Prospective Study Evaluating the Plasma Concentrations of Twenty-six Cytokines and Response to Morphine Treatment in Cancer Patients

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Abstract. *Cytokine signaling is involved in pain and opioid-receptor signaling. In this prospective study, we studied the plasma cytokine levels in order to identify candidate biomarkers for predicting resistance to morphine treatment in a cohort of opioid-treatment-naïve cancer patients. We analyzed pain rating and the plasma concentrations of 26 cytokines at baseline and after morphine treatment using a multiplex immunoassay system for the following cytokines: eotaxin, colony stimulating factor, granulocyte (G-CSF), colony stimulating factor granulocyte-macrophage (GM-CSF), interferon α2 (IFN-α2), IFN-γ, interleukin 1α (IL-1α), IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, tumor necrosis factor-α (TNF-α) and TNF-β. No correlation was observed between the clinical characteristics and the numerical rating scale for pain at baseline or among patients who developed resistance to morphine treatment. Interestingly, the plasma concentration of MIP-1α significantly decreased during morphine treatment (day 8 vs. baseline, p=0.03). Regarding the baseline plasma cytokine concentrations, none of the cytokine levels were correlated with the numerical rating scale for pain at baseline; however, the baseline plasma concentrations of eotaxin, IL-8, IL-12 (p40), IL-12 (p70), MIP-1α and MIP-1β were significantly lower in patients who required a high dose of morphine or who developed resistance to morphine treatment. In conclusion, this is the first report revealing that the plasma concentrations of several cytokines were significantly modulated during treatment and were correlated with treatment outcome of morphine. Our results suggest that plasma cytokine levels may be promising biomarkers for morphine treatment and that they warrant further study.*

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Approximately 80% of advanced-stage cancer patients suffer from pain as a result of their disease, and more than 10 million cancer patients are thought to be treated with opioids worldwide (1). Therefore, controlling chronic, severe pain caused by cancer is considered a very important issue for improving the quality of life of cancer patients. Since the degree of pain sensation and the outcome of morphine treatment varies widely among individuals, pharmacogenetic, pharmacokinetic and pharmacodynamic biomarkers of opioid treatment, such as genetic determinants, have been investigated intensively to improve the effectiveness of morphine treatment (2). Several genetic variants associated with varying pain sensitivity have been identified in the general population, including of the genes for μ-opioid receptor (*OPRM1*); δ-opioid receptor (*OPRD1*); catecholamine-O-methyltransferase (*COMT*); guanosine

triphosphate cyclohydrolase 1/DOPA-responsive dystonia (*GCH1*); melanocortin-1 receptor (*MC1R*); transient receptor potential cation channel, subfamily V, member 1 (*TRPV1*); and transient receptor potential cation channel, subfamily A, member 1 (*TRPA1*) (2, 3). With regard to morphine treatment, the most investigated genetic variant is *OPRM1* 118A>G. The *OPRM1* gene is the main target of morphine, and the 118A>G variant leads to a change in amino acids (asparagine to aspartic acid) at position 40 of the extracellular receptor region, affecting a putative glycosylation site of the receptor and suggesting that the different sensitizing to pain is biologically reasonable (2). Many studies have evaluated the correlation between *OPRM1* 118A>G and the outcome of morphine treatment; however, a recent meta-analysis showed no consistent associations between the *OPRM1* 118A>G genotype and most of the phenotypes in a heterogeneous set of eight clinical studies, except for weak evidence of an association with less nausea and increased opioid dosage requirements in homozygous carriers of the G allele (4). Other genetic variants (such as variations in *COMT*; *MC1R*; ATP-binding cassette, sub-family B, member 1 [*ABCB1*]; and UDP glucuronosyltransferase 2B7 [*UGT2B7*]) and combinations of such variants have been examined in several studies (2, 5). However, plasma cytokine levels have never been used as biomarkers for morphine treatment to date.

Meanwhile, emerging evidence has indicated that cytokine signaling is closely involved in pain and that bidirectional interactions exist between cytokine and opioid-receptor signaling (6, 7). In addition, the overexpression of cytokines and chemokines is frequently observed in many types of cancer (8, 9). However, few studies have evaluated the plasma concentrations of cytokines in association with pain scale ratings or the outcome of morphine treatment. Thus, we hypothesized that the plasma concentrations of some cytokines may be modulated or correlated with morphine treatment in cancer patients. In this prospective study, we examined the plasma concentrations of 26 cytokines to explore candidate biomarkers capable of predicting resistance to morphine treatment.

Materials and Methods

Patients. This prospective study started in July 2009 and enrollment was completed in March 2011 at the Kinki University Faculty of Medicine and Sakai Hospital, Kinki University Faculty of Medicine. Clinicopathological features including age, sex, ECOG performance status (PS), type of primary malignant neoplasm, metastatic sites, white blood cell count (WBC), hemoglobin (Hb) level, platelet count (PLT), and albumin (Alb) and C-reactive protein (CRP) levels were recorded. The numerical rating scale (NRS) for pain (10, 11) and the required doses of morphine were evaluated at baseline and on days 1 and 8 of morphine treatment. Morphine treatment was performed according to the standard method including titration (NCCN Guidelines™, Adult Cancer Pain) (12). Resistance to morphine treatment on day 1 (early phase) or on day 8 (stationary phase) was defined as the requirement of a high morphine dose (>30 mg) and the persistence of pain after morphine treatment (NRS ≥6) on days 1 or 8, respectively. The present study was approved by the Institutional Review Boards of both centers, and written informed consent was obtained from all the patients.

Table I. *Clinical characteristics of study patients*.

Characteristic		Total n=44	
		No. of patients	%
Age, years	Median	69	
	Range	40-85	
Gender	Male	22	50
	Female	22	50
PS	0	0	0
	1	9	20
	2	24	55
	3	10	23
	4	1	2
Cancer type	Lung	19	43
	CRC	8	18
	Gastric	4	9
	CUP	4	9
	Pancreatic	2	5
	Breast	2	5
	GB	1	2
	RCC	1	2
	Lymphoma	1	2
	PCC	1	2
	Skin	1	2
Metastatic Sites (n)	0	4	9
	1	19	43
	2	13	29
	3≥	8	18
WBC (/μl)	<5000	8	18
	5000-9999	22	50
	≥10000	14	32
Hb (g/dl)	<8.5	3	7
	8.5-11.9	27	61
	≥12	14	32
PLT (104/μl)	<10	0	0
	10-29	24	55
	≥30	20	45
Alb (g/dl)	<2.5	4	9
	2.5-3.4	20	45
	≥3.5	20	45
CRP (mg/dl)	<1	12	27
	1.0-4.9	16	36
	≥5	16	36

CRC, colorectal; CUP, cancer of unknown primary; GB, gallbladder; RCC, renal cell carcinoma; Lymphoma, malignant lymphoma; PCC, malignant pheochromocytoma.

Preparation of plasma samples. Blood samples were collected before the initiation of morphine treatment (baseline) and on day 8. The separated sera were stocked at –80˚C until further use.

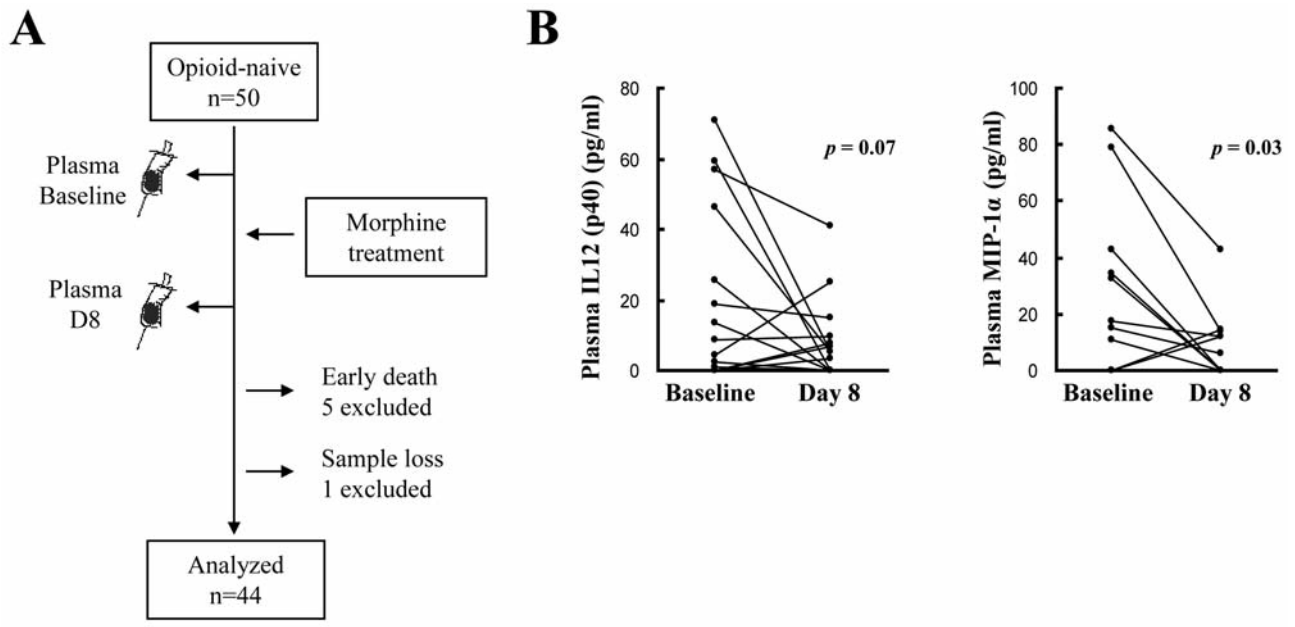

Figure 1. *A: Flow diagram of analyzed patients. B: Plasma concentrations of MIP-1α (left panel) and IL-12 (p40, right panel) at baseline and after morphine treatment (day 8).*

Antibody suspension bead array system. The plasma concentrations of 26 cytokines were determined using commercially available antibody suspension bead arrays (MILLIPLEX™ Human Panel 1 Pre-mixed 26 Plex #MPXHCYTO60KPMX26; Millipore, Billerica, MA, USA). The markers used in this panel were as follows: eotaxin, colony stimulating factor, granulocyte (G-CSF), colony stimulating factor, granulocyte-macrophage (GM-CSF), interferon, α2 (IFN-α2), IFN-γ, interleukin 1α (IL-1α), IL-1-β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, tumor necrosis factor-α (TNF-α) and TNF-β. Data was obtained using a Bio-Plex suspension array system® (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. The method has been previously described (13-14).

Statistical analysis. Statistical analyses were performed to test for differences between groups, using Student's *t*-test or Fisher's exact test. A *p*-value of <0.05 was considered statistically significant. All analyses were performed by JMP (SAS Institute, Cary, NC, USA).

Results

Patient results. A total of 50 patients with opioid-treatment-naïve and histologically confirmed malignant neoplasms, who were scheduled to undergo opioid treatment were eligible for enrollment in this study. Five patients were excluded from the analysis because of early cancer death within two weeks, and one patient was excluded because of absence of a plasma sample. Thus, 44 patients were included in the final analysis (Figure 1A). Of the 44 patients, 75% had a PS of 0–2 and 43% had advanced lung cancer (Table I). Forty-seven percent of the patients had metastatic lesions in two or more organs. The laboratory data for WBC, Hb, PLT, Alb and CRP are also shown (Table I).

Clinical characteristics and outcome of morphine treatment. We evaluated whether the clinical characteristics were associated with the NRS for pain at baseline or among patients who had developed resistance to morphine treatment on days 1 or 8. Age, sex, PS, tumor type, metastatic sites, WBC, Hb, PLT, Alb and CRP were examined (Table II). Twenty-five patients (57%) had severe pain (NRS ≥6) at baseline. Resistance to morphine treatment was observed in 11 patients (25%) on day 1 and 14 patients (32%) on day 8. None of the examined clinical characteristics were associated with the NRS for pain or the outcome of morphine treatment. These results suggest that predicting the outcome of morphine treatment based on clinical parameters may be difficult.

Plasma concentrations of cytokines at baseline and changes after morphine treatment. We examined the changes in the plasma concentrations of 26 cytokines at baseline and after morphine treatment (Table III). The baseline plasma concentrations seemed to vary widely among individuals; for example, the plasma concentrations of G-CSF and IL-6 varied from 0 to 2332 pg/ml and 0 to 1879 pg/ml, respectively. During morphine treatment, the plasma MIP-1α level decreased significantly (baseline: 7.2±19.3 pg/ml, day

Table II. *Relationship between clinical characteristics and resistance to morphine treatment.*

Characteristic		Pain scale* (Baseline)			Treatment outcome (day 1)			Treatment outcome (day 8)		
		Mild	Severe	*p*-value	Well controlled	Resistant**	*p*-value	Well controlled	Resistant**	*p*-value
Age (years)	65<	5	8	0.75	9	4	0.71	9	4	1.00
	65≥	14	17		24	7		21	10	
Gender	Male	11	11	0.54	16	6	1.00	14	8	0.75
	Female	8	14		17	5		16	6	
PS	0-2	12	21	0.16	24	9	0.70	21	12	0.46
	3-4	7	4		9	2		9	2	
Tumor types	Lung ca.	10	9	0.36	15	4	0.73	11	8	0.33
	Others	9	16		18	7		19	6	
Metastatic sites (n)	0-1	10	13	1.00	17	6	1.00	13	10	0.11
	2≥	9	12		16	5		17	4	
WBC (/μl)	<10000	14	16	0.53	24	6	0.29	21	9	0.74
	≥10000	5	9		9	5		9	5	
Hb (g/dl)	<10	6	13	0.23	15	4	0.73	14	5	0.53
	≥10	13	12		18	7		16	9	
PLT (104/μl)	<30	11	13	0.78	16	8	0.29	16	8	1.00
	≥30	8	12		17	3		14	6	
Alb (g/dl)	<3.5	10	14	1.00	21	3	0.08	16	8	1.00
	≥3.5	9	11		12	8		14	6	
CRP (mg/dl)	<5	13	15	0.75	19	9	0.28	20	8	0.74
	≥5	6	10		14	2		10	6	

Comparisons are between mild *vs*. severe pain groups and well-controlled *vs*. resistant to morphine treatment groups. *Pain was evaluated using the numerical rating scale for pain (NRS). Severe pain was defined as NRS ≥6. **Resistance group was defined as the requirement of a high dose of morphine (>30 mg) and persistent pain (NRS ≥6) after morphine treatment. The *p*-values were calculated using the Fisher's exact test.

8: 2.3±7.4 pg/ml, $p=0.03$). Although the difference was not significant, the plasma IL-12 (p40) level also decreased (baseline: 7.0±17.4 pg/ml, day 8: 2.7±7.6 pg/ml, $p=0.07$) (Figure 1B). Since the results were obtained from paired samples of the same individuals at baseline and after treatment, morphine treatment was thought to reduce these plasma concentrations. MIP-1α and IL-12 (p40) could be novel biomarkers for monitoring the effects of morphine treatment, although further studies are needed.

Baseline plasma cytokine concentrations and required dose of morphine. We analyzed whether the baseline plasma cytokine levels were associated with the required dose of morphine. IL-8, IL-12 (p40) and MIP-1α were significantly lower in patients who required a high dose (>30 mg) of morphine on day 1 after titration ($p=0.03$, $p=0.01$ and $p=0.02$, Table IV). Meanwhile, the concentration of eotaxin was significantly lower in patients who required a high dose of morphine on day 8 ($p=0.00026$).

Baseline plasma cytokines and outcome of morphine treatment. Finally, we analyzed whether the baseline plasma cytokine levels were associated with the outcome of morphine treatment. None of the cytokine levels were correlated with the NRS for pain at baseline (Table V). However, several cytokines, including IL-12 (p40), IL-12 (p70), MIP-1α and MIP-1β, were significantly lower in patients who developed resistance to morphine treatment on day 1 compared with the levels in patients whose pain was well controlled after morphine treatment ($p=0.03$, $p=0.03$, $p=0.02$ and $p=0.01$, respectively). Interestingly, the plasma concentrations of IL-12 (p40) and MIP-1α were identified by both changes and outcome of morphine treatment, suggesting that these cytokines may be closely involved in opioid signaling. Meanwhile, the concentration of eotaxin was significantly lower in patients with resistance to morphine treatment on day 8 (baseline: 53.8±26.0 pg/ml, day 8: 34.6±9.0 pg/ml, $p=0.0009$). Collectively, the baseline plasma concentrations of several cytokines were significantly associated with the outcome of morphine treatment.

Discussion

MIP-1α was identified as a macrophage inflammatory protein that has inflammatory and neutrophil chemokinetic properties (15). MIP-1α plays various roles in inflammatory responses by binding to receptors, including chemokine (C-C motif) receptor 1 (CCR1) and CCR5 (16). Cellular sources

Table III. *Plasma concentrations of cytokines at baseline and changes after morphine treatment. The plasma cytokine concentrations at baseline (before) and after (day 8) morphine treatment are shown as the minimum, maximum, and mean±SD. Comparisons between baseline and after treatment concentrations were evaluated using the t-test.*

Cytokine (pg/ml)	Baseline			After treatment*			Baseline *vs.* After
	Range		Mean±SD	Range		Mean±SD	
	Min	Max		Min	Max		*p*-value
Eotaxin	7.8	115.6	47.7±23.7	2.7	114.5	42.7±23.5	0.10
G-CSF	0.0	2331.9	265.1±363.4	12.0	4452.1	329.5±653.2	0.57
GM-CSF	0.0	13.5	2.6±2.5	0.0	17.7	2.7±2.9	0.75
IFN-α2	1.4	76.3	16.5±15.6	0.0	37.2	16.5±8.0	1.00
IFN-γ	0.0	86.6	7.7±16.9	0.0	68.2	6.4±12.2	0.62
IL-1α	0.0	581.9	82.8±118.5	0.0	594.8	88.4±113.8	0.63
IL-1β	0.0	4.9	0.5±1.3	0.0	4.5	0.3±1.0	0.40
IL-2	0.0	4.7	0.2±0.8	0.0	17.1	0.5±2.6	0.41
IL-3	0.0	2.1	0.1±0.3	0.0	0.0	0.0±0.0	0.28
IL-4	0.0	67.4	2.5±11.2	0.0	10.4	0.3±1.6	0.21
IL-5	0.0	1.4	0.1±0.2	0.0	0.8	0.1±0.2	0.89
IL-6	0.0	1878.7	49.2±282.4	0.0	75.2	13.4±20.1	0.41
IL-7	0.0	17.7	0.7±3.2	0.0	3.4	0.1±0.5	0.14
IL-8	0.0	280.7	24.7±52.5	0.0	247.5	29.8±53.9	0.48
IL-10	0.0	1880.7	50.5±284.4	0.0	751.7	22.1±112.9	0.28
IL-12 (p40)	0.0	70.7	7.0±17.4	0.0	40.8	2.7±7.6	0.07
IL-12 (p70)	0.0	31.3	3.1±6.9	0.0	33.9	2.7±6.9	0.55
IL-13	0.0	7.8	0.3±1.4	0.0	7.4	0.2±1.1	0.58
IL-15	0.0	10.5	0.8±2.1	0.0	15.1	1.2±3.1	0.34
IL-17	0.0	18.8	2.2±4.1	0.0	16.3	2.5±4.2	0.55
IP-10	199.9	18071.6	1303.0±2727.9	158.4	20000.0	1377.7±2965.7	0.53
MCP-1	92.3	2346.5	316.0±357.0	84.1	1772.6	346.7±346.2	0.67
MIP-1α	0.0	85.6	7.2±19.3	0.0	42.9	2.3±7.4	0.03
MIP-1β	0.0	90.4	16.7±20.3	0.0	56.5	13.8±13.9	0.44
TNF-α	0.4	175.2	12.3±29.3	0.2	98.5	9.5±14.9	0.37
TNF-β	0.0	9.9	0.7±1.7	0.0	3.0	0.3±0.7	0.15

and regulators of human MIP-1 are inducible in most mature hematopoietic cells and osteoblasts, astrocytes, microglia (fetal), epithelial cells, mesangial cells, fibroblasts, and vascular smooth muscle cells (16). We found that the plasma MIP-1α concentration decreased significantly during morphine treatment. In line with our findings, several studies have demonstrated that morphine directly down-regulates the expression of MIP-1β in leukocytes, astrocytes and astroglial cells *in vitro* (17-19). This effect was thought to be mediated through the opioid mu (μ) receptor (19). We hypothesized that morphine down-regulates the secretion of MIP-1α from mature hematopoietic cells, resulting in a decrease in the plasma concentrations of MIP-1α during morphine treatment. Thus, the concentration of MIP-1α may be useful as a pharmaco-dynamic biomarker of morphine treatment.

On the other hand, we found that the plasma concentrations of cytokines, including eotaxin, IL-8, IL-12 (p40), IL-12 (p70), MIP-1α and MIP-1β, are significantly correlated with the outcome of morphine treatment. The underlying mechanism explaining why these plasma concentrations of cytokines were lower in patients with resistance to morphine treatment remains unclear. Two possible hypotheses can be considered. Firstly, crosstalk between cytokine-signaling and opioid receptor-signaling may be involved. Accumulating evidence has indicated that stimulation of MIP-1α to its receptor CCR1 induced the internalization of μ-opioid receptors and severely impaired the μ-opioid receptor-mediated inhibition of cAMP accumulation in μ-opioid receptor/HEK293 cells (20). In addition, the prolonged activation of opioid receptors inhibited the function of chemokine receptors on leukocytes *via* a calcium-independent protein kinase C pathway (21). These studies indicate a direct link between these signaling pathways. Secondly, many leucocyte subpopulations in the peripheral blood, including lymphocytes, monocytes, and granulocytes, produce opioid peptides, such as met-enkephalin, β-endorphin, dynorphin, and endomorphins, in inflammatory peripheral tissue (22). Opioid peptides can bind to opioid receptors on sensory neurons and

Table IV. *Relationship between baseline plasma cytokine concentrations and required dose of morphine. The baseline plasma cytokine concentrations were analyzed between the groups according to required dose of morphine (>30 mg vs. 30 mg) using the t-test.*

Charasteristics	Base line plasma concentration			Base line plasma concentration		
	Required dose of morphine (after titration, day 1)			Required dose of morphine (day 8)		
(pg/ml)	>30 mg	30 mg	*p*-value	>30 mg	30 mg	*p*-value
Eotaxin	43.1±36.0	48.4±21.8	0.74	32.6±8.1	52.7±25.2	0.00026
G-CSF	243.7±244.2	268.5±381.2	0.84	294.8±254.9	255.2±395.9	0.70
GM-CSF	2.4±2.4	2.6±2.6	0.90	3.0±3.6	2.4±2.1	0.62
IFN-α2	11.5±8.6	17.3±16.4	0.21	17.1±21.1	16.3±13.7	0.90
IFN-γ	6.6±8.6	7.8±17.9	0.78	6.0±7.0	8.2±19.2	0.58
IL-1α	57.1±106.1	86.8±121.2	0.55	112.5±176.5	72.8±93.5	0.49
IL-1β	0.6±1.4	0.5±1.3	0.92	0.8±1.7	0.4±1.2	0.56
IL-2	0.0±0.0	0.2±0.8	0.10	0.4±1.4	0.1±0.3	0.46
IL-3	0.0±0.0	0.1±0.3	0.28	0.2±0.6	0.0±0.0	0.36
IL-4	0.0±0.0	2.8±12.1	0.16	0.0±0.0	3.3±12.9	0.16
IL-5	0.1±0.2	0.1±0.2	0.89	0.1±0.1	0.1±0.2	0.52
IL-6	1.5±3.6	56.8±303.7	0.27	5.7±9.6	63.7±326.0	0.31
IL-7	0.0±0.0	0.9±3.4	0.13	0.0±0.0	1.0±3.7	0.13
IL-8	6.6±5.0	27.5±56.0	0.03	41.8±84.5	18.9±36.5	0.40
IL-10	4.6±8.8	57.7±305.9	0.29	3.1±6.6	66.3±328.1	0.28
IL-12 (p40)	0.2±0.5	8.1±18.5	0.01	6.5±21.3	7.1±16.3	0.93
IL-12 (p70)	1.0±1.8	3.4±7.3	0.09	3.9±7.5	2.8±6.7	0.67
IL-13	0.0±0.0	0.4±1.5	0.13	0.1±0.3	0.4±1.6	0.32
IL-15	0.2±0.4	0.9±2.2	0.09	0.6±1.2	0.9±2.3	0.64
IL-17	2.2±3.2	2.2±4.2	0.99	1.9±2.5	2.3±4.5	0.70
IP-10	873.6±1167.8	1370.8±2903.3	0.47	2434.1±5265.6	926.0±865.0	0.37
MCP-1	209.0±102.7	332.9±380.2	0.11	215.2±99.6	349.6±404.4	0.09
MIP-1α	0.0±0.0	8.4±20.6	0.02	7.2±23.9	7.2±18.0	1.00
MIP-1β	7.7±9.8	18.1±21.3	0.07	16.0±21.4	17.0±20.3	0.90
TNF-α	5.8±4.6	13.3±31.4	0.18	6.0±3.8	14.4±33.6	0.17
TNF-β	0.6±1.5	0.7±1.8	0.95	0.7±1.5	0.6±1.8	0.82

elicit potent exogenous or endogenous analgesia in inflammatory tissue (23). Since chemokines regulate the migration of opioid peptide-containing leucocytes (23), the antinociceptive effects of chemokines may be involved in the outcome of morphine treatment.

Taken together, these results suggest that the plasma concentrations of several cytokines were correlated with resistance to morphine treatment. Our results provide novel insight into the relation between plasma cytokine levels and morphine treatment, which warrants for further study.

Disclosure Statement

All Authors declare they have no financial support or relationship that may pose conflict of interest.

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Table V. *Relationship between baseline plasma cytokine concentrations and resistance to morphine treatment.*

Cytokine	Base line plasma concentration								
	Pain scale*			Treatment outcome (day 1)			Treatment outcome (day 8)		
(pg/ml)	Mild	Severe	*p*-value	Well controlled	Resistant**	*p*-value	Well controlled	Resistant**	*p*-value
Eotaxin	49.2±23.2	46.5±24.6	0.71	49.6±22.3	41.9±28.1	0.42	53.8±26.0	34.6±9.0	0.0009
G-CSF	186.8±191.7	324.6±447.8	0.18	280.4±408.3	219.3±177.1	0.50	253.4±415.5	290.0±225.2	0.71
GM-CSF	2.9±3.4	2.3±1.6	0.45	2.7±2.7	2.2±1.9	0.56	2.6±2.1	2.6±3.3	0.98
IFN-α2	19.1±22.1	14.5±7.7	0.39	17.8±17.2	12.5±8.8	0.19	14.4±9.2	21.0±24.1	0.33
IFN-γ	10.6±24.3	5.4±7.7	0.38	8.6±19.1	4.9±7.0	0.35	8.3±20.1	6.2±6.7	0.61
IL-1α	78.7±102.8	85.9±131.2	0.84	71.6±91.5	116.1±178.9	0.44	76.3±97.5	96.6±158.0	0.66
IL-1β	0.5±1.3	0.5±1.3	0.85	0.6±1.4	0.3±1.0	0.51	0.4±1.2	0.7±1.5	0.53
IL-2	0.3±1.1	0.1±0.4	0.57	0.2±0.9	0.0±0.1	0.20	0.1±0.3	0.4±1.3	0.33
IL-3	0.1±0.5	0.0±0.0	0.37	0.1±0.4	0.0±0.0	0.28	0.0±0.0	0.2±0.6	0.36
IL-4	1.8±7.6	3.0±13.5	0.70	3.3±12.9	0.0±0.0	0.16	3.6±13.5	0.0±0.0	0.16
IL-5	0.1±0.3	0.1±0.1	0.76	0.1±0.3	0.1±0.1	0.58	0.1±0.3	0.1±0.1	0.75
IL-6	8.7±12.0	80.1±374.8	0.35	64.4±325.8	3.7±9.1	0.29	69.8±341.8	5.1±8.6	0.31
IL-7	1.6±4.7	0.1±0.7	0.21	1.0±3.7	0.0±0.0	0.13	1.1±3.8	0.0±0.0	0.13
IL-8	16.0±24.0	31.2±66.3	0.30	22.7±39.1	30.4±83.1	0.77	19.3±38.2	36.1±75.1	0.44
IL-10	100.5±431.1	12.5±45.2	0.39	66.3±328.1	3.2±6.5	0.28	72.8±343.9	2.6±5.8	0.27
IL-12 (p40)	10.6±22.2	4.2±12.4	0.27	9.0±19.7	0.9±2.6	0.03	6.0±14.2	9.2±23.3	0.64
IL-12 (p70)	4.3±8.8	2.1±4.9	0.34	3.8±7.8	0.7±1.3	0.03	3.0±7.1	3.3±6.7	0.89
IL-13	0.3±1.2	0.3±1.5	1.00	0.4±1.6	0.0±0.0	0.13	0.4±1.7	0.1±0.2	0.27
IL-15	1.0±2.8	0.6±1.3	0.53	0.9±2.3	0.5±1.2	0.50	0.9±2.4	0.5±1.1	0.39
IL-17	1.4±2.8	2.8±4.8	0.24	2.4±4.5	1.6±2.5	0.43	2.0±4.4	2.6±3.2	0.63
IP-10	950.7±893.8	1570.7±3544.0	0.41	902.9±845.3	2503.3±5253.4	0.34	948.8±904.7	2062.0±4677.3	0.39
MCP-1	333.7±229.2	302.5±434.2	0.76	346.5±405.8	224.3±93.8	0.12	366.6±420.8	207.5±89.7	0.06
MIP-1α	11.8±26.2	3.8±11.1	0.23	9.6±21.8	0.0±0.0	0.02	8.0±18.7	5.7±21.2	0.73
MIP-1β	19.8±22.0	14.3±19.1	0.39	19.8±22.1	7.5±9.3	0.01	16.8±21.1	16.6±19.3	0.97
TNF-α	15.2±39.0	10.0±19.7	0.60	14.2±33.7	6.3±4.0	0.19	15.3±35.2	5.8±3.4	0.16
TNF-β	0.3±0.6	0.9±2.2	0.19	0.7±1.9	0.4±1.1	0.50	0.6±1.9	0.8±1.4	0.73

The baseline plasma cytokine concentrations were analyzed between mild *vs*. severe pain groups and well-controlled *vs*. resistant to morphine treatment groups using the *t*-test. *Pain was evaluated using the numerical rating scale for pain (NRS). Severe pain was defined as NRS ≥6. **Resistance was defined as the requirement for a high dose of morphine (>30 mg) and persistent pain (NRS ≥6) after morphine treatment. The *p*-values were calculated using the *t*-test.

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