Contrast-enhanced harmonic endoscopic ultrasonography for differential diagnosis of pancreatic cysts

Background and study aim: Comparison of fundamental B-mode endoscopic ultrasonography (FB-EUS) and contrast-enhanced harmonic endoscopic ultrasonography (CH-EUS) in the differential diagnosis of pancreatic cysts according to presence of mural nodules.

Patients and methods: Between April 2007 and April 2012, FB-EUS and CH-EUS data were prospectively collected from 581 consecutive patients with pancreatic cysts, and were retrospectively analyzed from 70 with subsequent cyst resection. Presence and height of mural nodules as detected on FB-EUS and CH-EUS were evaluated, and thence accuracies of both methods for diagnosing mucinous versus nonmucinous and malignant versus benign cysts.

Results: On pathological examination 48 cysts were mucinous and 22 were nonmucinous; 30 cysts were malignant (high grade dysplasia or invasive carcinoma) and 40 were benign. If presence of a mural nodule was considered to indicate a mucinous cyst, FB-EUS and CH-EUS accuracies did not differ significantly (respectively: sensitivity 85 % vs. 79%; specificity 46 % vs. 96%; accuracy 73 % vs. 84 %, \( P = 0.057 \)). If presence of mural nodule was considered to indicate malignancy, CH-EUS was significantly more accurate than FB-EUS (respectively: sensitivity 97 % vs. 97 %; specificity 75 % vs. 40 %; accuracy 84 % vs. 64 %, \( P = 0.0001 \)). For diagnosing malignancy by evaluating mural nodule height, the area under the receiver operating characteristic (AUROC) was 0.84 and 0.93 for FB-EUS and CH-EUS, respectively (\( P = 0.028 \)). Presence of a mural nodule of height \( \geq 4 \) mm on CH-EUS was a sign of malignancy (false-positive fraction 0.2; true-positive fraction 0.93; odds ratio 56.0).

Conclusions: CH-EUS is more accurate than FB-EUS for diagnosing malignant pancreatic cysts.

Introduction

Pancreatic cyst is a frequent finding on cross-sectional imaging [1]. Mucinous cystic neoplasms (MCNs) and intraductal papillary mucinous neoplasms (IPMNs) have malignant potential; however, these lesions can be followed up without surgical resection when they are asymptomatic and present without high risk features [2]. Serous cystic neoplasms and non-neoplastic cysts are generally not considered at risk for malignant progression. Therefore, after identification a cyst, diagnosis of the cyst type and categorization of the cyst as benign, premalignant, or malignant is critical for guiding subsequent management decisions [3].

Endoscopic ultrasonography (EUS) is superior to other imaging methods in terms of spatial resolution and is widely used to evaluate pancreatic cysts [4–6]. Contrast-enhanced harmonic EUS (CH-EUS) is a new imaging modality that uses an ultrasonographic contrast agent to visualize blood flow in fine vessels and has been shown to be accurate for the differential diagnosis of solid pancreatic tumors [7–11]. CH-EUS may also aid in the diagnosis of pancreatic cysts by enabling assessment of the vascularity of structures such as cyst walls, septa, or mural nodules and the discrimination of contrast-enhancing mural nodules from nonenhancing mucus clots [7].

In this study, we evaluated fundamental B-mode EUS (FB-EUS) and CH-EUS in the diagnosis of mucinous versus nonmucinous and malignant versus nonmalignant pancreatic cysts, based on the evaluation of cyst mural nodules.
Patients and methods

Study design
This was a retrospective review of prospectively collected imaging, clinical, and pathological data. The aim was to determine and compare the diagnostic accuracies (and other test performance characteristics) of FB-EUS and CH-EUS for diagnosis of mucinous versus nonmucinous cysts and malignant versus benign cysts based on the presence and height of mural nodules. The “gold standard” for diagnosing pancreatic cystic lesions was histopathological diagnosis of specimens obtained by surgical resection.

Patients
A total of 581 consecutive patients with pancreatic cysts underwent FB-EUS followed by CH-EUS at the Kinki University School of Medicine between April 2007 and April 2012. Of these, 92 patients underwent surgical resection because of features seen on EUS and/or other imaging modalities that suggested malignancy (i.e., size ≥ 3 cm, thickened cyst walls, solid component within cyst, abrupt changes in caliber of pancreatic duct, and/or presence of concomitant pancreatic invasive cancer distinct from the cyst). The remaining 489 patients were managed by follow-up. Of the 92 patients with surgical resection, 22 patients with concomitant pancreatic invasive cancer distinct from the cyst (n = 11) or with multiple cysts (n = 11) were excluded from this study. Consequently, data were analyzed from 70 patients with solitary pancreatic cysts who underwent surgical resection. For the purpose of the study all surgical specimens were reviewed by expert pathologists to discriminate between mucinous versus nonmucinous and between malignant versus nonmalignant pancreatic cystic lesions. In line with the Japanese guidelines, EUS-guided fine needle aspiration (EUS-FNA) was not performed [12]. This study was performed with the approval of the ethics committee of the Kinki University School of Medicine.

FB-EUS and CH-EUS
An echoendoscope developed for CH-EUS (GF-UCT260; Olympus Medical Systems, Tokyo, Japan) was used. EUS images were analyzed using an Aloka ProSound SSD α-10 system (Aloka, Tokyo, Japan). After evaluation of all of the pancreas and the cyst using FB-EUS, the imaging mode was changed to the extended pure harmonic detection mode, which synthesized the filtered second-harmonic components with signals obtained from the phase shift for contrast-enhanced harmonic imaging. The transmitting frequency and mechanical index were 4.7 MHz and 0.3, respectively. The ultrasound contrast agent used for CH-EUS was Sonazoid (Daichi-Sankyo, Tokyo, Japan; GE Healthcare Milwaukee, Wisconsin, USA), which consists of perfluorobutane microbubbles surrounded by a lipid membrane. Immediately before performance of CH-EUS, the contrast agent was reconstituted with 2 mL of sterile water for injection, and a dose of 15 µL/kg body-weight was prepared in a 2-mL syringe. A bolus injection of the ultrasound contrast agent was administered at a speed of 1 mL/s through a 22-gauge cannula placed in the antecubital vein, followed by a 10-mL saline solution flush to ensure that all the contrast agent was introduced into the circulation. The CH-EUS examinations for the pancreatic cyst lasted for 60 s from injection of the contrast agent. Video sequences of 60 s were stored and then independently reviewed by two readers (M.K. and H.I.), who have each performed more than 1000 CH-EUS procedures. For this special review of the stored data, the readers were blinded to the final diagnosis. When the independent conclusions of the two reviewers were discordant regarding presence of a mural nodule, they re-evaluated the saved images together until agreement was reached.

Definitions
The reference standard was the pathological finding obtained after surgical resection. IPMNs and MCNs were classified as low, intermediate, or high grade dysplasia, or as invasive carcinoma [13]. Malignancy was defined as high grade dysplasia or invasive carcinoma. IPMNs and MCNs were considered to be mucinous cysts.

On FB-EUS, a mural nodule was defined as a EUS-detectable protrusion of the cyst wall into its lumen. On CH-EUS, a mural nodule was defined as a cyst wall protrusion that showed enhancement after contrast administration. The height of a mural nodule was measured from the nodule base to its top in the axis perpendicular to the cyst wall. Fig. 1 shows a representative image of a mural nodule as seen at FB-EUS and CH-EUS, and the histopathological appearance.

Fig. 1  Contrast-enhanced harmonic endoscopic ultrasound (CH-EUS) for differential diagnosis of pancreatic cysts: a side branch intrapapillary mucinous neoplasm (IPMN) with high grade dysplasia. a Fundamental B-mode endoscopic ultrasound (FB-EUS): the protruding component suspected of being a mural nodule is shown in the cystic lesion (arrow). b CH-EUS: the protruding component has vascularity (arrow). c Pathological examination of the resected specimen (hematoxylin and eosin staining) reveals high grade dysplasia in the part of the mural nodule detected by EUS (arrows).
Statistical analysis
McNemar’s test was used to compare the accuracies of FB-EUS and CH-EUS in the diagnosis of mucinous versus nonmucinous and malignant versus benign cysts. For interobserver agreement testing of FB-EUS and CH-EUS, kappa (κ) coefficients of >0.8, >0.6, and >0.4 were considered to indicate excellent, good, and moderate agreement, respectively. The optimal cutoff value of mural nodule height for malignant cysts was derived from the point closest to the top left corner in the receiver operating characteristic (ROC). The difference between FB-EUS and CH-EUS in area under the ROC was evaluated using DeLong’s test, which was performed using the R-3.0.3 software. All other analyses were performed using SAS 9.1.3 (SAS Institute, Cary, North Carolina, USA). Differences were considered to be significant when \( P < 0.05 \).

Table 1  Comparison of contrast-enhanced harmonic endoscopic ultrasound (CH-EUS) and fundamental B-mode endoscopic ultrasound (FB-EUS) for differential diagnosis of pancreatic cysts: patient characteristics and final diagnosis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FB-EUS</th>
<th>CH-EUS</th>
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<tbody>
<tr>
<td>Sex (male/female), n/n</td>
<td>31/39</td>
<td>31/39</td>
</tr>
<tr>
<td>Age, mean (range), years</td>
<td>62 (37–82)</td>
<td>62 (37–82)</td>
</tr>
<tr>
<td>Cyst size, median (range), mm</td>
<td>33 (10–82)</td>
<td>33 (10–82)</td>
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<tr>
<td>Cyst location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Body/tail</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous cystic neoplasm (MCN)</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Benign</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Malignant*</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Intraductal papillary mucinous neoplasm (IPMN) (branch-duct type)</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Benign</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Malignant*</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Serous cystic neoplasm</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Non-neoplastic cyst</td>
<td>18</td>
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* For the purpose of the study, both high grade dysplasia and invasive carcinoma were considered to be malignant.

Results ▼
Demographic characteristics and the final pathologic diagnosis of the 70 patients included in the study are shown in Table 1. Following surgical resection, 6 MCNs, 42 branch duct IPMNs, 4 serous cystic neoplasms, and 18 non-neoplastic cysts were diagnosed. Of the MCNs, 2 were mucinous cystic adenocarcinomas and 4 were adenomas. Of the IPMNs, 14 were benign (low grade dysplasia 14) and 28 were malignant (high grade dysplasia 9, invasive carcinoma 19). Of the non-neoplastic cysts, 6 were epithelium-lined true cysts and 12 were pseudocysts associated with acute or relapsing chronic pancreatitis.

Comparison of FB-EUS and CH-EUS for identifying mural nodules
Overall, FB-EUS detected mural nodules in 53 of 70 pancreatic cystic lesions (76%) (namely, in 2 MCNs, 39 IPMNs, and 12 non-neoplastic cysts), whereas CH-EUS detected mural nodules in 39 of 70 cases (56%) (in 1 MCN, 37 IPMNs, and 1 non-neoplastic cyst).

Both methods were in agreement on the absence or presence of a mural nodule in 17 and 39 cases, respectively (Fig. 2, Video 1). There was discrepancy between FB-EUS and CH-EUS in 14 cases; these comprised 1 of 6 MCNs (17%), 11 of 18 non-neoplastic cysts (61%) and 2 of 42 IPMNs (5%) (Fig. 3, Video 2). In all of these cases, FB-EUS detected nodules that were subsequently not confirmed on CH-EUS.

Testing of interobserver agreement between the two readers for detecting mural nodules revealed good reproducibility for FB-EUS (κ coefficient 0.69, \( P < 0.01 \)) and excellent reproducibility for CH-EUS (κ coefficient 0.83, \( P < 0.01 \)).

FB-EUS and CH-EUS for diagnosing mucinous and malignant cysts
When the presence of a mural nodule was considered indicative of a mucinous cyst, CH-EUS tended to be more accurate than FB-EUS; however, there was no statistically significant difference (Table 2).
When the presence of a mural nodule was considered indicative of a malignant cyst, CH-EUS was significantly more accurate than FB-EUS (● Table 3). For diagnosis of malignant cysts, the optimal cutoff value of mural nodule height for was 8 mm for FB-EUS and 4 mm for CH-EUS. The presence of a mural nodule ≥8 mm in height on FB-EUS was a sign of malignancy with a false-positive fraction 0.3, true-positive fraction 0.87, and odds ratio 15.17. By contrast, the presence of a mural nodule ≥4 mm in height on CH-EUS was a sign of malignancy with a false-positive fraction 0.2, true-positive fraction 0.93, and odds ratio 56.0. The areas under the ROCs for FB-EUS and CH-EUS were 0.84 and 0.93, respectively (P = 0.028) (● Fig. 4). This result suggested that CH-EUS was superior to FB-EUS in distinguishing between malignant and benign cysts.

**Discussion**

In this study, we assessed the abilities of FB-EUS and CH-EUS to diagnose mucinous versus nonmucinous and/or malignant versus nonmalignant pancreatic cysts by evaluation of mural nodules. Mural nodules, visualized as projections of epithelial cells, are commonly detected in mucinous cysts but not in serous cystic neoplasms. In our study, most mucinous cysts were IPMNs and mural nodules were detected by EUS in most cases of IPMN. However, there was a discrepancy between FB-EUS and CH-EUS in detecting mural nodules in 1 of 6 MCNs (17%), 11 of 18 non-neoplastic cysts (61%) and 2 of 42 (5%) IPMNs (● Fig. 3, ● Video 2), indicating that FB-EUS often misinterprets images of mucus clots, necrotic tissue, or sludge as mural nodules. In the current study, the presence of mural nodules distinguished mucinous and/or malignant cysts from other cystic lesions. Relative to FB-EUS, CH-EUS improved the diagnosis of these cystic lesions by more accurately identifying mural nodules.

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<th></th>
<th>FB-EUS</th>
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<th>CH-EUS</th>
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<tr>
<td>Patients, n/n % (95 %CI)</td>
<td>Sensitivity</td>
<td>41/48 85 % (78 % – 92 %)</td>
<td>38 /48 79 % (73 % – 81 %)</td>
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<tr>
<td>Specificity</td>
<td>10/22 46 % (30 % – 59 %)</td>
<td>21/22 96 % (82 % – 99 %)</td>
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<tr>
<td>PPV</td>
<td>41/53 77 % (71 % – 83 %)</td>
<td>38/39 97 % (90 % – 100 %)</td>
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<tr>
<td>NPV</td>
<td>10/17 59 % (39 % – 76 %)</td>
<td>21/31 68 % (58 % – 70 %)</td>
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<tr>
<td>Overall accuracy</td>
<td>51/70 73 % (63 % – 81 %)</td>
<td>59/70 84 % (76 % – 87 %)</td>
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CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

1 Mucinous cysts included branch duct intraductal papillary mucinous neoplasms and mucinous cystic neoplasms

2 P = 0.028 (McNemar’s test).

**Table 2** Mural nodule as a sign of mucinous cyst on fundamental B-mode endoscopic ultrasound (FB-EUS) and contrast-enhanced harmonic endoscopic ultrasound (CH-EUS).
Imaging information alone is often not sufficient to characterize pancreatic cystic lesions. In cases of diagnostic uncertainty, EUS-FNA may be valuable because of its ability to evaluate viscosity, cytology, chemistry, tumor markers, and molecular arrangement in the cyst fluid [14–25]. The American Society of Gastrointestinal Endoscopy and the International Association of Pancreatologists support the use of EUS-FNA for diagnosing pancreatic cysts [24, 25].

Surgical resection should be considered for pancreatic cystic lesions if a mural nodule is detected by CH-EUS, even if EUS-FNA does not show malignancy. In this way, the use of CH-EUS and EUS-FNA together may be useful for accurate diagnosis. In line with Japanese guidelines, we did not perform EUS-FNA in this study [12]. In addition, several studies have shown that IPMNs are frequently accompanied by carcinomas that are distinct from the IPMN both at the first medical examination and during follow-up [6, 26–28], and such concomitant pancreatic carcinomas may develop even if the IPMN lesion is smaller than 15 mm [6]. Thus, evaluation of the whole pancreas with EUS followed by CH-EUS may be helpful for identifying these carcinomas. However, we did not evaluate concomitant pancreatic cancers that were separate from the cyst in this study. These cases were excluded from the study because the main purpose of surgery was resection of the concomitant pancreatic cancers, rather than the cystic lesions. The utility of CH-EUS for follow-up of pancreatic cystic lesions should be evaluated in a further prospective multicenter study.

Several studies have evaluated the presence or size of mural nodules with regard to diagnosis of malignant IPMN [29–35]. Anand et al. reported that the presence of a mural nodule was a predictor of malignancy with an odds ratio of 9.3 [29]. In another report, multivariate analysis revealed that the presence of a mural nodule was a good predictor of malignancy (P = 0.002) [30]. Kawai et al. measured mural nodule size by ultrasonography or EUS and reported that the odds ratio of mural nodules larger than 10 mm as a predictor of malignancy was 198 (P < 0.0001) and, when the results of cytology were considered in addition to those of mural nodule size, the sensitivity, specificity, and accuracy were 88%, 98%, and 97%, respectively [31]. Hirono et al. reported that a mural nodule of more than 5 mm in height was an independent factor associated with malignancy [32]. In the present study, detection of a mural nodule of height ≥8 mm (odds ratio 15.17) by FB-EUS or ≥4 mm (odds ratio 5.60) by CH-EUS indicated malignancy, and these results are similar to those of previous reports.

The present study is the first to compare CH-EUS with FB-EUS for diagnosis of pancreatic cystic lesions by evaluation of mural nodule height. CH-EUS identified mural nodules more accurately than FB-EUS. On the other hand, Ohno et al. classified mural nodule vascularity into four patterns using contrast-enhanced EUS, and reported that papillary and invasive nodule patterns are associated with malignancy [33]. Kurihara et al. also evaluated the vascularity of mural nodules of 10 mm or more and reported that a branch-shaped pattern was associated with carcinoma [34]. Several studies, including the present one, have reported that the presence and height of mural nodules are associated with malignancy of IPMNs. However, Koshiba et al. have reported on flat-type IPMNs without mural nodules that had a higher recurrence rate and a poorer 5-year survival rate [35]. Therefore, during follow-up a focus may be needed on IPMNs without mural nodules.

This study has several limitations. The study was retrospective; however most of the data used for analysis were derived from prospectively collected databases. In addition, the pathological
diagnoses were verified by review of all resected specimens by expert pathologists. Verification bias cannot be excluded, because the FB-EUS and CH-EUS results might have influenced the decision regarding cyst resection. Only 70 patients with a solitary cyst who underwent surgical resection were enrolled in the study; 22 patients who underwent surgical resection were excluded because they either had multiple cysts, some of which differed in pathological features in individual patients, or had synchronous pancreatic carcinoma distinct from the cyst. For the purpose of the study cysts with high grade dysplasia as well as those with invasive carcinoma were considered to be malignant, and this may explain the high proportion of malignant branch-duct type IPMNs. The presence and height of mural nodules were analyzed in a subjective manner. There was potential bias resulting from the fact that the readers who assessed the FB-EUS and CH-EUS images might have known that there was high suspicion of malignancy in all patients included in the study because all of those patients underwent surgical resection. Moreover, the results of the analysis for the optimal cutoff value for mural nodule height were not validated. In this study, we did not perform EUS-FNA because we followed Japanese guidelines [12]; therefore, we could not compare CH-EUS with EUS-FNA for diagnosis of pancreatic cystic lesions. Further studies comparing the outcomes of CH-EUS and EUS-FNA would elucidate the utility of CH-EUS for managing pancreatic cystic lesions.

In conclusion, our study shows that, compared with FB-EUS, CH-EUS is better at discriminating mural nodules from mucus clots and more accurately distinguishes between malignant and benign pancreatic cysts. These advantages may help to reduce the number of unnecessary surgical procedures in patients with non-malignant cysts.

Competing interests: None

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