a surveillance program for early detection of hepatocellular carcinoma. *Cancer* 1996; **78:** 977–85.
24 Yuen MF, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *Hepatology* 2000; **31:** 330–5.
25 Bolondi L, Sofia S, Siringo S *et al*. Surveillance programme of cirrhotic patients for early diagnosis and treatment of hepatocellular carcinoma: a cost effectiveness analysis. *Gut* 2001; **48:** 251–9.
26 Chen TH, Chen CJ, Yen MF *et al*. Ultrasound screening and risk factors for death from hepatocellular carcinoma in a high risk group in Taiwan. *Int J Cancer* 2002; **98:** 257–61.
27 Danta M, Barnes E, Dusheiko G. The surveillance and diagnosis of hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 2005; **17:** 491–6.
28 Ryder SD. Guidelines for the diagnosis and treatment of hepatocellular carcinoma (HCC) in adults. *Gut* 2003; **52** (Suppl 3): iii1–8.
29 Bruix J, Sherman M, Llovet JM *et al*. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35:** 421–30.
30 Kokudo N, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: the J-HCC guidelines. *J Gastroenterol* 2009; **44** (Suppl 19): 119–21.
31 Thompson Coon J, Rogers G, Hewson P *et al*. Surveillance of cirrhosis for hepatocellular carcinoma: systematic review and economic analysis. *Health Technol Assess* 2007; **11** (34): 1–206.
32 Makuuchi M for the group formed to establish Guidelines for evidence-based clinical practice for the treatment of liver cancer. Clinical practice guidelines for hepatocellular carcinoma: Tokyo, Kanehara; 2005 (in Japanese).
33 Kew MC, Purves LR, Bersohn I. Serum alpha-fetoprotein levels in acute viral hepatitis. *Gut* 1973; **14:** 939–42.
34 Alpert E, Feller ER. Alpha-fetoprotein (AFP) in benign liver disease. Evidence that normal liver regeneration does not induce AFP synthesis. *Gastroenterology* 1978; **74** (5 Pt 1): 856–8.
35 Eleftheriou N, Heathcote J, Thomas HC, Sherlock S. Serum alpha-fetoprotein levels in patients with acute and chronic liver disease. Relation to hepatocellular regeneration and development of primary liver cell carcinoma. *J Clin Pathol* 1977; **30:** 704–8.
36 Taketa K. Alpha-fetoprotein: reevaluation in hepatology. *Hepatology* 1990; **12** (6): 1420–32.
37 Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. *Cancer* 1998; **82:** 1643–8.
38 Tsai SL, Huang GT, Yang PM, Sheu JC, Sung JL, Chen DS. Plasma des-gamma-carboxyprothrombin in the early stage of hepatocellular carcinoma. *Hepatology* 1990; **11:** 481–8.
39 Shimauchi Y, Tanaka M, Kuromatsu R *et al*. A simultaneous monitoring of Lens culinaris agglutinin A-reactive alpha-fetoprotein and des-gamma-carboxy prothrombin

as an early diagnosis of hepatocellular carcinoma in the follow-up of cirrhotic patients. *Oncol Rep* 2000; **7:** 249–56.
40 Nomura F, Ishijima M, Kuwa K, Tanaka N, Nakai T, Ohnishi K. Serum des-gamma-carboxy prothrombin levels determined by a new generation of sensitive immunoassays in patients with small-sized hepatocellular carcinoma. *Am J Gastroenterol* 1999; **94** (3)**:** 650–4.
41 Kawata S, Murakami T, Kim T *et al*. Multidetector CT: diagnostic impact of slice thickness on detection of hypervascular hepatocellular carcinoma. *AJR Am J Roentgenol* 2002; **179:** 61–6.
42 Ichikawa T, Erturk SM, Araki T. Multiphasic contrast-enhanced multidetector-row CT of liver: contrast-enhancement theory and practical scan protocol with a combination of fixed injection duration and patients' body-weight-tailored dose of contrast material. *Eur J Radiol* 2006; **58:** 165–76.
43 Noguchi Y, Murakami T, Kim T *et al*. Detection of hepatocellular carcinoma: comparison of dynamic MR imaging with dynamic double arterial phase helical CT. *AJR Am J Roentgenol* 2003; **180** (2)**:** 455–60.
44 Kim SH, Lee J, Kim MJ *et al*. Gadoxetic acid-enhanced MRI versus triple-phase MDCT for the preoperative detection of hepatocellular carcinoma. *AJR Am J Roentgenol* 2009; **192** (6)**:** 1675–81.
45 Dohr O, Hofmeister R, Treher M, Schweinfurth H. Preclinical safety evaluation of Gd-EOB-DTPA (Primovist). *Invest Radiol* 2007; **42:** 830–41.
46 Bartolozzi C, Crocetti L, Lencioni R, Cioni D, Della Pina C, Campani D. Biliary and reticuloendothelial impairment in hepatocarcinogenesis: the diagnostic role of tissue-specific MR contrast media. *Eur Radiol* 2007; **17** (10)**:** 2519–30.
47 Di Martino M, Marin D, Guerrisi A *et al*. Intraindividual comparison of gadoxetic acid (Gd-EOB-DTPA) enhanced MR imaging and multiphasic 64-slice CT for the detection of hepatocellular carcinoma (HCC) in patients with cirrhosis. *B-096, RNSA* 2008.
48 Luca A, Grazioli L, Caruso S *et al*. A two-centre study for the comparison of Gd-EOB-DTPA (PRIMOVIST)-enhanced MRI verrsus triple-phase MDCT for the detection of hepatocellular carcinoma in cirrhosis. *B-097, RNSA* 2008.
49 Sugiura NTK, Ohto M *et al*. Ultrasound image-guided percutaneous intratumor ethanol injection for small hepatocellular carcinoma. *Kanzo* 1983; **24:** 920.
50 Livraghi T, Festi D, Monti F, Salmi A, Vettori C. US-guided percutaneous alcohol injection of small hepatic and abdominal tumors. *Radiology* 1986; **161:** 309–12.
51 Shiina S, Yasuda H, Muto H *et al*. Percutaneous ethanol injection in the treatment of liver neoplasms. *AJR Am J Roentgenol* 1987; **149:** 949–52.
52 Seki T, Wakabayashi M, Nakagawa T *et al*. Percutaneous microwave coagulation therapy for patients with small hepatocellular carcinoma: comparison with percutaneous ethanol injection therapy. *Cancer* 1999; **85** (8)**:** 1694–702.
53 Shiina S, Teratani T, Obi S, Hamamura K, Koike Y, Omata M. Nonsurgical treatment of hepatocellular carcinoma: from percutaneous ethanol injection therapy and percutaneous microwave coagulation therapy to radiofrequency ablation. *Oncology* 2002; **62** (Suppl 1)**:** 64–8.
54 Shiina S, Tagawa K, Unuma T *et al*. Percutaneous ethanol injection therapy for hepatocellular carcinoma. A histopathologic study. *Cancer* 1991; **68:** 1524–30.
55 Nakashima Y, Nakashima O, Tanaka M, Okuda K, Nakashima M, Kojiro M. Portal vein invasion and intrahepatic micrometastasis in small hepatocellular carcinoma by gross type. *Hepatol Res* 2003; **26:** 142–7.
56 Okusaka T, Okada S, Ueno H *et al*. Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. *Cancer* 2002; **95** (9)**:** 1931–7.
57 Hatanaka K, Chung H, Kudo M *et al*. Usefulness of the post-vascular phase of contrast-enhanced ultrasonography with Sonazoid in the evaluation of gross types of hepatocellular carcinoma. *Oncology* 2010 (in press).
58 Burgener FA, Hamlin DJ. Contrast enhancement of focal hepatic lesions in CT: effect of size and histology. *AJR Am J Roentgenol* 1983; **140:** 297–301.
59 Arii S, Yamaoka Y, Futagawa S *et al*. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. *Hepatology* 2000; **32** (6)**:** 1224–9.
60 Huang GT, Lee PH, Tsang YM *et al*. Percutaneous ethanol injection versus surgical resection for the treatment of small hepatocellular carcinoma: a prospective study. *Ann Surg* 2005; **242** (1)**:** 36–42.
61 Ryu M, Shimamura Y, Kinoshita T *et al*. Therapeutic results of resection, transcatheter arterial embolization and percutaneous transhepatic ethanol injection in 3225 patients with hepatocellular carcinoma: a retrospective multicenter study. *Jpn J Clin Oncol* 1997; **27:** 251–7.
62 Livraghi T, Bolondi L, Buscarini L *et al*. No treatment, resection and ethanol injection in hepatocellular carcinoma: a retrospective analysis of survival in 391 patients with cirrhosis. Italian Cooperative HCC Study Group. *J Hepatol* 1995; **22:** 522–6.
63 Castells A, Bruix J, Bru C *et al*. Treatment of small hepatocellular carcinoma in cirrhotic patients: a cohort study comparing surgical resection and percutaneous ethanol injection. *Hepatology* 1993; **18** (5)**:** 1121–6.
64 Shiina S, Teratani T, Obi S *et al*. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129:** 122–30.
65 Lencioni RA, Allgaier HP, Cioni D *et al*. Small hepatocellular carcinoma in cirrhosis: randomized comparison of

radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003; **228** (1)**:** 235–40.
66 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma < or =4 cm. *Gastroenterology* 2004; **127** (6)**:** 1714–23.
67 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54:** 1151–6.
68 Chen MS, Li JQ, Zheng Y *et al*. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243:** 321–8.
69 Llovet JM, Di Bisceglie AM, Bruix J *et al*. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100:** 698–711.
70 The Japanese Liver Transplantation Society. Liver transplantation in Japan in 2006 (part 2)-Registry by the Japanese Liver Transplantation Society. *Ishoku* 2008; **43:** 45–55, (in Japanese).
71 Mazzaferro V, Regalia E, Doci R *et al*. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334:** 693–9.
72 Iwatsuki S, Starzl TE, Sheahan DG *et al*. Hepatic resection versus transplantation for hepatocellular carcinoma. *Ann Surg* 1991; **214:** 221–8, discussion 228–229.
73 Penn I. Hepatic transplantation for primary and metastatic cancers of the liver. *Surgery* 1991; **110:** 726–34, discussion 734–725.
74 Todo S, Furukawa H, Tada M. Extending indication: role of living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2007; **13** (11 Suppl 2)**:** S48–54.
75 Mazzaferro V, Llovet JM, Miceli R *et al*. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10** (1)**:** 35–43.
76 Yao FY, Ferrell L, Bass NM *et al*. Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology* 2001; **33** (6)**:** 1394–403.
77 Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. *Dig Dis* 2007; **25:** 310–12.
78 Lee SG, Hwang S, Moon DB *et al*. Expanded indication criteria of living donor liver transplantation for hepatocellular carcinoma at one large-volume center. *Liver Transpl* 2008; **14:** 935–45.
79 Ito T, Takada Y, Ueda M *et al*. Expansion of selection criteria for patients with hepatocellular carcinoma in living donor liver transplantation. *Liver Transpl* 2007; **13:** 1637–44.
80 Duffy JP, Vardanian A, Benjamin E *et al*. Liver transplantation criteria for hepatocellular carcinoma should be expanded: a 22-year experience with 467 patients at UCLA. *Ann Surg* 2007; **246:** 502–9, discussion 509–511.
81 Yamamoto J, Iwatsuki S, Kosuge T *et al*. Should hepatomas be treated with hepatic resection or transplantation? *Cancer* 1999; **86:** 1151–8.
82 Figueras J, Jaurrieta E, Valls C *et al*. Resection or transplantation for hepatocellular carcinoma in cirrhotic patients: outcomes based on indicated treatment strategy. *J Am Coll Surg* 2000; **190:** 580–7.
83 Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30** (6)**:** 1434–40.
84 Llovet JM, Real MI, Montana X *et al*. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359** (9319)**:** 1734–9.
85 Lo CM, Ngan H, Tso WK *et al*. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35** (5)**:** 1164–71.
86 Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. *Hepatology* 2003; **37** (2)**:** 429–42.
87 Bruix J, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* 2004; **127** (5 Suppl 1)**:** S179–88.
88 Makuuchi M, Kokudo N. Clinical practice guidelines for hepatocellular carcinoma: the first evidence based guidelines from Japan. *World J Gastroenterol* 2006; **12:** 828–9.
89 Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology* 2007; **72:** S2–15.
90 Ikai I, Arii S, Okazaki M *et al*. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007; **37:** 676–91.
91 Takayasu K, Arii S, Ikai I *et al*. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; **131** (2)**:** 461–9.
92 Ueno S, Tanabe G, Nuruki K *et al*. Prognostic performance of the new classification of primary liver cancer of Japan (4th edition) for patients with hepatocellular carcinoma: a validation analysis. *Hepatol Res* 2002; **24:** 395–403.
93 Marelli L, Stigliano R, Triantos C *et al*. Treatment outcomes for hepatocellular carcinoma using chemoembolization in combination with other therapies. *Cancer Treat Rev* 2006; **32:** 594–606.
94 Veltri A, Moretto P, Doriguzzi A, Pagano E, Carrara G, Gandini G. Radiofrequency thermal ablation (RFA) after

transarterial chemoembolization (TACE) as a combined therapy for unresectable non-early hepatocellular carcinoma (HCC). *Eur Radiol* 2006; **16** (3)**:** 661–9.
95 Buscarini L, Buscarini E, Di Stasi M, Quaretti P, Zangrandi A. Percutaneous radiofrequency thermal ablation combined with transcatheter arterial embolization in the treatment of large hepatocellular carcinoma. *Ultraschall Med* 1999; **20** (2)**:** 47–53.
96 Yamakado K, Nakatsuka A, Akeboshi M, Shiraki K, Nakano T, Takeda K. Combination therapy with radiofrequency ablation and transcatheter chemoembolization for the treatment of hepatocellular carcinoma: short-term recurrences and survival. *Oncol Rep* 2004; **11:** 105–9.
97 Koda M, Ueki M, Maeda Y *et al*. The influence on liver parenchymal function and complications of radiofrequency ablation or the combination with transcatheter arterial embolization for hepatocellular carcinoma. *Hepatol Res* 2004; **29** (1)**:** 18–23.
98 Obi S, Yoshida H, Toune R *et al*. Combination therapy of intraarterial 5-fluorouracil and systemic interferon-alpha for advanced hepatocellular carcinoma with portal venous invasion. *Cancer* 2006; **106** (9)**:** 1990–7.
99 Ando E, Tanaka M, Yamashita F *et al*. Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. *Cancer* 2002; **95:** 588–95.
100 Lin CP, Yu HC, Cheng JS *et al*. Clinical effects of intra-arterial infusion chemotherapy with cisplatin, mitomycin C, leucovorin and 5-flourouracil for unresectable advanced hepatocellular carcinoma. *J Chin Med Assoc* 2004; **67:** 602–10.
101 Sumie S, Yamashita F, Ando E *et al*. Interventional radiology for advanced hepatocellular carcinoma: comparison of hepatic artery infusion chemotherapy and transcatheter arterial lipiodol chemoembolization. *AJR Am J Roentgenol* 2003; **181** (5)**:** 1327–34.
102 Tzoracoleftherakis EE, Spiliotis JD, Kyriakopoulou T, Kakkos SK. Intra-arterial versus systemic chemotherapy for non-operable hepatocellular carcinoma. *Hepatogastroenterology* 1999; **46** (26)**:** 1122–5.
103 Court WS, Order SE, Siegel JA *et al*. Remission and survival following monthly intraarterial cisplatinum in non-resectable hepatoma. *Cancer Invest* 2002; **20** (5–6)**:** 613–25.
104 Okuda K, Tanaka M, Shibata J *et al*. Hepatic arterial infusion chemotherapy with continuous low dose administration of cisplatin and 5-fluorouracil for multiple recurrence of hepatocellular carcinoma after surgical treatment. *Oncol Rep* 1999; **6:** 587–91.
105 Tanioka H, Tsuji A, Morita S *et al*. Combination chemotherapy with continuous 5-fluorouracil and low-dose cisplatin infusion for advanced hepatocellular carcinoma. *Anticancer Res* 2003; **23** (2C)**:** 1891–7.
106 Park JY, Ahn SH, Yoon YJ *et al*. Repetitive short-course hepatic arterial infusion chemotherapy with high-dose 5-fluorouracil and cisplatin in patients with advanced hepatocellular carcinoma. *Cancer* 2007; **110:** 129–37.
107 Chung YH, Song IH, Song BC *et al*. Combined therapy consisting of intraarterial cisplatin infusion and systemic interferon-alpha for hepatocellular carcinoma patients with major portal vein thrombosis or distant metastasis. *Cancer* 2000; **88** (9)**:** 1986–91.
108 Kaneko S, Urabe T, Kobayashi K. Combination chemotherapy for advanced hepatocellular carcinoma complicated by major portal vein thrombosis. *Oncology* 2002; **62** (Suppl 1)**:** 69–73.
109 Llovet JM, Ricci S, Mazzaferro V *et al*. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359:** 378–90.
110 Cheng AL, Kang YK, Chen Z *et al*. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10** (1)**:** 25–34.
111 Kudo M. Hepatocellular carcinoma 2009 and beyond: from the surveillance to molecular targeted therapy. *Oncology* 2008; **75:** S1–12.
112 Kudo M. The 2008 Okuda lecture: management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. *J Gastroenterol Hepatol* 2010; **25** (3)**:** 439–52.

Hepatology Research

Hepatology Research 2010; **40**: 686–692 doi: 10.1111/j.1872-034X.2010.00674.x

Special Report

Response Evaluation Criteria in Cancer of the Liver (RECICL) proposed by the Liver Cancer Study Group of Japan (2009 Revised Version)

Masatoshi Kudo, Shouji Kubo, Kenichi Takayasu, Michiie Sakamoto, Masatoshi Tanaka, Iwao Ikai, Junji Furuse, Kenji Nakamura, Masatoshi Makuuchi, for The Liver Cancer Study Group of Japan (Committee for Response Evaluation Criteria in Cancer of the Liver, Liver Cancer Study Group of Japan)

The Liver Cancer Study Group of Japan, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

The World Health Organization (WHO) criteria and Response Evaluation Criteria in Solid Tumors (RECIST) are inappropriate to assess the direct effects of treatment on the hepatocellular carcinoma (HCC) by locoreginal therapies such as radiofrequency ablation (RFA) and transcatheter arterial chemoembolization (TACE). Therefore, establishment of response evaluation criteria solely devoted for HCC is needed urgently in the clinical practice as well as in the clinical trials of HCC treatment, such as molecular targeted therapies, which cause necrosis of the tumor. Response Evaluation Criteria in Cancer of the Liver (RECICL) was revised in 2009 by Liver Cancer Study Group of Japan based on the 2004 version of RECICL, which was commonly used in Japan. Major revised points of the RECICL 2009 is to provide TE4a (Complete response with enough ablative margin) and TE4b (complete response without enough ablative margin) for local ablation therapy. Second revised point is that setting the timing at which the overall treatment effects are assessed. Third point is that emergence of new lesion in the liver is regarded as progressive disease, different from 2004 version. Finally, 3 tumor markers including alpha-fetoprotein (AFP) and AFP-L3 and des-gamma-carboxy protein (DCP) were also added for the overall treatment response. We hope this new treatment response criteria, RECICL, proposed by Liver Cancer Study Group of Japan will benefit the HCC treatment response evaluation in the setting of the daily clinical practice and clinical trials as well not only in Japan, but also internationally.

Key words: Response Evaluation Criteria, hepatocellular carcinoma, WHO criteria, RECIST, Liver Cancer, Liver Cancer Study Group of Japan

INTRODUCTION

THE WORLD HEALTH Organization (WHO) criteria[1] and Response Evaluation Criteria in Solid Tumors (RECIST),[2] which are response evaluation criteria for solid tumors after chemotherapy, are commonly used for the evaluation of liver cancer treatment in Western countries. However, it is well known and obvious that both the WHO criteria and RECIST are inappropriate to assess the direct effects of treatment on the liver cancer lesions by ablative treatment and transcatheter arterial chemoembolization (TACE). Although effective treatments may exhibit a necrotizing effect on hepatocellular carcinoma (HCC) with deprivation of its blood flow, the WHO criteria and RECIST do not consider such necrotizing effects to be "effective"; instead, both criteria use only tumor size reduction as measures of effect. It has been shown that the tumor size reduction rate according to the WHO criteria and RECIST following TACE with lipiodol (Lip-TACE) is not correlated with the pathological necrosis rate.[3] When lipiodol is accumulated densely within the tumor, the early arterial staining is masked, and tumor size is not increased, the tumor is completely necrotized as confirmed by histology.[3] Even though the tumor is completely necrotized, it takes a long time to result in reduction of size. The nodule with complete necrosis after Lip-TACE can be seen for several years as a lipiodol more densely

Correspondence: Professor Masatoshi Kudo, The Liver Cancer Study Group of Japan, 377-2, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Ohno-Higashi, Osaka-Sayama, Osaka 589-8511, Japan. Email: m-kudo@med.kindai.ac.jp
Received 24 December 2009; revision 3 March 2010; accepted 9 March 2010.

accumulated nodules than 2 weeks after the intervention. In case of radiofrequency ablation (RFA), the phenomenon is the same with Lip-TACE, though lipiodol accumulation is not seen.

Moreover, the WHO criteria are originally based on bi-dimensional measurement, which was changed to a uni-dimensional measurement in RECIST. Even if tumor necrosis is considered in the response evaluation criteria, uni-dimensional measurement is inappropriate for assessment of the direct treatment effect. Therefore, establishment of response evaluation criteria solely devoted for HCC is needed urgently in the clinical practice as well as in the clinical trials of HCC. The current report describes the newly established response evaluation criteria for HCC by revising the previously existing criteria established by the Liver Cancer Study Group of Japan.

CONCEPT OF THE RESPONSE EVALUATION CRITERIA IN CANCER OF THE LIVER (RECICL)

THE FIRST EDITION of Criteria for the Evaluation of Direct Treatment Effects in Hepatocellular Carcinoma was published in 1994.[4] The revised edition was published in 2004,[5] and is commonly used in Japan, but several problems remained in the revised criteria. Thus, a third revision was carried out before publishing the English edition of the General Rules for the Clinical and Pathological Study of Primary Liver Cancer edited by the Liver Cancer Study Group of Japan (third edition).

Current response evaluation criteria focuses on the following points: (i) development of simple criteria that are sufficiently applicable in routine clinical practice centering on local treatment (ethanol injection therapy, microwave coagulation therapy, RFA) and transcatheter arterial therapy, radiotherapy and systemic chemotherapy can also be included; (ii) assessment of direct treatment effects on intrahepatic target lesions and overall effects are described separately; and (iii) the criteria follow the fifth edition of the General Rules for the Clinical and Pathological Study of Primary Liver Cancer edited by the Liver Cancer Study Group of Japan.[6]

Considering the biological characteristics of HCC, high frequencies of "intrahepatic metastatic recurrence" and "multicentric carcinogenesis", it may not necessarily be appropriate for liver cancer to be indiscriminately diagnosed as "progressive disease" based on the appearance of "a new lesion" alone because such "a new lesion" has not been treated by ablation or TACE when the recurrent nodule exists outside of the treated area. Thus, evaluation of the direct effects of treatment on target lesions should focus on the direct therapeutic effect on the target lesions, and the overall evaluation should be investigated with close association with the prognosis.

Although the chemotherapeutic agent permeates through the liver in chemotherapy, the therapeutic effect of TACE and ablative treatments is limited only to the target lesion or the area fed by embolized artery with the tumor. Treatment is not done for the new lesions appearing outside the area where the ablation or TACE are performed. After the same treatment is carried out on the targeted new lesion, a similar treatment effect may be expected on the formerly treated lesion. Accordingly, when "a new lesion" appears in a region outside the treatment area, the new lesion (intrahepatic metastasis or multicentric carcinogenesis) may not directly indicate the prognosis. The basic concept of the 2004 version of the Japanese response evaluation criteria[5] was to exclude the new lesions from the evaluation of treatment effect on the formerly treated lesions. In other words, the emergence of a new lesion is regarded as out of the evaluation of the treatment effect for the former lesions, which is the most marked difference from the WHO criteria or RECIST.

Therefore, these criteria established by the Liver Cancer Study Group of Japan are exclusively specified for the Evaluation of Therapeutic Effects on Liver Cancer, and differ from other evaluation criteria for solid tumor regarding the various points described above.

The 2004 version of the Criteria for the Evaluation of Direct Treatment Effects in Hepatocellular Carcinoma are superior to the WHO criteria or RECIST because it considers the biological characteristics of HCC as follows: (i) tumor necrosis is regarded as a direct effect of treatment on the target lesion as well as tumor size reduction even though it is minimal; (ii) tumors are measured in two dimensions; (iii) the dense accumulation of lipiodol is regarded as necrosis;[3] and (iv) the emergence of a new lesion is not regarded as a "progressive disease" in evaluation of the treated nodule.

However, several problems remained in the 2004 version: (i) assessment of direct treatment effects was performed at 3 months, while the overall evaluation was performed at 6 months; and (ii) even though the direct effects on target nodules varies among treatment methods, the timing of assessment was not described. To overcome these limitations, some minor changes were made in this 2009 revised version. These criteria may be suitable mainly for local treatment and transcatheter arterial therapy, but are also applicable for radiotherapy and chemotherapy in combination with

the WHO criteria and RECIST. Whether or not some criteria are superior to others will be investigated in future studies. We expect that the 2009 revised edition of Response Evaluation Criteria in Cancer of the Liver (RECICL), will be widely used in clinical practice as well as in the clinical trial settings, not only in our country but also worldwide, as the criteria are clearer and may be more suitable in response evaluation for liver cancer than WHO criteria or RECIST.

MAJOR REVISED POINTS OF THE RESPONSE EVALUATION CRITERIA IN THE 2009 VERSION

FIRST, WE HAVE clarified the direct effect of local treatments on target nodules. When the non-stained low-density area in local ablation therapy such as ethanol injection therapy, microwave coagulation therapy and RFA covers all parts of the low-density area in the late phase of dynamic computed tomography (CT) scan before treatment, the lesion is regarded as 100% necrotized and described as treatment effect 4 (TE4), even though the size of the nodule does not decrease in the follow-up CT scan or multiple resonance imaging (MRI). However, when the non-stained low-density area does not cover the low-density area before the treatment, the risk of local recurrence is high.[7–9] Therefore, for ethanol injection therapy, microwave coagulation therapy and RFA, when the non-stained low-density area is slightly wider across the entire circumference than the low-density area in the late phase of dynamic CT scan before treatment, the lesion is regarded as 100% necrotized (TE4a). When only hypervascularity has disappeared without a slightly wider non-stained region than the low-density area on dynamic CT scan, the condition is judged as TE4b (Table 1).

Second, we have settled the timing at which the overall treatment effects are assessed: (i) the maximum response within 3 months is regarded as the overall treatment effect; (ii) for transcatheter arterial therapy with lipiodol, it is desirable to assess the effect after at least 1 month; (iii) local ablative treatment can be assessed immediately after the treatment; and (iv) for radiotherapy, the maximum response within 6 months may be regarded as the overall effect.

Third, regarding the criteria for "progressive disease" in the overall evaluation, the emergence of a new lesion is regarded as "progressive disease", similar to that advocated in the WHO criteria or RECIST, as shown in the Appendix. However, new lesions are separately described in consideration of the biological characteristics of HCC and the description may contribute to a future review of the criteria, particularly for: (i) intrahepatic solitary lesions (whether it is in the treated area or outside of the treated area by ablation or TACE); (ii) intrahepatic multiple lesions; and (iii) vascular invasion/extrahepatic spread.

Fourth, the RECIST and WHO criteria may be appropriate for radiotherapy and systemic chemotherapy including molecular targeted agents because these are currently used internationally,[10–13] however, we recommend evaluation using the RECICL criteria in combination with the WHO criteria or RECIST in order to clarify which criteria among the three are the most appropriate in future studies. This point is described in the detailed regulation section.

Fifth, in the detailed regulation section, the lowest levels of three tumor markers (α-fetoprotein [AFP], AFP-L3 and Protein induced by vitamine K absence or antagonist [PIVKA-II] or des-gamma-carboxy prothrombin [DCP]) should be measured and described within 3 months and considered with reference to the overall evaluation. It may be useful to prospectively investigate whether there is a difference in the prognosis between complete response (CR) based on imaging alone and CR on imaging in combination with response of tumor markers.

Finally, we include a comparison between the WHO criteria, RECIST[14,15] and RECICL established by the Liver Cancer Study Group of Japan.

Table 1 Treatment effect (TE) on the target nodule

TE4:	The tumor-necrotizing effect is 100% or the tumor size reduction rate is 100%.*
TE4a:	Necrotized area with larger ablated area than original nodule.*
TE4b:	Necrotized area of same size with original nodule.
TE3:	The tumor-necrotizing effect or tumor size reduction rate is between 50% and <100%.*
TE2:	Effects other than TE3 and TE1.
TE1:	The tumor enlarged by >25% regardless of the necrotizing effect.

*For ethanol injection therapy, microwave coagulation therapy, and radiofrequency ablation, when the non-stained low-density area is slightly wider across the entire circumference than the low-density area in the late phase of dynamic computed tomography (CT) scan before treatment, the lesion is regarded as 100% necrotized (TE4a). When only hypervascularity has disappeared without a slightly wider non-stained region than the low-density area on dynamic CT scan, the condition is judged as TE4b. In transcatheter arterial chemoembolization (TACE), the tendency of reduction of tumor size, without tumor staining by CT scan with contrast enhancement, and denser uniform accumulation of lipiodol than just after lipiodol TACE when lipiodol is used, are classified to be TE4.

DESCRIPTION OF RECICL PROPOSED BY LIVER CANCER STUDY GROUP OF JAPAN

Subjects

THE SUBJECTS ARE patients who are treated initially and for recurrence. Because responses to treatment are evaluated, as a rule, by dynamic CT, intrahepatic lesions with hypervascular tumors are the principle targets of the RECICL criteria. It is essential that tumors can be clearly visualized using an imaging technique.

Detailed description

Description of past medical history

1 Methods and date when definitive diagnosis of liver cancer was made.
2 Previous treatment modality (as described in "c. Description of treatment modalities").
3 Dates of initiation and completion of previous treatment.
4 Methods and date when recurrence was diagnosed.

Descriptions of liver cancer at the time of the initiation of treatment

These issues are based on the second English Edition of the General Rules for the Clinical and Pathological Study of Primary Liver Cancer (edited by the Liver Cancer Study Group of Japan).[16] The following items should be noted:

1 Tumor location.
2 Tumor size, number, and vascular invasion. The tumor size is presented as the major axis and maximum diameter crossing the major axis at a right angle.
3 Macroscopic types.[16,17]
4 Macroscopic staging. Even for tumors that are only assessable by imaging, staging should be described following the rules for surgical findings and the resected specimen.[16,17]
5 Histological grading when biopsy is performed.[16,17]

Description of treatment modalities

1 Name of treatment: transcatheter hepatic arterial therapy (transcatheter arterial infusion chemotherapy, transcatheter arterial embolization, TACE), local treatment (ethanol injection therapy, microwave coagulation therapy, RFA), radiotherapy such as Liniac, γ-knife, or proton beam line, systemic chemotherapy.
2 Details of treatment: for treatments using drugs, the name of drugs* (anticancer drugs, Lipiodol, etc.), route of administration, treatment interval and single dose, and the total number of administrations and total dose should be described. For other treatment methods, the details should be described appropriately. When the treatment is discontinued, the reason for discontinuation and the presence or absence of adverse effects should be described. (*In addition to the chemotherapeutic drugs, any drugs directly injected into the tumor to necrotize it, such as ethanol, and/or embolizing materials, should be described.)
3 Dates of initiation and completion or termination of treatment.

Assessment of direct treatment effect on target nodule

1 On assessment of the direct effect of treatment on the target nodule, the tumor-necrotizing effect and tumor size reduction rate are calculated based on the size reduction or disappearance of hypervascularity of the nodule on dynamic CT. Findings of dynamic MRI, and/or contrast-enhanced ultrasonography can substitute dynamic CT.
2 The necrotizing effect is assessed by imaging. The percent ratio of the necrotized area to the cross-sectional area of the tumor should be calculated.* (*When various cross-sections are obtained for a single tumor, the total sum of the necrotic area should be used; however, when the maximum cross-section represents the entire findings of the tumor, assessment may be made based on the maximum cross-sectional area.)
3 The size reduction rate is calculated using the equation below, after calculating the product of the major axis of the maximum cross-section by the maximum diameter crossing the major axis at a right angle: size reduction rate = ([product before treatment] – [product after treatment]) / (product before treatment) × 100.
4 Direct treatment effect (TE) on target nodule: effects on individual lesions are categorized into four degrees based on the tumor-necrotizing effect observed within a fixed term* after the initiation of treatment or the maximum tumor size reduction rate, as shown in Table 1. (*For local treatments [such as ethanol injection therapy, microwave coagulation therapy, RFA], the effects are assessed immediately after treatment. For transcatheter arterial chemotherapy using lipiodol, transcatheter arterial embolization and transcatheter arterial chemoembolization, it is desirable to assess the effect after at least 1 month. For radiotherapy, the effect assessed based on the maximum response within 6 months.)
5 When multiple lesions are present in the liver, TE is determined in individual lesions.

Table 2 Overall response evaluation (effect of treatment on all intrahepatic lesions at 3 months; radiotherapy can be evaluated at 6 months)

Overall evaluation of treatment effect	Effect of treatment on the tumor
CR (complete response)	100% tumor-necrotizing effect or 100% tumor size reduction rate
PR (partial response)	The tumor-necrotizing effect or tumor size reduction rate is between 50% and <100%
SD (stable disease)	Effects other than PR and PD
PD (progressive disease)	The tumor growth >25% regardless of the necrotizing effect, or emergence of a new lesion*

*With regard to the emergence of new lesions, the lesion should be classified as either: (i) intrahepatic solitary lesion (within or outside the treatment area); (ii) intrahepatic multiple lesions (within or outside the treatment area); or (iii) vascular invasion (the portal vein, hepatic vein, bile duct)/extrahepatic spread.

OVERALL EVALUATION OF THE TREATMENT RESPONSE

1 The overall evaluation is determined, based on the effect in the entire liver and its persistence, and categorized as CR, partial response (PR), stable disease (SD) and progressive disease (PD), as defined in Table 2.
2 To use this method to predict the prognosis, TE is determined and recorded at 3 months when retreatment is not performed after the initiation of treatment, as an overall response evaluation, except for radiotherapy, in which the overall evaluation is performed at 6 months.
3 When multiple lesions are present, but the assessment of all of the lesions is difficult, evaluation of the five largest lesions may be considered to represent the overall evaluation of the entire liver, but it is not regarded as CR. In addition, CR should not be given when the findings of the maximum cross-section is regarded to represent the entire tumor. Tumors may only be described as CR when all of the intrahepatic lesions are assessable as well as the effect shown in Table 2 (100% tumor-necrotizing effect or 100% tumor size reduction rate) is obtained.

DETAILED REGULATIONS

THE NECROTIZING EFFECT is assessed based on the response evaluation criteria of treatment on target nodules.

1 The presence, on dynamic CT with an i.v. bolus injection, of a non-stained low-density area after treatment is regarded as a necrotizing effect. A non-stained low-density area represents an apparently lower level than that in the surrounding liver parenchyma in the early and late phases* of dynamic CT with an i.v. bolus injection. Usually, the CT attenuation value of a non-stained low-density area does not increase on dynamic imaging. (*The early phase represents the arterial dominant phase of dynamic CT. The late phase represents the equilibrium phase of dynamic CT.)
2 When lipiodol is used, the presence of a region retaining lipiodol homogeneously and densely in the tumor shown on CT 1 month after therapy is regarded as a necrotizing effect. Dynamic MRI, Doppler ultrasonography and contrast-enhanced ultrasonography can be also used.
3 The effects of radiotherapy, systemic chemotherapy (including treatment with molecular targeted agents) and hepatic arterial chemotherapy should be described by both RECIST and present criteria, RECICL.
4 The lowest levels of three tumor markers (AFP, AFP-L3 fraction, PIVKA-II or DCP) should be recorded as reference values for the overall response evaluation.

REFERENCES

1 WHO. *WHO Handbook for Reporting Results of Cancer Treatment*. Vol. 48, Geneva (Switzerland): World Health Organization Offset Publication, 1979.
2 Therasse P, Arbuck SG, Eisenhauer EA *et al*. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92:** 205–16.
3 Takayasu K, Arii S, Matsuo N *et al*. Comparison of CT findings with resected specimens after chemoembolization with iodized oil for hepatocellular carcinoma. *Am J Roentgenol* 2000; **175:** 699–704.
4 The Liver Cancer Study Group of Japan. The criteria for the evaluation of direct treatment effects in hepatocellular carcinoma. *Acta Hepatol Jpn* 1994; **35:** 193–205, (in Japanese).
5 The Liver Cancer Study Group of Japan. The criteria for the evaluation of direct treatment effects in hepatocellular carcinoma. *Acta Hepatol Jpn* 2004; **45:** 380–85, (in Japanese).
6 The Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, 5th edn. Tokyo: Kanehara, 2008; (in Japanese).

7 Okusaka T, Okada S, Ueno H *et al*. Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. *Cancer* 2002; **95:** 1931–7.
8 Kudo M. Local ablation therapy for hepatocellular carcinoma: current status and future perspectives. *J Gastroenterol* 2004; **39:** 205–14.
9 Nishijima N, Osaki Y, Kita R *et al*. Proposal of the radicality grading as a criterion for therapeutic effectiveness of RFA against hepatocellular carcinoma, in relation to the local recurrence rate. *Acta Hepatol Jpn* 2008; **49:** 192–99, (in Japanese).
10 Green S, Weiss GR. Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. *Invest New Drugs* 1992; **10:** 239–53.
11 Gehan EA, Tefft MC. Will there be resistance to the RECIST (Response Evaluation Criteria in Solid Tumors)? *J Natl Cancer Inst* 2000; **92:** 179–81.
12 Llovet JM, Beaugrand M. Hepatocellular carcinoma: present status and future prospects. *J Hepatol* 2003; **38** (Suppl 1)**:** S136–49.4
13 Llovet JM, Di Bisceglie Am, Bruix J *et al*. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100:** 698–711.
14 James K, Eisenhauer E, Christian M *et al*. Measuring response in solid tumors: unidimensional versus bidimensional measurement. *J Natl Cancer Inst* 1999; **91:** 523–8.
15 Park JO, Lee SI, Song SY *et al*. Measuring response in solid tumors: comparison of RECIST and WHO response criteria. *Jpn J Clin Oncol* 2003; **33:** 533–7.
16 The Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, 2nd English edn, Tokyo: Kanehara, 1997.
17 The Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*. 5th Edn, Revised Version, Tokyo: Kanehara, 2009; (in Japanese).

APPENDIX I

TOVERALL EVALUATION OF treatment effects on liver cancer: a comparison between the World Health Organization (WHO) criteria, Response Evaluation Criteria in Solid Tumors (RECIST) and Response Evaluation Criteria in Cancer of the Liver (RECICL)

	WHO criteria (after 4 weeks)	RECIST (after 4 weeks)	RECICL (after 3 months)
Lesion evaluated	All evaluable lesions	All measurable lesions, target lesions (five lesions, a maximum of 10 lesions when lesions are present over 2 or more organs)	Target lesions (a maximum of five lesions when more than 5 lesions are present)
Evaluation method	Bi-dimensional measurement (changes in the product of the major axis and the diameter crossing the major axis at a right angle). Sum of the all lesions.	Uni-dimensional measurement (changes in the sum of the major axis)	Bi-dimensional measurement (changes in the product of the major axis and the diameter crossing the major axis at a right angle, non-stained regions on dynamic CT and/or lipiodol-deposited regions are measured as necrosis). Sum of the all target lesions.
Overall evaluation			
CR (complete response)	Disappearance of all lesions	Disappearance of all target lesions	100% tumor-necrotizing effect or 100% tumor size reduction rate
PR (partial response)	50% or greater disappearance of all lesions	30% or greater reduction of target lesions	A tumor-necrotizing effect or tumor size reduction rate between 50% and <100%
SD (stable disease)	Effects other than PR and PD	Effects other than PR and PD	Effects other than PR and PD
PD (progressive disease)	≥25% enlargement of a lesion or appearance of a new lesion	≥20% increase or appearance of a new lesion	≥25% enlargement of the tumor regardless of the necrotizing effect or appearance of a new lesion (categorized into three groups: intrahepatic solitary lesion, intrahepatic multiple lesions, and vascular invasion/extrahepatic spread).

APPENDIX II

Example RECICL Evaluation Sheet

Patient Age Male/female ID

1. **Description of Liver Cancer**
   (1) Past medical history
      (i) Method and date of definite diagnosis of liver cancer
      (ii) Past treatment history (only patients treat for recurrence)
   (2) Condition of liver cancer
      Tumor location, number and size of lesions, vascular invasion, macroscopic classification, macroscopic staging, histological type or degree of differentiation

2. **Description of Treatment Method**
   (1) Initial treatment or treatment for recurrence
   (2) Name of treatment (describe all treatments when multiple treatments were performed)
   (3) Details of treatment, including the reason for the discontinuation and the presence or absence of an adverse event when treatment is discontinued
   (4) Dates of initiation and completion of treatment

3. **Treatment Effect on Target Nodule (TE1, 2, 3, 4)**[*1] _____

(Describe TE4a or 4b for local ablation) Assessment results: Lesion 1 / Lesion 2 / Lesion 3 / Lesion 4 / Lesion 5

4. **Overall Evaluation (CR, PR, SD, PD)**[*2] _____

Assessment results: _____

When a new lesion appears in PD (new lesion: a, b, c)

Additional notes: tumor markers

Name of tumor marker	Before treatment	Lowest level within 3 months Time point ( )	6 months (only for radiotherapy)
AFP			
AFP-L3 fraction			
PIVKA-II (DCP)			

*1: Refer to Table 1. *2: Refer to Table 2.

Endoscopic findings of intestinal Behçet's disease complicated with toxic megacolon

A 73-year-old woman was admitted to our hospital because of diarrhea and inveterate oral ulcers. Upon admission, her body temperature was 38.2 °C. The abdominal examination revealed mild tenderness without guarding. Initial blood tests revealed a white blood cell count of 10 500/μL, hemoglobin level of 7.8 g/dL, C-reactive protein level of 21.7 mg/dL, and positivity for HLA-B52. The day after admission, massive bloody stools appeared. Colonoscopy of the descending colon revealed punched-out ulcers scattered throughout the rectum up to the descending colon (**Fig. 1 a**). While genital ulcers appeared after admission, skin lesions and eye inflammation were not detected. The patient was consequently diagnosed as having intestinal Behçet's disease. She was treated with mesalazine (3 g/day) but her condition worsened gradually, and therefore intravenous prednisolone pulse therapy (1000 mg/day) was performed. However, bloody stools continued to appear. Second-look colonoscopy detected deep longitudinal ulcers in the transverse colon that exposed the muscular layer (**Fig. 1 b**). The patient was treated with infliximab (5 mg/kg) but complained 3 days later of severe abdominal pain and showed guarding in the left lower abdominal area. Computed tomography revealed a dilated transverse colon and perforation of the sigmoid colon with free air (**Fig. 1 c, d**). Consequently, the patient underwent surgery (**Fig. 2**), but unfortunately she died of disseminated intravascular coagulation after surgery.

In Behçet's disease, while involvement of the gastrointestinal tract is relatively uncommon [1], the rate of perforation is relatively high [2,3]. However, complication of Behçet's disease with a toxic megacolon is extremely rare [4,5]. Nevertheless, our case indicates that we should pay attention to patients with extensive deep ulcers. While toxic megacolon occurs in ulcerative colitis, it is not a complication peculiar to ulcerative colitis as it can occur in any case of an ulcerative lesion with inflammation that penetrates the entire colonic wall, such as can occur in intestinal Behçet's disease.

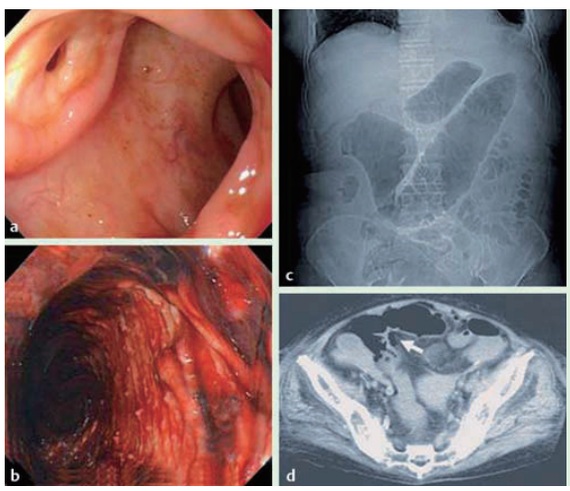


Fig. 1 **a, b** Endoscopic findings. **a** Punched-out ulcer in the descending colon. **b** Extensive deep longitudinal ulcers in the transverse colon that exposed the muscular layer. **c, d** Computed tomography. **c** Dilated transverse colon, similar to that seen in ulcerative colitis complicated with a toxic megacolon. **d** Perforation of the sigmoid colon with free air (arrow).

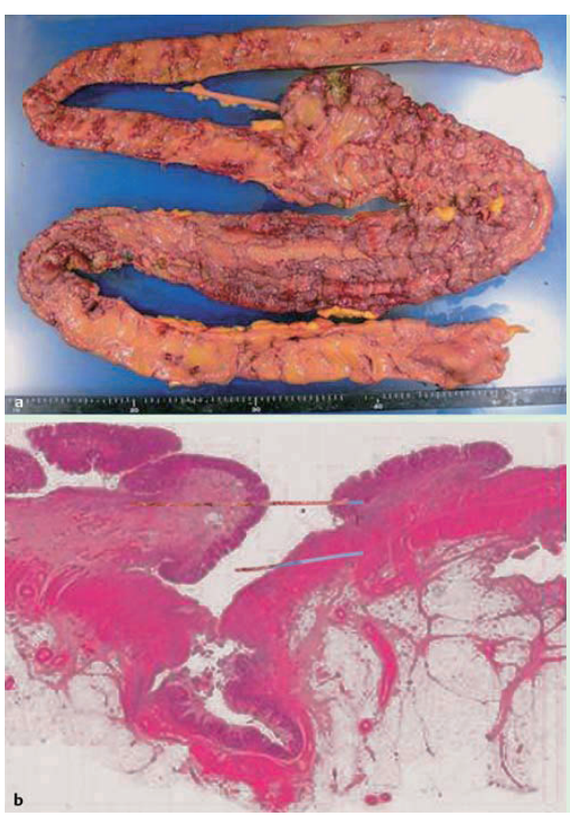


Fig. 2 **a, b** Surgical specimen (subtotal colectomy and ileectomy). **a** Multiple deep ulcers in the entire colon and ileum. **b** Deep ulcer and severe infiltration of inflammation cells in the entire wall. Moreover, vasculitis and fibrinoid necrosis of the vascular wall were observed that were consistent with intestinal Behçet's disease.

Endoscopy_UCTN_Code_CCL_1AD_2AD

Y. Umehara, M. Kudo, M. Kawasaki
Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, Osaka-Sayama, Japan

References

1 *Wallace GR, Verity DH, Delamaine LJ et al.* MIC-A allele profiles and HLA class I associations in Behçet's disease. Immunogenetics 1999; 49: 613 – 617
2 *Kasahara Y, Tanaka S, Nishino M et al.* Intestinal involvement in Behçet's disease: review of 136 surgical cases in the Japanese literature. Dis Colon Rectum 1981; 24: 103 – 106
3 *Lee CR, Kim WH, Cho YS et al.* Colonoscopic findings in intestinal Behçet's disease. Inflamm Bowel Dis 2001; 7: 243 – 249
4 *Adorian C, Khoury G, Tawil A et al.* Behçet's disease complicated by toxic megacolon. Dig Dis Sci 2003; 48: 2366 – 2368
5 *Roenspies U, Saegesser F.* Behcet's disease and toxic megacolon. Schweiz Med Wochenschr 1975; 105: 99 – 204

Bibliography
DOI 10.1055/s-0028-1103446
Endoscopy 2010; 42: E173 – E174

ISSN 0013-726X

Corresponding author
M. Kudo, MD, PhD
Division of Gastroenterology and Hepatology
Department of Internal Medicine
Kinki University School of Medicine
377-2 Ohno-Higashi
Osaka-Sayama 589-8511
Japan
Fax: +81-723-672880
m-kudo@med.kindai.ac.jp

World Journal of Radiology

Online Submissions: http://www.wjgnet.com/1949-8470office
wjr@wjgnet.com
doi:10.4329/wjr.v2.i7.249

World J Radiol 2010 July 28; 2(7): 249-256
ISSN 1949-8470 (online)

EDITORIAL

Hepatic malignancies: Correlation between sonographic findings and pathological features

Yasunori Minami, Masatoshi Kudo

Yasunori Minami, Masatoshi Kudo, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama 589-8511, Japan

Author contributions: Minami Y wrote the manuscript; Kudo M revised the article for important intellectual content.

Correspondence to: Masatoshi Kudo, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama 589-8511, Japan. m-kudo@med.kindai.ac.jp

Telephone: +81-72-3660221 Fax: +81-72-3672880

Received: March 30, 2010 Revised: May 27, 2010

Accepted: June 3, 2010

Published online: July 28, 2010

Abstract

Ultrasonography (US) findings are inevitably based on pathological features. Knowledge of the pathological features of hepatic malignancies such as hepatocellular carcinoma (HCC), liver metastasis and intrahepatic cholangiocarcinoma is essential for correct US diagnosis and appropriate management. One type of hepatocarcinogenesis is step-wise development from a low-grade dysplastic nodule (DN), high-grade DN, high-grade DN with malignant foci, and well-differentiated HCC, to classical HCC. The intranodular blood supply changes in accordance with this progression. Moreover, the malignant potential tends to change as the macroscopic configuration progresses. Therefore, typical US findings of advanced HCC are a mosaic pattern, septum formation, peripheral sonolucency (halo), lateral shadow produced by fibrotic pseudocapsule, posterior echo enhancement, arterial hypervascularity with dilated intratumoral blood sinusoids, and perinodular daughter nodule formation. Bull's eye appearance is a common presentation of metastases from gastrointestinal (GI) adenocarcinomas, and represents histological findings that show an area of central necrosis surrounded by a zonal area of viable tumor. Thick zonal area reflects the layer of viable cells that are fed by minute tumor vessels. US imaging features of liver metastases from the GI tract are as follows: Bull's eye appearance, multiple masses, irregular tumor border, arterial rim-like enhancement, and hypoenhancement in the late vascular phase. Most intrahepatic cholangiocarcinomas are ductal adenocarcinomas. The bile ducts peripheral to the tumor are usually dilated because of obstruction by tumors. US imaging features of mass-forming cholangiocarcinoma are as follows: peripheral bile duct dilatation, irregular tumor border, arterial enhancement due to minute intratumoral blood sinusoids, and hypoenhancement in the late vascular phase.

Key words: Hepatocellular carcinoma; Liver metastasis; Intrahepatic cholangiocarcinoma; Sonography; Pathology

Peer reviewer: Hui-Xiong Xu, MD, PhD, Professor, Department of Medical Ultrasonics, Institute of Diagnostic and Interventional Ultrasound, Sun Yat-Sen University, 58 Zhongshan Road 2, Guangzhou 510080, Guangdong Province, China

Minami Y, Kudo M. Hepatic malignancies: Correlation between sonographic findings and pathological features. *World J Radiol* 2010; 2(7): 249-256 Available from: URL: http://www.wjgnet.com/1949-8470/full/v2/i7/249.htm DOI: http://dx.doi.org/10.4329/wjr.v2.i7.249

INTRODUCTION

Recent advances in digital technologies have resulted in remarkable developments in the field of imaging modalities[1]. Ultrasonography (US) is one of the diagnostic tools that have shown significant improvement within the last decade[2-7]. For the diagnosis of liver tumors, US examination has the advantages of real-time observation, simple technique and non-invasiveness[8-13]. This modality is being used worldwide with high frequency as a reli-

able method for the initial diagnosis of liver tumors[14-19]. Color Doppler and power Doppler have increased the sensitivity for hepatic lesion detection compared to that of gray-scale US[20-25]. Furthermore, the application of microbubble contrast agents provides details of the hemodynamics, which are useful for the detection and characterization of liver tumors[26-31].

Even if advances in imaging technology increase further, US findings are inevitably based on the pathological features. Therefore, knowledge of disease conditions and pathological features is essential to comprehend the findings on US images.

This paper reviews the diagnosis of hepatic malignancies contrasted between US images and pathological features.

HEPATOCELLULAR CARCINOMA

Disease conditions and pathological features

Hepatocellular nodules associated with liver cirrhosis are divided into six categories according to the classification proposed by the International Working Party of World Congress of Gastroenterology in 1994: namely, large regenerative nodule, low-grade dysplastic nodule (DN), high-grade DN, high-grade DN with malignant foci, well-differentiated hepatocellular carcinoma (HCC), and HCC with Edmondson Ⅱ or higher, which is called classical HCC[32-36]. Among these nodules, two models of hepatocarcinogenesis are now hypothesized. One is *de novo* carcinogenesis, and the other is stepwise development from low-grade DN, high-grade DN, high-grade DN with malignant foci, and well-differentiated HCC, to classical HCC. The intranodular blood supply changes with the progression of human hepatocarcinogenesis from DN to overt HCC[32-36] (Figure 1). The portal tracts, including the portal vein and hepatic artery, decrease with the increasing grade of malignancy and are virtually absent in nodules. In contrast, abnormal arteries due to tumor angiogenesis develop in atypical adenomatous hyperplasia (high-grade DN) during the course of hepatocarcinogenesis, and are markedly increased in number in moderately differentiated HCCs. The intranodular vasculature changes in a stepwise manner as the grade of malignancy increases[32-36]. In the course of hepatocarcinogenesis, arterial and portal supply decreases (due to a decrease in the portal tracts), and arterial supply returns to a level equivalent (due to newly formed abnormal arteries) to the surrounding liver, while portal supply continues to decrease, and finally portal supply vanishes and only arterial blood (from newly formed abnormal arteries) supplies the lesion (classical HCC). Therefore, high-grade DN is usually hypodense relative to the surrounding liver. However, early-stage well-differentiated HCC often shows isodensity because the increased abnormal arterial supply compensates for the decreased normal hepatic arterial supply[34-36].

Macroscopic configurations of HCC

In the classification proposed by the Liver Cancer Study Group of Japan, macroscopic configurations of HCC are divided into five types, namely the small nodular type with indistinct margins, simple nodular type, simple nodular type with extranodular growth, confluent multinodular type, and infiltrative type[37,38] (Figure 2). The malignant potentials tend to change in accordance with the progression of macroscopic configurations[38].

In the small nodular type with indistinct margins, approximately 85% of nodules consist of well-differentiated cancerous tissue with a replacing growth at the boundary, and many portal tracts are retained in the tumor with immature fibrous capsule formation. When such tumors reach 1.5-2.0 cm in diameter, moderately or poorly differentiated cancer tissues develop within the well-differentiated cancer tissue. When less differentiated cancerous tissues within the well-differentiated cancer nodules proliferate in an expansive fashion, a "nodule-in-nodule" appearance is frequently seen.

The simple nodular type exhibits a round or nearly round shape, and demonstrates a clear boundary with non-cancerous tissue, and it often has an obvious capsule. Some tumors appear intersected by thin fibrous septa, but these tumors are round, and have the appearance of a single expanding nodule. Moderately differentiated cancerous tissue accounts for approximately 75% of the simple nodular type of HCC, and approximately 20% show portal invasion on histology.

In the simple nodular type with extranodular growth,

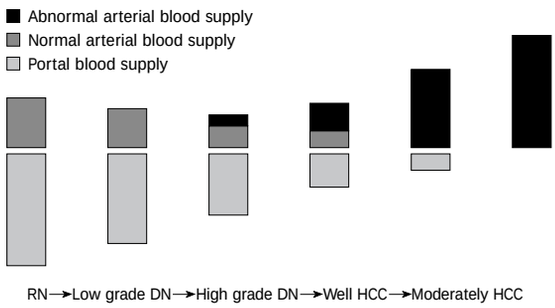


Figure 1 Changes in intranodular blood supply with the progression of hepatocarcinogenesis from dysplastic nodule to hepatocellular carcinoma. RN: Regenerative nodule; DN: Dysplastic nodule; HCC: Hepatocellular carcinoma.

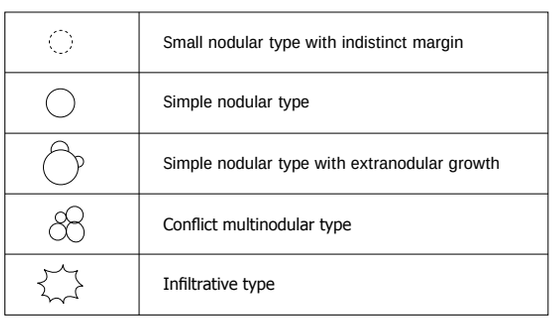


Figure 2 Macroscopic configurations of hepatocellular carcinoma.

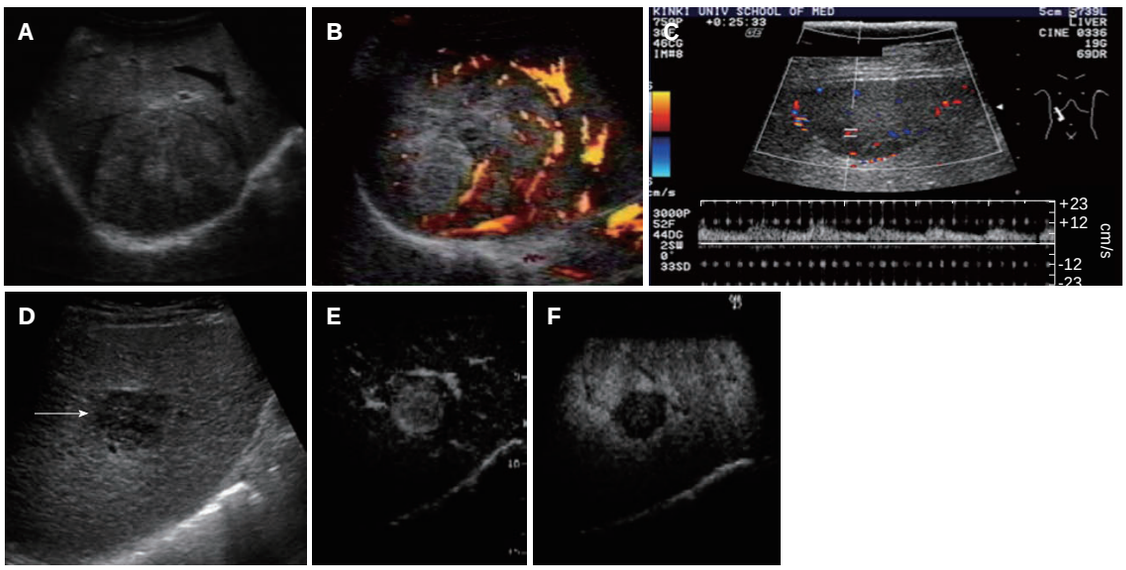


Figure 3 Advanced hepatocellular carcinoma. A: The nodule had a halo image and mosaic pattern in segment 8 of the liver on B-mode ultrasonography; B: Power Doppler imaging showed hypervascularity of the tumor; C: Color Doppler imaging showed intratumoral blood flow. Arterial pulsatile waveforms could be detected by pulsed Doppler images; D: The image of a simple nodular type with extranodular growth (arrow) was obtained on B-mode ultrasonography (US); E: Contrast harmonic US showed enhancement of hepatocellular carcinoma in early vascular phase after administration of perfluorocarbon microbubbles; F: Contrast harmonic US depicted the defect image in the post-vascular phase.

well-differentiated histological characteristics are present in only 12.5% of cases. The simple nodular type of HCC with extranodular growth is well developed in contrast to the delicate fibrous septa that are occasionally present in the small nodular type with indistinct margins. Most of the simple nodular type with extranodular growth demonstrates moderately differentiated HCC.

Confluent multinodular type tumor is formed by several small contiguous tumor nodules. In confluent multinodular type tumors, the margin of the whole tumor appears rugged because of the projection of each nodule. In addition, the internodular fibrous tissue of confluent multinodular type tumors is well developed. This unique appearance probably reflects the extension of tumor by growth replacement from one pseudolobule into its neighbors. Most of the confluent multinodular type is classified as moderately to poorly differentiated HCC.

The infiltrative type of tumor is classified as the poorly demarcated nodular type. The most important reason for classifying this tumor separately is that grossly the entire border is obscure. Histologically, the entire boundary between cancerous and non-cancerous parenchyma is composed of small nests of cancer cells that infiltrate the interlobular septa, similar to the infiltrative pattern of adenocarcinoma. Most infiltrative type tumor is diagnosed in poorly differentiated HCC or mixed HCC/cholangiocarcinoma.

US imaging of advanced HCC

Typical US findings of advanced HCC are a mosaic pattern, septum formation, peripheral sonolucency (halo), lateral shadow produced by fibrotic pseudocapsule, posterior echo enhancement, arterial hypervascularity with dilated intratumoral blood sinusoids, and perinodular daughter nodule formation (Figure 3).

Internal mosaic architecture and capsule formation are major macroscopic features of typical moderately differential HCCs[39-41]. The halo sign and lateral shadows correspond to the thin fibrous capsule of the HCC. Correspondence between sonographic halo sign and histological capsule has been reported as 90.1%, and that between the presence of extracapsular invasion on US and that on histology as 88.0%. The presence of extracapsular invasion on US is a predisposing factor for the development of tumor recurrence.

Posterior echo enhancement is due to the softness of the HCC[42]. However, posterior echo enhancement is not specific for HCC, as this finding is also observed with similarly frequency in hemangiomas.

The spread of HCC along the portal vein results in daughter nodule formation. In HCC, the hepatic artery is the feeding vessel and the portal vein serves mainly as an efferent vessel. Tumor cells invade efferent vessels by budding, extension to the vascular cavity, and then extending beyond the capsule to the portal vein branches. In more advanced cases of HCC, portal tumor thrombi, biliary invasion, and hepatic vein invasion are also observed, which strongly indicates a diagnosis of HCC.

A basket pattern on color Doppler images and/or power Doppler images has been described; this pattern represents a fine network of arterial vessels that surrounds the tumor nodules[43,44]. Typical color Doppler findings in advanced HCC are afferent pulsatile waveform signals, intratumoral pulsatile waveform signals associated with intratumoral

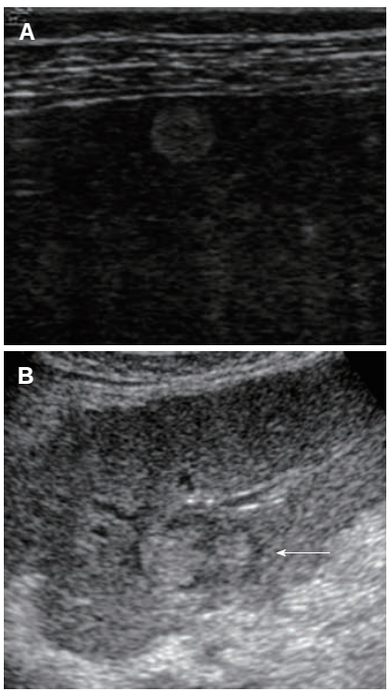


Figure 4 Early hepatocellular carcinoma. A: A nodule that was 1.5 cm in diameter in segment 5 of the liver was shown as highly echoic because of fatty changes in the nodule; B: A nodule-in-nodule appearance (arrow) was demonstrated as a hyperechoic tumor within a hypoechoic nodule.

continuous waveform signals, and efferent continuous waveform signals. Of the several parameters that can be obtained with Doppler spectral analysis, maximum flow velocity and pulsatility index (PI) are very important in the differential diagnosis of hepatic tumors. The PI in HCC is much higher than that in hemangioma.

Hepatic carcinogenesis is described as a multi-step process in which progressive arterialization and gradual loss of portal vessels are the principal features[45,46]. It is evident that vascular enhancement is related to the evolution of the lesion. Thus, during the arterial phase, DNs or early HCC usually appear hypo or isovascularized, while advanced HCCs are hypervascularized. Contrast-enhanced US can show selective enhancement in the arterial phase, which differentiates HCCs from regenerative nodules and DNs[4-6].

US imaging of early HCC

The imaging features of early HCC (highly well-differentiated HCC) are as follows: bright loop appearance, arterial hypovascularity and internal portal tracts or portal blood supply. Hypervascular foci in the nodule occasionally demonstrate a nodule-in-nodule appearance (Figure 4).

Bright loop appearance is defined as hypoechoic nodules in a hyperechoic tumor[47]. A nodule-in-nodule appearance might also appear as a hyperechoic tumor within a hypoechoic nodule[25,46]. Hyperechoic HCC nodules represent well-differentiated HCC with fatty changes, whereas an inner hypoechoic lesion represents moderately differentiated HCC without fatty changes. On US screening for HCC, these appearances are often observed in HCC nodules that measure 11-20 mm in diameter. Histological examination has demonstrated that bright loop appearance and nodule-in-nodule appearance of HCC on US are associated with tumor progression and dedifferentiation to moderately differentiated HCC within well-differentiated HCC with fatty changes.

Typical findings of early HCC are afferent continuous waveform signals, which reflect a feeding portal flow, which is rarely associated with pulsatile wave-form signals[48]. Thus, with afferent blood flow on Doppler US imaging, constant waveform signals that reflect portal inflow are a characteristic finding in DNs and early well-differentiated HCC.

Half of the well-differentiated HCCs wash out slowly during the late phase on contrast-enhanced US and the average washout time was significantly different from that of moderately to poorly differentiated HCCs[17,21,23,46]. Microbubbles continuously infusing the tumor through the portal vein could be the pathological basis of slow washout. Furthermore, well-differentiated HCCs consist of a trabecular pattern of cell cords and abundant sinusoids that may cause stagnation and slow clearance of microbubbles. Conversely, less differentiated HCCs contain fewer sinusoids and are mainly fed by the hepatic artery, which could cause the difference in washout time compared with that of well-differentiated HCCs.

LIVER METASTASIS

Disease conditions and pathological features

The liver is the organ second most commonly affected by metastatic disease. The most common primary sites are the gastrointestinal (GI) tract, lung, breast and head and neck[49-52]. Therefore, liver metastases vary in size, shape, vascularity, and growth pattern. However, most liver metastases are multiple and show the so-called "cluster sign". In 77% of patients with liver metastases, both lobes are involved, whereas metastasis is solitary in only 10% of cases[53]. Most metastatic tumors of the liver are expansive or infiltrative. Although most metastases are generally hypovascular, metastases often show the same degree of vascularity as the primary tumor[54]. Calcification can be seen in metastases from colon, stomach, breast, and other organs. Fundamentally, liver metastases occur in patients without liver cirrhosis[54].

In metastases from the GI tract, intratumoral fibrous septum and capsule formation are macroscopically rare. Hypervascular metastases are uncommon, however, arterial vascularity of metastases develops finely near the border. Large metastases often outgrow their blood supply and subsegment hypoxia causes a necrotic region at the center of the tumor[54].

In addition, metastatic carcinoma from the breast or pancreas induces an intense fibrous or sclerosing reaction around the tumor, which leads to fibrous scar formation[55]. In 7%-15% of patients, tumor thrombi occlude

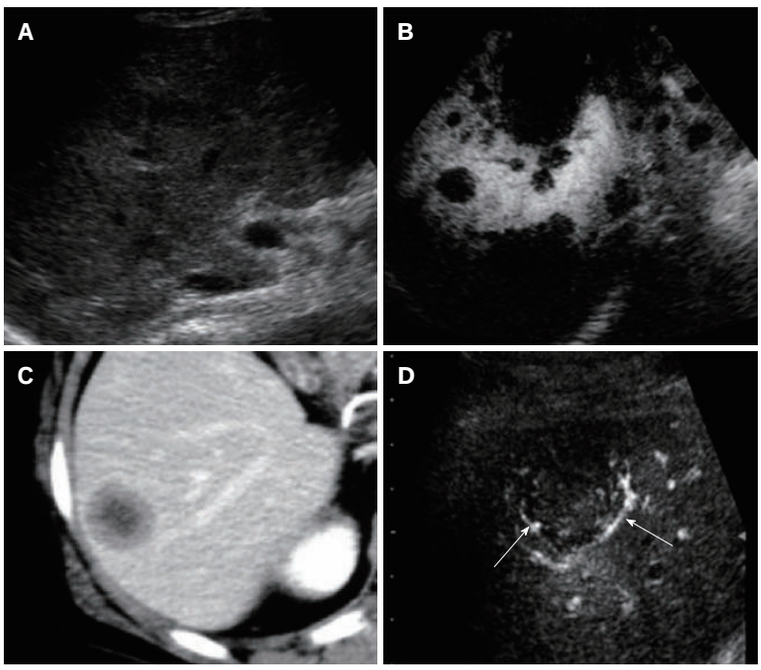


Figure 5 Liver metastasis. A: Multiple masses were seen in the liver by B-mode ultrasonography (US); B: Multiple defects were seen by Sonazoid-enhanced US in the post-vascular phase; C: Portal phase dynamic scan detected a hypoenhanced nodule in segment 6 of the liver; D: Peripheral enhancement of the nodule (arrows) was obtained by Sonazoid-enhanced ultrasonography in the early vascular phase.

the portal vein, the hepatic vein, or both. In the presence of mucin secretion, necrosis, and phosphate activity, metastases can develop calcification that is detectable radiographically.

US imaging

US imaging features of liver metastases from the GI tract are as follows: Bull's eye appearance, multiple masses, absence of liver cirrhosis, irregular tumor border, arterial rim-like enhancement, and hypoenhancement in the late vascular phase (Figure 5).

Bull's eye or target lesion is a common presentation of metastases from the GI tract[56]. Sonography also shows multiple round and/or hypoechoic masses with irregular borders[57]. A Bull's eye appearance represents histological findings of an area that shows central coagulative necrosis surrounded by a zonal area of viable tumor. The surrounding zonal area appears thick, and reflects a layer of viable cells. Calcified metastases might demonstrate shadows when they are densely echogenic. Then, colon cancer is the most likely cause in a patient with unknown primary tumor when calcified liver metastases are demonstrated by US.

Color or power Doppler US can show intratumoral vascularity in the peripheral hypoechoic zone, in which viable tumor cells are proliferating[58]. Actually, these signals appear to be poor because the density of tumor vasculature is lower than that of moderately differentiated HCC. However, in patients with metastasis from renal cell carcinoma or sarcoma, intratumoral flow can be demonstrated because of its hypervascularity.

Contrast-enhanced US of the liver with SonoVue provides a significantly higher sensitivity in the detection of liver metastases compared to that of unenhanced sonography, and identifies up to 40% more metastases[59-61]. It has been reported that the presence of rim-like enhancement with peripheral tumor vessels (sensitivity, 88.1%; specificity, 100%) is the typical pattern[62]. Contrast-enhanced US in the late phase provides a marked improvement in the detection of hepatic metastases as areas of hypoenhancement, and can be advantageous in detecting small metastases compared with computed tomography and magnetic resonance imaging[63-65].

INTRAHEPATIC CHOLANGIOCARCINOMA

Disease conditions and pathological features

Intrahepatic cholangiocarcinoma is a slow-growing ductal adenocarcinoma in the liver; it is relatively rare and comprises 3%-7% of malignant liver tumors[66-69]. The bile ducts are dilated because of obstruction by tumors[70,71]. Intrahepatic cholangiocarcinoma, unlike HCC, is not usually related to liver cirrhosis. However, hepatitis C virus infection has also been reported to be a risk factor for cholangiocarcinoma[72]. The Liver Cancer Study Group of Japan has proposed a classification of intrahepatic cholangiocarcinoma based on macroscopic features: mass-forming, periductal infiltrating, and intraductal, or mixed mass-forming, and periductal infiltrating[73,74]. More than half of intrahepatic cholangiocarcinomas are classified as the mass-forming type.

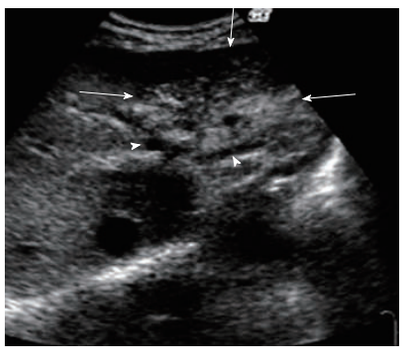


Figure 6 Intrahepatic cholangiocarcinoma (mass-forming type). B-mode ultrasonography showed mixed echogenicity with irregular borders (arrows) in the left lateral lobe. The intrahepatic bile duct peripheral to the tumor was dilated (arrowheads).

A mass-forming intrahepatic cholangiocarcinoma is usually large. On gross specimens, the tumor is firm and whitish gray because of its large amount of fibrous stroma[75]. The margin is well circumscribed and wavy or lobulated. Central necrosis might be present. Multicentricity, especially around the main tumor, is common, probably because of the propensity of the tumor to invade the adjacent peripheral branches of the portal vein[75-77]. It easily spreads to the lymph nodes. Most of the mass-forming intrahepatic cholangiocarcinomas are poorly or moderately differentiated[75].

US imaging

US imaging features of mass-forming cholangiocarcinoma are as follows: peripheral bile duct dilatation, absence of liver cirrhosis, irregular tumor border, arterial enhancement due to minute intratumoral blood sinusoids, and hypoenhancement in the late vascular phase (Figure 6).

The bile ducts peripheral to the tumor are usually dilated because of obstruction by the tumor, however, the US findings of intrahepatic cholangiocarcinoma are fundamentally very similar to those described in liver metastases[71]. Mass-forming cholangiocarcinomas can be hypoechoic, hyperechoic, or have mixed echogenicity, with irregular borders. Peripheral cholangiocarcinoma can appear as a solitary mass or as diffusely abnormal liver echotexture. Because of their nonspecific symptomatology, mass-forming lesions are generally far advanced when detected by US. In addition, mass-forming lesions might mimic HCC or metastases on B-mode US[70].

Color Doppler US typically shows a poor color signal in cholangiocarcinoma compared with HCC, which is hypervascular. Hence, color Doppler US is helpful in differentiating vessels from dilated ducts and can provide information regarding the status of vessels. It is considered highly accurate in detecting neoplastic involvement of the portal vein. In the study by Neumaier *et al*[78], the sensitivity of color Doppler US for portal vein occlusion was 100% and that for portal vein infiltration was 83%, with 100% specificity. However, there was poor sensitivity in detecting infiltration of the hepatic artery (43%) and metastases to regional lymph nodes (37%), liver (66%), and peritoneum (33%)[78].

Although the imaging findings of peripheral cholangiocarcinoma showed certain characteristics on low-mechanical index (MI) contrast-enhanced sonography, contrast-enhanced US findings are fundamentally similar to those described for liver metastases. It has been reported[79] that all peripheral cholangiocarcinomas show inhomogeneous enhancement during the arterial phase, such as irregular, peripheral, rim-like hyperenhancement (44.4%), inhomogeneous hyperenhancement (11.1%), or inhomogeneous hypoenhancement (44.4%). However, all cholangiocarcinomas show hypoenhancement in the late vascular phase.

CONCLUSION

Worldwide, US imaging plays an important role not only in screening, evaluating, staging and monitoring disease, but also in surveillance following tumor ablation. Advances in imaging techniques have increased our ability to detect and characterize focal liver lesions. The gross appearances of hepatic malignancies correlate with the pathological and US imaging findings, therefore, the macroscopic types can be a significant independent prognostic factor. Knowledge of the pathological features of liver tumors is essential for correct US diagnosis and appropriate management. Some pathological images can enhance our understanding of liver tumors.

REFERENCES

1 **Maruyama K**, Yoshikawa M, Yokosuka O. Contrast-enhanced ultrasonography: a recent application for the diagnosis and treatment of hepatocellular carcinoma. *JNMA J Nepal Med Assoc* 2008; **47**: 156-166

2 **Kudo M**. Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. *Semin Liver Dis* 1999; **19**: 297-309

3 **Kudo M**. Contrast harmonic ultrasound is a breakthrough technology in the diagnosis and treatment of hepatocellular carcinoma. *J Med Ultrason* 2001; **28**: 79-81

4 **Ding H**, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K. Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual-display mode. *Radiology* 2001; **220**: 349-356

5 **Ding H**, Kudo M, Maekawa K, Suetomi Y, Minami Y, Onda H. Detection of tumor parenchymal blood flow in hepatic tumors: value of second harmonic imaging with a galactose-based contrast agent. *Hepatol Res* 2001; **21**: 242-251

6 **Ding H**, Kudo M, Onda H, Suetomi Y, Minami Y, Chung H, Kawasaki T, Maekawa K. Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. *Radiology* 2001; **221**: 721-730

7 **Minami Y**, Kudo M, Kawasaki T, Kitano M, Chung H, Maekawa K, Shiozaki H. Transcatheter arterial chemoembolization of hepatocellular carcinoma: usefulness of coded phase-inversion harmonic sonography. *AJR Am J Roentgenol* 2003; **180**: 703-708

8 **Wen YL**, Kudo M, Zheng RQ, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. Radiofrequency ablation of hepatocellular carcinoma: therapeutic response using contrast-enhanced coded phase-inversion harmonic sonography. *AJR Am J Roentgenol* 2003; **181**: 57-63

9 **Wen YL**, Kudo M, Maekawa K, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T. Contrast advanced dynamic flow imaging and contrast pulse subtraction imaging: Preliminary results in hepatic tumors. *J Med Ultrason* 2002; **29**: 195-204

10 **Wen YL**, Kudo M, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. Contrast-enhanced agent detection imaging: Early experience in hepatocellular carcinoma. *J Med Ultrason* 2003; **30**: 77-84

11 **Wen YL**, Kudo M, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. Value of new contrast harmonic technique for detecting tumor vascularity in hepatocellular carcinoma: Preliminary results. *J Med Ultrason* 2003; **30**: 85-92

12 **Wen YL**, Kudo M, Minami Y, Chung H, Suetomi Y, Onda H, Kitano M, Kawasaki T, Maekawa K. Detection of tumor vascularity in hepatocellular carcinoma with contrast-enhanced Dynamic Flow imaging: Comparison with contrast-enhanced power Doppler imaging. *J Med Ultrason* 2003; **30**: 141-151

13 **Wang WP**, Ding H, Qi Q, Mao F, Xu ZZ, Kudo M. Characterization of focal hepatic lesions with contrast-enhanced C-cube gray scale ultrasonography. *World J Gastroenterol* 2003; **9**: 1667-1674

14 **Wen YL**, Kudo M, Zheng RQ, Ding H, Zhou P, Minami Y, Chung H, Kitano M, Kawasaki T, Maekawa K. Characterization of hepatic tumors: value of contrast-enhanced coded phase-inversion harmonic angio. *AJR Am J Roentgenol* 2004; **182**: 1019-1026

15 **Wen YL**, Zhou P, Kudo M. Detection of intratumoral vascularity in small hepatocellular carcinoma by coded phase inversion harmonics. *Intervirology* 2004; **47**: 169-178

16 **Zheng RQ**, Zhou P, Kudo M. Hepatocellular carcinoma with nodule-in-nodule appearance: demonstration by contrast-enhanced coded phase inversion harmonic imaging. *Intervirology* 2004; **47**: 184-190

17 **Kudo M**. Early detection and curative treatment of early-stage hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2005; **3**: S144-S148

18 **Zheng RQ**, Kudo M. Hepatic angiomyolipoma: identification of an efferent vessel to be hepatic vein by contrast-enhanced harmonic ultrasound. *Br J Radiol* 2005; **78**: 956-960

19 **Zheng RQ**, Zhang B, Kudo M, Sakaguchi Y. Hemodynamic and morphologic changes of peripheral hepatic vasculature in cirrhotic liver disease: a preliminary study using contrast-enhanced coded phase inversion harmonic ultrasonography. *World J Gastroenterol* 2005; **11**: 6348-6353

20 **Inoue T**, Kitano M, Kudo M, Sakamoto H, Kawasaki T, Yasuda C, Maekawa K. Diagnosis of gallbladder diseases by contrast-enhanced phase-inversion harmonic ultrasonography. *Ultrasound Med Biol* 2007; **33**: 353-361

21 **Kudo M**. New sonographic techniques for the diagnosis and treatment of hepatocellular carcinoma. *Hepatol Res* 2007; **37** Suppl 2: S193-S199

22 **Zhou P**, Kudo M, Minami Y, Chung H, Inoue T, Fukunaga T, Maekawa K. What is the best time to evaluate treatment response after radiofrequency ablation of hepatocellular carcinoma using contrast-enhanced sonography? *Oncology* 2007; **72** Suppl 1: 92-97

23 **Kudo M**, Hatanaka K, Maekawa K. Sonazoid-enhanced ultrasound in the diagnosis and treatment of hepatic tumors. *J Med Ultrasound* 2008; **16**: 130-139

24 **Kudo M**, Hatanaka K, Maekawa K. Defect reperfusion imaging, a newly developed novel technology using Sonazoid in the treatment of hepatocellular carcinoma. *J Med Ultrasound* 2008; **16**: 169-176

25 **Inoue T**, Kudo M, Hatanaka K, Takahashi S, Kitai S, Ueda T, Ishikawa E, Hagiwara S, Minami Y, Chung H, Ueshima K, Maekawa K. Imaging of hepatocellular carcinoma: qualitative and quantitative analysis of postvascular phase contrast-enhanced ultrasonography with sonazoid. Comparison with superparamagnetic iron oxide magnetic resonance images. *Oncology* 2008; **75** Suppl 1: 48-54

26 **Xia Y**, Kudo M, Minami Y, Hatanaka K, Ueshima K, Chung H, Hagiwara S, Inoue T, Ishikawa E, Kitai S, Takahashi S, Tatsumi C, Ueda T, Hayaishi S, Maekawa K. Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: the usefulness of sonazoid-enhanced harmonic sonography. *Oncology* 2008; **75** Suppl 1: 99-105

27 **Kudo M**, Minami Y. Radiofrequency ablation therapy under harmonic imaging guidance for the recurring cancer after local therapy for HCC: a randomized controlled study with RFA under B-mode guidance. *Ultrasound Med Biol* 2003; **29**: S145

28 **Minami Y**, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H. Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. *AJR Am J Roentgenol* 2004; **183**: 153-156

29 **Minami Y**, Kudo M, Chung H, Kawasaki T, Yagyu Y, Shimono T, Shiozaki H. Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR Am J Roentgenol* 2007; **188**: 489-494

30 **Minami Y**, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H. Percutaneous radiofrequency ablation guided by contrast-enhanced harmonic sonography with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. *AJR Am J Roentgenol* 2004; **182**: 1224-1226

31 **Kono Y**, Lucidarme O, Choi SH, Rose SC, Hassanein TI, Alpert E, Mattrey RF. Contrast-enhanced ultrasound as a predictor of treatment efficacy within 2 weeks after transarterial chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2007; **18**: 57-65

32 **Kanai T**, Hirohashi S, Upton MP, Noguchi M, Kishi K, Makuuchi M, Yamasaki S, Hasegawa H, Takayasu K, Moriyama N. Pathology of small hepatocellular carcinoma. A proposal for a new gross classification. *Cancer* 1987; **60**: 810-819

33 **Nakashima O**, Kojiro M. Recurrence of hepatocellular carcinoma: multicentric occurrence or intrahepatic metastasis? A viewpoint in terms of pathology. *J Hepatobiliary Pancreat Surg* 2001; **8**: 404-409

34 **Kojiro M**. 'Nodule-in-nodule' appearance in hepatocellular carcinoma: its significance as a morphologic marker of dedifferentiation. *Intervirology* 2004; **47**: 179-183

35 **Kojiro M**. Diagnostic discrepancy of early hepatocellular carcinoma between Japan and West. *Hepatol Res* 2007; **37** Suppl 2: S121-S124

36 **Kojiro M**, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. *Semin Liver Dis* 2005; **25**: 133-142

37 **Shimada M**, Rikimaru T, Hamatsu T, Yamashita Y, Terashi T, Taguchi K, Tanaka S, Shirabe K, Sugimachi K. The role of macroscopic classification in nodular-type hepatocellular carcinoma. *Am J Surg* 2001; **182**: 177-182

38 **Hui AM**, Takayama T, Sano K, Kubota K, Akahane M, Ohtomo K, Makuuchi M. Predictive value of gross classification of hepatocellular carcinoma on recurrence and survival after hepatectomy. *J Hepatol* 2000; **33**: 975-979

39 **Makuuchi M**, Hasegawa H, Yamazaki S, Bandai Y, Watanabe G, Ito T. Ultrasonic characteristics of the small hepatocellular carcinoma. *Ultrasound Med Biol* 1983; **Suppl 2**: 489-491

40 **Matsui O**. Detection and characterization of small hepatocellular carcinoma. *J Gastroenterol Hepatol* 2004; **19**: S266-S269

41 **Shibata T**, Sakahara H, Kawakami S, Konishi J. Sonographic characteristics of recurrent hepatocellular carcinoma. *Eur Radiol* 1996; **6**: 443-447

42 **Choi BI**, Kim CW, Han MC, Kim CY, Lee HS, Kim ST, Kim YI. Sonographic characteristics of small hepatocellular carcinoma. *Gastrointest Radiol* 1989; **14**: 255-261

43 **Tochio H**, Kudo M. Afferent and efferent vessels of premalignant and overt hepatocellular carcinoma: observation by color Doppler imaging. *Intervirology* 2004; **47**: 144-153

44 **Kudo M**, Tochio H. Intranodular blood supply correlates well with biological malignancy grade determined by tumor growth rate in pathologically proven hepatocellular carci-

noma. *Oncology* 2008; **75** Suppl 1: 55-64
45 **Tanaka S**, Kitamura T, Imaoka S, Sasaki Y, Taniguchi H, Ishiguro S. Hepatocellular carcinoma: sonographic and histologic correlation. *AJR Am J Roentgenol* 1983; **140**: 701-707
46 **Kudo M**. Multistep human hepatocarcinogenesis: correlation of imaging with pathology. *J Gastroenterol* 2009; **44** Suppl 19: 112-118
47 **Ogata R**, Majima Y, Tateishi Y, Kuromatsu R, Shimauchi Y, Torimyra T, Tanaka M, Kumashiro R, Kojiro M, Sata M. Bright loop appearance; a characteristic ultrasonography sign of early hepatocellular carcinoma. *Oncol Rep* 2000; **7**: 1293-1298
48 **Tochio H**, Tomita S, Kudo M, Iwasaki N, Tamura S, Nakamura H, Soga T, Fukunaga T, Okabe Y, Kashida H, Hirasa M, Ibuki Y, Morimoto Y, Orino A. The efferent blood flow of early hepatocellular carcinoma and borderline lesions: Demonstration by color Doppler imaging. *J Med Ultrason* 2002; **29**: 205-209
49 **Zavadsky KE**, Lee YT. Liver metastases from colorectal carcinoma: incidence, resectability, and survival results. *Am Surg* 1994; **60**: 929-933
50 **Roukos DH**. Current advances and changes in treatment strategy may improve survival and quality of life in patients with potentially curable gastric cancer. *Ann Surg Oncol* 1999; **6**: 46-56
51 **Lazaridis G**, Pentheroudakis G, Fountzilas G, Pavlidis N. Liver metastases from cancer of unknown primary (CUPL): a retrospective analysis of presentation, management and prognosis in 49 patients and systematic review of the literature. *Cancer Treat Rev* 2008; **34**: 693-700
52 **Roach H**, Whipp E, Virjee J, Callaway MP. A pictorial review of the varied appearance of atypical liver metastasis from carcinoma of the breast. *Br J Radiol* 2005; **78**: 1098-1103
53 **Robinson PJ**. Imaging liver metastases: current limitations and future prospects. *Br J Radiol* 2000; **73**: 234-241
54 **Marchal GJ**, Pylyser K, Tshibwabwa-Tumba EA, Verbeken EK, Oyen RH, Baert AL, Lauweryns JM. Anechoic halo in solid liver tumors: sonographic, microangiographic, and histologic correlation. *Radiology* 1985; **156**: 479-483
55 **Machi J**, Isomoto H, Kurohiji T, Yamashita Y, Shirouzu K, Kakegawa T, Sigel B, Zaren HA, Sariego J. Accuracy of intraoperative ultrasonography in diagnosing liver metastasis from colorectal cancer: evaluation with postoperative follow-up results. *World J Surg* 1991; **15**: 551-556; discussion 557
56 **Choti MA**, Kaloma F, de Oliveira ML, Nour S, Garrett-Mayer ES, Sheth S, Pawlik TM. Patient variability in intraoperative ultrasonographic characteristics of colorectal liver metastases. *Arch Surg* 2008; **143**: 29-34; discussion 35
57 **Yoshida T**, Matsue H, Okazaki N, Yoshino M. Ultrasonographic differentiation of hepatocellular carcinoma from metastatic liver cancer. *J Clin Ultrasound* 1987; **15**: 431-437
58 **Choi BI**, Kim TK, Han JK, Chung JW, Park JH, Han MC. Power versus conventional color Doppler sonography: comparison in the depiction of vasculature in liver tumors. *Radiology* 1996; **200**: 55-58
59 **Konopke R**, Kersting S, Bergert H, Bloomenthal A, Gastmeier J, Saeger HD, Bunk A. Contrast-enhanced ultrasonography to detect liver metastases : a prospective trial to compare transcutaneous unenhanced and contrast-enhanced ultrasonography in patients undergoing laparotomy. *Int J Colorectal Dis* 2007; **22**: 201-207
60 **Nicolau C**, Vilana R, Catalá V, Bianchi L, Gilabert R, García A, Brú C. Importance of evaluating all vascular phases on contrast-enhanced sonography in the differentiation of benign from malignant focal liver lesions. *AJR Am J Roentgenol* 2006; **186**: 158-167
61 **Furuse J**, Nagase M, Ishii H, Yoshino M. Contrast enhancement patterns of hepatic tumours during the vascular phase using coded harmonic imaging and Levovist to differentiate hepatocellular carcinoma from other focal lesions. *Br J Radiol* 2003; **76**: 385-392
62 **Hatanaka K**, Kudo M, Minami Y, Ueda T, Tatsumi C, Kitai S, Takahashi S, Inoue T, Hagiwara S, Chung H, Ueshima K, Maekawa K. Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. *Intervirology* 2008; **51** Suppl 1: 61-69
63 **Hatanaka K**, Kudo M, Minami Y, Maekawa K. Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. *Oncology* 2008; **75** Suppl 1: 42-47
64 **Dietrich CF**, Ignee A, Trojan J, Fellbaum C, Schuessler G. Improved characterisation of histologically proven liver tumours by contrast enhanced ultrasonography during the portal venous and specific late phase of SHU 508A. *Gut* 2004; **53**: 401-405
65 **Albrecht T**, Hoffmann CW, Schmitz SA, Schettler S, Overberg A, Germer CT, Wolf KJ. Phase-inversion sonography during the liver-specific late phase of contrast enhancement: improved detection of liver metastases. *AJR Am J Roentgenol* 2001; **176**: 1191-1198
66 **Nakajima T**, Kondo Y, Miyazaki M, Okui K. A histopathologic study of 102 cases of intrahepatic cholangiocarcinoma: histologic classification and modes of spreading. *Hum Pathol* 1988; **19**: 1228-1234
67 **Clemett AR**. Carcinoma of the major bile ducts. *Radiology* 1965; **84**: 894-903
68 **Khan SA**, Davidson BR, Goldin R, Pereira SP, Rosenberg WM, Taylor-Robinson SD, Thillainayagam AV, Thomas HC, Thursz MR, Wasan H. Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. *Gut* 2002; **51** Suppl 6: VI1-VI9
69 **Yamanaka N**, Okamoto E, Ando T, Oriyama T, Fujimoto J, Furukawa K, Tanaka T, Tanaka W, Nishigami T. Clinicopathologic spectrum of resected extraductal mass-forming intrahepatic cholangiocarcinoma. *Cancer* 1995; **76**: 2449-2456
70 **Colli A**, Cocciolo M, Mumoli N, Cesarini L, Prisco A, Gaffuri I, Martinez E. Peripheral intrahepatic cholangiocarcinoma: ultrasound findings and differential diagnosis from hepatocellular carcinoma. *Eur J Ultrasound* 1998; **7**: 93-99
71 **Sainani NI**, Catalano OA, Holalkere NS, Zhu AX, Hahn PF, Sahani DV. Cholangiocarcinoma: current and novel imaging techniques. *Radiographics* 2008; **28**: 1263-1287
72 **Donato F**, Gelatti U, Tagger A, Favret M, Ribero ML, Callea F, Martelli C, Savio A, Trevisi P, Nardi G. Intrahepatic cholangiocarcinoma and hepatitis C and B virus infection, alcohol intake, and hepatolithiasis: a case-control study in Italy. *Cancer Causes Control* 2001; **12**: 959-964
73 Liver Cancer Study Group of Japan. 1st ed. Tokyo: Kanehara Shuppan, 1997
74 **Yamamoto M**, Takasaki K, Yoshikawa T, Ueno K, Nakano M. Does gross appearance indicate prognosis in intrahepatic cholangiocarcinoma? *J Surg Oncol* 1998; **69**: 162-167
75 **Lim JH**. Cholangiocarcinoma: morphologic classification according to growth pattern and imaging findings. *AJR Am J Roentgenol* 2003; **181**: 819-827
76 **Yoon KH**, Ha HK, Kim CG, Roh BS, Yun KJ, Chae KM, Lim JH, Auh YH. Malignant papillary neoplasms of the intrahepatic bile ducts: CT and histopathologic features. *AJR Am J Roentgenol* 2000; **175**: 1135-1139
77 **Terada T**, Kida T, Nakanuma Y, Noguchi T. Extensive portal tumor thrombi with portal hypertension in an autopsy case of intrahepatic cholangiocarcinoma. *Am J Gastroenterol* 1992; **87**: 1513-1518
78 **Neumaier CE**, Bertolotto M, Perrone R, Martinoli C, Loria F, Silvestri E. Staging of hilar cholangiocarcinoma with ultrasound. *J Clin Ultrasound* 1995; **23**: 173-178
79 **Xu HX**, Lu MD, Liu GJ, Xie XY, Xu ZF, Zheng YL, Liang JY. Imaging of peripheral cholangiocarcinoma with low-mechanical index contrast-enhanced sonography and SonoVue: initial experience. *J Ultrasound Med* 2006; **25**: 23-33

S- Editor Cheng JX **L- Editor** Kerr C **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i33.4187

World J Gastroenterol 2010 September 7; 16(33): 4187-4192
ISSN 1007-9327 (print)

BRIEF ARTICLE

Diagnostic sensitivity of imaging modalities for hepatocellular carcinoma smaller than 2 cm

Keiji Mita, Soo Ryang Kim, Masatoshi Kudo, Susumu Imoto, Taisuke Nakajima, Kenji Ando, Katsumi Fukuda, Toshiyuki Matsuoka, Yoko Maekawa, Yoshitake Hayashi

Keiji Mita, Soo Ryang Kim, Susumu Imoto, Taisuke Nakajima, Kenji Ando, Katsumi Fukuda, Department of Gastroenterology, Kobe Asahi Hospital, Kobe 653-0801, Japan
Masatoshi Kudo, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osakasayama 589-8511, Japan
Toshiyuki Matsuoka, Department of Radiology, Osaka City University Medical School, Osaka 558-8585, Japan
Yoko Maekawa, Department of Surgery, Hyogo Cancer Center, Akashi 673-8558, Japan
Yoshitake Hayashi, Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan
Author contributions: Mita K, Nakajima T and Ando K designed and performed the majority of imaging examinations; Maekawa Y and Fukuda K were involved in editing the manuscript; Imoto S provided the patient data; Kudo M and Matsuoka T carried out and reviewed the imaging studies; Kim SR and Hayashi Y made the pathological diagnosis of hepatocellular carcinoma; Kim SR wrote the paper.
Correspondence to: Dr. Soo Ryang Kim, Department of Gastroenterology, Kobe Asahi Hospital, 3-5-25 Bououji-cho, Nagata-ku, Kobe 653-0801, Japan. asahi-hp@arion.ocn.ne.jp
Telephone: +81-78-6125151 Fax: +81-78-6125152
Received: March 12, 2010 Revised: May 4, 2010
Accepted: May 11, 2010
Published online: September 7, 2010

Abstract

AIM: To compare the imaging results with histology and to evaluate the diagnostic sensitivity of imaging modalities for hepatocellular carcinoma (HCC) smaller than 2 cm.

METHODS: Nodules smaller than 2 cm (n = 34) revealed by ultrasonography (US) in 29 patients with liver cirrhosis were analyzed. Histological diagnosis of HCC was performed by ultrasonographic guidance: moderately-differentiated HCC (n = 24); well-differentiated HCC (n = 10). The patterns disclosed by the four imaging modalities defined the conclusive diagnosis of HCC: (1) contrast-enhanced computed tomography (CECT), hypervascularity in the arterial phase and washout in the equilibrium phase; (2) Sonazoid contrast-enhanced US (CEUS), hypervascularity in the early vascular phase and defect in the Kupffer phase; (3) gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MRI), hypervascularity in the arterial phase and/or defect in the hepatobiliary phase; and (4) CT arterioportal angiography: hypervascularity by CT during arteriography and/or perfusion defect by CT during arterial portography.

RESULTS: Overall, the sensitivity of diagnosing HCC smaller than 2 cm was 52.9% (18/34) (95% CI: 35.1-70.2) by CECT; 67.6% (23/34) (95% CI: 49.5-82.6) by Sonazoid CEUS; 76.5% (26/34) (95% CI: 58.8-89.3) by Gd-EOB-DTPA MRI; and 88.2% (30/34) (95% CI: 72.5-96.7) by CT arterioportal angiography. The diagnostic sensitivity of detecting moderately-differentiated HCC by CECT, Sonazoid CEUS, Gd-EOB-DTPA MRI and CT arterioportal angiography was 62.5% (15/24) (95% CI: 40.6-81.2), 79.2% (19/24) (95% CI: 57.8-92.9), 75.0% (18/24) (95% CI: 53.3-90.2) and 95.8% (23/24) (95% CI: 78.9-99.9), respectively. A significant difference (P < 0.05) was observed between CECT and CT arterioportal angiography in all nodules. There was no difference between Sonazoid CEUS, Gd-EOB-DTPA MRI, and CT arterioportal angiography. The combined sensitivity of Sonazoid CEUS and Gd-EOB-DTPA MRI was 94.1% (32/34).

CONCLUSION: Changing the main diagnostic modality for HCC smaller than 2 cm from CT arterioportal angiography to Sonazoid CEUS and Gd-EOB-DTPA MRI is recommended.

Key words: Computed tomography arterioportal angiography; Contrast-enhanced computed tomography; Diagnostic sensitivity; Gd-EOB-DTPA-enhanced magnetic

resonance imaging; Hepatocellular carcinoma smaller than 2 cm: Sonazoid contrast-enhanced ultrasonography

Peer reviewers: Søren Rafaelsen, MD, Consultant Radiologist, Associate Professor, Department of Radiology, Vejle Hospital, Vejle, 7100, Denmark; Bernardo Frider, MD, Professor, Department of Hepatology, Hospital General de Agudos Cosme Argerich, Alte Brown 240, Buenos Aires 1155, Argentina

Mita K, Kim SR, Kudo M, Imoto S, Nakajima T, Ando K, Fukuda K, Matsuoka T, Maekawa Y, Hayashi Y. Diagnostic sensitivity of imaging modalities for hepatocellular carcinoma smaller than 2 cm. *World J Gastroenterol* 2010; 16(33): 4187-4192 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i33/4187.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i33.4187

INTRODUCTION

The definitive diagnosis of nodular lesions, detected by imaging techniques in the liver with cirrhosis, remains a critical challenge for clinicians. The issue is particularly complicated for small (1-2 cm) nodules, many of which may be preneoplastic with uncertain malignant potential[1], such as macroregenerative nodules, low-grade dysplastic nodules (LGDN) or high-grade dysplastic nodules (HGDN), or more rarely, hemangiomas that are found in up to 42% of explanted livers[2-4].

Recently, clinicians have been able to conduct computed tomography (CT) scanning during angiography, thereby acquiring data on lesions and intranodular blood flow simultaneously[5,6]. To resolve the areas of uncertainty, we have previously reported on the superiority of CT arterioportal angiography [including CT during arteriography (CTA) and CT during arterial portography (CTAP)], concluding that it is superior to contrast-enhanced CT (CECT) and magnetic resonance imaging (MRI) in the diagnosis of hepatocellular carcinoma (HCC) nodules smaller than 2 cm[7].

Moreover, development of the newly introduced diagnostic imaging techniques, Sonazoid contrast-enhanced ultrasonography (CEUS) and gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced MRI have provided higher degrees of detectability for small HCC. In this study, we compared the diagnostic sensitivity of CECT, Sonazoid CEUS, Gd-EOB-DTPA MRI, and CT arterioportal angiography in diagnosing HCC in nodules smaller than 2 cm.

MATERIALS AND METHODS

Patients

From April 2008 to December 2009, we analyzed 34 nodules smaller than 2 cm [8-20 mm; mean ± SD 12.7 ± 3.71 mm; the interquartile range (IQR) 10-15 mm] detected by US in 29 patients (13 men and 16 women; aged 55-84 years; mean ± SD 70.5 ± 7.96 years; IQR 67-76 years) with liver cirrhosis related to hepatitis B virus in 1, hepatitis C virus (HCV) in 24, and alcohol in 4. α-fetoprotein (AFP) measured less than 20 ng/mL in 21 patients and was above 21 ng/mL in 8 (Table 1). In this study, one nodule that was not histologically diagnosed as HCC irrespective of compatibility by imaging studies was excluded, and two nodules were excluded because of inconsistency between readers in the imaging results. The study was approved by the Ethics Committee in Kobe Asahi Hospital.

Table 1 Data and characteristics of 29 patients and 34 nodules

Age (yr), range (mean ± SD)	55-84 (70.5 ± 7.96) IQR 67-76
Sex (M/F)	13/16
Cause	
HBV	1
HCV	24
Alcohol	4
AFP (ng/mL)	
< 20	21
> 21	8
Nodule characteristics (mm), range (mean ± SD)	8-20 (12.7 ± 3.71) IQR 10-15
Histological diagnosis of the 34 nodules	
Moderately-differentiated HCC	24
Well-differentiated HCC	10

IQR: Interquartile range; HBV: Hepatitis B virus; HCV: Hepatitis C virus; AFP: α-fetoprotein; HCC: Hepatocellular carcinoma.

CECT

CECT was conducted with the use of helical CT (Siemens, Germany) with precontrast and postcontrast triple-phase (arterial, portal, venous, and equilibrium phases) scans, after the injection of 120 mL of nonionic contrast medium at 3 mL/s; the scans were carried out in a craniocaudal direction with a 5 mm collimation in the other phases. Acquisition of the arterial and equilibrium phases was automatically started at 30 and 180 s, respectively, after the intravenous injection.

CEUS

Ultrasonography was performed using a SSA-660A (Toshiba Medical Systems, Tochigi, Japan). The vascular findings on phase-inversion harmonic US were shown as tumor vessel flow in the early vascular phase about 15-40 s after injection of Sonazoid (GE HealthCare, Piscataway, NJ, USA). The real-time replenishing images were obtained during the vascular phase (< 2 min after the injection) by release burst imaging. Images of the liver parenchyma were obtained in the postvascular Kupffer phase, at least 10 min after the intravenous injection of Sonazoid. Hepatic malignances were visualized as defects in the postvascular phase. An additional contrast agent was injected to confirm tumor vessel flow in the defect, a technique known as defect reperfusion imaging[8].

Gd-EOB-DTPA MRI

Images by MRI scans (Phillips, Netherlands) were obtained by the 1.0-T superconducting system (Gyroscan 10T-NT, Phillips, Netherlands). Enhanced MRI was used to obtain

Table 2 Imaging patterns for the conclusive diagnosis of hepatocellular carcinoma by the four modalities

Modality	Imaging pattern
Contrast-enhanced CT	Hypervascularity in the arterial phase and washout in the equilibrium phase
Sonazoid contrast-enhanced ultrasonography	Hypervascularity in the early vascular phase and defect in the Kupffer phase
Gd-EOB-DTPA magnetic resonance imaging	Hypervascularity in the arterial phase and/or defect in the hepatobiliary phase
CT arterioportal angiography	Hypervascularity by CTA and/or perfusion defect by CTAP

CT: Computed tomography; CTA: CT during arteriography; CTAP: CT during arterial portography; Gd-EOB-DTPA: Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid.

coronal images by the gradient-echo technique (FFG) at 150/3.5 ms TR/TE, 80° flip angle, and 168 × 256 matrix. In each sequence, the respiration suspension time was 20-30 s. Gd-EOB-DTPA (Primovist; Bayer HealthCare, Osaka, Japan) at a dose of 0.025 mmol/kg body weight was injected intravenously as a rapid bolus at 2 mL/s. Dynamic contrast-enhanced MRI was initiated at 30 s, 70 s, 2-3 min and 20 min after the start of the bolus injection to obtain multiphasic (arterial, portal, late, and hepatobiliary) images.

CT arterioportal angiography (CTA and CTAP)

CTA: At angiography, 45 mL of diluted contrast medium was injected through a catheter at 2 mL/s into the common hepatic artery. The whole liver was then scanned at intervals of 5 to 10 mm.

CTAP: At angiography, 115 mL of diluted contrast medium was injected through a catheter at 2 mL/s into the superior mesenteric artery, according to the scanning time of the entire liver using a power injector during sequential scanning of the liver with incremental changes in the position of the table. Infusion of contrast material was initiated 20 s before CTAP. The whole liver was then scanned at intervals of 5 to 10 mm.

US-guided biopsy

US-guided biopsy was carried out with the use of a 21 gauge Majima needle (Top, Japan). The diagnosis of HCC was made by two operators [a physician (K.S.) and a pathologist (Y.H.)] using the same specimen.

Histological diagnosis

Specimens were routinely processed and stained with hematoxylin and eosin and by the Masson trichromatic method. The diagnosis of HCC was made according to the criteria of the International Working Party[1].

Imaging patterns for the conclusive diagnosis of HCC by the four modalities

The following patterns disclosed by the four imaging modalities were defined as the conclusive diagnosis of HCC. (1) CECT: hypervascularity in the arterial phase and washout in the equilibrium phase; (2) Sonazoid CEUS: hypervascularity in the early vascular phase and defect in the Kupffer phase; (3) Gd-EOB-DTPA MRI: hypervascularity in the arterial phase and/or defect in the hepatobiliary phase; and (4) CT arterioportal angiography: hypervascularity by CTA and/or perfusion defect by CTAP (Table 2).

Imaging studies

To minimize differences in the results between the operators, imaging studies were carried out and reviewed by two operators [a physician (M.K.) and a radiologist (T.M.)] using the same examination protocol.

Statistical analysis

The sensitivity for detecting tumors was indicated by the 95% CI. The 95% CI was estimated by F distribution. The level of significance was set at $P < 0.05$.

RESULTS

The 34 nodules were histologically diagnosed as moderately-differentiated (24 nodules) and well-differentiated (10 nodules) HCC (Table 1). For HCC smaller than 2 cm, the overall diagnostic sensitivity was 52.9% (18/34) (95% CI: 35.1-70.2) by CECT; 67.6% (23/34) (95% CI: 49.5-82.6) by Sonazoid CEUS; 76.5% (26/34) (95% CI: 58.8-89.3) by Gd-EOB-DTPA MRI; and 88.2% (30/34) (95% CI: 72.5-96.7) by CT arterioportal angiography, with a significant difference ($P < 0.05$) between CECT and CT arterioportal angiography. The combined sensitivity of Sonazoid CEUS and Gd-EOB-DTPA MRI was 94.1% (32/34). In diagnosing moderately-differentiated HCC, the diagnostic sensitivity of CECT, Sonazoid CEUS, Gd-EOB-DTPA MRI and CT arterioportal angiography was 62.5% (15/24) (95% CI: 40.6-81.2), 79.2% (19/24) (95% CI: 57.8-92.9), 75.0% (18/24) (95% CI: 53.3-90.2) and 95.8% (23/24) (95% CI: 78.9-99.9), respectively. There was no difference between CECT, Sonazoid CEUS, Gd-EOB-DTPA MRI, and CT arterioportal angiography in moderately differentiated HCC. The sensitivity of well-differentiated HCC was not analyzed because of the paucity of cases (Table 3).

Representative cases

Case No. 1: Detection by Gd-EOB-DTPA MRI and arterioportal angiography: In a 67-year-old woman with HCV-related liver cirrhosis (AFP 9.0 ng/mL; PIVKA Ⅱ 21 mAU/mL), US revealed a 12 mm hyperechoic nodule in segment eight (Figure 1A). Sonazoid CEUS revealed no hypervascularity in the early vascular phase and no defect in the Kupffer phase. CECT revealed no hypervascularity in the arterial phase and washout in the equilibrium phase.

Table 3 Diagnostic sensitivity of hepatocellular carcinoma by the four modalities

Modality	Diagnostic sensitivity			
	All nodules (n = 34)		Moderately-differentiated HCC (n = 24)	
	n (%)	95% CI	n (%)	95% CI
Contrast-enhanced computed tomography	18 (52.9)	35.1-70.2	15 (62.5)	40.6-81.2
Sonazoid contrast-enhanced ultrasonography	23 (67.6)	49.5-82.6	19 (79.2)	57.8-92.9
Gd-EOB-DTPA magnetic resonance imaging	26 (76.5)	58.8-89.3	18 (75.04)	53.3-90.2
Computed tomography arterioportal angiography	30 (88.2)	72.5-96.7	23 (95.8)	78.9-99.9

Gd-EOB-DTPA: Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid; HCC: Hepatocellular carcinoma.

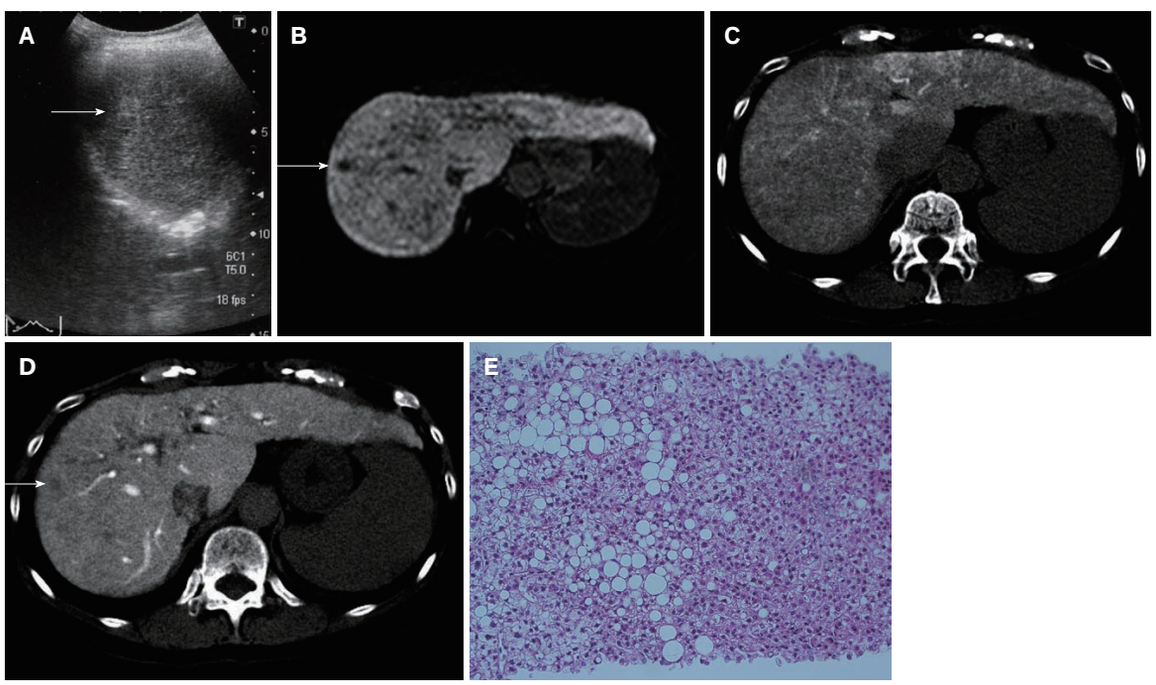


Figure 1 Case No. 1: detection by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging and computed tomography arterioportal angiography. Imaging and histological findings of the nodule in segment eight. A: Ultrasonography (US) reveals a 12 mm hyperechoic nodule (arrow); B: Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging reveals a defect (arrow) in the hepatobiliary phase; C, D: Computed tomography during arteriography reveals isodensity (C) and computed tomography during arterial portography (D) reveals a perfusion defect (arrow); E: The nodule is diagnosed as moderately-differentiated hepatocellular carcinoma by US-guided biopsy.

Gd-EOB-DTPA MRI revealed no hypervascularity in the arterial phase, but a defect in the hepatobiliary phase (Figure 1B). CTA revealed isodensity (Figure 1C), and CTAP a perfusion defect (Figure 1D). US-guided biopsy revealed moderately-differentiated HCC (Figure 1E).

Case No. 2: Detection by Gd-EOB-DTPA MRI: In a 74-year-old woman with HCV-related liver cirrhosis (AFP 7.1 ng/mL, PIVKA Ⅱ 42 mAU/mL), US revealed an 8 mm hyperechoic nodule in segment six (Figure 2A). Sonazoid CEUS revealed no hypervascularity in the early vascular phase and no defect in the Kupffer phase. CECT revealed isodensity in both the arterial phase and the equilibrium phase. MRI revealed isointensity. Gd-EOB-DTPA MRI revealed no hypervascularity in the early phase, but disclosed a defect in the hepatobiliary phase (Figure 2B). CTA revealed no hypervascularity and CTAP no perfusion defect. US-guided biopsy revealed well-differentiated HCC (Figure 2C).

DISCUSSION

Confirmation of arterial hypervascularity by three imaging modalities (triphasic CT, triphasic MRI, and CEUS), even in the absence of a significant (> 400 ng/mL) rise in AFP, is recommended by the European Association for the Study of the Liver (EASL) as diagnostic criteria for HCC nodules larger than 2 cm in patients with cirrhosis[9]. These recommendations for the management of HCC provide a rational approach to the problem but leave some areas of uncertainty, particularly those regarding the interpretation of discordant vascularity, the use of imag-

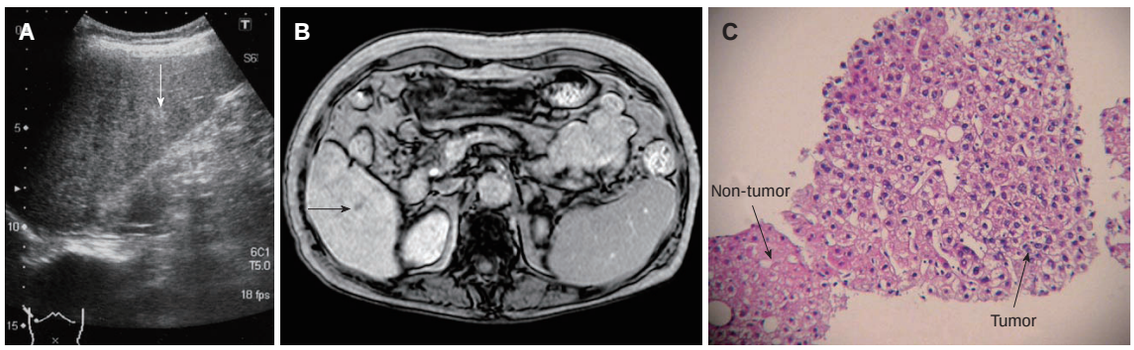


Figure 2 Case No. 2: detection by gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging. Imaging and histological findings of the nodule in segment six. A: Ultrasonography (US) reveals an 8 mm hyperechoic nodule (arrow); B: Gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid magnetic resonance imaging reveals a defect (arrow) in the hepatobiliary phase; C: The nodule is diagnosed as well-differentiated hepatocellular carcinoma by US-guided biopsy, showing cellularity more than two-fold that of the non-tumorous area.

ing techniques in nodules smaller than 2 cm, the meaning of truly hypovascular nodules, and the management of those diagnosed with LGDN or HGDN at guided biopsy.

The American Association for the Study of Liver Diseases[2] recommends that the diagnosis of HCC should be made without biopsy when characteristic arterial vascularization and venous washout are observed on three imaging modalities: triphasic CT scan, triphasic MRI and contrast-enhanced harmonic US.

Nevertheless, these recommendations have not been tested and validated except by Bolondi *et al*[10] and Forner *et al*[11]. According to Bolondi *et al*[10], the noninvasive EASL criteria with CEUS and CECT for the diagnosis of HCC are satisfied in only 44% of nodules smaller than 2 cm in cirrhosis. Forner *et al*[11] reported that the diagnostic sensitivity of MRI and CEUS in the diagnosis of HCC (smaller than 2 cm) is 67%.

The main characteristics of Sonazoid, a newly introduced second-generation US contrast agent exclusively approved in Japan in 2007, are that it facilitates real-time blood flow images at low acoustic power and stable Kupffer phase imaging from 10 to 120 min after its injection. In vascular imaging, Sonazoid is considered more effective than Levovist and easy to use; it allows visualization, even with the use of non-high-end equipment and, therefore, reduces dependence on the operator's skills/equipment, all of which may promote the widespread use of CEUS. As stated earlier, Sonazoid CEUS provides very stable postvascular phase images for up to 60-120 min[12], which has resulted in the invention of the breakthrough method, defect reperfusion imaging that is an innovative technology that will greatly change the daily practices of HCC management. In our study, the diagnostic sensitivity of Sonazoid CEUS was 67.6% in all HCC, and 79.2% in moderately-differentiated HCC.

Kudo *et al*[8,13,14] have recently developed defect reperfusion imaging (using the properties of very stable Kupffer phase images and real-time fine blood flow images obtained with Sonazoid) for typical HCC, which is depicted by CT but not by B mode scanning. The method is a breakthrough for accurate localization and treatment guidance[8]: dramatic resolution of many limitations in the diagnosis and treatment of HCC, such as detection of small HCCs[15], evaluation of treatment response[16], and needle insertion guidance; additionally, detection is even more sensitive than with MDCT[15].

A newly introduced contrast agent, Gd-EOB-DTPA, approved in Japan in 2008, is a hepatocyte-specific MRI contrast medium with a different mechanism that utilizes neither dynamic nor Kupffer cell imaging. It is useful in cases which would be difficult to diagnose by techniques such as dynamic MRI or SPIO-MRI. Typical HCC shows high intensity with Gd-EOB-DTPA in the arterial-dominant phase and low intensity in the portal-dominant phase and thereafter. The imaging diagnosis of HCC can be made approximately 10-20 min after the injection of Gd-EOB-DTPA. In our study, the diagnostic sensitivity of Gd-EOB-DTPA MRI was 76.5% in all nodules and 75.0% in moderately-differentiated HCC.

Previously, we had concluded that CT arterioportal angiography was superior to CECT and Gadolinium-enhanced MRI for diagnosing HCC in nodules smaller than 2 cm[7]. In this study, the diagnostic sensitivity of CT arterioportal angiography was 88.2% in all nodules and 95.8% in moderately-differentiated HCC. We observed a significant difference between CECT and CT arterioportal angiography ($P < 0.05$) in all nodules. However, there was no difference between Sonazoid CEUS, Gd-EOB-DTPA MRI, and CT arterioportal angiography. The combined sensitivity of Sonazoid CEUS and Gd-EOB-DTPA MRI in all nodules was 94.1%, due to improvement in the diagnostic capabilities of Sonazoid CEUS and Gd-EOB-DTPA MRI. This improvement in these two imaging modalities with the use of the newly introduced contrast agents provided higher sensitivity for the diagnosis of nodules smaller than 2 cm with Sonazoid CEUS and Gd-EOB-DTPA MRI than with Sonovue CEUS and CECT reported by Bolondi *et al*[10], or with Sonovue CEUS and Gadolinium-enhanced MRI reported by Forner *et al*[11].

These results, considered together with the invasiveness of CT arterioportal angiography, suggest that the principal diagnostic modality for HCC smaller than 2 cm

should be changed from CT arterioportal angiography to Sonazoid CEUS and Gd-EOB-DTPA MRI.

COMMENTS

Background

In spite of the recent advances in imaging techniques, the definitive diagnosis of nodular lesions detected by imaging modalities in the liver with cirrhosis remains a critical challenge for clinicians. The issue is particularly complicated for small (1-2 cm) nodules, many of which may be preneoplastic with uncertain malignant potential. We undertook this study to evaluate the effectiveness of imaging techniques in the diagnosis of hepatocellular carcinoma (HCC) smaller than 2 cm on the basis of histologic findings. Four imaging modalities were compared: contrast-enhanced computed tomography (CECT), Sonazoid contrast-enhanced ultrasonography (CEUS), gadolinium-ethoxybenzyl-diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) magnetic resonance imaging (MRI), and CT arterioportal angiography.

Research frontiers

The authors compared the imaging results with histology and evaluated the diagnostic sensitivity of the 4 imaging modalities.

Innovations and breakthroughs

Previously, the authors had concluded that CT arterioportal angiography was superior to CECT and gadolinium-enhanced MRI for diagnosing HCC in nodules smaller than 2 cm. In this study, the sensitivity of diagnosing 34 HCCs smaller than 2 cm was 52.9% by CECT; 67.6% by Sonazoid CEUS; 76.5% by Gd-EOB-DTPA MRI; and 88.2% by CT arterioportal angiography. A significant difference was observed between CECT and CT arterioportal angiography ($P < 0.05$). There was no difference between Sonazoid CEUS, Gd-EOB-DTPA MRI, and CT arterioportal angiography, and the combined sensitivity of Sonazoid CEUS and Gd-EOB-DTPA MRI was 94.1%, due to improvement in the diagnostic sensitivity of Sonazoid CEUS and Gd-EOB-DTPA MRI. This improvement in these two imaging modalities with the use of the newly introduced contrast agents provided higher sensitivity for the diagnosis of nodules smaller than 2 cm with Sonazoid CEUS and Gd-EOB-DTPA MRI than with Sonovue CEUS and CECT reported by Bolondi *et al*, or with Sonovue CEUS and Gadolinium-enhanced MRI reported by Forner *et al*.

Applications

These results, considered together with the invasiveness of CT arterioportal angiography, suggest that the principal diagnostic modality for HCC smaller than 2 cm should be changed from CT arterioportal angiography to Sonazoid CEUS and Gd-EOB-DTPA MRI.

Peer review

The major strength of the study is that there are many patients with small tumors. The patients have also been applied to new equipment and new contrast substances. It's a very interesting paper.

REFERENCES

1 Terminology of nodular hepatocellular lesions. International Working Party. *Hepatology* 1995; **22**: 983-993

2 **Theise ND**, Schwartz M, Miller C, Thung SN. Macroregenerative nodules and hepatocellular carcinoma in forty-four sequential adult liver explants with cirrhosis. *Hepatology* 1992; **16**: 949-955

3 **Mion F**, Grozel L, Boillot O, Paliard P, Berger F. Adult cirrhotic liver explants: precancerous lesions and undetected small hepatocellular carcinomas. *Gastroenterology* 1996; **111**: 1587-1592

4 **Burrel M**, Llovet JM, Ayuso C, Iglesias C, Sala M, Miquel R, Caralt T, Ayuso JR, Solé M, Sanchez M, Brú C, Bruix J. MRI angiography is superior to helical CT for detection of HCC prior to liver transplantation: an explant correlation. *Hepatology* 2003; **38**: 1034-1042

5 **Hayashi M**, Matsui O, Ueda K, Kawamori Y, Gabata T, Kadoya M. Progression to hypervascular hepatocellular carcinoma: correlation with intranodular blood supply evaluated with CT during intraarterial injection of contrast material. *Radiology* 2002; **225**: 143-149

6 **Tajima T**, Honda H, Taguchi K, Asayama Y, Kuroiwa T, Yoshimitsu K, Irie H, Aibe H, Shimada M, Masuda K. Sequential hemodynamic change in hepatocellular carcinoma and dysplastic nodules: CT angiography and pathologic correlation. *AJR Am J Roentgenol* 2002; **178**: 885-897

7 **Kim SR**, Ando K, Mita K, Fuki S, Ikawa H, Kanbara Y, Imoto S, Matsuoka T, Hayashi Y, Kudo M. Superiority of CT arterioportal angiography to contrast-enhanced CT and MRI in the diagnosis of hepatocellular carcinoma in nodules smaller than 2 cm. *Oncology* 2007; **72** Suppl 1: 58-66

8 **Kudo M**, Hatanaka K, Chung H, Minami Y, Maekawa K. A proposal of novel treatment-assist technique for hepatocellular carcinoma in the sonazoid-enhanced ultrasonography: value of defect re-perfusion imaging (in Japanese). *Kanzo* 2007; **48**: 299-301

9 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430

10 **Bolondi L**, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, Venturi AM, Piscaglia F. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005; **42**: 27-34

11 **Forner A**, Vilana R, Ayuso C, Bianchi L, Solé M, Ayuso JR, Boix L, Sala M, Varela M, Llovet JM, Brú C, Bruix J. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: Prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. *Hepatology* 2008; **47**: 97-104

12 **Inoue T**, Kudo M, Hatanaka K, Takahashi S, Kitai S, Ueda T, Ishikawa E, Hagiwara S, Minami Y, Chung H, Ueshima K, Maekawa K. Imaging of hepatocellular carcinoma: qualitative and quantitative analysis of postvascular phase contrast-enhanced ultrasonography with sonazoid. Comparison with superparamagnetic iron oxide magnetic resonance images. *Oncology* 2008; **75** Suppl 1: 48-54

13 **Kudo M**, Hatanaka K, Maekawa K. Defect reperfusion imaging, a newly developed novel technology using sonazoid in the treatment of hepatocellular carcinoma. *J Med Ultrasound* 2008; **16**: 169-176

14 **Kudo M**, Hatanaka K, Maekawa K. Sonazoid-enhanced ultrasound in the diagnosis and treatment of hepatic tumors. *J Med Ultrasound* 2008; **16**: 130-139

15 **Hatanaka K**, Kudo M, Minami Y, Maekawa K. Sonazoid-enhanced ultrasonography for diagnosis of hepatic malignancies: comparison with contrast-enhanced CT. *Oncology* 2008; **75** Suppl 1: 42-47

16 **Xia Y**, Kudo M, Minami Y, Hatanaka K, Ueshima K, Chung H, Hagiwara S, Inoue T, Ishikawa E, Kitai S, Takahashi S, Tatsumi C, Ueda T, Hayaishi S, Maekawa K. Response evaluation of transcatheter arterial chemoembolization in hepatocellular carcinomas: the usefulness of sonazoid-enhanced harmonic sonography. *Oncology* 2008; **75** Suppl 1: 99-105

S- Editor Wang JL **L- Editor** Webster JR **E- Editor** Zheng XM

MAJOR ARTICLE

Hepatitis C Virus Core Protein Induces Homotolerance and Cross-Tolerance to Toll-Like Receptor Ligands by Activation of Toll-Like Receptor 2

Hobyung Chung,[1] Tomohiro Watanabe,[2] Masatoshi Kudo,[1] and Tsutomu Chiba[2]

[1]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Osaka, and [2]Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, Kyoto, Japan

Background. Hepatitis C virus (HCV) activates host innate immune responses mediated by retinoic acid inducing gene-I (RIG-I) and Toll-like receptors (TLRs). Although the nonstructural protein 3/4A (NS3/4A) of HCV disrupts interferon responses by inhibiting RIG-I signaling, the effects of TLR activation by HCV-associated proteins on host innate immune responses are poorly understood.

Methods. Proinflammatory cytokine responses to various TLR ligands in human antigen-presenting cells (APCs) were examined either with or without prestimulation by HCV core protein.

Results. TLR2 activation by the HCV core protein leads to a decrease in interleukin 6 (IL-6) production by human APCs after subsequent stimulation with TLR2 (homotolerance) ligands and TLR4 (cross-tolerance) ligands. This hyporesponsiveness induced by preexposure to the HCV core protein was partially mediated by the negative regulation of nuclear factor-κB activation by the induction of IRAK-M. TLR ligand-induced IL-6 production was significantly reduced in peripheral blood monocytes isolated from HCV-infected patients, compared with those of healthy control subjects. Alloantigen presentation by monocytes isolated from HCV-infected patients results in impaired production of interleukin 17 by naive $CD4^+$ T cells in the presence of TLR ligands.

Conclusions. Chronic stimulation of APCs with HCV core protein is associated with hyporesponsiveness in TLR-mediated innate immunity.

Hepatitis C virus (HCV) is a successful pathogen that establishes persistent infection and causes chronic liver disease in the host [1, 2]. The mechanisms by which HCV avoids elimination by the host immune system are poorly understood. One proposed mechanism accounting for the high rate of persistent infection is that HCV infection inhibits the production of type I interferons that constitute the antiviral host defense [3]. HCV RNA is recognized by innate virus-sensing molecules, such as retinoic acid inducing gene-I and Toll-like receptor 3 (TLR3), which then induce rapid interferon responses [4–6]. The HCV nonstructural protein 3/4A protease is reported to blunt the innate antiviral interferon responses mediated by these virus-sensing molecules [5, 7]. However, defective interferon responses are not sufficient to explain the development of the abnormal immunological environments permissive to persistent HCV infection. This notion is supported by the clinical outcome showing that only ~50% of patients with HCV infection are successfully treated with pegylated type I interferon and ribavirin [8].

Several bacterial infections such as sepsis and cellulitis are more common in HCV-infected patients than in those without HCV infection [9–11]. Because TLR-mediated proinflammatory cytokine responses are necessary for host defenses against bacteria [12], it is likely that chronic HCV infection generates an immune environment in which TLR-mediated proinflammatory cytokine production is impaired after exposure to bacterial antigens. With respect to TLR activation by HCV-associated antigens, the core protein activates TLR2 on antigen-presenting cells (APCs) to induce cytokine re-

Received 1 December 2009; accepted 14 April 2010; electronically published 2 August 2010.

Potential conflicts of interest: none reported.

Financial support: This work was supported in part by grants from the Viral Hepatitis Research Foundation of Japan, the Takeda Science Foundation, the Yakult Bioscience Foundation, the Cell Science Research Foundation, and the Uehara Memorial Foundation (to T.W.).

Reprints or correspondence: Dr Tomohiro Watanabe, Department of Gastroenterology and Hepatology, Kyoto University Graduate School of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan (tmhrwtnb@kuhp.kyoto-u.ac.jp).

The Journal of Infectious Diseases 2010;202(6):853–861

0022-1899/2010/20206-0006$15.00
DOI: 10.1086/655812

sponses through nuclear translocation of nuclear factor-κB (NF-κB) subunits [13, 14]. However, the type of immune response that is finally generated by TLR activation in HCV-infected patients remains largely unknown. If the HCV core protein stimulates TLR2, then this TLR pathway will be constantly activated in peripheral blood APCs in HCV-infected patients. Preactivation of a single TLR pathway results in reduced cytokine responses after restimulation with TLR ligands [15]. Therefore, this study investigated whether the activation of TLR2 by the HCV core protein induces tolerogenic cytokine responses after subsequent stimulation with TLR ligands. We found that core protein–mediated activation of TLR2 in human APCs reduces interleukin 6 (IL-6) production by these cells after restimulation with TLR4 ligands (cross-tolerance), or TLR2 ligands (homotolerance). More importantly, IL-6 production mediated by these TLR ligands is significantly reduced in the peripheral blood monocytes of chronic HCV patients compared with healthy control subjects, resulting in impaired allogeneic interleukin 17 (IL-17) production.

METHODS

Stimulation of cell lines with core protein. Human embryonic kidney 293 (HEK293) cells (ATCC) and isolated clones of HEK293 cells stably expressing the human TLR2 gene (HEK293-TLR2; InvivoGen) or TLR4 and MD2 genes (HEK293-TLR4-MD2; InvivoGen) were stimulated with core protein (2 or 20 μg/mL; Biodesign International), peptidoglycan (10 μg/mL; Fluka), Pam_3CSK4 (10 μg/mL; InvivoGen) or lipopolysaccharide (1 μg/mL; Sigma-Aldrich). The purity of the core protein was >95%. The human monocytic cell line MonoMac 6 (MM6; 1×10^6 cells/mL) [16] was stimulated with core protein (5 μg/mL), peptidoglycan (10 μg/mL), Pam_3CSK4 (10 μg/mL), or lipopolysaccharide (1 μg/mL) in the presence of a neutralizing anti-TLR2 monoclonal antibody (T2.5; 2 or 20 μg/mL; eBioscience) or mouse IgG1 control antibody (eBioscience). Cells were cultured for 24 h, and supernatants were analyzed for production of IL-6 and interleukin 8 (IL-8).

Prestimulation of cells with core protein. Human monocyte-derived dendritic cells (DCs) from healthy control subjects were generated as described elsewhere [17]. DCs or MM6 cells (1×10^6 cells/mL) were incubated with core protein (10 μg/mL) or medium alone for 24 h. The cells were then washed 3 times and restimulated with microbial antigens as described above. Culture supernatants were collected 24 h after restimulation and analyzed for cytokine production.

Flow cytometry. MM6 cells (1×10^6 cells/mL) were incubated with core protein (10 μg/mL) or culture medium alone for 24 h. Cell surface expression of TLR2 and TLR4 was analyzed using a PE-conjugated anti-human TLR2 monoclonal antibody (TL2.1; eBioscience), an anti-TLR4 monoclonal antibody (HTA125; eBioscience) or a PE-conjugated mouse IgG2a control antibody (eBioscience). Apoptotic cell death was assessed using an Annexin V assay as described elsewhere [18]. Cell-surface expression of CD80 and CD86 was analyzed by using a PE-conjugated anti-human CD80 or CD86 monoclonal antibody (eBioscience) as described elsewhere [19].

Enzyme-linked immunosorbent assay. The concentrations of cytokines and chemokines were determined by enzyme-linked immunosorbent assay kits for human IL-6, IL-8, interleukin 10 (IL-10), interleukin 12p40 (IL-12p40), interferon γ (IFN-γ) (BD Bioscience), and IL-17 (eBioscience) as described elsewhere [20].

NF-κB activation assay. Nuclear extracts were prepared from MM6 cells (1×10^6 cells/mL) preincubated with either core protein (10 μg/mL) or medium for 24 h and then stimulated with core protein (5 μg/mL) or lipopolysaccharide (1 μg/mL) for 1 h. The binding of the nuclear extract (30 μg/well) to NF-κB consensus oligonucleotides was measured using a Mercury Transfactor kit (BD Bioscience) as described elsewhere [21].

Immunoblot analysis. Immunoblot analysis was performed as described elsewhere [21]. The blotted membranes were incubated with anti-MyD88 (Active Motif), anti–interferon regulatory factor 3 (IRF3; Santa Cruz Biotechnology), anti–interferon regulatory factor 5 (IRF5; Abcam), anti-IRAK-M (Cell Signalling), or anti-actin (Santa Cruz Biotechnology) antibodies.

Assays with small interfering RNA specific to IRAK-M. MM6 cells (5×10^5 cells/mL) were transfected with either IRAK-M small interfering RNA (siRNA) (Santa Cruz Biotechnology) or control siRNA (25 nmol/L) using the TransIT-TKO transfection reagent (Mirus), followed by stimulation with core protein (10 μg/mL) for 24 h and restimulation with core protein (5 μg/mL) and lipopolysaccharide (1 μg/mL).

Studies using peripheral blood cells from patients. Ethical permission for this study was granted by the review board of Kinki University. Healthy control subjects ($n = 10$) and treatment-naive patients with chronic HCV infection ($n = 10$) were enrolled in this study after informed consent was obtained. Peripheral blood monocytes (1×10^6 cells/mL) isolated from each patient were stimulated with HCV-associated proteins and TLR ligands as described. Monocytes were purified from peripheral blood mononuclear cells (PBMCs) using a monocyte isolation kit (Miltenyi Biotec). Culture supernatants were collected after 24 h and analyzed for cytokine production. In some experiments, monocytes (1×10^6 cells/mL) isolated from HCV patients or healthy control subjects were cocultured for 7 days with naive $CD4^+$ T cells (1×10^6 cells/mL) isolated from PBMCs of healthy control subjects. Culture supernatants were then analyzed for cytokine production. Naive $CD4^+$ T cells were purified using a naive $CD4^+$ T cell isolation kit (Miltenyi Biotec).

This figure is available in its entirety in the online version of the *Journal of Infectious Diseases*.

Figure 1. Hepatitis C virus (HCV) core protein is a specific activator of Toll-like receptor 2 (TLR2).

Statistical analysis. A Student *t* test was used to evaluate statistical significance. Statistical analysis was performed using the StatView software, version 4.5 (Abacus Concepts). A value of $P < .05$ was regarded as statistically significant.

RESULTS

Activation of TLR2 by core protein. Our initial studies determined whether core protein functions as a specific activator of TLR2. Core protein induced IL-8 production only in HEK293-TLR2 cells, which suggests that this protein activates TLR2 (Figure 1*A*). We next used a different approach to confirm this finding in the human monocytic cell line, MM6. Production of IL-6 and IL-8 induced by core protein was reduced by the addition of an anti-TLR2 monoclonal antibody as in the case of stimulation with a conventional TLR2 ligand, peptidoglycan (Figure 1*B*). To further confirm the activation of TLR2 by core protein, we used splenocytes lacking MyD88, a downstream effector molecule of the TLR2 pathway [12]. MyD88-deficient splenocytes failed to produce IL-6 after stimulation with core protein (Figure 1*C*). These data suggest that the core protein is a specific activator of the TLR2-MyD88 signaling pathway.

Induction of cross-tolerance by core protein. Although the ligation of TLRs on APCs induces proinflammatory responses, preexposure to TLR2 or TLR4 ligands has been shown to desensitize APCs to subsequent stimulation by TLRs [22–25]. In the case of the TLR4 signaling pathway, preexposure of APCs to lipopolysaccharide reduces responsiveness not only to lipopolysaccharide (homotolerance) but also to TLR2 ligands (cross-tolerance) [22–25]. It remains controversial whether preactivation of TLR2 leads to cross-tolerance in the TLR4 signaling pathway [22–25]. Identification of core protein as a specific TLR2 activator prompted us to address whether preexposure of APCs to core protein leads to tolerogenic responses not only to TLR2 ligands, but also to TLR4 ligands. For this purpose, MM6 cells were incubated with core protein, followed by stimulation with core protein, peptidoglycan, Pam_3CSK4, or lipopolysaccharide, to measure production of proinflammatory cytokines. Production of IL-6 and IL-8 induced by core protein was markedly reduced in cells prestimulated with core protein compared with cells that were not prestimulated (Figure 2*A*). Interestingly, the production of IL-6 and IL-8 induced by a TLR4 ligand (lipopolysaccharide), as well as TLR2 ligands (peptidoglycan or Pam_3CSK4), was markedly reduced in MM6 cells preincubated with core protein. Preexposure of cells to core protein did not alter the production of IL-12p40 after restimulation with TLR ligands (data not shown). Thus, exposure of MM6 cells to core protein induces homotolerance and cross-tolerance after subsequent stimulation with TLR ligands. We next examined whether this was the case with primary human APCs. Human monocyte-derived DCs from healthy control subjects were activated with core protein and then restimulated with TLR ligands. The production of IL-6 and IL-8 was markedly reduced in DCs after restimulation with TLR2 and TLR4 ligands (Figure 2*B*). Moreover, induction of cross-tolerance by exposure to core protein was impaired by blockade of the TLR2 pathway, because IL-6 production was significantly increased in MM6 cells treated with an anti-TLR2 monoclonal antibody (Figure 2*C*). Taken together, these data strongly suggest that preactivation of TLR2 on human APCs by core protein induces both homotolerance and cross-tolerance after subsequent stimulation with TLR ligands.

Expression of TLRs and activation markers in core protein–tolerant cells. We investigated whether preactivation by core protein alters the expression of costimulatory molecules and TLRs. No significant alteration in the expression of CD80, CD86, TLR2, or TLR4 was seen in MM6 cells by preactivation with core protein (Figure 3*A* and 3*C*). In addition, the expression of CD80 and CD86 was not changed by treatment with core protein after restimulation with core protein or lipopolysaccharide (Figure 3*A*). It is unlikely that the inhibition of TLR responses by core protein prestimulation is due to the induction of apoptotic cell death because stimulation of MM6 cells with core protein did not alter the percentage of Annexin V^+ apoptotic cells (Figure 3*B*).

Molecular mechanisms of cross-tolerance by core protein. NF-κB is a crucial transcription factor for the regulation of IL-6 and IL-8 gene expression. Tolerogenic responses seen after preexposure to Pam_3CSK4 or lipopolysaccharide have been shown to be mediated by a reduction in NF-κB activation [22, 23]. Figure 4*A* shows that after restimulation with either core protein or lipopolysaccharide, the binding of the NF-κB subunits, p65 and p50, from nuclear extracts isolated from MM6 cells preincubated with core protein to consensus sequences is markedly reduced compared with that of cells that had not been preincubated with core protein. In contrast, there was no significant difference in the binding of c-Rel in the nuclear extracts either with, or without, core protein prestimulation. Furthermore, no difference was seen in the binding activity of any of the NF-κB subunits when cells were restimulated with phorbol myristate acetate, which activates NF-κB independent of TLR signaling. Neither the core protein-induced nor lipopolysaccharide-induced binding activity of c-Fos and c-Jun in the nuclear extracts was affected by preincubation with core protein (data not shown). Moreover, p65 nuclear translocation

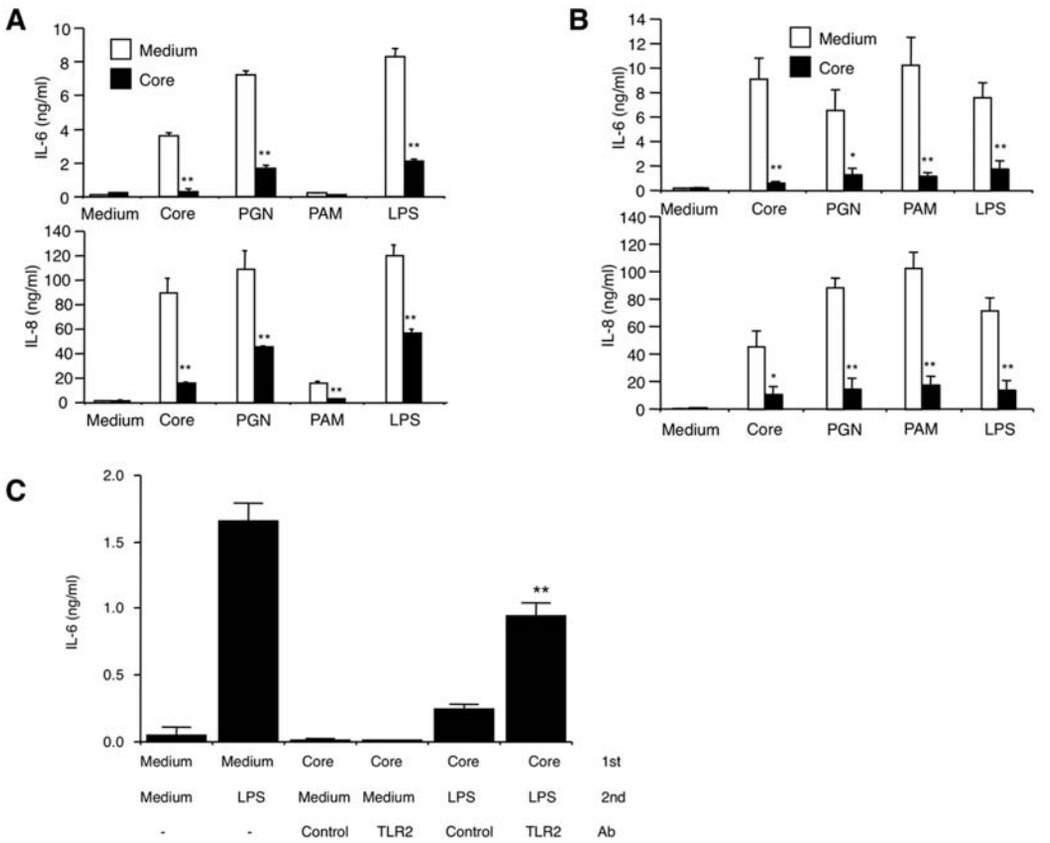


Figure 2. Induction of cross-tolerance to Toll-like receptor (TLR) ligands by hepatitis C virus (HCV) core protein. MM6 cells (1×10^6 cells/mL) (panels *A* and *C*) or human monocyte-derived dendritic cells (DCs) (1×10^6 cells/mL) (panel *B*) were incubated with HCV core protein (10 μg/mL) or culture medium alone for 24 h, washed 3 times, and then stimulated with HCV core protein (5 μg/mL), peptidoglycan (PGN) (10 μg/mL), Pam_3CSK4 (PAM) (10 μg/mL), or lipopolysaccharide (LPS) (1 μg/mL). In some experiments, MM6 cells were treated with anti-TLR2 monoclonal antibody (mAb) (50 μg/mL) or control antibody (Ab) (50 μg/mL) for 12 h before stimulation with core protein. Culture supernatants were collected 24 h later, and production of interleukin 6 (IL-6) and interleukin 8 (IL-8) was measured. *A, C*, Results represent 1 of 3 independent experiments and are shown as mean ± standard deviation. *B*, Results are shown as pooled DCs isolated from 4 healthy control subjects and are expressed as mean ± standard error. $^*P < .05$, $^{**}P < .01$ compared with cells preincubated with medium alone (panels *A* and *B*). $^{**}P < .01$, compared with cells treated with control Ab (panel *C*).

was restored in MM6 cells stimulated with core protein followed by lipopolysaccharide in the presence of an anti-TLR2 monoclonal antibody (Figure 4*B*). These data suggest that core protein prestimulation reduces subsequent activation of NF-κB by TLR ligands.

We performed immunoblot analyses to determine the expression levels of the signaling molecules, or negative regulators, involved in TLR signaling pathways [15] in cells incubated either with, or without, core protein. As shown in Figure 5*A*, there was no difference in the expression of TLR signaling molecules, such as MyD88, IRF3, or IRF5, in cells with or without core protein stimulation. In contrast, core protein clearly enhanced the expression of the negative regulator IRAK-M. A significant reduction in IRAK-M expression was seen in cells treated with an anti-TLR2 monoclonal antibody. We could not detect the expression of other negative regulators such as IRF4 or SOCS-1 by immunoblotting (data not shown). Because IRAK-M is responsible for the induction of endotoxin tolerance [26], we looked at whether IRAK-M expression induced by core protein is involved in the induction of homotolerance and cross-tolerance. We investigated whether gene silencing of IRAK-M expression by siRNA [17] abrogates core protein–mediated inhibitory effects. Figure 5*B* shows that transfection of MM6 cells with IRAK-M siRNA substantially reduced the expression of IRAK-

This figure is available in its entirety in the online version of the *Journal of Infectious Diseases.*

Figure 3. Cell-surface expression of costimulatory molecules and Toll-like receptors (TLRs) in cells stimulated with hepatitis C virus (HCV) core protein.

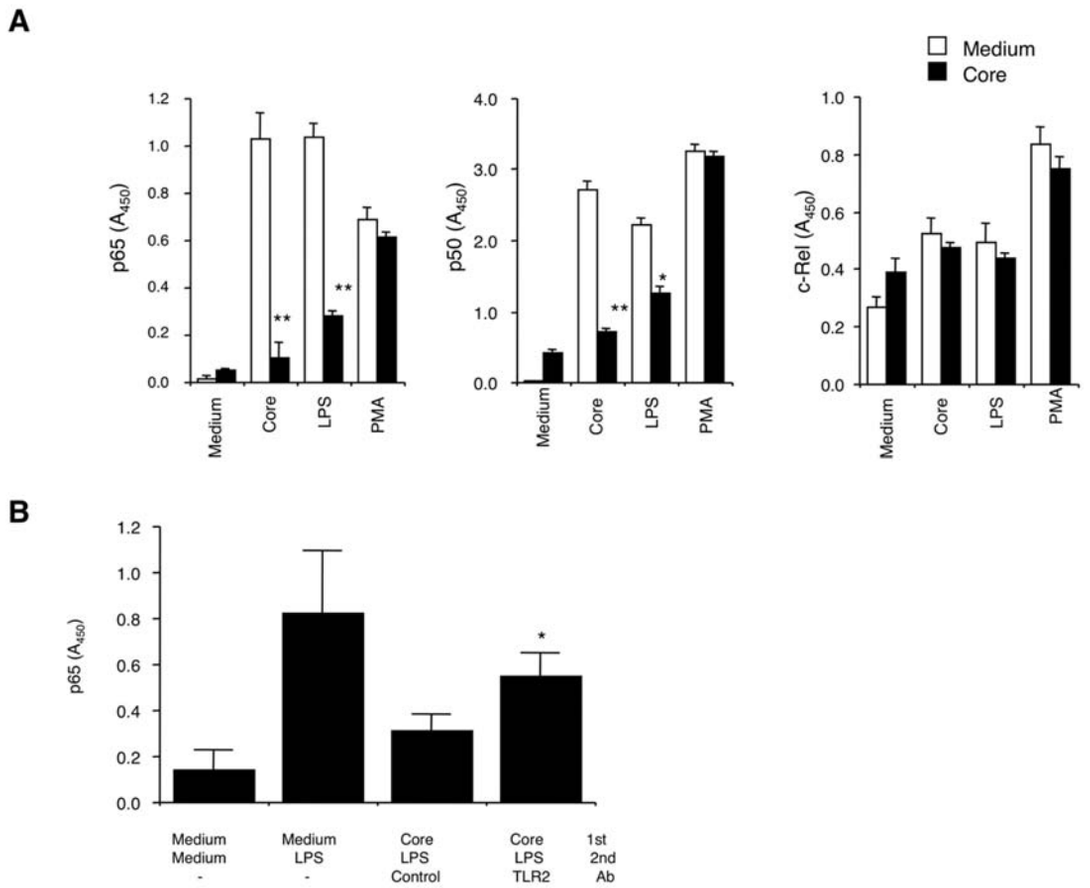


Figure 4. Activation of NF-κB in cells preincubated with hepatitis C virus (HCV) core protein and stimulated with core protein or lipopolysaccharide (LPS). *A*, MM6 cells (1×10^6 cells/mL) were incubated with HCV core protein (10 μg/mL) or culture medium alone for 24 h and then stimulated with HCV core protein (5 μg/mL), LPS (1 μg/mL), or phorbol myristate acetate (PMA) (50 ng/mL) for 1 h. Nuclear extracts were isolated, and the expression of NF-κB subunits was determined. *B*, Nuclear extracts were isolated from MM6 cells treated with an anti-TLR2 monoclonal antibody (mAb), or control antibody (Ab), followed by stimulation with HCV core protein and lipopolysaccharide. The expression of p65 was determined. Results shown are representative of 2 experiments (panel *B*) or 3 experiments (panel *A*) and are expressed as mean ± standard deviation. * $P < .05$, ** $P < .01$ compared with cells preincubated with medium alone (panel *A*). * $P < .05$, compared with cells treated with control Ab (panel *B*).

M at the protein level in core protein-stimulated MM6 cells. Transfection of IRAK-M siRNA led to a significant increase in IL-6 production in core protein–prestimulated MM6 cells after subsequent stimulation with either core protein or lipopolysaccharide, and these effects were associated with restored nuclear translocation of p65 (Figure 5*C* and 5*D*). These studies clearly show that induction of IRAK-M expression is involved in the inhibitory effects mediated by core protein prestimulation.

Production of IL-6 by monocytes from HCV-infected patients. Given the fact that circulating peripheral blood APCs in patients with HCV infection are exposed to core protein in the blood, it is interesting to examine whether core protein modulates the responsiveness of APCs by the mechanisms outlined above. To address this, we stimulated peripheral blood monocytes isolated from patients with HCV infection with core protein and TLR ligands and measured the production of IL-6 and IL-8. The production of IL-6 and IL-8 by monocytes isolated from HCV-infected patients was significantly reduced compared with that of healthy control subjects when cells were stimulated with core protein, peptidoglycan, Pam_3CSK4, or lipopolysaccharide (Figure 6). In contrast, no difference was seen in IL-12p40 production between the 2 populations. Thus, the continuous activation of TLR2 by core protein results in reduced cytokine responses to TLR ligands in monocytes from HCV-infected patients.

Impaired production of IL-17 by $CD4^+$ T cells cocultured with monocytes from HCV-infected patients in the presence of TLR ligands. Because APC-derived IL-6 is essential for Th17 differentiation [27], it is possible that chronic activation by core protein induces development of APCs with limited

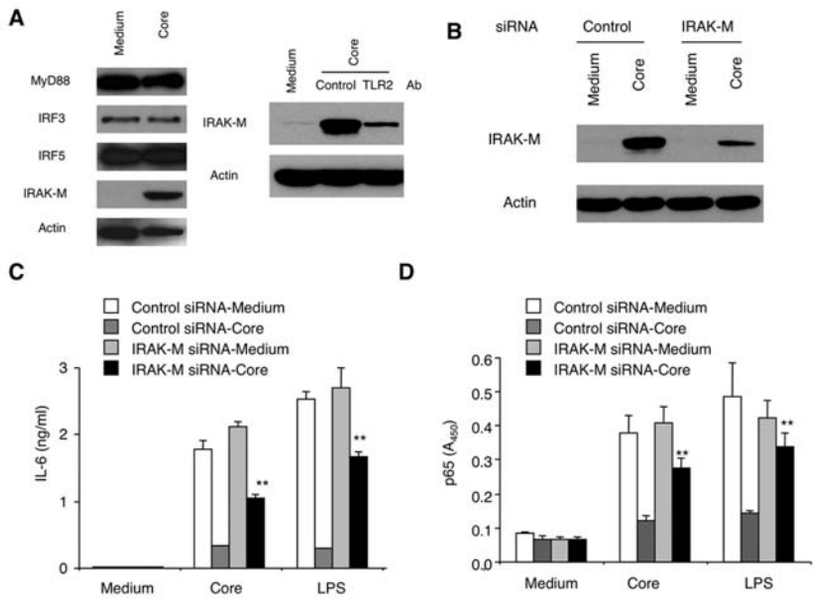


Figure 5. Core protein stimulation is associated with IRAK-M expression. *A*, Whole extracts were prepared from MM6 cells (1×10^6 cells/mL) stimulated with hepatitis C virus (HCV) core protein (10 μg/mL) or culture medium alone for 24 h. Whole extracts were immunoblotted with antibodies against the indicated proteins. The expression of IRAK-M after treatment with anti-TLR2 monoclonal antibody (mAb) (50 μg/mL) or control antibody (Ab) (50 μg/mL) is shown in the right panel. *B*, IRAK-M expression in MM6 cells transfected with IRAK-M small interfering RNA (siRNA). MM6 cells (5×10^5 cells/mL) were transfected with IRAK-M siRNA or control siRNA (25 nmol/L), followed by stimulation with HCV core protein (10 μg/mL) for 48 h. Whole extracts were subjected to immunoblot analysis. *C*, MM6 cells (5×10^5 cells/mL) transfected with IRAK-M siRNA or control siRNA (25 nmol/L) were incubated with HCV core protein (10 μg/mL) for 24 h and then stimulated with HCV core protein (5 μg/mL) or lipopolysaccharide (LPS) (1 μg/mL) for another 24 h. Culture supernatants were analyzed for interleukin 6 (IL-6) production. *D*, Nuclear extracts were isolated from MM6 cells transfected with siRNAs after stimulation with HCV core protein and LPS for 1 h. The expression of p65 was determined. Results shown are representative of 2 experiments (panel *D*) or 3 experiments (panels *A*, *B*, and *C*) and are expressed as mean $\pm$ standard deviation. $^{**}P < .01$, compared with cells preincubated with core protein and treated with control siRNA.

ability to drive Th17 differentiation in response to TLR ligands. To address this issue, we examined allospecific adaptive immune responses in naive $CD4^+$ T cells cocultured with monocytes isolated from HCV-infected patients or healthy control subjects in the presence of TLR ligands. Figure 7 shows that IL-17 production is markedly enhanced during antigen presentation by monocytes isolated from healthy control subjects in the presence of core protein, Pam_3CSK4, and lipopolysaccharide. Thus, the stimulation of TLR signaling in monocytes leads to increased production of IL-17 by $CD4^+$ T cells. In contrast, this enhancement of IL-17 production was absent in $CD4^+$ T cells stimulated by monocytes isolated from HCV-infected patients. Therefore, alloantigen presentation by monocytes from HCV-infected patients decreases IL-17 production by T cells in the presence of TLR ligands. Interestingly, IFN-γ production was similarly enhanced by alloantigen presentation by monocytes from both healthy control subjects and HCV-infected patients in the presence of TLR ligands. Furthermore, this reduction of IL-17 production was not due to counter-regulation of immunosuppressive cytokines, because the production of IL-10 or TGF-β was not increased by coculture with monocytes isolated from HCV-infected patients (Figure 7) (data not shown). Therefore, these data suggest that impaired production of IL-6 by monocytes isolated from HCV-infected patients is associated with a defective IL-17 response by $CD4^+$ T cells in the presence of TLR ligands.

DISCUSSION

This study demonstrates that activation of TLR2 by core protein induces not only homotolerance to subsequent TLR2 stimulation but also cross-tolerance to TLR4 stimulation. Consistent with this is the finding that monocytes isolated from HCV-infected patients show defective production of IL-6 after stimulation with TLR ligands, presumably due to chronic exposure to core protein. Impaired production of IL-6 by monocytes from HCV-infected patients is associated with reduced production of IL-17 by allogeneic T cells in the presence of TLR ligands. These results are supported by those of Villacres et al [28], who report a reduced IL-6 response to TLR ligands by PBMCs isolated from patients with HCV infection. They found that IL-6 production by PBMCs from patients with HCV in-

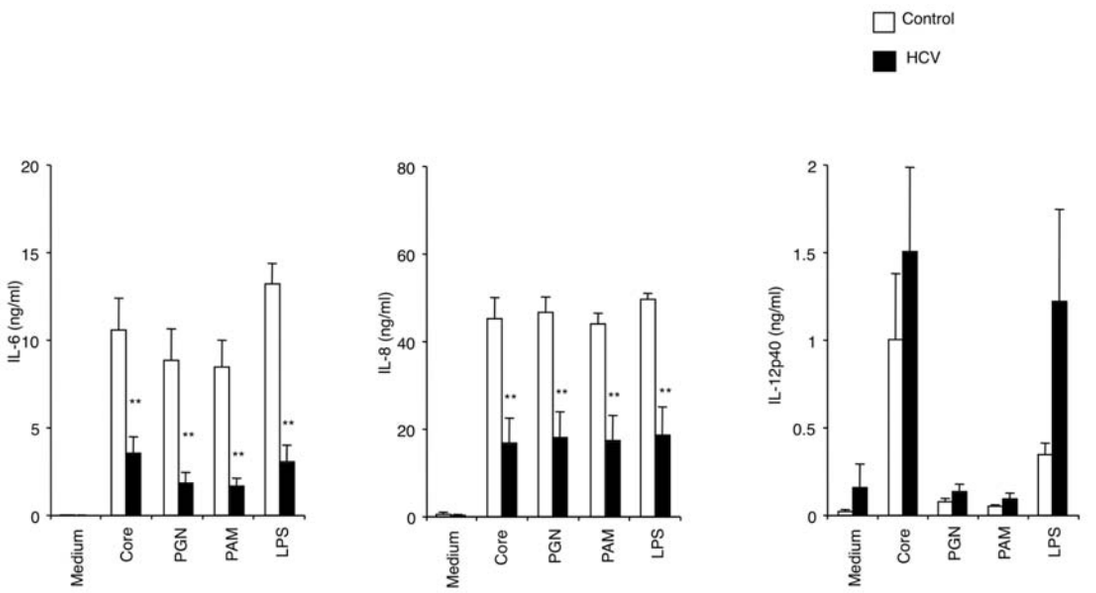


Figure 6. Production of interleukin 6 (IL-6) and interleukin 8 (IL-8) by monocytes isolated from patients infected with hepatitis C virus (HCV). Monocytes were isolated from 10 patients with HCV infection or 10 healthy control subjects. Monocytes (1×10^6/mL) were stimulated with HCV core protein (5 μg/mL), peptidoglycan (PGN) (10 μg/mL), Pam_3CSK4 (PAM) (10 μg/mL), or lipopolysaccharide (LPS) (1 μg/mL) for 24 h. Culture supernatants were analyzed for production of IL-6, IL-8, and interleukin 12p40 (IL-12p40). Results are expressed as mean ± standard deviation. $^{**}P < .01$, compared with monocytes from healthy control subjects.

fection was significantly decreased after stimulation with TLR4 ligands [28]. In addition, another report shows that DCs isolated from HCV-infected patients exhibit an impaired production of TNF-α in response to TLR4 ligands [29]. These results, taken together with our data, show impaired cytokine responses to TLR2 and TLR4 ligands in APCs isolated from HCV-infected patients.

We clearly show that antigen presentation by APCs isolated from HCV-infected patients affects T helper (Th) cell differentiation in the presence of TLR ligands. Chronic exposure to core protein results in the development of APCs with a limited ability to drive Th17 differentiation in the presence of TLR ligands. IL-17 (but not IFN-γ) production by allogeneic naive $CD4^+$ T cells was markedly reduced when T cells were cocultured with monocytes from HCV-infected patients and with TLR ligands. This selective impairment of the adaptive IL-17 response can be explained by profiles of cytokine production by these monocytes. IL-6 production induced by core protein and TLR ligands was significantly reduced in monocytes from HCV-infected patients compared with those from healthy control subjects, whereas IL-12p40 production was comparable in monocytes from both populations. Consistent with the results of the patient study (Figure 6), preincubation of APCs with core protein results in reduced production of IL-6 (but not IL-12p40) after restimulation with TLR ligands (Figure 2). Because IL-6 and IL-12 play an essential role for Th17 and Th1 differentiation, respectively [18, 27, 30], the defective IL-17 response seen in allogeneic $CD4^+$ T cells may be due to impaired IL-6 production by APCs from HCV-infected patients. Thus, chronic exposure to core protein appears to impair the adaptive IL-17 response (through the development of APCs with a limited ability to produce IL-6 after stimulation with TLR ligands) without affecting adaptive IFN-γ or TGF-β responses. However, it should be noted that we cannot exclude the involvement of TGF-β in reduced IL-17 production by $CD4^+$ T cells in the presence of TLR ligands and monocytes from HCV-infected patients. Rowan et al [31] show the indispensable role played by virus-induced TGF-β in the suppression of HCV-specific Th17 cells. This discrepancy regarding the role played by TGF-β may be explained by the differences in target antigens, responses to virus-specific antigens [31] or to allogeneic antigens, or by the difference in types of TGF-β tested—bioactive form [31], cell-surface, [31] or total.

Activation of NF-κB is impaired in APCs prestimulated with core protein after subsequent restimulation with TLR ligands. Nuclear translocation of p65 and p50 is reduced in cells prestimulated with core protein, whereas the translocation of c-Rel is not. Impaired nuclear translocation of p65 and p50 is responsible for a marked decrease in production of IL-6, because transcription of IL-6 is mediated by activation of the p65-p50 heterodimer [32]. In contrast, APCs that were prestimulated with core protein produced comparable levels of IL-12p40,

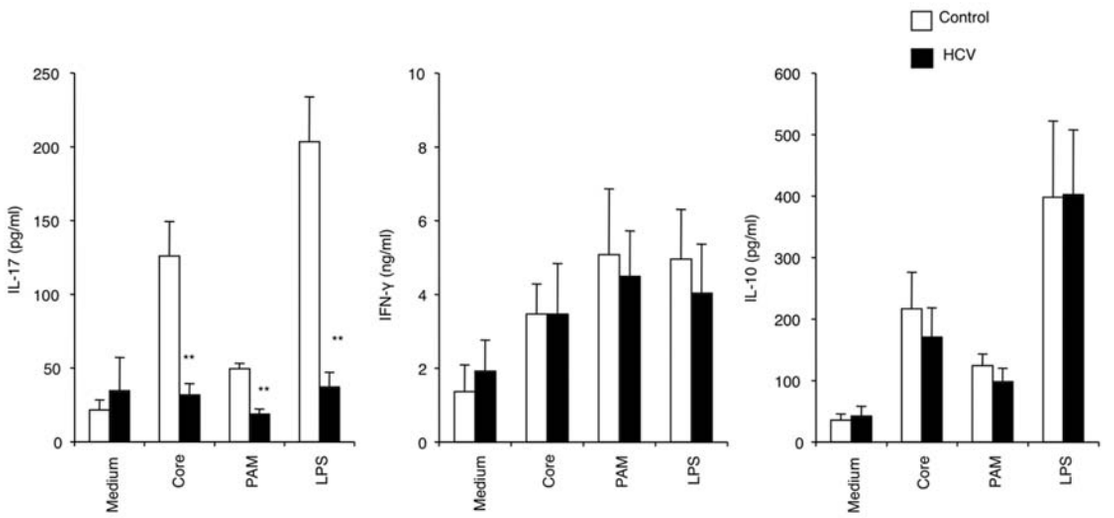


Figure 7. Production of interleukin 17 (IL-17) by allogeneic naive CD4+ T cells in the presence of Toll-like receptor (TLR) ligands and monocytes from patients infected with hepatitis C virus (HCV). Naive CD4+ T cells were isolated from the peripheral blood of healthy control subjects. Naive CD4+ T cells (1×10^6 cells/mL) were cocultured with peripheral blood monocytes (1×10^6 cells/mL) from 8 HCV-infected patients and 8 healthy control subjects in the presence of HCV core protein (5 μg/mL), Pam_3CSK4 (PAM) (10 μg/mL), or lipopolysaccharide (LPS) (1 μg/mL) for 7 days. Culture supernatants were analyzed for production of interleukin 17 (IL-17), interferon γ (IFN-γ), and interleukin 10 (IL-10). Results are expressed as means $\pm$ standard deviation. $^{**}P < .01$, compared with culture with monocytes from healthy control subjects.

the transcription of which depends on activation of the c-Rel subunit [21]. Therefore, preexposure of APCs to core protein results in reduced production of IL-6 because of the impaired nuclear translocation of p65 and p50 subunits. Impaired activation of NF-κB by prestimulation with core protein is associated with up-regulation of IRAK-M. Our results show that core protein–mediated activation of TLR2 leads to IRAK-M expression and that knockdown of IRAK-M expression by specific siRNA restores production of IL-6 by APCs prestimulated with core protein. Because IRAK-M is one of the most important negative regulators in TLR signaling [15], these data suggest that IRAK-M expression, induced by core protein–mediated TLR2 activation, modulates the cytokine responses mediated by multiple TLR ligands by the inhibition of NF-κB activation. However, it should be noted that transfection of IRAK-M siRNA did not completely restore the production of IL-6 by APCs prestimulated with core protein. Thus, other mechanisms of negative regulation of TLR signaling may also operate in the induction and maintenance of homotolerance and cross-tolerance by HCV core protein.

The impaired production of proinflammatory cytokines mediated by TLR2 and TLR4 might be involved in persistent infection by HCV. In fact, the activation of TLR2 and TLR4 plays a protective role in the case of respiratory syncytial virus and cytomegalovirus infection [33]. However, the reduction in IL-6 production by monocytes isolated from HCV-infected patients did not correlate with the HCV load in the serum (data not shown). This finding may be explained by the fact that the doses of core protein used in this study are much higher than those in the serum of HCV-infected patients. Indeed, serum levels of IL-6 are comparable between HCV-infected patients and healthy control subjects [34, 35]. Similarly, Shiina et al [36] report that infectious cell culture-produced HCV did not inhibit TLR4-mediated IL-6 production by DCs, which suggests that the dose of core protein in this system is not enough to cause cross-tolerance.

Given the fact that TLR2 and TLR4 play critical roles in host defense against microbial infection [12], and that Th17 cells are involved in host defense against bacterial infection [37], our results suggest that TLR2 activation by core protein may contribute to an increased susceptibility to microbial infection in individuals with chronic HCV infection. However, most patients with HCV infection are asymptomatic, although bacterial infections are more common among HCV-infected patients than among those without HCV infection [9–11]. Thus, impaired proinflammatory responses through TLRs might be compensated by other mechanisms in patients with HCV infection. In this regard, Foster et al [38] report that proinflammatory cytokine responses and antimicrobial effectors are differently regulated by TLR-induced chromatin modifications. Therefore, it is possible that antimicrobial effectors rather than proinflammatory cytokines play an important role in host defense against bacterial infection in HCV-infected patients.

References

1. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med **2001**; 345:41–52.
2. Dustin LB, Rice CM. Flying under the radar: the immunobiology of hepatitis C. Annu Rev Immunol **2007**; 25:71–99.
3. Keller BC, Johnson CL, Erickson AK, Gale M, Jr. Innate immune evasion by hepatitis C virus and West Nile virus. Cytokine Growth Factor Rev **2007**; 18:535–544.
4. Foy E, Li K, Sumpter R Jr, et al. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. Proc Natl Acad Sci U S A **2005**; 102:2986–2991.
5. Meylan E, Curran J, Hofmann K, et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature **2005**; 437:1167–1172.
6. Li K, Foy E, Ferreon JC, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proc Natl Acad Sci U S A **2005**; 102:2992–2997.
7. Loo YM, Owen DM, Li K, et al. Viral and therapeutic control of IFN-beta promoter stimulator 1 during hepatitis C virus infection. Proc Natl Acad Sci U S A **2006**; 103:6001–6006.
8. Dienstag JL, McHutchison JG. American Gastroenterological Association technical review on the management of hepatitis C. Gastroenterology **2006**; 130:231–264.
9. El-Serag HB, Anand B, Richardson P, Rabeneck L. Association between hepatitis C infection and other infectious diseases: a case for targeted screening? Am J Gastroenterol **2003**; 98:167–174.
10. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet **2008**; 371:838–851.
11. Riordan SM, Williams R. The intestinal flora and bacterial infection in cirrhosis. J Hepatol **2006**; 45:744–757.
12. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol **2004**; 4:499–511.
13. Dolganiuc A, Oak S, Kodys K, et al. Hepatitis C core and nonstructural 3 proteins trigger Toll-like receptor 2-mediated pathways and inflammatory activation. Gastroenterology **2004**; 127:1513–1524.
14. Duesberg U, von dem Bussche A, Kirschning C, Miyake K, Sauerbruch T, Spengler U. Cell activation by synthetic lipopeptides of the hepatitis C virus (HCV)–core protein is mediated by Toll-like receptors (TLRs) 2 and 4. Immunol Lett **2002**; 84:89–95.
15. Liew FY, Xu D, Brint EK, O'Neill LA. Negative regulation of Toll-like receptor-mediated immune responses. Nat Rev Immunol **2005**; 5:446–458.
16. Fichtner-Feigl S, Strober W, Kawakami K, Puri RK, Kitani A. IL-13 signaling through the IL-13α2 receptor is involved in induction of TGF-beta1 production and fibrosis. Nat Med **2006**; 12:99–106.
17. Watanabe T, Asano N, Murray PJ, et al. Muramyl dipeptide activation of nucleotide-binding oligomerization domain 2 protects mice from experimental colitis. J Clin Invest **2008**; 118:545–559.
18. Watanabe T, Katsukura H, Shirai Y, et al. A liver tolerates a portal antigen by generating CD11c+ cells, which select Fas ligand+ Th2 cells via apoptosis. Hepatology **2003**; 38:403–412.
19. Watanabe T, Yoshida M, Shirai Y, et al. Administration of an antigen at a high dose generates regulatory CD4+ T cells expressing CD95 ligand and secreting IL-4 in the liver. J Immunol **2002**; 168:2188–2199.
20. Watanabe T, Katsukura H, Chiba T, Kita T, Wakatsuki Y. Periportal and sinusoidal liver dendritic cells suppressing T helper type 1-mediated hepatitis. Gut **2007**; 56:1445–1451.
21. Watanabe T, Kitani A, Murray PJ, Strober W. NOD2 is a negative regulator of Toll-like receptor 2-mediated T helper type 1 responses. Nat Immunol **2004**; 5:800–808.
22. Sato S, Nomura F, Kawai T, et al. Synergy and cross-tolerance between Toll-like receptor (TLR) 2- and TLR4-mediated signaling pathways. J Immunol **2000**; 165:7096–7101.
23. Siedlar M, Frankenberger M, Benkhart E, et al. Tolerance induced by the lipopeptide Pam3Cys is due to ablation of IL-1R–associated kinase-1. J Immunol **2004**; 173:2736–2745.
24. Dobrovolskaia MA, Medvedev AE, Thomas KE, et al. Induction of in vitro reprogramming by Toll-like receptor (TLR)2 and TLR4 agonists in murine macrophages: effects of TLR "homotolerance" versus "heterotolerance" on NF-kappa B signaling pathway components. J Immunol **2003**; 170:508–519.
25. Jacinto R, Hartung T, McCall C, Li L. Lipopolysaccharide- and lipoteichoic acid-induced tolerance and cross-tolerance: distinct alterations in IL-1 receptor-associated kinase. J Immunol **2002**; 168:6136–6141.
26. Kobayashi K, Hernandez LD, Galan JE, Janeway CA Jr, Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. Cell **2002**; 110:191–202.
27. McGeachy MJ, Cua DJ. Th17 cell differentiation: the long and winding road. Immunity **2008**; 28:445–453.
28. Villacres MC, Literat O, DeGiacomo M, Du W, Frederick T, Kovacs A. Defective response to Toll-like receptor 3 and 4 ligands by activated monocytes in chronic hepatitis C virus infection. J Viral Hepat **2008**; 15: 137–144.
29. Rodrigue-Gervais IG, Jouan L, Beaule G, et al. Poly(I:C) and lipopolysaccharide innate sensing functions of circulating human myeloid dendritic cells are affected in vivo in hepatitis C virus-infected patients. J Virol **2007**; 81:5537–5546.
30. Watanabe T, Kitani A, Murray PJ, Wakatsuki Y, Fuss IJ, Strober W. Nucleotide binding oligomerization domain 2 deficiency leads to dysregulated TLR2 signaling and induction of antigen-specific colitis. Immunity **2006**; 25:473–485.
31. Rowan AG, Fletcher JM, Ryan EJ, et al. Hepatitis C virus-specific Th17 cells are suppressed by virus-induced TGF-beta. J Immunol **2008**; 181: 4485–4494.
32. Matsusaka T, Fujikawa K, Nishio Y, et al. Transcription factors NF-IL6 and NF-kappa B synergistically activate transcription of the inflammatory cytokines, interleukin 6 and interleukin 8. Proc Natl Acad Sci U S A **1993**; 90:10193–10197.
33. Saito T, Gale M Jr. Principles of intracellular viral recognition. Curr Opin Immunol **2007**; 19:17–23.
34. Feldmann G, Nischalke HD, Nattermann J, et al. Induction of interleukin-6 by hepatitis C virus core protein in hepatitis C-associated mixed cryoglobulinemia and B-cell non-Hodgkin's lymphoma. Clin Cancer Res **2006**; 12:4491–4498.
35. Grungreiff K, Reinhold D, Ansorge S. Serum concentrations of sIL-2R, IL-6, TGF-beta1, neopterin, and zinc in chronic hepatitis C patients treated with interferon-α. Cytokine **1999**; 11:1076–1080.
36. Shiina M, Rehermann B. Cell culture-produced hepatitis C virus impairs plasmacytoid dendritic cell function. Hepatology **2008**; 47:385–395.
37. Iwakura Y, Nakae S, Saijo S, Ishigame H. The roles of IL-17A in inflammatory immune responses and host defense against pathogens. Immunol Rev **2008**; 226:57–79.
38. Foster SL, Hargreaves DC, Medzhitov R. Gene-specific control of inflammation by TLR-induced chromatin modifications. Nature **2007**; 447:972–978.

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2010;40(Supplement 1)i19–i27
doi:10.1093/jjco/hyq123

Liver Cancer Working Group Report

Masatoshi Kudo[1,*], Kwang Hyub Han[2], Norihiro Kokudo[3], Ann-Lii Cheng[4], Byung Ihn Choi[5], Junji Furuse[6], Namiki Izumi[7], Joong-Won Park[8], Ronnie T. Poon[9] and Michiie Sakamoto[10]

[1]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Japan, [2]Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Republic of Korea, [3]Department of Hepatobiliary and Pancreatic Surgery, University of Tokyo Graduate School of Medecine, Tokyo, Japan, [4]Department of Oncology, National Taiwan University Hospital, Taipei, Taiwan, [5]Department of Radiology, Seoul National University College of Medicine, Seoul, Republic of Korea, [6]Department of Medical Oncology, Kyorin University School of Medicine, Japan, [7]Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan, [8]Center for Liver Cancer, National Cancer Center, Korea, [9]Department of Surgery, Queen Mary Hospital, University of Hong Kong, Hong Kong and [10]Department of Pathology, Keio University School of Medicine, Tokyo, Japan

*For reprints and all correspondence: Masatoshi Kudo, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, 377-2, Ohono-Higashi, Osaka-Sayama, Osaka, Japan.
E-mail: m-kudo@med.kindai.ac.jp

Hepatocellular carcinoma is a highly prevalent disease in many Asian countries, accounting for 75–80% of victims worldwide. The incidence of hepatocellular carcinoma varies enormously across Asia, but tends to follow the incidences of hepatitis B infection and liver cirrhosis. The incidence and etiology of hepatocellular carcinoma in Japan are different from the rest of Asia, but similar to that in Western countries because hepatitis C infection is the main etiological factor in Japan. Hepatitis B virus vaccination programs are showing great success in reducing hepatitis B virus-related hepatocellular carcinoma. Screening program improves detection of early hepatocellular carcinoma and has some positive impact on survival, but the majority of hepatocellular carcinoma patients in Asia still present with advanced hepatocellular carcinoma. Long-term outcomes following treatment of even early/intermediate or advanced disease are often unsatisfactory because of a lack of effective adjuvant and systemic therapies. Various clinical practice guidelines for hepatocellular carcinoma have been established and are in use. Clinical diagnosis of hepatocellular carcinoma by imaging diagnosis is replacing diagnosis of hepatocellular carcinoma by pathological confirmation. New imaging and treatment techniques are continuously being developed and guidelines should be updated every 3 or 4 years, incorporating new evidence. New molecularly targeted therapies hold great promise. Sorafenib is the first systemic therapy to demonstrate prolonged survival vs. the placebo in patients with advanced hepatocellular carcinoma. Various other new molecularly targeted agents are currently under investigation.

Key words: liver cancer – epidemiology – etiology – diagnosis – treatment

INTRODUCTION

The Liver Cancer Working Group report was divided into seven topics: (i) epidemiology and etiology in Asian countries; (ii) proportions of early, intermediate and advanced stages of hepatocellular carcinoma (HCC); (iii) surveillance systems and prediction of HCC development; (iv) recent developments in imaging diagnosis; (v) pathological development of early HCC, especially consensus between Asia and the West; (vi) current status of treatment strategies; (vii) future perspectives, especially in regard to sorafenib; and other molecularly targeted agents.

Epidemiology and Etiology

Liver cancer, or HCC, is endemic in Asia. It is expected that around 75–80% of HCC cases worldwide develop in Asia (Fig. 1) (1). In most Asian countries, HCC is ranked from number 1 to number 5 among the leading causes of death. In

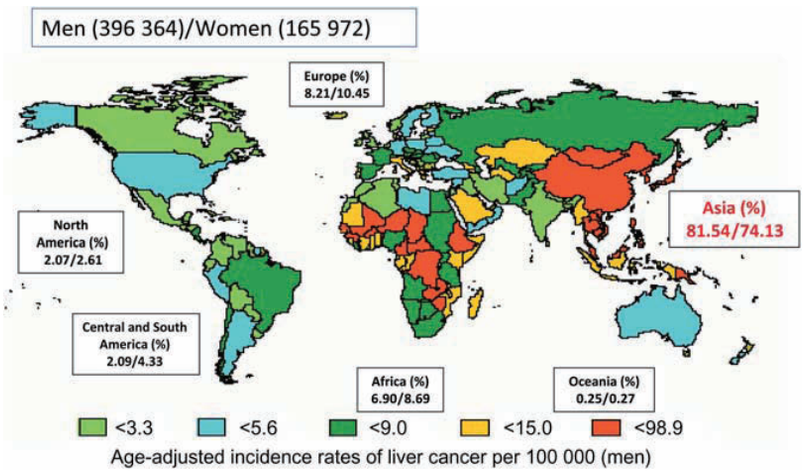


Figure 1. Liver cancer in the world (Curado et al. IARC Press, 2010).

Mainland China and Taiwan, the incidence of HCC has been increasing in the past 30 years, but in Japan, the incidence has been relatively stable during that period (2). In Korea, particularly in the male population, the incidence of HCC decreased slightly in the past 10 years. The primary etiological factor in Asia is hepatitis B. As exemplified by Korea, hepatitis B virus (HBV) accounts for 70–75% of HCC cases and hepatitis C virus (HCV) accounts for 10–15% (3). In Hong Kong, 80% of HCC cases are caused by HBV, and around 7% are caused by HCV. Japan is unique in the etiology of HCC in Asia because almost two-thirds of cases are caused by HCV and only 15% are related to HBV (2,4–6). Taiwan appears to be in between. In the early 1980s, HBV was the dominant cause of HCC in Taiwan, accounting for 88% (4), but in the past 30 years, HCV increased significantly and now accounts for more than 30%. HBV remains the predominant cause, but because of a vaccination program that was started in 1984, Taiwanese younger than 25 years old will have a carrier rate of around 1%. Thirty years from now, HBV-related HCC will decrease dramatically in Taiwan and in other countries that have adopted a nationwide HBV vaccination program (7). Regarding the age distribution of HCC, in all countries in which HBV is the dominant cause, the median age is around 55 years old. Statistics for Japan, which is characterized by HCV, show that the median age is about 10 years older.

In conclusion, HCC in the Asia-Pacific region accounts for 75–80% of victims worldwide. The incidence of HCC is on the rise in some countries, such as mainland China and Taiwan, but it is plateauing and decreasing slightly in some countries, like Japan. Except in Japan, HBV is the major etiology of HCC. The proportion of HCV has increased significantly in the past 30 years in Taiwan. Because of successful vaccination, the incidence of HBV-related HCC will decrease dramatically by 2040 (8).

Proportions of Early, Intermediate and Advanced HCC

There are various staging systems for HCC, with each system having its pros and cons and no consensus regarding which system is the best. The Barcelona Clinic of Liver Cancer, BCLC, system (9,10) is quite widely used in the West and in many clinical trials. The BCLC system stages patients into very early stage, early stage, intermediate stage, advanced stage and end stage according to the tumor size, vascular invasion, the tumor nodule number and the presence of metastasis. The BCLC system also provides a guideline for treatment according to the stage of HCC. Basically, patients with very early-stage or early-stage HCC are considered for curative treatment, either resection, liver transplantation or local ablation. Patients with intermediate-stage HCC, mainly those with multinodular disease, will be eligible for transarterial chemoembolization (TACE), and patients with advanced-stage disease showing portal invasion or distant metastasis will be considered for sorafenib or recruitment to clinical trials.

In addition to the BCLC, the Japanese TNM staging system (11) is quite widely used in Japan and Korea. This staging system takes into account three criteria for the T stage, i.e. whether the tumor is solitary or multiple, the tumor size, $\leq$2 cm or $>$2 cm, and the presence of any vascular or bile duct invasion. Patients are thus classified as T1, T2, T3 or T4. For N and M, it is similar to other TNM staging systems, based on the presence of lymph node or distant metastasis. By integrating Japanese TNM stage and Child–Pugh grade, Japan Integrated Staging system was developed (12) and widely used in Japan and Korea.

The current distribution of HCC based on the BCLC system is quite similar in Hong Kong and Korea, with about 30–40% of patients having early-stage disease, about 20–30% having intermediate-stage disease and about 30% having advanced-stage disease. In Japan, the proportion of early-stage HCC is very high: about 65%, whereas only 5% of

patients present with advanced-stage disease (5). Japan is thus quite different from the rest of the Asia-Pacific region, probably because of its very well-established surveillance system.

But even within a country, there can be a significant variation between regions, as exemplified by Taiwan. In northern Taiwan, about 58% of patients have early-stage HCC, whereas in the southern part, the rate is only 35.2%. This is probably related to differences in the popularity of surveillance due to cultural, social and economic differences between the populations in the north and south of Taiwan. Data generated in Japan and Korea, using the Japanese TNM staging system, are similar to the BCLC staging results and show that Japan has a higher number of patients with early-stage HCC compared with Korea.

The disease stage obviously affects the treatment modality. For early-stage cancers, curative treatments like surgery or ablation are generally implemented, whereas TACE is performed for intermediate-stage disease and systemic therapy for advanced disease. Comparison between Hong Kong and Japan shows a dominance of ablation and surgery in Japan, whereas in Hong Kong, the percentage of patients amenable to ablation is limited. Even for TACE, the proportion of patients is higher in Japan than in Hong Kong, where a large proportion of patients have advanced disease and receive systemic therapy. For early-stage disease, curative treatment is the first choice, and about 38% of patients in Hong Kong and 65% in Japan are amenable to curative treatments. For intermediate-stage HCC, the rates are 22% in Hong Kong and 30% in Japan, and for advanced-stage disease, the rates are 40% in Hong Kong and 5% in Japan.

BCLC staging has important predictive power for overall survival. Data for more than 3000 patients in Hong Kong show very good stratification of overall survival in terms of the stage. Survival data from Yonsei University (Korea) show a very similar stratification. For patients with early HCC, the 5-year survival rate is now more than 50%, whereas for patients with advanced-stage disease, the 5-year survival is $<5\%$, showing a great difference in the survival outcomes. In some countries, like Korea, evidence points to some recent improvement in the overall survival of HCC patients: comparison between 1993 and 2005 shows that the 5-year survival has improved from 10.7% to 18.9% in the most recent 5-year period.

In conclusion, there is a significant variation in the distribution of early, intermediate and advanced stages of HCC among Asia-Pacific countries, with the highest proportion of early HCC in Japan. Curative treatment for early-stage HCC is associated with the 5-year survival $>50\%$, while the prognosis of advanced-stage HCC remains dismal. These results underscore the importance of early diagnosis by means of surveillance of high-risk patients.

Surveillance Systems and Prediction of HCC

A Hong Kong study proved that a screening program can improve survival by increasing the chance of treatment in the screened group (13). Unfortunately, in Hong Kong, the percentage of patients with HCC diagnosed by screening is low, but it has increased slightly, from 29% in 1991–1997 to 33% in 1998–2004 (14). There is no government-funded surveillance program for HCC in Hong Kong or other parts of China. Korea, however, established a national surveillance program in 2003, with the target population being those over 40 years of age, with liver cirrhosis or an HBV or HCV carrier (15). Taiwan has a similar surveillance program in place, and a different testing interval is applied depending on whether the subject has cirrhosis or not: 3–6 months for cirrhosis, but 6–12 months for non-cirrhosis. There is no age limitation for surveillance of HBV carriers in Taiwan, but in Korea, the government recommends over 40 years. The surveillance program in Japan is slightly different: it selects super high-risk patients, meaning liver cirrhosis B or C, and applies a shorter interval for examination, every 3 or 4 months, and test for more tumor markers (three tumor markers, including AFP, AFP-L3 and DCP) (16,17). The surveillance programs in Korea and China prefer a 6-month interval. Japanese surveillance program also recommends CT or MRI every 6–12 months for improving sensitivity. Thus, there are some differences in HCC surveillance among Asia-Pacific countries, including the candidates for surveillance and the age limit for HBV carriers. As surveillance tools, ultrasonography and AFP are still the standards, but there is a need to know whether more tumor markers will improve the sensitivity. A study investigated whether the surveillance interval is important for improving the survival. The group with a surveillance interval of within 6 months showed better survival than that of more than 6 months.

It is important to predict the development of HCC by quantitative risk estimation. An individualized prediction model is possible by combining multiple risk factors into a comprehensive risk expression. A study identified eight independent risk factors, and a special formula was established to calculate the relative risk factors. This model enables identification of the high- and low-risk groups.

In conclusion, HCC surveillance can detect early tumors and increase the chance of a curative approach. All patients at risk of developing HCC with potentially curative treatment available are recommended for regular surveillance. At present, ultrasonography and the serum AFP test at 6-month intervals are the standard surveillance tools. To improve the detection rate of early-stage HCC, the benefit of additional tests and a shorter surveillance interval should be confirmed by a randomized clinical trial in Asia. The application of individualized prediction model to surveillance programs may improve the cost-effectiveness by focusing on the high-risk group.

Recent Developments in Imaging Diagnosis

Various clinical practice guidelines for HCC are being implemented around the world, including in Europe, Korea, America, Japan and the Asia-Pacific region. In accordance

with those guidelines, the use of dynamic imaging, such as contrast-enhanced ultrasound (US), CT and MRI, is increasing and becoming more important, whereas application of biopsy is decreasing. Angiography and fusion imaging are other imaging tools that are available for the diagnosis of HCC. These tools are based on different imaging techniques. US is the first step for imaging diagnosis of HCC in accordance with the guidelines. If a nodule is found by US examination, the next technique to be used depends on the size of the mass. For a nodule that is <1 cm in diameter, follow-up study is usually recommended. If the nodule is >2 cm in diameter, one further imaging examination, such as contrast-enhanced US, CT or MRI, is sufficient to make a diagnosis of HCC with specific findings. Specific findings consist of a hypervascular nature in the arterial phase of imaging, and a washout pattern in the equilibrium phase. Diagnosis of HCC by dynamic imaging (contrast-enhanced ultrasonography, CT or MRI) is based on the enhancement pattern according to time sequence or phase. Overt HCC shows high attenuation in the arterial phase, indicating the hypervascular nature of the tumor, iso-attenuation in the portal-venous phase and low attenuation in the equilibrium phase, indicating a rapid washout pattern. These comprise very specific findings for the diagnosis of HCC.

In the APASL Guideline 2009 for imaging diagnosis of HCC, US is a screening test, not a diagnostic test for confirmation. US can detect a nodule but cannot characterize it. However, contrast-enhanced US is as sensitive as dynamic CT or dynamic MRI for the diagnosis of HCC (18). When using a US contrast agent for the diagnosis of HCC, the arterial phase and equilibrium phase show a rapid wash-in and washout pattern, which are characteristic findings for overt HCC. Dynamic CT or dynamic MRI is recommended as a first-line diagnostic tool for HCC when a screening test is abnormal. The hallmark of HCC in a CT scan or MRI is the presence of arterial enhancement followed by washout of the tumor in the portal-venous and/or delayed phases. In the diagnostic algorithm for hypervascular masses, typical HCC can be diagnosed by imaging regardless of the size of the detected tumor if a typical vascular pattern—arterial enhancement with portal-venous washout—is obtained on dynamic CT, dynamic MRI or contrast-enhanced US. In the diagnostic algorithm for hypervascular nodules, US is the initial screening method. If a nodule is detected by US, the nodule is then characterized by dynamic CT or MRI. Further characterization is usually performed by Kupffer cell imaging, including Sonazoid-enhanced US, or gadolinium-ethoxybenzyl-diethylene triamine pentaacetic acid (Gd-EOB-DTPA) MRI (Fig. 2) (19). In the diagnostic algorithm for hypovascular masses, nodular lesions showing an atypical imaging pattern, such as iso- or hypovascularity in the arterial phase, or arterial hypervascularity alone without portal-venous washout, should undergo further examination or close follow-up (Fig. 3). Recently, new imaging techniques are being developed, including volume US using various contrast agents, US elastography (20), volume CT, dual energy CT for perfusion CT, diffusion-weighted MRI, MRI elastography, etc. The efficacy of these techniques in diagnosing HCC is being evaluated.

In conclusion, various clinical practice guidelines including diagnostic algorithm for HCC have been established and

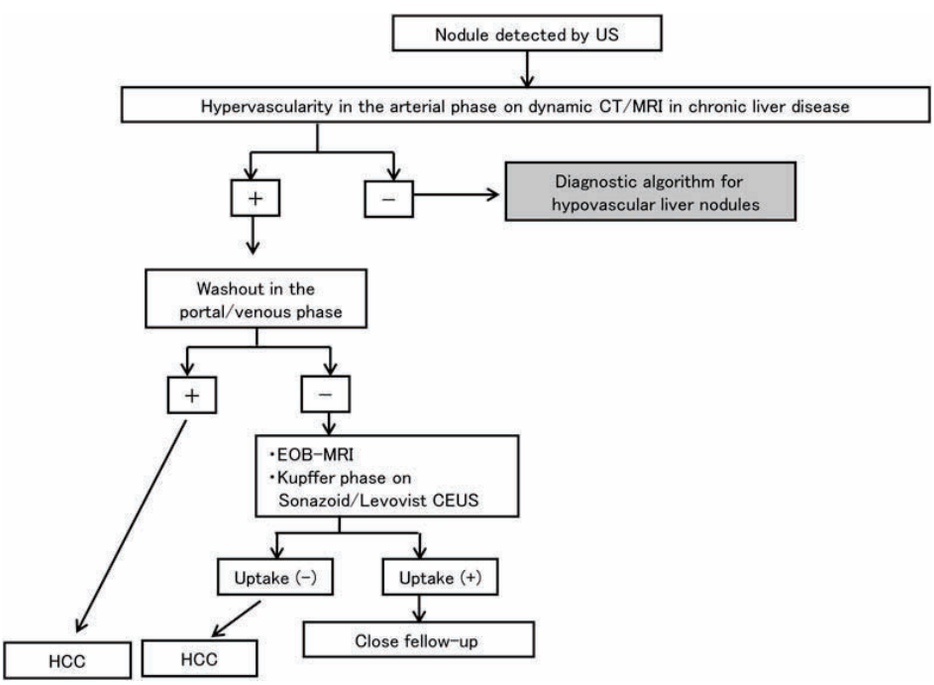


Figure 2. Diagnostic algorithm for hypervascular nodule (APASL Guideline). US, ultrasound; HCC, hepatocellular carcinoma.

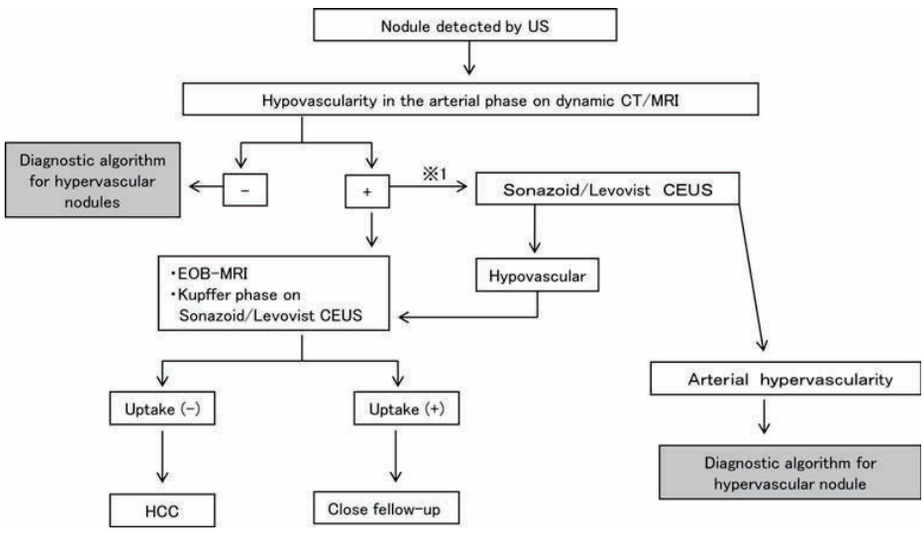


Figure 3. Diagnostic algorithm for hypovascular nodule (APASL Guideline). ※1: When the nodule is hypovascular on dynamic CT or dynamic MRI, Sonazoid-enhanced contrast US is recommended to confirm whether it is truly a hypovascular nodule.

are in use. Use of imaging diagnosis is increasing, whereas the use of biopsy is decreasing. New imaging techniques are continuously being developed. Practice guidelines should be updated to reflect the development of new imaging techniques.

Pathological Diagnosis of Early HCC

In 2009, pathologists from all over the world made great progress by reaching a consensus on the pathological diagnosis of early HCC. A consensus paper was published in the journal, *Hepatology* (21). The main topic of the consensus paper was histopathological definition of early HCC, together with premalignant lesions, dysplastic nodules and progressed HCC. Representative early HCC is a small, well-differentiated tumor, of vaguely nodular type. Microscopically, the border is unclear, and very well-differentiated cancer cells show a replacing growth pattern. They also frequently show stromal invasion, which is quite useful for making a diagnosis of cancer. However, histological atypia or histological alteration is usually very slight in early HCC, which is quite similar to the case of early cancers in other organs. Biopsy diagnosis of early HCC is especially difficult. In an example case, a slight increase in chromatin staining with substantial increase in the nuclear density is seen. Several standard techniques reveal slight changes or alterations in the tumor portion, such as a decrease in reticulin and a slight increase in proliferative activity. However, the use of some new markers, such as heat shock protein (HSP) 70, clearly highlights the tumor portion, making it more easily recognized. Greater use of tumor markers, including glypican 3 and HSP70, is likely and will increase the accuracy of diagnosis of early HCC.

Much has been learned about early HCC, but various problems remain. We know that cancer development is a multistep process, especially when there are cirrhotic changes. Early HCC grows very slowly and has a favorable outcome, whereas progressed, small HCC has a greater likelihood of showing intrahepatic spread and a worse prognosis. It is necessary to recognize that there is a gray zone between precancerous lesion and early HCC. Liver biopsy is recommended for small, equivocal lesions. Also, molecular markers are expected to raise the diagnostic accuracy, especially in the case of biopsy diagnosis of HCC. At the same time, controversy remains regarding which lesions should be examined by biopsy, and there is a risk of overdiagnosis of early cancer.

Current Treatment Strategies

Since 2001, when the Barcelona group published their consensus guideline, at least eight other guidelines have been released worldwide regarding the diagnosis and/or treatment of HCC. In 2003, the Korean guidelines were published, and in 2005, the Japanese guidelines for evidence-based clinical practice (Fig. 4) (16) were released. Clinical practice guidelines should be evidence-based, and they should represent the consensus of expert committees. Sometimes, it is very difficult to reach a consensus in the field of HCC. Guidelines must also take into consideration the socioeconomic status and current daily practice in the country or region. The socioeconomic background and daily practice regarding HCC were compared among Europe and the USA, Asia (Korea) and Japan. The major etiology of HCC is HCV in Europe, the USA and Japan, but HBV in Asia (Korea). A surveillance system has been established in Japan, is being

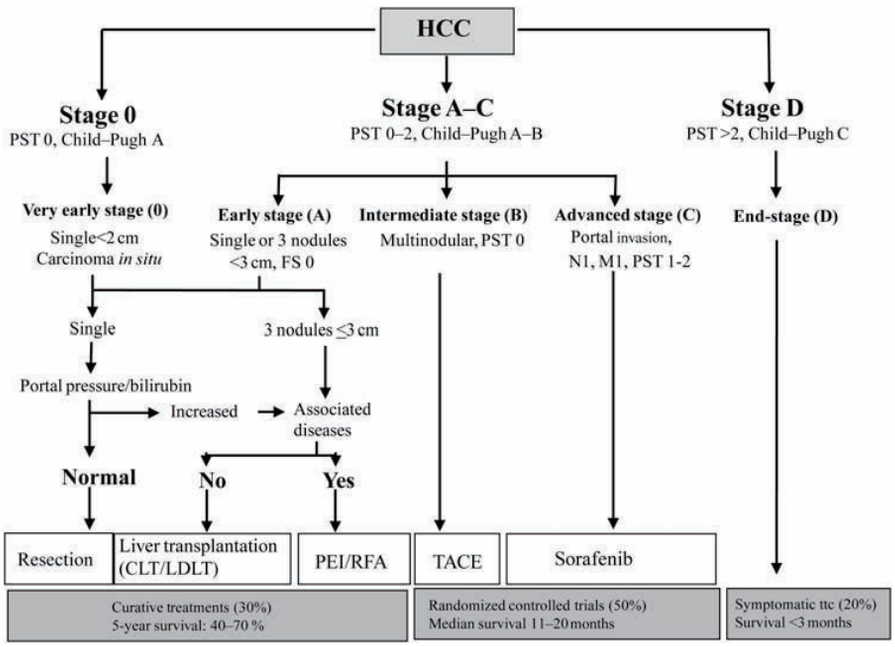


Figure 4. BCLC staging [Llovet et al. (10)]. BCLC, Barcelona Clinic of Liver Cancer; PST, performance status; CLT, cadaveric liver transplantation; LDLT, living donor liver transplantation; PEI, percutaneous ethanol injection; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

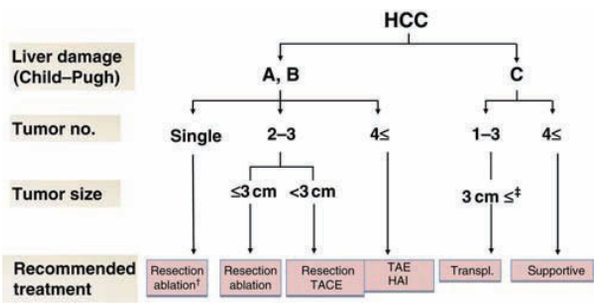


Figure 5. EBM-based algorithm for HCC treatment (J-HCC Guidelines 2009). Resection or transarterial chemoembolization (TACE) may be selected for liver damage A patients with vascular invasion. Chemotherapy may be selected for extrahepatic HCC. LT is only for ≤65 years old. †Recommended for Child B; ‡<2 cm for solitary lesion. HAI, hepatic arterial infusion.

developed in Asia (Korea), but does not exist in the Western countries. As a result, most HCC patients are diagnosed in an early stage in Japan, but at a very advanced stage in Western countries. As tumor markers, only AFP is measured in Western countries, whereas three tumor markers are measured in Japan. The risk of treatment of HCC must also be considered. The mortality of liver resection is as high as 4–5% in Western countries, but only 0.7% in Japan. Brain-dead donors for liver transplantation are very rare in Japan, but common in Western countries (22). These factors must be considered for development of treatment strategies for HCC.

The BCLC guidelines to staging and treatment of HCC are probably the most popular treatment algorithm in Western countries, but not in Asia. The Japanese guidelines were just revised in 2009, are very simple and cover a majority of early- and intermediate-stage HCC patients (Fig. 5). A Japanese consensus-based algorithm for HCC covers even very advanced-stage HCC, including patients with extrahepatic spread and vascular invasion (Fig. 6) (17,19). Sorafenib is recommended for such advanced disease with good liver function, and an ongoing trial is evaluating its use as an adjuvant therapy. The Korean guideline for management of HCC was initially published in 2003, after which they accumulated evidence, held a nationwide forum for revision of the guidelines and created a revision committee. As a result, their updated guidelines were published in 2009 (23). The algorithm for the Korean HCC treatment plan lists hepatic resection, liver transplantation, radiofrequency ablation and ethanol injection as curative treatments. There is no evidence showing which treatment is superior for cure of HCC in each patient, so the guideline recommends that the physician decide which treatment will be used. The APASL Consensus on Treatment of HCC (24) was published in 2010 and may be utilized in the Asian region.

In conclusion, several practice guidelines presenting treatment strategies for HCC in Asia have been developed. They were created based on evidence-based medicine methodology and consensus among experts in the region. They also reflect the socioeconomic status and current daily practice in the region. A number of ongoing clinical trials aim to

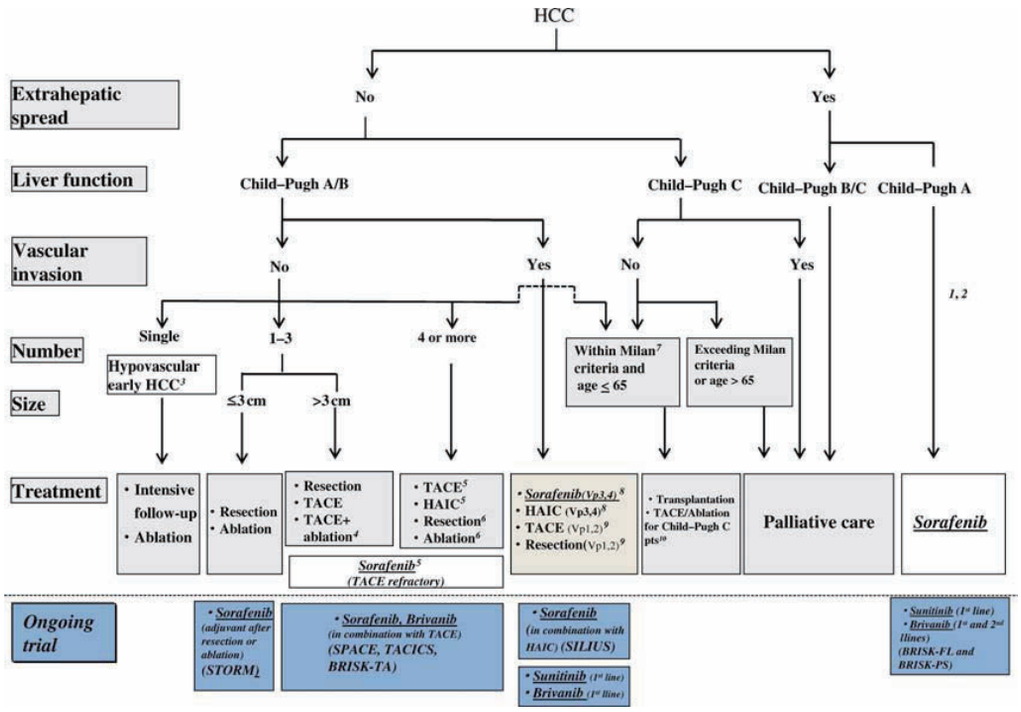


Figure 6. Consensus-based treatment algorithm for HCC proposed by Japan Society of Hepatology (JSH) 2009 revised in 2010. *1*, Treatment should be performed as if extrahepatic spread is negative, when extrahepatic spread is not regarded as a prognostic factor. *2*, Sorafenib is the first choice of treatment in this setting as a standard of care. *3*, Intensive follow-up observation is recommended for hypovascular nodules by the Japanese Evidence-Based Clinical Practice Guidelines. However, local ablation therapy is frequently performed in the following case: (i) when the nodule is diagnosed pathologically as early HCC, (ii) when the nodules show decreased uptake on gadolinium-ethoxybenzyl-diethylene triamine pentaacetic acid or (iii) when the nodules show decreased portal flow by CTAP, since these nodules are known to frequently progress to the typical advanced HCC. *4*, Even for HCC nodules exceeding 3 cm in diameter, combination therapy of TACE and ablation is frequently performed when resection is not indicated. *5*, TACE is the first choice of treatment in this setting. Hepatic arterial infusion chemotherapy (HAIC) using an implanted port is also recommended for TACE refractory patients. The regimen for this treatment is usually low-dose FP (5FU + CDDP) or intra-arterial 5FU infusion combined with systemic interferon therapy. Sorafenib is also recommended for TACE refractory patients. *6*, Resection is sometimes performed even when numbers of nodules are over 4. Furthermore, ablation is sometimes performed in combination with TACE. *7*, Milan criteria: Tumor size $\leq$3 cm and tumor numbers $\leq$3; or solitary tumor $\leq$5 cm. Even when liver function is good (Child–Pugh A/B), transplantation is sometimes considered for frequently recurring HCC patients. *8*, Sorafenib and HAIC are recommended for HCC patients with Vp3 (portal invasion at the first portal branch) or Vp4 (portal invasion at the main portal branch). *9*, Resection and TACE are frequently performed when portal invasion is minimum such as Vp1 (portal invasion at the third or more peripheral portal branch) or Vp2 (portal invasion at the second portal branch). *10*, Local ablation therapy or subsegmental TACE is performed even for Child–Pugh C patients when transplantation is not indicated when there is no hepatic encephalopathy, no uncontrollable ascites and a low bilirubin level ($<$3.0 mg/dl). However, it is regarded as an experimental treatment since there is no evidence of its survival benefit in Child–Pugh C patients. A prospective study is necessary to clarify this issue.

generate evidence for a better treatment algorithm. Guidelines should be updated every 3 or 4 years, incorporating new evidence.

Future Perspectives, Especially In Regard To Sorafenib

There was no established systemic chemotherapy for HCC. However, sorafenib has become a standard systemic treatment for advanced HCC. This section addresses the future perspectives for sorafenib and beyond sorafenib. Two randomized control studies have shown the survival benefit of sorafenib in advanced HCC patients with good liver function of Child–Pugh A. The SHARP trial (25), carried out mainly in European countries, and an Asia-Pacific trial (26) both showed that sorafenib provides a survival benefit in advanced HCC patients. Both trials yielded similar hazard ratio of 0.69 and 0.68, respectively, in favor of sorafenib over placebo. Other published reports on sorafenib for HCC include a Phase II trial conducted in Western countries (27), a Phase I Japanese study (28), a Korean study (29) and a Phase 2 Hong Kong study (30). The studies had various differences in patient background, such as involvement of HBV, HCV or others, liver function of Child–Pugh A and B, and the ECOG performance status. Those differences affected the survival outcomes in the four studies like outcomes after other treatment modalities.

Although sorafenib has become a standard systemic treatment for advanced HCC, there are still issues to be investigated with regard to this agent, including its efficacy and safety in patients with Child–Pugh B moderate liver

function, combination therapy with other treatment methods, and the need to identify predictive factors and markers for sorafenib. Various studies are currently attempting to elucidate those issues. The Phase III STORM global trial will evaluate sorafenib as an adjuvant therapy after surgery or radiofrequency ablation. A Japanese Phase II study will evaluate the efficacy and safety of sorafenib in patients with Child–Pugh A and B, with investigation of biomarkers. A global trial of combination of sorafenib with TACE is ongoing, while two Japanese Phase I studies of combination of sorafenib with hepatic arterial infusion are in progress (19). Arterial infusion chemotherapy is a very common and useful treatment in Japan (31), and one of these studies combines sorafenib with cisplatin, whereas the other combines sorafenib with 5-FU and cisplatin. It is anticipated that these trials will lead to Phase III studies.

Other Molecularly Targeted Agents

Sorafenib is the first systemic therapy approved for advanced-stage HCC, and widely used. Sorafenib prolongs time to progression and overall survival in patients with advanced HCC; however, predictive factors are unknown at the present. Good responders show a good response, but how can they be identified in advance? Researchers are currently looking for biomarkers that will identify good responders and lead to modification of the treatment algorithm. Also, a 'good response' has limitations. How can a 'complete response' be attained? Combination therapy and some adjuvant treatment, after palliative or curative treatment, will be needed. There are also many poor responders. How can a poor response be overcome? Second-line agents are necessary, as is combination therapy. Various targeted agents in addition to sorafenib are under development for HCC. They include brivanib, bevacizumab, cediranib, erlotinib, gefitinib, lapatinib, RAD001, sunitinib, thalidomide and TSU-68. These agents have similar yet slightly different mechanisms of action. The results of various clinical studies of these molecular targeted therapy agents were summarized in *Hepatology* (32). The results look good, and many Phase II and Phase III trials are ongoing. The trials can be categorized into three types: first-line or combination studies, second-line studies and adjuvant studies.

First-line or combination studies are being carried out as Phase III trials of sunitinib vs. sorafenib (terminated in 2010 because of severe adverse effect); brivanib vs. sorafenib; lilifanib vs. sorafenib; erlotinib plus sorafenib vs. sorafenib; and erlotinib plus bevacizumab vs. sorafenib. The results of these trials should be available in 2 or 3 years. There are also many first-line Phase II studies. There are two second-line Phase II studies, of brivanib vs. the placebo and RAD001 vs. the placebo, for patients who failed to respond to sorafenib. There are three Phase III adjuvant studies. The STORM study investigates sorafenib vs. placebo after resection or ablation. A second adjuvant study investigated sorafenib vs. placebo after TACE; this is already finished and the results were presented at ASCO-GI in 2010 (33). The third Phase III adjuvant study compares brivanib vs. placebo after TACE. In a first-line Phase II study of brivanib, 46% of the patients showed stable disease, and in the second-line Phase II study, 43% showed stable disease (34,35). These results were promising, and at least three trials are now ongoing for brivanib.

In conclusion, molecularly targeted therapy (MTT) has emerged as a promising approach for advanced HCC. Sorafenib impacted on MTT agents in HCC, but the benefits of sorafenib were reported to be relatively modest. Several MTT agents for first- and second-line treatments are undergoing clinical trials. The advantages of MTT agents are being explored in combination treatments as well as adjuvant therapy with resection, local ablation, radiation, hepatic arterial infusion chemotherapy and TACE.

CONCLUSION

HCC is a highly prevalent disease in many Asian countries and incidence of HCC varies enormously across Asia, but tends to follow incidences of hepatitis B infection and liver cirrhosis. Incidence and etiology of HCC in Japan is different from the rest of Asia, but similar to Western countries since hepatitis C infection is the main etiological factor. Screening program improves detection of early HCC and has some positive impact on survival, but the majority of HCC patients in Asia still present with advanced HCC. Long-term outcomes following treatment of early, intermediate or advanced disease are still unsatisfactory because of lack of effective adjuvant or systemic therapies. Sorafenib is the first systemic therapy to demonstrate prolonged survival vs. placebo in patients with advanced HCC. New molecular targeting therapies hold great promise. Many new agents are under investigation and their results are awaited.

Conflict of interest statement

The author, Joong-Won Park, participated in phase II and phase III clinical studies sponsored by Bristol-Myers Squibb, Pfizer Inc., Bayer Healthcare and Bukwang Pharmaceutical Co. He is also a member of BMS Brivanib study steering committee, Pfizer Sunitinib advisory committee, and Bukwang Pharmacetutical Co. advisory committee.

References

1. Curado MP, Edwards B, Shin HR, Storm H, Ferlay J. Cancer Incidence in Five Continents. France: IARC Scientific Publications 2010.
2. Chung H, Ueda T, Kudo M. Changing trends in hepatitis C infection over the past 50 years in Japan. *Intervirology* 2010;53:39–43.
3. Han KH, Kim JK. Liver cancer in Korea. *Hepatol Res* 2007;37(Suppl. 2):S106–9.
4. Yu MC, Yuan JM, Govindarajan S, Ross RK. Epidemiology of hepatocellular carcinoma. *Can J Gastroenterol* 2000;14:703–9.

5. Ikai I, Arii S, Okazaki M, Okita K, Omata M, Kojiro M, et al. Report of the 17th Nationwide Follow-up Survey of Primary Liver Cancer in Japan. *Hepatol Res* 2007;37:676–91.
6. Kim SR, Kudo M, Hino O, Han KH, Chung YH, Lee HS. Epidemiology of hepatocellular carcinoma in Japan and Korea. A review. *Oncology* 2008;75(Suppl. 1):13–6.
7. Yuen MF, Hou JL, Chutaputti A. Hepatocellular carcinoma in the Asia Pacific region. *J Gastroenterol Hepatol* 2009;24:346–53.
8. Ni YH, Huang LM, Chang MH, Yen CJ, Lu CY, You SL, et al. Two decades of universal hepatitis B vaccination in Taiwan: impact and implication for future strategies. *Gastroenterology* 2007;132: 1287–93.
9. Llovet JM, Bru C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. *Semin Liver Dis* 1999;19:329–38.
10. Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008;100:698–711.
11. Minagawa M, Ikai I, Matsuyama Y, Yamaoka Y, Makuuchi M. Staging of hepatocellular carcinoma: assessment of the Japanese TNM and AJCC/UICC TNM systems in a cohort of 13,772 patients in Japan. *Ann Surg* 2007;245:909–22.
12. Kudo M, Chung H, Haji S, Osaki Y, Oka H, Seki T, et al. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004;40:1396–405.
13. Yuen MF, Cheng CC, Lauder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *Hepatology* 2000;31:330–5.
14. Chan AC, Poon RT, Ng KK, Lo CM, Fan ST, Wong J. Changing paradigm in the management of hepatocellular carcinoma improves the survival benefit of early detection by screening. *Ann Surg* 2008;247:666–73.
15. Amarapurkar D, Han KH, Chan HL, Ueno Y. Application of surveillance programs for hepatocellular carcinoma in the Asia-Pacific Region. *J Gastroenterol Hepatol* 2009;24:955–61.
16. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008;38:37–51.
17. Kudo M, Okanoue T. Management of hepatocellular carcinoma in Japan: consensus-based clinical practice manual proposed by the Japan Society of Hepatology. *Oncology* 2007;72:S2–15.
18. Hatanaka K, Kudo M, Minami Y, Ueda T, Tatsumi C, Kitai S, et al. Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. *Intervirology* 2008;51:S61–9.
19. Kudo M. The 2008 Okuda lecture: management of hepatocellular carcinoma: from surveillance to molecular targeted therapy. *J Gastroenterol Hepatol* 2010;25:439–52.
20. Tatsumi C, Kudo M, Ueshima K, Kitai S, Ishikawa E, Yada N, et al. Non-invasive evaluation of hepatic fibrosis for type C chronic hepatitis. *Intervirology* 2010;53:76–81.
21. Kojiro M, Wanless I, Alves V, Badve S, Balabaud C, Bedossa P, et al. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. *Hepatology* 2009;49:658–64.
22. Todo S, Furukawa H. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. *Ann Surg* 2004;240:451–9, discussion 9–61.
23. Practice guidelines for management of hepatocellular carcinoma 2009. *Korean J Hepatol* 2009;15:391–423.
24. Omata M, Lesmana L, Tateishi R, Chen P, Lin S, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int* 2010; Epub ahead of print.
25. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378–90.
26. Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, et al. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009;10:25–34.
27. Abou-Alfa GK, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, et al. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006;24:4293–300.
28. Furuse J, Ishii H, Nakachi K, Suzuki E, Shimizu S, Nakajima K. Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. *Cancer Sci* 2008;99:159–65.
29. Shim JH, Park JW, Choi JI, Park BJ, Kim CM. Practical efficacy of sorafenib monotherapy for advanced hepatocellular carcinoma patients in a hepatitis B virus-endemic area. *J Cancer Res Clin Oncol* 2009;135:617–25.
30. Yau T, Chan P, Ng KK, Chok SH, Cheung TT, Fan ST, et al. Phase 2 open-label study of single-agent sorafenib in treating advanced hepatocellular carcinoma in a hepatitis B-endemic Asian population: presence of lung metastasis predicts poor response. *Cancer* 2009;115:428–36.
31. Arii S, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, et al. Management of hepatocellular carcinoma: Report of consensus meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010;40:667–85.
32. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. *Hepatology* 2008;48:1312–27.
33. Okita K, Imanaka K, Chiba N, Tak W, Nakachi K, Takayama T, et al. Phase III study of sorafenib in patients in Japan and Korea with advanced hepatocellular carcinoma (HCC) treated after transarterial chemoembolization. *ASCO Gastrointestinal Cancers Symposium Proceedings* 2010;89 (LBA128).
34. Park JW, Walters I, Raoul JL, Harris R, Cai C, Thomas M. Phase II open-label study of brivanib alaniante in patients with hepatocellular carcinoma. *ILCA.* 2008; abstract #O-013.
35. Raoul JL, Finn RS, Kang WK, Park JW, Harris R, Coric V, et al. Phase 2 study of first- and second-line treatment with brivanib in patients with hepatocellular carcinoma. *ILCA.* 2009; abstract #P-0111.

Hepatology Research

Hepatology Research 2010; **40**: 1043–1059 doi: 10.1111/j.1872-034X.2010.00731.x

Special Report

Report of the 18th follow-up survey of primary liver cancer in Japan

Iwao Ikai, Masatoshi Kudo, Shigeki Arii, Masao Omata, Masamichi Kojiro, Michiie Sakamoto, Kenichi Takayasu, Norio Hayashi, Masatoshi Makuuchi, Yutaka Matsuyama and Morito Monden

The Liver Cancer Study Group of Japan, Osaka, Japan

In the 18th Nationwide Follow-Up Survey of Primary Liver Cancer in Japan, 20 753 people were newly registered as patients with primary liver cancer at 544 medical institutions over a period of 2 years (from 1 January 2004 to 31 December 2005). Of these patients, 94.0% had hepatocellular carcinoma (HCC) and 4.4% had intrahepatic cholangiocarcinoma (ICC). In addition, 30 677 follow-up patients were registered in the survey. Epidemiological and clinicopathological factors, diagnosis and treatment were investigated in the newly registered patients. Compared with the 17th follow-up survey, this follow-up survey in HCC indicated an increase in elder patients and women, a decrease in patients positive for hepatitis B surface antigen and hepatitis C virus antibody, and a decrease in tumor size at the clinical diagnosis. In the local ablation therapy, ratio of radio frequency ablation therapy was increasing. The cumulative survival rates of newly-registered patients between 1994 and 2005 were calculated for each histological type (HCC, ICC, and combined HCC and ICC) and stratified by background factors and treatment. The cumulative survival rates of newly-registered patients between 1978 and 2005 divided into three groups (1978–1985, 1986–1995 and 1996–2005) were also calculated. The data obtained in this follow-up survey should contribute to future research and medical practice for primary liver cancer.

Key words: combined hepatic carcinoma, cumulative survival rate, follow-up survey, hepatocellular carcinoma, intrahepatic cholangiocarcinoma

INTRODUCTION

SINCE 1969, THE Liver Cancer Study Group of Japan (LCSGJ) has conducted 17 nationwide follow-up surveys of primary liver cancer in patients in member hospitals and cooperative institutions in Japan, with the goal of promoting research and clinical treatment of liver cancer.[1–17] The 18th Nationwide Follow-up Survey of Primary Liver Cancer was conducted over a 2-year period from 1 January 2004 to 31 December 2005, and 20 753 patients with primary liver cancer were newly registered at 544 institutions. In addition, 30 677 registered patients were followed up with a valid response rate of 74.2%. Items related to epidemiological and clinicopathological factors, diagnosis and treatment were investigated in the newly-registered patients. Cumulative survival rates of newly-registered patients between 1994 to 2005 were calculated for each histological type and based on background factors and treatment.

METHODS

Basic statistics

THE SUBJECTS WERE 20 753 patients with primary liver cancer who were diagnosed clinically or by autopsy and underwent treatment or autopsy during a 2-year period from 1 January 2004 to 31 December 2005 at 544 institutions in Japan. Doctors in each institution completed a form developed by the Follow-up

Correspondence: Dr Iwao Ikai, The Liver Cancer Study Group of Japan, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osakasayama, Osaka 589-8511, Japan. Email: kangan@ nihon-kangan.jp
Received 21 June 2010; revision 3 August 2010; accepted 19 August 2010.

Table 1 Classification of primary liver cancer

Diagnosis	Male (n = 14 601)	Female (n = 6152)	Total (n = 20 753)
HCC	13 805	5 694	19 499 (94.0%)
ICC	561	344	905 (4.4%)
Combined	119	41	160 (0.8%)
Cystadenocarcinoma	14	13	27 (0.1%)
Hepatoblastoma	5	9	14 (0.1%)
Sarcoma	7	2	9 (0.0%)
Undifferentiated carcinoma	6	2	8 (0.0%)
Others	84	47	131 (0.6%)

Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

Survey Committee of the Liver Cancer Study Group of Japan (chairperson, Masatoshi Kudo). In cases with an inconsistency between the clinical, pathological and autopsy diagnoses, the autopsy and pathological diagnoses were given first and second priority, respectively. Of the 20 753 patients, 94.0% had hepatocellular carcinoma (HCC) and 4.4% had intrahepatic cholangiocarcinoma (ICC) (Table 1). The results in the tables are categorized into HCC, ICC, and combined HCC and ICC, for which more than 100 newly-registered cases appeared in the current follow-up survey. The abbreviations in the tables conform to *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, 2nd English edition and *Response Evaluation Criteria in Cancer of Liver* proposed by the Liver Cancer Study Group of Japan.[18,19]

Cumulative survival rate

The cumulative survival rates of newly-registered patients in the 13th to 18th follow-up surveys between 1994 and 2005 whose final prognosis was determined to be survival or death (excluding patients with unknown outcomes) were calculated for each histological type (HCC, ICC, and combined HCC and ICC) and based on different background factors and treatment, including hepatectomy, local ablation therapy and transcatheter arterial embolization. The cumulative survival rates of newly-registered patients between 1978 and 2005 divided into three groups (1978–1985, 1986–1995 and 1996–2005) were also calculated. In this report, patients who had died from either liver-related or liver-unrelated causes were considered to be uncensored cases in estimating cumulative survival rates.

RESULTS

Basic statistics

Causes of death during the study period

FOR HCC, THE mortality of newly-registered patients during the study period was 15.7%: the death rate due to cancer was 55.8% and death rates due to hepatic failure, gastrointestinal bleeding and rupture of esophagogastric varices were 18.8%, 2.1% and 4.1%, respectively. Of the patients who did not survive, 42 died within 30 days after surgery; these patients represented 0.7% of the 5794 patients who underwent surgery. For ICC, the mortality of newly-registered patients during the study period was 35.5% and death rates due to cancer and hepatic failure were 78.5% and 8.3%, respectively (Table 2).

Past history

Of patients with HCC, 76.2% and 60.0% had a past history of chronic hepatitis and liver cirrhosis, respectively, whereas only 19.9% and 9.4% of ICC patients had this history. Interferon therapy had been given to 15.7% of HCC patients due to concomitant chronic hepatitis, and 26.9% and 24.5% of HCC patients and 9.1% and 15.7% of ICC patients had a past history of blood transfusion and habitual alcohol intake, respectively.

Clinical diagnosis

Clinical diagnosis of primary liver cancer in patients with HCC was made at a mean age of 66.4 years in men and 69.9 years in women. For patients with ICC, the corresponding mean ages were 67.2 years in men

Table 2 Causes of death of patients with primary liver cancer

	HCC		ICC		Combined	
Alive	15 885		567		110	
Total deaths of between 2004 and 2005	2 952		312		46	
Cancer death	1 646	(55.8%)	245	(78.5%)	35	(76.1%)
Hepatic failure	554	(18.8%)	26	(8.3%)	7	(15.2%)
Gastrointestinal bleeding	62	(2.1%)	2	(0.6%)	0	(0.0%)
Rupture of esophageal varices	122	(4.1%)	2	(0.6%)	0	(0.0%)
Rupture of tumor	166	(5.6%)	0	(0.0%)	0	(0.0%)
Operative death	42	(1.4%)	4	(1.3%)	0	(0.0%)
Other causes	360	(12.2%)	33	(10.6%)	4	(8.7%)
Unknown	612		22		4	

Combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

and 66.6 years in women. The male : female ratios for HCC and ICC patients were 2.41 and 1.67, respectively.

In patients with HCC, the level of liver injury at the time of diagnosis, based on the liver damage classification of the LCSGJ, was class A, B and C in 60.4%, 32.2% and 7.4% of patients, respectively, whereas 71.0%, 23.6% and 5.4% of HCC patients were in the Child–Pugh class A, B and C categories, respectively (Table 3). Of the HCC patients, 37.1%, 36.3% and 26.6% had serum α-fetoprotein (AFP) levels of less than 15 ng/mL, 15–199 ng/mL and 200 ng/mL or more, respectively, and 64.3%, 5.2% and 30.6% of patients with HCC had serum levels of lectin-reactive AFP-L_3 of less than 10%, 10.0–14.9% and 15% or more, respectively. Of the HCC patients, 40.5%, 14.4% and 45.0% had a protein induced by vitamin K absence or antagonist-II (PIVKA-II) level of less than 40 mAU/mL, 40–99 mAU/mL and 100 mAU/mL or more, respectively. In patients with ICC, 60.0%, 13.9% and 26.2% had a carcinoembryonic antigen level of less than 5.0 ng/mL, 5.0–9.9 ng/mL and 10 ng/mL or more, respectively, and 30.5%, 18.0% and 51.4% had a carbohydrate antigen 19-9 level of less than 37 U/mL, 37–99 U/mL and 100 U/mL or more, respectively (Table 3).

Of the patients with HCC, ICC, and combined HCC and ICC, those who were positive for hepatitis B virus surface antigen comprised 15.0%, 6.3% and 18.9%, respectively. The percentages of anti-hepatitis C virus antibody positive patients were 67.7%, 18.8% and 46.7%, respectively (Table 4).

Tumor size was determined using diagnostic imaging. Of patients with HCC, 33.5% and 45.5% had tumors of 2.0 cm or less and 2.1–5.0 cm, respectively. The corresponding numbers for patients with ICC were 9.3% and 48.8%, respectively (Table 5). Of the tumors, 57.7% and 73.7% were solitary in patients with HCC and ICC, respectively. In patients with HCC, 93.2% had a tumor stain, 2.5% exhibited tumor rupture and 40.4% had esophagogastric varices of F2 or RC_1 or higher.

Major treatment

Of patients with HCC, 31.7%, 30.6% and 31.7% had undergone surgery (hepatectomy and liver transplantation), local ablation therapy and transcatheter arterial embolization, respectively. In patients with ICC, 67.1% and 26.5% had undergone surgery (hepatectomy) and chemotherapy, respectively, and in patients with combined HCC and ICC, 63.8% and 13.5% had undergone surgery (hepatectomy) and transcatheter arterial chemoembolization, respectively (Table 6). Among the HCC patients, 74.5%, 23.2% and 2.2% who underwent surgery, 60.6%, 34.7% and 4.7% of those treated with local ablation therapy, and 57.7%, 36.0% and 6.2% of those treated with transcatheter arterial embolization were in liver damage classes A, B and C, respectively.

Surgery

Of patients with HCC, 5646 underwent hepatectomy and 148 received a liver transplantation. Macroscopic analysis of the resected specimens showed that 59.0% of cases were of the single nodular type. Of patients with ICC, 492 underwent hepatectomy and two received a liver transplantation, and 63.1% of these cases were of the mass-forming type.

Table 3 Clinical profile of patients with primary liver cancer

	HCC		ICC		Combined	
Diagnosis	*n* = 35 472		*n* = 1693		*n* = 301	
Computed tomography	15 275	(43.1%)	701	(41.4%)	124	(41.2%)
Magnetic resonance imaging	2 815	(7.9%)	221	(13.1%)	30	(10.0%)
Ultrasonography	9 305	(26.2%)	378	(22.3%)	76	(25.2%)
Selective angiography	6 388	(18.0%)	186	(11.0%)	37	(12.3%)
Histopathological finding	1 504	(4.2%)	162	(9.6%)	29	(9.6%)
Others	185	(0.5%)	45	(2.7%)	5	(1.7%)
performance status	*n* = 16 364		*n* = 741		*n* = 137	
PS0	13 224	(80.8%)	575	(77.6%)	108	(78.8%)
PS1	2 100	(12.8%)	105	(14.2%)	18	(13.1%)
PS2	616	(3.8%)	30	(4.0%)	6	(4.4%)
PS3	273	(1.7%)	14	(1.9%)	4	(2.9%)
PS4	151	(0.9%)	17	(2.3%)	1	(0.7%)
Encephalopathy	*n* = 18 188		*n* = 813		*n* = 146	
None	17 494	(96.2%)	808	(99.4%)	145	(99.3%)
Mild	490	(2.7%)	3	(0.4%)	0	(0.0%)
Coma occasionally	204	(1.1%)	2	(0.2%)	1	(0.7%)
Ascites	*n* = 18 509		*n* = 830		*n* = 154	
Absent	16 135	(87.2%)	769	(92.7%)	138	(89.6%)
Slight	1 474	(8.0%)	19	(2.3%)	7	(4.5%)
Moderate	900	(4.9%)	42	(5.1%)	9	(5.8%)
Serum bilirubin (mg/mL)	*n* = 18 614		*n* = 852		*n* = 153	
0.0–0.9	10 342	(55.6%)	518	(60.8%)	104	(68.0%)
1.0–1.9	6 383	(34.3%)	195	(22.9%)	38	(24.8%)
2.0–3.0	1 140	(6.1%)	32	(3.8%)	4	(2.6%)
≥3.1	749	(4.0%)	107	(12.6%)	7	(4.6%)
Serum albumin (g/dL)	*n* = 18 481		*n* = 825		*n* = 152	
<2.8	1 470	(8.0%)	37	(4.5%)	9	(5.9%)
2.8–2.9	967	(5.2%)	23	(2.8%)	4	(2.6%)
3.0–3.5	5 255	(28.4%)	160	(19.4%)	40	(26.3%)
>3.5	10 789	(58.4%)	605	(73.3%)	99	(65.1%)
ICG R_{15} (%)	*n* = 10 794		*n* = 487		*n* = 106	
≤14	3 875	(35.9%)	341	(70.0%)	62	(58.5%)
15–24	3 286	(30.4%)	103	(21.1%)	31	(29.2%)
25–40	2 409	(22.3%)	32	(6.6%)	11	(10.4%)
>40	1 224	(11.3%)	11	(2.3%)	2	(1.9%)
Prothrombin activity (%)	*n* = 17 538		*n* = 775		*n* = 145	
<40	278	(1.6%)	15	(1.9%)	1	(0.7%)
40–49	372	(2.1%)	7	(0.9%)	1	(0.7%)
50–70	3 876	(22.1%)	70	(9.0%)	19	(13.1%)
71–80	3 900	(22.2%)	119	(15.4%)	31	(21.4%)
>80	9 112	(52.0%)	564	(72.8%)	93	(64.1%)
Platelet count ($\times 10^4/mm^3$)	*n* = 18 374		*n* = 847		*n* = 154	
<3.0	145	(0.8%)	4	(0.5%)	1	(0.6%)
3.0–4.9	942	(5.1%)	5	(0.6%)	0	(0.0%)
5.0–9.9	5 979	(32.5%)	53	(6.3%)	24	(15.6%)
10.0–14.9	5 419	(29.5%)	114	(13.5%)	46	(29.9%)
15.0–19.9	3 119	(17.0%)	216	(25.5%)	36	(23.4%)
20.0–99.9	2 697	(14.7%)	453	(53.5%)	47	(30.5%)
>100	73	(0.4%)	2	(0.2%)	0	(0.0%)

Table 3 *Continued*

	HCC		ICC		Combined	
Liver damage classification by LCSGJ	*n* = 15 574		*n* = 706		*n* = 138	
A	9 400	(60.4%)	596	(84.4%)	100	(72.5%)
B	5 016	(32.2%)	82	(11.6%)	35	(25.4%)
C	1 158	(7.4%)	28	(4.0%)	3	(2.2%)
Child–Pugh classification	*n* = 18 032		*n* = 790		*n* = 149	
A	12 799	(71.0%)	667	(84.4%)	121	(81.2%)
B	4 254	(23.6%)	101	(12.8%)	21	(14.1%)
C	979	(5.4%)	22	(2.8%)	7	(4.7%)
AFP (ng/mL)	*n* = 17 804		*n* = 562		*n* = 145	
<15	6 608	(37.1%)	449	(79.9%)	59	(40.7%)
≤199	6 466	(36.3%)	77	(13.7%)	38	(26.2%)
≤399	1 000	(5.6%)	11	(2.0%)	7	(4.8%)
≤999	994	(5.6%)	7	(1.2%)	11	(7.6%)
≤9 999	1 549	(8.7%)	12	(2.1%)	17	(11.7%)
≤99 999	761	(4.3%)	3	(0.5%)	9	(6.2%)
≥100 000	426	(2.4%)	3	(0.5%)	4	(2.8%)
AFP-L_3 (%)	*n* = 7904		*n* = 126		*n* = 62	
ND	2 661	(33.7%)	71	(56.3%)	14	(22.6%)
<5.0	1 785	(22.6%)	21	(16.7%)	10	(16.1%)
≤9.9	634	(8.0%)	4	(3.2%)	1	(1.6%)
≤14.9	411	(5.2%)	0	(0.0%)	3	(4.8%)
≤19.9	250	(3.2%)	0	(0.0%)	3	(4.8%)
≥20.0	2 163	(27.4%)	30	(23.8%)	31	(50.0%)
PIVKA-II (mAU/mL)	*n* = 16 114		*n* = 389		*n* = 140	
<40	6 531	(40.5%)	311	(79.9%)	61	(43.6%)
≤99	2 327	(14.4%)	32	(8.2%)	17	(12.1%)
≤299	1 998	(12.4%)	12	(3.1%)	18	(12.9%)
≤499	781	(4.8%)	6	(1.5%)	7	(5.0%)
≤999	842	(5.2%)	6	(1.5%)	11	(7.9%)
≤2 999	1 087	(6.7%)	5	(1.3%)	9	(6.4%)
≤9 999	975	(6.1%)	8	(2.1%)	8	(5.7%)
≥10 000	1 573	(9.8%)	9	(2.3%)	9	(6.4%)
CEA (ng/mL)	*n* = 6 192		*n* = 758		*n* = 113	
<2.5	2 329	(37.6%)	236	(31.1%)	38	(33.6%)
≤4.9	2 319	(37.5%)	219	(28.9%)	34	(30.1%)
≤9.9	1 219	(19.7%)	105	(13.9%)	27	(23.9%)
≤19.9	223	(3.6%)	60	(7.9%)	6	(5.3%)
≤49.9	57	(0.9%)	58	(7.7%)	0	(0.0%)
≤99.9	19	(0.3%)	27	(3.6%)	1	(0.9%)
≥100	26	(0.4%)	53	(7.0%)	7	(6.2%)
CA 19-9 (U/mL)	*n* = 4 807		*n* = 737		*n* = 108	
<37	3 023	(62.9%)	225	(30.5%)	49	(45.4%)
≤99	1 224	(25.5%)	133	(18.0%)	26	(24.1%)
≤299	422	(8.8%)	110	(14.9%)	15	(13.9%)
≤999	95	(2.0%)	82	(11.1%)	9	(8.3%)
≤2999	24	(0.5%)	51	(6.9%)	4	(3.7%)
≤9999	12	(0.2%)	64	(8.7%)	2	(1.9%)
≥10 000	7	(0.1%)	72	(9.8%)	3	(2.8%)

AFP, α-fetoprotein; AFP-L_3, lectin-reactive α-fetoprotein; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; combined, combined hepatocellular and cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; ICG R_{15}, indocyanine green retention rate at 15 min; LCSGJ, Liver Cancer Study Group o Japan; ND, not detectable; PIVKA, protein induced by vitamin K absence or antagonist.

Table 4 Hepatitis B and C virus-associated antigen and antibody

	HCC		ICC		Combined	
HBsAg	*n* = 18 317		*n* = 809		*n* = 148	
Negative	15 550	(84.9%)	758	(93.7%)	120	(81.1%)
Positive	2 754	(15.0%)	51	(6.3%)	28	(18.9%)
Undetermined	13	(0.1%)	0	(0.0%)	0	(0.0%)
HBsAb	*n* = 5 436		*n* = 219		*n* = 62	
Negative	4 293	(79.0%)	181	(82.6%)	46	(74.2%)
Positive	1 107	(20.4%)	38	(17.4%)	16	(25.8%)
Undetermined	36	(0.7%)	0	(0.0%)	0	(0.0%)
HBcAb	*n* = 4 731		*n* = 160		*n* = 55	
Negative	2 200	(46.5%)	105	(65.6%)	28	(50.9%)
Positive	2 515	(53.2%)	54	(33.8%)	27	(49.1%)
Undetermined	16	(0.3%)	1	(0.6%)	0	(0.0%)
HBeAg	*n* = 3 410		*n* = 94		*n* = 42	
Negative	2 829	(83.0%)	91	(96.8%)	38	(90.5%)
Positive	570	(16.7%)	3	(3.2%)	3	(7.1%)
Undetermined	11	(0.3%)	0	(0.0%)	1	(2.4%)
HBeAb	*n* = 3 338		*n* = 84		*n* = 39	
Negative	1 723	(51.6%)	50	(59.5%)	16	(41.0%)
Positive	1 580	(47.3%)	31	(36.9%)	23	(59.0%)
Undetermined	35	(1.0%)	3	(3.6%)	0	(0.0%)
HCVAb	*n* = 18 624		*n* = 828		*n* = 150	
Negative	5 998	(32.2%)	671	(81.0%)	80	(53.3%)
Positive	12 610	(67.7%)	156	(18.8%)	70	(46.7%)
Undetermined	16	(0.1%)	1	(0.1%)	0	(0.0%)

Combined, combined hepatocellular and cholangiocarcinoma; HBcAb, antibody to hepatitis B core antigen; HbeAb, antibody to hepatitis B e antigen; HbeAg, hepatitis B e-antigen; HbsAb, hepatitis B surface antibody; HbsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCVAb, hepatitis C virus antibody; ICC, intrahepatic cholangiocarcinoma.

Macroscopic results from the resected specimens are shown in Table 7. In the HCC patients who underwent hepatectomy, tumors of size 2.0 cm or less, 2.1–5.0 cm and 5.1–10.0 cm were found in 17.7%, 54.9% and 20.2% of patients, respectively, and 74.3% of the tumors were solitary. Vascular invasion in the portal vein, hepatic vein and bile duct were found in 16.2%, 7.3% and 2.7% of the patients, respectively. Regarding findings in non-cancerous parts of the liver, normal liver, chronic hepatitis/liver fibrosis and liver cirrhosis were found in 9.0%, 49.0% and 42.1% of the patients, respectively. The extent of surgical resection was Hr0, HrS, Hr1, Hr2 and Hr3 in 30.7%, 23.4%, 22.6%, 20.8% and 2.5% of the patients, respectively (Table 7).

In patients with ICC, tumors of size 2.0 cm or less, 2.1–5.0 cm and 5.1–10.0 cm were found in 9.3%, 52.1% and 33.9% of patients, respectively, and 83.8% of the tumors were solitary.

Local ablation therapy

Of patients with HCC, 6673 underwent local ablation therapy. Ethanol injection therapy, microwave coagulation therapy and radiofrequency ablation therapy were given to 18.6%, 8.5% and 72.1% of these patients, respectively, suggesting a marked increase in the use of radiofrequency ablation therapy (Table 8). Percutaneous treatment was given in 86.3% of these cases, and of these patients, 71.2% had one tumor, 59.3% had a tumor of size 2.0 cm or less, and 28.5% had a tumor of 2.1–3.0 cm. Treatment outcomes of complete response (CR) and partial response (PR) at 6 months after treatment occurred in 80.3% and 9.9% of patients, respectively.

Transcatheter arterial embolization

Transcatheter arterial embolization was conducted in 8188 patients with HCC. Of these patients, lipiodol

Table 5 Tumor characteristics by imaging studies

	HCC		ICC		Combined	
Tumor size by imaging studies (cm)	*n* = 17 804		*n* = 746		*n* = 137	
Image ≤1	855	(4.8%)	11	(1.5%)	0	(0.0%)
Image ≤2	5 106	(28.7%)	58	(7.8%)	17	(12.4%)
Image ≤3	4 272	(24.0%)	133	(17.8%)	29	(21.2%)
Image ≤5	3 833	(21.5%)	231	(31.0%)	43	(31.4%)
Image ≤10	2 743	(15.4%)	269	(36.1%)	33	(24.1%)
Image ≤15	723	(4.1%)	40	(5.4%)	13	(9.5%)
Image ≤20	176	(1.0%)	4	(0.5%)	2	(1.5%)
Image ≤25	67	(0.4%)	0	(0.0%)	0	(0.0%)
Image >25	29	(0.2%)	0	(0.0%)	0	(0.0%)
No. tumors by imaging studies	*n* = 18 255		*n* = 792		*n* = 145	
Image 1	10 539	(57.7%)	584	(73.7%)	79	(54.5%)
Image 2	3 157	(17.3%)	55	(6.9%)	23	(15.9%)
Image 3	1 437	(7.9%)	25	(3.2%)	7	(4.8%)
Image 4	577	(3.2%)	11	(1.4%)	6	(4.1%)
Image 5	281	(1.5%)	4	(0.5%)	2	(1.4%)
Image ≥6	2 264	(12.4%)	113	(14.3%)	28	(19.3%)
Portal vein invasion by imaging studies	*n* = 17 455		*n* = 727		*n* = 139	
Image Vp0	15 170	(86.9%)	477	(65.6%)	98	(70.5%)
Image Vp1	532	(3.0%)	58	(8.0%)	11	(7.9%)
Image Vp2	485	(2.8%)	49	(6.7%)	8	(5.8%)
Image Vp3	689	(3.9%)	110	(15.1%)	19	(13.7%)
Image Vp4	579	(3.3%)	33	(4.5%)	3	(2.2%)
Hepatic vein invasion by imaging studies	*n* = 16 688		*n* = 694		*n* = 130	
Image Vv0	15 961	(95.6%)	600	(86.5%)	121	(93.1%)
Image Vv1	269	(1.6%)	31	(4.5%)	4	(3.1%)
Image Vv2	229	(1.4%)	42	(6.1%)	4	(3.1%)
Image Vv3	229	(1.4%)	21	(3.0%)	1	(0.8%)
Bile duct invasion by imaging studies	*n* = 16 536		*n* = 691		*n* = 126	
Image B0	16 134	(97.6%)	403	(58.3%)	108	(85.7%)
Image B1	181	(1.1%)	81	(11.7%)	5	(4.0%)
Image B2	96	(0.6%)	66	(9.6%)	8	(6.3%)
Image B3	74	(0.4%)	101	(14.6%)	0	(0.0%)
Image B4	51	(0.3%)	40	(5.8%)	5	(4.0%)
Distant metastases by imaging studies						
Lung	302		44		8	
Bone	207		15		6	
Adrenal gland	66		5		0	
Lymph node	228		152		21	
Brain	19		2		0	
Peritoneum	30		20		3	
Others	52		8		0	
Esophageal or gastric varices	*n* = 5 251		*n* = 33		*n* = 22	
F1, RC^-	2 766	(52.7%)	22	(66.7%)	12	(54.5%)
F2 or RC^+	2 123	(40.4%)	10	(30.3%)	10	(45.5%)
Rupture	362	(6.9%)	1	(3.0%)	0	(0.0%)

B0, absence of invasion of the bile ducts; B1, invasion of (or tumor thrombus in) the third order or more peripheral branches of the bile duct, but not of second order branches; B2, invasion of (or tumor thrombus in) the second order branches of the bile duct; B3, invasion of (or tumor thrombus in) the first order branches of the bile duct; B4, invasion of (or tumor thrombus in) the common hepatic duct; combined, combined hepatocellular and cholangiocarcinoma; HCC: hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; Vp0, absence of invasion of (or tumor thrombus in) the portal vein; Vp1, invasion of (or tumor thrombus in) distal to the second order branches of the portal vein, but not of the second order branches; Vp2, invasion of (or tumor thrombus in) second order branches of the portal vein; Vp3, invasion of (or tumor thrombus in) first order branches of the portal vein; Vp4, invasion of (or tumor thrombus in) the main trunk of the portal vein and/or contralateral portal vein branch to the primarily involved lobe; Vv0, absence of invasion of (or tumor thrombus in) the hepatic vein; Vv1, invasion of (or tumor thrombus in) peripheral branches of the hepatic vein; Vv2, invasion of (or tumor thrombus in) the right, middle, or left hepatic vein, the inferior right hepatic vein, or the short hepatic vein; Vv3: invasion of (or tumor thrombus in) the inferior vena cava.

Table 6 Major treatment of patients with primary liver cancer

	HCC		ICC		Combined	
Treatment for tumor	*n* = 17 986		*n* = 732		*n* = 141	
Surgery	5 698	(31.7%)	491	(67.1%)	90	(63.8%)
Local ablation therapy	5 500	(30.6%)	18	(2.5%)	12	(8.5%)
Transcatheter arterial chemoembolization	5 693	(31.7%)	13	(1.8%)	19	(13.5%)
Chemotherapy	997	(5.5%)	194	(26.5%)	20	(14.2%)
Others	98	(0.5%)	16	(2.2%)	0	(0.0%)
Best supportive care	*n* = 1 388		*n* = 158		*n* = 16	

Combined, combined hepatocellular and cholangiocarcinoma; HCC: hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

alone, gelatin sponge alone, and lipiodol plus gelatin sponge were used in 20.6%, 2.6% and 75.6% of cases, respectively (Table 9), with concomitant administration of anticancer agents in 93.2% of these patients. Regarding the extent of embolization, less than one segment, one segment to one lobe, more than one lobe and the whole liver were treated in 36.0%, 40.5%, 17.5% and 6.0% of patients, respectively. Treatment outcomes of CR and PR at 6 months occurred in 40.5% and 27.6% of patients, respectively.

Chemotherapy

Chemotherapy was given to 1862 patients with HCC, and of these patient 85.8%, 4.6% and 7.9% received chemotherapy intra-arterially, i.v. and p.o., respectively; treatment outcomes of CR and PR at 6 months occurred in 13.5% and 25.5% of patients, respectively. Of the patients with ICC, 232 underwent chemotherapy, and of these patients 22.4%, 55.2% and 15.9% received chemotherapy intra-arterially, i.v. and p.o., respectively; treatment outcomes of CR and PR at 6 months occurred in 4.0% and 11.9% of patients, respectively.

Pathological diagnosis

Pathological diagnosis was conducted in 40.4% of patients with HCC, whereas 59.6% of patients were not diagnosed pathologically. The percentage of diagnoses by biopsy alone, resected specimens alone, and both biopsy and resected specimens was 25.8%, 71.5% and 2.7%, respectively. Microscopic pathological results from biopsy and resected specimens are shown in Table 10. Well, moderately and poorly differentiated tumor types were found in 27.3% ($n = 1842$), 60.3% ($n = 4063$) and 11.6% ($n = 784$) of patients with HCC, respectively, whereas well, moderately and poorly differentiated tumor types were found in 23.3% ($n = 115$), 54.1% ($n = 268$) and 19.8% ($n = 98$) of patients with ICC, respectively. Regarding microscopic pathological findings in noncancerous parts of the liver, normal liver, chronic hepatitis/liver fibrosis and liver cirrhosis were found in 6.5%, 48.0% and 45.6% of patients with HCC, respectively, and in 65.0%, 24.4% and 10.6% of patients with ICC, respectively.

Recurrence

During the period of this survey (<2 years after diagnosis), 28.8% of patients with HCC experienced recurrence of the disease. Transcatheter arterial embolization and local therapy were given to 58.3% and 27.2% of these patients, respectively, as treatment for recurrence in the liver. The most frequent organ of distant metastasis was the lung, followed by bone and lymph nodes. Radiation therapy, systemic chemotherapy and resection were chosen as treatment for distant organ metastasis.

Autopsy

Autopsy in 280 patients of primary liver cancer were registered, 238 of whom were patients with HCC. Liver cirrhosis was found in 81.5% of the autopsied patients with HCC, invasion of the portal vein, hepatic vein or bile duct was found in 72.4%, 42.7% and 25.8%, respectively, and distant metastasis was found most frequently in the lung. In patients with ICC, the most frequent distant metastasis site was also the lung.

Cumulative survival rates

The cumulative survival rates of newly-registered patients in the 13th to 18th follow-up surveys (1994–2005) whose final prognosis was defined as survival

Table 7 Operative findings or macroscopic pathological characteristics of surgical specimen (hepatic resection)

	HCC		ICC		Combined	
Tumor size (cm)	*n* = 5277		*n* = 451		*n* = 85	
≤1	91	(1.7%)	8	(1.8%)	0	(0.0%)
≤2	846	(16.0%)	34	(7.5%)	10	(11.8%)
≤3	1360	(25.8%)	79	(17.5%)	16	(18.8%)
≤5	1534	(29.1%)	156	(34.6%)	31	(36.5%)
≤10	1066	(20.2%)	153	(33.9%)	18	(21.2%)
≤15	304	(5.8%)	15	(3.3%)	8	(9.4%)
≤20	57	(1.1%)	4	(0.9%)	1	(1.2%)
≤25	16	(0.3%)	2	(0.4%)	1	(1.2%)
>25	3	(0.1%)	0	(0.0%)	0	(0.0%)
No. of tumors	*n* = 5336		*n* = 458		*n* = 85	
1	3966	(74.3%)	384	(83.8%)	50	(58.8%)
2	792	(14.8%)	28	(6.1%)	16	(18.8%)
3	258	(4.8%)	9	(2.0%)	4	(4.7%)
4	96	(1.8%)	7	(1.5%)	3	(3.5%)
5	36	(0.7%)	6	(1.3%)	1	(1.2%)
≥6	188	(3.5%)	24	(5.2%)	11	(12.9%)
Tumor extent	*n* = 5189		*n* = 465		*n* = 85	
Hs	2099	(40.5%)	70	(15.1%)	25	(29.4%)
H1	1458	(28.1%)	138	(29.7%)	17	(20.0%)
H2	1284	(24.7%)	210	(45.2%)	32	(37.6%)
H3	259	(5.0%)	39	(8.4%)	9	(10.6%)
H4	89	(1.7%)	8	(1.7%)	2	(2.4%)
Growth type	*n* = 5105		*n* = 424		*n* = 83	
Eg	4731	(92.7%)	196	(46.2%)	60	(72.3%)
Ig	374	(7.3%)	228	(53.8%)	23	(27.7%)
Capsule formation	*n* = 5047		*n* = 416		*n* = 80	
Fc^-	1147	(22.7%)	379	(91.1%)	54	(67.5%)
Fc^+	3900	(77.3%)	37	(8.9%)	26	(32.5%)
Capsule infiltration	*n* = 4702		*n* = 288		*n* = 65	
$Fc\text{-}Inf^-$	2768	(58.9%)	265	(92.0%)	52	(80.0%)
$Fc\text{-}Inf^+$	1934	(41.1%)	23	(8.0%)	13	(20.0%)
Septum formation	*n* = 4968		*n* = 398		*n* = 79	
Sf^-	2313	(46.6%)	374	(94.0%)	51	(64.6%)
Sf^+	2655	(53.4%)	24	(6.0%)	28	(35.4%)
Serosal invasion	*n* = 5016		*n* = 429		*n* = 81	
S0	4022	(80.2%)	254	(59.2%)	52	(64.2%)
S1	755	(15.1%)	130	(30.3%)	21	(25.9%)
S2	161	(3.2%)	45	(10.5%)	7	(8.6%)
S3	78	(1.6%)	0	(0.0%)	1	(1.2%)
Lymph node metastasis	*n* = 4910		*n* = 449		*n* = 83	
Absent	4858	(98.9%)	312	(69.5%)	70	(84.3%)
Present	52	(1.1%)	137	(30.5%)	13	(15.7%)
Portal vein invasion	*n* = 5228		*n* = 445		*n* = 86	
Vp0	4384	(83.9%)	286	(64.3%)	52	(60.5%)
Vp1	481	(9.2%)	66	(14.8%)	20	(23.3%)
Vp2	166	(3.2%)	37	(8.3%)	7	(8.1%)
Vp3	126	(2.4%)	48	(10.8%)	6	(7.0%)
Vp4	71	(1.4%)	8	(1.8%)	1	(1.2%)
Hepatic vein invasion	*n* = 5088		*n* = 434		*n* = 82	
Vv0	4719	(92.7%)	354	(81.6%)	72	(87.8%)
Vv1	253	(5.0%)	36	(8.3%)	10	(12.2%)
Vv2	84	(1.7%)	30	(6.9%)	0	(0.0%)
Vv3	32	(0.6%)	14	(3.2%)	0	(0.0%)
Hepatic arterial invasion	*n* = 5057		*n* = 429		*n* = 82	
Va0	5020	(99.3%)	382	(89.0%)	81	(98.8%)
Va1	36	(0.7%)	26	(6.1%)	1	(1.2%)
Va2	1	(0.0%)	13	(3.0%)	0	(0.0%)
Va3	0	(0.0%)	8	(1.9%)	0	(0.0%)

Table 7 *Continued*

	HCC		ICC		Combined	
Bile duct invasion	*n* = 5184		*n* = 436		*n* = 84	
B0	5049	(97.4%)	214	(49.1%)	73	(86.9%)
B1	70	(1.4%)	72	(16.5%)	4	(4.8%)
B2	21	(0.4%)	60	(13.8%)	4	(4.8%)
B3	29	(0.6%)	70	(16.1%)	1	(1.2%)
B4	15	(0.3%)	20	(4.6%)	2	(2.4%)
Intrahepatic metastasis	*n* = 5187		*n* = 450		*n* = 85	
Im0	4076	(78.6%)	346	(76.9%)	55	(64.7%)
Ims	215	(4.1%)	14	(3.1%)	5	(5.9%)
Im1	353	(6.8%)	38	(8.4%)	8	(9.4%)
Im2	362	(7.0%)	37	(8.2%)	9	(10.6%)
Im3	181	(3.5%)	15	(3.3%)	8	(9.4%)
Peritoneal dissemination	*n* = 5164		*n* = 449		*n* = 84	
Absent	5132	(99.4%)	432	(96.2%)	83	(98.8%)
Present	32	(0.6%)	17	(3.8%)	1	(1.2%)
Surgical margin	*n* = 5174		*n* = 447		*n* = 85	
Presence of cancer invasion	320	(6.2%)	56	(12.5%)	10	(11.8%)
Absence of cancer invasion	4854	(93.8%)	391	(87.5%)	75	(88.2%)
Non-cancerous portion	*n* = 5146		*n* = 436		*n* = 84	
Normal liver	461	(9.0%)	309	(70.9%)	15	(17.9%)
Chronic hepatitis / liver fibrosis	2519	(49.0%)	90	(20.6%)	41	(48.8%)
Liver cirrhosis	2166	(42.1%)	37	(8.5%)	28	(33.3%)
Extent of hepatic resection	*n* = 5148		*n* = 467		*n* = 86	
Hr0	1579	(30.7%)	32	(6.9%)	13	(15.1%)
HrS	1203	(23.4%)	35	(7.5%)	23	(26.7%)
Hr1	1163	(22.6%)	61	(13.1%)	12	(14.0%)
Hr2	1072	(20.8%)	294	(63.0%)	32	(37.2%)
Hr3	131	(2.5%)	45	(9.6%)	6	(7.0%)
Lymph node dissection	*n* = 4925		*n* = 457		*n* = 84	
Not performed	4807	(97.6%)	185	(40.5%)	67	(79.8%)
Performed	118	(2.4%)	272	(59.5%)	17	(20.2%)
Residual cancer	*n* = 5078		*n* = 442		*n* = 79	
Absent	4800	(94.5%)	397	(89.8%)	69	(87.3%)
Present	278	(5.5%)	45	(10.2%)	10	(12.7%)
Distant metastases	*n* = 5214		*n* = 452		*n* = 86	
Absent	5175	(99.3%)	440	(97.3%)	84	(97.7%)
Present	39	(0.7%)	12	(2.7%)	2	(2.3%)
TNM stage by LCSGJ	*n* = 5268		*n* = 452		*n* = 84	
I	689	(13.1%)	24	(5.3%)	3	(3.6%)
II	2647	(50.2%)	121	(26.8%)	21	(25.0%)
III	1342	(25.5%)	149	(33.0%)	34	(40.5%)
IV A	534	(10.1%)	43	(9.5%)	20	(23.8%)
IV B	56	(1.1%)	115	(25.4%)	6	(7.1%)

B0–B4, described in Table 5; combined, combined hepatocellular and cholangiocarcinoma; Eg, expansive growth, well-demarcated border; Fc^{-}, absence of capsule formation; Fc^{+}, presence of capsule formation; Fc-Inf^{-}, absence of cancerous infiltration of the tumor capsule, Fc-Inf^{+}, presence of cancerous infiltration of the tumor capsule; HCC, hepatocellular carcinoma; Hs, cancer limited to one subsegment; H1, cancer limited to one segment; H2, cancer limited to two segments; H3, cancer limited to three segments; H4, cancer involving more than three segments; Hr0, resection of less than one subsegment (Couinaud's segment); HrS, resection of one subsegment (Couinaud's segment); Hr1, resection of one segment (anterior, posterior, medial or left lateral segmentectomy); Hr2, resection of two segments (right or left lobectomy or central bisegmentectomy); Hr3, resection of three segments (right or left trisegmentectomy); Ig, infiltrative growth, poorly demarcated border; Im0, absence of intrahepatic metastasis; Ims, intrahepatic metastasis within the subsegment in which the principal tumor is located; Im1, intrahepatic metastasis within the subsegment in which the principal tumor is located; Im2, intrahepatic metastasis in two segments; Im3, intrahepatic metastasis to three or more segments; LCSGJ, Liver Cancer Study Group of Japan; Sf^{-}, absence of formation of a fibrous septum within the tumor; Sf^{+}, presence of fibrous septum within the tumor; S0, absence of invasion of the serosa; S1, tumor invasion of the serosa; S2, tumor invasion of adjacent organs; S3, tumor rupture with intraperitoneal bleeding; Va0, absence of invasion of the hepatic artery; Va1, invasion distal to the second order branches of the hepatic artery, but not of the second order branches; Va2, invasion to the second order branches of the hepatic artery; Va3, invasion to the left or right hepatic artery, or the proper hepatic artery; Vp0–Vp4, described in Table 5; Vv0–Vv3, described in Table 5.

Table 8 Local ablation therapy

	HCC		ICC		Combined	
	n = 17 794		*n* = 734		*n* = 147	
Not performed	11 121	(62.5%)	704	(95.9%)	132	(89.8%)
Performed	6 673	(37.5%)	30	(4.1%)	15	(10.2%)
EIT	1 241	(18.6%)	6	(20.0%)	3	(20.0%)
MCT	565	(8.5%)	2	(6.7%)	0	(0.0%)
RFA	4 812	(72.1%)	21	(70.0%)	12	(80.0%)
Others	55	(0.8%)	1	(3.3%)	0	(0.0%)
Percutaneous or not	*n* = 6 488		*n* = 29		*n* = 14	
Percutaneous	5 597	(86.3%)	21	(72.4%)	13	(92.9%)
Others	891	(13.7%)	8	(27.6%)	1	(7.1%)
No. tumors	*n* = 6 518		*n* = 29		*n* = 15	
1	4 643	(71.2%)	21	(72.4%)	11	(73.3%)
2	1 219	(18.7%)	6	(20.7%)	3	(20.0%)
3	412	(6.3%)	0	(0.0%)	1	(6.7%)
4	123	(1.9%)	2	(6.9%)	0	(0.0%)
5	56	(0.9%)	0	(0.0%)	0	(0.0%)
≥6	65	(1.0%)	0	(0.0%)	0	(0.0%)
Tumor size (cm)	*n* = 6 326		*n* = 27		*n* = 14	
≤1	560	(8.9%)	2	(7.4%)	0	(0.0%)
≤2	3 189	(50.4%)	10	(37.0%)	7	(50.0%)
≤3	1 800	(28.5%)	11	(40.7%)	4	(28.6%)
≤5	688	(10.9%)	4	(14.8%)	3	(21.4%)
≤10	89	(1.4%)	0	(0.0%)	0	(0.0%)
≤15	0	(0.0%)	0	(0.0%)	0	(0.0%)
≤20	0	(0.0%)	0	(0.0%)	0	(0.0%)
≤25	0	(0.0%)	0	(0.0%)	0	(0.0%)
>25	0	(0.0%)	0	(0.0%)	0	(0.0%)
Modalities combined with local ablation therapy	*n* = 6 500		*n* = 28		*n* = 14	
None	4 096	(63.0%)	20	(71.4%)	10	(71.4%)
Transcatheter arterial embolization	2 182	(33.6%)	5	(17.9%)	4	(28.6%)
others	222	(3.4%)	3	(10.7%)	0	(0.0%)
Efficacy evaluation at 6 months	*n* = 5 378		*n* = 23		*n* = 11	
CR	4 318	(80.3%)	9	(39.1%)	10	(90.9%)
PR	530	(9.9%)	4	(17.4%)	1	(9.1%)
SD	160	(3.0%)	5	(21.7%)	0	(0.0%)
PD	370	(6.9%)	5	(21.7%)	0	(0.0%)

Combined, combined hepatocellular and cholangiocarcinoma; CR, complete response; EIT, ethanol injection therapy; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; MCT, microwave coagulation therapy; MR, minor response; NC, no change; PD, progressive disease; PR, partial response; RFA, radiofrequency ablation therapy.

or death (excluding cases of unknown outcome) were calculated for cases of HCC, ICC, and combined HCC and ICC.

HCC

The 3-, 5- and 10-year cumulative survival rates in all patients with HCC were 55.0%, 37.9% and 16.5%, respectively. Cumulative survival rates for patients with HCC were also stratified by initial treatment, which included hepatectomy (Table 11), local ablation therapy (ethanol injection therapy, microwave coagulation therapy and radiofrequency ablation therapy) (Table 12), and transcatheter arterial embolization (Table 13). In newly-registered patients in the 16th and 17th surveys, the liver damage classification by

Table 9 Transcatheter arterial embolization

	HCC		ICC		Combined	
	n = 17 898		*n* = 736		*n* = 149	
Not performed	9 710	(54.3%)	707	(96.1%)	113	(75.8%)
Performed	8 188	(45.7%)	29	(3.9%)	36	(24.2%)
Embolic materials	*n* = 7 850		*n* = 28		*n* = 37	
Lipiodol	1 621	(20.6%)	8	(28.6%)	16	(43.2%)
Gelatin sponge	205	(2.6%)	1	(3.6%)	0	(0.0%)
Lipiodol + gelatin sponge	5 936	(75.6%)	18	(64.3%)	21	(56.8%)
Others	88	(1.1%)	1	(3.6%)	0	(0.0%)
Extent of embolization	*n* = 7 157		*n* = 26		*n* = 34	
Less than one segment	2 578	(36.0%)	8	(30.8%)	6	(17.6%)
One segment to one lobe	2 896	(40.5%)	8	(30.8%)	16	(47.1%)
More than one lobe	1 252	(17.5%)	4	(15.4%)	7	(20.6%)
Whole liver	431	(6.0%)	6	(23.1%)	5	(14.7%)
Efficacy evaluation at 6 months	*n* = 5 448		*n* = 13		*n* = 24	
CR	2 208	(40.5%)	4	(30.8%)	3	(12.5%)
PR	1 502	(27.6%)	1	(7.7%)	5	(20.8%)
SD	632	(11.6%)	3	(23.1%)	6	(25.0%)
PD	1 106	(20.3%)	5	(38.5%)	10	(41.7%)

Combined, combined hepatocellular and cholangiocarcinoma; CR, complete response; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; MR, minor response; NC, no change; PD, progressive disease; PR, partial response.

LCSGJ was estimated from data collected in the surveys.

ICC and combined HCC and ICC

For ICC, cumulative survival rates were calculated for all patients and based on various background factors. For combined HCC and ICC, cumulative survival rates were calculated for all patients (Tables 14,15).

Changes in the cumulative survival rates of HCC patients

The cumulative survival rates of newly-registered HCC patients in the 5th to 18th follow-up surveys (1978–2005) whose final prognosis was defined as survival or death (excluding cases of unknown outcome) divided into three groups (1978–1985, 1986–1995 and 1996–2005) were also calculated (Fig. 1). The 3- and 5-year cumulative survival rates were 15.7% and 9.5% in patients between 1978 and 1985 (*n* = 7852), 42.1% and 26.8% between 1986 and 1995 (*n* = 51 719), and 56.6% and 39.3% between 1996 and 2005 (*n* = 88 590), respectively.

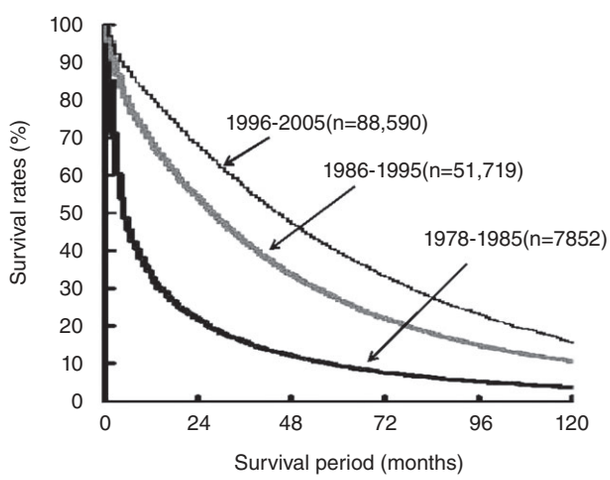


Figure 1 Cumulative survival rates of newly-registered patients in the 5th to 18th follow-up surveys (1978–2005) divided into three groups (1978–1985, 1986–1995 and 1996–2005) are shown. The 3- and 5-year cumulative survival rates were 15.7%, 9.5% in patients between 1978 and 1985 (*n* = 7852), 42.1% and 26.8% between 1986 and 1995 (*n* = 51 719), and 56.6% and 39.3% between 1996 and 2005 (*n* = 88 590), respectively.

Table 10 Microscopic pathological findings of surgical or biopsy specimens

	HCC		ICC		Combined	
Capsule formation	*n* = 5221		*n* = 406		*n* = 84	
Fc$^-$	1293	(24.8%)	386	(95.1%)	54	(64.3%)
Fc$^+$	3928	(75.2%)	20	(4.9%)	30	(35.7%)
Capsule infiltration	*n* = 3850		*n* = 16		*n* = 30	
Fc-inf$^-$	1264	(32.8%)	8	(50.0%)	8	(26.7%)
Fc-inf$^+$	2586	(67.2%)	8	(50.0%)	22	(73.3%)
Septum formation	*n* = 4983		*n* = 372		*n* = 83	
Sf$^-$	1930	(38.7%)	348	(93.5%)	41	(49.4%)
Sf$^+$	3053	(61.3%)	24	(6.5%)	42	(50.6%)
Serosal invasion	*n* = 4959		*n* = 409		*n* = 82	
S0	4267	(86.0%)	267	(65.3%)	61	(74.4%)
S1	537	(10.8%)	96	(23.5%)	15	(18.3%)
S2	84	(1.7%)	44	(10.8%)	5	(6.1%)
S3	71	(1.4%)	2	(0.5%)	1	(1.2%)
Lymph node metastasis	*n* = 3984		*n* = 427		*n* = 70	
Absent	3938	(98.8%)	257	(60.2%)	57	(81.4%)
Present	46	(1.2%)	170	(39.8%)	13	(18.6%)
Portal vein invasion	*n* = 5368		*n* = 430		*n* = 87	
vp0	3971	(74.0%)	223	(51.9%)	41	(47.1%)
Vp1	1019	(19.0%)	137	(31.9%)	33	(37.9%)
Vp2	167	(3.1%)	37	(8.6%)	6	(6.9%)
Vp3	138	(2.6%)	31	(7.2%)	7	(8.0%)
Vp4	73	(1.4%)	2	(0.5%)	0	(0.0%)
Hepatic vein invasion	*n* = 5320		*n* = 423		*n* = 84	
Vv0	4714	(88.6%)	304	(71.9%)	61	(72.6%)
Vv1	499	(9.4%)	85	(20.1%)	23	(27.4%)
Vv2	77	(1.4%)	24	(5.7%)	0	(0.0%)
Vv3	30	(0.6%)	10	(2.4%)	0	(0.0%)
Hepatic arterial invasion	*n* = 5160		*n* = 402		*n* = 82	
Va0	5103	(98.9%)	377	(93.8%)	79	(96.3%)
Va1	54	(1.0%)	18	(4.5%)	2	(2.4%)
Va2	2	(0.0%)	3	(0.7%)	1	(1.2%)
Va3	1	(0.0%)	4	(1.0%)	0	(0.0%)
Bile duct invasion	*n* = 5279		*n* = 403		*n* = 87	
B0	5095	(96.5%)	184	(45.7%)	66	(75.9%)
B1	108	(2.0%)	91	(22.6%)	15	(17.2%)
B2	37	(0.7%)	50	(12.4%)	3	(3.4%)
B3	21	(0.4%)	61	(15.1%)	1	(1.1%)
B4	18	(0.3%)	17	(4.2%)	2	(2.3%)
Intrahepatic metastasis	*n* = 5206		*n* = 430		*n* = 86	
Im0	4147	(79.7%)	322	(74.9%)	52	(60.5%)
Ims	238	(4.6%)	17	(4.0%)	5	(5.8%)
Im1	384	(7.4%)	39	(9.1%)	11	(12.8%)
Im2	299	(5.7%)	34	(7.9%)	10	(11.6%)
Im3	138	(2.7%)	18	(4.2%)	8	(9.3%)
Surgical margin	*n* = 5104		*n* = 434		*n* = 84	
Presence of cancer invasion	408	(8.1%)	80	(18.4%)	13	(15.5%)
Absence of cancer invasion	4696	(91.9%)	354	(81.6%)	71	(84.5%)
Non-cancerous portion	*n* = 5395		*n* = 414		*n* = 84	
Normal liver	349	(6.5%)	269	(65.0%)	9	(10.7%)
Chronic hepatitis or liver fibrosis	2587	(48.0%)	101	(24.4%)	46	(54.8%)
Liver cirrhosis	2459	(45.6%)	44	(10.6%)	29	(34.5%)
Liver fibrosis	*n* = 3153		*n* = 169		*n* = 49	
F0 (normal)	184	(5.8%)	82	(48.5%)	5	(10.2%)
F1	429	(13.6%)	39	(23.1%)	3	(6.1%)
F2	532	(16.9%)	14	(8.3%)	12	(24.5%)
F3	578	(18.3%)	13	(7.7%)	12	(24.5%)
F4 (liver cirrhosis)	1430	(45.4%)	21	(12.4%)	17	(34.7%)

B0–B4, described in Tables 5 and 7; combined, combined hepatocellular and cholangiocarcinoma; Fc, Fc-inf, described in Table 7; F1, fibrosis expansion of portal tract; F2, bridging fibrosis formation; F3, bridging fibrosis formation accompanying lobular distortion; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; Im0–Im3, described in Table 7; Sf, S0–S3 described in Table 7; Va0–Va3, described in Table 7; Vp0–Vp4, Vv0–Vv3, described in Tables 5 and 7.

Table 11 Cumulative survival rates (%) of HCC patients treated with hepatic resection (1994–2005)

		n	Year									
			1	2	3	4	5	6	7	8	9	10
All cases		25 066	88.2%	78.4%	69.5%	61.7%	54.2%	48.1%	42.0%	36.9%	32.5%	29.0%
Tumor size (cm)	≤2	4 363	95.8%	91.1%	85.4%	78.2%	69.4%	61.7%	53.4%	46.5%	40.5%	35.5%
	2–5	12 801	91.9%	82.9%	73.2%	65.0%	56.8%	50.2%	43.9%	38.8%	34.2%	30.6%
	5–10	4 802	82.3%	68.7%	58.5%	50.2%	44.0%	39.1%	34.0%	29.8%	26.0%	23.6%
	>10	2 044	66.5%	50.6%	42.5%	36.7%	32.1%	29.5%	25.9%	22.6%	20.3%	18.5%
Tumor number	1	17 531	91.0%	82.9%	74.8%	67.7%	60.2%	54.0%	47.5%	42.1%	37.5%	33.2%
	2	3 692	87.3%	75.3%	64.8%	55.9%	48.0%	40.3%	34.8%	28.5%	24.6%	22.7%
	≥3	3 010	75.7%	59.6%	48.1%	38.4%	30.6%	26.3%	22.0%	19.3%	15.3%	13.7%
Portal vein invasion	Vp0	20 195	92.2%	83.7%	74.9%	67.0%	59.0%	52.4%	45.5%	40.1%	35.3%	31.3%
	Vp1	1 978	79.3%	64.9%	54.2%	45.7%	39.1%	34.3%	31.9%	28.1%	24.2%	22.9%
	Vp2	820	61.0%	45.4%	33.6%	27.6%	23.3%	22.8%	20.6%	17.0%	16.0%	16.0%
	Vp3 or Vp4	1 021	52.1%	33.6%	26.4%	22.4%	18.3%	16.6%	14.8%	13.1%	10.5%	8.4%
Non-cancerous portion	Normal liver	1 801	86.2%	76.2%	68.9%	63.6%	59.1%	55.7%	51.1%	46.9%	43.4%	37.6%
	Chronic hepatitis/ liver fibrosis	9 581	90.4%	81.5%	73.4%	67.0%	60.8%	55.8%	50.2%	45.6%	41.7%	39.0%
	Liver cirrhosis	10 401	87.3%	77.0%	67.3%	58.3%	49.1%	42.1%	35.1%	30.2%	25.4%	22.1%
Liver damage classification by LCSGJ	A	16 963	90.0%	81.5%	73.3%	66.0%	59.0%	52.9%	46.3%	41.5%	36.7%	33.2%
	B	6 478	85.6%	73.8%	63.6%	54.8%	45.3%	39.2%	33.8%	28.6%	25.1%	21.3%
	C	454	73.4%	56.0%	44.9%	39.8%	35.0%	32.1%	30.9%	22.9%	21.7%	21.7%
TNM Stage by LCSGJ	I	2 846	96.9%	93.6%	88.7%	81.8%	73.0%	66.1%	57.6%	51.3%	45.4%	38.1%
	II	12 458	92.7%	84.1%	75.3%	67.4%	59.7%	53.4%	46.1%	40.4%	35.9%	32.5%
	III	4 223	82.2%	68.1%	56.1%	47.2%	39.5%	34.1%	30.6%	26.9%	23.6%	21.4%
	IV A	1 398	60.3%	42.4%	31.9%	25.9%	21.4%	19.7%	17.8%	15.3%	12.5%	11.9%
	IV B	253	53.1%	33.6%	24.2%	21.7%	16.5%	14.1%	14.1%	14.1%	14.1%	14.1%

HCC, hepatocellular carcinoma; LCSGJ, Liver Cancer Study Group of Japan; TNM, Tumor–Node–Metastasis; Vp0–V94, described in Tables 5 and 7.

Table 12 Cumulative survival rates (%) of HCC patients treated with local ablation therapy (1994–2005)

		n	Year									
			1	2	3	4	5	6	7	8	9	10
All cases		27 150	92.8%	81.4%	68.6%	56.5%	45.6%	37.1%	29.8%	23.9%	19.5%	15.7%
Liver damage	A	14 370	95.5%	87.2%	76.3%	65.5%	54.2%	44.4%	36.6%	30.3%	25.0%	19.9%
classification	B	9 751	92.4%	78.5%	63.4%	50.0%	38.7%	31.0%	24.2%	18.0%	14.7%	12.4%
by LCSGJ	C	1 757	77.2%	56.2%	41.2%	28.1%	21.6%	16.9%	12.3%	9.4%	7.1%	5.4%
Tumor number	1	16 883	94.2%	84.5%	73.2%	62.3%	51.9%	42.8%	35.1%	28.8%	23.8%	19.4%
	2	5 638	92.4%	79.8%	65.9%	51.8%	39.6%	32.8%	24.4%	18.7%	15.5%	11.7%
	3	2 307	91.6%	76.8%	60.9%	46.3%	35.0%	25.8%	20.6%	15.7%	11.5%	10.9%
	4	812	88.5%	72.6%	55.3%	41.1%	30.8%	21.4%	17.7%	13.1%	6.9%	3.4%
	≥5	1 079	82.9%	62.1%	44.0%	33.0%	23.4%	20.4%	13.9%	12.2%	9.1%	7.2%
Tumor size	≤1	1 792	96.6%	90.6%	81.6%	72.1%	60.1%	49.8%	44.6%	38.4%	31.1%	25.7%
(cm)	1–2	12 253	95.2%	86.4%	75.1%	63.7%	52.7%	43.0%	34.0%	27.3%	22.0%	18.1%
	2–3	7 714	93.0%	79.7%	64.8%	51.9%	40.0%	32.1%	25.5%	20.1%	16.4%	13.4%
	3–5	3 257	88.2%	71.0%	55.7%	41.8%	32.4%	26.1%	20.8%	17.6%	15.3%	8.7%
	>5	809	76.9%	58.9%	43.6%	33.7%	25.5%	21.0%	15.6%	10.6%	9.1%	–

HCC, hepatocellular carcinoma; LCSGJ, Liver Cancer Study Group of Japan.

Newly-registered patients were increasing and their survival rates were improving.

DISCUSSION

PRIMARY LIVER CANCER is the fourth leading cause of cancer death in Japanese people, following tracheal–bronchial–lung, gastric and colorectal cancers; more than 34 000 people die annually due to liver cancer. In the 18th Nationwide Follow-Up Survey of Primary Liver Cancer, approximately 30% of patients with primary liver cancer were newly registered. Compared with the 17th follow-up survey,[11] this follow-up survey in HCC indicated an increase in elder patients and women, a decrease in patients positive for hepatitis B surface antigen and hepatitis C virus antibody, and a decrease in tumor size at the clinical diagnosis. In the local ablation therapy, ratio of radio frequency ablation therapy was increasing. Advance in diagnostic and therapeutic modalities were considered to have contributed to an improvement in survival of patients with HCC between 1978 and 2005.

We hope that the results of this follow-up survey will contribute to research and improved medical practice for primary liver cancer.

ACKNOWLEDGMENTS

WE WOULD LIKE to express our sincere gratitude to the doctors of the 544 medical institutions that participated in this follow-up survey, and to Mrs T. Idutsu and M. Ogawa for data compilation.

REFERENCES

1 Okuda K, The Liver Cancer Study Group of Japan. Primary liver cancers in Japan. *Cancer* 1980; **45:** 2663–9.
2 The Liver Cancer Study Group of Japan. Primary liver cancer in Japan. *Cancer* 1984; **54:** 1747–55.
3 The Liver Cancer Study Group of Japan. Primary liver cancer in Japan-Sixth report. *Cancer* 1987; **60:** 1400–11.
4 The Liver Cancer Study Group of Japan. Primary liver cancer in Japan. *Ann Surg* 1990; **211:** 277–87.
5 Primary Liver Cancer in Japan. Tobe T *et al*. Springer-Verlag Tokyo, Berlin, Heidelberg, New York, London, Paris, Hong Kong, Barcelona 1992.
6 The Liver Cancer Study Group of Japan. Predictive factors for long term prognosis after partial hepatectomy for patients with hepatocellular carcinoma in Japan. *Cancer* 1994; **74:** 2772–80.
7 Arii S, Yamaoka Y, Futagawa S *et al*. Results of surgical and nonsurgical treatment for small-sized hepatocellular carci-

Table 13 Cumulative survival rates (%) of HCC patients treated with transcatheter arterial embolization (1994–2005)

		n	Year									
			1	2	3	4	5	6	7	8	9	10
All cases		3955	51.7%	35.1%	28.5%	23.7%	20.3%	18.1%	16.7%	14.5%	12.5%	12.5%
Liver damage classification by LCSGJ	A	1658	72.0%	53.1%	43.9%	36.2%	31.3%	27.6%	26.1%	23.7%	21.2%	21.2%
	B	2294	36.3%	21.6%	17.1%	14.4%	12.2%	11.0%	9.6%	5.8%	0.0%	–
	C	137	88.3%	81.3%	75.2%	67.9%	62.8%	59.8%	59.8%	54.8%	54.8%	54.8%
Tumor number	1	738	77.8%	58.9%	49.4%	40.1%	32.3%	26.5%	25.3%	23.6%	18.2%	18.2%
	2	547	63.7%	43.4%	33.4%	29.0%	26.7%	24.9%	23.6%	20.4%	18.1%	18.1%
	3	129	55.3%	32.8%	28.6%	22.2%	19.0%	14.2%	14.2%	14.2%	14.2%	14.2%
	4	1272	76.3%	58.8%	49.4%	41.2%	36.3%	32.2%	30.8%	28.4%	24.8%	24.8%
	≥5	102	75.7%	49.4%	36.5%	31.3%	27.8%	27.8%	22.2%	22.2%	22.2%	22.2%

HCC, hepatocellular carcinoma; LCSGJ, Liver Cancer Study Group of Japan.

Table 14 Cumulative survival rates (%) of ICC patients (1994–2005)

			n	Year									
				1	2	3	4	5	6	7	8	9	10
All cases			3955	51.7%	35.1%	28.5%	23.7%	20.3%	18.1%	16.7%	14.5%	12.5%	12.5%
Hepatic resection		Performed	1658	72.0%	53.1%	43.9%	36.2%	31.3%	27.6%	26.1%	23.7%	21.2%	21.2%
		Not performed	2294	36.3%	21.6%	17.1%	14.4%	12.2%	11.0%	9.6%	5.8%	0.0%	–
Cases of hepatic resection	Tumor size (cm)	≤2	137	88.3%	81.3%	75.2%	67.9%	62.8%	59.8%	59.8%	54.8%	54.8%	54.8%
		2–5	738	77.8%	58.9%	49.4%	40.1%	32.3%	26.5%	25.3%	23.6%	18.2%	18.2%
		5–10	547	63.7%	43.4%	33.4%	29.0%	26.7%	24.9%	23.6%	20.4%	18.1%	18.1%
		>10	129	55.3%	32.8%	28.6%	22.2%	19.0%	14.2%	14.2%	14.2%	14.2%	14.2%
	Tumor number	1	1272	76.3%	58.8%	49.4%	41.2%	36.3%	32.2%	30.8%	28.4%	24.8%	24.8%
		2	102	75.7%	49.4%	36.5%	31.3%	27.8%	27.8%	22.2%	22.2%	22.2%	22.2%
		≥3	186	42.2%	19.5%	16.6%	12.3%	6.3%	4.2%	4.2%	2.1%	2.1%	2.1%
	Residual tumor	Absent	784	77.7%	59.3%	50.6%	43.1%	37.6%	35.6%	33.6%	30.2%	26.5%	26.5%
		Present	608	64.4%	41.4%	31.3%	22.1%	20.6%	20.6%	10.3%	10.3%	10.3%	–
	Lymph node metastasis	Absent	1046	80.6%	64.6%	54.5%	45.3%	39.9%	36.2%	33.8%	30.2%	28.8%	28.8%
		Present	497	55.9%	29.8%	22.8%	17.9%	15.3%	10.7%	10.7%	10.7%	8.0%	8.0%

ICC, intrahepatic cholangiocarcinoma.

Table 15 Cumulative survival rates (%) of combined HCC and ICC (1994–2005)

		n	Year									
			1	2	3	4	5	6	7	8	9	10
All cases		653	58.6%	40.5%	29.7%	23.4%	19.8%	17.8%	15.7%	14.5%	12.7%	12.7%
Hepatic resection	Performed	354	70.7%	50.5%	40.7%	31.0%	28.2%	26.1%	21.9%	20.0%	20.0%	20.0%
	Not performed	299	44.2%	28.8%	16.9%	14.2%	10.6%	8.9%	8.9%	8.9%	0.0%	–

HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma.

nomas: a retrospective and nationwide survey in Japan. The liver cancer study group of Japan. *Hepatology* 2000; **32:** 1224–9.
8 Ikai I, Itai Y, Okita K *et al*. Report of the 15th follow-up survey of primary liver cancer. *Hepatol Res* 2004; **28:** 21–9.
9 Ikai I, Arii S, Kojiro M *et al*. Re-evaluation of prognostic factors for survival after liver resection in patients with hepatocellular carcinoma in a Japanese nationwide survey. *Cancer* 2004; **101:** 796–802.
10 Ikai I, Arii S, Ichida T *et al*. Report of the 16th follow-up survey of primary liver cancer. *Hepatol Res* 2005; **32:** 163–72.
11 Ikai I, Arii S, Okazaki M *et al*. Report of the 17th nationwide follow-up survey of primary liver cancer. in Japan. *Hepatol Res* 2007; **37:** 676–91.
12 Takayasu K, Arii S, Ikai I *et al*. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; **131:** 461–9.
13 Ikai I, Takayasu K, Omata M *et al*. A modified Japan integrated stage score for prognostic assessment in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41:** 884–92.
14 Minagawa M, Ikai I, Matsuyama Y *et al*. Staging of Hepatocellular Carcinoma: assessment of the Japanese TNM and AJCC/UICC TNM systems in a cohort of 13 772 patients in Japan. *Ann Surg* 2007; **245:** 909–22.
15 Eguchi S, Kanematsu T, Arii S *et al*. Comparison of the outcomes between an anatomical subsegmentectomy and a non-anatomical minor hepatectomy for single hepatocellular carcinomas based on a Japanese nationwide survey. *Surgery* 2008; **143:** 469–75.
16 Hasegawa K, Makuuchi M, Takayama T *et al*. Surgical resection vs. percutaneous ablation for hepatocellular carcinoma: a preliminary report of the Japanese nationwide survey. *J Hepatol* 2008; **49:** 589–94.
17 Takayasu K, Arii S, Ikai I *et al*. Liver Cancer Study Group of Japan. Overall survival after transarterial lipiodol infusion chemotherapy with or without embolization for unresectable hepatocellular carcinoma: propensity score analysis. *AJR Am J Roentgenol* 2010; **194:** 830–7.
18 Liver Cancer Study Group of Japan. *General Rules for the Clinical and Pathological Study of Primary Liver Cancer, Second English Edition*. Tokyo: Kanehara & Co., Ltd., 2003.
19 Kudo M, Kubo S, Takayasu K *et al*. Response evaluation criteria in cancer of the liver (RECICL) proposed by the liver cancer study group of Japan (2009 revised version). *Hepatol Res* 2010; **40:** 686–62.

World Journal of Radiology

Online Submissions: http://www.wjgnet.com/1949-8470office
wjr@wjgnet.com
doi:10.4329/wjr.v2.i11.417

World J Radiol 2010 November 28; 2(11): 417-424
ISSN 1949-8470 (online)

EDITORIAL

Radiofrequency ablation of hepatocellular carcinoma: Current status

Yasunori Minami, Masatoshi Kudo

Yasunori Minami, Masatoshi Kudo, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2 Ohno-Higashi Osaka-Sayama, 589-8511, Japan
Author contributions: Minami Y drafted the manuscript and wrote the final version of the manuscript; Kudo M reviewed and approved the last version of the manuscript.
Correspondence to: Masatoshi Kudo, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2 Ohno-Higashi Osaka-Sayama, 589-8511, Japan. m-kudo@med.kindai.ac.jp
Telephone: +81-72-3660221 Fax: +81-72-3672880
Received: September 13, 2010 Revised: October 14, 2010
Accepted: October 21, 2010
Published online: November 28, 2010

Key words: Hepatocellular carcinoma; Radiofrequency ablation; Transcatheter arterial chemoembolization

Peer reviewers: Yicheng Ni, MD, PhD, Professor, Biomedical Imaging, Interventional Therapy and Contrast Media Research, Department of Radiology, University Hospitals, K.U. Leuven, Herestraat 49, B-3000, Leuven, Belgium; Sergio Sartori, MD, Department of Internal Medicine, Section of Interventional Ultrasound, St. Anna Hospital, I-44100 Ferrara, Italy

Minami Y, Kudo M. Radiofrequency ablation of hepatocellular carcinoma: Current status. *World J Radiol* 2010; 2(11): 417-424 Available from: URL: http://www.wjgnet.com/1949-8470/full/v2/i11/417.htm DOI: http://dx.doi.org/10.4329/wjr.v2.i11.417

Abstract

Ablation therapy is one of the best curative treatment options for malignant liver tumors, and can be an alternative to resection. Radiofrequency ablation (RFA) of primary and secondary liver cancers can be performed safely using percutaneous, laparoscopic, or open surgical techniques, and RFA has markedly changed the treatment strategy for small hepatocellular carcinoma (HCC). Percutaneous RFA can achieve the same overall and disease-free survival as surgical resection for patients with small HCC. The use of a laparoscopic or open approach allows repeated placements of RFA electrodes at multiple sites to ablate larger tumors. RFA combined with transcatheter arterial chemoembolization will make the treatment of larger tumors a clinically viable treatment alternative. However, an accurate evaluation of treatment response is very important to secure successful RFA therapy. Since a sufficient safety margin (at least 0.5 cm) can prevent local tumor recurrences, an accurate evaluation of treatment response is very important to secure successful RFA therapy. To minimize complications of RFA, clinicians should be familiar with the imaging features of each type of complication. Appropriate management of complications is essential for successful RFA treatment.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common solid cancers worldwide, with an estimated annual incidence of at least one million new patients[1-4]. Furthermore, the liver is second only to lymph nodes as a common site of metastasis from other solid cancers[5-8]. Surgery is the only curative option for HCC; however, the majority of primary liver cancers are not suitable for curative resection at the time of diagnosis. Difficulties in surgical resection may be related to size, site, and number of tumors, vascular and extrahepatic involvement as well as the general condition and liver function of the patient[9-12]. There is, therefore, a need to develop a simple and effective technique for the treatment of unresectable tumors within the liver. In recent years, local ablative techniques [percutaneous ethanol injection (PEI), microwave coagulation therapy (MCT) and radiofrequency ablation (RFA)] have emerged in clinical practice to expand the pool of patients considered for liver-directed therapies[13-16].

Localized application of thermal energy induces tumor cell destruction. When tumor cells are heated above

45-50℃, intracellular proteins are denatured and cell membranes are destroyed through the dissolution and melting of lipid bilayers[17]. RFA is a localized thermal treatment technique designed to produce tumor destruction by heating tumor tissue to temperatures that exceed 60℃[17]. The alternating current of radiofrequency waves passing down from an uninsulated electrode tip into the surrounding tissues generates changes in the direction of ions and creates ionic agitation and frictional heating. This tissue heating then drives extracellular and intracellular water out of the tissue, resulting in tissue destruction by coagulative necrosis[18,19]. Currently, RFA has gained popularity based on the ease of use, safety, reasonable cost and applicability to minimally invasive techniques. This paper reviews the current status of RFA for HCC.

EQUIPMENT

RFA electrodes and generators

Three types of RF electrodes are currently available commercially: two brands of retractable needle electrodes (model 70 and model 90 Starburst XL needles, RITA Medical Systems, Mountain View, CA, USA; LeVeen needle electrode, Boston Scientific, Boston, MA, USA) and an internally cooled electrode (Cool-Tip RF electrode; Radionics, Burlington, MA, USA)[15].

The needle electrodes of RITA consist of a 14-gauge insulated outer needle that houses nine retractable curved electrodes of various lengths. When the electrodes are extended, the device assumes the approximate configuration of a Christmas tree. Nine of the electrodes are hollow and contain thermocouples in their tips in order to measure the temperature of adjacent tissue. The alternating electric current generator comes in a 250-W model at 460 kHz (Model 1500X RF Generator, RITA Medical Systems). The ablation algorithm is based on temperature at the tips of the electrodes. After the ablation cycle is completed, a temperature reading from the extended electrodes in excess of 50℃ at 1 min is considered to indicate satisfactory ablation.

Another RFA device (LeVeen Needle Electrode; Radiotherapeutics) has retractable curved electrodes and an insulated 17-gauge outer needle that houses 10 solid retractable curved electrodes that, when deployed, assume the configuration of an umbrella. The electrodes are manufactured in different lengths (2- to 4.0-cm umbrella diameter). The alternating electric current generator is 200 W operated at 480 kHz (RF 3000; Boston Scientific). The ablation algorithm is based on tissue impedance, and ablation is considered successful if the device impedes out.

The third RFA device (Cool-Tip radiofrequency electrode; Radionics) has an insulated hollow 17-gauge needle with an exposed needle tip of variable length (2- or 3-cm). The tip of the needle contains a thermocouple to record the temperature of adjacent tissue. The shaft of the needle has two internal channels to allow the needle to be perfused with chilled water. In an attempt to further increase the size of the ablation area, the manufacturer placed three of the cooled needles in a parallel triangular cluster with a common hub. The generator has a peak power output of 200 W and is operated at 480 kHz (CC-1; Radionics). The ablation algorithm is based on tissue impedance, and ablation is considered successful if the device impedes out. As a result, successful ablations usually increase the temperature of the ablated tissue to above 60℃.

Selection criteria of patients with HCC

In patients with HCC, exclusion criteria should include evidence of extrahepatic metastases and/or lobar and local portal venous thrombosis or uncontrolled liver disease decompensation, patients with clotting impairment, renal failure, or Child-Pugh class C cirrhosis. In the EASL Consensus Conference criteria[20], all patients that had tumor nodules with a maximum diameter of 3 cm and not more than three in number with contraindications for surgery are included.

Assessment of technical effectiveness

The technical effectiveness of ablation is commonly assessed by findings on contrast-enhanced computed tomography (CT) or magnetic resonance imaging. A tumor was considered to have been successfully ablated when there were no longer any enhanced regions within the entire tumor during the arterial phase and at least a 0.5 cm margin of apparently normal hepatic tissue surrounding the tumor during the portal phase (Figure 1)[21-23]. This safety margin for RFA therapy is necessary from the perspective of partial volume effect. Failure to establish a sufficient ablative safety margin was shown to be an independently significant risk factor for local tumor progression on multivariate analysis[24]. Part of the tumor was diagnosed as remaining viable when images of the ablated area showed nodular peripheral enhancement[25].

CLINICAL OUTCOMES

Percutaneous approach

A randomized control trial (RCT) has shown that RFA achieved survival rates similar to those achieved following resection[26] (Table 1). Chen *et al*[26] conducted a RCT on 180 patients with a solitary HCC ≤ 5 cm indicated to receive either percutaneous RFA or surgical resection. This study showed percutaneous RFA achieved the same overall and disease-free survival rates as surgical resection for patients with small solitary HCC. The 1- and 4-year overall survival rates after percutaneous RFA and surgery were 95.8%, 67.9% and 93.3%, 64.0%, respectively. The corresponding disease-free survival rates were 85.9%, 46.4% and 86.6%, 51.6%, respectively. However, in cases of primary liver cancer in which local curative therapy was achieved by securing a safety margin, the 4-year survival rate was relatively high, at 66%-82% (results in Japan)[27,28]. Percutaneous RFA has an advantage over liver resection in providing a better short-term postoperative result because local ablative therapy is a less invasive procedure. Although

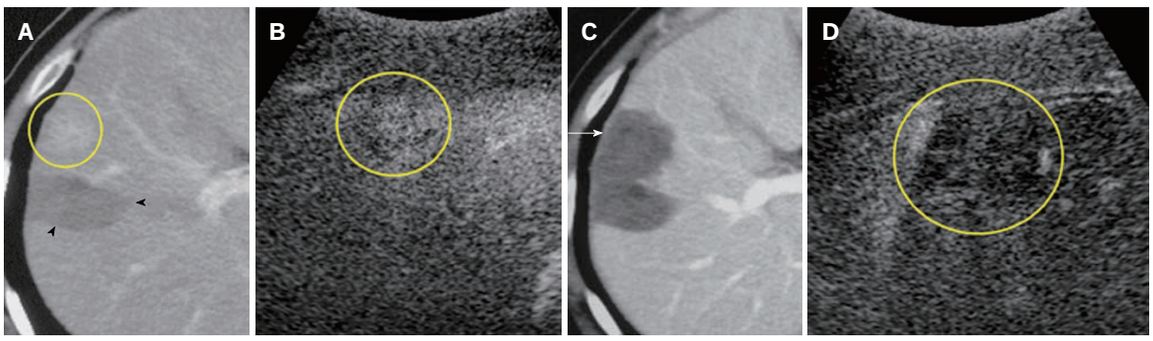


Figure 1 A 61-year-old man with 1.5-cm recurrent hepatocellular carcinoma after ablation therapy in segment 5 of the liver. A: Early-phase dynamic computed tomography (CT) scan shows recurrent tumor (circle). Non-enhanced area (arrowheads) was previously treated by radiofrequency ablation (RFA); B: Contrast harmonic ultrasound (US) using Levovist shows enhancement of viable focus of a hepatocellular carcinoma (HCC) nodule (circle); C: Portal-phase dynamic CT scan, which was obtained 3 d after RFA shows that the tumor was not enhanced, indicating complete necrosis of the lesion (arrow); D: Contrast harmonic US, which was obtained 3 d after ablation shows non-enhanced area (circle).

Table 1 Studies comparing radiofrequency ablation *vs* hepatic resection for hepatocellular carcinoma

Author, yr	Study type	RFA/resection	RFA/resection (mean tumor size, cm)	RFA *vs* resection (%) (overall survival)	*P*
Chen, 2006	RCT	90/90	-/-	65.9 *vs* 64.0 (4-yr)	NS
Takayama, 2009	Retrospective	1315/1235	1.6/1.8	95 *vs* 94 (2-yr)	0.28
Ueno, 2009	Retrospective	123/110	2.0/2.7	63 *vs* 80 (5-yr)	0.06
Hiraoka, 2008	Retrospective	105/59	-/-	59.3 *vs* 59.4 (5-yr)	NS
Abu-Hilal, 2008	Retrospective	34/34	3.0/3.8	57 *vs* 56 (5-yr)	0.3
Gnglielmi, 2008	Retrospective	23/33	-/-	45 *vs* 55 (5-yr)	0.7
Wakai, 2006	Retrospective	64/85	-/-	30 *vs* 53 (10-yr)	0.012
Ogihara, 2005	Retrospective	40/47	4.6/7.4	39 *vs* 31 (5-yr)	0.79
Montorsi, 2005	Prospective	58/40	-/-	30 *vs* 53 (4-yr)	0.018
Vivarelli, 2004	Retrospective	79/79	-/-	33 *vs* 65 (3-yr)	0.002

RFA: Radiofrequency ablation; RCT: Randomized control trial; NS: Not significant.

promising, these data need to be confirmed in larger RCTs before local ablative therapy can replace partial hepatectomy in the treatment of good surgical candidates.

RFA has also been investigated for treating patients with large or multifocal tumors. However, the size and number of tumors are important factors determining the local recurrence rate after RFA[29]. Apart from the larger tumor volume, large liver cancers more frequently have irregular borders and satellite lesions. Therefore, precise tailoring of the size and shape of the thermal lesion is important in RFA for large liver cancers. A number of precisely calculated overlapping coagulation zones are necessary to treat large liver cancers. To increase the size of the coagulation zone in RFA, investigators tried using vascular occlusion during RFA[30,31]. Temporary interruption of hepatic blood flow using vascular occlusion techniques (e.g. balloon catheter occlusion of the hepatic artery, transcatheter arterial embolization (TAE), or transcatheter arterial chemoembolization (TACE) has been shown to increase the efficacy of interstitial thermotherapy due to a significant increase in lesion volume. Vascular occlusion causes a reduction of heat dispersion, thus increasing the range of therapeutic thermal coagulation. Peng *et al*[32] reported a series of 120 patients with HCC, and the 1-, 2-, 3-, and 5-year overall survival rates for the TACE-RFA and RFA groups were 93%, 83%, 75%, 50%, and 89%, 76%, 64%, 42%, respectively ($P = 0.045$).

Ultrasound (US)-guided procedures are necessary but have limited use when the tumor is located under the diaphragm. However, saline solution injection into the pleural cavity can separate the lung and liver on B-mode US, i.e. artificial pleural effusion acts as an acoustic media. There are reports on the feasibility and safety of RFA with artificially induced pleural effusion for HCC located in the right subphrenic region[33-36]. In a series of 24 patients with HCC located in the hepatic dome, 200-1100 mL of 5% glucose solution was infused intrathoracically to separate the lung and liver, thus, complete tumor necrosis in a single session was achieved in 96.4% of patients[36].

Multiple RFA sessions for locally progressive HCCs were previously required because it is frequently difficult to distinguish viable tumors from necrotic tissue on B-mode US[37]. However, contrast-enhanced harmonic US imaging is able to evaluate small hypervascular HCCs even when B-mode US cannot adequately characterize the tumors[38-43]. In particular, contrast harmonic US has been improved by the development of second-generation contrast agents such as sulfur hexafluoride microbubbles (So-

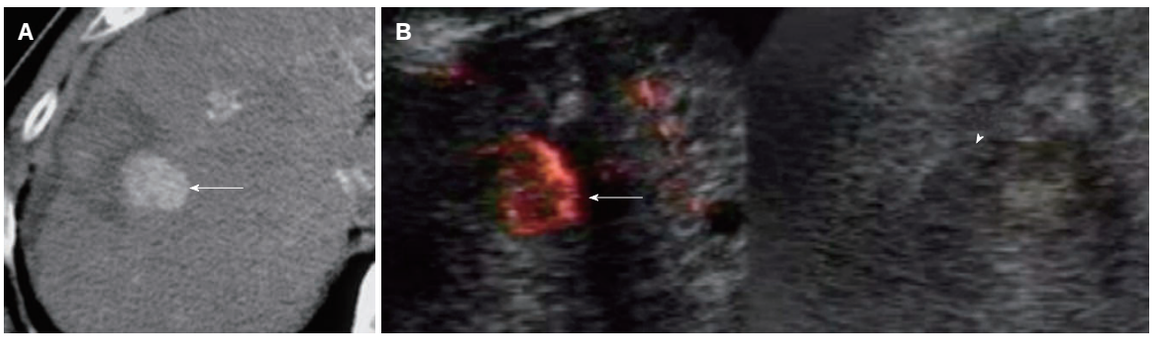


Figure 2 A 71-year-old man with 2.0 cm local tumor progression of hepatocellular carcinoma after radiofrequency ablation therapy in segment 8 of the liver. A: Early-phase dynamic computed tomography (CT) scan shows outgrowth pattern of locally progressive hepatocellular carcinoma (HCC) (arrow). The lesion borders an unenhanced area, which was previously treated; B: Left: Contrast harmonic Doppler ultrasound (US) using Levovist shows enhancement of local tumor progression of HCC (arrow). Therefore, an enhanced lesion can be identified as a target for the insertion of a single RF electrode; Right: B-mode US shows a HCC nodule demonstrated as a low echoic lesion with an unclear border (arrowhead).

noVue; Bracco SpA, Milan, Italy), perflutren lipid microbubbles (Definity; Bristol- Myers Squibb, North Billerica, MA, USA), perflutren protein microbubbles (Optison; GE Healthcare, Buckinghamshire, UK), and perfluorocarbon microbubbles (Sonazoid; Daiichi-Sankyo, Tokyo, Japan). These microbubbles provide stable nonlinear oscillation in a low power acoustic field due to the hard shell of these bubbles, producing great detail in the harmonic signals in real-time[44-49]. It has been reported that contrast harmonic sonography-guided RFA is an efficient approach for guiding further ablation of hepatic malignancies that are not clearly demarcated by B-mode US (Figure 2)[50-54].

Laparoscopic/open surgical approach

The use of a laparoscopic or open approach allows repeated placements of RFA electrodes at multiple sites to ablate larger tumors. The laparoscopic approach appears to be the safest and most effective method for small tumors on the liver surface, and offers the advantages of laparoscopic US, which provides better resolution of the number and location of liver tumors[55,56]. Moreover, a hand-assisted technique can be applied safely and effectively to laparoscopic liver surgery[57-59]. An intraoperative US probe is inserted into the peritoneal cavity together with the surgeon's hand through a hand-access device. An RF electrode can be subcostally or intercostally advanced into a liver tumor under direct guidance by intraoperative US. Therefore, a hand-assisted laparoscopic US-guided method has advantages for both laparoscopic and open surgical approaches. The postoperative recovery of patients was shorter compared with that after an open surgical approach. Ishiko *et al*[57] reported that the surgical procedures consisted of 5 RFA to tumors in the caudate lobe with hand-assisted laparoscopic surgery (HALS), and a postoperative CT scan demonstrated sufficient ablation in all patients and there was no surgical mortality. The HALS approach has several advantages; it facilitates and expedites the procedure, reduces the stress factor on the surgeon, greatly improves exposure, and facilitates immediate and efficient control of bleeding vessels with the internal hand. The hand-access device, which essentially consists of a cuff with a spiral inflatable valve, enables withdrawal and reinsertion of the hand without loss of pneumoperitoneum during the procedure. However, the local treatment failure rate of the laparoscopic approach was higher in patients with HCC nodules situated deep within the liver and measuring 4 cm or more in diameter[60]. Great difficulty can be encountered during treatment of lesions located close to the gallbladder or in contact with the diaphragm.

Although more invasive, open RFA can be performed more easily and the puncture course of the RF needle can be more widely selected than that during the laparoscopic approach. It has been reported that patients undergoing radical open RFA demonstrated few ablation site recurrences even though the nodules measured more than 4 cm in diameter and/or there were more than three nodules[61,62]. Open RFA can be indicated for patients who are considered suitable for open surgery with large, numerous, or deeply located tumors that cannot be accurately accessed by a laparoscopic approach. Furthermore, when patients have synchronous liver metastases, open surgical RFA can be performed in conjunction with resection of the primary cancer.

Local controllability (local tumor progression)

The local recurrence rate after RFA for HCC ranged from 1.7% to 41%[63-70] (Table 2). As reported by Kudo[28], in a series of 141 HCC patients who underwent curative RFA therapy, local tumor progression was observed in 9 cases (local tumor progression rate, 6.3%), whereas the cumulative local tumor progression rate, calculated by the Kaplan-Meier method, was 12% at 4 years. The rate may have depended on the size of nodules treated and the skill of the surgeons. There has not been any definitive report of local recurrence of nodules measuring 2-cm or smaller, and we ourselves have not encountered any case showing such recurrence, suggesting that recurrence in such cases is exceptional. The risk of local tumor progression increases with size, but the local tumor progression rate

Table 2 Studies comparing local tumor progression rates of radiofrequency ablation for hepatocellular carcinoma

Author	Yr	*n*	Tumor size (mean, cm)	Follow-up period (mean, mo)	Local tumor progression rate (%)
Rossi *et al*[63]	1996	41	2.3	22.6	5.0
Buscarini *et al*[64]	2001	60	-	26.8	14
Choi *et al*[65]	2004	53	2.1	23	21
Lu *et al*[66]	2005	87	2.5	12.7	5.8
Shiina *et al*[67]	2005	118	-	34.8	1.7
Solmi *et al*[68]	2006	63	2.8	32.3	41
Hänsler *et al*[69]	2007	21	4.2	-	21
Waki *et al*[70]	2010	88	-	36	4.8

differs markedly depending on whether or not a circumferential 5-mm safety margin is secured. In a meta-analysis of RFA *vs* PEI in HCC, the survival rate showed a significant benefit for RFA over PEI at 1, 2, 3 and 4 years[71]. The survival advantage increased over time with Relative Risk values of: 1.28 (95% CI: 1.12-1.45) and 1.24 (95% CI: 1.05-1.48) for RFA *vs* PEI at 3- and 4-years, respectively. Likewise, RFA achieved significantly lower rates of local recurrence (RR: 0.37, 95% CI: 0.23-0.59)[71].

Complications

Complications reported following percutaneous RFA of malignant liver tumors in 2320 patients treated at 41 different hospitals in Italy indicate that the mortality rate was 0.3% with an overall complication rate of 7.1%[72,73]. The authors described major complications (2.4% incidence) including death, hemorrhage, RFA needle-track seeding, RFA lesion abscess, perforation of gastrointestinal viscus, liver failure, biloma, biliary stricture, portal vein thrombosis, and hemothorax or pneumothorax requiring drainage, and minor complications (4.7% incidence) including pain, fever, and asymptomatic pleural effusion. Another recent review indicated that complication rates for percutaneous, laparoscopic, and open RFA of hepatic tumors in 3670 patients were 7.2%, 9.5%, and 9.9%, respectively[74]. Complications directly related to the liver included bleeding (1.6%), intrahepatic abscess (1.1%), biliary or hepatic vascular injury (1.7%), and liver failure (0.8%). Complications that arose in less than 1% of hepatic tumor RFA patients included pulmonary problems (pneumothorax, hydrothorax, pleural effusion), grounding pad skin burn, myoglobinemia or myoglobinuria, renal failure, coagulopathy, tumor seeding of the needle track, excessive hormone release from treated neuroendocrine tumors, cardiac problems (myocardial infarction, arrhythmia), and injury to the diaphragm or adjacent viscera. Although Llovet *et al*[75] reported that dissemination along the puncture route was observed in 12.5% of their patients, only a few such cases have been reported in Japan, and dissemination may not occur at such a high frequency. This complication was almost absent in many reports from Japan[28]. Overall, the frequency of major complications of percutaneous RFA was 0.6%-8.9%, which was higher than that of PEI, but generally lower than that of MCT[28].

Some investigators have suggested that tumor location is closely related to the risk of major complications. Central tumors close to the hepatic hilum were reported to be unsuitable for percutaneous RFA because of the risk of injuring adjacent bile ducts[15]. It was also suggested that RFA for nodules adjacent to large vessels might often result in incomplete necrosis because of a heat sink effect. In addition, peripheral tumors adjacent to extrahepatic organs were also suggested to be unsuitable because of the risk of heat injuries, such as intestinal perforation and pleural effusion[72,76]. Thus, there may be difficulty with RFA of nodules in such high-risk locations, possibly resulting in complications or preventing adequate treatment. However, Teratani *et al*[77] reported that there was no difference in early complication rates according to tumor location. The effort to achieve thorough ablation increased the total number of electrode insertions, and this may have led to an increase in complications.

To minimize complications of RFA for malignant liver tumors, knowledge of risk factors and prevention methods is required. In addition, because early and accurate diagnosis is necessary for the proper management of complications, not only radiologists but also hepatologists and surgeons should be familiar with the imaging features of each type of complication. Appropriate management of complications is essential for successful treatment with RFA.

CONCLUSION

RFA can be performed safely using percutaneous, laparoscopic, or open surgical techniques, and has markedly changed the treatment strategy for small HCC. RFA combined with TACE will likely make the treatment of larger tumors a clinically viable treatment alternative. Moreover, an accurate evaluation of treatment response is very important to secure successful RFA therapy. A sufficient safety margin can prevent local tumor recurrences. However, surgery is still the recommended treatment modality for patients with both primary hepatic malignancies. For inoperable lesions, RFA will likely play a significant role with a potential curative intent. Currently, the important clinical issue is that follow-up studies need to be performed for the early detection and treatment of recurrence, either locally or at different sites after RFA.

REFERENCES

1 **El-Serag HB**. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34
2 **Kiyosawa K**, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, Tanaka E. Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; **127**: S17-S26
3 **Di Bisceglie AM**. Epidemiology and clinical presentation of hepatocellular carcinoma. *J Vasc Interv Radiol* 2002; **13**: S169-S171
4 **McCaughan GW**, Koorey DJ, Strasser SI. Hepatocellular carcinoma: current approaches to diagnosis and management. *Intern Med J* 2002; **32**: 394-400
5 **Zavadsky KE**, Lee YT. Liver metastases from colorectal carci-

noma: incidence, resectability, and survival results. *Am Surg* 1994; **60**: 929-933
6 **Liu LX**, Zhang WH, Jiang HC. Current treatment for liver metastases from colorectal cancer. *World J Gastroenterol* 2003; **9**: 193-200
7 **Tsim NC**, Frampton AE, Habib NA, Jiao LR. Surgical treatment for liver cancer. *World J Gastroenterol* 2010; **16**: 927-933
8 **Michalski CW**, Erkan M, Hüser N, Müller MW, Hartel M, Friess H, Kleeff J. Resection of primary pancreatic cancer and liver metastasis: a systematic review. *Dig Surg* 2008; **25**: 473-480
9 **Cho YK**, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. *Hepatology* 2010; **51**: 1284-1290
10 **Rust C**, Gores GJ. Locoregional management of hepatocellular carcinoma. Surgical and ablation therapies. *Clin Liver Dis* 2001; **5**: 161-173
11 **Lee WS**, Yun SH, Chun HK, Lee WY, Kim SJ, Choi SH, Heo JS, Joh JW, Choi D, Kim SH, Rhim H, Lim HK. Clinical outcomes of hepatic resection and radiofrequency ablation in patients with solitary colorectal liver metastasis. *J Clin Gastroenterol* 2008; **42**: 945-949
12 **Mulier S**, Ruers T, Jamart J, Michel L, Marchal G, Ni Y. Radiofrequency ablation versus resection for resectable colorectal liver metastases: time for a randomized trial? An update. *Dig Surg* 2008; **25**: 445-460
13 **Bartolozzi C**, Lencioni R. Ethanol injection for the treatment of hepatic tumours. *Eur Radiol* 1996; **6**: 682-696
14 **Okada S**. Local ablation therapy for hepatocellular carcinoma. *Semin Liver Dis* 1999; **19**: 323-328
15 **McGhana JP**, Dodd GD 3rd. Radiofrequency ablation of the liver: current status. *AJR Am J Roentgenol* 2001; **176**: 3-16
16 **Shiina S**, Teratani T, Obi S, Hamamura K, Koike Y, Omata M. Nonsurgical treatment of hepatocellular carcinoma: from percutaneous ethanol injection therapy and percutaneous microwave coagulation therapy to radiofrequency ablation. *Oncology* 2002; **62** Suppl 1: 64-68
17 **McGahan JP**, Brock JM, Tesluk H, Gu WZ, Schneider P, Browning PD. Hepatic ablation with use of radio-frequency electrocautery in the animal model. *J Vasc Interv Radiol* 1992; **3**: 291-297
18 **McGahan JP**, Browning PD, Brock JM, Tesluk H. Hepatic ablation using radiofrequency electrocautery. *Invest Radiol* 1990; **25**: 267-270
19 **Goldberg SN**, Gazelle GS, Halpern EF, Rittman WJ, Mueller PR, Rosenthal DI. Radiofrequency tissue ablation: importance of local temperature along the electrode tip exposure in determining lesion shape and size. *Acad Radiol* 1996; **3**: 212-218
20 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430
21 **Ni Y**, Mulier S, Miao Y, Michel L, Marchal G. A review of the general aspects of radiofrequency ablation. *Abdom Imaging* 2005; **30**: 381-400
22 **Ni Y**, Chen F, Mulier S, Sun X, Yu J, Landuyt W, Marchal G, Verbruggen A. Magnetic resonance imaging after radiofrequency ablation in a rodent model of liver tumor: tissue characterization using a novel necrosis-avid contrast agent. *Eur Radiol* 2006; **16**: 1031-1040
23 **Mori K**, Fukuda K, Asaoka H, Ueda T, Kunimatsu A, Okamoto Y, Nasu K, Fukunaga K, Morishita Y, Minami M. Radiofrequency ablation of the liver: determination of ablative margin at MR imaging with impaired clearance of ferucarbotran--feasibility study. *Radiology* 2009; **251**: 557-565
24 **Kim YS**, Rhim H, Cho OK, Koh BH, Kim Y. Intrahepatic recurrence after percutaneous radiofrequency ablation of hepatocellular carcinoma: analysis of the pattern and risk factors. *Eur J Radiol* 2006; **59**: 432-441
25 **Lim HK**, Choi D, Lee WJ, Kim SH, Lee SJ, Jang HJ, Lee JH, Lim JH, Choo IW. Hepatocellular carcinoma treated with percutaneous radio-frequency ablation: evaluation with follow-up multiphase helical CT. *Radiology* 2001; **221**: 447-454
26 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328
27 **Kudo M**. Radiofrequency ablation for hepatocellular carcinoma: updated review in 2010. *Oncology* 2010; **78** Suppl 1: 113-124
28 **Kudo M**. Local ablation therapy for hepatocellular carcinoma: current status and future perspectives. *J Gastroenterol* 2004; **39**: 205-214
29 **Lau WY**, Lai EC. Hepatocellular carcinoma: current management and recent advances. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 237-257
30 **Yamasaki T**, Kurokawa F, Shirahashi H, Kusano N, Hironaka K, Okita K. Percutaneous radiofrequency ablation therapy for patients with hepatocellular carcinoma during occlusion of hepatic blood flow. Comparison with standard percutaneous radiofrequency ablation therapy. *Cancer* 2002; **95**: 2353-2360
31 **Yamakado K**, Nakatsuka A, Akeboshi M, Shiraki K, Nakano T, Takeda K. Combination therapy with radiofrequency ablation and transcatheter chemoembolization for the treatment of hepatocellular carcinoma: Short-term recurrences and survival. *Oncol Rep* 2004; **11**: 105-109
32 **Peng ZW**, Chen MS, Liang HH, Gao HJ, Zhang YJ, Li JQ, Zhang YQ, Lau WY. A case-control study comparing percutaneous radiofrequency ablation alone or combined with transcatheter arterial chemoembolization for hepatocellular carcinoma. *Eur J Surg Oncol* 2010; **36**: 257-263
33 **Uehara T**, Hirooka M, Ishida K, Hiraoka A, Kumagi T, Kisaka Y, Hiasa Y, Onji M. Percutaneous ultrasound-guided radiofrequency ablation of hepatocellular carcinoma with artificially induced pleural effusion and ascites. *J Gastroenterol* 2007; **42**: 306-311
34 **Koda M**, Ueki M, Maeda Y, Mimura K, Okamoto K, Matsunaga Y, Kawakami M, Hosho K, Murawaki Y. Percutaneous sonographically guided radiofrequency ablation with artificial pleural effusion for hepatocellular carcinoma located under the diaphragm. *AJR Am J Roentgenol* 2004; **183**: 583-588
35 **Minami Y**, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H. Percutaneous radiofrequency ablation guided by contrast-enhanced harmonic sonography with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. *AJR Am J Roentgenol* 2004; **182**: 1224-1226
36 **Minami Y**, Kudo M, Kawasaki T, Chung H, Ogawa C, Inoue T, Sakaguchi Y, Sakamoto H, Shiozaki H. Percutaneous ultrasound-guided radiofrequency ablation with artificial pleural effusion for hepatocellular carcinoma in the hepatic dome. *J Gastroenterol* 2003; **38**: 1066-1070
37 **Cioni D**, Lencioni R, Rossi S, Garbagnati F, Donati F, Crocetti L, Bartolozzi C. Radiofrequency thermal ablation of hepatocellular carcinoma: using contrast-enhanced harmonic power doppler sonography to assess treatment outcome. *AJR Am J Roentgenol* 2001; **177**: 783-788
38 **Kudo M**. Imaging diagnosis of hepatocellular carcinoma and premalignant/borderline lesions. *Semin Liver Dis* 1999; **19**: 297-309
39 **Ding H**, Kudo M, Onda H, Suetomi Y, Minami Y, Maekawa K. Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual-display mode. *Radiology* 2001; **220**: 349-356
40 **Ding H**, Kudo M, Maekawa K, Suetomi Y, Minami Y, Onda

H. Detection of tumor parenchymal blood flow in hepatic tumors: value of second harmonic imaging with a galactose-based contrast agent. *Hepatol Res* 2001; **21**: 242-251
41 **Kudo M**. Contrast harmonic ultrasound is a breakthrough technology in the diagnosis and treatment of hepatocellular carcinoma. *J Med Ultrasonics* 2001; **28**: 79-81
42 **Ding H**, Kudo M, Onda H, Suetomi Y, Minami Y, Chung H, Kawasaki T, Maekawa K. Evaluation of posttreatment response of hepatocellular carcinoma with contrast-enhanced coded phase-inversion harmonic US: comparison with dynamic CT. *Radiology* 2001; **221**: 721-730
43 **Minami Y**, Kudo M, Kawasaki T, Kitano M, Chung H, Maekawa K, Shiozaki H. Transcatheter arterial chemoembolization of hepatocellular carcinoma: usefulness of coded phase-inversion harmonic sonography. *AJR Am J Roentgenol* 2003; **180**: 703-708
44 **Meloni MF**, Goldberg SN, Livraghi T, Calliada F, Ricci P, Rossi M, Pallavicini D, Campani R. Hepatocellular carcinoma treated with radiofrequency ablation: comparison of pulse inversion contrast-enhanced harmonic sonography, contrast-enhanced power Doppler sonography, and helical CT. *AJR Am J Roentgenol* 2001; **177**: 375-380
45 **Quaia E**, Calliada F, Bertolotto M, Rossi S, Garioni L, Rosa L, Pozzi-Mucelli R. Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence. *Radiology* 2004; **232**: 420-430
46 **Jang HJ**, Kim TK, Burns PN, Wilson SR. Enhancement patterns of hepatocellular carcinoma at contrast-enhanced US: comparison with histologic differentiation. *Radiology* 2007; **244**: 898-906
47 **Wang Z**, Tang J, An L, Wang W, Luo Y, Li J, Xu J. Contrast-enhanced ultrasonography for assessment of tumor vascularity in hepatocellular carcinoma. *J Ultrasound Med* 2007; **26**: 757-762
48 **Leen E**, Angerson WJ, Yarmenitis S, Bongartz G, Blomley M, Del Maschio A, Summaria V, Maresca G, Pezzoli C, Llull JB. Multi-centre clinical study evaluating the efficacy of SonoVue (BR1), a new ultrasound contrast agent in Doppler investigation of focal hepatic lesions. *Eur J Radiol* 2002; **41**: 200-206
49 **Kono Y**, Lucidarme O, Choi SH, Rose SC, Hassanein TI, Alpert E, Mattrey RF. Contrast-enhanced ultrasound as a predictor of treatment efficacy within 2 weeks after transarterial chemoembolization of hepatocellular carcinoma. *J Vasc Interv Radiol* 2007; **18**: 57-65
50 **Kudo M**, Minami Y. Radiofrequency ablation therapy under harmonic imaging guidance for the recurring cancer after local therapy for HCC: a randomized controlled study with RFA under B-mode guidance. *Ultrasound Med Biol* 2003; **29**: S145
51 **Minami Y**, Kudo M, Kawasaki T, Chung H, Ogawa C, Shiozaki H. Treatment of hepatocellular carcinoma with percutaneous radiofrequency ablation: usefulness of contrast harmonic sonography for lesions poorly defined with B-mode sonography. *AJR Am J Roentgenol* 2004; **183**: 153-156
52 **Minami Y**, Kudo M, Chung H, Kawasaki T, Yagyu Y, Shimono T, Shiozaki H. Contrast harmonic sonography-guided radiofrequency ablation therapy versus B-mode sonography in hepatocellular carcinoma: prospective randomized controlled trial. *AJR Am J Roentgenol* 2007; **188**: 489-494
53 **Minami Y**, Kudo M. Contrast-enhanced harmonic ultrasound imaging in ablation therapy for primary hepatocellular carcinoma. *World J Radiol* 2009; **1**: 86-91
54 **Minami Y**, Kudo M, Hatanaka K, Kitai S, Inoue T, Hagiwara S, Chung H, Ueshima K. Radiofrequency ablation guided by contrast harmonic sonography using perfluorocarbon microbubbles (Sonazoid) for hepatic malignancies: an initial experience. *Liver Int* 2010; **30**: 759-764
55 **Santambrogio R**, Podda M, Zuin M, Bertolini E, Bruno S, Cornalba GP, Costa M, Montorsi M. Safety and efficacy of laparoscopic radiofrequency ablation of hepatocellular carcinoma in patients with liver cirrhosis. *Surg Endosc* 2003; **17**: 1826-1832
56 **Okabayashi T**, Kobayashi M, Akimori T, Akisawa N, Iwasaki S, Onishi S, Araki K. Usefulness of laparoscopic radiofrequency ablation of hepatocellular carcinoma. *Surg Technol Int* 2005; **14**: 177-181
57 **Ishiko T**, Beppu T, Sugiyama S, Masuda T, Takahashi M, Komori H, Takamori H, Hirota M, Kanemitu K, Baba H. Radiofrequency ablation with hand-assisted laparoscopic surgery for the treatment of hepatocellular carcinoma in the caudate lobe. *Surg Laparosc Endosc Percutan Tech* 2008; **18**: 272-276
58 **Schumacher G**, Eisele R, Spinelli A, Schmidt SC, Jacob D, Pratschke J, Neuhaus P. Indications for hand-assisted laparoscopic radiofrequency ablation for liver tumors. *J Laparoendosc Adv Surg Tech A* 2007; **17**: 153-159
59 **Hirooka M**, Kisaka Y, Uehara T, Ishida K, Kumagi T, Watanabe Y, Abe M, Matsuura B, Hiasa Y, Onji M. Efficacy of laparoscopic radiofrequency ablation for hepatocellular carcinoma compared to percutaneous radiofrequency ablation with artificial ascites. *Dig Endosc* 2009; **21**: 82-86
60 **Santambrogio R**, Opocher E, Montorsi M. Laparoscopic radiofrequency ablation of hepatocellular carcinoma: A critical review from the surgeon's perspective. *J Ultrasound* 2008; **11**: 1-7
61 **Minami Y**, Kawasaki T, Kudo M, Haji S, Shiraishi O, Kawabe T, Yasuda C, Nakai T, Takeyama Y, Shiozaki H. Treatment of large and/or multiple hepatic malignancies: open surgical approaches of radiofrequency ablation. *Hepatogastroenterology* 2007; **54**: 2358-2360
62 **Tanaka S**, Shimada M, Shirabe K, Taketomi A, Maehara S, Tsujita E, Ito S, Kitagawa D, Maehara Y. Surgical radiofrequency ablation for treatment of hepatocellular carcinoma: an endoscopic or open approach. *Hepatogastroenterology* 2009; **56**: 1169-1173
63 **Rossi S**, Di Stasi M, Buscarini E, Quaretti P, Garbagnati F, Squassante L, Paties CT, Silverman DE, Buscarini L. Percutaneous RF interstitial thermal ablation in the treatment of hepatic cancer. *AJR Am J Roentgenol* 1996; **167**: 759-768
64 **Buscarini L**, Buscarini E, Di Stasi M, Vallisa D, Quaretti P, Rocca A. Percutaneous radiofrequency ablation of small hepatocellular carcinoma: long-term results. *Eur Radiol* 2001; **11**: 914-921
65 **Choi D**, Lim HK, Kim MJ, Lee SH, Kim SH, Lee WJ, Lim JH, Joh JW, Kim YI. Recurrent hepatocellular carcinoma: percutaneous radiofrequency ablation after hepatectomy. *Radiology* 2004; **230**: 135-141
66 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137
67 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130
68 **Solmi L**, Nigro G, Roda E. Therapeutic effectiveness of echo-guided percutaneous radiofrequency ablation therapy with a LeVeen needle electrode in hepatocellular carcinoma. *World J Gastroenterol* 2006; **12**: 1098-1104
69 **Hänsler J**, Frieser M, Tietz V, Uhlke D, Wissniowski T, Bernatik T, Hothorn T, Hahn EG, Strobel D. Percutaneous radiofrequency ablation of liver tumors using multiple saline-perfused electrodes. *J Vasc Interv Radiol* 2007; **18**: 405-410
70 **Waki K**, Aikata H, Katamura Y, Kawaoka T, Takaki S, Hiramatsu A, Takahashi S, Toyota N, Ito K, Chayama K. Percutaneous radiofrequency ablation as first-line treatment for small hepatocellular carcinoma: results and prognostic factors on

long-term follow up. *J Gastroenterol Hepatol* 2010; **25**: 597-604
71 **Bouza C**, López-Cuadrado T, Alcázar R, Saz-Parkinson Z, Amate JM. Meta-analysis of percutaneous radiofrequency ablation versus ethanol injection in hepatocellular carcinoma. *BMC Gastroenterol* 2009; **9**: 31
72 **Livraghi T**, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. *Radiology* 2003; **226**: 441-451
73 **Curley SA**, Marra P, Beaty K, Ellis LM, Vauthey JN, Abdalla EK, Scaife C, Raut C, Wolff R, Choi H, Loyer E, Vallone P, Fiore F, Scordino F, De Rosa V, Orlando R, Pignata S, Daniele B, Izzo F. Early and late complications after radiofrequency ablation of malignant liver tumors in 608 patients. *Ann Surg* 2004; **239**: 450-458
74 **Mulier S**, Mulier P, Ni Y, Miao Y, Dupas B, Marchal G, De Wever I, Michel L. Complications of radiofrequency coagulation of liver tumours. *Br J Surg* 2002; **89**: 1206-1222
75 **Llovet JM**, Vilana R, Brú C, Bianchi L, Salmeron JM, Boix L, Ganau S, Sala M, Pagès M, Ayuso C, Solé M, Rodés J, Bruix J. Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. *Hepatology* 2001; **33**: 1124-1129
76 **Meloni MF**, Goldberg SN, Moser V, Piazza G, Livraghi T. Colonic perforation and abscess following radiofrequency ablation treatment of hepatoma. *Eur J Ultrasound* 2002; **15**: 73-76
77 **Teratani T**, Yoshida H, Shiina S, Obi S, Sato S, Tateishi R, Mine N, Kondo Y, Kawabe T, Omata M. Radiofrequency ablation for hepatocellular carcinoma in so-called high-risk locations. *Hepatology* 2006; **43**: 1101-1108

S- Editor Cheng JX **L- Editor** Webster JR **E- Editor** Zheng XM

The Oncologist®

The Challenge of Prognosis and Staging for Hepatocellular Carcinoma

JORGE A. MARRERO,[a] MASATOSHI KUDO,[b] JEAN-PIERRE BRONOWICKI[c]

[a]Multidisciplinary Liver Tumor Program, University of Michigan, Ann Arbor, Michigan, USA; [b]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan; [c]Department of Gastroenterology and Hepatology, INSERM U954, University Hospital of Nancy, University Henri Poincaré, Vandœuvre-lès-Nancy, France

Key Words. Hepatocellular carcinoma • Tumor staging • Prognosis • Classification

Disclosures: Jorge A. Marrero: *Honoraria:* Bayer/Onyx; *Research funding/contracted research:* Bayer; **Masatoshi Kudo:** None; **Jean-Pierre Bronowicki:** *Honoraria:* Bayer Pharma, Bristol-Myers Squibb.

The content of this article has been reviewed by independent peer reviewers to ensure that it is balanced, objective, and free from commercial bias. No financial relationships relevant to the content of this article have been disclosed by the independent peer reviewers.

ABSTRACT

Hepatocellular carcinoma (HCC) is a heterogeneous condition, with multiple confounding factors making patient assessment extremely complex. Tumor burden, the presence of symptoms, liver function, and comorbidities must all be considered to ensure accurate patient assessment, thereby providing physicians with a common language on which to base treatment decisions and guide research. Although many staging classifications have been developed, there is no consensus on the best classification to use. The Barcelona Clinic Liver Cancer system is a promising candidate for a standard western classification, because it has been externally validated and is endorsed by the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases. Similarly, the biomarker-combined Japanese Integrated Staging (JIS) score is the most promising candidate for a standard Asia-Pacific classification, because it has been externally validated and shown to be superior to conventional JIS. Because risk factors vary significantly by region, so too does the predictive power of current staging classifications; any standard global staging classification would need to be validated in both western and Asia-Pacific patients. To date, no such globally validated classification exists. Findings from scientific research have improved our understanding of HCC and enabled us to refine current classifications. The role of tumor markers to predict survival was recently reported, and α-fetoprotein, *lens culinaris* agglutinin-reactive α-fetoprotein, and des-γ-carboxyprothrombin have now been incorporated into some classifications. Molecular markers have also been linked with poor outcomes and will likely play a role in future classifications. Although more work is required, it is hoped that these and other ongoing research efforts will eventually enable the development of a global staging classification. ***The Oncologist*** 2010;15(suppl 4):23–33

Correspondence: Jorge A. Marrero, M.D., M.S., Multidisciplinary Liver Tumor Program, University of Michigan, 3912 Taubman Center SPC 5362, Ann Arbor, Michigan, 48109, USA. Telephone: 734-615-4628; Fax: 734-936-7392; e-mail: jmarrero@umich.edu Received January 21, 2010; accepted for publication July 23, 2010. 1083-7159/2010/$30.00/0 doi: 10.1634/theoncologist.2010-S4-23

INTRODUCTION

Hepatocellular carcinoma (HCC) is a heterogeneous condition with multiple variables that vary from region to region, complicating diagnosis, prognosis, and treatment recommendations. The presence of comorbidities is a common confounding factor that can compromise liver function and affect outcomes. For example, 80% of patients present with liver cirrhosis [1] and 85.5% of patients are carriers of either hepatitis B virus (HBV), which is particularly prevalent in Africa and Asia, or hepatitis C virus (HCV), prevalent in western countries and Japan [2, 3]. The characteristics of HCC also vary with geographic location. In rural South Africa, HCC is commonly diagnosed at a more advanced stage than in North America [4]. Because HBV is often acquired at an early age in Africa and Asia, HCC may also develop in younger patients and in the absence of liver cirrhosis [5]. Conversely, in North America, many patients have long-term liver cirrhosis and subsequently develop HCC. Clinical presentation in these patients is therefore dominated by complications of cirrhosis. These confounding factors mean that multiple variables must be considered when assessing patients with HCC.

The aims of HCC staging classifications are to: stratify patients to determine their overall survival (OS) probability prior to treatment, facilitate treatment, and enable objective comparison among the outcomes of research studies. What separates HCC from other solid tumors is that the presence of chronic liver disease and cirrhosis affects OS and the ability to treat this tumor. Therefore, liver disease is a very important variable, together with the overall health of the patient [6, 7]. In considering all these variables, it is hoped that accurate and consistent assessment of all patients can be achieved, thereby providing a common language for physicians as well as the broader multidisciplinary team. This, in turn, should facilitate appropriate treatment selection and ensure optimum patient management. However, with >15 HCC staging classifications available, each measuring a range of different factors and developed in different patient populations, physicians are faced with the complicated task of choosing which classification to use.

In this article, we review the major HCC staging classifications used globally and examine the factors assessed, as well as how each of the staging classifications was developed and validated. We also provide an overview of comparisons among various staging classifications reported in the literature. The paper does not aim to assess the relative values of individual classifications, nor to provide any endorsement of one system over another. However, we suggest possible areas for improvements that are necessary if we are to achieve a globally applicable HCC staging classification.

STAGING CLASSIFICATIONS IN HCC

The factors influencing the development of HCC and its disease course vary considerably from region to region. As a result, various staging classifications have been developed that take into account a range of factors (Table 1), and although some classifications appear to be effective across broad regions, such as western or Asia-Pacific patient populations, others have been evaluated only in a single country. However, there is no globally applicable staging classification, and thus no common language on which to base treatment decisions and guide research.

Tumor–Node–Metastasis Staging System

The first staging classification for solid tumors was developed >50 years ago by the French surgeon Pierre Denoix [8]. In 1968, his recommendations for various tumors were compiled and published by the International Union Against Cancer and the American Joint Committee on Cancer in the first edition of the tumor–node–metastasis (TNM) staging system. Since then, this staging classification has undergone several amendments, and the most recent, sixth edition, was published in 2003 [9, 10].

The TNM staging classification provides an assessment of solid tumors based only on size and extent of invasion. This is measured according to the size of the primary tumor (T), presence of tumor in the regional lymph nodes (N), and presence of metastatic spread beyond the lymph nodes (M). Assessment of TNM staging can be prior to treatment (clinical staging) or after surgery (pathologic staging) [8]. Clinical staging is performed using imaging procedures, but in patients with HCC, the presence of cirrhosis and/or swelling of the lymph nodes as a result of chronic liver disease may prevent accurate assessment. Pathologic staging is therefore needed, but this may not be possible in the majority of patients because very few undergo surgical therapies that allow appropriate sampling.

The prognostic value of the sixth edition of the TNM staging system was compared with three other staging classifications (the Okuda, Cancer of the Liver Italian Program [CLIP], and Chinese University Prognostic Index [CUPI] classifications) in 234 patients with HCC who underwent curative resection at the Southwestern Hospital in China. Both the Okuda and the TNM systems were better at stratifying patients according to survival than the CLIP or CUPI system. However, the TNM classification was also better for predicting prognosis than the three other classifications, and was significantly better than the CLIP score ($p < .05$) [11]. The sixth edition of the TNM staging system also proved to be more effective than six other classifications (the Okuda, Barcelona Clinic Liver Cancer [BCLC], Japanese Integrated Staging [JIS], CLIP, and Groupe d'Etude et

Table 1. Key characteristics of various staging classifications available to assess the prognosis of patients with hepatocellular carcinoma

	Variables measured					
Staging classification	**Tumor staging**	**Liver function**	**Performance status**	**Serum tumor markers**	**Year published**	**Study**
CLIP	Tumor morphology (uninodular and extension ≤50%, multinodular and extension ≤50%, massive or extension >50%), portal vein thrombosis	Child-Pugh	No	AFP	1998	CLIP Investigators [16]
BCLC	Tumor size, number of nodules, portal vein thrombosis	Child-Pugh, bilirubin, portal hypertension	PST	No	1999	Llovet et al. [24]
GRETCH	Portal vein thrombosis	Bilirubin, alkaline phosphatase	Karnofsky	AFP	1999	Chevret et al. [34]
U.S. nomogram	Resection margin status, tumor size >5 cm, satellite lesions, vascular invasion	No	Age, operative blood loss	AFP	2008	Cho et al. [45]
Okuda	Tumor size (</>50% of liver)	Ascites, albumin, bilirubin	No	No	1985	Okuda et al. [13]
CUPI	TNM fifth edition	Ascites, bilirubin, alkaline phosphatase	Presence of symptoms	AFP	2002	Leung et al. [37]
JIS	Japanese TNM fourth edition	Child-Pugh	No	No	2003	Kudo et al. [23]
bm-JIS	Japanese TNM fourth edition	Child-Pugh	No	AFP, AFP-L3, DCP	2008	Kitai et al. [39]
SLiDe	Stage and liver damage categories from the Japanese TNM fourth edition	No	No	DCP	2004	Omagari et al. [41]
Tokyo	Size and number of tumors	Albumin, bilirubin	No	No	2005	Tateishi et al. [42]
BALAD	No	Albumin, bilirubin	No	AFP, AFP-L3, DCP	2006	Toyoda et al. [44]
ALCPS	Tumor size, portal vein thrombosis, lung metastases	Ascites, Child-Pugh, alkaline phosphatase, bilirubin, urea	Abdominal pain, weight loss	AFP	2008	Yau et al. [46]

Abbreviations: AFP, α-fetoprotein; AFP-L3, *lens culinaris* agglutinin-reactive AFP; ALCPS, Advanced Liver Cancer Prognostic System; BALAD, bilirubin, albumin, AFP-L3, AFP, DCP; BCLC, Barcelona Clinic Liver Cancer; bm-JIS, biomarker-combined JIS; CLIP, Cancer of the Liver Italian Program; CUPI, Chinese University Prognostic Index; DCP, des-γ-carboxyprothrombin; GRETCH, Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire; JIS, Japanese Integrated Staging; PST, performance status test; SLiDe, Stage, Liver damage, DCP; TNM, tumor–node–metastasis.
From Meier V, Ramadori G. Clinical staging of hepatocellular carcinoma. Dig Dis 2009;27:131–141. Reproduced with permission from S. Karger AG, Basel, Switzerland.

de Traitement du Carcinome Hépatocellulaire [GRETCH] classifications) at assessing prognosis in 163 patients with HCC following resection in a retrospective study at a single institution in Korea [12]. Those studies were limited to the postsurgery setting, and evaluation in a larger sample size and broader patient population is still required.

Okuda Classification

The Okuda classification was published in 1985 and was the first staging system to include parameters related to tumor size (>50% versus <50% of the liver involved) and liver function (albumin, ascites, bilirubin) [13]. Its ability to predict prognosis according to treatment was evaluated as part of a retrospective analysis among 850 patients treated in three different institutes in Japan, with patients stratified into three stages (I, not advanced; II, moderately advanced; III, very advanced). These findings showed that surgically treated patients had a longer survival time than medically treated patients, and that medical treatment prolonged survival in stage II and stage III patients but not in stage I patients. However, because stage at diagnosis as well as the available medical interventions have moved on since the time this staging classification was developed, stratifying patients to receive radical or palliative therapies using this system alone would not be appropriate. Moreover, although its simplicity makes it clinically attractive, its ability to predict prognosis is relatively modest [5]. Indeed, in a retrospective study in Canada, the Okuda classification failed to identify two thirds of the 37 patients with a poor prognosis who were identified by the CLIP criteria [14]. Furthermore, in an evaluation of staging systems for HCC patients undergoing surgery, the Okuda system was not superior to TNM staging [15].

CLIP Scoring

The CLIP scoring system was derived from a retrospective analysis of 435 patients with HCC from 16 Italian institutions and was published in 1998 [16]. Here, four independent predictive factors of survival were identified (Child-Pugh score, tumor morphology, α-fetoprotein [AFP], and

portal vein thrombosis), and a simple linear scoring system (0, 1, or 2) was assigned to the covariates in order to give patients a total score of 0–6. This scoring system was subsequently validated by the same group in a prospective trial of 196 patients with HCC and cirrhosis [17] and was also shown to be effective in predicting survival among a group of 145 patients in the Middle East [18] and in 662 Japanese patients [19]. However, whereas the median survival time associated with each CLIP score (0–6) appears to be similar between patients included in the prospective validation conducted by the founding group and those included in the study conducted in the Middle East [18, 20], the median survival times reported for Japanese patients were higher for all CLIP scores [19], and it has been suggested that these findings could compromise the external validation of the CLIP scoring system [21].

In a comparison of the CLIP, BCLC, and Okuda staging systems using a pooled database from two randomized trials of French patients with mainly alcoholic HCC, the performances of all three systems were disappointing; different systems performed differently according to patient populations and for individual prognostic factors. None clearly emerged as an unquestionable reference [22]. However, for all statistics, the CLIP system had better prognostic ability. The authors concluded that the CLIP staging seems to be most adapted to the palliative setting and that it could be improved by associating World Health Organization performance status.

A number of limitations of the CLIP scoring system have been reported [23]. First, the tumor morphology categories used may be too general to be globally applicable, particularly in countries such as Japan, where more patients are diagnosed with very small solitary tumors, largely because of the established screening programs in place. Secondly, although patient populations with different CLIP scores appear to be well discriminated from each other, there is no clear difference among patient populations with CLIP scores of 4–6 [17]. Indeed, in the prospective validation of this scoring system performed by the founding group [20], they grouped patients with a CLIP score of 4–6 into one group. Finally, all studies evaluating the CLIP score reported to date show that a high proportion of patients are categorized as CLIP score 0–2, suggesting poor stratification ability with this system.

Taken together, these findings suggest that, although the CLIP scoring system is associated with a good prognostic ability, this staging system may not be sensitive enough to be applicable to all patient populations and cannot easily be applied to a patient's management.

BCLC Staging

The BCLC staging classification was proposed by Llovet and colleagues in 1999 [24]. One of the most important observations for the development of the BCLC staging system came from the follow-up of patients with nonresectable and nontransplantable HCC who were randomized to placebo in two different clinical trials [25]. In that study, the multivariate analysis identified performance status, constitutional syndrome, vascular invasion, and extrahepatic spread as independent predictors for mortality. The authors showed that the 1-, 2-, and 3-year survival rates for the 48 patients without predictors of mortality (i.e., intermediate stage) were 80%, 65%, and 50%, respectively, and these were 29%, 16%, and 8% in the 54 patients with at least one adverse factor (i.e., advanced stage). This has been externally validated [26]. This allowed patients to be divided into different categories based on tumor stage (tumor size, number of nodules, and presence of portal vein thrombosis), liver function (Child-Pugh score, portal hypertension, bilirubin level), physical status (performance status test), and cancer-related symptoms. Furthermore, four categories were created (A, early; B, intermediate; C, advanced; D, end-stage disease). It is also unique in that it is the only system that provides treatment recommendations for each of the assigned stages based on the best treatment options currently available. The BCLC staging classification has been externally validated in the U.S. [6], Europe [27, 28], and Taiwan [29] and has demonstrated superior survival stratification and prognosis prediction over a range of other classifications, including the Okuda, TNM, CLIP, GRETCH, CUPI, and JIS classifications [28, 30]. Moreover, BCLC staging is endorsed by both the European Association for the Study of the Liver (EASL) [5] and the American Association for the Study of Liver Diseases (AASLD) [31], and it is emerging as a standard staging classification in western populations [32]. The most important aspect of this staging classification is that it is linked to an evidence-based treatment algorithm and can easily be used in a clinical setting. However, it should be noted that, in a study investigating which of the available staging systems was the most informative for the medical oncologist [33], the BCLC system was found to be less informative than the GRETCH and CLIP classifications when ranked using a concordance index, a likelihood ratio, and the Akaike information criterion. However, that study mostly evaluated patients with advanced tumors and may not be generally applicable.

GRETCH Scoring

The GRETCH scoring system was based on findings from a prospective study among 761 patients from 24 western medical centers and was published in 1999 [34]. The aim of

the study was to compile a classification system for predicting survival among these patients using a multivariate Cox model. Five prognostic factors were selected (Karnofsky index <80%, bilirubin >50 μmol/l, alkaline phosphatase ≥2× the upper limit of normal, AFP >25 μg/l, and ultrasonographic portal vein obstruction) in order to divide patients in the study training sample ($n = 506$) into three prognostic classification groups (A, B, C). The 1-year survival rates associated with these three groups were derived (72%, 34%, and 7% for groups A, B, and C, respectively) and independently validated in the study test sample (79%, 31%, and 4% for groups A, B, and C, respectively; $n = 255$). This system has not been validated in nonwestern patient populations. Furthermore, because this system originated from a multivariate analysis, it may not be reproducible or easily used in clinical practice.

Liver Cancer Study Group of Japan TNM Staging

In 1965, the Liver Cancer Study Group of Japan (LCSGJ) started a nationwide registration of clinicopathologic and prognostic data from patients with primary liver cancer, and using data collected in this database they introduced the Japanese version of the TNM staging system in 1983. This has subsequently undergone a number of revisions, and in 2007 the LCSGJ evaluated data from their database of 63,736 patients with primary liver cancer, 13,772 of whom underwent curative resection, in order to present evidence to develop and validate this staging classification [35]. Based on univariate and multivariate survival analyses, they selected three factors (vascular or bile duct invasion, tumor diameter ≤2 cm versus >2 cm, and number of tumors—single versus multiple), and classified patients as T1–T4 based on the number of adverse factors present (patients with none were considered T1, those with one were T2, those with two were T3, and those with three were T4). Significant survival differences were demonstrated among patients in each of the four assigned stages, with 5-year survival rates of 70% (T1), 58% (T2), 41% (T3), and 24% (T4) ($p < .0001$). A potential weakness of the LCSGJ staging system is that it assumes equal weight for growth pattern, size, and vascular or bile duct invasion. No external validation has been reported to date.

The Vauthey Simplified Staging System

In 2002, Vauthey and colleagues evaluated the efficacy of using the TNM's T categories to stratify patients according to survival and assessed a range of independent prognostic factors among 557 patients undergoing resection [36]. Independent predictors of death in that study were major vascular invasion, microvascular invasion, severe fibrosis/cirrhosis of the liver, multiple tumors, and tumors >5 cm. Based on these findings, Vauthey and colleagues proposed a simplified model of patient stratification using vascular invasion, tumor number and size, and the effect of fibrosis on survival. Patients were divided into three stages (I, II, III) and these were associated with a significant survival difference, with 5-year survival rates of 55% (I), 37% (II), and 16% (III) ($p < .001$) [36]. This is limited to postsurgery patients and has not been externally validated.

CUPI Score

The CUPI score was developed at the Chinese University in Hong Kong and was published in 2002 [37]. In that study, 19 potential prognostic factors were evaluated in a multivariate analysis using a Cox regression model among 926 Chinese patients, mostly with HBV-associated HCC. From this, five additional prognostic factors (asymptomatic disease at presentation, AFP, total bilirubin, alkaline phosphatase, and ascites) were added to the fifth edition of the TNM staging classification. Patients were divided into three risk groups (high, medium, and low risk for dying within 3 months), and highly significant differences in survival were observed among these groups ($p < .00001$). Findings from that study also showed that the CUPI system was better at classifying patients into different risk groups than the TNM staging system alone, or the Okuda or CLIP scoring systems, although the authors advise that validation across broader patient populations is needed. In a more recent study, the CUPI staging system was compared with the Okuda, CLIP, and sixth edition of the TNM staging systems among 234 Chinese patients who underwent resection [11]. The authors concluded that the TNM sixth edition was superior in discriminating survival among patients stratified into different stages, and suggested that a possible limitation of the CUPI score is that it is based on the fifth edition of the TNM. The CUPI system has not been externally validated.

JIS Score

In 2003, an integrated prognostic classification system was published by Kudo and colleagues [23]. This scoring system combines the Japanese TNM staging (stages I, II, III, and IV are converted to scores 0, 1, 2, and 3, respectively) and the conventional Child-Pugh (stages A, B, and C are converted to scores 0, 1, and 2, respectively) to produce a JIS score of 0–5. This scoring system was evaluated in 722 Japanese patients with HCC, and statistically significant differences were observed in the survival curves among JIS scores of 0–3, but not among scores of 4–6 [23]. It has been noted that the JIS system may be limited in its ability to stratify patients with advanced scores because it uniformly

assigns tumor stage and liver function [35]. However, this system has been externally validated [38] and it appears to be one of the most promising candidates for a standard classification system across the Asia-Pacific region. However, it has not been validated in a western patient population.

The JIS staging classification was further modified by Kitai and colleagues to include evaluation of three tumor markers for HCC, namely AFP, *lens culinaris* agglutinin-reactive AFP (AFP-L3), and des-γ-carboxyprothrombin (DCP). This biomarker-combined JIS (bm-JIS) scoring system was evaluated in 1,924 patients with HCC, and findings published in 2008 showed that the bm-JIS scoring system had superior stratification ability and was a better predictor of prognosis than the conventional JIS scoring system [39]. This system has now been externally validated but still requires validation in a western patient population [40].

Stage, Liver Damage, DCP Staging System

The stage, liver damage, DCP (SLiDe) staging system was established in 2004 when Omagari and colleagues evaluated a range of prognostic markers in univariate and multivariate analyses using the medical records of 177 patients with HCC from the Nagasaki University School of Medicine in Japan [41]. In that analysis, only the "stage" and "liver damage" categories from the fourth edition of the Japanese TNM staging classification, as well as serum DCP, remained significant prognostic factors of survival. Thus, in the SLiDe staging system, patients were assigned a score based on these covariates (0, 1, 2, or 3), and findings from this retrospective analysis showed that there was clear discrimination among the survival curves plotted for patients with different SLiDe scores [41]. Although the authors concluded that this is a useful system to assess the prognosis of patients, they also advised that, because the Japanese TNM staging classification must be used, which includes some parameters that are not routinely assessed in other parts of the world, external validation in a large patient population would be needed before this system could be adopted.

Tokyo Classification

In a study published in 2005, 403 patients with HCC treated with percutaneous ablation at the University of Tokyo were used as a training sample to identify prognostic factors and to develop the Tokyo score based on four factors (albumin, bilirubin, and size and number of tumors) [42]. Prognostic factors were then analyzed in a testing sample of 203 patients with HCC who had undergone resection. Clear survival differences were demonstrated among Tokyo scores, with 5-year survival rates of 78.7% (0), 62.1% (1), 40.0% (2), 27.7% (3), and 14.3% (4–6). This system was validated by the same group, whereby it showed similar predictive ability to the CLIP scoring system and superior predictive ability to the BCLC staging classification. However, in a comparison of the JIS, BCLC, and Tokyo classifications in a Japanese cohort of HCC patients mainly with early-stage disease treated with radical therapy, the JIS score provided the best prognostic stratification [43]. Further external validation of the Tokyo classification in different patient populations is needed.

Bilirubin, Albumin, AFP-L3, AFP, DCP Score

The bilirubin, albumin, AFP-L3, AFP, DCP (BALAD) score, published by Toyoda and colleagues in 2006 [44], is a staging classification devised using only serum markers (bilirubin, albumin, AFP-L3, AFP, DCP). This scoring system, calculated as the sum of the remnant liver function score (i.e., albumin and bilirubin scoring, as devised by Tateishi and colleagues [42]) plus the tumor progression score (measured as the number of elevated tumor markers), was evaluated among 2,600 patients with HCC from five institutions. Patients were divided into six groups on the basis of the five laboratory values, with clear survival differences observed among the groups. Toyoda and colleagues also compared the BALAD scoring system with two staging classifications that consider both tumor progression and liver function factors (the JIS and CLIP classifications). They demonstrated that all three systems showed comparable prediction and discrimination of patient survival [44]. However, in a study comparing the BALAD scoring system with the JIS and bm-JIS systems conducted by Kitai and colleagues [40], there were significant differences between the BALAD and bm-JIS scores and the BALAD and JIS scores, even though all three systems effectively predicted patient survival. The authors concluded that the bm-JIS classification was superior to both the JIS and BALAD scoring systems, especially among patients with a good prognosis [40].

A U.S.-Based Prognostic Nomogram

In a recent study published in 2008, 184 patients with HCC undergoing resection at a single institution in the U.S. were classified according to eight staging classifications [45]. The ability of these classifications to predict postoperative survival was evaluated in randomly selected pairs using Harrell's concordance index. A novel nomogram was then developed using age, AFP level, operative blood loss, surgical resection margin status, tumor size, satellite lesions, and vascular invasion. Using this nomogram, survival could be predicted with a higher concordance level between randomly tested pairs than with any of the eight conven-

tional classification systems tested (concordance index of 0.74 for the nomogram versus 0.54–0.59 for the eight staging classifications tested) [45]. That analysis relied on a single institutional data set of HCC patients, which may introduce selection bias. These findings have not yet been externally validated and this nomogram is not currently used clinically.

Advanced Liver Cancer Prognostic System Score

Because patients with advanced HCC who are not amenable to locoregional therapy are candidates for inclusion in clinical trials providing they have a good 3-month survival probability, the advanced liver cancer prognostic system (ALCPS) scoring system was devised to objectively predict the 3-month survival probability among these patients [46]. In a study by Yau and colleagues published in 2008, the prognostic significance of a range of factors was evaluated by univariate and multivariate Cox regression analyses in a training set of 1,109 patients. From this, 11 significant prognostic factors were identified (ascites, abdominal pain, weight loss, Child-Pugh score, alkaline phosphatase, total bilirubin, AFP, urea level, tumor size, portal thrombosis, and lung metastases) and assessed to provide patients with a score of 0–39 (with a higher score being associated with a lower survival probability). These scores were then divided into three groups in order to categorize patients as having a good (ALCPS score, 0–8), intermediate (ALCPS score, 9–15), or poor (ALCPS score, 16–39) probability of surviving at least 3 months. Patients assessed in the training set were stratified according to their ALCPS score, and Kaplan–Meier estimates for each group showed clear survival differences, with median OS times of 7.9 months, 3.2 months, and 1.4 months for the good, intermediate, and poor groups, respectively. In the same study, ALCPS scores were subsequently assessed in a validation sample of 320 patients, and outcomes very similar to the testing sample were reported (median OS time, 7.5 months, 3.2 months, and 1.2 months for the good, intermediate, and poor groups, respectively) [46]. Moreover, patients in the validation set were also assessed by the Okuda and CLIP scoring systems, and the discriminatory ability of each prognostic scoring system, assessed by constructing receiver-operating characteristic curves, showed that the ALCPS scoring system had significantly better predictive power than either the Okuda (area under the curve [AUC], 0.77 versus 0.66 for the ALCPS and Okuda classifications, respectively; $p < .001$) or CLIP (AUC, 0.77 versus 0.71 for ALCPS and CLIP classifications, respectively; $p = .002$) scoring systems. It must be noted that the data set used to construct ALCPS system was from a single institute, consisting predominantly of an HBV-prevalent Chinese population. It is not known whether ALCPS system can be applied to other populations.

Summary of Staging Classifications: What Is the Best System Available?

The number of staging classifications for HCC has increased in recent years, and more recent classifications have demonstrated better prognostic ability than earlier systems (Table 2). However, improvements are still ongoing and there is no agreement on a standard classification that could be used globally.

Earlier classifications, such as the TNM staging system, only considered tumor staging factors, and as such their prognostic ability was regarded as limited. Given the impact that HCC and common comorbidities such as cirrhosis, HBV, and HCV have on liver function, most classifications now consider both tumor staging factors and liver function to predict patient outcomes. In recent years, there has been increasing interest in the role of biomarkers to predict survival. However, although adding further parameters to staging classifications may help improve the accuracy of these systems, it is important to ensure we do not create systems that are overly complex, because this may limit their clinical utility.

One of the goals of staging systems today is to provide an evidence-based treatment guide [6, 7, 21]. Although all staging classifications have been designed to predict prognosis, the BCLC staging classification is currently the only system that also provides a recommended treatment algorithm linked to each stage of disease [24]. However, the main strength of the BCLC staging system is that the four categories of patients have distinct natural histories and it is easy to apply clinically. Whether the treatment that is linked to each BCLC stage is used will depend on factors such as institutional strength and patient selection.

Because most patients with HCC present with advanced disease, many of the staging classifications, including the CUPI, CLIP, GRETCH, and ALCPS classifications, were constructed among this patient group [16, 34, 37, 46]. This could represent a limitation of these systems in terms of the accuracy of predicting prognosis in patients with earlier-stage HCC. Thus, systems such as the Japanese TNM staging system, which was constructed based on a large database of clinicopathologic data from patients at all stages of disease, including 13,772 who were eligible for curative resection, may be more appropriate for assessing patients with earlier-stage disease [35].

Because there are significant regional differences in HCC in terms of tumor morphology and the presence of comorbidities, which affect the disease course and ultimately patient prognosis, a staging classification needs to be validated in both western and Asia-Pacific patient populations

Table 2. Comparison of externally validated staging classifications available for hepatocellular carcinoma

Staging classification	Region developed	*n* of patients	Validation studies	Comparator staging classifications used	*n* of patients	Main outcomes
CLIP [16]	Italy	435	Italy [17, 20]	CLIP, Okuda	196	CLIP demonstrated greater survival predictive power than Okuda
			Middle East [18]	CLIP, Okuda	145	CLIP was more reliable than Okuda in predicting survival
			Japan [19]	CLIP, TNM, Okuda	662	CLIP had the highest stratification ability. Median survival times greater in this study than two previous studies
BCLC [24]	Spain	239	USA [6]	Okuda, TNM, BCLC, CLIP, GRETCH, CUPI, JIS	239	BCLC demonstrated the best independent predictive power for survival
			Italy [28]	Okuda, CLIP, Child-Pugh, BCLC, CUPI	187	BCLC was the best prognostic system among patients suitable for resection or ablation
			Italy [30]	Okuda, TNM, BCLC, CLIP, GRETCH, CUPI, JIS	112	BCLC showed superior discriminatory power among a group of patients who underwent radiofrequency ablation therapy
JIS [23]	Japan	722	Japan [38]	JIS, CLIP	4,525	The prognostic predictive power of JIS was superior to that of CLIP JIS score was simple to obtain and remember
bm-JIS [39]	Japan	1,924	Japan [40]	JIS, bm-JIS, BALAD	1,173	bm-JIS score showed good stratification ability and was superior in predicting prognosis, especially among patients with a good prognosis

Abbreviations: BALAD, bilirubin, albumin, *lens culinaris* agglutinin-reactive α-fetoprotein, α-fetoprotein, des-γ-carboxyprothrombin; BCLC, Barcelona Clinic Liver Cancer; bm-JIS, biomarker-combined JIS; CLIP, Cancer of the Liver Italian Program; CUPI, Chinese University Prognostic Index; GRETCH, Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire; JIS, Japanese Integrated Staging; TNM, tumor–node–metastasis.

before it can be considered globally applicable. Unfortunately, none of the staging classifications currently available has been validated in all these patient populations, and as such none can be recommended for worldwide use. However, the BCLC system has been validated in the U.S., Europe, and Taiwan, and it is the only system that has so far been validated in three continents.

A number of studies have been conducted to compare various staging classifications in the same patient population (Table 3), and findings suggest that the staging classification to show superior predictive power depends on the region. In western patient populations, the BCLC staging system appears to be superior based on findings in separate studies (two conducted in Italy, one in Taiwan, and one in North America) [6, 28, 29, 30]. In Japan, Kudo and colleagues demonstrated that the JIS scoring system was superior to the CLIP classification among 4,525 patients with HCC [38]. However, it has not been validated outside Japan.

Taken together, these findings show that, as our knowledge of this complex disease improves, staging classifications continue to be refined. As more is known about the pathogenesis of HCC and molecular markers, better staging systems will be developed.

Conclusions

HCC is a heterogeneous condition, with multiple confounding factors making assessment of these patients extremely complex. Many elements, including tumor burden, the presence of symptoms, liver function, comorbidities, and the likely effect of treatment, need to be considered in order to ensure accurate and consistent assessment of all patients, thereby providing physicians with a common language on which to base treatment decisions and guide research. This review examines each classification but does not assess their relative value. Although many different staging classifications have been developed and there is currently no consensus on the best classification to use, the BCLC staging classification is emerging as a promising candidate for a standard classification in western regions, because it has been externally validated [6, 28, 30] and it is also endorsed

Table 3. Comparison of studies evaluating different staging classifications in the same hepatocellular carcinoma patient population

Study	*n* of patients	Region	Staging classifications compared	Superior classification identified
Choi et al. [12]	163	Korea	TNM (fifth and sixth editions), Okuda, BCLC, CLIP, GRETCH, JIS	TNM sixth edition
Lu et al. [11]	234	China	Okuda, CLIP, TNM, CUPI	TNM sixth edition
Cillo et al. [28]	187	Italy	Okuda, BCLC, CLIP, GRETCH, CUPI	BCLC
Guglielmi et al. [30]	112	Italy	Okuda, TNM, BCLC, CLIP, GRETCH, CUPI, JIS	BCLC
Marrero et al. [6]	239	USA	Okuda, TNM, BCLC, CLIP, GRETCH, CUPI, JIS	BCLC
Kitai et al. [40]	1,173	Japan	JIS, bm-JIS, BALAD	bm-JIS
Kudo et al. [38]	4,525	Japan	CLIP, JIS	JIS

Abbreviations: BALAD, bilirubin, albumin, *lens culinaris* agglutinin-reactive α-fetoprotein, α-fetoprotein, des-γ-carboxyprothrombin; BCLC, Barcelona Clinic Liver Cancer; bm-JIS, biomarker-combined JIS; CLIP, Cancer of the Liver Italian Program; CUPI, Chinese University Prognostic Index; GRETCH, Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire; JIS, Japanese Integrated Staging; TNM, tumor–node–metastasis.

by both the EASL [5] and the AASLD [31]. However, because risk factors vary significantly from region to region, any standard global staging classification needs to be validated in both western and Asia-Pacific patient populations; to date, no such staging classification exists.

Continued research efforts have improved our understanding of this complex disease, which has allowed us to refine staging classifications and improve our therapeutic approach. In recent years, a significant amount of research has reported on the role of tumor markers to predict survival in HCC, and the markers AFP, AFP-L3, and DCP have now been incorporated into some staging classifications. In addition, molecular markers such as hepatocyte growth factor, vascular endothelial growth factor, and transforming growth factor β 1 have been linked with poor outcomes in HCC patients [47], and so may play a role in helping us to further improve staging classifications. In addition to the added information that tumor and molecular markers bring, data from ongoing studies may contribute. The Global Investigation of therapeutic DEcisions in hepatocellular carcinoma and Of its treatment with sorafeNib (GIDEON) study is a large global, noninterventional study of patients with unresectable HCC receiving sorafenib (Nexavar®; Onyx Pharmaceuticals, Inc., Emeryville, CA; Bayer HealthCare Pharmaceuticals, Inc., Wayne, NJ; Bayer Schering Pharma AG, Berlin, Germany) therapy. That study will collect details of local, regional, and global methods of patient evaluation, diagnosis, and follow-up, and assess comorbidities and their influence on treatment and outcome. Information collected in this database may be of value in further refining current staging classifications. However, further research efforts are needed for us to gain a full understanding of the factors that affect the prognosis of patients with HCC.

Acknowledgments

The authors take full responsibility for the scope, direction, and content of the manuscript and have approved the submitted manuscript. They would like to thank Karen Brayshaw, Ph.D., at Complete HealthVizion for her assistance in the preparation and revision of the draft manuscript, based on detailed discussion and feedback from all the authors. Editorial assistance was supported by a grant from Bayer HealthCare Pharmaceuticals.

Author Contributions

Conception/Design: Jean-Pierre Bronowicki, Masatoshi Kudo, Jorge A. Marrero
Data analysis and interpretation: Jean-Pierre Bronowicki, Masatoshi Kudo, Jorge A. Marrero
Manuscript writing: Jean-Pierre Bronowicki, Masatoshi Kudo, Jorge A. Marrero
Final approval of manuscript: Jean-Pierre Bronowicki, Masatoshi Kudo, Jorge A. Marrero

References

1 Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003; 362:1907–1917.

2 Parkin DM, Bray F, Ferlay J et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.

3 Poon D, Anderson BO, Chen LT et al. Management of hepatocellular carcinoma in Asia: Consensus statement from the Asian Oncology Summit 2009. Lancet Oncol 2009;10:1111–1118.

4 Rilling WS, Drooz A. Multidisciplinary management of hepatocellular carcinoma. J Vasc Interv Radiol 2002;13:S259–S263.

5 Bruix J, Sherman M, Llovet JM et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.

6 Marrero JA, Fontana RJ, Barrat A et al. Prognosis of hepatocellular carcinoma: Comparison of 7 staging systems in an American cohort. Hepatology 2005;41:707–716.

7 Llovet JM, Fuster J, Bruix J et al. The Barcelona approach: Diagnosis, staging, and treatment of hepatocellular carcinoma. Liver Transpl 2004;10(2 suppl):S115–S120.

8 Greene FL, Sobin LH. The staging of cancer: A retrospective and prospective appraisal. CA Cancer J Clin 2008;58:180–190.

9 Green F. Liver (including intrahepatic bile ducts). In: Green F, Page D, Fleming I, eds. AJCC Cancer Staging Handbook, Sixth Edition. New York: Springer, 2002:131–144.

10 Sobin LH. TNM, sixth edition: New developments in general concepts and rules. Semin Surg Oncol 2003;21:19–22.

11 Lu W, Dong J, Huang Z et al. Comparison of four current staging systems for Chinese patients with hepatocellular carcinoma undergoing curative resection: Okuda, CLIP, TNM and CUPI. J Gastroenterol Hepatol 2008;23: 1874–1878.

12 Choi SB, Lee JG, Kim KS et al. The prognosis and survival analysis according to seven staging systems of hepatocellular carcinoma following curative resection. Hepatogastroenterology 2008;55:2140–2145.

13 Okuda K, Ohtsuki T, Obata H et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer 1985;56:918–928.

14 Levy I, Sherman M; Liver Cancer Study Group of the University of Toronto. Staging of hepatocellular carcinoma: Assessment of the CLIP, Okuda, and Child-Pugh staging systems in a cohort of 257 patients in Toronto. Gut 2002;50:881–885.

15 Huang YH, Chen CH, Chang TT et al. Evaluation of predictive value of CLIP, Okuda, TNM and JIS staging systems for hepatocellular carcinoma patients undergoing surgery. J Gastroenterol Hepatol 2005;20:765–771.

16 The Cancer of the Liver Italian Program (CLIP) Investigators. A new prognostic system for hepatocellular carcinoma: A retrospective study of 435 patients. Hepatology 1998;28:751–755.

17 Llovet JM, Bruix J. Prospective validation of the Cancer of the Liver Italian Program (CLIP) score: A new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatology 2000;32:679–680.

18 Siddique I, El-Naga HA, Memon A et al. CLIP score as a prognostic indicator for hepatocellular carcinoma: Experience with patients in the Middle East. Eur J Gastroenterol Hepatol 2004;16:675–680.

19 Ueno S, Tanabe G, Sako K et al. Discrimination value of the new western prognostic system (CLIP score) for hepatocellular carcinoma in 662 Japanese patients. Cancer of the Liver Italian Program. Hepatology 2001;34: 529–534.

20 The Cancer of the Liver Italian Program (CLIP) Investigators. Prospective validation of the CLIP score: A new prognostic system for patients with cirrhosis and hepatocellular carcinoma. Hepatology 2000;31:840–845.

21 Pons F, Varela M, Llovet JM. Staging systems in hepatocellular carcinoma. HPB (Oxford) 2005;7:35–41.

22 Collette S, Bonnetain F, Paoletti X et al. Prognosis of advanced hepatocellular carcinoma: Comparison of three staging systems in two French clinical trials. Ann Oncol 2008;19:1117–1126.

23 Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): Its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging score (JIS score). J Gastroenterol 2003;38:207–215.

24 Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: The BCLC staging classification. Semin Liver Dis 1999;19:329–338.

25 Llovet JM, Bustamante J, Castells A et al. Natural history of untreated nonsurgical hepatocellular carcinoma: Rationale for the design and evaluation of therapeutic trials. Hepatology 1999;29:62–67.

26 Beaugrand M, Sala M, Degos F et al. Treatment of advanced hepatocellular carcinoma (HCC) by seocalcitol (a Vit D analogue): An international randomized double-blind placebo-controlled study in 747 patients [abstract 37]. J Hepatol 2005;42(suppl 2):17.

27 Cillo U, Vitale A, Grigoletto F et al. Prospective validation of the Barcelona Clinic Liver Cancer staging system. J Hepatol 2006;44:723–731.

28 Cillo U, Bassanello M, Vitale A et al. The critical issue of hepatocellular carcinoma prognostic classification: Which is the best tool available? J Hepatol 2004;40:124–131.

29 Wang JH, Changchien CS, Hu TH et al. The efficacy of treatment schedules according to Barcelona Clinic Liver Cancer staging for hepatocellular carcinoma: Survival analysis of 3892 patients. Eur J Cancer 2008;44:1000–1006.

30 Guglielmi A, Ruzzenente A, Pachera S et al. Comparison of seven staging systems in cirrhotic patients with hepatocellular carcinoma in a cohort of patients who underwent radiofrequency ablation with complete response. Am J Gastroenterol 2008;103:597–604.

31 Bruix J, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005;42:1208–1236.

32 Meier V, Ramadori G. Clinical staging of hepatocellular carcinoma. Dig Dis 2009;27:131–141.

33 Huitzil FD, Capanu M, O'Reilly E et al. Ranking and improvement of staging systems (SS) in advanced hepatocellular carcinoma (AHCC) [abstract 210]. Presented at the American Society of Clinical Oncology Gastrointestinal Cancers Symposium, Orlando, FL, January 25–27, 2008.

34 Chevret S, Trinchet JC, Mathieu D et al. A new prognostic classification for predicting survival in patients with hepatocellular carcinoma. J Hepatol 1999;31:133–141.

35 Minagawa M, Ikai I, Matsuyama Y et al. Staging of hepatocellular carcinoma: Assessment of the Japanese TNM and AJCC/UICC TNM systems in a cohort of 13,772 patients in Japan. Ann Surg 2007;245:909–922.

36 Vauthey J-N, Lauwers GY, Esnaola NF et al. Simplified staging for hepatocellular carcinoma. J Clin Oncol 2002;20:1527–1536.

37 Leung TWT, Tang AMY, Zee B et al. Construction of the Chinese University Prognostic Index for hepatocellular carcinoma and comparison with the TNM staging system, the Okuda staging system, and the Cancer of the Liver Italian Program staging system: A study based on 926 patients. Cancer 2002;94:1760–1769.

38 Kudo M, Chung H, Haji S et al. Validation of a new prognostic staging system for hepatocellular carcinoma: The JIS score compared with the CLIP score. Hepatology 2004;40:1396–1405.

39 Kitai S, Kudo M, Minami Y et al. A new prognostic staging system for hepatocellular carcinoma: Value of the biomarker combined Japan Integrated Staging score. Intervirology 2008;51(suppl 1):86–94.

40 Kitai S, Kudo M, Minami Y et al. Validation of a new prognostic staging system for hepatocellular carcinoma: A comparison of the biomarker-combined Japan Integrated Staging score, the conventional Japan Integrated Staging score and the BALAD score. Oncology 2008;75(suppl 1): 83–90.

41 Omagari K, Honda S, Kadokawa Y et al. Preliminary analysis of a newly proposed prognostic scoring system (SLiDe score) for hepatocellular carcinoma. J Gastroenterol Hepatol 2004;19:805–811.

42 Tateishi R, Yoshida H, Shiina S et al. Proposal of a new prognostic model

for hepatocellular carcinoma: An analysis of 403 patients. Gut 2005;54: 419–425.

43 Chung H, Kudo M, Takahashi S et al. Comparison of three current staging systems for hepatocellular carcinoma: Japan Integrated Staging score, new Barcelona Clinic Liver Cancer staging classification, and Tokyo score. J Gastroenterol Hepatol 2008;23:445–452.

44 Toyoda H, Kumada T, Osaki Y et al. Staging hepatocellular carcinoma by a novel scoring system (BALAD score) based on serum markers. Clin Gastroenterol Hepatol 2006;4:1528–1536.

45 Cho CS, Gonen M, Shia J et al. A novel prognostic nomogram is more accurate than conventional staging systems for predicting survival after resection of hepatocellular carcinoma. J Am Coll Surg 2008;206:281–291.

46 Yau T, Yao TJ, Chan P et al. A new prognostic score system in patients with advanced hepatocellular carcinoma not amendable to locoregional therapy: Implication for patient selection in systemic therapy trials. Cancer 2008; 113:2742–2751.

47 Gomaa AI, Khan SA, Leen ELS et al. Diagnosis of hepatocellular carcinoma. World J Gastroenterol 2009;15:1301–1314.

see related editorial on page 2607

EUS-Guided Broad Plexus Neurolysis Over the Superior Mesenteric Artery Using a 25-Gauge Needle

Hiroki Sakamoto, MD, PhD[1], Masayuki Kitano, MD, PhD[1], Ken Kamata, MD[1], Takamitsu Komaki, MD, PhD[1], Hajime Imai, MD[1], Takaaki Chikugo, MD, PhD[2], Yoshifumi Takeyama, MD, PhD[3] and Masatoshi Kudo, MD, PhD[1]

OBJECTIVES: **Endoscopic ultrasonography (EUS)-guided celiac plexus neurolysis (EUS-CPN) is safe and effective but not beneficial for some patients with extended abdominal cancer. We compared the effectiveness of standard EUS-CPN and EUS-guided broad plexus neurolysis (EUS-BPN) that extends over the superior mesenteric artery (SMA) using a 25-gauge needle.**

METHODS: **Consecutive patients referred to our quaternary EUS centers were eligible for inclusion. To evaluate the neurolytic spread, contrast was mixed with the neurolytic agent and post-procedure computed tomography scanning was performed. The regions containing the celiac, superior, and inferior mesenteric arteries were divided on the frontal plane into six areas: upper right and left, middle right and left, and lower right and left. The number of contrast-bearing areas after EUS-CPN and EUS-BPN were related to the degree of pain relief achieved.**

RESULTS: **A total of 67 patients with advanced abdominal cancer were included (34 EUS-CPN and 33 EUS-BPN). The qualitative variables of the two groups did not differ significantly. The EUS-BPN group had more patients with six contrast-bearing areas (42%) than the EUS-CPN group (0%). These patients had significantly better short-term and long-lasting pain relief than patients with less than five contrast-bearing areas. EUS-BPN patients exhibited significantly greater reductions in days 7 and 30 visual analog pain scale scores than EUS-CPN patients.**

CONCLUSIONS: **Our preliminary data suggested that EUS-BPN using a 25-gauge needle provides patients with advanced abdominal cancer with better pain relief than standard EUS-CPN, and without incurring serious complications. Moreover, it seems that broad neurolysis over the SMA may provide superior analgesia.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg Video content online

Am J Gastroenterol 2010; 105:2599–2606; doi:10.1038/ajg.2010.339; published online 7 September 2010

INTRODUCTION

Pancreatic cancer commonly induces severe refractory pain (1). First-line therapy with nonsteroidal anti-inflammatory agents is frequently ineffective, and opioid administration is often needed. Although opioids provide pain relief, they are associated with a dry mouth, constipation, nausea, vomiting, drowsiness, and impaired immune function (2,3). An alternative is celiac plexus neurolysis (CPN), which is an established and effective nonpharmacological method of pain control for patients with pancreatic cancer (4–10). Endoscopic ultrasonography (EUS) guided-CPN (EUS-CPN) is a procedure in which a linear echoendoscope is used to inject the neurolytic agent into the region of the celiac ganglion (11–16). EUS-CPN is theoretically safer than posterior percutaneous CPN as EUS provides detailed Doppler imaging of the blood vessels, particularly of those surrounding the gastrointestinal tract lumen. EUS-CPN has been reported to relieve pain in ~70–80% of patients (11–16). However, a significant proportion of cancer patients achieve only suboptimal pain relief after EUS-CPN. Such post-neurolysis residual pain may be due to technical failure, the failure of the needle to reach the celiac plexus (CP), or the presence of undiagnosed nonvisceral pain caused by the cancerous invasion of muscle and connective tissue. It is also possible that nociceptive impulses from the abdominal viscera cannot be intercepted by standard EUS-CPN in cases in which

[1]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osakasayama City, Osaka, Japan; [2]Department of Pathology, Kinki University School of Medicine, Osakasayama City, Osaka, Japan; [3]Department of Surgery, Kinki University School of Medicine, Osakasayama City, Osaka, Japan. **Correspondence:** Hiroki Sakamoto, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2 Ohno-Higashi, Osakasayama City, Osaka, 589-8511, Japan. E-mail: hiroki.sakamoto@nifty.com
Received 1 October 2009; accepted 23 March 2010

a cancer has expanded extensively within the abdominal cavity beyond the reach of the CP.

In this study, we compared the pain-relieving effectiveness of standard EUS-CPN and EUS-guided broad plexus neurolysis (EUS-BPN) that employs a 25-gauge needle and extends over the superior mesenteric artery (SMA) in patients with advanced abdominal cancer. For this purpose, post-procedure computed tomography (CT) scanning was used to assess the spread of the neurolytic agent (which was mixed with contrast) in the celiac, superior, and inferior mesenteric areas, and the relationship between the pain relief that was achieved and the number of contrast-bearing areas was determined.

METHODS

Patients

Seventy-six patients with upper abdominal pain due to nonresectable pancreatic cancer or intra-abdominal metastatic cancer, and were offered EUS-guided pain therapy between September 2004 and May 2008 were eligible for inclusion. Of these, 37 and 39 were offered EUS-CPN and EUS-BPN, respectively. Three of the EUS-CPN group patients did not receive EUS-CPN because of direct invasion of the pancreatic cancer to the stomach ($n=1$) or interference from a collateral vessel ($n=2$). Six of the EUS-BPN patients did not receive EUS-BPN because of interference from a collateral vessel ($n=1$), the inability to identify the SMA postoperatively ($n=1$), or an anatomical impediment ($n=4$). The remaining 67 patients, of whom 34 and 33 received EUS-CPN and EUS-BPN, respectively, were enrolled in this study. Written informed consent to the procedure and the collection of data was obtained from all patients. The study was approved by the Institutional Review Board of the Kinki University of School of Medicine, Japan.

Treatment allocation

Before September 2004, standard EUS-CPN was used routinely for all patients referred to our hospital. During this period our study endosonographer (MK) performed more than 50 bilateral EUS-CPN procedures using a 22-gauge needle. We then introduced EUS-BPN using a 25-gauge needle to determine whether the response rate could be improved. After conducting technical challenges of EUS-BPN in five patients with abdominal cancer pain, all of whom were excluded from analysis, we performed EUS-BPN using a 25-gauge needle on all subsequently referred patients. This study therefore compares the response rates before and after the introduction of the EUS-BPN procedure.

EUS-CPN and EUS-BPN techniques

Patients were hydrated with intravenous saline solution (200–500 ml) before the procedure to minimize the risk of hypotension. They were placed in the left lateral decubitus position and sedated with intravenous midazolam and propofol. The level of sedation was titrated to optimize the tolerance to the procedure without compromising respiration. Patients were continuously monitored during the procedure with an automated noninvasive blood pressure device, electrocardiogram tracing, and pulse oximetry. EUS-CPN and EUS-BPN were both performed using a convex array echoendoscope (GF-UC 240P-AL5, GF-UCT 240-AL5, Olympus, Tokyo, Japan). After the patient was sedated, the scope was passed transorally into the esophagus. Under endoscopic visualization, the scope was advanced to the gastroesophageal junction. When the scope was in the stomach, attention was turned to the ultrasonographic imaging. Suction was used to remove the air from the stomach and the scope tip was deflected upward so that ultrasound probe came into contact with the gastric wall. Subsequently, the scope was torqued so that the aorta was identified in an elongated cross-section. The scope was then advanced slowly while being gently torqued to maintain a proper view of the aorta. The scope was advanced in order that the celiac trunk was seen to branch anteriorly and inferiorly from the aorta. At 1–2 cm inferior to the celiac trunk, the SMA branch of the aorta could be seen in a similar manner. The vascular landmarks were confirmed by color Doppler imagery (**Figure 1**).

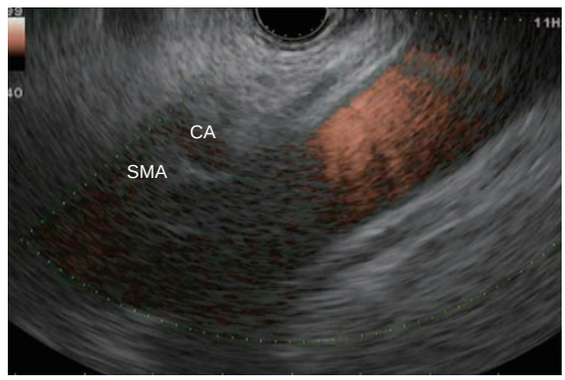


Figure 1. An endoscopic ultrasonography (EUS) image from the proximal stomach demonstrates a longitudinal view of the aorta at the level of the celiac and superior mesenteric arteries. The vascular landmarks were confirmed by color Doppler imagery. CA, celiac artery; SMA, superior mesenteric artery.

For EUS-CPN at the level of the celiac artery (CA), the probe was rotated clockwise toward the patient's left until the CA origin could no longer be visualized but the aorta could still be seen. For EUS-BPN at the level of the SMA, the probe was rotated clockwise toward the patient's left until the SMA origin could no longer be visualized but the aorta could still be seen (**Figure 2**). A 22- or 25-gauge needle (Echo Tip, Wilson-Cook Medical, Wiston-Salem, NC, or NA-200H-8022, Olympus) filled with 0.9% saline solution was prepared and introduced through the biopsy channel and affixed to the hub. In the EUS-CPN procedure, the 22-gauge needle was placed under direct EUS visualization adjacent and anterior to the lateral aspect of the aorta at the level of over or next to the CA trunk. In the EUS-BPN procedure, a 25-gauge needle was placed under direct EUS visualization adjacent and anterior to the lateral aspect of the aorta over the level of the SMA trunk (**Figure 3**, **Supplementary video** online). An aspiration test was then performed. A volume of 3 ml of 1% Lidocain was injected to prevent transient neurolysis agent-induced pain. As moderate

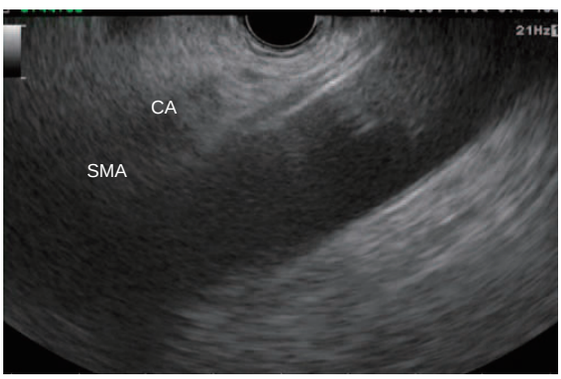


Figure 2. The echoendoscope is rotated clockwise toward the patient's left relative to the view in **Figure 1** until the superior mesenteric artery (SMA) origin can no longer be visualized. CA, celiac artery.

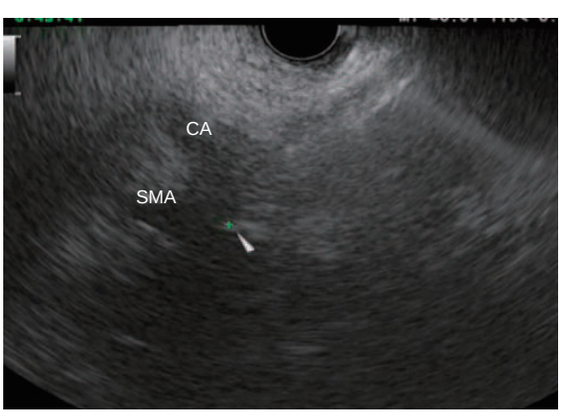


Figure 3. A 25-gauge needle is advanced along the lateral aspect over the superior mesenteric artery (SMA) of the aorta. The arrowhead indicates the needle tip. CA, celiac artery.

resistance should be observed, any lack of resistance might suggest that the vascular space has been punctured. In this case, the syringe plunger should be withdrawn to apply negative pressure and one should not proceed if blood is returned. Subsequently, a mixed solution of 9 ml of pure alcohol and 1 ml of contrast material (Iopamiron, Schering AG, Germany) was injected. One should be aware that there is significant resistance to injection of the BPN solution via the 25-gauge needle. It is also very important to remember that while injecting the absolute alcohol solution for CPN/BPN, the visibility of the needle is lost because of the hyperechoic appearance of the injected alcohol, which results in a "white-out" or "snowstorm" effect (**Figure 4**). This event emphasizes how necessary it is to place the needle tip accurately before completing the injection of the alcohol. The needle was then withdrawn from the patient and flushed with 0.9% saline solution and the same process was performed on the opposite side of the aorta (counter-clockwise rotation). All patients were given 250–500 ml of 0.9%

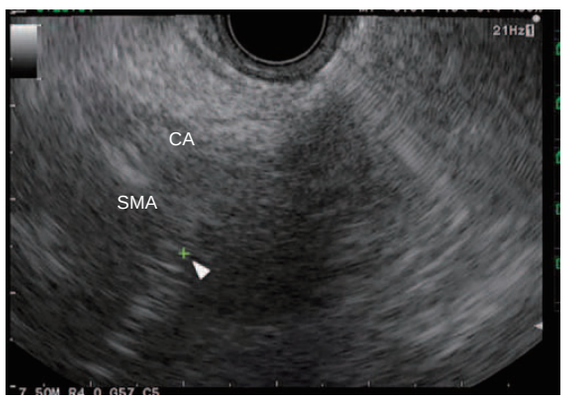


Figure 4. Lidocain and a mixed solution of alcohol and contrast material are injected. The hyper-echoic appearance of the injected alcohol results in a "white-out" or "snowstorm" effect. The entire process is then repeated on the opposite side of the aorta. CA, celiac artery; SMA, superior mesenteric artery.

saline solution intravenously during the procedure. Blood pressure was measured after the procedure. Procedure-related complications were assessed at 2, 7, 14, and 30 days after the procedure.

CT assessment

CT scanning was performed immediately after the procedure to confirm the injection site. Serial CT images were obtained from positions above and below the injection site, between the upper and lower limits of the neurolytic/contrast spread. To evaluate the spread pattern, the region containing the celiac, superior, and inferior mesenteric arteries was divided on the frontal plane into six areas: upper right and left (upper CA), middle right and left (between the CA and the SMA), and lower right and left (between the SMA and inferior mesenteric artery (IMA)) (**Figures 5–7**). The CP is located in the upper and middle areas, whereas the abdominal aortic plexus (AP) and part of the inferior mesenteric plexus (IMP) are located in the lower areas. The relationship between the pattern of neurolytic/contrast spread and the subsequent level of pain relief was then analyzed. For this, the results were expressed in terms of the number of areas into which the neurolytic/contrast solution had spread. We also evaluated how the location of the cancer affected the pain relief achieved by EUS-CPN and EUS-BPN. For this purpose, the cancers were categorized as upper cancers (indicating a localized cancer that had not spread beyond the SMA) and lower cancers (indicating a cancer that had expanded extensively within the abdominal cavity beyond the SMA) (**Figures 6** and **7**).

Pain scores

Pain intensity was measured according to a standardized visual 11-point continuous analog scale (VAS) in which 0 indicated no pain, 5 indicated moderate pain, and 10 indicated the worst pain ever experienced (8). If the VAS score dropped by ≥3 and <3 points after the procedure, this was judged to reflect good and

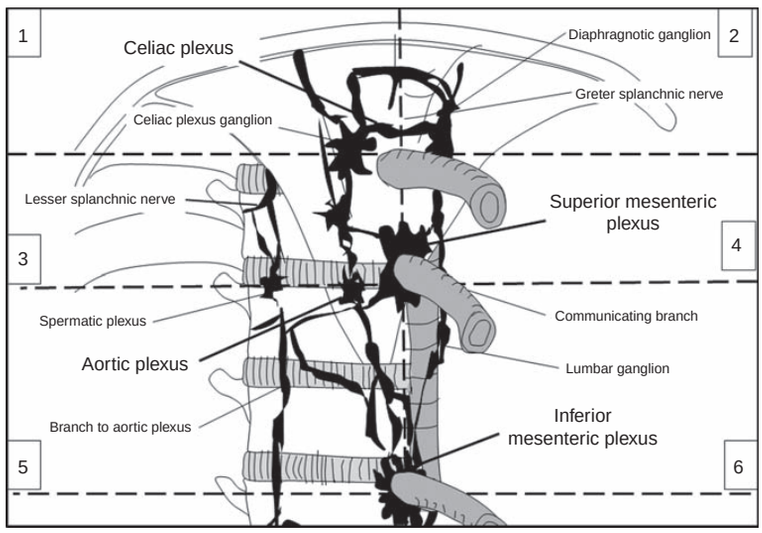


Figure 5. The division of the celiac, superior mesenteric, and inferior mesenteric regions into six areas: two upper areas (1 and 2), two middle areas (3 and 4), and two lower areas (5 and 6).

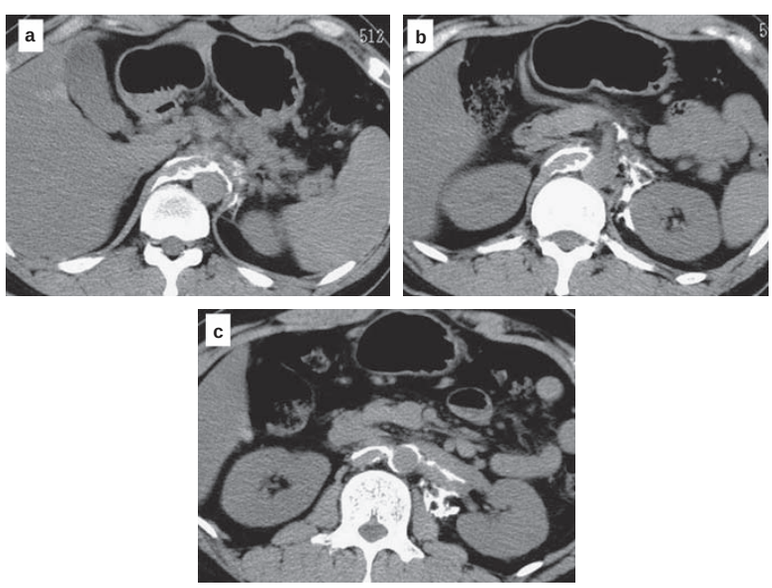


Figure 6. Computed tomography (CT) images at the level (**a**) T11-T12 above the celiac plexus (**b**), T 12-L1 in the center of the celiac plexus, and (**c**) L2-L3 above the inferior mesenteric plexus in a case with pancreatic cancer categorized as upper cancer. The contrast material was distributed over all six areas (areas 1–6 of **Figure 5**).

poor pain relief, respectively. To minimize subjective variations in the evaluation of VAS scores, the same physician explained the scoring pain intensity scale to all patients. At each follow-up contact, detailed instructions explaining how to assess the VAS were read aloud and the patient then informed the physician of the VAS score that best reflected their pain status. The pain relief achieved on days 7 (short-term pain relief) and 30 (long-lasting pain relief) was analyzed relative to the number of areas that had been shown to contain contrast during post-procedure CT scanning. Evaluation of pain relief was continued until day 30, which was the duration for which the complete medical records of the study patients were available.

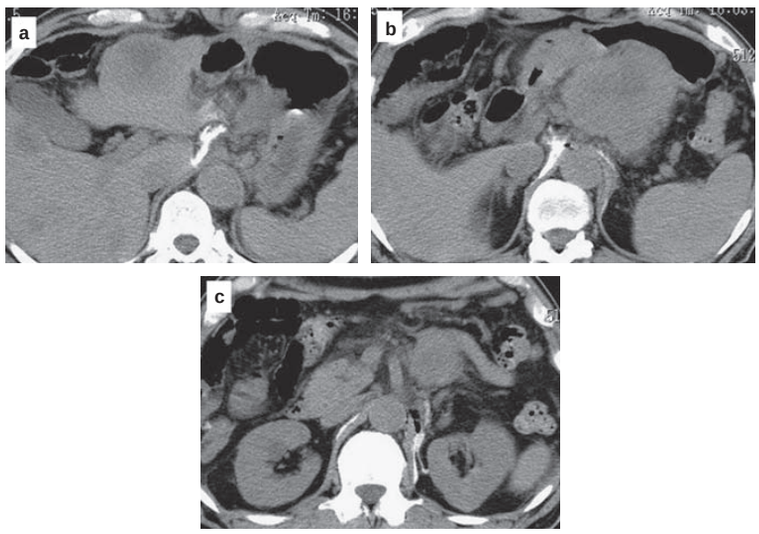


Figure 7. Computed tomography (CT) images at the level of (**a**) T11-T12 above the celiac plexus (**b**), T 12-L1 in the center of the celiac plexus, and (**c**) L2-L3 above the inferior mesenteric plexus in a case with pancreatic cancer categorized as lower cancer. The contrast material was distributed over two areas (areas 1 and 3 of **Figure 5**).

Statistical analysis

Student's *t*-test was used to compare the EUS-CPN and EUS-BPN groups in terms of the change in VAS scores on days 7 and 30 relative to the preprocedure scores. The Kruskal–Wallis test and Mann–Whitney *U*-test were used to analyze the qualitative parameters. Differences in pain relief achieved by day 30 between the patients divided according to their cancer location (upper and lower) were analyzed by using the χ^2-test. A *P* value of <0.05 was considered to indicate statistical significance.

RESULTS

The EUS-CPN and EUS-BPN groups did not differ significantly in terms of age, gender ratio, type of disease, or therapy (**Table 1**).

Comparison of the EUS-CPN and EUS-BPN groups in terms of neurolytic/contrast spread

Figure 8 depicts the pattern of neurolytic spread (expressed as the number of areas with contrast) and how the contrast spread differed between the EUS-CPN and EUS-BPN groups. The EUS-BPN groups had a significantly higher frequency of patients with contrast in all six areas (14/33, 42%) than the EUS-CPN group (0/34, 0%; $P<0.05$). The EUS-BPN groups also had a significantly higher percentage of patients with contrast in five areas (9/33, 28%) than the EUS-CPN group (3/34, 9%; $P<0.05$). Conversely, the percentage of patients with contrast in four areas was higher in the EUS-CPN group (12/34, 34%) than in the EUS-BPN group (5/33, 15%), although no significant difference was found between the both groups ($P=0.058$). The EUS-CPN group also had a higher frequency of patients with contrast in just one, two, or three areas (57%) than the EUS-BPN group (15%).

Table 1. **The pre-block patient characteristics**

	EUS-CPN group (*n*=34)	EUS-BPN group (*n*=33)	*P* value
Age (median, years)	64.1	65.2	0.6
Gender (percentage of female)	41.2	48.5	0.7
Initial pain score (0–10) 95% CI	7.74	7.79	0.3
Preintervention narcotic use (percentage of patients)	55.9	60.7	0.6
Diagnosis			0.5
Pancreatic cancer (percentage of patients)	29 (85.3)	31 (93.3)	—
Other GI cancer (percentage of patients)	5 (14.7)	2 (6.7)	—
Pancreatic cancer location (percentage of patients)			0.7
Head	18 (52.9)	19 (57.6)	—
Body/tail	16 (47.1)	14 (42.4)	—

CI, confidence interval; EUS-BPN, endoscopic ultrasonography-guided broad plexus neurolysis; EUS-CPN, endoscopic ultrasonography-guided celiac plexus neurolysis; GI, gastrointestinal.

Relationship between overall VAS scores and the number of contrast-bearing areas

Table 2 shows the VAS pain scores of all 67 patients divided according to the number of areas with contrast. The reduction in VAS score on days 7 and 30 was found to correlate with the number of areas with contrast. Thus, on day 30 (long-term pain relief), patients with contrast in four and five areas showed significantly greater reduc-

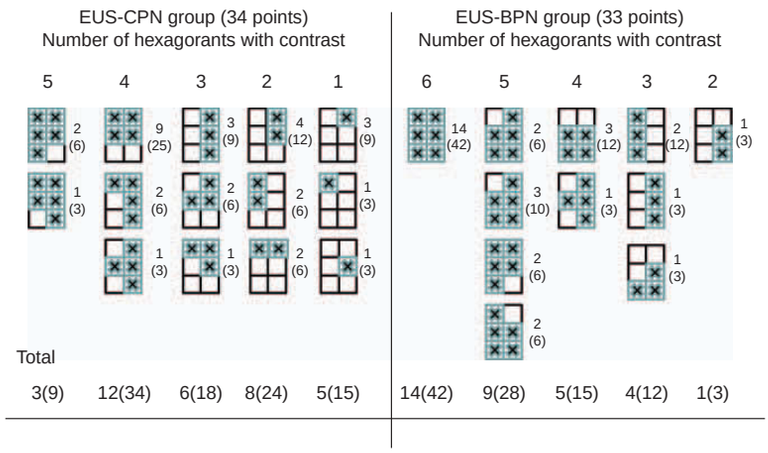


Figure 8. Degree of spread of the contrast material in the celiac area (see **Figure 5**) depending on whether endoscopic ultrasonography-guided celiac plexus neurolysis (EUS-CPN) (**a**) or EUS-guided broad plexus neurolysis (EUS-BPN) (**b**) was performed. Patient numbers are shown, with percentages indicated in brackets.

Table 2. Correlations between VAS scores and number of area with contrast

Number of areas containing contrast	Number of patients	Mean ±s.d. pre-block VAS scores	Mean ±s.d. day 7 VAS scores	Mean ±s.d. day 30 VAS scores
6	14	7.8±1.0	1.4±1.0	1.7±1.6
5	12	7.8±1.0	2.4±1.6	3.4±1.8
4	17	7.6±1.4	2.8±0.9	3.5±1.3
3	10	7.8±1.0	4.3±1.3	5.6±1.9
2	9	7.8±1.0	3.9±1.1	6.1±1.7
1	5	7.8±1.1	6.8±1.1	7.6±1.4

$P<0.05$ $P<0.05$ $P<0.05$ $P<0.05$ $P<0.05$

VAS, visual analog pain scale.

tions in VAS scores than patients with contrast in three or less areas. In addition, patients with six areas with contrast had significantly greater reductions in VAS scores than the patients with five or fewer areas with contrast. On day 7 (short-term pain relief), the patients with contrast in six areas showed significantly greater reductions in VAS scores than patients with contrast in three or fewer areas. Notably, the spread of contrast to just one or two areas did not lead to significant reductions in VAS scores on day 7 or 30.

In terms of long-term relief, of the 14 patients who exhibited the spread of contrast to all areas, 13 experienced good pain relief (93%). As the number of contrast-bearing areas decreased so did the frequency of patients who experienced good pain relief. Thus, 83 (10/12), 77 (12/17), 50 (5/10), 33 (3/9), and 0% (0/5) of the patients who exhibited the spread of contrast to five, four, three, two, and one areas experienced good pain relief, respectively.

Comparison of EUS-CPN and EUS-BPN in terms of VAS scores and subsequent narcotic use

For the EUS-CPN group, the mean VAS scores before the procedure and on days 7 and 30 after the procedure were 7.8±1.1, 3.9±2.0, and 4.8±2.2, respectively. The equivalent mean scores for the EUS-BPN group were 7.8±1.2, 2.5±1.9, and 3.4±2.5, respectively. The EUS-BPN group exhibited a significantly greater reduction in the VAS scores on days 7 and 30 than the EUS-CPN group ($P<0.05$).

Of the 19 EUS-CPN patients whose narcotic use on days 7 and 30 had been recorded, the narcotic use of three patients (16%) had increased by day 7; none of these three had decreased their narcotic use by day 30. In eight patients (42%), narcotic use remained the same on day 7; by day 30, one and two of those eight patients had decreased and increased their narcotic use, respectively. The narcotic use of the remaining five stayed the same. The narcotic use had decreased in 6 of the 19 EUS-CPN patients (32%) by day 7; by day 30, one and two of those six patients had decreased and increased their narcotic use, respectively. The narcotic use of the remaining three patients stayed the same. The remaining two patients (10%) were able to discontinue narcotic use altogether, although one of these (50%) had to restart opioid use on day 30. Of the 20 EUS-BPN patients whose narcotic use on days 7 and 30 had been was recorded, one patient

(5%) had increased their narcotic use by day 7; the narcotic use of that patient remained the same on day 30. Five patients (25%) had not changed their narcotic use by day 7; by day 30, one had increased their narcotic use, but the narcotic use in the remaining four patients was unchanged. In nine patients (45%), the narcotic use had decreased by day 7; of these nine, one discontinued and one increased their narcotic use by day 30. The remaining seven patients did not change their pain relief regimen. The remaining five patients (25%) were able to discontinue narcotic use, although one (20%) had to restart opiate use on day 30.

Efficacy of the EUS-CPN and EUS-BPN procedures relative to the location of the cancer

Of the EUS-CPN patients, 18 (53%) had upper cancer and the remaining 16 (47%) had lower cancer. In the EUS-BPN group, 14 (42%) had upper cancer and the remaining 19 (58%) had lower cancer. The two groups did not differ significantly in terms of tumor location.

For the upper cancer patients, the EUS-CPN and EUS-BPN procedures did not differ in terms of efficacy as these procedures yield good long-lasting pain relief in 78 (14/18) and 72% (10/14) of the patients, respectively. However, the EUS-BPN procedure was significantly more effective for the lower cancer patients as 79% (15/19) achieved good long-lasting pain relief after EUS-BPN, whereas only 19% (3/16) of the EUS-CPN patients achieved this ($P<0.05$).

Complications associated with the EUS-CPN and EUS-BPN procedures

No serious complications involving organ puncture or neurological disturbance occurred in this study. In addition, neither procedure was associated with development of late complications such as intra-abdominal abscess or neuritis. There were also no cases of prolonged hospitalization.

DISCUSSION

The CP is adjacent to the aorta and extends down from the origin of the CA to the origin of the SMA. The upper IMP, the abdominal AP, and the lumbar ganglion are situated on the lateral and anterior aspects of the aorta between the origins of the SMA and the IMA. The IMP surrounds the IMA and is mainly derived from the AP. The abdominal AP has connections to both the CP and the IMP. It is formed by branches that are derived on either side from the CP and ganglia, and it receives filaments from some of the lumbar ganglia. There are various plexuses like this in the upper and middle abdominal cavity, and they are composed of a network of nerve fibers that originate from both the sympathetic and parasympathetic nervous systems. These plexuses also receive parasympathetic fibers from the vagus nerve. The autonomic nerves of the CP supply the liver, pancreas, gallbladder, stomach, spleen, kidneys, and ascending colon. Hence, CPN provides pain relief for visceral pain caused by cancer in the upper abdomen. The IMP supplies the descending colon and sigmoid colon. Hence, IMP neurolysis block provides pain relief for left-side abdominal pain that is due to extensions from upper and lower abdominal cancers (10). We hypothesized that pain relief might be achieved in patients with unresectable abdominal cancer by using EUS-guided broad neurolysis over the SMA, because this could interrupt extensive nociceptive impulses from the abdomen. In this study, we used post-procedure CT scanning to assess the spread of the neurolytic agent mixed with contrast in the celiac, superior, and inferior mesenteric areas after either EUS-CPN or EUS-BPN, and compared the spreading patterns with the pain relief that was achieved by the procedures.

EUS-CPN can be performed by injecting at the base of the celiac axis (the central technique) or by injecting at the base, as well as on either side of the celiac axis (the bilateral technique). When Sahai *et al.* (13) compared the efficacy of central and bilateral EUS-CPN in 160 patients with upper abdominal pain secondary to abdominal disease, they found that bilateral EUS-CPN was significantly more effective in reducing pain (mean percent pain reductio $n=70.4\%$) than central EUS-CPN (45.9%). In this study, the bilateral technique was used for all patients who received EUS-CPN.

In this study, EUS-BPN resulted in a significantly higher frequency of patients who had contrast in six and five areas than EUS-CPN. These findings suggest that broad neurolysis over the SMA under EUS guidance ensures neurolysis of the abdominal AP and part of the IMP in addition to neurolysis of the CP.

We also found that the spread of contrast to four or five areas provided good and long-lasting pain relief, irrespective of the site of needle insertion, but that complete spread (i.e., to all six areas), which was achieved by EUS-BPN only, provided even more effective long-lasting pain relief. Furthermore, complete spread provided both long-lasting and early pain relief. In contrast, the spread of contrast to only one area did not even ensure short-term pain relief. Therefore, complete spread ensures more effective analgesia in patients with severe abdominal pain, whereas poor pain relief should be expected when the neurolytic spread is confined to just one or two areas. The tendency of EUS-BPN to induce more extensive neurolytic spread, explains why we found that EUS-BPN resulted in greater VAS score reductions on days 7 and 30 than EUS-CPN.

We observed that EUS-BPN induced good long-lasting pain relief in lower cancer patients more frequently than EUS-CPN, whereas the two techniques did not differ significantly for upper cancer patients. This suggests that EUS-BPN blocks a broad range of plexuses that extend from the CP to plexuses below, such as the abdominal AP and part of the IMP between the SMA and the IMA. Consequently, EUS-BPN is more likely than EUS-CPN to provide patients with abdominal cancer with good pain relief, especially in cases in which the cancer has expanded extensively within the abdominal cavity beyond the distribution of the CP. In addition, it would be very useful in such cases to confirm the injection site by CT after EUS-CPN and EUS-BPN.

EUS-CPN-related complications have been reported to affect between 0 and 10% of patients (11–16). Most of these complications are not severe and include transient diarrhea, drunkenness, increasing pain, and hypotension after neurolysis. Significantly, there was no increase in the rate of procedure-related complications in this study. In particular, no serious procedure-related complications occurred. This may reflect some precautions that

we took. Sahai *et al.* (13) reported that bilateral EUS-CPN using a 19-gauge needle had led on one occasion to a serious bleeding complication caused by trauma to the left adrenal artery. As a consequence, we used a 22-gauge needle in the EUS-CPN to minimize the risk of such bleeding. Notably, during the EUS-BPN procedure, it is necessary to insert the needle deeply over the level of the SMA. We used 25-gauge needles for EUS-BPN because they are thinner and more flexible than a 22- or 19-gauge needle and thus provide greater safety and flexibility during insertion into the target area (17). In this study, it was possible to insert the 25-gauge needle smoothly and deeply around the CP and the SMP, and this was not associated with any bleeding complications. However, as stronger resistance is encountered upon injection of the neurolytic solution through a 25-gauge needle and deep needle insertion is required, this procedure can be more technically difficult than standard EUS-CPN. Indeed, there were some cases in which it was not possible to perform EUS-BPN because the SMA could not be identified.

One potential limitation of this study is the lack of double blinding, as EUS-CPN was used in the first half of the study period and EUS-BPN in the last half. The second limitation of our study is that the operator became increasingly more experienced during the course of this study, which means that the procedures performed later on, specifically the BPN procedures, were more effective. This may have biased the efficacies of the two methods. The third limitation is the categorization of cancer location into two locations (upper and lower cancers), as CT is not always accurate in defining the exact extension of the cancer. Given these limitations, our study should be seen as a pilot study that provides preliminary data regarding the early- and late-term efficacy and safety of EUS-BPN and EUS-CPN for patients with abdominal cancer pain.

In conclusion, EUS-BPN using a 25-gauge needle may be more effective than standard EUS-CPN for patients with advanced abdominal cancer-related abdominal pain because it allows the neurolytic agent to be administered to a larger number of ganglia, without incurring serious complications. Moreover broad neurolysis over the SMA appears to guarantee more effective analgesia. To confirm our observations, prospective comparative and large-scale multicenter trials are needed.

CONFLICT OF INTEREST

Guarantor of the article: Masayuki Kitano, MD, PhD.

Specific author contributions: Hiroki Sakamoto and Masayuki Kitano contributed equally to this work. Masayuki Kitano performed the EUS-CPNs and EUS-BPNs. Hiroki Sakamoto, Masayuki Kitano, Takaaki Chikugo, and Yoshifumi Takeyama clinically managed the patients and discussed their cases. Hajime Imai and Ken Kamata clinically managed the patients by performing CT assessments. Takamitsu Komaki was in charge of the pain scale follow-ups of the patients. Masatoshi Kudo performed the statistical analysis. Hiroki Sakamoto wrote the paper.

Financial support: This research was supported by the Japan Society for the Promotion of Science and the Japanese Foundation for Research and Promotion of Endoscopy.

Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Endoscopic ultrasonography (EUS) provides effective pain relief for 70–80% of cancer patients.
- ✓ However, some patients do not benefit from it.

WHAT IS NEW HERE

- ✓ This study suggests that EUS-guided broad plexus neurolysis using a 25-gauge needle provides more effective pain relief for patients with advanced cancer than EUS-guided celiac plexus neurolysis, and without incurring serious complications.
- ✓ Broad neurolysis over the superior mesenteric artery appears to ensure more effective analgesia.

REFERENCES

1. Ischia S, Ischia A, Polati E *et al.* Three posterior percutaneous celiac plexus block techniques. A prospective, randomized study in 61 patients with pancreatic cancer pain. Anesthesiology 1992;76:534–40.
2. Regan JM, Peng P. Neurophysiology of cancer pain. Cancer Control 2000;7:111–9.
3. Firdousi FH, Sharma D, Raina VK. Palliation by coceliac plexus block for upper abdominal visceral cancer pain. Trop Doct 2002;32:224–6.
4. Ducable G, Menguy E, Jouini S *et al.* Celiac plexus block: value of x-ray computed guidance. Agressologie 1991;32:267–9.
5. Gress F, Schmitt C, Sherman S *et al.* A prospective randomized comparison of endoscopic ultrasound-and computed tomography-guided celiac plexus block for managing chronic pancreatitis pain. Am J Gastroenterol 1999;94:900–5.
6. Fujita Y. CT-guided neurolytic splanchnic nerve block with alcohol. Pain 1993;55:363–6.
7. Eisenberg E, Carr DB, Chalmers TC. Neurolytic celiac plexus block for treatment of cancer pain: a meta-analysis. Anesth Analg 1995;80: 290–5.
8. Pusceddu C, Mameli S, Pili A *et al.* Percutaneous neurolysis of the celiac plexus under CT guidance in the invasive treatment of visceral pain caused by cancer. Tumori 2003;89:286–91.
9. Cariati M, Henriquet F, Fiorentini F *et al.* Computerized tomography-guided neurolytic block of the splanchnic nerve. Radiol Med 1997;93: 739–42.
10. Kitoh T, Tanaka S, Ono K *et al.* Combined neurolytic block of celiac, inferior mesenteric, and superior hypogastric plexuses for incapacitating abdominal and/or pelvic cancer pain. J Anesth 2005;19:328–32.
11. Gunaratnam NT, Sarma AV, Norton ID *et al.* A prospective study of EUS-guided celiac plexus neurolysis for pancreatic cancer pain. Gastrointest Endosc 2001;54:316–24.
12. Wiersema MJ, Wiersema LM. Endosoonography-guided celiac plexus neurolysis. Gastrointest Endosc 1996;44:656–62.
13. Sahai AV, Lemelin V, Lam E *et al.* Central vs. bilateral endoscopic ultrasound-guided celiac plexus block or neurolysis: a comparative study of short-term effectiveness. Am J Gastroenterol 2009;104:326–9.
14. Sakamoto H, Kitano M, Nishio T *et al.* Value of computed tomography for evaluating the injection site in endosonography guided celiac plexus neurolysis. Digestive Endosc 2006;18:206–11.
15. Kaapis M. Erfahrungen mit local anathesie bie bauchoperationen. Vehr Dtsch Gesellsch Chir 1914; 43:87-9. O'Toole TM, Schmulewitz N. Complication rates of EUS-guided celiac plexus blockade and neurolysis: results of a large case series. Endoscopy 2009;41:593–7.
16. Ramirez-Luna M, Chavez-Tapia N, Franco-Gunzman A *et al.* Endoscopic ultrasound-guided celiac plexus neurolysis in patients with unresectable pancreatic cancer. Rev Gastroenterol Mex 2008;73:63–7.
17. Sakamoto H, Kitano M, Komaki T *et al.* Prospective comparative study of the EUS guided 25-gauge FNA needle with the 19-gauge Trucut needle and 22-gauge FNA needle in patients with solid pancreatic masses. J Gastroenterol Hepatol 2009;24:384–90.

Characterization of intra-abdominal lesions of undetermined origin by contrast-enhanced harmonic EUS (with videos)

Yu Xia, MD, PhD, Masayuki Kitano, MD, PhD, Masatoshi Kudo, MD, PhD, Hajime Imai, MD, Ken Kamata, MD, Hiroki Sakamoto, MD, PhD, Takamitsu Komaki, MD, PhD

Osaka-sayama, Japan

Background: The diagnosis of intra-abdominal lesions of undetermined origin is often a challenge for endoscopists and radiologists.

Objective: To evaluate the microvasculature of benign and malignant intra-abdominal lesions by contrast-enhanced harmonic EUS (CEH-EUS) and to investigate its usefulness for discriminating between malignant and benign lesions.

Design: The vascularity of intra-abdominal lesions of undetermined origin was observed by using CEH-EUS. The lesions were classified according to their vascular patterns. The effectiveness of CEH-EUS in differentiating malignant from benign lesions was evaluated.

Setting: Kinki University School of Medicine, Osaka, Japan.

Patients: Forty-three patients, each with a lesion of undetermined origin, were evaluated prospectively by CEH-EUS between March 2007 and March 2009.

Interventions: CEH-EUS was performed by using a prototype echoendoscope and the extended pure harmonic detection mode (a specific mode for contrast harmonic imaging).

Main Outcome Measurements: The lesions were categorized by 2 physicians as having no, homogeneous, or heterogeneous enhancement. A consensus was reached for each case offline. How the benign and malignant groups differed in terms of their enhancement patterns was examined.

Results: The κ coefficient of the interobserver agreement test was 0.953 ($P < .001$). Of the 27 malignant lesions, 26 (96.3%) exhibited heterogeneous enhancement. The 1 remaining malignant lesion (3.7%) showed homogeneous enhancement. Of the 16 benign lesions, none displayed heterogeneous enhancement, and 12 (75%) and 4 (25%) exhibited homogeneous and no enhancement, respectively. The malignant and benign lesion groups differed significantly in terms of homogeneous and heterogeneous enhancement ($P < .001$). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy with which CEH-EUS differentiated malignant from benign lesions were 96.3%, 100%, 100%, 94.1%, and 97.6%, respectively.

Limitations: A single medical unit with a limited number of patients.

Conclusions: CEH-EUS depicted the microvasculature of intra-abdominal lesions of undetermined origin very clearly and may be useful for characterizing such lesions.

Abbreviations: CEH-EUS, contrast-enhanced harmonic EUS; GIST, GI stromal tumor.

DISCLOSURE: *The prototype echoendoscope was provided by Olympus Medical Systems. Drs. Kitano and Kudo were supported by grants from the Japan Society for Promotion of Science, the Program of the Japan Society of Ultrasonics in Medicine, and the Japanese Foundation for the Research and Promotion of Endoscopy. All authors disclosed no financial relationships relevant to this publication.*

0016-5107/$36.00

doi:10.1016/j.gie.2010.04.013

Received January 11, 2010. Accepted April 13, 2010.

Current affiliations: Department of Gastroenterology and Hepatology (M. Kitano, M. Kudo, H.I., K.K., H.S., T.K.), Kinki University School of Medicine, Osaka, Japan, Department of Ultrasound (Y.X.), Peking Union Medical College Hospital, Beijing, China.

Reprint requests: Masayuki Kitano, MD, PhD, Department of Gastroenterology and Hepatology, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-sayama 589-8511, Japan.

The diagnosis of intra-abdominal lesions of undetermined origin is often challenging for endoscopists and radiologists.[1] Although imaging-guided FNA has made cytopathological diagnosis possible,[2] noninvasive evaluation remains important. This is especially true for cases in which a lesion cannot be accessed for FNA[3] or FNA is not successful in obtaining adequate material for analysis. In addition, noninvasive methods could facilitate FNA by helping to select the most promising lesion. One such noninvasive method is EUS, which has a high spatial resolution.[4-6] Moreover, the development of contrast-enhanced power Doppler EUS has significantly improved the detection of vessels in lymph nodes.[3,7] However, it is still difficult to image the microvasculature by EUS.

US technology was recently revolutionized by the invention of second-generation US contrast agents that, when combined with low mechanical index US imaging techniques, make it possible to depict microvasculature in real time.[8] Recently, this novel perfusion imaging technique has been combined with EUS, yielding contrast-enhanced harmonic EUS (CEH-EUS).[9,10] In this study, the microvasculature of intra-abdominal lesions of undetermined origin was observed by CEH-EUS, and the lesions were classified according to their vascular patterns. The ability of this latest imaging technique to characterize intra-abdominal lesions of undetermined origin was assessed. In particular, its ability to differentiate malignant from benign lesions was determined.

PATIENTS AND METHODS

Patients

Between March 2007 and March 2009, 43 patients (27 men, 16 women; median age 65 years; age range 35-82 years), each of whom had an intra-abdominal lesion of undetermined origin, were enrolled. Conventional imaging techniques such as CT, magnetic resonance imaging, or transabdominal US could not determine the origin of any of the lesions. All lesions had the EUS features of malignant lymph nodes: a hypoechoic lesion, a sharp border, a rounded shape, and greater than 10 mm in size.[11,12] The median lesion size was 19 mm (size range 10-80 mm). All lesions were investigated by CEH-EUS and then subjected to EUS-guided FNA.

The study was approved by the Institutional Review Board of Kinki University School of Medicine. All patients provided informed consent with regard to the procedure and participation in the study. The final diagnoses of all 43 lesions were determined by histological and/or cytological analyses of the samples that were obtained by EUS-guided FNA. Patients were followed clinically for at least 6 months after the procedure.

Equipment

A prototype echoendoscope developed for CEH-EUS (Olympus GF-UE260-AL5; Olympus Medical Systems, Tokyo, Japan) was used. US imaging was performed with an ALOKA ProSound SSD α-10 (Aloka Co Ltd, Tokyo, Japan). The extended pure harmonic detection mode, which combines receiving frequencies of filtered fundamental and second harmonic components with a transmitting frequency of 4.7 MHz, was used for CEH-EUS. Standardized presets were established for EUS and CEH-EUS.

US contrast

Sonazoid (Daiichi-Sankyo, Tokyo, Japan; GE Healthcare, Milwaukee, Wisc) was used as the US contrast agent. Sonazoid is a second-generation US contrast agent composed of perfluorobuthane microbubbles with a median diameter of 2 to 3 μm.[13] Sonazoid was reconstituted with 2 mL of sterile water for injection. A dose of 0.015 mL/kg body weight was used.

CEH-EUS

Each CEH-EUS was performed by 2 physicians (M.K., H.S.). One was responsible for endoscopic manipulation and scanning, and the other for operating the US scanner. Each study was performed by using the same protocol. Both endosonographers (who were qualified by the Japan Gastroenterological Endoscopy Society) are experienced in EUS and CEH-EUS, having performed more than 3000 and 100 of these procedures, respectively. Each study was performed by using the same protocol. First, the ideal imaging plane of each lesion for CEH-EUS was determined. A bolus injection of Sonazoid was administered at a speed of 1 mL/s through a 22-gauge cannula placed in the antecubital vein. This was followed by a 10-mL saline solution flush to ensure that all contrast was administered into the circulation system. All clips were stored in the hard disk of the scanner for offline investigation.

Image Analysis

Based on the enhancement area and previous research,[3,7] the enhancement patterns were classified as no enhancement, homogenous enhancement, or heterogeneous enhancement (Fig. 1, Videos 1-3, available online at www.giejournal.org). All enhancement patterns were initially determined and noted on-site independently by 2 physicians (M.K., Y.X.), each of whom had at least 5 years experience with contrast harmonic sonography. A consensus on the enhancement pattern of each lesion was later reached offline.

EUS-guided FNA

After CEH-EUS, the lesions were immediately punctured with a 22- or 25-gauge FNA system (Cook Medical, Bloomington, Ind) under EUS guidance. The specimens were subjected to pathological diagnosis.

Statistical Analyses

Data analysis was performed by using SAS software version 8.2 (SAS Institute, Cary, NC). The enhancement

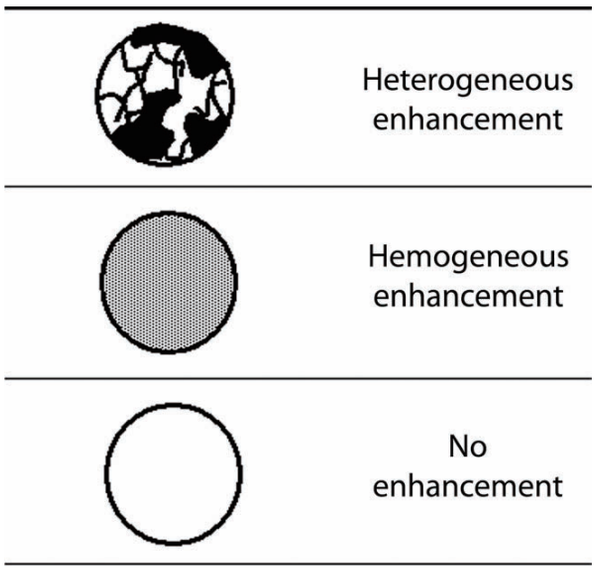


Figure 1. Classification of lesions according to their vascular structure as determined by CEH-EUS.

patterns of benign and malignant samples were categorized based on the consensus reached by the readers. A κ coefficient of >0.8 was considered to indicate very good agreement. The Fisher exact test in $R \times C$ contingency tables was applied to test differences between the enhancement patterns of the benign and malignant groups. A difference with $P < .05$ was regarded as significant. The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy with which CEH-EUS differentiated malignant from benign lesions were calculated.

RESULTS

CEH-EUS was performed on all patients successfully and associated side effects were not observed. Of the 43 intra-abdominal lesions studied, 27 were malignant and 16 were benign, as indicated by histological and/or cytological analyses of the samples obtained by EUS-guided FNA. The final pathological diagnoses were 21 malignant metastatic lymphadenopathies, 3 GI stromal tumors (GISTs), 2 malignant lymphomas, 1 paraganglioma, 11 reactive lymphadenopathies, 3 abscesses, 1 schwannoma, and 1 sarcoidosis.

All 43 lesions yielded high-quality dynamic images on CEH-EUS (Videos 1-3). Interobserver agreement testing revealed very good reproducibility between the 2 readers (κ coefficient: 0.953, $P < .001$). There were 2 cases for which the readers had different opinions: 1 schwannoma and 1 malignant metastatic lymphadenopathy. Reader A defined the former case as no enhancement, whereas reader B defined it as heterogeneous enhancement; after discussion, the enhancement pattern was defined as no enhancement. Reader A defined the latter case as heterogeneous enhancement and Reader B defined it as homogeneous enhancement; after discussion, the pattern was defined as heterogeneous enhancement.

Table 1 lists the number and percentage of lesions in the benign and malignant lesion groups that had each of the 3 enhancement patterns. Of the 27 malignant lesions, 26 (96.3%) presented with heterogeneous enhancement, in which the distorted tumor vessels could be clearly visualized (Fig. 2, Video 1); these included the 21 malignant metastatic lymphadenopathies, 1 lymphoma, 3 GISTs, and 1 paraganglioma. The remaining lymphoma (3.7%) presented with homogeneous enhancement. Of the 16 benign lesions, 12 (75%) presented with homogeneous enhancement (Fig. 3, Video 2); these included the 11 reactive lymphadenopathies and 1 sarcoidosis. The remaining 4 lesions (1 schwannoma and 3 abscesses) presented with no enhancement (Fig. 4, Video 3). The benign and malignant lesion groups differed significantly in terms of the frequency of homogeneous and heterogeneous enhancement ($P < .001$). The sensitivity, specificity, positive predictive value, negative predictive value, and accuracy with which CEH-EUS differentiated malignant from benign lesions were 96.3%, 100%, 100%, 94.1%, and 97.6%, respectively.

DISCUSSION

Since the invention of US contrast, advances in US contrast agents and US imaging techniques have facilitated the depiction of tumor vascularity.[14,15] The use of second-generation US contrast agents combined with low mechanic index imaging techniques has led to the use of contrast-enhanced US for perfusion imaging.[15,16] For technical reasons, it was not possible to combine contrast-enhanced harmonic imaging techniques with endoscopy until 2008, when CEH-EUS was developed. Compared with power Doppler imaging, CEH-EUS allows lesion microvasculature to be imaged without introducing blooming or motion artifacts.[9] In this study, CEH-EUS successfully depicted the vasculature of intra-abdominal lesions of undetermined origin and demonstrated excellent ability to differentiate malignant from benign lesions.

Heterogeneous enhancement was only observed in malignant masses; none of the benign lesions displayed this pattern. This is consistent with the observation of a pathology-based study,[17] namely, the vascular architecture of malignant lymph nodes is characterized by caliber fluctuations, an irregular course, sinusoid formation, and arteriovenous shunts. Sawhney et al[4] also reported that when tumor infiltration and neovascularization occur, the centrally located vessels found in normal lymph nodes are distorted; indeed, they found that the absence of a central intranodal blood vessel was a strong and independent predictor of metastatic lymphadenopathy.

TABLE 1. The number and percentage of the lesions in the benign and malignant groups that exhibited heterogeneous, homogeneous, and no enhancement on contrast-enhanced harmonic EUS

	Number (%) with each enhancement pattern			
Final diagnosis	**Heterogeneous**	**Homogeneous**	**None**	**Total**
Malignant lesion	26 (96.3)	1 (3.7)	0 (0)	27 (100)
Benign lesion	0 (0)	12 (75)	4 (25)	16 (100)
Total	26 (60.5)	13 (30.2)	4 (9.3)	43 (100)

In the current study, 1 lymphoma exhibited homogeneous enhancement and did not differ from the benign group lesions that exhibited the same pattern. Hocke et al[3] previously reported that contrast-enhanced US was unable to discriminate between benign lesions and malignant lymphomas. In contrast, findings of another study of 10 lymphomas suggested that different lymphoma types may have different enhancement patterns on contrast-enhanced US and that the homogeneous enhancement of diffuse B–type lymphoma may be caused by homogeneous invasion.[18] However, given the limited case numbers that were involved in the previous studies and our study, additional research will be needed to confirm this possibility.

None of the lesions originated from any intra-abdominal organs, and their features on conventional EUS suggested strongly that they were malignant lymph nodes. However, the final pathological results showed that only 35 lesions (35/43, 81%) were lymph nodes. The other 8 lesions (8/43, 19%) had diverse origins; they were abscesses, a schwannoma, a paraganglioma, and GISTs. The 3 GISTs that were assessed were primary lesions that originated from the mesenteric region rather than from the GI tract. Further research evaluating GI mesenchymal tumors in the GI tract may demonstrate the usefulness of CEH-EUS in predicting the risk of malignancy of such tumors.

The κ test was used in this study to test the reproducibility of the enhancement patterns on CEH-EUS. The κ value for the 3 enhancement patterns was 0.953 ($P < .001$), which indicates that there was very good agreement. This good reproducibility is probably attributable to the high sensitivity with which CEH-EUS depicted the microvasculature, the simplicity of the enhancement pattern classification system, and perhaps also the real-time nature of CEH-EUS. There were 2 discordant cases, namely a schwannoma and a metastatic lymphadenopathy. The discrepancy in the first case may have arisen because the enhancement pattern classification system was too simplistic to accurately reflect the subtle enhancement seen in the schwannoma. Although it was finally agreed that the pattern of the latter case was heterogeneous, both readers acknowledged that the enhancement pattern was difficult to discern. This situation indicates that

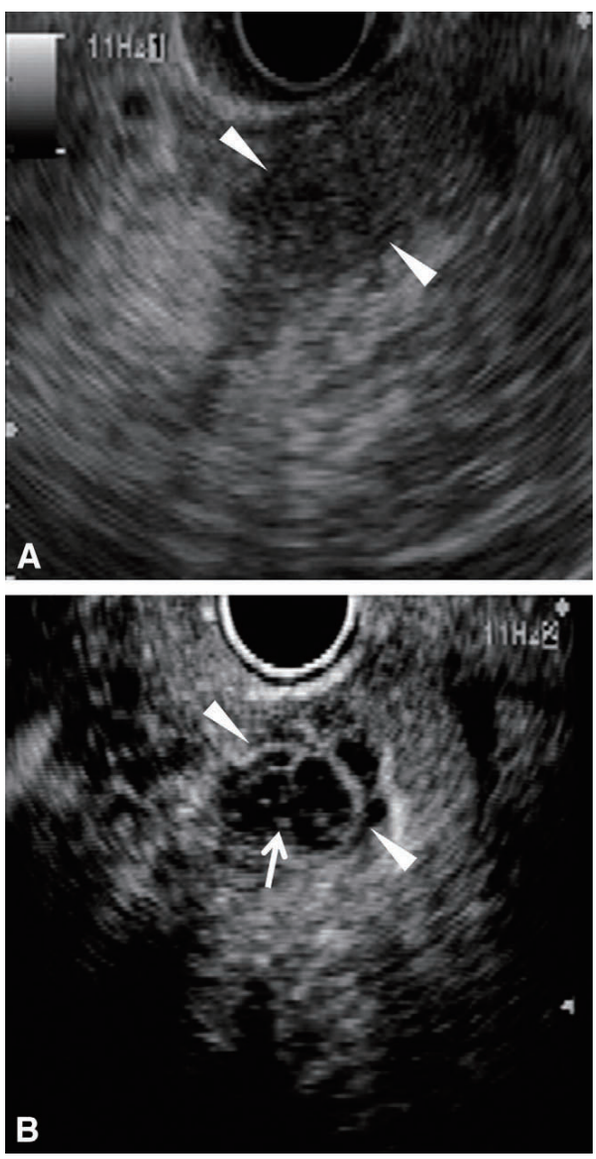


Figure 2. A typical lesion with heterogeneous enhancement (a malignant metastatic lymphadenopathy; adenocarcinoma). **A**, Fundamental B mode (monitor mode). A hypoechoic mass with an irregular rim can be seen around the pancreatic head (*arrowheads*). **B**, Extended pure harmonic detection mode. Heterogeneous enhancement is observed in the lesion (*arrowheads*). The lesion includes hypovascular parts with slow flow (*arrow*).

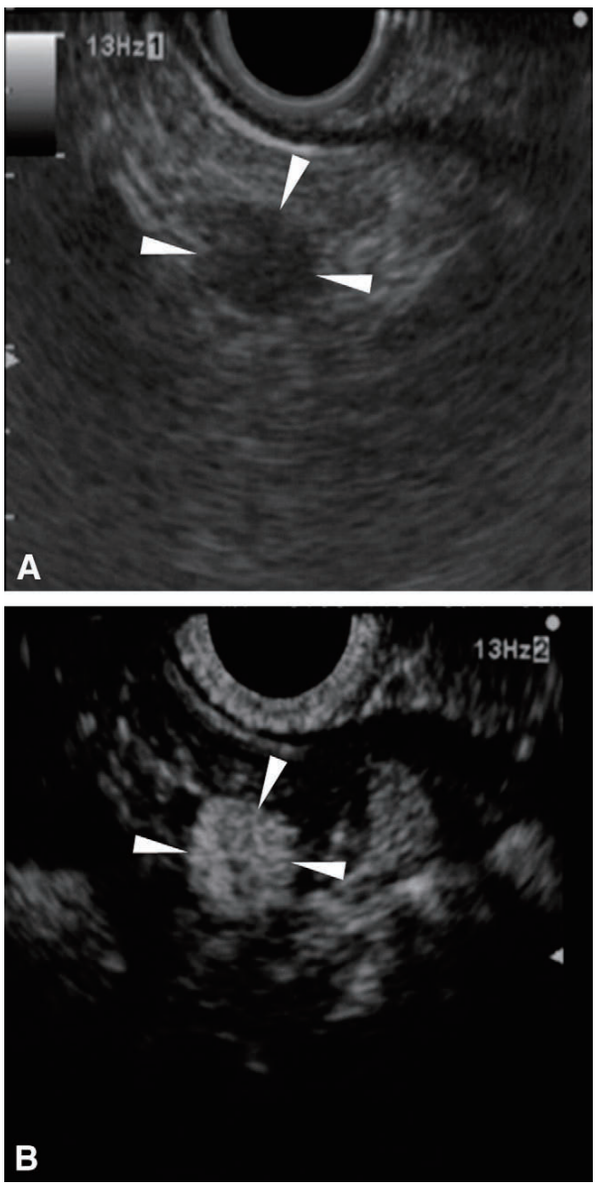


Figure 3. A typical lesion with homogeneous enhancement (a reactive lymphadenopathy). **A**, Fundamental B mode (monitor mode). A hypoechoic mass (*arrowheads*) can be seen around the pancreatic tail. **B**, Extended pure harmonic detection mode. Homogeneous enhancement is observed in the lesion (*arrowheads*).

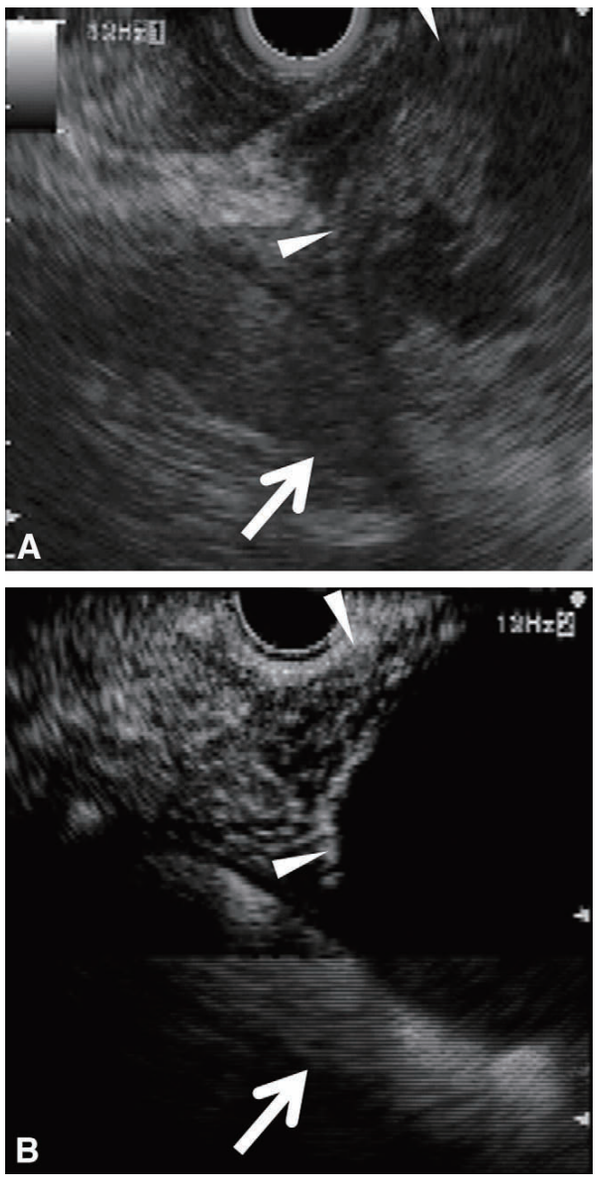


Figure 4. A typical lesion with no enhancement (an abscess). **A**, Fundamental B mode (monitor mode). An echogenic mass (*arrowheads*) can be seen in front of the abdominal aorta (*arrow*). The mass includes cystic parts. **B**, Extended pure harmonic detection mode. No enhancement is observed in the lesion (*arrowheads*).

although the categorization of CEH-EUS enhancement patterns as no, homogeneous, and heterogeneous enhancement was helpful for the differential and final diagnosis, this classification system is still based on the subjective opinion of the readers. Enhancement determination is not yet an objective measure.

Sonazoid-related major side effects were never observed in either this study or our previous study using Sonazoid in 214 patients with hepatocellular carcinomas.[19] We used Sonazoid as the US contrast agent because it is the only agent that is available in Japan. A previous CEH-EUS study using SonoVue as the US contrast agent was performed in Germany.[9] Although both agents enable clear vascular imaging, the echo intensity in the normal pancreas 60 seconds after SonoVue and Sonazoid infusion was about half[9] and 70% (unpublished observation) of the maximum level, respectively. Thus, Sonazoid seems superior to SonoVue in terms of the duration of contrast enhancement, although a prospective comparative study that tests the 2 agents is needed to confirm this.

In conclusion, CEH-EUS depicted the microvasculature of intra-abdominal lesions of undetermined origin very clearly and permitted the enhancement patterns to be interpreted with good agreement by different readers. Thus, it may be a useful modality for characterizing lesions. However, given that the sample size of this study was relatively small and all CEH-EUS procedures were performed in a single medical unit, an additional study that confirms the value of CEH-EUS for characterizing intra-abdominal lesions of undetermined origin is warranted.

REFERENCES

1. Sharma A, Fidias P, Hayman LA, et al. Patterns of lymphadenopathy in thoracic malignancies. Radiographics 2004;24:419-39.
2. Jhala NC, Jhala DN, Chhieng DC, et al. Endoscopic ultrasound-guided fine-needle aspiration. A cytopathologist's perspective. Am J Clin Pathol 2003;120:351-67.
3. Hocke M, Menges M, Topalidis T, et al. Contrast-enhanced endoscopic ultrasound in discrimination between benign and malignant mediastinal and abdominal lymph nodes. J Cancer Res Clin Oncol 2008;134:473-80.
4. Sawhney MS, Debold SM, Kratzke RA, et al. Central intranodal blood vessel: a new EUS sign described in mediastinal lymph nodes. Gastrointest Endosc 2007;65:602-8.
5. Eloubeidi MA, Wallace MB, Reed CE, et al. The utility of EUS and EUS-guided fine needle aspiration in detecting celiac lymph node metastasis in patients with esophageal cancer: a single-center experience. Gastrointest Endosc 2001;54:714-9.
6. Bhutani MS, Hawes RH, Hoffman BJ. A comparison of the accuracy of echo features during endoscopic ultrasound (EUS) and EUS-guided fine-needle aspiration for diagnosis of malignant lymph node invasion. Gastrointest Endosc 1997;45:474-9.
7. Kanamori A, Hirooka Y, Itoh A, et al. Usefulness of contrast-enhanced endoscopic ultrasonography in the differentiation between malignant and benign lymphadenopathy. Am J Gastroenterol 2006;101:45-51.
8. Quaia E, Calliada F, Bertolotto M, et al. Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence. Radiology 2004;232:420-30.
9. Kitano M, Sakamoto H, Matsui U, et al. A novel perfusion imaging technique of the pancreas: contrast-enhanced harmonic EUS (with video). Gastrointest Endosc 2008;67:141-50.
10. Kitano M, Kudo M, Sakamoto H, et al. Preliminary study of contrast-enhanced harmonic endosonography with second-generation contrast agents. J Med Ultrasonics 2008;35:11-8.
11. Catalano MF, Sivak MV Jr, Rice T, et al. Endosonographic features predictive of lymph node metastasis. Gastrointest Endosc 1994;40:442-6.
12. Vassallo P, Edel G, Roos N, et al. In-vitro high resolution ultrasonography of benign and malignant lymph nodes. A sonographic-pathologic correlation. Invest Radiol 1993;28:698-705.
13. Sontum PC, Ostensen J, Dyrstad K, et al. Acoustic properties of NC100100 and their relation with the microbubble size distribution. Invest Radiol 1999;34:268-75.
14. Burns PN, Wilson SR, Simpson DH. Pulse inversion imaging of liver blood flow: improved method for characterizing focal masses with microbubble contrast. Invest Radiol 2000;35:58-71.
15. Ding H, Kudo M , Onda H, et al. Hepatocellular carcinoma: depiction of tumor parenchymal flow with intermittent harmonic power Doppler US during the early arterial phase in dual-display mode. Radiology 2001; 220:349-56.
16. Park BK, Kim B, Kim SH, et al. Assessment of cystic renal masses based on Bosniak classification: comparison of CT and contrast-enhanced US. Eur J Radiol 2007;61:310-4.
17. Shubik P. Vascularization of tumors: a review. J Cancer Res Clin Oncol 1982;103:211-26.
18. Nakase K, Yamamoto K, Hiasa A, et al. Contrast-enhanced ultrasound examination of lymph nodes in different types of lymphoma. Cancer Detect Prev 2006;30:188-91.
19. Hatanaka K, Kudo M, Minami Y, et al. Differential diagnosis of hepatic tumors: value of contrast-enhanced harmonic sonography using the newly developed contrast agent, Sonazoid. Intervirology 2008;51: S61-9.

Cancer Science
The official journal of the Japanese Cancer Association

Phase I/II study of the pharmacokinetics, safety and efficacy of S-1 in patients with advanced hepatocellular carcinoma

Junji Furuse,[1,2,6] Takuji Okusaka,[3] Shuichi Kaneko,[4] Masatoshi Kudo,[5] Kohei Nakachi,[1] Hideki Ueno,[3] Tatsuya Yamashita[4] and Kazuomi Ueshima[5]

[1]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital East, Kashiwa; [2]Medical Oncology Division, Kyorin University School of Medicine, Mitaka-shi; [3]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo; [4]Department of Gastroenterology, Kanazawa University Hospital, Kanazawa, Ishikawa; [5]Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka, Japan

(Received April 26, 2010/Revised August 17, 2010/Accepted August 18, 2010/Accepted manuscript online August 26, 2010/Article first published online October 14, 2010)

S-1, an oral fluoropyrimidine derivative, has been shown to be clinically effective against various solid tumors, and preclinical studies have demonstrated activity against hepatocellular carcinoma. We conducted a phase I/II study in patients with advanced hepatocellular carcinoma to examine the pharmacokinetics, recommended dose, safety and efficacy of S-1. In phase I, the administered dose of S-1 was approximately 64 mg/m^2 per day in three patients (level 1) and approximately 80 mg/m^2 per day in six patients (level 2). There was no dose-limiting toxicity at level 1, but two patients had dose-limiting toxicity at level 2 (grade 3 anorexia and grade 2 rash requiring eight or more consecutive days of rest). The recommended dose was finally estimated to be 80 mg/m^2 per day. There were no significant differences in the pharmacokinetics of S-1 between patients with Child-Pugh A and those with B. In phase II, five of 23 patients (21.7%) had partial responses. The median progression-free survival and overall survival were 3.7 and 16.6 months, respectively. The most common toxicities of grade 3 or 4 were elevated serum aspartate aminotransferase levels, hypochromia and thrombocytopenia. In conclusion, S-1 showed an acceptable toxicity profile and promising antitumor activity for hepatocellular carcinoma, warranting further evaluation in randomized clinical trials. (*Cancer Sci* 2010; 101: 2606–2611)

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Outcomes remain poor because the disease is usually advanced and associated with hepatic impairment at diagnosis, and because of the high rate of recurrence resulting from either intrahepatic metastases from the primary tumor or multicentric lesions. As for therapy, surgical resection and percutaneous ethanol injection (PEI) or radiofrequency ablation (RFA) are considered the mainstays of treatment in patients with potentially curable disease. Transcatheter arterial chemoembolization (TACE) is the treatment of choice for noncurative HCC. Despite numerous clinical trials of a wide variety of cytotoxic agents, survival remains dismal in HCC.[1] Recently, sorafenib, an oral multi-kinase inhibitor that targets mainly Raf kinases and receptor tyrosine kinases associated with angiogenesis (vascular endothelial growth factor receptor [VEGFR]-2/-3 and platelet-derived growth factor receptor [PDGFR]-β), provided a significant survival benefit in patients with advanced HCC enrolled in placebo-controlled, randomized, phase III trials, including Asian as well as European subjects.[2,3] An initial phase I study in Japanese patients with HCC associated mainly with hepatitis C virus (HCV) infection showed promising antitumor activity and a favorable tolerability profile.[4] However, further improvement in the treatment of advanced HCC is essential.

S-1 is a novel, orally administered drug that combines tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP) and oteracil potassium (Oxo) in a molar concentration ratio of 1:0.4:1.[5] CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase (DPD), a metabolizing enzyme of 5-fluorouracil (5-FU) that is expressed in the liver. Inhibition of DPD by CDHP results in prolonged effective concentrations of 5-FU in plasma and tumor tissue.[6] Oxo, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, thereby reducing serious 5-FU-related gastrointestinal toxicity.[7] Clinically, S-1 has been shown to be effective against a variety of solid tumors, with response rates ranging 21–49% in late phase II studies conducted in Japan.[8] S-1 has yet to be evaluated in patients with HCC. However, in nude rats with human HCC xenografts, S-1 has been confirmed to have antitumor activity.[9]

Patients with HCC usually have various degrees of liver dysfunction because of associated liver disease and replacement of liver tissue by tumor, leading to pathophysiological changes that influence drug disposition. Decreased hepatic blood flow, extrahepatic and intrahepatic blood shunting and hepatocyte loss also alter drug metabolism, and decreased protein synthesis reduces drug binding to plasma proteins. In fact, the maximal tolerated dose (MTD) of 5-FU given as a 5-day continuous infusion in patients with HCC is approximately 50% of that in patients with normal organ function, and patients with cirrhosis have significantly lower clearance of 5-FU than those without cirrhosis.[10] We therefore conducted a multicenter phase I/II study to evaluate the pharmacokinetics, safety and efficacy of S-1 monotherapy in patients with advanced HCC.

Materials and Methods

Eligibility. Eligible patients had histologically or cytologically proved HCC that was not amenable to treatment by resection, liver transplantation, RFA, PEI or percutaneous microwave coagulation therapy (PMCT) and was not expected to respond to TACE. A hypervascular mass on computed tomography (CT) or magnetic resonance imaging (MRI) associated with a serum alpha-fetoprotein level or a serum protein induced by vitamin K absence or antagonist (PIVKA-II) level of more than the upper limit of normal (ULN) was considered a sufficient non-invasive diagnostic criterion for HCC. At least one measurable lesion on CT or MRI (not including necrotic lesions caused by prior treatment) was required. Other eligibility criteria included: age of at least 20 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; estimated life expectancy of at least 60 days; adequate

[6]To whom correspondence should be addressed. E-mail: jfuruse@ks.kyorin-u.ac.jp
Clinical trial registration: this trial was not registered in the clinical trial database because it was an early phase trial and not a controlled study.

doi: 10.1111/j.1349-7006.2010.01730.x

hematological function (white blood cells [WBC] ≥3000/mm^3, hemoglobin ≥ 9.0 g/dL, platelets ≥ 7.0 × 10^4/mm^3); adequate hepatic function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] ≤5 times the ULN, total bilirubin ≤2.0 mg/dL, serum albumin ≥2.8 g/dL, prothrombin activity ≥40%); adequate renal function (serum creatinine ≤ULN); and a Child-Pugh class of A or B. Prior treatment for HCC, such as resection, liver transplantation, RFA, PEI, PMCT and TACE was permitted if the treatment had been performed 30 or more days before registration in the study. Patients were excluded if they had: tumor involving more than 50% of the liver; brain or bone metastasis or vascular invasion of the main trunk and first-order branch(es) of the portal vein, hepatic veins, hepatic arteries or bile duct; severe complications; other malignancies; or inability to comply with the protocol requirements. Written informed consent was obtained from each patient. The study was approved by the local institutional review boards at all participating centers.

Study design. S-1 was supplied by Taiho Pharmaceutical Co., Ltd (Tokyo, Japan) in capsules containing 20 or 25 mg of FT. Individual doses were calculated according to body surface area. The calculated dose was rounded to derive the daily dose and the number of capsules to be dispensed per patient. At each dose level, S-1 was administered orally twice daily (after breakfast and dinner) for 28 consecutive days, followed by a 14-day recovery period. Each treatment cycle was 42 days. If grade 3 or higher hematological toxicity, grade 2 or higher non-hematological toxicity, grade 3 or higher elevations of AST or ALT, or grade 2 or higher increases in the serum creatinine concentration occurred, treatment with S-1 was temporarily suspended, the dose of S-1 was reduced, or both (minimum dose, 50 mg/day). Treatment continued until there was evidence of disease progression, or if the recovery period exceeded 28 days, the patient requested treatment to be discontinued or unacceptable toxicity developed and treatment was terminated at the discretion of the investigator. Drug compliance and accountability were carefully monitored; patients were requested to record their intake of S-1 and other medications in a diary.

During phase I, the starting dose of S-1 (level 1) was approximately 64 mg/m^2 per day twice daily (80% of the standard dose), level 2 was approximately 80 mg/m^2 per day and level 0 was approximately 50 mg/m^2 per day (80% of level 1). Patients were enrolled in cohorts of three for each dose level. The dose was escalated according to the cohort and was not increased in the same patient. If none of the first three patients had dose-limiting toxicity (DLT) during the first cycle, the dose was increased to level 2. If one or two of the first three patients had DLT, three additional patients were entered at the same dose level; if only one or two of the first six patients at level 1 had DLT, the dose was increased to level 2; if all of the first three patients or three or more of the first six patients had DLT, the dose was decreased to level 0; if none of the first three patients had DLT at level 0 or level 2, three additional patients were assigned to receive the same dose level. The DLT was defined as any of the following: (i) hematological toxicity ≥grade 4; (ii) non-hematological toxicity ≥grade 3; (iii) AST, ALT ≥15 times the ULN; or (iv) a rest period of 8 or more consecutive days was required. The recommended dose (RD) determined in the phase I part of this study was used in phase II.

Pharmacokinetics. Blood samples (5 mL) were obtained from each patient assigned to receive level 2 in the phase I part of the study. The samples were taken before and 1, 2, 4, 6, 8, 10 and 12 h after administration of S-1 on days 1 and 8 of the first treatment cycle. Plasma was separated from the whole-blood samples by centrifugation and stored at −20°C until analysis. Plasma FT concentrations were measured by high-performance liquid chromatography with ultraviolet detection. Plasma concentrations of 5-FU, CDHP and Oxo were measured by gas chromatography-negative ion chemical ionization mass spectrometry, as described previously.[11]

Pharmacokinetic data, including the maximum plasma concentration (C_{max}, ng/mL), time to reach C_{max} (T_{max}, h), area under the plasma-concentration–time curve for 0–12 h (AUC_{0-12}, ng h/mL) and the elimination half-life ($T_{1/2}$, h) were calculated by noncompartment model analysis using WinNonlin software, version 4.1 (Pharsight, Cary, NC, USA).

Assessment of efficacy and toxicity. All patients who received at least one dose of the study drug were included in the evaluations of response and toxicity. During each course of treatment, tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) by computed tomography (CT) or magnetic resonance imaging (MRI), with a slice thickness of no more than 5 mM.[12] The primary efficacy end-point in the phase II part of this study was the overall response rate, assessed on the basis of changes in tumor dimensions. The other end-points were overall survival (OS) and progression-free survival (PFS). The PFS was defined as the interval between the date of initiating treatment and the date on which disease progression was first confirmed or the date of death from any cause. Overall survival was defined at the interval from the date of initiating treatment to the date of death from any cause. Median OS and median PFS were

Table 1. Patient characteristics

	Level 1 (*n* = 3)	Level 2 (*n* = 23)
	n (%)	*n* (%)
Median age (range) (years)	67.0 (63–68)	68.0 (45–78)
Gender		
Male	2 (66.7)	21 (91.3)
Female	1 (33.3)	2 (8.7)
Virus marker		
HBs (+)	1 (33.3)	3 (13.0)
HCV (+)	1 (33.3)	14 (60.9)
HBs(−), HCV(−)	1 (33.3)	6 (26.1)
Child-Pugh classification		
A	3 (100)	16 (69.6)
B	0 (0)	7 (30.4)
Stage		
Stage II	1 (33.3)	3 (13.0)
Stage III	1 (33.3)	10 (43.5)
Stage IVB	1 (33.3)	10 (43.5)
Vascular invasion	0 (0)	2 (8.7)
ECOG PS		
0	3 (100)	21 (91.3)
1	0 (0)	2 (8.7)
Pretreatment		
TA(C)E	2 (66.7)	17 (73.9)
Surgery	1 (33.3)	8 (34.8)
RFA	0 (0)	7 (30.4)
HAI	2 (66.7)	6 (26.1)
PEI	0 (0)	4 (17.4)
Radiation	0 (0)	4 (17.4)
PMCT	0 (0)	3 (13.0)
Systemic chemotherapy	0 (0)	3 (13.0)
BCLC staging		
Early	0 (0)	1 (4.3)
Intermediate	2 (66.7)	11 (47.8)
Advanced	1 (33.3)	11 (47.8)

BCLC, Barcelona Clinic Liver Cancer Group; ECOG, Eastern Cooperative Oncology Group; HAI, hepatic arterial infusion; HBs, hepatitis B surface antigen; HCV, hepatitis C virus antibody; PEI, percutaneous ethanol injection; PMCT, percutaneous microwave coagulation therapy; PS, performance status; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

Table 2. Toxic effects

Toxicity	Level 1 (*n* = 3)		Level 2 (*n* = 23)		Child Pugh A (*n* = 16)		Child Pugh B (*n* = 7)	
	All grades	≥G3	All grades	≥G3	All grades	≥G3	All grades	≥G3
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
All adverse events	3 (100.0)	0 (0.0)	23 (100.0)	10 (43.5)	16 (100.0)	8 (50.0)	7 (100.0)	2 (28.6)
Hematological								
Erythropenia	1 (33.3)	0 (0.0)	21 (91.3)	1 (4.3)	14 (87.5)	1 (6.3)	7 (100.0)	0 (0.0)
Hypochromia	1 (33.3)	0 (0.0)	19 (82.6)	4 (17.4)	12 (75.0)	4 (25.0)	7 (100.0)	0 (0.0)
Leukopenia	2 (66.7)	0 (0.0)	18 (78.3)	1 (4.3)	12 (75.0)	1 (6.3)	6 (85.7)	0 (0.0)
Lymphopenia	2 (66.7)	0 (0.0)	12 (52.2)	3 (13.0)	7 (43.8)	3 (18.8)	5 (71.4)	0 (0.0)
Neutropenia	1 (33.3)	0 (0.0)	17 (73.9)	1 (4.3)	12 (75.0)	1 (6.3)	5 (71.4)	0 (0.0)
Reduced hematocrit	1 (33.3)	0 (0.0)	19 (82.6)	1 (4.3)	12 (75.0)	1 (6.3)	7 (100.0)	0 (0.0)
Reduced prothrombin content	1 (33.3)	0 (0.0)	19 (82.6)	0 (0.0)	14 (87.5)	0 (0.0)	5 (71.4)	0 (0.0)
Thrombocytopenia	1 (33.3)	0 (0.0)	18 (78.3)	4 (17.4)	12 (75.0)	4 (25.0)	6 (85.7)	0 (0.0)
Non-hematological								
Elevated alkaline phosphatase	0 (0.0)	0 (0.0)	8 (34.8)	1 (4.3)	7 (43.8)	1 (6.3)	1 (14.3)	0 (0.0)
Elevated lactate dehydrogenase	0 (0.0)	0 (0.0)	15 (65.2)	0 (0.0)	9 (56.3)	0 (0.0)	6 (85.7)	0 (0.0)
Elevated serum AST	1 (33.3)	0 (0.0)	8 (34.8)	4 (17.4)	6 (37.5)	3 (18.8)	2 (28.6)	1 (14.3)
Elevated serum bilirubin	0 (0.0)	0 (0.0)	18 (78.3)	3 (13.0)	13 (81.3)	2 (12.5)	5 (71.4)	1 (14.3)
Hyponatremic	0 (0.0)	0 (0.0)	8 (34.8)	0 (0.0)	5 (31.3)	0 (0.0)	3 (42.9)	0 (0.0)
Reduced cholinesterase	2 (66.7)	0 (0.0)	18 (78.3)	0 (0.0)	13 (81.3)	0 (0.0)	5 (71.4)	0 (0.0)
Reduced serum albumin	0 (0.0)	0 (0.0)	18 (78.3)	2 (8.7)	12 (75.0)	1 (6.3)	6 (85.7)	1 (14.3)
Reduced total protein	0 (0.0)	0 (0.0)	11 (47.8)	0 (0.0)	8 (50.0)	0 (0.0)	3 (42.9)	0 (0.0)
Anorexia	1 (33.3)	0 (0.0)	18 (78.3)	2 (8.7)	13 (81.3)	1 (6.3)	5 (71.4)	1 (14.3)
Ascites	0 (0.0)	0 (0.0)	7 (30.4)	0 (0.0)	3 (18.8)	0 (0.0)	4 (57.1)	0 (0.0)
Diarrhea	0 (0.0)	0 (0.0)	10 (43.5)	0 (0.0)	8 (50.0)	0 (0.0)	2 (28.6)	0 (0.0)
Fatigue	0 (0.0)	0 (0.0)	19 (82.6)	2 (8.7)	13 (81.3)	2 (12.5)	6 (85.7)	0 (0.0)
Pigmentation	0 (0.0)	0 (0.0)	20 (87.0)	0 (0.0)	14 (87.5)	0 (0.0)	6 (85.7)	0 (0.0)
Rash	0 (0.0)	0 (0.0)	8 (34.8)	0 (0.0)	5 (31.3)	0 (0.0)	3 (42.9)	0 (0.0)
Stomatitis	0 (0.0)	0 (0.0)	7 (30.4)	0 (0.0)	5 (31.3)	0 (0.0)	2 (28.6)	0 (0.0)

Dosage level, level 1, 2 (*n* = 3, 23); AST, aspartate aminotransferase.

Table 3. Efficacy in patients who received dose level 2

	Child-Pugh A (*n* = 16)	Child-Pugh B (*n* = 7)	Total (*n* = 23)
Partial response†	4	1	5
Stable disease‡	5	2	7
Progressive disease	7	3	10
Not evaluable	0	1	1
Response rate (90%CI)§ (%)	–	–	23.1 (9.0–40.4)
Response rate (95%CI) (%)	25.0 (7.3–52.4)	14.3 (0.4–57.9)	23.1 (7.5–43.7)
Median PFS (95% CI) (months)	3.3 (2.3–5.1)	3.7 (2.5–7.4)	3.7 (2.5–5.1)
Median OS (95% CI) (months)	17.8 (14.0–NA)	14.5 (9.6–18.7)	16.6 (14.0–24.5)
1-year survival (95% CI) (%)	–	–	69.6 (50.8–88.4)
1.5-years survival (95% CI) (%)	–	–	43.0 (22.6–63.5)
Disease control rate¶			
6W (95% CI) (%)	–	–	47.8 (26.8–69.4)
12W (95% CI) (%)	–	–	26.1 (10.2–48.4)
24W (95% CI) (%)	–	–	21.7 (7.5–43.7)

†Partial response was re-evaluated after at least 4 weeks in patients with a partial response. ‡Stable disease was reassessed after at least 6 weeks. §Response rate (90% CI) is a primary end-point. ¶Disease control rates were respectively estimated by dividing the number of patients with no disease progression by the total number of patients. Disease control was defined as a response of complete response, partial response or stable disease. CI, confidence interval; NA, not available; OS, overall survival; PFS, progression-free survival.

estimated using the Kaplan–Meier method. Physical findings and the results of serum chemical and urine analyses were assessed at 2-week intervals; vital signs were assessed as necessary. Patients were observed until death or at least 1 year after registration to determine survival status. The severity of all adverse events was evaluated according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE, Ver. 3.0). The duration of all adverse events and their relation to S-1 were initially assessed by the attending physicians. Subsequently, an independent review committee reviewed data on objective response and adverse events.

Statistical considerations. With the response rate as the primary end-point, a total sample size of at least 23 patients was estimated to be required in the phase II portion to allow the

doi: 10.1111/j.1349-7006.2010.01730.x

study to have a one-sided 5% significance level of 0.05 and a power of 70%, assuming a threshold response rate of 5% and an expected response rate of 20%.

Results

Patient characteristics and treatment. Between May 2006 and April 2007, a total of 26 patients (nine in phase I and 17 in phase II) were enrolled at four centers in Japan. All patients were eligible for the evaluation of toxicity and efficacy. The first six patients who received dose level 2 (80 mg/m^2 per day) during the phase I part of this study were included in the phase II assessment, along with the 17 other patients (a total of 23 patients in the phase II assessment). The characteristics of patients are summarized in Table 1. At the study entry, 11 of 26 (42.3%) had metastatic disease. Six patients (23.1%) had single extrahepatic metastases (lung metastases, three patients; lymph node metastasis, three patients). Four patients had two sites of metastases, including the lung, lymph nodes and adrenal glands. Of the 26 patients, 23 had received some prior treatment, including three who had received systemic chemotherapy.

Dose-limiting toxicity and RD. None of the three patients who received dose level 1 (64 mg/m^2 per day) in the phase I part of the study had DLT. At dose level 2 (80 mg/m^2 per day), one patient with Child-Pugh class B had grade 3 anorexia during the first course of treatment, but the other two patients in the same cohort had no DLT. Three additional patients were enrolled to confirm safety, and one patient with Child-Pugh class B had a grade 2 rash; recovery required eight or more consecutive days of rest. Because two of the six patients who received level 2 had DLT, level 2 was defined as the RD for the phase II part of the study.

Treatment delivered. Twenty-three patients received a total of 85 cycles of treatment at dose level 2 (median, three cycles per patient; range, 1–15). The dose of S-1 was reduced in seven patients (30.4%) or a total of nine cycles (10.6%). The most common reasons for dose reductions were rash in four patients, and elevated serum bilirubin concentrations and anorexia in two patients each (some overlap among patients). Treatment was delayed because of toxicity in 12 patients (20 cycles), most often in cycles 1 or 2. The most common reasons for toxicity-related treatment delays were fatigue (five patients), rash (four patients) and elevated serum bilirubin concentrations (three patients). The reasons for terminating treatment were progressive disease in 19 patients (82.6%), adverse reactions in two patients (8.7%) and other reasons in two patients (8.7%; one required 28 or more consecutive days of rest, and one withdrew consent).

Toxicity. Drug-related adverse events occurring in all 26 patients in the phase I/II portion of the study are shown in Table 2. Treatment with S-1 was generally well tolerated throughout the study. Grade 3 or 4 toxicity occurred in 10 of the 23 patients (43.5%) who received level 2. Most toxic effects were laboratory abnormalities. There was no grade 3 or 4 toxicity at level 1. The most common grade 3 or 4 hematological toxic effects were hypochromia (17.4%), thrombocytopenia (17.4%) and lymphopenia (13.0%); the most common grade 3 or 4 nonhematological toxic effects were elevated serum AST levels (17.4%) and elevated serum bilirubin concentrations (13.0%).

Efficacy. A response could be evaluated in 26 patients in the phase I/II portion of the study. In the phase I part of the study (dose level 1), one patient had a partial response, one had progressive disease and the other was not evaluable. Of the 23 patients in the phase II part of the study, five (21.7%; 90% confidence interval [CI], 9.0–40.4%) responded to treatment Among the 23 patients in whom a response could be evaluated, five had a partial response, seven had stable disease, and 10 had progressive disease (Table 3). The remaining patient underwent imaging studies, but treatment was completed after one course, and continuation of stable disease for at least 6 weeks could not be

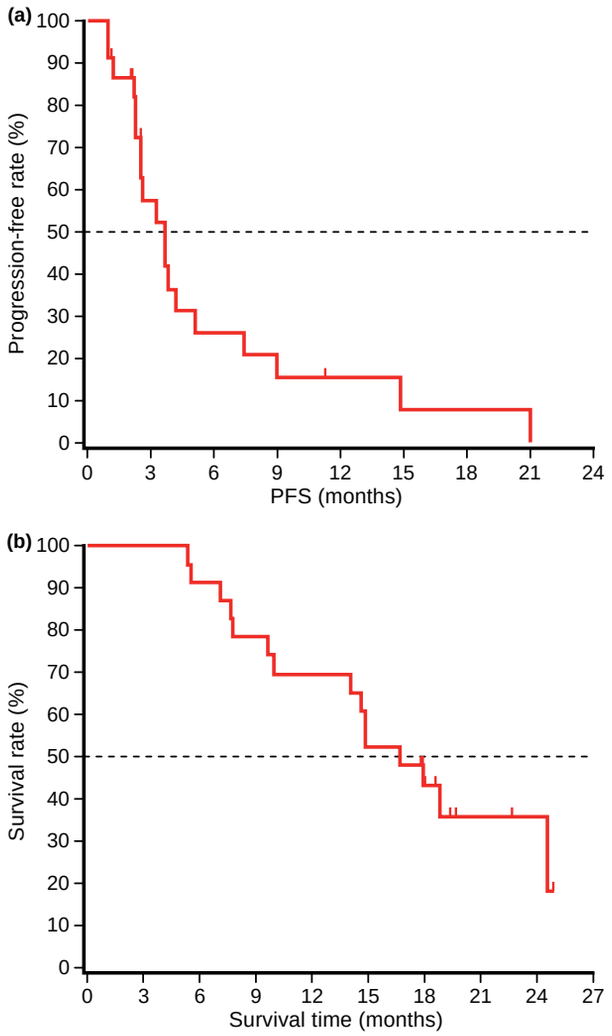


Fig. 1. Progression-free survival (PFS) (a) and overall survival (b) in patients who received dose level 2 of S-1 (n = 23). The median progression-free survival and overall survival were 3.7 and 16.6 months, respectively.

Table 4. Pharmacokinetics of FT, 5-FU, CDHP and Oxo on day 1 and day 8 in patients with HCC who received dose level 2

		C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}12}$ (ng h/mL)	$T_{1/2}$ (h)
FT	Day 1	2032 ± 437	3.3 ± 1.0	17070 ± 5139	10.1 ± 2.8
	Day 8	4365 ± 1712	3.7 ± 0.8	42399 ± 18137	12.7 ± 5.0
5-FU	Day 1	114.5 ± 35.5	4.3 ± 0.8	695.3 ± 223.6	2.3 ± 1.0
	Day 8	145.8 ± 31.4	4.3 ± 0.8	936.6 ± 292.3	2.4 ± 1.0
CDHP	Day 1	267.2 ± 76.8	3.3 ± 1.0	1424.8 ± 414.2	3.3 ± 0.9
	Day 8	281.0 ± 113.8	3.3 ± 1.0	1694.4 ± 603.5	3.4 ± 0.9
Oxo	Day 1	38.5 ± 1.8	3.7 ± 0.8	231.6 ± 69.8	4.0 ± 2.1
	Day 8	33.4 ± 9.5	4.0 ± 0.0	241.5 ± 115.6	4.0 ± 2.0

Parameters are represented as mean ± SD. CDHP, 5-chloro-2,4-dihydroxypyridine; 5-FU, 5-fluorouracil; FT, tegafur; Oxo, oteracil potassium.

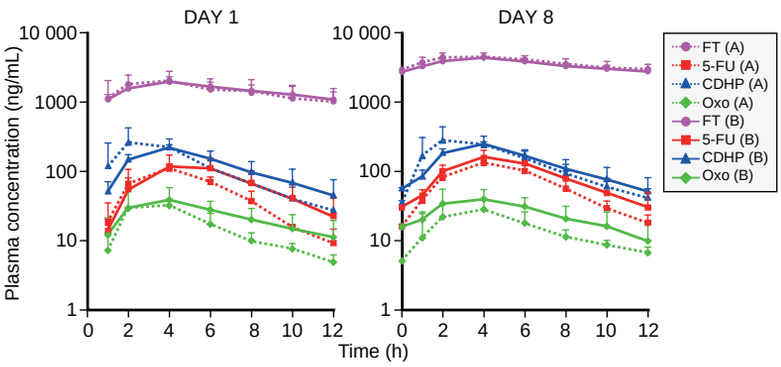


Fig. 2. Plasma-concentration–time profiles of tegafur (FT), 5-fluorouracil (5-FU), 5-chloro-2,4-dihydroxypyridine (CDHP) and oteracil potassium (Oxo) on day 1 and day 8 were similar in patients with Child-Pugh class A (n = 3) and those with Child-Pugh class B (n = 3).

confirmed. The duration of the five responses was 42, 147, 188, 238 and 371 days, respectively.

The median PFS was 3.7 months (95% CI, 2.5–5.1 months). The disease control rates at 6, 12 and 24 weeks were 47.8% (95% CI, 26.8–69.4%), 26.1% (95% CI, 10.2–48.4%) and 21.7% (95% CI, 7.5–43.7%), respectively. The PFS and OS are shown in Figure 1. The median OS was 16.6 months (95% CI, 14.0–24.5 months). Survival rates were 69.6% (95% CI, 50.8–88.4%) at 1 year and 43.0% (95% CI, 22.6–63.5%) at 1.5 years.

Pharmacokinetic analysis. Table 4 shows the pharmacokinetic data for the components of S-1 and 5-FU at level 2 on days 1 and 8. Compared with day 1, the C_{max} and $AUC_{0\text{-}12}$ of FT increased markedly on day 8; however, these increases were within the expected range given the slow elimination of FT, and repeated administration of S-1 had no effect on the T_{max} or $T_{1/2}$ of FT. There was no evidence of accumulation of 5-FU, CDHP or Oxo on day 8.

Figure 2 compares the plasma-concentration–time profiles of S-1 components and 5-FU between patients with Child-Pugh class A and those with Child-Pugh class B on days 1 and 8. The plasma-concentration–time profiles of FT, 5-FU, CDHP and Oxo were similar in patients with Child-Pugh class A and those with Child-Pugh class B on both days.

Discussion

There has been no established standard therapy for patients with advanced HCC refractory to surgery, transplantation, local ablation and TACE.[13,14] Some cytotoxic regimens have produced encouraging response rates, but survival benefits have been minimal compared with control groups, at the cost of clinically unacceptable adverse effects.[1,15]

S-1 is an anticancer drug consisting of FT, CDHP and Oxo. The conversion of FT to 5-FU is mediated mainly by hepatic cytochrome CYP2A6.[16] 5-FU is rapidly metabolized by DPD in the liver after the intravenous administration of 5-FU alone, but S-1, which includes a DPD inhibitor (i.e. CDHP), produces prolonged, effective concentrations of 5-FU in the blood. Thus, the liver plays an important role in the metabolism of FT.

The RD of S-1 in patients with HCC was estimated to be 80 mg/m^2 per day in phase I, which is similar to the dose recommended for the treatment of other solid tumors. However, in patients with HCC, Ueno *et al.*[10] reported that the DLT of 5-FU administered as a 5-day continuous infusion was stomatitis. Moreover, the MTD was equivalent to approximately 50% of that of 5-FU in patients with normal organ function,[10] suggesting that 5-FU-related gastrointestinal toxicity was reduced by Oxo in the formulation of S-1. We did not determine the MTD in this study because S-1 was approved for the treatment of other cancers. The pharmacokinetic properties of S-1 components and 5-FU in patients with HCC were similar to those in patients with pancreatic cancer or biliary tract cancer.[17,18]

Hematological toxic effects and symptomatic events such as pigmentation (87.0%), fatigue (82.6%), anorexia (78.3%) and ascites (30.4%) were more common than previously reported for S-1 in patients with other cancers. Nonetheless, severe toxic effects were comparable among patients with HCC and those with other cancers. Nonhematological toxic effects related to hepatic function were also more frequent than reported previously for S-1 in patients with other types of cancer, but such effects may have been caused by differences in underlying liver disease.

The pharmacokinetics of S-1 did not obviously differ between patients with Child-Pugh class A and those with Child-Pugh class B, suggesting that hepatic dysfunction associated with Child-Pugh class B did not affect the pharmacokinetics of S-1 components or 5-FU. The sample size of the pharmacokinetic evaluations was small because the primary end-point was to determine the RD as the evaluation of DLT in phase I. At dose level 2, DLT occurred in two patients with Child-Pugh class B (Grade 3 anorexia in one, and a Grade 2 rash requiring 8 or more consecutive days of rest in the other). There was no DLT at level 1 (given only to patients with Child-Pugh class A). However, the patient who had DLT of grade 3 anorexia had renal dysfunction at baseline, and the plasma 5-FU concentrations in this patient on day 8 were higher than those in other patients, perhaps contributing to the development of DLT (data not shown). In addition, there were no obvious differences in the incidence or grade of drug-related adverse events between patients with Child-Pugh class A and those with Child-Pugh class B, consistent with the results of pharmacokinetic analysis. These results suggested that there were no clinically meaningful differences in pharmacokinetics or safety according to Child-Pugh class or between patients with HCC and those with other cancers, and that S-1 was well tolerated in patients with HCC, similar to patients with other cancers. However, our study had several limitations: only a very small number of patients with Child-Pugh class B were included; among the patients with Child-Pugh class B, the score was heterogeneous, ranging from 7 to 9; and only patients with better scores were studied. Therefore, extra care should be taken when S-1 is given to patients with Child-Pugh class B.

As for efficacy, five of 23 patients had partial responses at dose level 2. Compared with previously reported response rates obtained with single-agent chemotherapy in patients with HCC, our results are good. In particular, the median OS appeared to be longer than that obtained with other agents in non-Japanese studies. The reason for the better OS in Japanese patients might be similar to that previously reported for sorafenib.[4] The median OS in our study was similar to that in a Japanese phase I study of sorafenib.[4] In studies of sorafenib in non-Japanese and

doi: 10.1111/j.1349-7006.2010.01730.x

Japanese patients with HCC, the median TTP and response rates were comparable, but the median OS was 15.6 months in Japanese patients compared with only 9.2 months in non-Japanese patients.[4] Differences in various treatments, including hepatic arterial infusion chemotherapy, and the palliative care of patients with progressive disease who had conditions such as hepatic decompression and variceal bleeding might be related to the longer survival time in Japanese rather than non-Japanese patients with HCC.

In conclusion, our results suggested that S-1 is effective and has an acceptable toxicity profile in patients with advanced HCC. Nonetheless, S-1 should be used with caution in the presence of liver dysfunction. Sorafenib has been established to be a standard treatment for advanced HCC. Perhaps, systemic chemotherapy with S-1 plus molecular-targeted therapies such as sorafenib will further improve survival in patients with advanced HCC or monotherapy with S-1 will be useful as a second-line regimen for chemotherapy.

Acknowledgments

We thank Drs T. Taguchi, M. Kurihara, K. Tanaka and K. Aiba for their kind advice, and Drs N. Moriyama, J. Tanaka and W. Koizumi for their extramural review. The authors are indebted to Peter Star of Medical Network K.K., Tokyo, Japan for his review of this manuscript. This study was supported by Taiho Pharmaceutical Co., Ltd.

Disclosure Statement

J. Furuse received honoraria for lecture fees from Taiho Pharmaceutical; T. Okusaka, S. Kaneko, M. Kudo, K. Nakachi, H. Ueno, T. Yamashita and K. Ueshima have no conflict of interest.

References

1 Zhu AX. Systemic therapy of advanced hepatocellular carcinoma: how hopeful should we be? *Oncologist* 2006; **11**: 790–800.
2 Cheng AL, Kang YK, Chen Z *et al.* Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25–34.
3 Llovet JM, Ricci S, Mazzaferro V *et al.* Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378–90.
4 Furuse J, Ishii H, Nakachi K, Suzuki E, Shimizu S, Nakajima K. Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. *Cancer Sci* 2008; **99**: 159–65.
5 Shirasaka T, Shimamato Y, Ohshimo H *et al.* Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. *Anticancer Drugs* 1996; **7**: 548–57.
6 Tatsumi K, Fukushima M, Shirasaka T, Fujii S. Inhibitory effects of pyrimidine, barbituric acid and pyridine derivatives on 5-fluorouracil degradation in rat liver extracts. *Jpn J Cancer Res* 1987; **78**: 748–55.
7 Shirasaka T, Shimamoto Y, Fukushima M. Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. *Cancer Res* 1993; **53**: 4004–9.
8 Shirasaka T. Development history and concept of an oral anticancer agent S-1 (TS-1): its clinical usefulness and future vistas. *Jpn J Clin Oncol* 2009; **39**: 2–15.
9 Yamashita T, Kaneko S, Furuse J, *et al. Experimental and Early Clinical Studies of S-1, a Novel Oral DPD Inhibitor, Chemotherapy for Advanced Hepatocellular Carcinoma*. San Francisco: The American Association for the Study of Liver Diseases, 2008; Publication Number 1442.
10 Ueno H, Okada S, Okusaka T, Ikeda M, Kuriyama H. Phase I and pharmacokinetic study of 5-fluorouracil administered by 5-day continuous infusion in patients with hepatocellular carcinoma. *Cancer Chemother Pharmacol* 2002; **49**: 155–60.
11 Matsushima E, Yoshida K, Kitamura R, Yoshida K. Determination of S-1 (combined drug of tegafur, 5-chloro-2,4-dihydroxypyridine and potassium oxonate) and 5-fluorouracil in human plasma and urine using high-performance liquid chromatography and gas chromatography-negative ion chemical ionization mass spectrometry. *J Chromatogr B Biomed Sci* 1997; **691**: 95–104.
12 Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
13 Couto OF, Dvorchik I, Carr BI. Causes of death in patients with unresectable hepatocellular carcinoma. *Dig Dis Sci* 2007; **52**: 3285–9.
14 Ng KK, Poon RT, Lo CM, Yuen J, Tso WK, Fan ST. Analysis of recurrence pattern and its influence on survival outcome after radiofrequency ablation of hepatocellular carcinoma. *J Gastrointest Surg* 2008; **12**: 183–91.
15 Thomas M. Molecular targeted therapy for hepatocellular carcinoma. *J Gastroenterol* 2009; **44**: 136–41.
16 Ikeda K, Yoshisue K, Matsushima E *et al.* Bioactivation of tegafur to 5-fluorouracil is catalyzed by cytochrome P-450 2A6 in human liver microsomes in vitro. *Clin Cancer Res* 2000; **6**: 4409–15.
17 Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C. Phase II study of S-1 in patients with advanced biliary tract cancer. *Br J Cancer* 2004; **91**: 1769–74.
18 Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C. An early phase II study of S-1 in patients with metastatic pancreatic cancer. *Oncology* 2005; **68**: 171–8.

World Journal of Radiology

Online Submissions: http://www.wjgnet.com/1949-8470office
wjr@wjgnet.com
doi:10.4329/wjr.v2.i8.289

World J Radiol 2010 August 28; 2(8): 289-297
ISSN 1949-8470 (online)

EDITORIAL

Diagnosis of subepithelial tumors in the upper gastrointestinal tract by endoscopic ultrasonography

Hiroki Sakamoto, Masayuki Kitano, Masatoshi Kudo

Hiroki Sakamoto, Masayuki Kitano, Masatoshi Kudo, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, Osaka-Sayama 589-8511, Japan
Author contributions: Sakamoto H wrote this manuscript; Kitano M and Kudo M revised the manuscript.
Supported by The Japan Society for Promotion of Science, Research and Development Committee Program of The Japan Society of Ultrasonics in Medicine, Japan Research Foundation for Clinical Pharmacology, and Japanese Foundation for Research and Promotion of Endoscopy
Correspondence to: Hiroki Sakamoto, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kinki University School of Medicine, 377-2, Ohno-Higashi, Osaka-Sayama 589-8511, Japan. hiroki.sakamoto@nifty.com
Telephone: +81-72-3660221 Fax: +81-72-3672880
Received: June 11, 2010 Revised: July 29, 2010
Accepted: August 5, 2010
Published online: August 28, 2010

Abstract

Endoscopic ultrasonography (EUS) is the most accurate procedure for detecting and diagnosing subepithelial tumors, due to its higher sensitivity and specificity than other imaging modalities. EUS can characterize lesions by providing information on echogenic origin, size, borders, homogeneity, and the presence of echogenic or anechoic foci. Linear echoendoscopes, and recently also electronic radial echoendoscopes, can be used with color Doppler or power Doppler to assess the vascular signals from subepithelial masses, and thus permit the differentiation of vascular structures from cysts, as well as the assessment of the tumor blood supply. However, the diagnostic accuracy of EUS imaging alone has been shown to be low in subepithelial lesions with 3rd and 4th layers. It is also difficult to differentiate exactly between benign and malignant tumors and to gain an accurate picture of histology using EUS. On the other hands, EUS guided fine needle aspiration (EUS-FNA) can provide samples for cytologic or histologic analysis. Hypoechoic lesions of the 3rd and the 4th EUS layers, more than in 1 cm diameter are recommended, and histologic confirmation using endoscopic submucosal resection or EUS-FNA should be obtained when possible. Therefore, EUS-FNA plays an important role in the clinical management of subepithelial tumors. Furthermore improvements in endoscopic technology are expected to be more useful modalities in differential diagnosis and discrimination between benign and malignant subepithelial tumors.

Key words: Endoscopic ultrasonography; Submucosal tumor; Subepithelial tumor

Peer reviewers: Alain Chapel, PhD, Institut de Radioprotection et de S reté Nucléaire, DPHD, IRSN. B.P. no 17, F-92262 Fontenay-Aux-Roses, France; Antonio Pinto, MD, PhD, Department of Radiology, Cardarelli Hospital, Via Posillipo 168/D, I-80123, Naples, Italy

Sakamoto H, Kitano M, Kudo M. Diagnosis of subepithelial tumors in the upper gastrointestinal tract by endoscopic ultrasonography. *World J Radiol* 2010; 2(8): 289-297 Available from: URL: http://www.wjgnet.com/1949-8470/full/v2/i8/289.htm DOI: http://dx.doi.org/10.4329/wjr.v2.i8.289

INTRODUCTION

Submucosal masses or lesions often referred to as 'submucosal tumors', represent a growth underneath the mucosa of the gastrointestinal (GI) tract whose etiology cannot be determined by GI endoscopy or barium studies[1]. However, the term 'submucosal tumor' is inappropriate, because many of these lesions do not arise from the submucosa and many of them are not tumors[2-5]. Thus, 'subepithelial' is a more appropriate term than 'submucosal'. Hence, other authors call these abnormalities subepithelial lesions, because they are covered by normal mucosa[6]. These can

be caused by external compression by the neighboring organs or by intramural lesions. However, submucosal is still recognized and used.

The majority of subepithelial tumors do not cause symptoms and are discovered incidentally during endoscopic or radiologic examinations. The overlying mucosa usually appears smooth and normal at endoscopy. If symptoms do occur, they are nonspecific such as abdominal pain, obstruction, hemorrhage and intussusceptions[7,8]. Large submucosal neoplasms may outgrow their blood supply, ulcerate through the mucosa, and present as GI bleeding. Firm subepithelial tumors may also present with obstructive symptoms, especially if they are located near the cardia or the pylorus. Subepithelial tumors obstructing the major or minor papilla may cause jaundice or pancreatitis. Pain and weight loss, often associated with large submucosal GI stromal tumors (GISTs), are symptoms that suggest malignancy[7,9].

Endoscopic ultrasonography (EUS) is the most sensitive imaging procedure for the characterization of subepithelial tumors and it can also diagnose them, especially small ones[10-14]. Linear echoendoscopes and electronic radial echoendoscopes can be used with color Doppler or power Doppler to assess the vascular signals from subepithelial masses, and thus permit the differentiation of vascular structures from cysts, as well as the assessment of the tumor blood supply[11,12,15]. Furthermore, Catheter US (miniprobes), if available, may be particularly useful for evaluating subepithelial tumors because they permit sonographic examination of the tumor while the patient is having a diagnostic endoscopy[16,17]. In addition to being convenient, catheter-type US probes are particularly useful for imaging small subepithelial tumors that are difficult to identify with dedicated echoendoscopes. They are also useful in imaging subepithelial tumors in the colon[7], however, miniprobes are not useful if the subepithelial lesions are over 2 cm in diameter because of the limited penetrating depths. Therefore, EUS is performed as the second intervention following standard endoscopy[14]. On the other hand, it is difficult to differentiate exactly between benign and malignant tumors and to gain an accurate picture of histology using EUS. EUS guided fine needle aspiration (EUS-FNA) can be used to provide samples for cytologic or histologic analysis. Therefore, EUS-FNA plays an important role in the clinical management of subepithelial tumors. This review will focus on EUS appearances of common subepithelial GI tract tumors, the diagnostic accuracy of EUS-FNA, and surveillance by EUS, highlighting their relative advantages and their complementary roles in clinical practice.

EUS IMAGING

Optimal imaging of subepithelial lesions requires submersion under water, which sometimes requires repositioning of the patient after the GI lumen has been filled with water. Endosonographically, the wall of the GI tract consists of 5 layers of alternating echogenicity (Figure 1). The 1st layer is hyperechoic and represents the superficial layer of the mucosa. The 2nd layer is hypoechoic and constitutes of the deep layer of the mucosa, including the muscularis mucosa. The 3rd, hyperechoic layer is the submucosa, the 4th hypoechoic the muscularis propria and the 5th hyperechoic is the serosa/adventitia[18]. For subepithelial tumors that are intrinsic to the GI wall, it is important to characterize the layer(s) of origin or involvement, the echogenicity of the tumor, the smoothness of the border and any internal feature (Table 1). Inflation of the balloon covering the transducer with water may improve the ultrasonic contact. However, this may compress the GI tract wall and distort the EUS image. This is the reason why the esophagus and duodenum are sometimes visualized with only three layers, with the first hyperechoic layer corresponding to the balloon-mucosa-submucosa together with the submucosa-muscularis-propria interface.

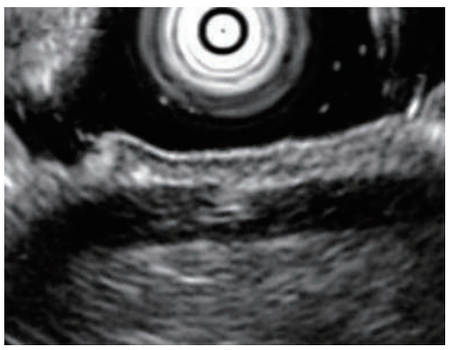


Figure 1 Normal structure of the gastric wall with five endoscopic ultrasonography layers present.

Extrinsic compressions

An enlarged left atrium, left hepatic lobe, and spleen may commonly masquerade as a subepithelial tumor of the esophagus and stomach during endoscopy[19-21]. A recent international multicenter study reported that the sensitivity and the specificity of extramural compression with endoscopy alone were 87% and 29%, respectively[2]. The EUS characterization of these organs is useful in the evaluation of extraluminal organs which compress the GI tract lumen, 100% accurate for the differential diagnosis and superior to transabdominal ultrasound or CT scans (Figure 2). Pancreatic pseudocysts or tumors can also be identified when assessing subepithelial tumors by EUS.

Varices

Occasionally, large gastric varices may be polypoid[3-5,22]. EUS imaging of gastric varices demonstrates characteristic anechoic serpiginous structures in the third hyperechoic layer. Flow within the varix can be demonstrated by Doppler examination.

Lipomas

Lipomas are generally soft, exhibiting a pillow sign when

Table 1 Endoscopic ultrasonography feature of subepithelial tumors

	EUS layer	Organ	EUS appearance
Varices	3rd	Fundus	Anechoic
Lipomas	3rd	Stomach, duodenum, rectum	Hyperechoic, smooth margins
Ectopic pancreas	3rd, 4th (2nd, 5th)	Antrum	Hypoechoic, heterogeneous (possible ductal structure)
Cysts	3rd	Esophagus, stomach, duodenum	Anechoic, compressible, round or oval (3rd or 5th layer are suggestive of duplication cyst)
Inflammatory fibroid polyp	2nd	Antrum, duodenum	Polypoid, hypoechoic, covered by a thin mucosa
Granular cell tumor	2nd, 3rd, 4th	Esophagus	Hypoechoic, oval, heterogeneous,
Leiomyoma	4th (2nd)	Esophagus, cardia	Hypoechoic, round or oval, well demarcated
Schwannoma	4th (3rd)	Stomach	Hypoechoic, round or oval, well demarcated
Gastrointestinal stromal tumor	4th (2nd, 3rd, 5th)	Stomach, small intestine	Hypoechoic, round (large tumors > 4 cm, homogeneous, irregular border, cystic areas of echogenic foci: borderline or malignant)
Leiomyosarcoma	2nd, 4th	Esophagus, stomach	Hypoechoic, heterogeneous, irregular extraluminal border or invasiveness of the neighbouring organs
Carcinod	2nd, 3rd	Fundus, rectum	Hypoechoic
Lymphoma	2nd, 3rd, 4th	Stomach	Hypoechoic
Metastases	1st-5th or all	All	Hypoechoic, heterogeneous, irregular margin

EUS: Endoscopic ultrasonography.

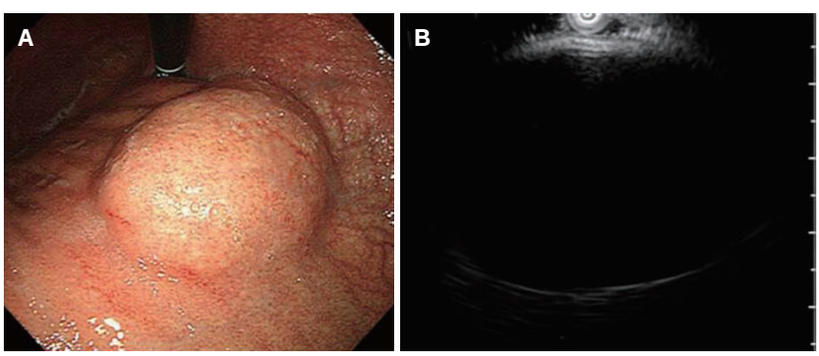


Figure 2 Endoscopic and endoscopic ultrasonography finding of extrinsic compression. A: Endoscopic view of subepithelial lesion of the gastric angle; B: Endoscopic ultrasonography shows an extramural compression by a liver cyst.

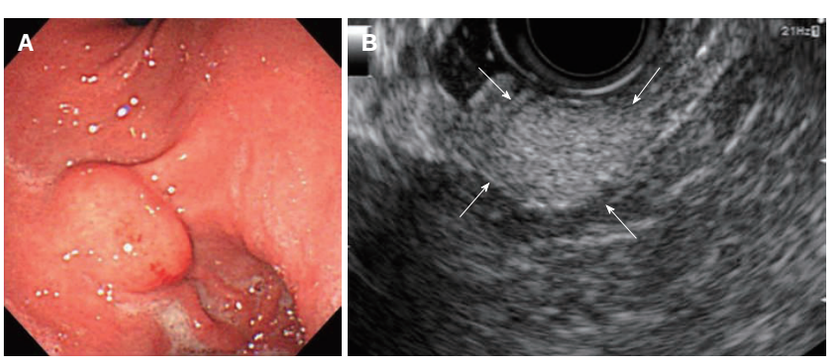


Figure 3 Endoscopic and endoscopic ultrasonography finding of lipomas. A: Endoscopic view of 1.5 cm subepithelial lesion of the anterior part of the gastric angle; B: Endoscopic ultrasonography shows an typical aspect of an 1.6 cm lipoma of the gastric angle (arrows).

probed, and have a yellowish hue. EUS demonstrates lipomas as hyperechoic, homogeneous, well-circumscribed ovoid masses in the 3rd layer (Figure 3)[3-5].

Cysts/duplication cyst

Cysts typically appear as round or ovoid, smooth anechoic compressible structures located within the 3rd layer. The wall of the duplication cyst may appear as a three or a five layer structure[23,24].

Ectopic pancreas

Ectopic pancreas, also called heterotopic or aberrant pan-

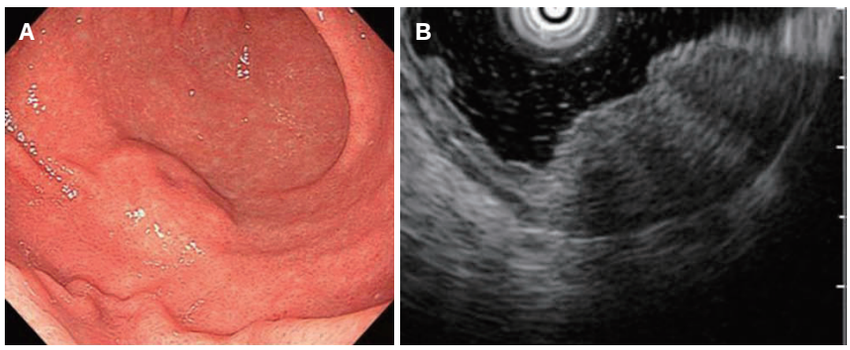


Figure 4 Endoscopic and endoscopic ultrasonography finding of ectopic pancreas. A: Endoscopic view of a subepithelial lesion of the greater curvature of the gastric antrum, covered with normal mucosa, with a central depression; B: Endoscopic ultrasonography shows indistinct margin, hypoechoic tumor developed within the 4th layer.

creas, is defined as pancreatic tissue lying outside its normal location and lacking anatomic or vascular connection with the pancreas. Ectopic pancreas, which usually does not cause symptoms, is found incidentally in the stomach, duodenum, and small intestine. Gastric lesions are discovered in the antrum in 85%-90%, either on the posterior or anterior wall, being more common along the greater curvature. The frequency of ectopic pancreas has been estimated as 1 case per 500 explorations of the upper abdomen or 0.6% to 13.7% of autopsies. The endoscopic appearance of a pancreatic rest is usually that of a firm, slightly irregular nodule in the stomach or elsewhere in the GI tract (Figure 4A). The mucosa over the nodule may have a central depression or dimpling, and ducts may empty into the lumen at this site. Usually, the characteristic EUS demonstrates an indistinct margin, hypoechoic or mixed echogenicity, a heterogeneous lesion, and most locations are within either the 3rd or 4th layers or only in the 3rd layer (Figure 4B)[3-5].

Granular cell tumor

Granular cell tumors are benign neoplasms. Typically they are located in the distal part of the esophagus with a yellowish appearance; EUS demonstrates a heterogeneous mass with smooth borders located in the 3rd layer[25,26].

Submucosa cancer/metastases

Subepithelial primary carcinoma, lymphoma or metastases may rarely involve the submucosa. EUS show a hypoechoic, heterogeneous lesion in any or all of the EUS layers[1,7]. The most frequent primary tumors that result in GI metastases are breast cancer, melanoma and lung cancer[18].

Gastric inflammatory fibroid polyp

Inflammatory fibroid polyp (IFP) appears as a 2 cm almost-pedunculated polyp on the antrum when analysed using endoscopy. The polyp is covered mostly by normal mucosa, with whitish exudates. The appearance of IFPs on EUS is characterized by an indistinct margin, hypoechoic homogeneous lesion and location within the 2nd and/or 3rd layer with an intact 4th layer[27].

Mesenchymal tumor

Mesenchymal tumors of the GI tract are classified in three type tumors, GIST, leiomyoma, and schwannoma. Pathologically, most of these tumors are completely or partly composed of spindle cells and have a light microscopic appearance suggestive of smooth muscle or nerve sheath differentiation. These tumors therefore have been presumed to be of smooth muscle origin and often labeled as leiomyomatous or Schwann cell tumors[28,29]. In recent years, with the advance of immunohistochemical[30,31] and ultrastructural[32] studies, it has been shown that most gastric and small intestinal mesenchymal tumors are neither leiomyoma nor schwannoma but GIST derived from the interstitial cells of Caja. GISTs are the most common GI mesenchymal tumors, now defined as KIT-positive mesenchymal tumors. Leiomyoma tumors demonstrate α-smooth muscle actin, desmin protein on immunohistochemistry, but not KIT expression. Schwannoma tumors demonstrate S100 protein on immunohistochemistry, but not KIT expression[30-32].

Leiomyoma

Leiomyomas are benign tumors without malignant potential which arise from the muscularis mucosa or the muscularis propria. They are found in the esophagus, but are rare in the stomach and small intestine. EUS demonstrates a hypoechoic, well-circumscribed, homogeneous lesion, developed in the 2nd or 4th layer (Figure 5A).

Schwannoma

The GI schwannoma to GISTs (the most frequent GI SMTs) ratio is approximately 1:50-100[33]. Therefore, GI schwannomas are rare. The schwannoma appearance is similar to that of leiomyoma or GISTs (Figure 5B)[34-36].

GIST

GISTs occur most frequently in the stomach (65%) and in the small bowel (25%), rarely in the rectum and the colon. They are exceptional in the esophagus (1%)[1,7,33-36]. Approximately 10%-30% of GISTs are clinically malignant, although the fact that all GISTs are considered to have some degree of malignant potential should be kept

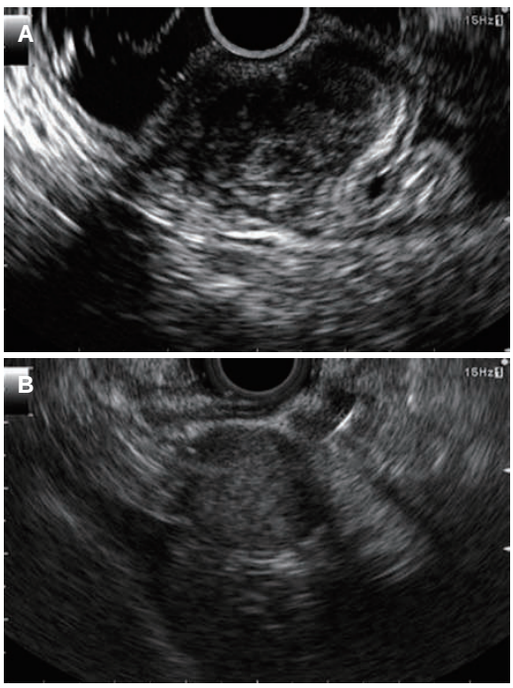


Figure 5 Hypoechoic tumor developed within the 4th layer. A: Leiomyoma of the esophagus (30 mm); B: Schwannoma of the stomach (22 mm).

in mind. GISTs in the small intestine are more aggressive that those located in the stomach[37]. EUS demonstrates a hypoechoic tumor contiguous with the 4th layer and well-delineated lesion (Figure 6). However recent reports also indicate the presence of GISTs in the 3rd layer[1-7,34] contiguous with the muscularis mucosa[38-41].

Differentiation between leiomyomas, schwannomas and GISTs is extremely difficult by imaging modalities, even EUS. Recently, Okai *et al*[42] tried to differentiate between 19 GISTs, 3 leiomyomas, and 2 schwannomas by EUS. A complete or incomplete marginal hypoechoic halo was found in more than half of the patients with GISTs and schwannomas, whereas a distinct marginal halo was not seen in leiomyomas. It was also demonstrated that the echogenicities of GISTs were generally low but slightly higher than that of the normal surrounding proper muscle layer, whereas the level of leiomyomas was nearly equal to that of the surrounding normal proper muscle layer and that of schwannoma was extremely low. Accordingly, the difference in echogenicities among the three mesenchymal tumors might reflect the pathologic differences of cellularity and structural components of the tumor. Although the number of patients enrolled in their study was too small to make a comparison, these EUS findings may be helpful for differentiation between these gastric mesenchymal tumors.

DIFFERENTIAL DIAGNOSIS

We have described the EUS appearance of each subepithelial tumor. Determination of the histologic layer and the echotexture of the lesion can significantly narrow the

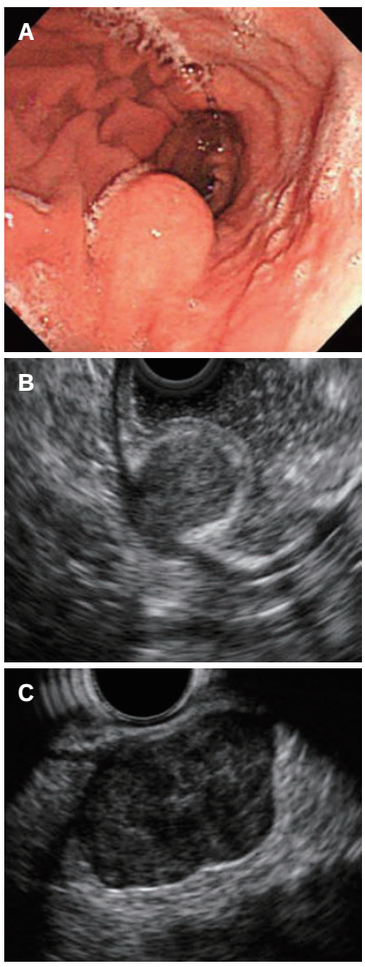


Figure 6 Endoscopic and endoscopic ultrasonography finding of gastrointestinal stromal tumor. A: Endoscopic view of subepithelial lesion of the posterior side of the greater curvature of the gastric body; B: Endoscopic ultrasonography shows hypoechoic, homogeneous 2 cm tumor developed within the 4th layer (low risk gastrointestinal stromal tumor of the stomach); C: 35 mm hypoechoic, heterogeneous, lobulated submucosal lesion with exogastric growth developed within the 4th layer (high risk gastrointestinal stromal tumor of the stomach).

differential diagnosis. However, the differential diagnosis of a hypoechoic 4th layer lesion is broad and includes benign, premalignant, and malignant lesions[43]. EUS performs better than other modalities in evaluating GI subepithelial lesion, but the diagnostic accuracy of EUS imaging alone has been shown to be as low as 43% in subepithelial lesions with 3rd and 4th layers[2]. Hwang *et al*[2] prospectively evaluated the performance characteristics of EUS in the diagnosis of GI subepithelial masses. Most incorrect EUS diagnoses occurred with hypoechoic 3rd and 4th layer masses with two of the cases demonstrating malignancies. One case was an invasive squamous cell carcinoma invading the esophagus that on EUS coincided with the 4th EUS layers and was hypoechoic with internal hyperechoic foci, and had an irregular appearing margin. The 2nd case was a gastric adenocarcinoma with EUS demonstrating the lesion coincided with the 3rd EUS layers and was

hypoechoic with internal hyperechoic foci, with smooth margins. Therefore, hypoechoic lesions of the 3rd and the 4th EUS layer were considered. Histologic confirmation by using endoscopic submucosal resection or EUS-FNA should be obtained when possible.

DIFFERENTIAL DIAGNOSIS BETWEEN BENIGN AND MALIGNANT TUMORS

In 1992, Rösch *et al*[10] compared the EUS features of benign with malignant tumors in SMT of the upper GI tract, and concluded there was no single reliable criterion that would enable a differential diagnosis. However, they proposed larger, echo-inhomogeneous masses with irregular outer borders are suggestive of malignancy whereas smaller (< 3 cm) echo-homogeneous subepithelial tumors with a smooth margin are likely to be benign. Chak *et al*[16] found that features predictive of malignant subepithelial tumors were diameter > 4 cm, irregular extraluminal border, echogenic foci, and cystic space. When the presence of at least two of the following three features were used as malignancy determinants, sensitivity ranged from 80% to 100%, depending on the endosonographer. Recently, it has been considered that subepithelial tumors are mostly gastric GISTs, and there are some reports that assess EUS characteristics for predicting the malignant potential of GISTs[44]. Tumor size (more than 3 to 5 cm depending on the study) was the most the important. The predictive value of other features, such as irregular borders, echogenic foci, cystic spaces, ulcerated mucosae, lymph nodes and exogastric growths with malignant pattern, is unclear (Figure 6C)[1,7,34-36,42]. However, those studies are retrospective and included small numbers of tumor samples, thus somewhat conflicting results that have not been validated in prospective series have been obtained. Therefore, larger study numbers and prospective multicenter studies are needed.

With the use of EUS, subepithelial lesions can be further characterized by demonstrating the location of the mass, size, and echogenicity[8,20,21]. Furthermore, if a lesion is intramural, EUS can demonstrate the histologic layer of origin within the GI wall. Determination of the histologic layer and the echotexture of the lesions can significantly narrow the differential diagnosis and may be diagnostic in some cases.

In addition, studies have shown interobserver agreement to be poor, and the diagnostic accuracy to depend heavily on the experience of the endosonographer[45].

EUS-FNA

EUS-FNA is a safe and effective technique for obtaining samples for cytologic or histologic examinations either as a primary procedure or in cases where biopsy techniques have failed (Figure 7). Williams *et al*[46] reported that the overall sensitivity, specificity and accuracy of EUS-FNA for the diagnosis of malignancy were 85%, 100% and 89%, respectively, for lymph nodes; 82%, 100%, and 85%, respectively, for pancreatic lesions; 88%, 100%, and 90%, respectively, for perirectal masses; and 50%, 25%, and 38%, respectively, for intramural lesions. They suggested that when providing accurate diagnosis of pancreatic and perirectal malignancies, the technique is less useful for intramural lesions. Similarly, Wiersema *et al*[47] reported that EUS-FNA sensitivity, specificity, and accuracy were 92%, 93%, and 92%, respectively, for lymph nodes, 88%, 95%, and 90%, respectively, for extraluminal masses, and 61%, 79%, and 67%, respectively, for GI wall lesions. Therefore, from those previous reports, EUS-FNA for subepithelial tumors has not had high reliability and sufficient diagnostic accuracy. Recently, there are some reports that the diagnostic yield of EUS-FNA depends on site, size and characteristics of the tumor as well as technical and procedural factors (type of needle, biopsy technique and material processing). Other weighting factors include expertise, training and interaction between the endosonographer and cytopathologist[41,44]. Another factor that appears to affect the accuracy of EUS-FNA is the presence of an on-site pathologist since, in most studies that reported high levels of EUS-FNA diagnostic accuracy, a cytopathologist was present during the procedure to ensure that adequate cytological specimens were obtained[48,49]. When a cytopathologist is present during EUS-FNA, it appears that the diagnostic yield increases by 10%[50,51]. Vander Noot *et al*[52] reported that the sensitivity, specificity, and diagnostic accuracy of EUS-FNA on-site cytological evaluation during FNA procedure in diagnosing GI tract neoplastic lesions were 89%, 88%, and 89%, respectively. When specimens with suspicious cytologic diagnoses were classified as being positive for malignancy the sensitivity and specificity became 96% and 81%, respectively, and the diagnostic accuracy improved to 92%. It is noteworthy that the results of this study were better than those reported in the literature. They suggested that one possible explanation is a cytopathologist is always present on site to assess specimen adequacy and to determine whether additional material should be obtained for ancillary studies, such as flow cytometric and immunocytochemical analyses. Klapman *et al*[53] observed that an EUS center with on-site cytologic

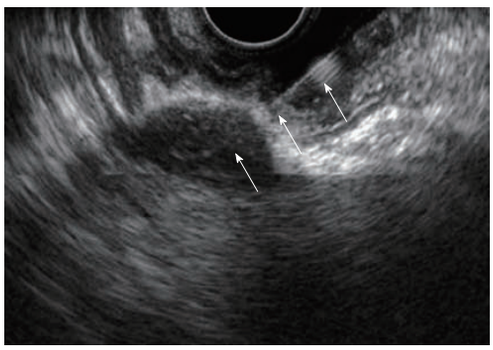


Figure 7 Endoscopic ultrasonography-guided fine needle aspiration of a 20 mm hypoechoic subepithelial tumor of the stomach, using a 25-gauge (arrows).

interpretation had significantly lower rates of unsatisfactory specimens and a higher rate of positive or negative cytologic diagnoses for malignancy compared with an EUS center without on-site cytologic interpretation. False-positive diagnosis of malignancy in EUS-guided biopsy is also rare. Jenssen *et al*[54] reported that the high prognostic and therapeutic relevance of the cytopathological diagnoses resulting from EUS-guided biopsy calls for a shared responsibility of an endosonographer and a cytologist.

For EUS-guided biopsy predictors of malignancy GIST, several factors have been studied in an effort to provide preoperative cytologic risk assessment. Ando *et al*[55] reported that the presence of mitoses in specimens collected by fine-needle aspiration was associated with malignant GISTs. However, mitoses are seldom seen on smears. The same study also found that a high Ki-67 labeling index, a protein marker of cell proliferation, was significantly associated with malignant lesion. Okubo *et al*[56] reported that the presence of an MIB-1 labeling index of more than 5% indicated a high-grade malignancy, with a diagnostic accuracy of 85.7%. KIT and PDGFRA mutation analysis has been proven possible using EUS-guided cell block specimens[57-59]. As KIT mutation analysis has prognostic importance and can be predictive of response to treatment[60-63], its preoperative determination may help to guide the approach to treatment in locally advanced and metastatic disease. The clinical role of such testing is currently being investigated.

EUS-FNA is a safe and precise non-invasive procedure for the diagnosis of subepithelial upper GI tract tumors. Furthermore, utilization of sampling material by EUS-FNA has been expected to improve treatment and management in clinical practice. However, recently, two cases of tumor seeding after percutaneous biopsy for malignant GIST were reported[48,49]. Although there have been no reports of seeding after EUS-FNA for malignant subepithelial tumors, obtaining samples by EUS-FNA from small tumors and from tumors with exogastric growth may result in high peritoneal seeding risk because the FNA needle may easily penetrate not only the tumor but also the whole gastric wall, reaching the peritoneal side and seeding tumor cells along the way. Therefore, during sampling by EUS-FNA in such cases we must play attention to the needle in order not to penetrate the tumor.

SURVEILLANCE BY EUS

For management of subepithelial tumors, EUS is recommended for subepithelial tumors more than 1 cm in diameter, and histologic evaluation, such as EUS-FNA, is recommended for hypoechoic subepithelial tumors less than 3 cm in diameter. Surgery is recommended for subepithelial tumors more than 3 cm in diameter[64]. Although these procedures are helpful in a categorizing a lesion, they cannot absolutely determine the type of lesion or determine if a lesion is benign or malignant[7]. The American Gastroenterological Association recommends periodic endoscopic or endosonographic follow-up or surgical resection for small (less than 3 cm), hypoechoic, 3rd and 4th layer masses, which are most likely GISTs[22]. GISTs are most commonly identified intramural subepithelial tumors in the upper GI tract[7]. Small GISTs (less than 2 cm) have very low malignant potential according to the classification system proposed by the National Institutes of Health Consensus Conference[65]. The recommended duration of follow-up is very variable. Hwang *et al*[43] suggested a 1 year follow-up interval and suggested that the interval between surveillance examinations be extended if the lesion remained unchanged for 2 consecutive follow-up EUS. Guidelines in Japan recommended endoscopic examination once or twice per year for subepithelial lesions less than 2 cm in diameter[66].

CONCLUSION

EUS imaging is essential for the evaluation of subepithelial tumors, because EUS performs better than other modalities in evaluating GI subepithelial lesions. However, the diagnostic accuracy of EUS imaging alone has been shown to be low in subepithelial lesions with a 3rd and 4th layer. In the case of hypoechoic lesions of the 3rd and the 4th EUS layers that are more than in 1 cm diameter, histologic confirmation by using EUS-FNA should be obtained when possible. Although EUS-FNA is a safer and more accurate non-invasive method than other methods of getting samples of the subepithelial tumor, even EUS-FNA is not always accurate enough to determine malignancy, especially determination of malignant GISTs. Furthermore improvements in endoscopic technology are expected to be more useful modalities in differential diagnosis and discrimination between benign and malignant subepithelial tumors.

REFERENCES

1 **Chak A**. EUS and natural orifice transluminal endoscopic surgery. *Gastrointest Endosc* 2009; **69**: S210-S211
2 **Hwang JH**, Saunders MD, Rulyak SJ, Shaw S, Nietsch H, Kimmey MB. A prospective study comparing endoscopy and EUS in the evaluation of GI subepithelial masses. *Gastrointest Endosc* 2005; **62**: 202-208
3 **Polkowski M**, Butruk E. Submucosal lesions. *Gastrointest Endosc Clin N Am* 2005; **15**: 33-54, viii
4 **Nickl N**. Endoscopic approach to gastrointestinal stromal tumors. *Gastrointest Endosc Clin N Am* 2005; **15**: 455-466, viii
5 **Polkowski M**. Endoscopic ultrasound and endoscopic ultrasound-guided fine-needle biopsy for the diagnosis of malignant submucosal tumors. *Endoscopy* 2005; **37**: 635-645
6 **Landi B**, Palazzo L. The role of endosonography in submucosal tumours. *Best Pract Res Clin Gastroenterol* 2009; **23**: 679-701
7 **Chak A**. EUS in submucosal tumors. *Gastrointest Endosc* 2002; **56**: S43-S48
8 **Caletti G**, Zani L, Bolondi L, Brocchi E, Rollo V, Barbara L. Endoscopic ultrasonography in the diagnosis of gastric submucosal tumor. *Gastrointest Endosc* 1989; **35**: 413-418
9 **Hsu CC**, Chen JJ, Changchien CS. Endoscopic features of metastatic tumors in the upper gastrointestinal tract. *Endoscopy* 1996; **28**: 249-253
10 **Rösch T**, Lorenz R, Dancygier H, von Wickert A, Classen M. Endosonographic diagnosis of submucosal upper gastrointestinal tract tumors. *Scand J Gastroenterol* 1992; **27**: 1-8

11 **Shim CS**, Jung IS. Endoscopic removal of submucosal tumors: preprocedure diagnosis, technical options, and results. *Endoscopy* 2005; **37**: 646-654
12 **Hizawa K**, Matsumoto T, Kouzuki T, Suekane H, Esaki M, Fujishima M. Cystic submucosal tumors in the gastrointestinal tract: endosonographic findings and endoscopic removal. *Endoscopy* 2000; **32**: 712-714
13 **Hünerbein M**, Dohmoto M, Haensch W, Schlag PM. Endosonography-guided biopsy of mediastinal and pancreatic tumors. *Endoscopy* 1998; **30**: 32-36
14 **Frank N**, Grieshammer B, Zimmermann W. A new miniature ultrasonic probe for gastrointestinal scanning: feasibility and preliminary results. *Endoscopy* 1994; **26**: 603-608
15 **Fockens P**. Current endosonographic possibilities in the upper gastrointestinal tract. *Baillieres Clin Gastroenterol* 1994; **8**: 603-619
16 **Chak A**, Canto M, Stevens PD, Lightdale CJ, Van de Mierop F, Cooper G, Pollack BJ, Sivak MV Jr. Clinical applications of a new through-the-scope ultrasound probe: prospective comparison with an ultrasound endoscope. *Gastrointest Endosc* 1997; **45**: 291-295
17 **Buscarini E**, Stasi MD, Rossi S, Silva M, Giangregorio F, Adriano Z, Buscarini L. Endosonographic diagnosis of submucosal upper gastrointestinal tract lesions and large fold gastropathies by catheter ultrasound probe. *Gastrointest Endosc* 1999; **49**: 184-191
18 **Wiech T**, Walch A, Werner M. Histopathological classification of nonneoplastic and neoplastic gastrointestinal submucosal lesions. *Endoscopy* 2005; **37**: 630-634
19 **Boyce GA**, Sivak MV Jr, Rösch T, Classen M, Fleischer DE, Boyce HW Jr, Lightdale CJ, Botet JF, Hawes RH, Lehman GA. Evaluation of submucosal upper gastrointestinal tract lesions by endoscopic ultrasound. *Gastrointest Endosc* 1991; **37**: 449-454
20 **Yasuda K**, Nakajima M, Yoshida S, Kiyota K, Kawai K. The diagnosis of submucosal tumors of the stomach by endoscopic ultrasonography. *Gastrointest Endosc* 1989; **35**: 10-15
21 **Motoo Y**, Okai T, Ohta H, Satomura Y, Watanabe H, Yamakawa O, Yamaguchi Y, Mouri I, Sawabu N. Endoscopic ultrasonography in the diagnosis of extraluminal compressions mimicking gastric submucosal tumors. *Endoscopy* 1994; **26**: 239-242
22 **Hwang JH**, Rulyak SD, Kimmey MB. American Gastroenterological Association Institute technical review on the management of gastric subepithelial masses. *Gastroenterology* 2006; **130**: 2217-2228
23 **Seidal T**, Edvardsson H. Expression of c-kit (CD117) and Ki67 provides information about the possible cell of origin and clinical course of gastrointestinal stromal tumours. *Histopathology* 1999; **34**: 416-424
24 **Franquemont DW**. Differentiation and risk assessment of gastrointestinal stromal tumors. *Am J Clin Pathol* 1995; **103**: 41-47
25 **Palazzo L**, Landi B, Cellier C, Roseau G, Chaussade S, Couturier D, Barbier J. Endosonographic features of esophageal granular cell tumors. *Endoscopy* 1997; **29**: 850-853
26 **Tada S**, Iida M, Yao T, Miyagahara T, Hasuda S, Fujishima M. Granular cell tumor of the esophagus: endoscopic ultrasonographic demonstration and endoscopic removal. *Am J Gastroenterol* 1990; **85**: 1507-1511
27 **Matsushita M**, Hajiro K, Okazaki K, Takakuwa H. Gastric inflammatory fibroid polyps: endoscopic ultrasonographic analysis in comparison with the histology. *Gastrointest Endosc* 1997; **46**: 53-57
28 **Pidhorecky I**, Cheney RT, Kraybill WG, Gibbs JF. Gastrointestinal stromal tumors: current diagnosis, biologic behavior, and management. *Ann Surg Oncol* 2000; **7**: 705-712
29 **Miettinen M**, Sarlomo-Rikala M, Lasota J. Gastrointestinal stromal tumors: recent advances in understanding of their biology. *Hum Pathol* 1999; **30**: 1213-1220
30 **Sarlomo-Rikala M**, Kovatich AJ, Barusevicius A, Miettinen M. CD117: a sensitive marker for gastrointestinal stromal tumors that is more specific than CD34. *Mod Pathol* 1998; **11**: 728-734
31 **Miettinen M**, Sobin LH, Sarlomo-Rikala M. Immunohistochemical spectrum of GISTs at different sites and their differential diagnosis with a reference to CD117 (KIT). *Mod Pathol* 2000; **13**: 1134-1142
32 **Kindblom LG**, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am J Pathol* 1998; **152**: 1259-1269
33 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001; **438**: 1-12
34 **Shen EF**, Arnott ID, Plevris J, Penman ID. Endoscopic ultrasonography in the diagnosis and management of suspected upper gastrointestinal submucosal tumours. *Br J Surg* 2002; **89**: 231-235
35 **Caletti G**, Fusaroli P, Bocus P. Endoscopic ultrasonography. *Endoscopy* 1998; **30**: 198-221
36 **Lambert R**, Caletti G, Cho E, Chang KJ, Fusaroli P, Feussner H, Fockens P, Hawes RH, Inui K, Kida M, Lightdale CJ, Matos C, Napoleon B, Palazzo L, Rösch T, Van Dam J. International Workshop on the clinical impact of endoscopic ultrasound in gastroenterology. *Endoscopy* 2000; **32**: 549-584
37 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: review on morphology, molecular pathology, prognosis, and differential diagnosis. *Arch Pathol Lab Med* 2006; **130**: 1466-1478
38 **Kawamoto K**, Yamada Y, Furukawa N, Utsunomiya T, Haraguchi Y, Mizuguchi M, Oiwa T, Takano H, Masuda K. Endoscopic submucosal tumorectomy for gastrointestinal submucosal tumors restricted to the submucosa: a new form of endoscopic minimal surgery. *Gastrointest Endosc* 1997; **46**: 311-317
39 **Kameyama H**, Niwa Y, Arisawa T, Goto H, Hayakawa T. Endoscopic ultrasonography in the diagnosis of submucosal lesions of the large intestine. *Gastrointest Endosc* 1997; **46**: 406-411
40 **Kojima T**, Takahashi H, Parra-Blanco A, Kohsen K, Fujita R. Diagnosis of submucosal tumor of the upper GI tract by endoscopic resection. *Gastrointest Endosc* 1999; **50**: 516-522
41 **Waxman I**, Saitoh Y, Raju GS, Watari J, Yokota K, Reeves AL, Kohgo Y. High-frequency probe EUS-assisted endoscopic mucosal resection: a therapeutic strategy for submucosal tumors of the GI tract. *Gastrointest Endosc* 2002; **55**: 44-49
42 **Okai T**, Minamoto T, Ohtsubo K, Minato H, Kurumaya H, Oda Y, Mai M, Sawabu N. Endosonographic evaluation of c-kit-positive gastrointestinal stromal tumor. *Abdom Imaging* 2003; **28**: 301-307
43 **Hwang JH**, Kimmey MB. The incidental upper gastrointestinal subepithelial mass. *Gastroenterology* 2004; **126**: 301-307
44 **Săftoiu A**, Vilmann P, Ciurea T. Utility of endoscopic ultrasound for the diagnosis and treatment of submucosal tumors of the upper gastrointestinal tract. *Rom J Gastroenterol* 2003; **12**: 215-229
45 **Gress F**, Schmitt C, Savides T, Faigel DO, Catalano M, Wassef W, Roubein L, Nickl N, Ciaccia D, Bhutani M, Hoffman B, Affronti J. Interobserver agreement for EUS in the evaluation and diagnosis of submucosal masses. *Gastrointest Endosc* 2001; **53**: 71-76
46 **Williams DB**, Sahai AV, Aabakken L, Penman ID, van Velse A, Webb J, Wilson M, Hoffman BJ, Hawes RH. Endoscopic ultrasound guided fine needle aspiration biopsy: a large single centre experience. *Gut* 1999; **44**: 720-726
47 **Wiersema MJ**, Vilmann P, Giovannini M, Chang KJ, Wiersema LM. Endosonography-guided fine-needle aspiration biopsy: diagnostic accuracy and complication assessment. *Gastroenterology* 1997; **112**: 1087-1095
48 **Erickson RA**, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. *Gastrointest Endosc* 2000; **51**: 184-190

49 **Chang KJ**, Katz KD, Durbin TE, Erickson RA, Butler JA, Lin F, Wuerker RB. Endoscopic ultrasound-guided fine-needle aspiration. *Gastrointest Endosc* 1994; **40**: 694-699

50 **Mertz H**, Gautam S. The learning curve for EUS-guided FNA of pancreatic cancer. *Gastrointest Endosc* 2004; **59**: 33-37

51 **Eisen GM**, Dominitz JA, Faigel DO, Goldstein JA, Petersen BT, Raddawi HM, Ryan ME, Vargo JJ 2nd, Young HS, Wheeler-Harbaugh J, Hawes RH, Brugge WR, Carrougher JG, Chak A, Faigel DO, Kochman ML, Savides TJ, Wallace MB, Wiersema MJ, Erickson RA. Guidelines for credentialing and granting privileges for endoscopic ultrasound. *Gastrointest Endosc* 2001; **54**: 811-814

52 **Vander Noot MR 3rd**, Eloubeidi MA, Chen VK, Eltoum I, Jhala D, Jhala N, Syed S, Chhieng DC. Diagnosis of gastrointestinal tract lesions by endoscopic ultrasound-guided fine-needle aspiration biopsy. *Cancer* 2004; **102**: 157-163

53 **Klapman JB**, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol* 2003; **98**: 1289-1294

54 **Jenssen C**, Dietrich CF. Endoscopic ultrasound-guided fine-needle aspiration biopsy and trucut biopsy in gastroenterology - An overview. *Best Pract Res Clin Gastroenterol* 2009; **23**: 743-759

55 **Ando N**, Goto H, Niwa Y, Hirooka Y, Ohmiya N, Nagasaka T, Hayakawa T. The diagnosis of GI stromal tumors with EUS-guided fine needle aspiration with immunohistochemical analysis. *Gastrointest Endosc* 2002; **55**: 37-43

56 **Okubo K**, Yamao K, Nakamura T, Tajika M, Sawaki A, Hara K, Kawai H, Yamamura Y, Mochizuki Y, Koshikawa T, Inada K. Endoscopic ultrasound-guided fine-needle aspiration biopsy for the diagnosis of gastrointestinal stromal tumors in the stomach. *J Gastroenterol* 2004; **39**: 747-753

57 **Gomes AL**, Bardales RH, Milanezi F, Reis RM, Schmitt F. Molecular analysis of c-Kit and PDGFRA in GISTs diagnosed by EUS. *Am J Clin Pathol* 2007; **127**: 89-96

58 **Rader AE**, Avery A, Wait CL, McGreevey LS, Faigel D, Heinrich MC. Fine-needle aspiration biopsy diagnosis of gastrointestinal stromal tumors using morphology, immunocytochemistry, and mutational analysis of c-kit. *Cancer* 2001; **93**: 269-275

59 **Willmore-Payne C**, Layfield LJ, Holden JA. c-KIT mutation analysis for diagnosis of gastrointestinal stromal tumors in fine needle aspiration specimens. *Cancer* 2005; **105**: 165-170

60 **Debiec-Rychter M**, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, Blay JY, Leyvraz S, Stul M, Casali PG, Zalcberg J, Verweij J, Van Glabbeke M, Hagemeijer A, Judson I. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer* 2006; **42**: 1093-1103

61 **Heinrich MC**, Corless CL, Blanke CD, Demetri GD, Joensuu H, Roberts PJ, Eisenberg BL, von Mehren M, Fletcher CD, Sandau K, McDougall K, Ou WB, Chen CJ, Fletcher JA. Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. *J Clin Oncol* 2006; **24**: 4764-4774

62 **Heinrich MC**, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003; **21**: 4342-4349

63 **Singer S**, Rubin BP, Lux ML, Chen CJ, Demetri GD, Fletcher CD, Fletcher JA. Prognostic value of KIT mutation type, mitotic activity, and histologic subtype in gastrointestinal stromal tumors. *J Clin Oncol* 2002; **20**: 3898-3905

64 **Eckardt AJ**, Wassef W. Diagnosis of subepithelial tumors in the GI tract. Endoscopy, EUS, and histology: bronze, silver, and gold standard? *Gastrointest Endosc* 2005; **62**: 209-212

65 **Fletcher CD**, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002; **33**: 459-465

66 **Nishida T**, Hirota S, Yanagisawa A, Sugino Y, Minami M, Yamamura Y, Otani Y, Shimada Y, Takahashi F, Kubota T. Clinical practice guidelines for gastrointestinal stromal tumor (GIST) in Japan: English version. *Int J Clin Oncol* 2008; **13**: 416-430

S- Editor Cheng JX **L- Editor** O'Neill M **E- Editor** Zheng XM

Endoscopic ultrasound (EUS)-guided transluminal endoscopic removal of gallstones

Although laparoscopic cholecystectomy is the standard treatment for cholecystitis, including cholecystolithiasis [1,2], endoscopic ultrasound (EUS)-guided cholecystenterostomy is an alternative treatment for patients at high surgical risk [3–5]. Here we report on using the fistula created by the EUS-guided cholecystenterostomy to remove gallstones for successful radical treatment of cholecystolithiasis without cholecystectomy.

A 62-year-old man with significant dementia presented with severe acute cholecystitis. Cholecystectomy was deemed unsuitable because of the presence of sepsis, and EUS-guided cholecystoduodenostomy was carried out. An echoendoscope (GF-UCT240-AL5, Olympus, Tokyo, Japan) was introduced into the duodenum, and a 19-G needle (Echo-Tip; Cook, Winston-Salem, North Carolina, USA) was used to puncture the gallbladder (**Fig. 1**).

Cholecystocholangiography revealed gallstones and sludge in the gallbladder (**Fig. 2**).

A 0.035-inch guide wire (Revowave, Olympus, Tokyo, Japan) was passed through the needle until it was coiled within the gallbladder, and then 6, 7 and 9-Fr dilators (Soehendra Biliary Dilation Catheters, Cook, Winston-Salem, North Carolina, USA) were serially advanced over the guide wire to dilate the tract. A one-sided pigtail-type stent (Catex, Tokyo, Japan) was deployed in the gallbladder. By day 11 the cholecystitis had improved. However, on day 145 after the procedure, the patient had a relapse. Duodenoscopy (JF260V, Olympus, Tokyo, Japan) revealed obstruction of the stent and the need for reintervention to treat the cholecystitis. After the stent was extracted with a snare, a 0.035-inch guide wire was passed through the catheter (Swing Tip, Olympus Medical Systems, Tokyo, Japan) via the fistula until it reached the gallbladder. A covered metal stent (CMS) (Wallflex, diameter 10 mm; length 4 cm, Boston Scientific, Boston, Massachusetts, USA) was placed to bridge the gallbladder with the duodenum (**Fig. 3**), and the gallstones and sludge were discharged into the bowel tract by irrigating the gallbladder with saline (**Fig. 4**).

Finally, the CMS was removed with forceps, and a 7-Fr pigtail-type stent was de-

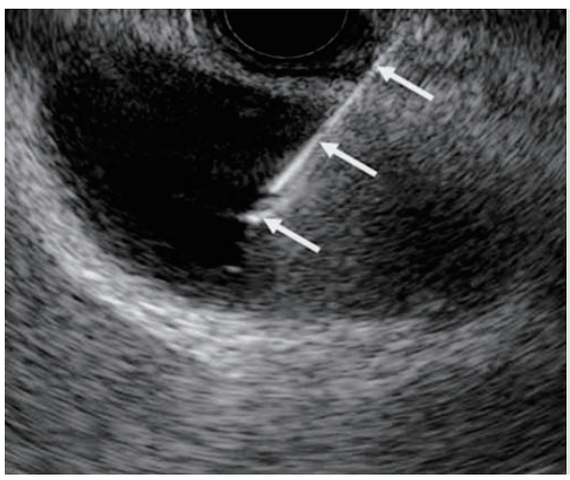


Fig. 1 Endosonographic image of the gallbladder puncture. Arrows indicate the puncture needle.

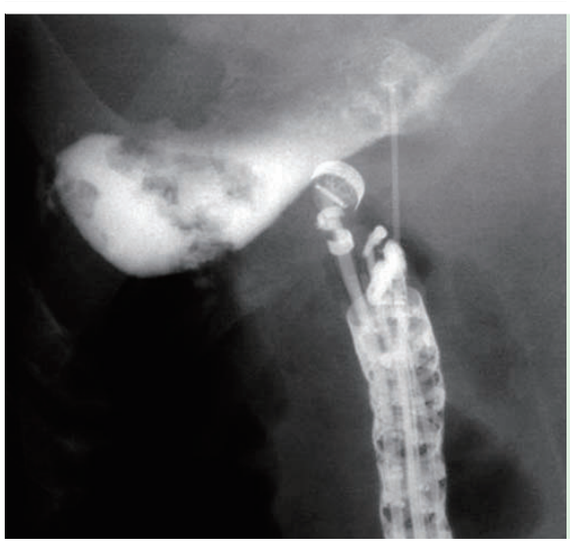


Fig. 2 Cholecystographic image of the gallbladder puncture.

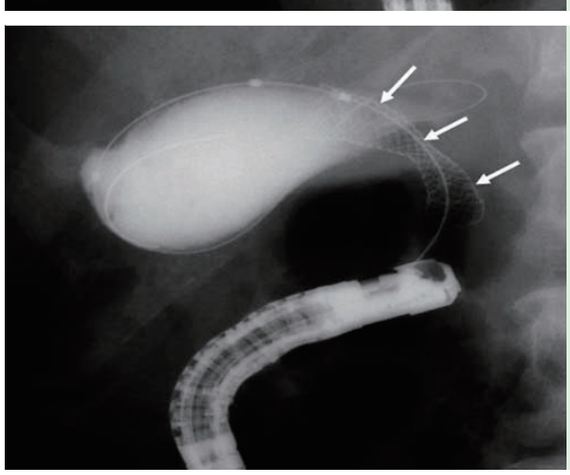


Fig. 3 Fluoroscopic image of the deployment of the covered metal stent (arrows).

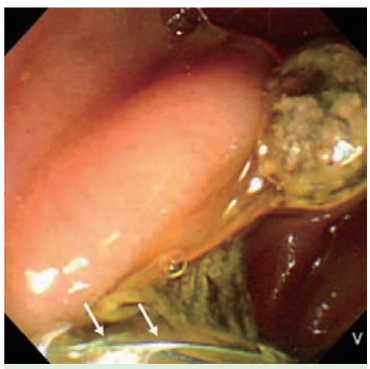


Fig. 4 Endoscopic image of a stone discharged from the gallbladder through the covered metal stent (arrows).

ployed in the gallbladder instead. On day 5 after the operation, the patient's condition improved and he could resume eating. The stent has been in place for 5 months now with no recurrence of symptoms.

Competing interests: None

Endoscopy_UCTN_Code_TTT_1AS_2AD

K. Kamata, M. Kitano, M. Kudo, H. Imai, H. Sakamoto, T. Komaki
Department of Gastroenterology and Hepatology, Kinki University School of Medicine, Osaka-Sayama, Japan

References

1 *Reddick EJ, Olsen DO.* Laparoscopic laser cholecystectomy. A comparison with mini-lap cholecystectomy. Surg Endosc 1989; 3: 131 – 133
2 *Dubois F, Lcard P, Berthelot G et al.* Coelioscopic cholecystectomy. Preliminary report of 36 cases. Ann Surg 1990; 212: 649 – 650
3 *Kwan V, Eisendrath P, Antaki F et al.* EUS-guided cholecysterostomy: a new technique. Gastrointest Endosc 2007; 66: 582 – 586
4 *Lee SS, Park DH, Hwang CY et al.* EUS-guided transmural cholecystostomy as rescue management for acute cholecystitis in elderly or high-risk patients: a prospective feasibility study. Gastrointest Endosc 2007; 66: 1008 – 1012
5 *Kamata K, Kitano M, Kudo M et al.* Transgastric endoscopic ultrasound (EUS)-guided gallbladder drainage for acute cholecystitis. Endoscopy 2009; 41: e315 – e316

Bibliography
DOI 10.1055/s-0030-1255941
Endoscopy 2010; 42: E331 – E332

ISSN 0013-726X

Corresponding author
M. Kitano
Department of Gastroenterology and Hepatology
Kinki University School of Medicine
377-2 Ohno-Higashi
Osaka-Sayama 589-8511
Japan
Fax: +81-723-67 2880
m-kitano@med.kindai.ac.jp

Digestive Endoscopy (2010) **22**, 192–199 doi:10.1111/j.1443-1661.2010.00983.x

ORIGINAL ARTICLE

DIAGNOSTIC ACCURACY OF PIT PATTERN AND VASCULAR PATTERN ANALYSES IN COLORECTAL LESIONS

YOSHIKI WADA,[1] HIROSHI KASHIDA,[1] SHIN-EI KUDO,[1] MASASHI MISAWA,[1] NOBUNAO IKEHARA[1] AND SHIGEHARU HAMATANI[2]

[1]*Digestive Disease Center and* [2]*Department of Pathology, Showa University Northern Yokohama Hospital, Yokohama, Japan*

Background: The aim of this prospective study is to compare the usefulness of magnifying narrow band imaging (NBI) and magnifying chromoendoscopy in the diagnosis of colorectal lesions.
Methods: The subjects were 1185 patients who underwent a complete colonoscopic examination and endoscopic or surgical treatment, from January 2006 to February 2008. A total of 1473 lesions were evaluated (53 hyperplastic polyps, 1317 adenomas, 103 submucosally invasive cancers). The digital images with NBI or chromoendoscopy were recorded and diagnosed independently from each other by two endoscopists who were blinded to the final pathological diagnosis.
Results: We could differentiate between neoplastic and non-neoplastic lesions with sensitivity of 88.9%, specificity of 98.5% and accuracy of 98.2% according to the vascular pattern. By recognizing an irregular or sparse pattern with NBI, massively invasive submucosal cancer could be diagnosed with the sensitivity and specificity of 94.9% and 76.0%. Using chromoendoscopy, we could differentiate between neoplastic and non-neoplastic lesions with sensitivity of 86.8% and specificity of 99.2%. We were able to differentiate between massively invasive cancers and slightly invasive cancers using the pit patterns with sensitivity of 89.7% and specificity of 88.0%. The specificity was superior to that of NBI colonoscopy.
Conclusion: Both NBI and chromoendoscopy can be useful for distinguishing between neoplastic and non-neoplastic lesions. In the diagnosis of submucosal cancer, pit pattern diagnosis was slightly superior to vascular pattern diagnosis. It is desirable to perform chromoendoscopy in addition to NBI for distinguishing between slightly and massively invasive submucosal cancer lesions and determining the treatment.

Key words: magnifying colonoscopy, narrow band imaging, pit pattern, vascular pattern.

INTRODUCTION

Magnifying colonoscopy has enabled the observation of minute surface structure of various lesions. The orifices of colonic mucosal glands are called pits; the specific shape and arrangement of pits in each lesion is called a pit pattern and can be observed with chromoendoscopy. The pit pattern of each lesion reflects its histological structural atypia and pit pattern analysis has been reported as useful for predicting the lesion's histological nature: neoplastic or non-neoplastic, invasive or non-invasive, etc.[1–3] Recently a new method of enhanced endoscopy, called narrow band imaging (NBI), has been developed. It emphasizes the superficial tissue structures, especially vasculature without using any dye.[4] We have named the vascular structure seen with NBI the 'vascular pattern'. The usefulness of vascular pattern analysis for predicting the histology of various lesions in the colorectum has been reported. The aim of this study is to compare the usefulness of magnified narrow band imaging (NBI) and magnifying chromoendoscopy in the diagnosis of colorectal lesions.

Correspondence: Yoshiki Wada, Digestive Disease Center, Showa University Northen Yokohama Hospital, 35-1, Chigasaki-Chuo, Tsuzuki, Yokohama 224-8503, Japan. Email: w-yoshi@mtj.biglobe.ne.jp

Received 18 February 2009; accepted 7 December 2009.

METHODS

We present a single-center study that took place at the Digestive Disease Center of Showa University Northern Yokohama Hospital from January 2006 to February 2008. The protocol was approved by the medical ethics committee of the hospital. All patients gave informed consent before participating in this study. In this prospective study, 1473 colorectal lesions from 1185 patients were enrolled and observed with conventional images, NBI and chromoendoscopy. All the lesions were detected with white light ordinary view. Chromoendoscopy was performed with 0.2% indigo-carmine dye and, in addition, 0.05% crystal violet dye as indicated. The score used in this study was CF-H260AZI (with a magnifying power of ×75 on the 19-inch monitor).

Of the 1185 patients, 680 were men and 505 were women, and the mean age was 61.5 years. We classified gross configuration according to the 'General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus' of the Japanese Society for Cancer of the Colon and Rectum[5] and the Paris classification.[6] The so-called laterally spreading tumors (LST) can be divided into some subgroups and are expressed as type IIa, IIc+IIa, or IIa+Is according to the categories of the Paris and Kyoto classification.[7] The lesions were resected endoscopically or surgically and sent for pathological assessment. According to the classification of the World Health Organization, adenomas are

defined by the presence of intraepithelial neoplasia.[8] Intramucosal cancer was included as adenoma in this study.

We defined massively invasive cancers (SMm) as those with invasion depth of 1000 μm or more, according to the above-mentioned role.[5] Measurement of the depth of submucosal invasion was measured as previously described.[9]

Endoscopic images were stored electronically and randomly allocated to two readers (Y. W., H. K.) for evaluation. When the NBI diagnosis was different for the same lesion between them, one final diagnosis was determined by discussion. We classified vascular patterns into normal, faint, network, dense, irregular and sparse[10] (Fig. 1). Our definitions for 'irregular' vascular pattern are: (i) interruption of network; (ii) tortuous course of vessels; and (iii) unusually large caliber of vessels (twice as large as that of surrounding vessels). As for the differences between Fig. 1C and Fig. 1D (network pattern and dense pattern, respectively), in the former the uniform thick vessels are present surrounding each regular pit, whereas in the latter many vessels exist crowdedly in the intervening area among the pits. Concerning the differences between Fig. 1B and Fig. 1F (faint pattern and sparse pattern, respectively), in the former the vessels are simply obscure and unrecognized. In the latter, vessel density is fairly low, but winding and noncontinuous vessels do exist in the lesion. When two or more vascular patterns were recognized in a lesion, we adopted the most advanced pattern as representative of the lesion. The vascular pattern was considered as more advanced in a descending order of sparse, irregular, dense, network and faint. The pit pattern was classified into I, II, III, IV, Vi (irregular) and Vn (non-structure) (Fig. 2) according to Kudo's classification.[1] Vi pit pattern was subdivided into Vi low-grade and Vi high-grade.[11] Our criteria for Vi high-grade pit pattern were irregular pits with rough margin and narrowed lumen (Fig. 3).

According to our previous study on NBI,[12] we decided that the lesions with the faint pattern should be diagnosed as hyperplastic polyps and those with the irregular or sparse pattern should be diagnosed as massively invading submucosal cancers. Concerning the pit pattern diagnosis, we considered the type II pit pattern as diagnostic of hyperplastic polyps and the types Vi high-grade and Vn as representative of SMm.[11] Sensitivity, specificity and diagnostic accuracy of NBI and chromoendoscopy were assessed by reference to histopathology. The efficacy of NBI was evaluated in the differential diagnosis between neoplastic and non-neoplastic lesion and between SMm (which is indicated for surgical operation) and non- or invasive neoplastic lesions (adenomas and slightly invasive cancers [SMs], which are indicated for endoscopic treatment).

SPSS for Windows Version 11.0 statistical software package was used for the data analysis. For the comparison of the demographic features of the subjects, the Student's *t*-test was applied. *P*-values of less than 0.05 were considered significant. For the assessment of diagnostic ability of NBI-enhanced endoscopy and chromoendoscopy, the χ^2-test was used and *P*-values of less than 0.01 were considered significant.

RESULTS

A total of 1473 lesions were evaluated in the 1185 patients. These included 53 hyperplastic polyps, 1317 adenomas and 103 submucosally invasive (T1) cancers. There was no significant difference in tumor size in hyperplastic polyp versus adenoma and adenoma versus cancer (Table 1). Endoscopic images including all of the conventional views, narrow band images and chromoendoscopic views were obtained in all cases. Subsequently an endoscopic or surgical therapy was performed. Among 1473 lesions, 1401 lesions were treated endoscopically and 72 were treated surgically. In submucosal cancers, 65 cases were treated surgically.

In this prospective study, the vascular pattern of hyperplastic polyps showed a faint pattern in most lesions (Table 2). The vascular patterns of adenomas and SMs were mainly network pattern and dense pattern. On the other hand, the major vascular patterns of SMm were irregular pattern and sparse pattern. Typically, protruded cancer with massive invasion showed irregular pattern (Fig. 4). In contrast, the depressed type lesions, especially depressed invasive cancers, were characterized by the sparse pattern (Fig. 5).

Using NBI, we could differentiate between neoplastic and non-neoplastic lesions with sensitivity of 88.9%, specificity of 98.5% and accuracy of 98.2% (Table 3). Vascular pattern analysis with NBI magnification was considered helpful in differentiating between neoplasia and non-neoplasia. Of the 21 adenomas that presented the faint pattern, 18 lesions were sessile serrated adenomas. Thus, sessile serrated adenomas are sometimes difficult to distinguish from hyperplastic polyps. We were able to differentiate between SMm and SMs by using the vascular patterns with sensitivity of 94.9% and specificity of 76.0% (Table 4).

The result of pit pattern analysis was similar to the previous reports (Table 5).[1] Most hyperplastic polyps showed a type II pit pattern. Almost all of the lesions with type IIIL, IIIs or IV pit pattern were shown to be adenomas (99.0%, 100% and 97.1%, respectively). The type Vi pattern was seen in 163 lesions. Type Vi low-grade was seen in 108 lesions and 78.7% of them (85/108) were adenomas. Type Vi high-grade was seen in 55 lesions and 87.3% of them (45/55) were SMm. All of the lesions with type Vn pit pattern lesions were SMm.

Using chromoendoscopy, we could differentiate between neoplastic and non-neoplastic lesions with sensitivity of 86.8% and specificity of 99.2% (Table 6). We were able to differentiate between SMm and SMs using the pit patterns with sensitivity of 89.7% and specificity of 88.0% (Table 7). The specificity was superior to that of NBI colonoscopy.

DISCUSSION

Chromoendoscopy with indigo-carmine or crystal violet is currently used for tissue characterization and differential diagnosis. Kudo *et al.*[13] reported that the magnifying chromoendoscopy provides an accurate and immediate assessment of the histology of colorectal tumors. Tobaru *et al.*[14] subdivided the type Vi pit pattern into the well-demarcated and poorly-demarcated subtypes and reported that Vi pit pattern sub-classification was a useful indicator of the depth of the colorectal tumor. Kanao *et al.*[15] divided the type Vi pit pattern into mildly and severely irregular and concluded that the subclassification of the type Vi pit pattern was useful for identifying dysplasias or lesions with invasion depth <1000 μm. In this study, Vi pit pattern was divided into Vi low-grade and Vi high-grade. The subclassification of Vi pit

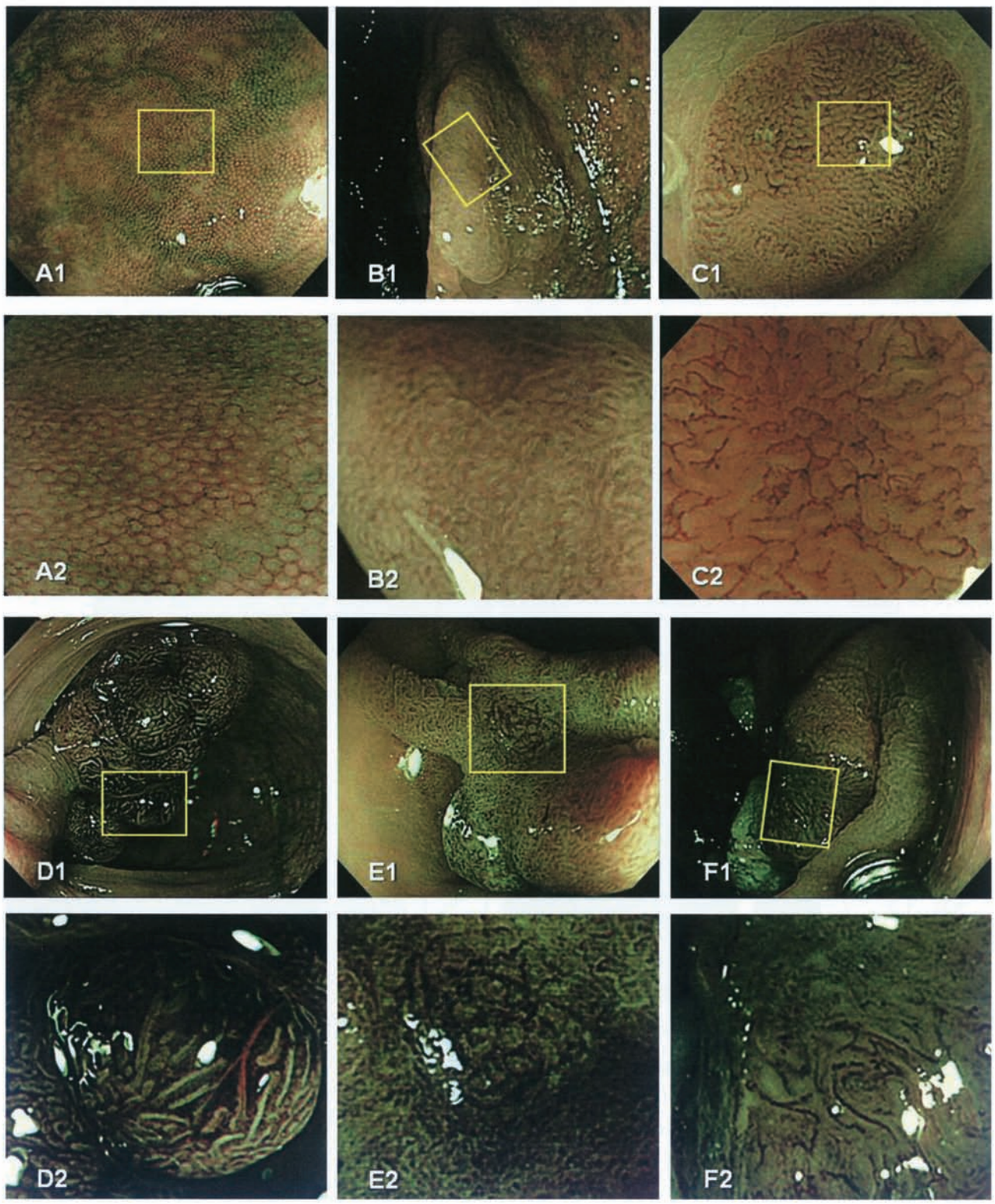


Fig. 1. Classification of vascular pattern (A,B,C). (A) Normal pattern (A1, unmagnified; A2, magnified). (B) Faint pattern (B1, unmagnified; B2, magnified). (C) Network pattern (C1, unmagnified; C2, magnified). Classification of vascular pattern (D,E,F). (D) dense pattern (D1, unmagnified; D2, magnified). (E) Irregular pattern (E1, unmagnified; E2, magnified). (F) Sparse pattern (F1, unmagnified; F2, magnified).

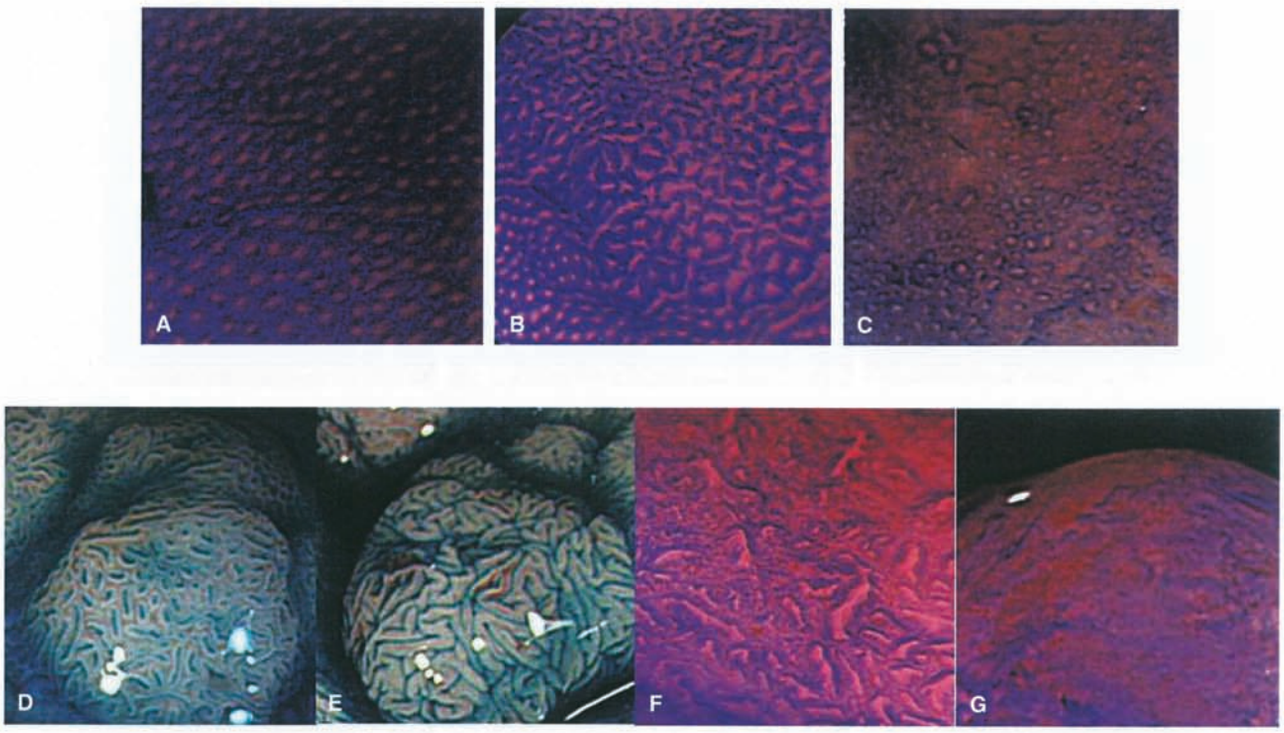


Fig. 2. Kudo's classification. (A) Type I pit pattern. (B) Type II pit pattern. (C) Type IIIs pit pattern. (D) Type IIIL pit pattern. (E) Type IV pit pattern. (F) Type Vi pit pattern. (G) Type Vn pit pattern.

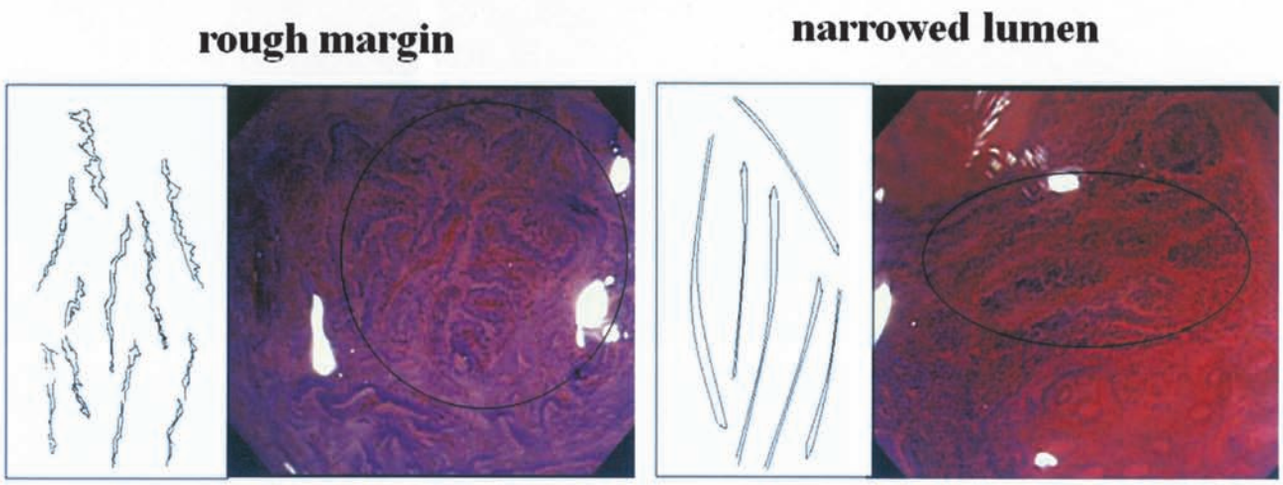


Fig. 3. Vi high-grade. Our definitions for Vi high-grade pit pattern were rough margin and narrowed lumen.

Table 1. Size and location in colorectum of materials

	Pathological diagnosis		
	Hyperplastic polyp	Adenoma	Cancer
Number	53	1317	103
Size	11.73 ± 5.22 (2–35)	13.14 ± 7.23 (2–60)	15.02 ± 9.43 (8–58)
Location			
Right colon	18	487	34
Left colon	17	340	25
Rectum	18	490	44

Mean size: hyperplastic polyp versus adenoma; adenoma versus cancer: not significant.

pattern was useful for distinguishing SMm from SMs. When we detect a colorectal localized lesion, we do not usually take a biopsy because if we did so we should have to wait for the pathological report and perform another colonoscopy when endoscopic treatment is indicated. In contrast, magnifying colonoscopy with dye staining enables us an appropriate prediction of the histology of the lesion on site and thus enables us to avoid the cost, time and risk of biopsy and repeated colonoscopy. Moreover precise prediction of histology with magnifying scopes can also avoid unnecessary polypectomy (in case of a hyperplastic polyp, for example).

Table 2. Comparison between vascular pattern and pathological diagnosis

Vascular pattern	Pathological diagnosis				Total
	Hyperplastic polyp	Adenoma	SMs	SMm	
Faint	47	21			68
Network	6	1018	14	1	1039
Dense		256	5	3	264
Irregular		18	1	38	57
Sparse		4	5	36	45
Total	53	1317	25	78	1473

SMm, massively invasive cancers; SMs, slightly invasive cancers.

Many researchers have reported that NBI is useful for detecting colorectal lesions.[16–18] On the other hand, Alder A *et al.* reported that the increased adenoma detection rate by means of NBI colonoscopy were not statistically significant.[19] Evaluation of the detectability of NBI was not the purpose of the present study. We are using NBI just for tissue characterization of the lesions, which were detected with ordinary white light view. NBI has some advantages over chromoendoscopy: (i) it does not require any staining agents because it is easily activated by a manual switch on the endoscope's handle; and (ii) it allows for inspection of the whole endoscopic field, whereas in chromoendoscopy the dye often does not distribute equally over the mucosa. In addition to these practical advantages, NBI shows a high-contrast image of the superficial vasculature, whereas with chromoendoscopy, the vasculature is often less visible.

Pit pattern analysis is useful for differentiating between neoplasia and non-neoplasia.[13] Some reports described the

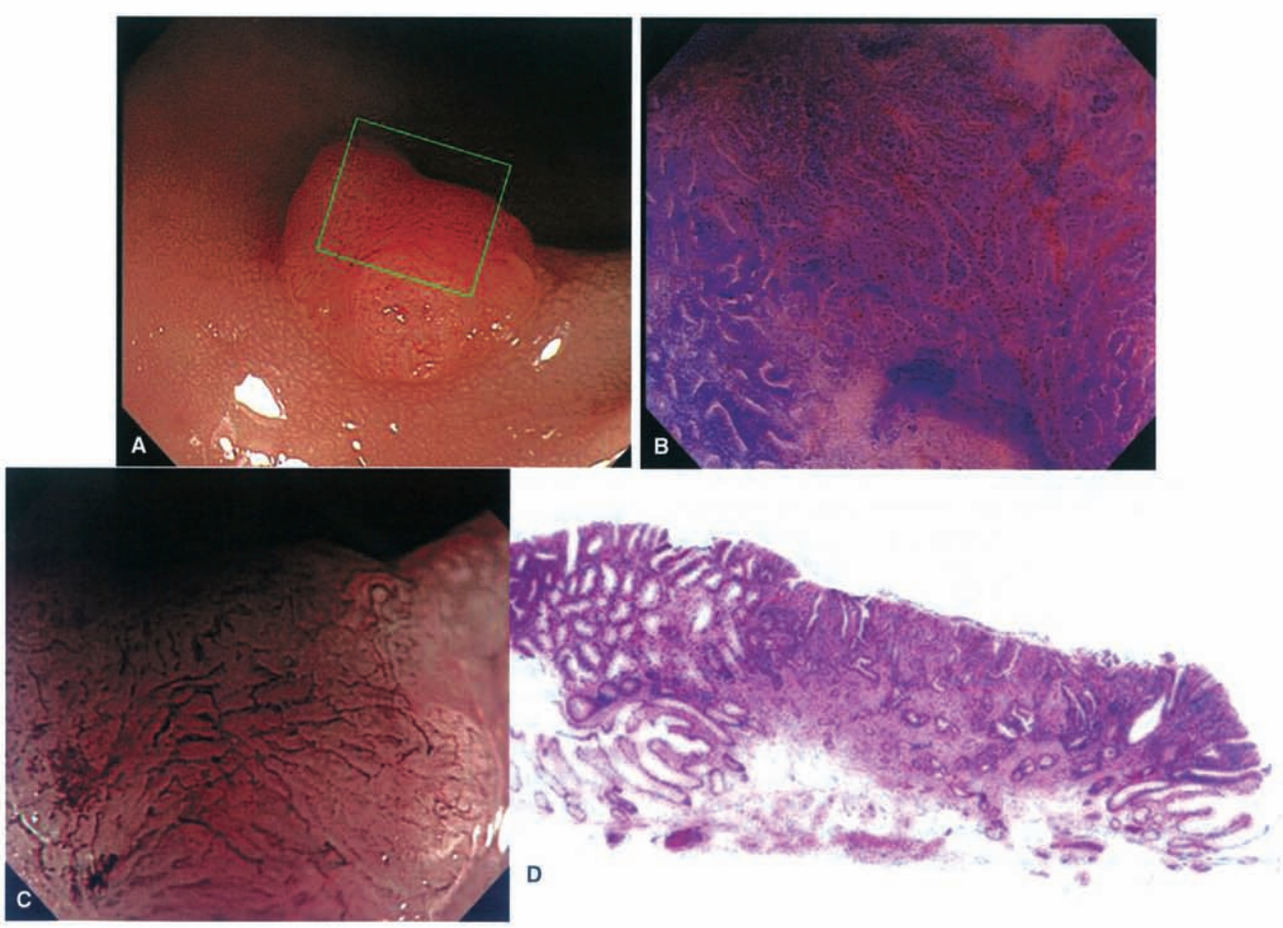


Fig. 4. Protruded lesion (Is) size: 9 mm. (A) Conventional view. (B) Magnified endoscopic view after crystal violet dye staining. (C) Magnified endoscopic view with narrow band imaging system. Thick and irregular vessels were observed. (D) Microscopic view of the resected specimen (hematoxylin–eosin). Well-differentiated tubular adenocarcinoma with adenoma, pSM (1500 μ) with lymphatic permeation (ly1) but without nodal metastasis (N0).

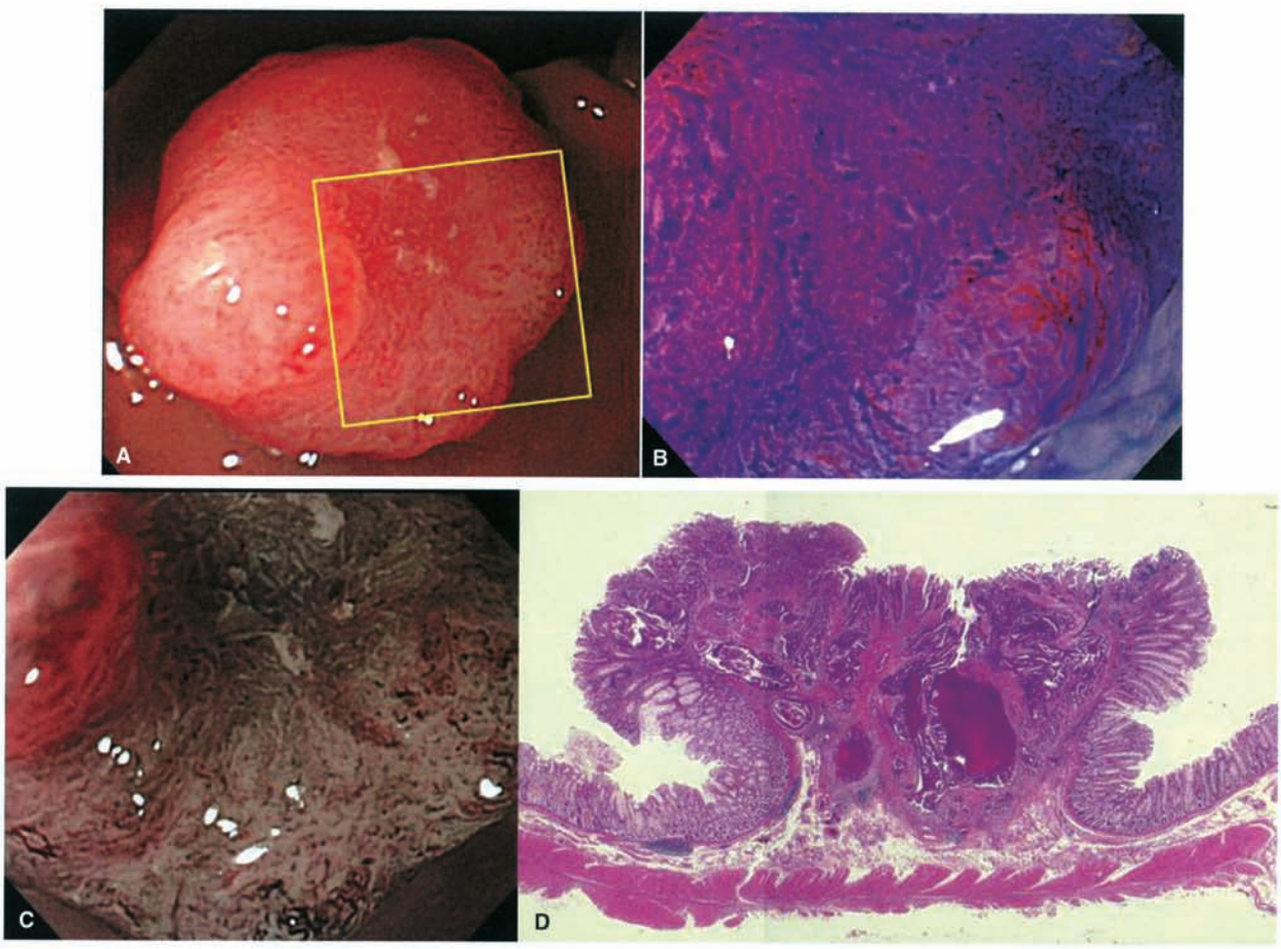


Fig. 5. Depressed lesion (IIa+IIc) size: 10 mm. (A) Conventional view. (B) Magnified endoscopic view after crystal violet dye staining. (C) Magnified endoscopic view with narrow band imaging system. The vessels were very thin and sparsely distributed. (D) Microscopic view of the resected specimen (hematoxylin–eosin). Well-differentiated tubular adenocarcinoma, pSM (3250 μ) with lymphatic permeation (ly1), vessel permeation (v2), nodal metastasis (N1) and liver metastasis.

Table 3. Differential diagnosis between hyperplastic polyp and neoplasia by vascular pattern

Vascular pattern	Pathological diagnosis		Total
	Hyperplastic polyp	Neoplasia	
Faint	47	21	68
Network / dense / irregular / sparse	6	1399	1405
Total	53	1420	1473

Table 4. Vascular patterns of submucosally invasive cancers

Vascular pattern	Pathological diagnosis		Total
	SMm	SMs	
Irregular / sparse	74	6	80
Network / dense	4	19	23
Total	78	25	103

SMm, massively invasive cancers; SMs, slightly invasive cancers.

efficiency of NBI in distinguishing neoplasia and non-neoplasia. Sano *et al.* reported that the NBI system may be sufficient to differentiate hyperplastic polyp from adenomatous polyp.[20,21] In this study, both NBI and chromoendoscopy could be useful for distinguishing between neoplastic and non-neoplastic lesions. Recently some reports have referred to the use of NBI in conjunction with magnification for predicting the invasive nature of the target lesions. Hirata *et al.* reported that NBI magnification was useful for the prediction of histological diagnosis and selection of therapeutic strategies of colorectal tumors.[22] East *et al.* reported that NBI is useful for finding dysplasia-associated lesions or masses and distinguishing dysplastic from non-dysplastic mucosa in ulcerative colitis.[23] We classified the vascular patterns of colorectal lesions into six distinct patterns and found that normal and faint patterns were characteristic of non-neoplasia. Network, dense, irregular, and sparse patterns were those in neoplasia. Irregular and sparse patterns were

Table 5. Comparison between pit pattern and pathological diagnosis

Pit pattern	Pathological diagnosis				Total
	Hyperplastic polyp	Adenoma	SMs	SMm	
II	46	12			58
IIIL	7	853	2		862
IIIs		14			14
IV		346	4	1	351
Vi low-grade		85	16	7	108
Vi high-grade		7	3	45	55
Vn				25	25
	53	1317	25	78	1473

SMm, massively invasive cancers; SMs, slightly invasive cancers.

Table 6. Differential diagnosis between hyperplastic polyp and neoplasia by pit pattern

Pit pattern	Pathological diagnosis		Total
	Hyperplastic polyp	Neoplasia	
II	46	12	58
IIIL / IIIs / IV / Vi / Vn	7	1408	1415
Total	53	1420	1473

Table 7. Pit patterns of submucosally invasive cancers

Vascular pattern	Pathological diagnosis		Total
	SMm	SMs	
Vn / Vi high-grade	70	3	73
Vi low-grade / IV / III	8	22	30
Total	78	25	103

SMm, massively invasive cancers; SMs, slightly invasive cancers.

useful for the diagnosis of SMm. Katagiri *et al.* classified the NBI findings of various colorectal lesions as 'capillary pattern (CP)' type I, II and III.[24] The corresponding histology in CP type I, II and III was predicted to be non-neoplasia, low-grade dysplasia and high-grade dysplasia to carcinoma, respectively. Recently, Ikematsu *et al.*[25] subclassified CP type III into IIIA and IIIB. They consider that CP type IIIA represents high-grade dysplasia to minimally invasive cancer and type IIIB corresponds to substantially invasive cancer. Kanao *et al.*[26] has recently classified the findings of NBI magnifying colonoscopy into A, B, C1, C2 and C3. Their classification seems to be based not only on the vascular pattern but also on the mucosal pattern. In the above-mentioned classifications and many other classifications in Japan the groups of vascular are expressed in numbers or letters whereas our classification utilizes a descriptive term for each group. Strictly speaking, the mucosal structure observed with NBI are not the pits themselves but also include the epithelial surface surrounding the opening of glands. Unlike Tanaka *et al.*,[26,27] we focus only on the vascular pattern and not on the mucosal pattern during the observation with NBI.

Now that we have an advanced technique for endoscopic resection, we can select the treatment option between surgical and endoscopic treatment according to the possibility of nodal/distant metastasis of the lesion. One of the most relevant factors for metastasis is the degree of invasion; SMs without vessel permeation almost never metastasizes, but SMm are associated with a significant possibility of metastasis.[9] Prediction of the invasion depth is very important for deciding a method of intervention. By recognizing an irregular or sparse pattern with NBI we could differentiate between SMm and SMs with sensitivity of 94.9% and specificity of 76.0%. Pit pattern type Vi high-grade or Vn pattern in chromoendoscopy is considered diagnostic for SMm in submucosal cancers, and we could differentiate between SMm and SMs with sensitivity of 89.7% and specificity of 88.0% (Table 4). The specificity was higher in the pit pattern than in the vascular pattern. The merits of NBI over chromoendoscopy are that it is easier and time-saving, but with the current diagnostic ability of NBI, we should also perform chromoendoscopy in addition to NBI for more precise diagnosis and appropriate selection of treatment.

In conclusion, both NBI and chromoendoscopy can be useful for distinguishing between neoplastic and non-neoplastic lesions. In the diagnosis of submucosal cancer, pit pattern diagnosis was slightly superior to vascular pattern diagnosis. It is desirable to perform chromoendoscopy in addition to NBI for distinguishing between SMs and SMm lesions and determining treatment selection, endoscopic or surgical.

REFERENCES

1. Kudo S, Hirota S, Nakajima T *et al.* Colorectal tumors and pit pattern. *J. Clin. Pathol.* 1994; **47**: 880–5.
2. Fu K-I, Sano Y, Kato S *et al.* Chromoendoscopy using indigo carmine dye spraying magnifying observation is the most reliable method for differential diagnosis between non-neoplastic and neoplastic colorectal lesions: A prospective study. *Endoscopy* 2004; **36**: 1089–93.
3. Tanaka S, Haruma K, Nagata S, Oka S, Chayama K. Diagnosis of invasion depth in early colorectal carcinoma by pit pattern analysis with magnifying endoscopy. *Dig. Endosc.* 2001; **13**: S2–5.
4. Gono K, Obi T, Yamaguchi M *et al.* Appearance of enhanced tissue features in narrow-band endoscopic imaging. *J. Biochemical. Opt.* 2004; **9**: 568–77.
5. Japanese Society for Cancer of the Colon and Rectum. *Japanese Society for Cancer of the Colon and Rectum*, 2nd English Edn. Tokyo: Kanehara & Co., 2009.
6. Lamber R, Lightdale C. The Paris endoscopic classification of superficial neoplastic lesions: Esophagus, stomach, and colon: November 30 to December 1, 2002. *Gastrointest. Endosc.* 2003; **58** (6 Suppl): S3–43.
7. Kudo S, Lambert R, Allen JI *et al.* Nonpolypoid neoplastic lesions of the colorectal mucosa. *Gastrointest. Endosc.* 2008; **68** (4 Suppl): S3–47.
8. Stanley R, Hamilton SR, Aaltonen LA (eds). *World Health Organization Classification of Tumors: Pathology and Genetics of Tumors of the Digestive System*. Lyon: International Agency for Research on Cancer, 2000; 103–119.
9. Kitajima K, Fujimori T, Fujii S *et al.* Correlations between lymph node metastasis and depth of submucosal invasion in

submucosal invasive colorectal carcinoma: A Japanese collaborative study. *J. Gastroenterol.* 2004; **39**: 534–43.
10. Wada Y, Kashida H, Ikehara N *et al.* The diagnosis of colorectal lesions with magnifying narrow band imaging system [abstract]. *Gastrointest. Endosc.* 2008; **67**: AB311–12.
11. Kashida H, Kudo S. Magnifying colonoscopy, early colorectal cancer, and flat adenomas. In: Waye JD, Rex DK, Williams CB (eds). *Colonoscopy: Principles and Practice*, 2nd edn. Malden, USA, Oxford, UK, Melbourne, Australia: Blackwell, 2009; 412–22.
12. Wada Y, Kudo S, Kashida H *et al.* The diagnosis of colorectal lesions with magnifying narrow-band imaging system. *Gastrointest. Endosc.* 2009; **70**: 522–31.
13. Kudo S, Tamura S, Nakajima T, Yamano H, Kusaka H, Watanabe H. Diagnosis of colorectal tumorous lesions by magnifying endoscopy. *Gastrointest. Endosc.* 1996; **44**: 8–14.
14. Tobaru T, Mitsuyama K, Tsuruta O, Kawano H, Sata M. Sub-classification of type VI pit patterns in colorectal tumors: Relation to the depth of tumor invasion. *Int. J. Oncol.* 2008; **33**: 503–8.
15. Kanao H, Tanaka S, Oka S *et al.* Clinical significance of type VI pit pattern subclassification in determining the depth of invasion of colorectal neoplasms. *World J. Gastroenterol.* 2008; **14**: 211–17.
16. Sano Y, Horimatsu T, Fu K-I *et al.* Magnified observation of architecture using narrow band imaging (NBI) for the differential diagnosis between non-neoplastic and neoplastic colorectal lesion. A prospective study. *Gastrointest. Endosc.* 2006; **63**: AB102.
17. Inoue T, Murano M, Murano N *et al.* Comparative study of conventional colonoscopy and pan-colonic narrow-band imaging system in the detection of neoplastic colonic polyps: A randomized controlled trial. *J. Gastroenterol.* 2008; **43**: 45–50.
18. Uraoka T, Saito Y, Matsuda T *et al.* Detectability of colorectal neoplastic lesions using a narrow-band imaging system: A pilot study. *J. Gastroenterol. Hepatol.* 2008; **23**: 1810–15.
19. Adler A, Pohl H, Papanikoloau IS *et al.* A prospective randomized study on narrow-band imaging versus conventional colonoscopy for adenoma detection: Does narrow-band imaging induce a learning effect? *Gut* 2008; **57**: 59–6420.
20. Sano Y, Horimatsu T, Fu KI, Katagiri A, Muto M, Ishikawa H. Optical/digital chromoendoscopy during colonoscopy using narrow-band imaging system. *Dig. Endosc.* 2005; **17**: 43–8.
21. Sano Y, Horimatsu T, Fu KI *et al.* Magnifying observation of microvascular architecture of colorectal lesions using a narrow-band imaging system. *Dig. Endosc.* 2006; **18**: S44–S51.
22. Hirata M, Tanaka S, Oka S *et al.* Evaluation of microvessels in colorectal tumors by narrow band imaging. *Gastrointest. Endosc.* 2008; **66**: 945–52.
23. East JE, Suzuki N, von Herbay A, Saunders BP. Narrow band imaging with magnification for dysplasia detection and pit pattern assessment in ulcerative colitis surveillance: A case with multiple dysplasia associated lesions or masses. *Gut* 2006; **55**: 1432–5.
24. Katagiri A, Fu K-I, Sano Y *et al.* Narrow band imaging with magnifying colonoscopy as diagnostic tool for predicting histology of early colorectal neoplasia. *Aliment. Pharmacol. Ther.* 2008; **27**: 1269–74.
25. Ikematsu H, Kaneko K, Fukuda D *et al.* Accuracy of capillary pattern classification using NBI magnification for diagnosis of colorectal lesions. *Gastrointest. Endosc.* 2008; **67**: AB311.
26. Kanao H, Tanaka S, Oka S, Hirata M, Yoshida S, Chayama K. Narrow-band imaging magnification predicts the histology and invasion depth of colorectal tumors. *Gastrointest. Endosc.* 2009; **69**: 631–27.
27. Tanaka S, Oka S, Hirata M, Yoshida S, Kaneko I, Chayama K. Pit pattern diagnosis for colorectal neoplasia using narrow band imaging magnification. *Dig. Endosc.* 2006; **18**: S52–6.

J Gastroenterol (2010) 45:1212–1218
DOI 10.1007/s00535-010-0288-3

ORIGINAL ARTICLE—ALIMENTARY TRACT

Detection of desmoplastic reaction in biopsy specimens is useful for predicting the depth of invasion of early colorectal cancer: a Japanese collaborative study

Motohiko Hirose · Hirokazu Fukui · Yoshinori Igarashi · Yukari Fujimori · Yoshinori Katake · Akira Sekikawa · Kazuhito Ichikawa · Shigeki Tomita · Johji Imura · Yoichi Ajioka · Hideki Ueno · Kazuo Hase · Yasuo Ohkura · Hiroshi Kashida · Kazutomo Togashi · Takashi Nishigami · Toshiyuki Matsui · Takashi Yao · Ryo Wada · Keiji Matsuda · Toshiaki Watanabe · Atsushi Ochiai · Tamotsu Sugai · Kenichi Sugihara · Takahiro Fujimori

Received: 21 January 2010 / Accepted: 21 June 2010 / Published online: 28 July 2010

Abstract
Background We have previously demonstrated a relationship between the depth of submucosal invasion (SM depth) and the frequency of lymph node metastasis in resected submucosal invasive colorectal cancers (SICRCs). Here, we assessed the desmoplastic reaction (DR) in pretreatment biopsy specimens of SICRC to predict the SM depth.

Methods A total of 359 patients with SICRCs, who had undergone surgical or endoscopic mucosal resection, were enrolled. The SM depth of the SICRC lesions was evaluated according to the procedure established by the Japanese Society for Cancer of the Colon and Rectum, and the patients' corresponding pretreatment biopsy specimens were examined histologically to evaluate the prevalence of DR.

M. Hirose · H. Fukui · Y. Fujimori · Y. Katake · A. Sekikawa · K. Ichikawa · S. Tomita · J. Imura · T. Fujimori (✉)
Department of Surgical and Molecular Pathology, Dokkyo University School of Medicine, 880 Kitakobayshi, Mibu, Shimotsuga, Tochigi 321-0293, Japan
e-mail: t-fuji@dokkyomed.ac.jp

M. Hirose · Y. Igarashi
Division of Gastroenterology and Hepatology, Department of Internal Medicine, Toho University Omori Medical Center, Tokyo, Japan

Y. Ajioka
Division of Molecular and Functional Pathology, Department of Cellular Function, Graduate School of Medical and Dental Sciences, Niigata University, Niigata, Japan

H. Ueno · K. Hase
Department of Surgery, National Defense Medical College, Tokorozawa, Japan

Y. Ohkura
Department of Pathology, Kyorin University School of Medicine, Mitaka, Japan

H. Kashida
Digestive Disease Center, Showa University Northern Yokohama Hospital, Yokohama, Japan

K. Togashi
Division of Endoscopy, Department of Surgery, Jichi Medical University, Tochigi, Japan

T. Nishigami
Department of Pathology, Hyogo College of Medicine, Nishinomiya, Japan

T. Matsui
Department of Gastroenterology, Fukuoka University Chikushi Hospital, Fukuoka, Japan

T. Yao
Department of Human Pathology, Juntendo University School of Medicine, Tokyo, Japan

R. Wada
Department of Pathology, Juntendo Shizuoka Hospital of Juntendo University School of Medicine, Shizuoka, Japan

K. Matsuda · T. Watanabe
Department of Surgery, Teikyo University School of Medicine, Tokyo, Japan

A. Ochiai
Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Japan

T. Sugai
Division of Diagnostic Molecular Pathology, Department of Pathology, Iwate Medical University, Morioka, Japan

K. Sugihara
Department of Surgical Oncology, Tokyo Medical and Dental University, Tokyo, Japan

Results For pedunculated SICRCs, the prevalence of DR in pretreatment biopsy specimens was significantly higher in moderately differentiated than in well-differentiated adenocarcinomas, but was not significantly related to SM depth. For nonpedunculated SICRCs, the prevalence of DR in pretreatment biopsy specimens was significantly related to histological type, tumor size, and SM depth. When nonpedunculated SICRCs were further divided using a specific cutoff value of 1000 μm for SM depth, the DR positivity rate in pretreatment biopsy specimens was significantly higher in SICRCs with an SM depth of ≧1000 μm (termed "SM massive CRCs") than in cases where the SM depth was <1000 μm (termed "SM slight CRCs").
Conclusions Detection of DR in pretreatment biopsy specimens is useful for the prediction of SM depth in nonpedunculated SICRCs, and may be useful for the selection of such cases that would be treatable by endoscopic mucosal resection and endoscopic submucosal dissection (EMR/ESD).

Keywords Desmoplastic reaction · Early colorectal cancer · Submucosal invasion · Biopsy

Introduction

Early colorectal cancer (CRC) is defined as a tumor whose invasion is limited to the mucosa or submucosa. Following recent advances in endoscopic instruments and techniques, endoscopic mucosal resection and endoscopic submucosal dissection (EMR/ESD) have become applicable for early CRC, thus avoiding surgery and allowing a good quality of life to be maintained. However, EMR is applicable only to intramucosal carcinoma, and additional surgery is required if the resected lesion reveals submucosal invasion histologically, as submucosal invasion may suggest the presence of lymph node metastasis [1–3], which significantly affects the prognosis of patients with CRC [4–7]. However, the prevalence of lymph node metastasis is only about 10% in patients with submucosal invasive CRC (SICRC) [1, 5, 8], suggesting that most of such patients do not require additional surgery after EMR if the lesions are completely resected. In this context, we have recently demonstrated that the depth of submucosal invasion (SM depth) is closely correlated with the prevalence of lymph node metastasis in patients with SICRC [1]. Therefore, we considered that it would be very useful to predict the depth of invasion of SICRC before EMR in order to precisely select cases that are suitable for EMR.

Desmoplastic reaction (DR), which is characterized by the infiltration of eosinophilic myofibroblasts in the stroma of invasive carcinoma [9, 10], is suggested to be a prognostic marker in CRC patients [11–13]. Indeed, from the viewpoint of pathology, the grade of DR in advanced CRC is positively correlated with invasion, although its significance in early CRC still remains unclear [12]. Interestingly, Nakada et al. [14] have preliminarily reported that the presence of DR in the surface of resected early CRC predicts deep invasion into the submucosa. Accordingly, in the present larger-scale study of SICRC, we investigated the relationship between SM depth and the presence of DR in biopsy specimens before treatment, and clarified whether the presence of DR in such specimens would be useful for the prediction of SM depth.

Methods

Patients and specimens

Fifteen institutions (Cancer Institute Hospital, Tokyo Medical and Dental University, Fukuoka University Chikushi Hospital, Niigata University, National Cancer Center Hospital East, Juntendo University, Teikyo University, Kyorin University, Hyogo College of Medicine, Showa University Northern Yokohama Hospital, National Defense Medical College, Jichi Medical University, Iwate Medical University, Toho University Omori Medical Center, and Dokkyo University School of Medicine) affiliated with the Japanese Society for Cancer of the Colon and Rectum participated in this collaborative study. In total, 359 SICRCs and their corresponding pretreatment biopsy specimens, obtained from 359 patients who had undergone surgical resection or EMR at these institutions, were analyzed retrospectively. Factors such as age, sex, and tumor location were determined for all patients by reference to the hospital records.

Histology

The examined SICRCs were classified macroscopically into pedunculated and nonpedunculated types, as previously reported [1]. The resected colonic tissues were cut for evaluating SM depth; the evaluation was done according to the *General rules for clinical and pathological studies on cancer of the colon, rectum and anus* of the Japanese Society for Cancer of the Colon and Rectum [15]. EMR specimens were cut at 2-mm intervals. On the other hand, surgically resected colonic tissues were cut along the long axis at 2- to 4-mm intervals. Pathological examinations were performed on sections stained with hematoxylin and eosin. The histological type in the deepest invasive portion was classified as well, moderately, or poorly differentiated adenocarcinoma in accordance with the World Health Organization classification [16]. SM depth was measured

according to the *General rules for clinical and pathological studies on cancer of the colon, rectum and anus* of the Japanese Society for Cancer of the Colon and Rectum [15]. The histological findings of DR were evaluated by pathologists at each respective institute. The presence of DR in pretreatment biopsy specimens was evaluated according to the findings described previously [15, 17]. A representative micrograph of a biopsy specimen with DR is shown in Fig. 1.

Statistical analysis

We performed χ^2analyses to determine the correlations between various pathological parameters, and Fisher's exact test was also used as necessary. SM depth was expressed as mean ± SEM, and the significance of differences between the DR-positive and DR-negative groups was assessed using Student's *t*-test at a significance level of $P < 0.05$.

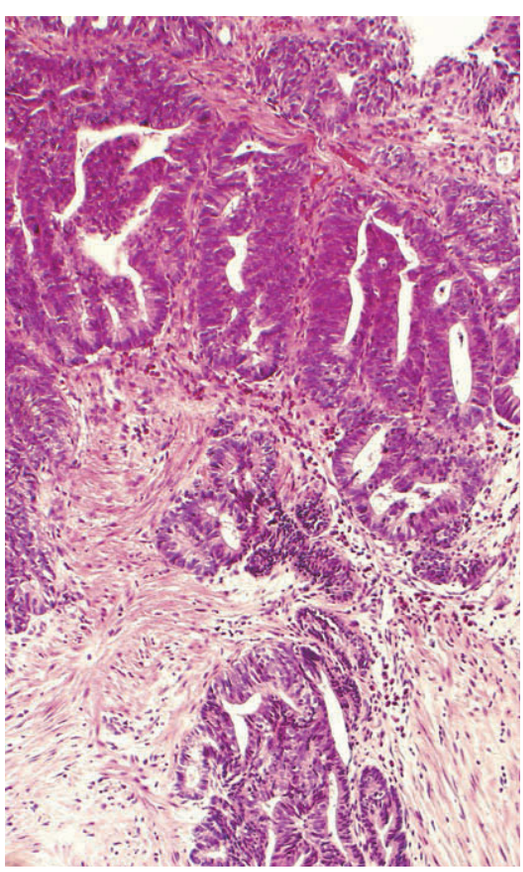


Fig. 1 Microphotograph showing the growth of eosinophilic spindle cells (myofibroblasts) in the submucosal invasive carcinoma component (H&E, ×100)

Results

Clinicopathological features of SICRCs

The clinicopathological features of the 359 patients (137 males, 222 females, mean age 65.2 ± 0.6 years) with SICRCs are summarized in Table 1. With regard to tumor location, 136 lesions (37.9%) occurred in the rectum and 105 (29.2%) in the sigmoid colon. With regard to macroscopic appearance, 32 lesions (8.9%) were pedunculated and 327 were non-pedunculated. DR was detectable in pretreatment biopsy specimens from 214 patients (59.6%). Mean tumor size was 21.5 ± 0.6 mm, and mean SM depth was 3193.6 ± 133.7 μm.

Relationship between clinicopathological features and presence of DR in biopsy specimens from patients with SICRC

According to the macroscopic type of SICRC, we divided the patients into two groups—those with pedunculated and non-pedunculated tumors, respectively—and investigated

Table 1 Clinicopathological features of 359 patients with submucosal invasive colorectal cancers (SICRCs)

Clinicopathological features	Number of patients (%)
Sex	
Male	137 (38.2)
Female	222 (61.8)
Tumor location	
Rectum	136 (37.9)
Sigmoid	105 (29.2)
Descending	19 (5.3)
Transverse	30 (8.4)
Ascending	51 (14.2)
Cecum	18 (5.0)
Histological type	
Well-differentiated	280 (78.0)
Moderately differentiated	76 (21.2)
por, sig	3 (0.8)
Macroscopic type	
Pedunculated	32 (8.9)
Nonpedunculated	327 (91.1)
Tumor size (mm)	21.5 ± 0.6
Depth of submucosal invasion (μm)	3193.6 ± 133.7 (0–25000)
Desmoplastic reaction in biopsy specimen	
Positive	214 (59.6)
Negative	145 (40.4)

por poorly differentiated; *sig* signet-ring cell

Table 2 Relationship between clinicopathological features and desmoplastic reaction (DR) in biopsy specimens of 32 patients with pedunculated SICRCs

Clinicopathological features	DR-negative (%) ($n = 16$)	DR-positive (%) ($n = 16$)	P value
Sex			
Male	8 (40.0)	12 (60.0)	0.144
Female	8 (66.7)	4 (33.3)	
Tumor location			
Rectum	3 (42.9)	4 (57.1)	
Sigmoid	9 (50.0)	9 (50.0)	
Descending	1 (25.0)	3 (75.0)	0.387
Transverse	1 (100)	0 (0.0)	
Ascending	2 (100)	0 (0.0)	
Cecum	0 (0.0)	0 (0.0)	
Histological type			
Well-differentiated	16 (64.0)	9 (36.0)	
Moderately differentiated	0 (0.0)	7 (100)	0.003
por, sig	0 (0.0)	0 (0.0)	
Tumor size (mm)	28.9 ± 6.0	23.8 ± 4.9	0.508
Depth of submucosal invasion (μm)	3320.6 ± 659.6	3139.4 ± 724.4	0.855

por poorly differentiated; *sig* signet-ring cell

Table 3 Relationship between clinicopathological features and DR in biopsy specimens of 327 patients with nonpedunculated SICRCs

Clinicopathological features	DR-negative (%) ($n = 129$)	DR-positive (%) ($n = 198$)	P value
Sex			
Male	88 (43.6)	114 (56.4)	0.053
Female	41 (32.8)	84 (67.2)	
Tumor location			
Rectum	45 (34.9)	84 (65.1)	
Sigmoid	29 (33.3)	58 (66.7)	
Descending	6 (40.0)	9 (60.0)	0.149
Transverse	15 (51.7)	14 (48.3)	
Ascending	24 (49.0)	25 (51.0)	
Cecum	10 (55.6)	8 (44.4)	
Histological type			
Well-differentiated	110 (43.1)	145 (56.9)	
Moderately differentiated	18 (26.1)	51 (73.9)	0.061
por, sig	1 (33.3)	2 (66.7)	
Tumor size (mm)	23.4 ± 1.1	19.5 ± 0.6	0.001
Depth of submucosal invasion (μm)	2732.6 ± 197.4	3488.0 ± 187.9	0.008

por poorly differentiated; *sig* signet-ring cell

the relationship between clinicopathological features and DR positivity in biopsy specimens separately.

DR was detectable in pretreatment biopsy specimens from 16 patients with pedunculated SICRCs (50.0%; Table 2), and the prevalence of DR positivity was significantly higher in moderately differentiated than in well-differentiated adenocarcinomas (100 and 36.0%, respectively; $P < 0.05$). However, none of the other factors, including age, sex, tumor size, tumor location, or SM depth, was significantly related to DR positivity in the pretreatment biopsy specimens of pedunculated SICRCs.

Analysis of patients with nonpedunculated SICRCs (Table 3) showed that the prevalence of DR was significantly related to histological type (moderately differentiated > well-differentiated; $P = 0.0102$). In this group, tumors for which the biopsy specimens were positive for DR were significantly smaller, whereas SM depth was significantly greater.

Relationship between depth of submucosal invasion and presence of DR in biopsy specimens from patients with SICRC

We further investigated the detection rate of DR in biopsy specimens in relation to SM depth. The number of pedunculated SICRCs was small ($n = 32$), and in those specimens we found no tendency for an association between the SM depth and the DR detection rate (Table 4). However, in non-pedunculated SICRCs, we found that the DR detection rate increased rapidly when the SM depth exceeded 2000 μm. Therefore, in relation to the DR detection rate in biopsy specimens, we attempted to establish a significant cutoff value (COV) for SM depth in nonpedunculated SICRCs. The patients with nonpedunculated SICRCs were divided into two groups—a "shallow" group and a "deep" group—using a specific COV for submucosal invasion, and then we compared the rates of DR positivity in their pretreatment biopsy specimens (Table 5). For a COV of up to 3000 μm, the rate of DR positivity was significantly lower in the "shallow" group. When considering EMR/ESD treatment for SICRCs, it is important to bear in mind that an SM depth of ≧1000 μm is associated with a high risk of lymph node metastasis [1]. We found that the DR positivity rate was significantly higher in SICRCs with an SM depth of ≧1000 μm (termed "SM massive CRCs") than in cases where the SM depth was <1000 μm (termed "SM slight CRCs"). The positive predictive value that the prevalence of DR predicts an SM massive CRC was 91.9%, and the negative predictive value that the absence of DR predicts an SM slight CRC was 23.3%.

Discussion

In the present study, we clarified that nonpedunculated SICRCs showing DR in biopsy samples had deeper SM invasion than DR-negative cases. Moreover, detailed analyses revealed that when the COV for SM depth was

Table 4 Relationship between depth of submucosal invasion and DR in biopsy specimens of patients with SICRCs

Depth of submucosal invasion (μm)	Pedunculated SICRC		Nonpedunculated SICRC	
	DR (−) (%)	DR (+) (%)	DR (−) (%)	DR (+) (%)
$X < 1000$	3 (37.5)	5 (62.5)	30 (65.2)	16 (34.8)
$1000 \leq X < 2000$	1 (50.0)	1 (50.0)	25 (56.8)	19 (43.2)
$2000 \leq X < 3000$	5 (100)	0 (0.0)	22 (27.5)	58 (72.5)
$3000 \leq X < 4000$	3 (33.3)	6 (66.7)	20 (29.9)	47 (70.1)
$4000 \leq X < 5000$	0 (0.0)	1 (100)	11 (31.4)	24 (68.6)
$5000 \leq X < 6000$	1 (100)	0 (0.0)	10 (43.5)	13 (56.5)
$6000 \leq X < 7000$	2 (100)	0 (0.0)	5 (55.6)	4 (44.4)
$7000 \leq X < 8000$	0 (0.0)	2 (100)	0 (0.0)	4 (100)
$8000 \leq X < 9000$	0 (0.0)	0 (0.0)	4 (44.4)	5 (55.6)
$9000 \leq X < 10000$	0 (0.0)	0 (0.0)	0 (0.0)	4 (100)
$10000 \leq X$	1 (50.0)	1 (50.0)	2 (33.3)	4 (66.7)

Table 5 Cutoff values for the depth of submucosal invasion and DR positivity in biopsy specimens of 327 patients with nonpedunculated SICRCs

Cutoff value (μm)	Shallow (<) DR-positive rate (*n*)	Deep (≥) DR-positive rate (*n*)	*P* value
1000	34.8 (46)	64.8 (281)	0.0001
2000	38.9 (90)	68.8 (237)	<0.0001
3000	54.7 (170)	66.9 (157)	0.0244
4000	59.1 (237)	64.4 (90)	0.3746
5000	60.3 (272)	61.8 (55)	0.8330
6000	60.0 (295)	65.6 (32)	0.5363
7000	59.5 (304)	73.9 (23)	0.1739
8000	60.1 (308)	68.4 (19)	0.4695
9000	59.9 (317)	80.0 (10)	0.2012
10000	60.4 (321)	66.7 (6)	0.7570

1000–3000 μm, the rate of DR positivity was significantly higher in nonpedunculated SICRCs with "deep" SM invasion, whereas the rate showed no significant difference between tumors with "deep" and "shallow" SM invasion when the COV for SM depth was more than 4000 μm. These findings suggest that the detection of DR in pretreatment biopsy specimens may be useful for the prediction of SM depth, especially in nonpedunculated SICRCs with slight submucosal invasion, for which EMR/ESD treatment may be applicable. However, if DR is used as a predictor of SM invasion in practice, we favor a COV of 1000 μm, because the rate of lymph node metastasis in SICRCs begins to increase when the SM depth exceeds 1000 μm [1].

In contrast to nonpedunculated SICRCs, we found no significant relationship between the SM depth and the DR positivity rate in patients with pedunculated SICRCs. Although it is difficult to explain this finding, we consider that several problems with DR evaluation using biopsy samples from pedunculated SICRCs may be responsible. For example, if a tumor has cancerous invasion into the stalk but its surface is covered with noncancerous components (adenoma or non-neoplastic epithelium), a biopsy sample may miss the area of cancerous invasion, yielding a false-negative result suggestive of stalk invasion with DR negativity. On the other hand, it is not possible to exclude false positivity suggestive of SM invasion with DR positivity in pedunculated SICRCs. The growth of myofibroblasts is affected not only by tumor progression but also by inflammatory or mechanical stimulation, and indeed, pedunculated colorectal tumors (such as pedunculated SICRCs [18] or polyps in juvenile polyposis [19], Peutz-Jeghers syndrome [20], and mucosal prolapse syndrome [21]) tend to have abundant myofibroblasts around the muscularis mucosae, regardless of histological malignancy [22]. In this context, the evaluation of DR in biopsy samples may not be useful for predicting the depth of invasion in pedunculated SICRCs. However, because the detection of DR is evidently predictive for the depth of invasion in nonpedunculated SICRCs, it appears to be important to divide SICRC lesions into pedunculated and nonpedunculated types.

We have recently investigated the relationship between SM depth and the prevalence of lymph node metastasis in patients with SICRC, and clarified that nonpedunculated SICRCs with an SM depth of <1000 μm (termed "SM slight CRCs") have no lymph node metastasis [1]. However, although such data are very useful for deciding whether EMR or surgery should be used to treat SICRC lesions, it is not possible to measure the SM depth in biopsy samples before treatment. Therefore, in the present study, we attempted to establish a method for the prediction of SM depth using biopsy samples from SICRC lesions. DR is frequently observed in gastrointestinal tumors [23, 24], and is thought to start increasing when carcinoma cells have invaded beyond the muscularis mucosae [17], suggesting that DR may be a good marker for predicting the depth of SM invasion in SICRCs. Indeed, in the present study,

we clearly demonstrated that the presence of DR was significantly correlated with SM depth in nonpedunculated SICRCs. Although it is still unclear whether DR represents a form of host defense against tumor invasion or metastasis [25, 26], the initiation of DR may be associated with destruction of the muscularis mucosae [17]. In support of this idea, DR is hardly ever observed in intramucosal carcinoma [14], but is known to be involved in the biological changes in the muscularis mucosae during tumor invasion [27, 28]. From the present data, together with the findings of these studies [17, 27, 28], it may be reasonable to suggest that DR has potential as a marker for predicting the depth of invasion of SICRCs.

In conclusion, the detection of DR in pretreatment biopsy specimens may be useful for the prediction of SM depth in nonpedunculated SICRCs that are potential candidates for EMR treatment. On the basis of the present findings, a collaborative prospective study of DR has been started in Japan. In the present study, we found that the detection of DR was not useful in pedunculated SICRCs and that the absence of DR in a biopsy specimen may not always indicate a submucosal invasion of <1000 μm in nonpedunculated SICRCs. Although these findings should be reconfirmed in a prospective study on a larger scale, we wish to emphasize that in endoscopic examinations it is important to divide SICRC lesions into pedunculated and nonpedunculated types accurately. Besides, we found it important to target an adequately representative cancerous portion for biopsy and, therefore, magnifying endoscopy may be recommended in future. Endoscopists with high skill levels might be able to predict the SM depth using magnifying endoscopy for endoscopic treatment, thus avoiding biopsy. However, the aim of this project is to establish an easy method for general endoscopists to predict the SM depth in SICRCs. Finally, we expect that an ongoing prospective study of DR will yield further details about the significance of DR in pretreatment biopsy specimens from SICRC patients.

Acknowledgments We thank Drs. Shigehiko Fujii (Kyoto-Katsura Hospital), Takahiro Fujii (TF Clinic), Yasushi Sano (Gastrointestinal Center, Sano Hospital), Shinji Tanaka (Department of Endoscopy, Hiroshima University), and Tetsuichiro Muto (Cancer Institute Hospital) for their valuable advice. This study was supported by the Japanese Society for Cancer of the Colon and Rectum.

Conflict of interest No conflicts of interest exist.

References

1. Kitajima K, Fujimori T, Fujii S, Takeda J, Ohkura Y, Kawamata H, et al. Correlations between lymph node metastasis and depth of submucosal invasion in submucosal invasive colorectal carcinoma: a Japanese collaborative study. J Gastroenterol. 2004; 39:534–43.
2. Coverlizza S, Risio M, Ferrari A, Fenoglio-Preiser CM, Rossini FP. Colorectal adenomas containing invasive carcinoma: pathologic assessment of lymph node metastatic potential. Cancer. 1989;64:1937–47.
3. Nivatvongs S, Rojanasakul A, Reiman HM, Dozois RR, Wolff BG, Pemberton JH, et al. The risk of lymph node metastasis in colorectal polyps with invasive adenocarcinoma. Dis Colon Rectum. 1991;34:323–8.
4. Netzer P, Forster C, Biral R, Ruchti C, Neuweiler J, Stauffer E, et al. Risk factor assessment of endoscopically removed malignant polyps. Gut. 1998;43:669–74.
5. Wang HS, Liang WY, Lin TC, Chen WS, Jiang JK, Yang SH, et al. Curative resection of T1 colorectal carcinoma: risk of lymph node metastasis and long-term prognosis. Dis Colon Rectum. 2005;48:1182–92.
6. Hassan C, Zullo A, Risio M, Rossini FP, Morini S. Histologic risk factors and clinical outcome in colorectal malignant polyp: a pooled-data analysis. Dis Colon Rectum. 2005;48:1588–96.
7. Berger AC, Sigurdson ER, LeVoyer T, Hanlon A, Mayer RJ, Macdonald JS, et al. Colon cancer survival is associated with decreasing ratio of metastatic to examined lymph nodes. J Clin Oncol. 2005;23:8706–12.
8. Kawamura YJ, Sakuragi M, Togashi K, Okuda M, Nagai H, Konishi F. Distribution of lymph node metastasis in T1 sigmoid colon carcinoma. Scand J Gastroenterol. 2005;40:858–61.
9. Schurch W, Seemayer TA, Gabbiani G. Myofibroblasts. In: Sternberg SS, editor. Histology for pathologists. 2nd ed. Philadelphia: Lippincott-Raven Publishers; 1997. p. 129–65.
10. Martin M, Pujuguet P, Martin F. Role of stromal myofibroblast. Pathol Res Pract. 1996;192:708–11.
11. Halvorsen TB, Seim EVA. Association between invasiveness, inflammatory reaction, desmoplasia and survival in colorectal cancer. J Clin Pathol. 1989;42:162–6.
12. Sis B, Sarioglu S, Sokmen S, Sakar M, Kupelioglu A, Fuzun M. Desmoplasia measured by computer assisted image analysis: an independent prognostic marker in colorectal carcinoma. J Clin Pathol. 2005;58:32–8.
13. Tsujino T, Seshimo I, Yamamoto H, Ngan CY, Ezumi K, Takemasa I, et al. Stromal myofibroblasts predict disease recurrence for colorectal cancer. Clin Cancer Res. 2007;13:2082–90.
14. Nakada I, Tasaki T, Ubukata H, Goto Y, Watanabe Y, Sato S, et al. Desmoplastic response in biopsy specimens of early colorectal carcinoma is predictive of deep submucosal invasion. Dis Colon Rectum. 1998;41:896–900.
15. Japanese Society for Cancer of the Colon and Rectum. General rules for clinical and pathological studies on cancer of the colon, rectum and anus. 7th ed., revised version. Tokyo: Kanehara; 2009. p. 41–2.
16. Jass JR, Sobin LH. Histological typing of intestinal tumors. In: World Health Organization, editor. International histological classification of tumors. 2nd ed. Berlin: Springer; 1989. p. 29–40.
17. Ohtani H, Sasano N. Stromal cell changes in human colorectal adenomas and carcinomas: an ultrastructural study of fibroblasts, myofibroblasts, and smooth muscle cells. Virchows Arch. 1983;401:209–22.
18. Yao T, Tsuneyoshi M. Significance of pericryptal fibroblasts in colorectal epithelial tumors: a special reference to the histologic features and growth patterns. Hum Pathol. 1993;24:525–33.
19. Jacoby RF, Schlack S, Cole CE, Skarbek M, Harris C, Meisner LF. A juvenile polyposis tumor suppressor locus at 10q22 is deleted from nonepithelial cells in the lamina propria. Gastroenterology. 1997;112:1398–403.
20. Kinzler KW, Vogelstein B. Landscaping the cancer terrain. Science. 1998;280:1036–7.

21. Brosens LAA, Montgomery EA, Bhagavan BS, Offerhaus GJA, Giardiello FM. Mucosal prolapse syndrome presenting as rectal polyposis. J Clin Pathol. 2009;62:1034–6.
22. Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts. II. Intestinal subepithelial myofibroblasts. Am J Physiol. 1999;277:C183–201.
23. Tiitta O, Shipponen P, Gould V, Virtanen I. Tenascin expression in inflammatory, dysplastic and neoplastic lesions of the human stomach. Virchows Arch. 1994;425:369–74.
24. Yoshiro M, Chung YS, Kubo T, Hato F, Sowa M. Differential responses of scirrhous and well-differentiated gastric cancer cells to orthotopic fibroblasts. Br J Cancer. 1996;74:1096–103.
25. Hewitt RE, Desmond G, Powe G, Carter I, Turner DR. Desmoplasia and its relevance to colorectal tumour invasion. Int J Cancer. 1993;53:62–9.
26. Conti JA, Kendall TJ, Bateman A, Armstrong TA, Papa-Adams A, Xu Q, et al. The desmoplastic reaction surrounding hepatic colorectal adenocarcinoma metastases aids tumor growth and survival via α_v integrin ligation. Clin Cancer Res. 2008; 14:6405–13.
27. Martin M, Pujuguet P, Martin F. Role of stromal myofibroblasts infiltrating colon cancer in tumor invasion. Pathol Res Pract. 1996;192:712–7.
28. Pujuguet P, Hammann A, Moutet M, Samuel JL, Martin F, Martin M. Expression of fibronectin ED-A+ abd ED-B+ isoforms by human and experimental colorectal cancer. Contribution of cancer cells and tumor-associated myofibroblasts. Am J Pathol. 1996;148:579–92.


週刊朝日MOOK
抗菌加工
本誌の表紙は、抗菌加工を施してあります。
がん、心臓病、脳疾患、放射線治療、
整形外科、眼、耳の病気など
疾病別のトップ病院を総力取材
全国4961
医療機関の
手術数一覧
執刀医、治療医名リスト4030人
手術数でわかる
いい病院
2010
全国&地方別データブック
信頼のランキングデータ
全国トップ977病院、
地方別トップ
1925病院を大公開
●特別対談
がん専門トップ病院の院長が語る
日本のがん医療はどこに向かうのか?
癌研有明病院名誉院長・武藤徹一郎医師×
国立がんセンター中央病院院長・土屋了介医師
●特別企画
編集部が独自に選んだ
わが街の「いい病院」
恵佑会札幌病院、仙台厚生病院、
県立静岡がんセンター、大阪市立大学病院、
厚生連佐久総合病院、済生会熊本病院
本誌は収益の一部を
日本の対がん活動のために
寄付します
朝日新聞出版

手術に代わる、からだにやさしい治療として普及

肝がんの治療法として急速に普及しているのがラジオ波だ。電極のついた針から放出される熱によってがん細胞を死滅させるもので、完全に焼き切れば根治も可能だ。

ラジオ波は手術のように開腹する必要がないため、からだの負担が小さい。そのため、肝切除術に代わる治療法として注目されている。

また、肝機能が不十分で手術ができない患者や再発した場合にも、繰り返し治療できるのが利点の一つだ。肝切除術の場合、術後に再発して再び手術ができるのは10～30％といわれているが、ラジオ波の場合は、89％（東京大学病院のデータ）の人が再びラジオ波で治療することができるという。

肝がんは、一度治療をしても再発を起こしやすいという特徴を持つ。そのため、再発時の治療法も重要な点になる。そのため、アルコールを注入してがん細胞を死滅させる「エタノール注入療法（ＰＥＩＴ）」や「肝動脈化学塞栓術（ＴＡＣＥ）」などとともに、からだへの負担が小さい内科的な治療法としてラジオ波が採り入れられてきた。

日本肝癌研究会刊行の『第17回全国原発性肝癌追跡調査報告』によると、ラジオ波で治療した肝細胞がん（５４７８例）の５年生存率は57・３％。肝切除術（２万７０６２例）の５年生存率53・４％と比べて変わらない結果となっている。

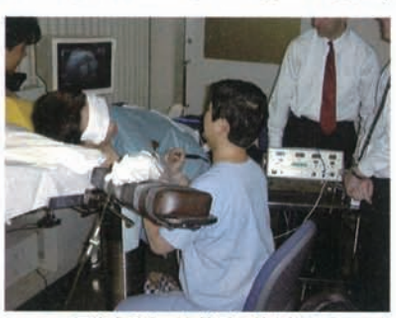
手軽に受けられる印象のラジオ波だが、今回もトップとなった**東京大学病院**消化器内科講師の椎名秀一朗医師は次のように話す。

「ラジオ波は簡単そうにみえるため、訓練なしに安易に実施している場合も少なくありません。しかし、手術以上に技術の差がある治療法なので、病院によって成績が大きく異なります」

東京大学病院は、これまでラジオ波を通算で約６千例実施。治療数は世界で最多数を誇る。

「原則としてラジオ波は、がんの大きさが３㌢以下、３個までが適応（治療の条件）とされていますが、厳密なものではなく、医師の経験による部分が非常に大きい治療といえます」（椎名医師）

治療成績に差が出やすいラジオ波だが、２位の**近畿大学病院**消化器内科教授の工藤正俊医師は、基本の大切さについて強調する。



エコー画像を見ながら慎重に針を押し込んでいく。治療時間は40分から1時間程度

「ラジオ波の基本は、まず超音波で腫瘍を描出できること、そして、（２～２・５㌢ほどの小さながんの場合）腫瘍のど真ん中に確実に針を刺せることです。単に超音波で見えている部分だけを治療するのでは焼き残しが出ます。というのも、肝がんで２㌢を超えるものは腫瘍の周囲５㍉以内に、小さな転移がすでに存在することがわかっているからです。だから、ＣＴ（コンピューター断層撮影）上、がんが消えているように見えていても、その周囲を十分に焼き切れていない場合、再発を起こすのです」

工藤医師は、造影剤（ソナゾイド）を使った独自の超音波造影法を編み出し、それまで超音波では見えなかった数㍉台の小さながんを映し出すことができるようになった。これによって、ラジオ波では困難といわれたごく早期のがんも治療することが可能となった。

「３㌢以下のがんの場合、再発率はラジオ波のほうが高いですが、５年生存率は肝切除術もラジオ波も変わりません。ラジオ波は肝機能が多少落ちていても、あるいは腫瘍の大きさや個数がある程度多くてもカバーしてくれますが、私は決してラジオ波にこだわっているわけではありません。肝機能がよくて３㌢を超える場合は、がん

写真提供：椎名秀一朗医師（東京大学病院）

国内の治療数は、年間約3万8千件にものぼる

肝がんラジオ波焼灼術

肝がんはC型やB型肝炎ウイルスに感染することで起こる肝炎や肝硬変などが原因で発症するため、手術で肝臓を切除したとしても再発を繰り返しやすいのが特徴だ。肝臓自体に肝硬変などの病変があるため肝機能が悪い人が多く、肝がんと診断された患者で手術を受けられるのは3割程度だ。

肝がんの三大治療は「肝切除術」「ラジオ波焼灼術」「肝動脈塞栓術」で、がんの大きさやできた場所、肝機能の状態などによって、治療法が決められる。

内科的療法の一つであるラジオ波焼灼術が日本に導入されたのは1999年のこと。ラジオ波は、超音波（エコー）で腹部の様子を確認しながら電極針を腫瘍の部分に刺し、約100度の熱でがんを焼き切る治療法だ。1回の治療で電極の周囲約3㌢を壊死させることができる。

肝がんは手術で切除しても5年以内には約8割という高い確率で再発を起こすため、これまで手術以外にも、アルコールを注入して腫瘍を壊死させる治療方法などが模索されてきた。その後導入されたのがラジオ波だが、最大の利点は、患者のからだへの負担が小さいということ。

肝がん手術後、再発した場合に再び手術ができる患者は1～3割程度。それに対し、全身麻酔の必要もなく、細い針を刺すだけで治療できるラジオ波は、何度も治療することが可能だ。

ラジオ波の対象となるのは、腫瘍の大きさが3㌢以下で個数は3個以内。原発性肝がんに対する治療として実施されるのが一般的である。だが、一部の病院では、大腸がんなどの転移性肝がんにも応用している。

患者の負担が大きい肝切除術が減少傾向にある一方で、ラジオ波による治療は増加傾向にある。

世界のラジオ波の年間実施数を見てみると、米国が約1万4400件、中国が約9500件、イタリアが約5610件であるのに対し、日本は約3万8千件ともっとも実施件数が多い。

だが、手術と同等の有効性があるかどうか、長期的な成績がまだ少ないこともあって、肝臓外科医の中にはラジオ波に批判的な医師もいる。そこで、肝切除術とラジオ波の有効性を比較する臨床試験が現在進められている。

また、病院間の技術差が大きく、ラジオ波で焼き切れずに再発して、再手術になったというケースも報告されている。

手軽な印象のラジオ波だが、血管を傷つけて出血したり、がんを焼き切るための熱で腸などに穴があいたりする危険がある点にも注意が必要だ。治療を受ける病院は慎重に選ぶ必要があるだろう。

肝がんラジオ波焼灼術 全国データ

順位	病院名	治療数		所在地	常勤医数	主な医師名	
❶	東京大学病院	924	東京都	文京区本郷７－３－１ ☎03－3815－5411	11	椎名秀一朗	建石良介
❷	近畿大学病院	391	大阪府	大阪狭山市大野東377－２ ☎072－366－0221	10	工藤正俊	土師誠二
❸	大阪赤十字病院	318	大阪府	大阪市天王寺区筆ケ崎町５－30 ☎06－6774－5111	10	大﨑往夫	木村　達
❹	関東中央病院	269	東京都	世田谷区上用賀６－25－１ ☎03－3429－1171	1	小池幸宏	
❺	NTT東日本関東病院	263	東京都	品川区東五反田５－９－22 ☎03－3448－6111	2	寺谷卓馬	竹内　卓
❻	松山赤十字病院	240	愛媛県	松山市文京町１ ☎089－924－1111	5	上甲康二	小林雄一
❼	三重大学病院	238	三重県	津市江戸橋２－174 ☎059－232－1111	14	山門亨一郎	田中秀明
❽	岐阜市民病院	230	岐阜県	岐阜市鹿島町７－１ ☎058－251－1101	4	西垣洋一	林　秀樹
❾	武蔵野赤十字病院	215	東京都	武蔵野市境南町１－26－１ ☎0422－32－3111	12	泉　並木	朝比奈靖浩
❿	和歌山県立医科大学病院	212	和歌山県	和歌山市紀三井寺811－１ ☎073－447－2300	8	玉井秀幸	河合信行
⓫	岡山済生会総合病院	205	岡山県	岡山市北区伊福町１－17－18 ☎086－252－2211	3	大澤俊哉	藤岡真一
⓬	済生会新潟第二病院	197	新潟県	新潟市西区寺地280－７ ☎025－233－6161	2	石川　達	窪田智之
⓭	岡山大学病院	183	岡山県	岡山市北区鹿田町２－５－１ ☎086－223－7151	10	貞森　裕	小林功幸
⓮	明和病院	178	兵庫県	西宮市上鳴尾町４－31 ☎0798－47－1767	9	相原　司	安井智明
⓮	熊本大学病院	178	熊本県	熊本市本荘１－１－１ ☎096－344－2111	6	別府　透	田中基彦
⓰	東邦大学医療センター大森病院	158	東京都	大田区大森西６－11－１ ☎03－3762－4151	5	飯田和成	和久井紀貴
⓱	横浜市立大学市民総合医療センター	157	神奈川県	横浜市南区浦舟町４－57 ☎045－261－5656	3	沼田和司	森本　学
⓲	大分県立病院	150	大分県	大分市豊饒476 ☎097－546－7111	1	池田哲夫	椛島　章
⓳	虎の門病院	145	東京都	港区虎ノ門２－２－２ ☎03－3588－1111	5	池田健次	川村祐介
⓳	姫路赤十字病院	145	兵庫県	姫路市下手野１－12－１ ☎079－294－2251	4	佐藤四三	甲斐恭平
㉑	香川県立中央病院	141	香川県	高松市番町５－４－16 ☎087－835－2222	4	高口浩一	永野拓也
㉒	愛媛県立中央病院	140	愛媛県	松山市春日町83 ☎089－947－1111	3	平岡　淳	上原貴秀
㉒	高知大学病院	140	高知県	南国市岡豊町小蓮185－１ ☎088－866－5811	5	小野正文	高橋昌也
㉔	岡山市立市民病院	136	岡山県	岡山市北区天瀬６－10 ☎086－225－3171	4	狩山和也	湧田暁子
㉕	市立広島市民病院	133	広島県	広島市中区基町７－33 ☎082－221－2291	3	植松周二	岩堂昭太
㉖	札幌厚生病院	131	北海道	札幌市中央区北３条東８丁目５ ☎011－261－5331	7	大村卓味	桑田靖昭
㉗	手稲渓仁会病院	130	北海道	札幌市手稲区前田１条12丁目１－40 ☎011－681－8111	7	辻　邦彦	松居剛志
㉗	名古屋市立大学病院	130	愛知県	名古屋市瑞穂区瑞穂町川澄１ ☎052－851－5511	6	野尻俊輔	宮木知克
㉙	北里大学東病院	127	神奈川県	相模原市麻溝台２－１－１ ☎042－748－9111	4	中澤貴秀	日高　央
㉚	九州厚生年金病院	126	福岡県	北九州市八幡西区岸の浦１－８－１ ☎093－641－5111	2	一木康則	上平幸史
㉛	北海道大学病院	125	北海道	札幌市北区北14条西５丁目 ☎011－716－1161	4	中馬　誠	中西　満
㉜	埼玉医科大学病院	124	埼玉県	毛呂山町毛呂本郷38 ☎049－276－1111	14	濱岡和宏	
㉝	岩手医科大学病院	123	岩手県	盛岡市内丸19－１ ☎019－651－5111	1	黒田英克	
㉞	三井記念病院	122	東京都	千代田区神田和泉町１ ☎03－3862－9111	1	大木隆正	
㉟	東京女子医科大学病院	120	東京都	新宿区河田町８－１ ☎03－3353－8111	4	斎藤明子	片桐　聡
㉟	倉敷中央病院	120	岡山県	倉敷市美和１－１－１ ☎086－422－0210	2	利國信行	詫間義隆
㊲	西神戸医療センター	119	兵庫県	神戸市西区糀台５－７－１ ☎078－997－2200	10	三村　純	井谷智尚
㊳	京都府立医科大学病院	118	京都府	京都市上京区河原町通広小路上ル梶井町465　☎075－251－5111	11	伊藤義人	南　祐仁
㊳	大阪市立大学病院	118	大阪府	大阪市阿倍野区旭町１－５－７ ☎06－6645－2121	13	坂口浩樹	岩井秀司
㊳	関西医科大学枚方病院	118	大阪府	枚方市新町２－３－１ ☎072－804－0101	2	池田広記	中橋佳嗣
㊳	鳥取大学病院	118	鳥取県	米子市西町36－１ ☎0859－33－1111	4	孝田雅彦	大山賢治

が広がっていることが多いので、この場合には連携がとれている外科での手術をすすめることもあります」（工藤医師）

からだへの負担が小さいラジオ波だが、電極針から放出される熱が肝臓の周囲にある腸や心臓などに伝わるため、潰瘍をつくったり、心臓の心膜を破って大出血を起こしたりと、危険をともなう治療なので注意も必要だ。

9位の**武蔵野赤十字病院**はより安全で確実な治療をするため、腹腔鏡を使ったラジオ波を実施している。腹腔鏡は、おなか（腹腔内）に挿入するカメラのことで、モニターに映し出された体内の映像を見ながら治療することができる。

「肝臓の表面にがんがある場合、ラジオ波の針を刺したときにがんが飛び散り転移することがあります。これを防ぐため当院では、腹腔鏡でおなかの中を直接見ながら、肝臓の表面を見る超音波と、ラジオ波の針を通す超音波の2本を挿入し、正確な位置を確かめて、がんを焼いています。そうすれば、がんを散らすことなく確実に治療することができます」

と、副院長で消化器科部長の泉並木医師は話す。

負担が少なく何度でも治療可

同院はさらに、最初に刺した細い針でがんの部位を正確にとらえた後にラジオ波の針を重ねる「二段階法」を採り入れているのも特徴だ。刺した部位がずれても針を刺しなおせるため、狙った場所に確実にラジオ波をあてることが可能となる。

ラジオ波で治療の対象となるのはおもに原発性肝がんだが、東京大学病院や4位の**関東中央病院**など、一部の病院では転移性肝がんへの治療としても採り入れられている。

「大腸がんなどの転移性肝がんは悪性度も高いため、基本的には切除という考えですが、手術ができず、抗がん剤しか治療法がない場合、あるいはほかに治療法がないといわれた人を対象にラジオ波による治療をしています。根治は望めないような場合でも、肝臓の中にあるがんを減らしたうえで、抗がん剤を併用して延命効果を図るのが目的です」

と、同院消化器内科医長の小池幸宏医師は話す。

ラジオ波による治療を一度でもしたことのある医療機関は国内に約千もあるという。しかし、そのすべてに専門医がいるわけではない。

「手軽に実施されすぎているのがラジオ波の問題です。どこでラジオ波を受けても同じ治療が受けられるようなシステムを作ることが、私たちの使命だと考えています」（前出の椎名医師）

肝がんはほかのがんと異なり、腫瘍を取り除いて終わりではない。

「C型やB型の慢性肝炎、肝硬変から肝がんは発生するため、C型ならインターフェロン、B型なら核酸アナログによる治療で肝硬変の進行を抑えることが重要です。肝がんの治療でもっとも大切なことは、切った後、あるいは焼いた後にどんな治療をするかです。肝硬変があってがんができるので、がんの治療をした後に肝硬変の治療をしなければ長期の生存はむずかしいのです。肝がん治療には病院の総合力が必要といえるでしょう」（前出の泉医師）

ライター・美奈川由紀

東京大学病院
消化器内科講師
椎名秀一朗医師

近畿大学病院
消化器内科教授
工藤正俊医師

武蔵野赤十字病院
副院長・消化器科部長
泉 並木医師

関東中央病院
消化器内科医長
小池幸宏医師

◀表の見方

厚生労働省が届け出義務を課す「肝切除術等」が10例以上の医療機関を対象に調査し、肝がんに対するラジオ波焼灼術の治療数（2008年1年間）でランキング。治療数は1人の患者に実施した一連のラジオ波焼灼術を1回と数えるのべ患者数とした。

1・2.緩和ケア室と、原先生が統括する緩和ケアチーム。近畿大学医学部附属病院ではがん治療の初期の段階から緩和ケアを実施し、肉体的・精神的・社会的な苦痛を緩和することで患者さんの生活の質を向上させるよう努めている

専門性の高い治療

がん治療の未来を担う“分子標的治療”

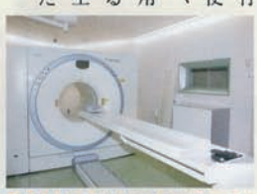
近畿大学医学部附属病院では全国に先駆けて腫瘍内科や放射線腫瘍科を設置するなど、がん治療に対する先進的な取組みが目覚ましい。中でもがん細胞の増殖を抑え従来では手のつけられなかった進行がんにも効果を発揮するという『分子標的治療』が、がん治療の行く末を担う新しい治療法として注目されている。「分子標的治療薬は既に治験が終わって厚労省の承認も進んでおり、今後は放射線治療、抗がん剤治療に取って代わる画期的な治療法として普及するでしょう。元々はがん細胞の増殖を抑えてがんと共生する、というコンセプトの薬だったのですが、血管新生を抑える作用ががん細胞を死滅させる働きを示し、複数の臓器に転移していたがんが全て消滅したという結果も報告されています」とのことで、外科手術が難しいケースにも応用できる。

肝臓疾患を専門とする工藤院長が代表世話人となって「日本肝がん分子標的治療研究会」を組織するなど、各病院の先生方と協力して分子標的治療の普及にも努めている

がん治療での地域連携

がんセンターを設置し診療所に施設利用の呼びかけ

その他のがん治療の取組みでは、今までは各科が別個で治療に当たっていたのを、それぞれが症例を持ち合って治療法を検討する場を設けたり、各科を横断的にラウンドできる緩和ケアチームの発足、ＮＳＴ（栄養サポートチーム）と連携しながらのがん患者の栄養管理、化学療法による通院治療の充実など、側面からサポートするシステムも構築している。そしてそれらの動きを統括し総合的に治療にあたるべく『がんセンター』を設置。また文部科学省の提唱による『がんプロフェッショナル養成プラン』において近畿エリアの基幹校となり、がん治療の専門医の育成にも尽力している。以上のがん治療に対する取組みから、平成19年には『がん診療連携拠点病院』に指定された。「がんの根治治療に関しては当院で行いますが、その後の経過観察や、進行がんの患者さんに対する痛みのコントロール、それ以前に早期発見などは地域の先生方の協力が必要です。そのため、医療従事者向けの学術講演会などの啓蒙活動を積極的に行い、ＰＥＴの使用に関しても、もっと有効利用していただけるよう地域の先生方に働きかけたいと思います」。



併設する高度先端総合医療センターではPET-CTを設置。使用手続きが簡略化され、地域の先生からの利用希望にも対応できる

工藤院長が目指す今後の展開

各地域の協力病院の拡大と南大阪における医師数の充実

地域医療の観点において、工藤院長はさらなる目標を掲げている。「がん診療連携拠点病院、ならびに肝疾患診療連携拠点病院として、病病連携のできる協力病院を増やしていきたい。当院はアクセスが不便ですし、患者さんの住む土地に近い病院に協力を仰ぎ、そこからさらに病診連携に広げられればと思います。また南大阪は北部に比べると医師不足が進んでいます。そのため魅力ある研修プログラムで医師を誘致し、ゆくゆくは南大阪地域の医師不足を解消できるぐらいの医師を確保できれば」と話し、南大阪の地域医療の充実を図っている。

かかりつけ医を持とう！

近畿大学医学部附属病院
病院長

工藤 正俊先生

先進的な治療を行う一方で地域医療の方向をリードするがん診療連携拠点病院

診療に加え研究、教育といった医療の未来をも支える近畿大学医学部附属病院。南大阪唯一の大学病院として専門性の高い先進的な医療と同時に地域密着という側面も充実させる同院の工藤院長にお話を伺った。

ドクター紹介ページ有り16・17P　医療詳細ページ有り106P

近畿大学医学部附属病院の現状と役割

特定機能病院の高度医療と市民病院のような地域密着性

低出生体重児や心疾患・外科疾患新生児の治療を行う新生児集中治療室（ＮＩＣＵ部）や、二次・二次救急患者を受け入れる救急診療部（ＥＲ部）の創設、ＰＥＴ診断と放射線治療に特化した高度先端総合医療センターを併設するなど、最先端の医療を提供し続ける近畿大学医学部附属病院。工藤院長は「いわゆる南大阪と呼ばれる大和川以南の地域では、大学病院はここだけ。そういう意味では南大阪の一番大きな拠点病院になります」と話す。また大学病院としての機能の一方で、市民病院のような地域に密着した機能も兼ね備えている。１日で平均２千５００人、多い時では３千人を上回る患者数からも、近畿大学医学部附属病院に対する信頼の篤さがうかがえるだろう。さらに特定機能病院の認定や日本医療機能評価機構からの施設認定も受け、遠方から通う患者さんも多く、地域の枠を超えた重要な役割を担っている。

夕刊フジ　2010年（平成22年）2月27日（26日発行）

ニッポン病院の実力

近畿大学医学部附属病院

早期発見＆治療で医学界をリード

99年に日本初のラジオ波焼灼療法が成功

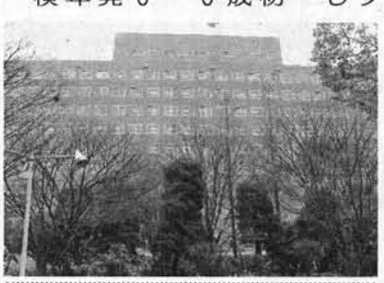
余命数カ月の患者への「分子標的薬」にも取り組む

ウイルス性肝炎の影響で増え続けている肝がん。その治療では、特殊な針を刺してがんを焼灼するラジオ波焼灼療法や、がんにつながる血管を塞ぐ肝動脈塞栓術などが広く普及しているが、かつては外科的手術しか根治できなかった。また、血管が張り巡らされた巨大な臓器ゆえに、早期の肝がんを見つけるのも困難。それを打破したのが、近畿大学医学部附属病院消化器内科だ。肝がんの早期発見と治療において、世界に先駆けた方法を開発し、リーダーとして君臨している。

こう話す同科の工藤正俊教授＝写真＝は、これまで数々の診断法や治療法を生み出し、標準技術として世に広めている。たとえば、肝硬変が進んだ状態の肝がんを手術で切除する場合には、正常な肝機能をできるだけ残す必要がある。どこまで切除できるのか。その肝機能の予備力を診断できる方法を見いだし、1989年にノーベル医学賞につながる米国核医学会バーソン・ヤロー賞を受賞した。

また、99年には日本初のラジオ波焼灼療法を成功させ、これまで3000例以上に行っている。肝動脈塞栓術は4000例以上。肝がんの早期発見法として、2007年には新たな造影超音波検査法も開発した。

「5㍉程度の肝がんは、超音波だけでは見つかりにくい。07年に承認された造影剤を2回用いると、再発したがんだけでなく、小さながんも100％見つかる」（工藤教授）



微小の肝がんを見つける方法はそれまでなかっただけに、学会で発表したところ大反響を呼んだという。今では、その造影剤を使える医療機器があれば、どこの病院でも行える検査法となっている。新たに見いだした検査法や治療法を瞬く間に広めてしまう〝工藤マジック〟。昨年には、北米肝臓学会で日本人としては20年ぶりに講演を行うなど、国内外での活動で休む暇もないほど。しかし、工藤教授は、「診断も治療も現状に満足していない」ときっぱりいう。

「今取り組んでいるのは、肝がんに対する分子標的薬です。がん細胞内のシグナル伝達を遮断する化学療法で、余命数カ月の肝がん患者さんを助けることができる。効く人と効かない人を見極めているところです」

治療や診断で壁にぶつかっても、元気になった患者の笑顔を支えに、乗り越え続ける。肝がんで亡くなる人がゼロになるまで、工藤マジックに終わりはない。

（安達純子）

<データ>2008年実績
★ラジオ波焼灼療法405件
★肝動脈塞栓療法332件
★動注化学療法102件
★造影超音波検査620件
★インターフェロン治療（新規導入件数）251例
★病床数985床
〔住所〕〒589―8511大阪府大阪狭山市大野東377の2 ☎072・366・0221

Session 3

Case study and Discussion –voting system

アンサーパッドによる集計結果は、P24～26をご参照ください。

討論者：

池田 健次 先生
（国家公務員共済組合連合会 虎の門病院 肝臓内科 部長）

池田 公史 先生
（国立がん研究センター東病院 肝胆膵腫瘍科 副科長）

泉　並木 先生
（武蔵野赤十字病院 副院長）

國土 典宏 先生
（東京大学大学院 肝胆膵・人工臓器移植外科 教授）

建石 良介 先生
（東京大学大学院 消化器内科 助教）

古瀬 純司 先生
（吉林大学 医学部腫瘍内科 教授）

松井　修 先生
（金沢大学大学院 医学系研究科 経血管診療学（放射線医学） 教授）

山下 竜也 先生
（金沢大学 地域医療教育学 特任教授）

司 会
工藤 正俊 先生
（近畿大学 医学部消化器内科学 教授）

15

STORM

A STUDY OF SORAFENIB AS ADJUVANT TREATMENT IN THE PREVENTION OF RECURRENCE OF HEPATOCELLULAR CARCINOMA

STORM Forecast

BAY 43-9006
12414
Aug / Sept 2010

Issue 13
Page 1

CURRENT STATUS

- All targeted sites have been activated
- 1550 subjects screened
- 1013 randomized

Current as of 21 Oct. 2010

Keep up the good work— just a few weeks left!

TOP RECRUITERS

Current as of 19 Oct. 2010

INVESTIGATOR	COUNTRY	RANDOMIZED
Mazzaferro	ITALY	42
Takayama	JAPAN	42
Gar-Yang	TAIWAN	27
Yang	CHINA	26
Han	KOREA	22
Kudo	JAPAN	21

In the Spotlight This Month....Patient Retention

Currently, our data shows that about 16% of patients randomized have discontinued prior to HCC recurrence or death, which is higher than the anticipated rate. This increase may negatively affect the timing of the final analysis of the study primary endpoint; RFS (Recurrence Free Survival), when 611 "events" (death or tumor recurrence) have occurred. Therefore, it is crucial that all reasonable precautions be taken to minimize potentially avoidable drop outs.

Non-fatal Adverse Events (25% of discontinuations)

Many AE's may be unavoidable; however, the most common sorafenib side effects can be minimized with proper prophylaxis and post-onset treatment. You have been provided with patient education materials to assist in your AE management efforts. These materials include the side effect management booklets for the patients and investigational site flipchart, AE management webcast, and site instructional materials. We encourage you to use these materials as appropriate to educate your patients in prevention and management of expected AE's.

Your protocol-specified telephone contacts between clinic visits are also an excellent opportunity to ensure that your patients feel supported by addressing their concerns and providing ongoing guidance on AE management.

Consent Withdrawal (15% of discontinuations)

Maintaining patient interest is essential in retaining research patients in studies with long-term commitments. Site personnel must be responsive to patients' ongoing needs and concerns and show recognition to the patients of the value of their dedication to the study. To assist in maintaining patient interest, birthday cards, holiday cards, patient newsletter and magnetic refrigerator calendars have been provided to sites where acceptable by your local regulations. Please ensure that you have received local ethics committee approval before distributing any of these materials to your patients.

Upcoming Patient Retention Survey

In order for the STORM team to determine the most productive ways to assist you with patient retention, we will be sending the investigational sites a link to a brief electronic survey in the next few weeks. Your completion of this survey will provide us valuable information to help narrow our focus to only the most effective patient retention strategies. Once the survey is completed, if you have any additional ideas about retaining patients, please do share them with your CRA.

Enrolment

Remaining enrolment time is rapidly ticking down...only a few weeks left to screen patients by end-October and randomize last the patient by end November 2010!

Please note that you will be notified in advance before enrolment is formally closed.

STORM WORLD CUP GLOBAL CHALLENGE

Congratulations to the most recent winners—Austria, Germany, Italy, Taiwan, China, US.

Sixteen Investigators have scored hat tricks. Congratulations on randomizing at least 3 subjects since the beginning of the Challenge. The following Investigators have randomized more than three subjects since the Challenge began—Drs. Jinwan Wang (+8), Liming Wang (+3), Ruocai Xu (+5), Jiamei Yang (+5), Qichang Zheng (+1), Paul Lai (+2), Tadatoshi Takayama (+12), Lee Ching-The (+6), Chau Gar-Yang (+7), Yan-Shen Shan (+3)—congratulations to all of you.

Voice of the Center..... Kinki University Hospital

Picture to the right:
Prof. Masatoshi Kudo, MD, PhD (PI)
Picture to the left:
Ms. Kayoko Ogawa (CRC)

Our site: Our PI, Prof. Masatoshi Kudo, is the head of the Department of Gastroenterology and Hepatology of Kinki University Hospital.

Prof. Kudo and his team are highly esteemed in advanced diagnosis and treatment of HCC. They pioneered RFA for HCC treatment in 1999 and now perform RFAs for 400 HCC patients a year. Their advanced diagnostic techniques, such as the contrast-enhanced Ultrasound and EOB MRI, contribute to early detection.

Patient recruitment: As a Clinical Research Coordinator, I review the medical records of all the HCC patients who will undergo RFA or resection. When I find a potential candidate, I contact the investigator to initiate pre-screening. I also participate in Prof. Kudo's weekly team meeting for patient information sharing.

Additionally, when Prof. Kudo goes on his hospital rounds, he checks if there are any potential candidates, which gives good encouragement to sub-investigators. Based on the strong network, referral letters are periodically distributed to surrounding hospitals/GPs (1,500 letters!). We have been making every effort for patient recruitment.

Patient care: I always encourage the study patients to call me as soon as they experience any signs or symptoms. I promptly report the information to the investigator and ask the patient to see the investigator, if necessary. With team effort, we maximize patient safety and minimize early discontinuations.

One of Prof. Kudo's mottos is 'Teamwork'. Collaborative efforts with surgery, radiology, and co-medicals are fully utilized for clinical trials as well.

I am very pleased to work in this team on this challenging global trial.

Data Management—EDC

- For those subjects that have discontinued, please ensure that you are entering all of your post study follow-up visits in the eCRF.
- When a patient completes the study please access the All Visits page in the eCRF and update all of the blank fields. Also remember to update the Concomitant Medication and Medical History pages where ongoing or stop dates are missing.

Perceptive

Discontinued Patients: Whenever patients are discontinued, Perceptive issues a query requesting verification that all study images have been submitted. Please respond promptly to these queries by either replying that all scans have been submitted or send in any outstanding scans and respond accordingly to the query. Please also ensure to record the discontinuation date and the reason for discontinuation in the IVRS in a timely manner.

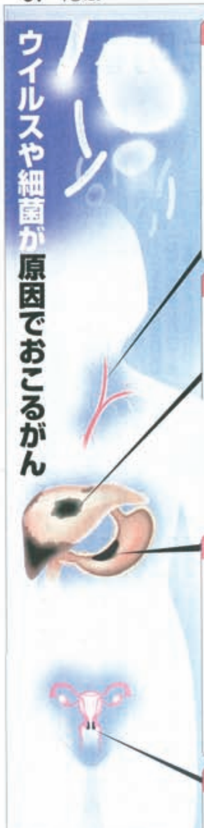
IVRS

Core ICF Amendment #5 – Withdrawn Consent

Please note the following clarifications regarding the latest ICF amendment and patients withdrawing consent:

- If patient wishes to end study treatment and study visits but agrees to post study follow up the IVRS Reason for Discontinuation should be recorded as "Other" and reason given "Patient refused to continue treatment but agreed to continue long term follow-up". The patient should sign the ICF amendment confirming agreement to long term follow-up.
- If patient does not wish to continue treatment and does not agree to be followed long term, the reason for discontinuation should be recorded in IVRS as "Withdrew Consent". No further information is allowed to be collected from this type of patient.
- If the reason for discontinuation falls under any other categories, such as an AE, it is assumed the patient will continue long term follow-up and will not be required to sign the additional consent form.

ウイルスや細菌が原因でおこるがん

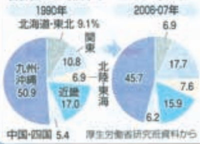


成人T細胞白血病

原因

母乳や性交渉でHTLV－1というウイルス感染による

首都圏などで広がるHTLV－1感染者



厚生労働省研究班資料から

予防と治療

妊娠時の検査で感染がわかれば、人工乳で育てる。発症したら、抗がん剤治療や造血幹細胞移植をする

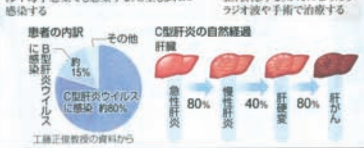
肝臓がん

原因

9割以上が、B、C型肝炎ウイルスの感染が原因。以前は輸血や注射針の使い回しなどでウイルスに感染していた。B型は性交渉や母子感染でも感染する。C型もまれに感染する

予防と治療

血液検査でウイルス感染を調べる。感染していれば、薬物治療でウイルスの増殖防止、排除する。がんになったらラジオ波や手術で治療する



胃がん

原因

ヘリコバクター・ピロリ菌が慢性胃炎を引き起こし、がんに進む

古賀泰裕・東海大学教授提供

予防と治療

呼気や便、尿などによる検査。見つかれば、除菌。がんかどうかは内視鏡検査で。がんは内視鏡や手術で切除

子宮頸がん

原因

ヒトパピローマウイルスの感染による

先進国の子宮頸がん検診受診率（2006年）

米国	83.5%
英国	79.4
スウェーデン	78.6
ノルウェー	75.6
カナダ	72.8
フランス	72.4
ニュージーランド	70.6
フィンランド	70.5
オランダ	69.6
デンマーク	69.4
ベルギー	65.3
オーストラリア	60.6
日本	24.5

経済協力開発機構ヘルスデータ2009から

予防と治療

ワクチンで感染予防。20歳以上は検診で細胞をとり、がんかどうかをみる。治療は手術、放射線、抗がん剤治療など

がんの部位別死亡者数

2009年 厚生労働省人口動態統計から

グラフィック・上村伸也 / The Asahi Shimbun

健康

■講演
東健・神戸大学大学院医学研究科消化器内科学分野教授
工藤正俊・近畿大学医学部付属病院長・消化器内科学教授
今野良・自治医科大学さいたま医療センター産婦人科教授
仁科亜季子さん（女優）

■司会
岩本愛吉・東京大学医科学研究所教授、日本感染症学会理事長
上田孝典・福井大学医学部長

子宮頸がん

原因ウィルス 女性の8割感染

自治医大さいたま医療センター教授
今野良さん

子宮頸がんは初期の段階では自覚症状がありません。しかし、症状がなくても検診を受けることで、がんになる前の段階「前がん病変」で発見できます。前がん病変や初期のがんなら、治療でほぼ100％治ります。子宮を残して、妊娠・出産も可能です。

がんが進行すると手術では取りきれず、放射線でも治りません。抗がん剤と放射線による治療を同時に行うことになります。子宮の周りを多く取ると、排尿障害が出ることもあります。

子宮頸がん検診の受診率は日本は20％台で、他の先進国が60～80％であるのに比べると高くありません。ただ、昨年実施された検診無料クーポンは非常に有効で、20歳では4倍、40歳でも2倍以上の受診率でした。検診無料クーポンのことを周りの方に教えてあげてください。

かつては30歳、40歳が子宮頸がん検診の受診者の中心でした。最近では60歳の方が中心、つまり30年ずっと検診を受け続けている人たちが受診者の多くを占めています。20代はわずか4％。1995年ごろを境に30歳、40歳の死亡が増えてきています。検診を受けなくなったからです。

子宮頸がんは性交渉でヒトパピローマウイルス（HPV）に感染することで起きます。女性の8割が生涯に1度は感染するとされ、性交渉の経験のあるすべての女性に感染の可能性があるわけです。ただ、HPVは非常におとなしいウイルスで、感染しても症状も起こさないうちに排除されることがほとんどです。

しかし、その中のごく一部ががんになります。がんになりやすいのは、免疫上の問題があってウイルスを排除できない方、それから喫煙者。そしてなによりも検診を受けていない女性です。検診について学校で教わっていないし、地域や家庭でも覚える機会がないため、若い方たちが検診を受けない。このままでは非常に困ったことになります。

ワクチンは、子宮頸がんの70％を占めるHPVの16、18型による感染を予防できます。中学1年なら70％、成人でも60％の予防効果があるとされています。ワクチン接種を公費で補助する自治体は約300。栃木県大田原市は学校での接種を市が全額負担し、98％の接種率でした。同じく全額負担の埼玉県志木市では中学3年で86％、新潟県の湯沢町では91％が接種しました。ポイントは全額補助と適切な情報提供です。

ワクチンでがんが予防でき、検診との両方でがんを抑制できる時代を迎えました。検診を受診することと、ワクチンを接種することの意義をご理解いただければ幸いです。

今できる予防は

ゲスト講演
仁科亜季子さん（女優）

私のがんが見つかったのは38歳のときです。食中毒で受診した内科の先生から勧められてついでに検診を受けたところ、子宮頸がんが見つかりました。5月でした。入院は子どもの1学期が終わって夏休みにでもと思っていたのですが、一刻も早くということで6月に入院しました。

最初は抗がん剤の治療でしたが、想像していた以上の吐き気に襲われました。しかも1回目が効かず、2回目の抗がん剤治療をしました。このときは見事に効いて、髪の毛も3日間でなくなってしまいました。

手術してからは放射線治療を受けました。放射線は週に5回を7クールぐらい。体が非常にだるくなり、皮膚がただれてしまいましたが、最後のほうになると金曜の夜からは外泊許可をいただいて家に帰れるようになりました。

6カ月と言われていた入院は、4カ月で退院できました。ただ、入院中は先生、看護師さんが見守ってくださいますが、退院後は1人で孤軍奮闘になります。患者としての寂しさ、不安、恐怖感は、退院してから増してくるものだと思います。

がんは、治療して終わる病気ではありません。一生の問題です。私も卵巣を取って女性ホルモンのバランスが崩れ、いきなり更年期障害のような症状が出ました。夏なのに寒さで震えたかと思うと、シャツが汗でびっしょりになることもあります。今もホルモン補充療法をしています。

子宮頸がんは女性特有な病気なので、手術をすると多くの割合で排尿障害が起こります。膀胱に尿がたまっていることがわからなくなります。

私も肉体的にも精神的にもつらい思いをしました。今でもロケや舞台の前には前日から水分を控えるなど、いつも気をつかいながら生活しています。

再発の不安もあります。体のどこかが痛いと思うと胸がどきどきしたり、免疫を高めるというサプリメントを飲んでみたりします。がんは精神的にも金銭的にも、時間的にもマイナスの多い病気です。

11～14歳ぐらいの女の子はぜひワクチンを受けてください。そして、20歳を過ぎたら子宮頸がん検診に行ってください。

ヒトパピローマウイルスは、人はみんな持っているウイルスです。男の方にもあります。だから男の方が知らん顔ではいけないのです。お父さんも、ワクチンや検診についてぜひ発信してください。

しかし、全国の自治体でワクチンの公費助成はまだ300カ所くらいしかありません。国にもっと助成をしてもらえるように、今年の初めから活動しています。中学に入学した女の子全員が、国からの贈り物として公平にワクチンを打ってもらえるよう願っています。

舞台の最後のことを「大詰め」と言います。私も人生の3分の2が過ぎてしまいました。残りの人生を私は五つの「気」を持ってがんばっていくつもりです。一つは元気。それから陽気、健気、やる気、そして勇気。五つの気を持って、最後までかわいい女性、かわいい女優でいきたいと思います。

主催 日本感染症学会 福井大学 朝日新聞社　後援 福井県 福井県医師会 坂井市

シンポジウム「感染症でおこるがん――がんの予防をめざして」

がんの芽を摘め

胃がんや肝臓がん、子宮頸がんなど細菌やウイルスが原因のがんは、除菌やワクチン接種、検診をこまめに受ければ予防でき、治すことも可能だ。市民公開シンポジウム「感染症でおこるがん――がんの予防をめざして」が10月30日、福井市のフェニックス・プラザで開かれた。専門家らは、検診による早期発見と早期治療の大切さを訴えた。

（コーディネーターは中村通子・朝日新聞編集委員）

感染症 がんの第一歩

福井大医学部長　上田孝典さん

感染症とがんは、診療する上でまったく縁がない病気ではありません。治療の仕方も似ています。抗がん剤として検討された薬が感染症のエイズの治療薬になったり、感染症に効かなかった薬が抗がん剤として効いたりという例もあります。しかし、一般的には体の外からかかる病気「感染症」と内側から起こる「がん」は、まったく違う病気と考えられています。

18世紀に天然痘の予防接種が始まり、感染症は予防や治療の可能性が大きい病気になりました。1980年に天然痘の根絶宣言が出たように、予防接種には一つの病気をなくしてしまうくらいの力があります。本来違う病気である感染症とがんですが、「感染症で起こるがんがある」とわかってきました。感染症で慢性の炎症が続くと遺伝子にも障害が及びます。がんの第一歩は遺伝子の障害なので、感染症でがん化が起こることがあるとわかってきたのです。

胃がんにつながるピロリ菌、ウイルスでは成人T細胞白血病のHTLV―1、肝臓がんのB型肝炎ウイルスとC型肝炎ウイルス、子宮頸がんのヒトパピローマウイルス（HPV）などがあります。子宮頸がんは若者にもおこり、罹患割合が高い。原因となる感染症の予防・治療でがんが予防できれば、多くの人が苦しまずに済むはずです。

がんには罹患の危険を除く1次予防と、検診で早く見つけて治療する2次予防があります。感染症で起こるがんは、感染症を予防すればかからないわけですから、1次予防が十分に可能で、それを知っていただくのが非常に大切だと思います。

胃がん　ピロリ菌除菌で予防可能

神戸大教授　東健さん

胃がんは、昔から日本が「世界一多い国」というレッテルをずっと張られています。男性では死者は肺がんに次いで、女性も大腸がん、肺がんに次いで多いです。

しかし、男女とも胃がんによる死者は昔に比べると減っています。ヘリコバクター・ピロリ菌の感染者が減っているのが大きな理由の一つです。ピロリ菌を除菌して検診を受ければ、多くの人は胃がんで命を落とさずに済む。予防可能ながんです。

しかし、予防の意識がまだ浸透していません。胃がん検診の受診率は低いのが現状です。検診で早期にがんを見つけられないと、我々医療従事者も治すことができません。

胃がんはピロリ菌が胃の中に巣くって、感染が続くことで発症します。最近の研究で、ピロリ菌の中にはいろいろな毒素を持った菌がいて、その毒素が胃がんの発症にかかわっていることがわかってきました。

ピロリ菌は子供のころに感染して定着します。5歳ぐらいまでの間に感染すると、除菌しない限りずっと胃の中に巣くいます。日本は衛生環境が良くなってきて感染率が低くなってきていますが、50年前は10代でも約8割が感染していました。

どうやって感染するのか。ピロリ菌に感染したお母さんが、赤ちゃんに口移しで食事を与えていたことが原因の一つといわれています。感染している子どもの嘔吐物などが口に入って感染することも考えられます。昔、内視鏡の洗浄が不十分なまま検査して感染した例もありました。

内視鏡検査では胃の組織の一部をとって、ピロリ菌の酵素などを調べます。内視鏡を用いない検査では呼気に含まれる成分を調べたり、血中または尿中のピロリ菌の抗体を調べたりします。便を調べる方法もあります。内視鏡による胃がん検診は、X線で見ることができない細かい病変も見ることができます。できれば、内視鏡の検査を受けるのがよいと思います。

検診で、胃の粘膜にとどまる早期の段階でがんを見つけることができれば、内視鏡でがんの部分を切り取ることができます。最近は直径10センチもある大きながんでも、早期なら内視鏡で取れます。

ピロリ菌に感染していないなら、胃がんになる危険性は低いです。念のために40～50歳の間に1度、内視鏡検査を受け、胃の粘膜がなくきれいな状態なら、その後の検査は必要ないくらいです。しかし、感染していたら必ず内視鏡検査を受け除菌する。取り除いても、その後も定期的に検診をしないといけません。そうすれば、胃がんでは命を落とすことはありません。

肝臓がん　肝炎の血液検査が重要

近畿大教授　工藤正俊さん

ウイルス性の慢性肝炎は肝臓がんになり、最終的に命を奪うということがわかっています。肝臓がんの患者のうち80%がC型、15%がB型肝炎ウイルスを持っています。肝臓がんになる危険性はC型肝炎はそうでない人の約500倍、B型では100倍高くなります。

血液検査で肝炎ウイルス陽性の人を見つけ、がんを予防することが重要です。ウイルスに持続的に感染しているキャリアかを調べ、キャリアならC型はインターフェロンとリバビリンの併用療法、B型は核酸アナログというウイルスを抑える薬で治療します。

キャリアになると10～30年の間に炎症、線維化が進み、肝硬変、肝がんへと進みます。B、C型肝炎の患者さんを数カ月ごとに超音波と腫瘍マーカーで検査することで肝臓がんが早期で見つかります。早期がんは切除したりラジオ波をあてたりして根治的に治療できます。さらに再発予防の治療で肝臓がんによる死者を減らせます。日本の肝臓がんでの死者はわずかに低下傾向にありますが、まだまだ急には減りません。今後も対策が必要です。

B型肝炎ウイルスの成人後の感染は以前は輸血が主な原因でしたが、今は検査しているのでなくなっています。ほかには性交渉や注射針刺し事故がありますが、成人で感染してもキャリアにはなりません。ただし、最近日本でも増えてきた外国種のB型肝炎ウイルスはまれに成人でも慢性化するので注意が必要です。

急性肝炎と違いキャリアの場合は通常、産道で感染する母子感染が主体となります。しかし、B型肝炎ウイルス陽性の妊婦から生まれる子どもへのワクチン接種が1986年から公費で行われ、母子感染もほとんどなくなりました。世界保健機関はすべての新生児にワクチン接種をすすめています。しかし、英国と日本だけが行っていません。この点が残された課題と思われます。

C型肝炎ウイルスのキャリアは200万人といわれています。89年にC型肝炎が見つかって以降対策がとられ、20歳以下のキャリアは微減。C型肝炎は急性肝炎を起こすと約8割が慢性化します。

C型肝炎はウイルスを完全に排除するためインターフェロンとリバビリンを併用して治療します。無治療だと15年で30～40%以上の確率でがんになりますが、治療の効果が出れば2%以下に抑えられます。

キャリアとわかったら、治療をしておくと肝臓がんを防げます。75歳以下は治療するよう日本肝臓学会が推奨しています。かかりつけ医と専門医との間で連携しあって、治療をすすめることが重要です。

パネルディスカッション


上田孝典さん


岩本愛吉さん


東健さん


工藤正俊さん


今野良さん

――感染症で起きるがんの征圧には、検査や検診を受けることと的確な治療を受けることが欠かせません。子宮頸がんの検診は何歳ぐらいまで受けたらいいのかという質問が会場から出ています。

今野　今行われている検診は、子宮の細胞をとってがんかどうかを調べるものです。日本では20歳以上で上限はありません。不安があれば、ずっと受けてもいいと思います。検診をきちんと受け続けて異常がなければ、65～70歳まででいいでしょう。

――ピロリ菌についての質問です。便の検査で陽性だったのが、呼気の検査で陰性でした。どちらの結果を信用すればいいですか。

東　どちらかが陽性ならば陽性ととらえていいです。内視鏡検査を受け、萎縮があれば年に1回は継続して検査を受けるべきです。

――20代のB型肝炎のキャリアの女性からの質問です。体内のウイルスは自然になくなりますか。

工藤　C型肝炎は体内から完全に排除できますが、B型は完全に排除できません。ただし、薬でウイルスを抑えて、血液中から検出できなくなるまでに減らすことはできます。90%のキャリアの人は、肝機能の数値が正常のまま何も起こらないことが多いです。ただ、発がんの危険性があるので、年に1回は検査を受けましょう。

仁科さん「子宮頸がん、無料検診知って」

――検査や検診はどんな人がいつ受けたらいいですか。

東　胃がんは40歳以上では検診を受けるのがよいです。X線検査より、早期のがんが分かりやすい内視鏡検査を勧めます。ピロリ菌の除菌は早ければ早いほどよいです。胃がんへの危険性はさらに少なくなります。母子感染を考えると、出産前に1度確認するのがよいと思います。

――肝炎、肝臓がんは、どんな検診態勢になっていますか。

工藤　多くの自治体がB型、C型肝炎ウイルスの検査の公費助成をしています。40歳や45歳で、必ず1回はチェックしましょう。C型は普通の生活をしている限り感染しないので、その後は調べる必要はありません。B型はタイプによって慢性化しやすいものがあるので、時々調べてもいいかもしれません。

――子宮頸がんは検診率が低いことが問題ですね。

仁科　子宮頸がん検診無料クーポン券は金券と同じ価値があるのに放っておかれています。検診を受けるのは権利です。妊産婦検診で、がんが見つかって赤ちゃんが宿った子宮ごと切除するような悲しいことにならないよう、ぜひ検診に行ってほしいです。

――ヒトパピローマウイルス（HPV）のワクチンは、何歳まで効きますか。

今野　臨床試験では、45歳までは予防効果が確認され、55歳までは安全性が証明されています。55歳までは打っていいでしょう。大事なのは大人になったら検診を受けることです。

――HPVのワクチンを男性が接種すると、パートナーへの感染予防になりますか。

今野　子宮頸がん予防のために男の子にも打つと、費用が倍かかります。子宮頸がんの予防を考えれば女子を優先すべきです。HPVは肛門がんや咽頭がんも引き起こします。欧米では男性の肛門がんや咽頭がんのワクチンによる予防がテーマになっていますが、日本はまず女性のことを考えていくべきです。

――ピロリ菌の除菌をすれば完全にがんは防げるのですか。除菌後、再び感染しますか。

東　完全に除菌した場合、今の日本の環境下であれば再度感染することはほとんどないです。外国など衛生環境の悪いところで再感染する例は散見されます。

岩本さん「ワクチン開発 政府は推進を」

――ワクチンの開発には時間もお金もかかります。政府の取り組みに対し、感染症学会はどう考えますか。

岩本　ワクチンは開発されてから、安全性と効果が確認されるのに長い時間が要ります。C型肝炎やピロリ菌はまだ基礎研究の段階。ワクチンが必要だという国民の理解が欠かせません。政府も推進していくべきです。

上田　私たちが治療している成人T細胞白血病は感染で起こるがんですが、早期発見しても治りにくいという状況です。しかし、肝臓がん、子宮頸がん、胃がんはワクチンや早期の除菌などで予防もできるので、ぜひそれを充実していくべきだと思います。

――仁科さんはワクチンについてどうお考えですか。

仁科　女性の場合、働いていれば社内で検診を受ける機会も多いですが、主婦など子育て中の人は、検診に行く機会を逃してしまうことが多いです。ワクチンを打つことが、大事だと身にしみてわかっているつもりです。私は自分の体験を元にワクチンの普及に努めていきたいです。

The Newsletter of the **JAPAN** Study

CRAD00102301

Vol.2

Nov 2010

EVOLVE-1

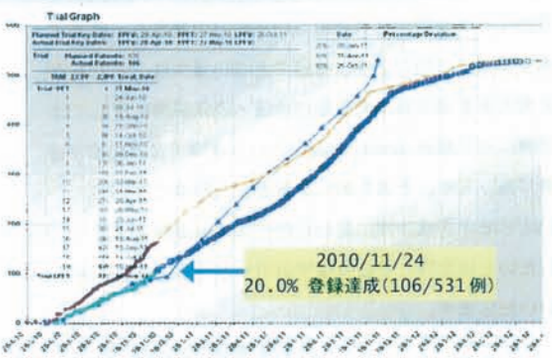
EVerOlimus for LiVer cancer Evaluation

日頃より CRAD00102301 試験（EVOLVE-1 試験）では格別の御高配賜り厚く御礼申し上げます。また、今月に行われたデータカットオフ対応では、お忙しいところご協力いただき誠に有難うございました。News letter vol.2 では、前号に引き続き EVOLVE-1 試験の進捗状況、Special message をお届け致します。

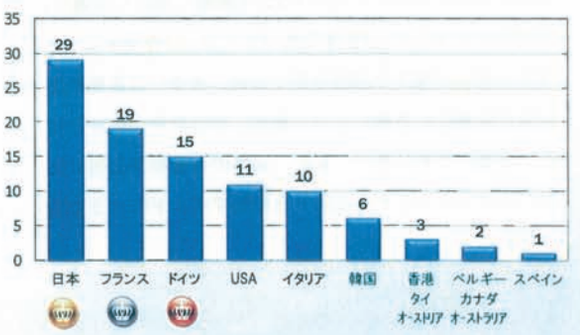
Current Recruitment Plan （2010/11/24 現在）

全世界における**投薬開始例数は106例**（目標症例数の20%！）が、**日本では29例**が登録されております。先生方のご協力により、登録症例数では首位の座を守っております。ただ、**2010/9/22から約2ヶ月間で日本は6例**が登録されておりますが、**フランス15例、イタリア7例、ドイツ6例、USA5例**と他国も徐々に進捗が進んできております。Global trialにおいて日本の存在感を示すチャンスと捉え、弊社としてもより一層努力していきたいと存じます。先生方におかれましても、今後ともEVOLVE-1試験への甚大なるご協力を宜しくお願い申し上げます。

GLOBAL



登録症例数 Ranking



JAPAN

5 例/月の Enrollment Plan を立てておりますが、9 月以降は 3 例/月と少し計画から遅れてきております。
候補患者様がいらっしゃいましたら、EVOLVE-1 試験へのご参加をご検討いただけますと幸いでございます。

	May-10	Jun-10	Jul-10	Aug-10	Sep-10	Oct-10	Nov-10	Dec-10	Jan-11	Feb-11
Amount(Plan)	1	5	12	20	25	30	35	40	45	50
Amount(Actual)	0	5	12	20	23	26	29			

Randomize 数 Total **29** 例

- **7** 例 近畿大学医学部附属病院
- **4** 例 飯塚病院
- **3** 例 九州医療センター
- **2** 例 国立がん研究センター東病院
- 横浜市立大学附属市民総合医療センター
- 千葉大学医学部附属病院
- 熊本大学医学部附属病院
- 杏林大学医学部付属病院

Confidential Information ▸For Principal Investigators and/or Study Personnel ▸Do not distribute to patients

Special Massage from Dr.Kudo

本試験の Global FPFV、及び登録例数第一位など EVOLVE-1 試験に多大なるご協力を頂いております、近畿大学医学部 消化器内科　工藤　正俊先生よりメッセージをいただきました。工藤先生、ご多忙のところ有難うございました。

2009年5月日本でも進行肝細胞癌に対してソラフェニブが適応追加となりました。ソラフェニブは世界で唯一 survival を延長させることが証明された分子標的薬であります。日本のガイドライン上は現在、脈管浸潤あるいは遠隔転移を有する進行肝細胞癌および TACE や動注不応の肝細胞癌に対してその適応が認められております。ソラフェニブは long SD を達成させる薬剤ではありますが、一方で極めて多彩な副作用が出現することも知られています。手足症候群や下痢、高血圧、倦怠感、食欲不振などがそれであります。また、SHARP study などの結果により約 20%程度の患者さんが薬剤の効果がなく PD となることも知られております。これらソラフェニブ不応および副作用による不耐の進行肝細胞癌例に対する 2nd line の治療法として現在 RAD001 の第 III 相臨床試験が開始されております（EVOLVE-1 試験）。御存じの通り、RAD001 は mTOR 阻害薬でありますが、ソラフェニブとは全く異なる機序で細胞増殖の抑制、あるいは血管新生の阻害効果などを有しており非常にその効果が期待されている薬剤であります。また、薬剤の有害事象も他の癌腫におけるデータを見る限り比較的マイルドのようであります。

その意味で、今回始まった EVOLVE-1 試験は肝細胞癌患者さんの予後をさらに延長させる可能性を有しており大いに期待されるところであります。日本は従来より肝細胞癌の治療では世界をリードしてきましたが国際共同治験であるこの試験でも是非、日本がリードして日本の presence を世界に示すと共にこの薬剤を一日でも早く臨床の現場で使用できるようにすべく先生方と一緒に努力していきたいと考えております。

最後に蛇足かもしれませんが、ソラフェニブの post TACE 試験が negative に終わったこと、この際に日本の副作用中止が外国に比して圧倒的に多いことが一因と考えられたことから、『肝細胞癌の治療を担ってきたこれまでの日本の肝癌治療医は一般に薬物療法に不慣れであり、また臨床試験に対する意識や quality の低さが若干世界的にみて疑問視され始めている』との懸念も一部ではあります。このことは、看過できない重大なことであると考えますので日本の肝癌治療医も臨床試験に対しては真剣に取り組み quality も高いという事実を世界に示す必要があります。そのためにも是非とも日本において EVOLVE-1 を成功裡に終わらせ日本の quality の高さを示したいと祈念致しておりますので、どうぞよろしくお願いいたします。

近畿大学医学部消化器内科
教授　工藤　正俊

近畿大学医学部附属病院　CRC のみなさま

いつもご協力いただき、有難うございます！（担当 CRA より）

主な医療機関の肝臓がん治療件数

①手術とラジオ波治療の合計(②と③の合計)
②手術③ラジオ波治療④転移性肝がんの手術

	①	②	③	④
北海道				
札幌厚生	219	61	158	17
手稲渓仁会	219	34	185	8
北海道大	202	72	130	25
市立旭川	79	0	79	3
帯広厚生	54	14	40	－
北海道消化器科	49	10	39	12
市立函館	46	13	33	3
青森				
県立中央	54	18	36	8
弘前大	53	22	31	22
岩手				
岩手医大	156	30	126	30
宮城				
仙台厚生	99	52	47	20
石巻市立	46	4	42	10
秋田				
秋田大	57	16	41	4
山形				
公立置賜総合	115	13	102	14
山形大	44	13	31	6
福島				
太田西ノ内	98	32	66	25
福島労災	48	9	39	9
茨城				
日立総合	91	13	78	22
県立中央	84	18	66	18
筑波大	74	29	45	14
東京医大茨城	58	11	47	3
栃木				
独協医大	100	76	24	39
自治医大	94	17	77	37
県立がんセ	50	26	24	15
群馬				
伊勢崎市民	81	2	79	8
済生会前橋	78	2	76	2
前橋赤十字	54	14	40	18
県立がんセ	46	25	21	7
埼玉				
埼玉医大	143	0	143	0
県立がんセ	95	45	50	48
さいたま赤十字	58	13	45	12
埼玉医大国際	50	48	2	30
埼玉医大総合	43	23	20	15
千葉				
千葉大	186	55	131	22
国立がん研究セ東	147	62	85	61
亀田総合	59	14	45	11
国・千葉医療セ	53	2	51	6
国保旭中央	50	23	27	16
県がんセ	45	30	15	13
成田赤十字	40	6	34	16
東京				
東京大	929	109	820	54
虎の門	349	88	261	35
武蔵野赤十字	257	3	254	13
ＮＴＴ東日本関東	236	5	231	9
東京女子医大	226	109	117	46
東京医大	150	12	138	14
関東中央	146	－	146	－
日大板橋	143	138	5	44
三井記念	141	8	133	2
国立がん研究セ中央	136	57	79	61
東京医科歯科大	130	69	61	24
都立駒込	125	24	101	37
日赤	118	66	52	54
癌研有明	97	55	42	107
昭和大	86	15	71	19
帝京大	85	16	69	14
慶応大	79	12	67	22
ＪＲ東京総合	77	2	75	3
日本医大	64	31	33	19
東邦大大橋	61	3	58	18
杏雲堂	60	0	60	0
杏林大	54	10	44	18
順天堂大練馬	54	4	50	9
順天堂大	52	52	0	41
東芝	51	0	51	6
国際医療福祉大三田	48	11	37	8
慈恵医大	44	41	3	11
町田市民	42	1	41	3
神奈川				
横浜市立大市民総合	171	11	160	13
北里大東	155	28	127	17
済生会横浜市南部	93	3	90	15
横浜市立大	88	24	64	36
聖マリアンナ医大	77	36	41	13
県立がんセ	76	37	39	17
東海大	55	23	32	11
平塚市民	52	16	36	3
済生会横浜市東部	43	9	34	23
新潟				
済生会新潟第二	183	5	178	13
県立新発田	56	8	48	9
富山				
県立中央	69	22	47	10
石川				
県立中央	54	3	51	12
福井				
福井大	37	13	24	10
山梨				
山梨大	139	48	91	12
県立中央	65	19	46	9
長野				
諏訪赤十字	68	38	30	18
佐久総合	62	25	37	21
岐阜				
岐阜市民	242	16	226	7
大垣市民	185	37	148	30
岐阜大	90	31	59	27
静岡				
県立総合	134	50	84	32
県立静岡がんセ	103	53	50	59
静岡市立清水	50	0	50	3
順天堂大静岡	48	6	42	5
磐田市立総合	47	21	26	11
愛知				
名古屋市立大	123	13	110	12
藤田保健衛生大	123	70	53	30
小牧市民	104	10	94	4
愛知医大	91	38	53	10
豊橋市民	71	29	42	22
名古屋共立	52	10	42	5
刈谷豊田総合	42	18	24	7
県がんセ中央	40	22	18	33
三重				
市立四日市	66	12	54	10
滋賀				
大津市民	33	7	26	5
京都				
京都大	189	75	114	16
国・京都	115	16	99	7
府立医大	108	30	78	10
京都桂	80	30	50	5
大阪				
近畿大	429	31	398	20
大阪赤十字	359	59	300	14
大阪市立大	281	126	155	21
市立池田	176	26	150	6
関西医大枚方	163	42	121	27
大阪大	155	53	102	16
府立成人病セ	139	35	104	23
国・大阪	133	26	107	29
大阪市立総合	122	57	65	16
市立岸和田市民	112	8	104	8
国・大阪南	111	25	86	7
大阪労災	107	32	75	21
済生会吹田	101	28	73	7
大阪医大	94	46	48	25
大阪警察	91	21	70	12
ＮＴＴ西日本大阪	84	15	69	5
関西電力	81	10	71	7
大阪市立十三市民	80	26	54	5
大阪厚生年金	80	8	72	10
貝面市立	75	5	70	8
東大阪市立総合	74	6	68	10
市立豊中	69	12	57	21
ＪＲ大阪鉄道	68	16	52	7
岸和田徳洲会	66	12	54	5
住友	64	3	61	7
府立急性期・総合	62	6	56	5
済生会中津	61	8	53	10
市立堺	55	33	22	20
八尾徳洲会総合	47	16	31	6
兵庫				
明和	264	88	176	20
姫路赤十字	230	68	162	11
兵庫医大	172	76	96	13
県立西宮	142	17	125	10
神戸市立中央市民	109	21	88	20
県立がんセ	104	59	45	24
西神戸	98	10	88	14
神戸大	93	68	25	20
神戸朝日※	91	0	91	0
神戸市立西市民	61	15	46	9
関西労災	53	19	34	13
奈良				
県立医大	103	25	78	14
天理よろづ相談所	100	29	71	3
大和高田市立	76	5	71	9
近畿大奈良	56	2	54	9
県立奈良	40	4	36	8
和歌山				
県立医大	276	58	218	15
済生会和歌山	70	5	65	2
鳥取				
鳥取大	104	22	82	6
島根				
県立中央	76	2	74	3
島根大	54	7	47	3
岡山				
岡山大	237	59	178	9
岡山済生会総合	227	58	169	26
倉敷中央	103	18	85	11
天和会松田	81	59	22	10
津山中央	71	19	52	7
倉敷成人病セ	59	2	57	3
川崎医大	42	12	30	－
広島				
中国労災	149	22	127	－
市立広島市民	144	43	101	15
広島大	142	88	54	19
ＪＡ尾道総合	97	21	76	6
国・呉	94	14	80	15
広島赤十字・原爆	93	78	15	6
福山市民	58	28	30	5
国・福山	55	18	37	7
県立広島	50	35	15	14
山口				
山口大	97	33	64	15
下関厚生	94	44	50	8
徳山中央	68	13	55	6
周東総合	42	6	36	3
徳島				
徳島大	218	58	160	17
県立中央	145	6	139	6
徳島市民	62	21	41	11
徳島赤十字	41	13	28	5
香川				
県立中央	148	28	120	14
香川大	118	20	98	5
ＫＫＲ高松	41	4	37	0
高松赤十字	40	10	30	21
愛媛				
松山赤十字	287	11	276	13
県立中央	151	34	117	10
国・四国がんセ	40	31	9	23
高知				
高知大	204	29	175	10
近森	69	3	66	2
高知医療セ	56	37	19	20
福岡				
飯塚	180	73	107	10
国・九州医療セ	169	29	140	12
福岡市民	130	47	83	8
福岡大	130	17	113	12
久留米大医療セ	115	15	100	20
久留米大	109	84	25	18
北九州市立医療セ	83	32	51	20
小倉記念	80	9	71	10
九州厚生年金	77	8	69	7
産業医大	70	36	34	5
福岡大筑紫	61	7	54	5
新古賀	53	25	28	0
戸畑共立	51	7	44	2
国・福岡東	49	19	30	1
浜の町	48	7	41	14
佐賀				
佐賀大	108	56	52	6
県立好生館	61	4	57	4
佐賀社会保険	50	3	47	2
長崎				
国・長崎医療セ	134	72	62	24
佐世保市立総合	129	104	25	6
長崎市立市民	108	6	102	3
長崎大	105	52	53	17
熊本				
ＮＴＴ西日本九州	45	15	30	5
大分				
大分大	105	17	88	12
大分赤十字	73	50	23	9
県立	73	15	58	4
市立中津市民	51	7	44	3
宮崎				
宮崎大	42	22	20	8
宮崎医療セ	41	5	36	0
鹿児島				
鹿児島厚生連	107	54	53	14
鹿児島大	100	53	47	13
鹿児島市立	82	25	57	5
南風	73	9	64	7
沖縄				
浦添総合	17	11	6	7

「国・」は独立行政法人国立病院機構、「セ」はセンター。「－」は無回答または不明。外科のみ、内科のみ回答の施設もある。
※神戸朝日病院は2009年4月～2010年3月実績

同門会　名簿

名前	施設	卒業年度	出身大学
工藤　正俊	近畿大学医学部	昭和53年	京都大学
樫田　博史	近畿大学医学部	昭和58年	京都大学
汐見　幹夫	近畿大学医学部	昭和55年	近畿大学
北野　雅之	近畿大学医学部	平成　2年	鳥取大学
西田　直生志	近畿大学医学部	昭和60年	大阪医科大学
松井　繁長	近畿大学医学部	平成　3年	近畿大学
上嶋　一臣	近畿大学医学部	平成　7年	神戸大学
櫻井　俊治	近畿大学医学部	平成　7年	京都大学
南　康範	近畿大学医学部	平成　9年	近畿大学
萩原　智	近畿大学医学部	平成 10年	近畿大学
井上　達夫	近畿大学医学部	平成 11年	近畿大学
矢田　典久	近畿大学医学部	平成 11年	京都大学
坂本　洋城	近畿大学医学部	平成 12年	近畿大学
朝隈　豊	近畿大学医学部	平成 14年	近畿大学
北井　聡	近畿大学医学部	平成 14年	近畿大学
小牧　孝充	近畿大学医学部	平成　7年	近畿大学
畑中　絹世	近畿大学医学部	平成 13年	川崎医科大学
川崎　正憲	近畿大学医学部	平成 15年	近畿大学
田北　雅弘	近畿大学医学部	平成 15年	近畿大学
永井　知行	近畿大学医学部	平成 16年	近畿大学
永田　嘉昭	近畿大学医学部	平成 16年	近畿大学
今井　元	近畿大学医学部	平成 17年	近畿大学
早石　宗右	近畿大学医学部	平成 18年	近畿大学
有住　忠晃	近畿大学医学部	平成 19年	近畿大学
鎌田　研	近畿大学医学部	平成 19年	近畿大学
峯　宏昌	近畿大学医学部	平成 19年	近畿大学
宮田　剛	近畿大学医学部	平成 19年	近畿大学
高山　政樹	近畿大学医学部	平成 19年	近畿大学
足立　哲平	近畿大学医学部	平成 18年	近畿大学
大本　俊介	近畿大学医学部	平成 18年	近畿大学
門阪　薫平	近畿大学医学部	平成 18年	近畿大学
工藤　可苗	近畿大学医学部	平成 12年	近畿大学
黒田　恵美(旧姓 石川)		平成 11年	近畿大学
岡田　無文		平成 13年	近畿大学
辰巳　千栄		平成 15年	近畿大学
上田　泰輔		平成 15年	近畿大学
辻　直子	近畿大学医学部堺病院	昭和60年	京都府立医科大学
山本　典雄	近畿大学医学部堺病院		
奥村　直己	近畿大学医学部堺病院		
高場　雄久	近畿大学医学部堺病院		
梅原　康湖	近畿大学医学部堺病院	平成 12年	近畿大学
川崎　俊彦	近畿大学医学部奈良病院	昭和58年	京都大学
岸谷　譲	近畿大学医学部奈良病院	昭和 62年	近畿大学

豊澤　昌子	近畿大学医学部奈良病院	平成 12年	近畿大学
宮部　欽生	近畿大学医学部奈良病院	平成 14年	近畿大学
茂山　朋広	近畿大学医学部奈良病院	平成 17年	近畿大学
奥田　英之	近畿大学医学部奈良病院		
加藤　玲明		平成11年	近畿大学
林　道友	近畿大学医学部奈良病院		
宮本　容子(旧姓 北口)	近畿大学医学部奈良病院	平成 12年	近畿大学
前川　清	近畿大学医学部超音波室		
上硲　俊法	近畿大学医学部臨床検査学	昭和60年	近畿大学
山本　俊夫(ご逝去)		昭和26年	京都大学
山本　健二	岡本クリニック		神戸大学
亀山　千晴	育和会記念病院	平成　7年	近畿大学
南野　達夫	なんの医院	昭和55年	近畿大学
中里　勝	上ヶ原病院		
水野　成人	神戸薬科大学医療薬学講座	昭和61年	京都府立医科大学
鍋島　紀滋	天理よろづ相談所病院	昭和61年	京都大学
井上　良一	吉川病院内科	昭和43年	京都大学
由谷　逸朗	高石藤井病院	昭和62年	近畿大学
遠田　弘一		平成 7年	近畿大学
遠田　由紀			
谷池　聡子	和歌山串本病院	平成 7年	奈良県立医科大学
川端　一史	川端内科クリニック	平成元年	近畿大学
米田　円	米田内科	平成 元年	近畿大学
小川　力	高松日赤病院	平成11年	近畿大学
渡邉　和彦	結核予防会大阪府支部相談診療所	平成　3年	近畿大学
森村　正嗣	森村医院	平成　3年	帝京大学
中岡　良介	山本病院	平成 8年	近畿大学
冨田　崇文	冨田病院	平成 14年	近畿大学
西尾　健	高石藤井病院	平成 14年	近畿大学
仲谷　達也	仲谷・飯山クリニック	平成　3年	近畿大学
福永　豊和	北野病院	平成　4年	京都大学
福田　信宏	朝日大学村上記念病院	平成 10年	近畿大学
坂口　康浩		平成 11年	近畿大学
永島　美樹		平成 12年	近畿大学
坂本　康明	坂本医院	平成 15年	近畿大学
市川　勉	市川クリニック	平成 12年	近畿大学
齊藤　佳寿(旧姓 野田)		平成 14年	近畿大学
高橋　俊介	市立堺病院	平成 14年	近畿大学
末冨　洋一郎	末冨放射線科医院	平成　8年	近畿大学
梅原　泰	辻腎太郎クリニック	平成 11年	近畿大学
鄭　浩柄	神戸市立中央センター中央市民病院	平成　8年	東京慈恵医科大学
鄭　扶美	近畿大学医学部　元秘書		
木村　由佳	近畿大学医学部　元秘書		
川辺　仁美	近畿大学医学部　元秘書		
西川　由佳	近畿大学医学部　元秘書		
二見　佳央里	近畿大学医学部　元秘書		

藤田　真紀	近畿大学医学部　教授秘書		
井上　真由美	近畿大学医学部　教授秘書		
村橋　亜季	近畿大学医学部　教授秘書		
弓削　公子	近畿大学医学部　教授秘書		
胡桃　由佳	近畿大学医学部　医局秘書		
朝隈　智	近畿大学医学部　医局秘書		
林　直子	近畿大学医学部　医局秘書		
田村　利恵	近畿大学医学部　臨床研究補佐員（肝癌研究会）		
前原　なつみ	近畿大学医学部　臨床研究補佐員（肝癌研究会）		
小川　佳良子	近畿大学医学部　臨床研究補佐員（CRC）		
原田　八千代	近畿大学医学部　基礎研究補佐員（実験助手）		
安井　章子	近畿大学医学部　基礎研究補佐員（実験助手）		

近畿大学消化器内科　同門会役員

会長　　工藤正俊

副会長　　北野雅之

幹事　　松井繁長

会計　　上嶋一臣

庶務　　鄭　浩柄

同門会誌作製　秘書一同

近畿大学医学部消化器内科教室同門会会則

第一条　名称

本会は近畿大学医学部消化器内科教室同門会と称する。

第二条　目的

本会は会員相互の親睦及び教室の隆盛を図ることを目的とする。

第三条　会員

会員は消化器内科教室出身者、教室員及び同教室の発展に寄与するものをもって構成される。

第四条　役員

１．　本会の運営を円滑にするために幹事会を設ける。幹事会は代表幹事を長とし、代表幹事が指名する教室員をもって構成する。尚、幹事会は代表幹事が随時召集するものとする。その他、会計をおく。

２．　会長

①　会長は現職主任教授より選出される。

②　会長退任後は名誉会長となる。また、名誉会長は主任教授経験者からも選出できる。

３．　顧問

本会の発展に寄与したもので、幹事会が推戴する。

４．　役員の選出

①　幹事は役員より選出する。

②　代表幹事は医局長が兼任する。

５．　幹事の任期は２年とする。但し再任を妨げない。

第五条　会議

１．　総会は年１回の開催とする。

２．　幹事会において仮決議された条件の最終決定権は総会に委ねられる。

３．　決議は総会出席者の多数決により成立する。

第六条　会計

１．　本会の経費は会費をもって充てる。

２．　本会の会費は年額壱萬円とする。

３．　会計年度は４月１日から翌年３月 31 までとし、会計担当者は年１回会計報告を行う。

第七条

事務局は近畿大学医学部消化器内科教室内に置く。

第八条　会則の改正

会則の改正は幹事会の仮決議を経て総会で議決されるものとする。

附則　除名規定

本会の名誉を毀損したものや、その他本会に不適当と考えられるものは幹事会の動議により総会にて除名が議決される。

編集後記

2010 年版の annual report がやっと完成しました。消化器内科の秘書も現在教授室 5 名、医局秘書 3 名、CRC 1 名、臨床研究補助 1 名の 10 名体制ですが、今年は 5 月に日本超音波医学会、11 月には日本消化器病学会市民公開講座など、その他こまごました研究会を含め、工藤教授が会長を務められた学会が多かったこと、ならびに各種学会の事務局業務（日本肝癌研究会事務局、NPO 法人日本肝がん臨床研究機構事務局、日本腹部造影エコー・ドプラ診断研究会事務局、日本肝がん分子標的治療研究会事務局）などの業務が忙しく、なかなか年報の作成に時間を要しました。本当に申し訳なく思っております。

秘書の体制ですが、現在、くるみさん、藤田さん、朝隈さん、井上さん、田村さん、前原さん、林さん、村橋さん、小川さん、弓削さんでの 10 人体制となっております。

来年 2011 年版はぜひもっと早い機会に出したいと思いますので、今後とも何卒宜しく御願い申し上げます。

秘書一同