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Prospective Study

IL-7, IL-18, MCP-1, MIP1-β, and OPG as Biomarkers for Pain Treatment Response in Patients with Cancer

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Background: Pain is one of the most common symptoms in patients suffering from advanced cancer and receiving palliative care and is often responsible for a poor quality of life. To date, there exists no published correlation between biological, measurable biomarkers and pain intensity.

Objectives: The primary objective was to search and identify pain-associated cytokines (biomarkers) correlating with changes in numeric rating scale (NRS) pain scores in patients with cancer before and after pain treatment. The secondary objectives were to assess cytokine serum level differences between patients and healthy controls and to evaluate possible relationships between pain entities, pain intensity (in NRS), gender, location of primary tumor, and the patients' cytokine baseline concentrations.

Study Design: Controlled, prospective study.

Setting: University medical center.

Methods: Eligible patients with exacerbated cancer-related pain (NRS ≥ 5) and healthy controls with no pain were included. Serum level changes of 19 cytokines were analyzed before and during opioid treatment.

Results: Of 19 analyzed biomarkers, 5 (IL-7, IL-18, MCP-1, MIP-1α, MIP-1β and OPG) turned out to correlate significantly with pain relief. In healthy controls, all analyzed cytokines showed no significant differences. In the secondary analysis, only one significant correlation was detected between OPG and pain entities. Furthermore, IL-4, IL-7, IFN-γ and OPG appeared to account for the ability to predict a patient's gender.

Limitations: Our findings should be considered as preliminary and need to be confirmed in further studies.

Conclusion: Our results provide preliminary evidence of a significant correlation of pain relief in patients with cancer and at least 5 cytokines. These biomarkers may serve as the basis for development of diagnostic tools for pain assessment and could serve as potential new targets for pain control.

Key words: Biomarker, cytokine, pain intensity, pain reduction, cancer-related pain, IL-7, IL-18, MCP-1, MIP-1α, MIP-1β, OPG.

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Pain is one of the most common symptoms in patients with cancer, even more frequent in patients suffering from advanced cancer and consequently receiving palliative care. It is estimated that 70-90% of patients with cancer who have a progression of their disease suffer from pain. Hence, pain is responsible for their poor quality of life (1). Tumor growth is associated with the expression of oncogenes and inactivation of tumor-suppressor genes. These proceedings lead to production of different growth factors, cyto- and chemokines. Therefore, the response of cyto- and chemokine levels in cancer is similar to inflammation and most tumor entities show elevated levels of different cytokines (2-4). Even if it is well known that several molecules, including cytokines, chemokines, and neuropeptides, play a role in the pathophysiology of pain (5,6), pain is an individual, subjective and multifactorial experience influenced by somatic (physical) and psychological factors. There are numerous studies describing pain assessment (7-10), though there exist only few published data on biomarkers for an assessment of pain based on biological/biochemical parameters or prospective studies analyzing cytokine levels within a defined period of time in correlation to pain reduction and/or analgesic treatment. There is only one published report revealing a correlation between plasma concentrations of several cytokines including MIP-1α and MIP-1β and the treatment outcome of morphine (11). Furthermore, there exist data that the administration of morphine and other opioids could have an influence on the blood level of cytokines in humans (12-14).

Objective

The aim of this study was to identify biological, measurable biomarkers in serum correlating with pain intensity in patients with cancer. We performed the following controlled, prospective study in a population of patients suffering from tumor-caused, advanced cancer pain by analyzing the serum concentration of selected cytokines before and during analgesic therapy.

Methods

This prospective, controlled study was approved by the local ethics committee as part of the EPCRC-Project (European Palliative Care Research Collaborative; 6th Framework Program of the European Union, Study no. LSHC_CT_2006-037777).

Study Population

Incurable patients with severe cancer-related pain (Numeric Rating Scale [NRS] $\geq$ 5; 0-10, 0 = no pain, 10 = worst pain imaginable) despite standardized predefined opioid therapy were recruited from August 2007 through July 2010. Since all patients required an improvement of analgesic therapy, they were hospitalized for at least 2 days. All patients signed written informed consent prior to inclusion. Only 2 physicians were allowed to identify suitable patients to decrease a potential inclusion bias. Inclusion and exclusion criteria are listed in Table 1.

As a control group, 20 healthy individuals without pain were recruited. Their exclusion criteria included existence or any clinical signs of current inflammation or pain; any history of chronic diseases and/or cancer; any major

Table 1. *Inclusion/exclusion criteria.*

Inclusion Criteria	Exclusion Criteria
Tumor-related pain	Non-tumor-related
Basic ongoing analgesic therapy for at least 3 days prior to study inclusion	Breakthrough pain during sufficient pain therapy
Pain which required improvement of analgesic therapy	Opioid-induced pain
Pain of NRS $\geq$ 5	Inflammatory or autoimmune disease (e.g., rheumatoid arthritis)
Capability to provide information on pain intensity, pain location, pain entity, and the adverse effects experienced during the study period	Steroid therapy administered within the last 24 hours and/or planned during the study period
Capability to provide informed consent	Chemotherapy administered within the last 14 days and/or planned during the study period
At least 18 years of age	Therapy and/or therapy planned during the study period with thalidomide, cytokines, and antibodies
Women patients had to be hysterectomized and/or postmenopausal at the time of inclusion	Radiotherapy within the last 14 days and/or planned during the study period
	Severe anemia (Hb < 8 g/dL)
	Patients unwilling or unable to comply with the protocol

surgery within the previous 3 months; any drugs administered within the previous 4 weeks; passive or active immunization within the previous 4 weeks; pregnancy.

Study Design and Objectives

The primary objective was to identify cytokines correlating with changes in total pain intensity of at least 3 NRS values in patients with cancer. Therefore, serum cytokine concentrations were assessed before and during morphine treatment. The secondary objectives were to assess cytokine serum level differences between patients and healthy controls and to evaluate possible relationships between pain entities, pain intensity (in NRS), gender, location of the primary tumor, and the patients' cytokine baseline concentrations.

Blood for cytokine analysis was collected before the study's morphine treatment (TP0) and one hour after reaching target pain relief (at least 3 NRS values), but not later than 3 hours after the first blood draw (TP2). In the healthy control population, blood was drawn using the same algorithm, with TP2 exactly 3 hours after the first blood draw (Fig. 1).

This study was divided into 2 parts. During the pilot phase, 20 patients and 20 healthy individuals were recruited. A broad panel of cytokines was analyzed comparing TP0 versus TP2, finalized by a statistical interim analysis. According to the protocol, the study should continue after recalculation of the sample size if at least one of the selected markers showed significant results. Otherwise, the study would have been closed at this point. In the second part, recruitment of patients should continue until the stipulated end of the study.

Pain entities were assessed by the Brief Pain Inventory and the Short Form McGill Pain Questionnaire and classified into the following entities: nociceptive, visceral, neuropathic, and bone pain.

Dosing and Time Schedule of Morphine Therapy

As analgesic bolusses, patients received one-sixth of the daily oral morphine equivalent of their current opioid medication. After 20 minutes the patients were assessed for their actual pain intensity. If the achieved pain relief was less than 3 NRS values, patients received their next bolus. This schedule was repeated until patients reported the target pain relief (TP2).

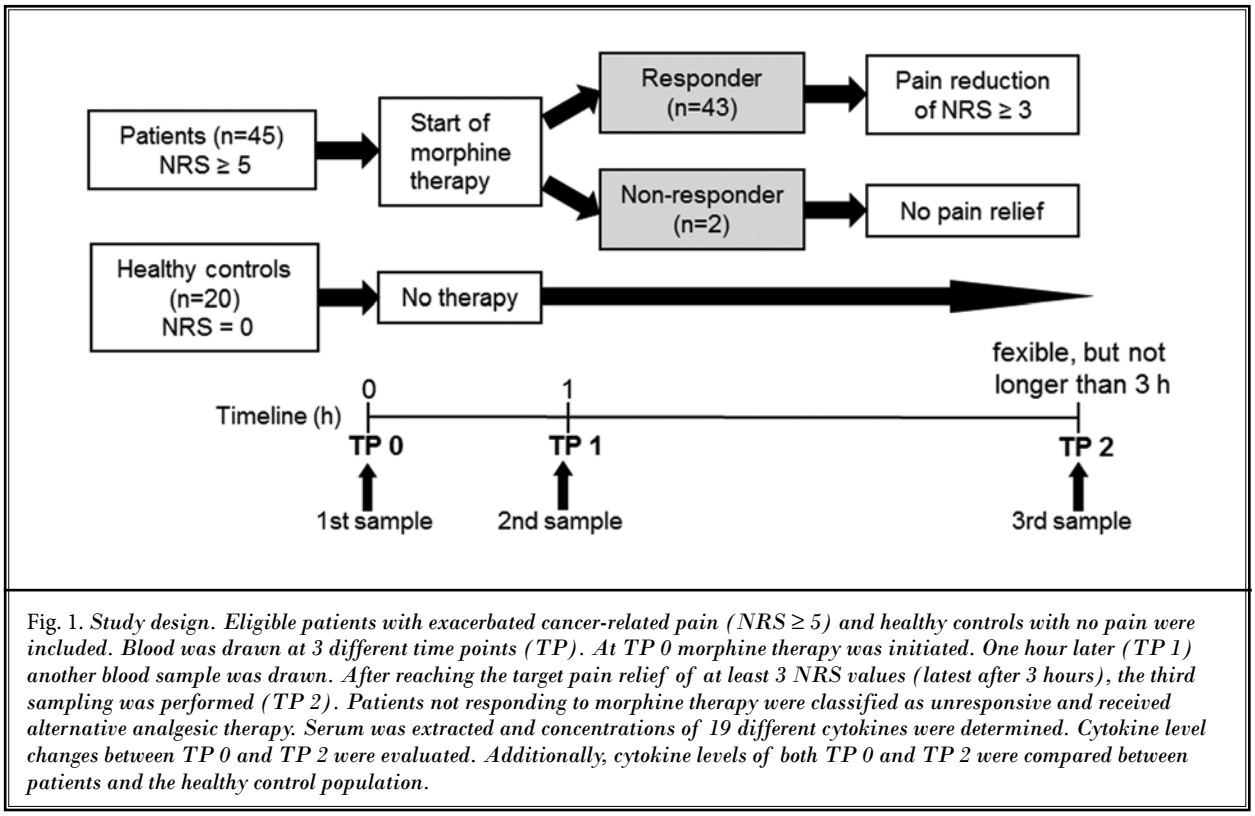

Fig. 1. *Study design. Eligible patients with exacerbated cancer-related pain (NRS ≥ 5) and healthy controls with no pain were included. Blood was drawn at 3 different time points (TP). At TP 0 morphine therapy was initiated. One hour later (TP 1) another blood sample was drawn. After reaching the target pain relief of at least 3 NRS values (latest after 3 hours), the third sampling was performed (TP 2). Patients not responding to morphine therapy were classified as unresponsive and received alternative analgesic therapy. Serum was extracted and concentrations of 19 different cytokines were determined. Cytokine level changes between TP 0 and TP 2 were evaluated. Additionally, cytokine levels of both TP 0 and TP 2 were compared between patients and the healthy control population.*

Patients with no satisfactory analgesic response at TP2 - not later than hour 3 - were considered as unresponsive and received an alternative analgesic therapy at the discretion of the responsible physician.

Blood Samples and Cytokine Analysis

Ten mL of blood was drawn and processed within one hour after sampling. Briefly, serum was collected by centrifugation at 3000 x g for 10 minutes at room temperature, aliquoted and stored at -70°C until the analysis day. Serum samples were tested by our EPCRC-partner Bender MedSystems GmbH, Vienna, Austria, for cytokine detection by enzyme-linked immunosorbent assay (ELISA) using eBioscience FlowCytomix assays. The following cytokines were analyzed: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, TNF-α, TNF-β, IFN-γ (assay no. BMS810FF), IL-1α, (assay no. BMS80243FF), IL-7 (assay no. BMS237inst), IL-13, (assay no. BMS8231FF), IL-18 (assay no. BMS8267FF), MCP-1 (assay no. BMS-8281FF), MIP-1α (assay no. BMS82029FF), MIP-1β (assay no. BMS82030FF), and Osteoprotegrin (OPG) (assay no. BMS82021FF).

Statistical Analysis

Since all cytokine values were not normally distributed (Shapiro-Wilk test), we used the Wilcoxon test for comparisons within and the Mann-Whitney-U-test between both patient groups. Cytokine values are presented as median, plus minimum and maximum. To control the false discovery rate, *P* values on cytokine concentrations were corrected for multiple testing using Bonferroni-Holm correction (15). Furthermore, we computed nonparametric Spearman rank correlations and multiple linear regression analysis of cytokine levels with the secondary parameters pain entity, pain intensity, gender, and location of the primary tumor. $P < 0.05$ was considered significant.

Epidemiological and secondary parameters were calculated descriptively and are presented as mean ± SD.

SigmaPlot (Version 12 Systat Software Inc., San Jose, CA) was used for statistical analysis. Sample size calculation was performed using multiple regression power analysis.

Results

Pilot Study: Interim Analysis

After inclusion of 20 healthy controls and 20 patients with cancer, one patient with cancer was excluded for being opioid unresponsive. The interim multivariate analysis of the remaining 19 patients with cancer revealed a significant correlation between the targeted pain relief and the decrease in the serum concentrations in 5 of 19 cytokines: IL-7, IL-18, MCP-1, MIP-1β, and OPG (Fig.1). In healthy controls, all analyzed cytokines showed no significant differences (data not presented). According to the protocol, the study continued with a new sample size of 45 patients achieving 90% power to detect a significant relation of R2 = 0.3 attributed to 5 independent variables with a significance level (alpha) of 0.05.

Final Analysis

Patient Characteristics

In total, we recruited 45 patients with cancer and 20 controls over the 3-year study period (Fig.2). Two unresponsive patients were excluded.

Of 38 analyzable patients (17 men and 21 women, mean age 63.1 ± 11.5 years, range 43 - 89 years), all had progressive metastatic disease (Table 2). Except for one, all patients had prior antitumor therapy at least more than 2 weeks before inclusion. All patients received concomitant medication due to concomitant diseases or symptoms. The mean NRS score before treatment was 6.9 (range 5-10); after treatment it was 3.2 (range 0-7). Nine patients (24%) reported nociceptive pain, 11 patients (29%) reported visceral pain, and 10 patients (26%) reported bone pain. Only 5 patients (13%) reported neuropathic pain, and 3 patients (8%) reported mixed pain. Mean time for the targed pain reduction of at least 3 NRS values was 61.8 minutes (range 20 -140 minutes), whereas 11 patients achieved the targeted pain response before TP1. The mean required morphine dose was 24.1mg (range 3-180 mg).

Of 20 recruited healthy individuals, all were eligible for analysis (10 women and 10 men; mean age 27.5 ± 4.3, range 25-38).

Comparing Cytokine Concentrations of Patients with Cancer Versus Healthy Individuals (TP0 vs. TP0; TP2 vs. TP2)

We observed a broad variability of the measured cytokines. Of 19 analyzed cytokines at TP0, the median baseline serum levels of 15 cytokines were elevated only in patients with cancer (Table 3). Of these 15 remaining cytokines, 14 cytokines still showed significant differences in median concentrations at TP2, except for MIP-1β.

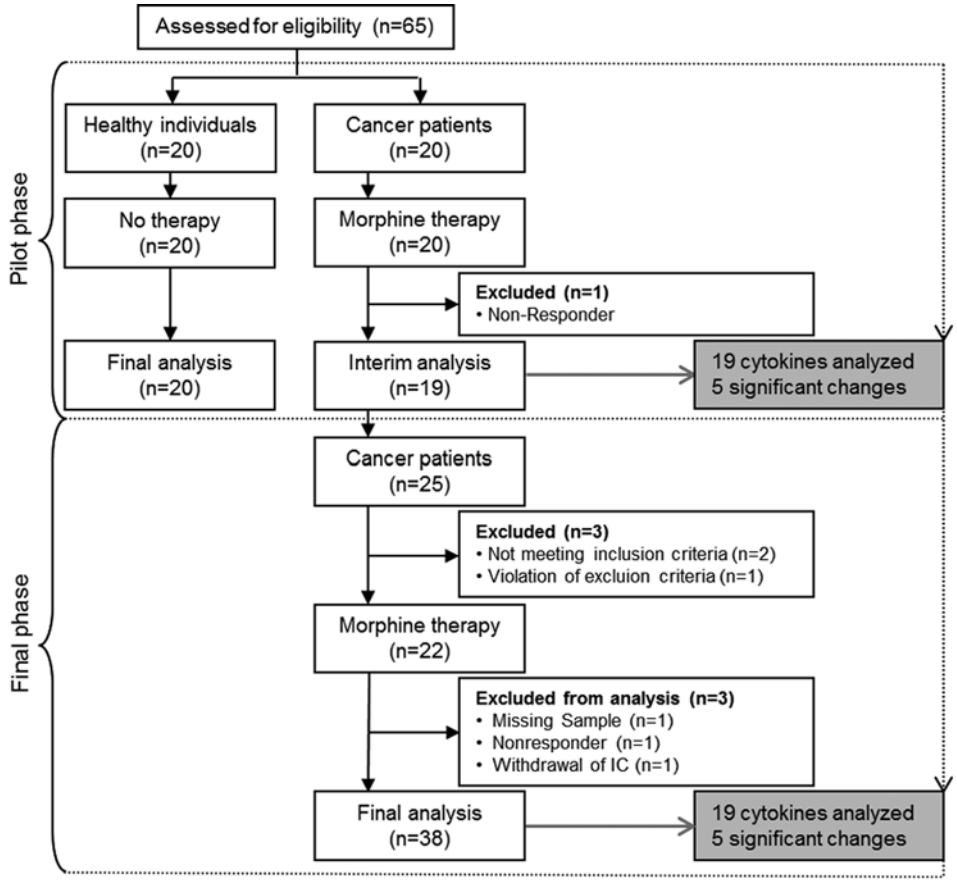

Fig. 2. *Consort diagram: Progress of all participating patients with cancer and healthy individuals.*

Correlation Between Changes in Cytokine Concentrations and Pain Relief in Cancer Patients (TP0 vs. TP2)

The final analysis of all 38 patients with cancer confirmed the correlation of the targeted pain relief with the median decrease in serum concentrations of the same 5 cytokines: IL-7, IL-18, MCP-1, MIP-1β and OPG (Table 3, Figs. 3a, 3b). Individually in morphine-responders, not all cytokine levels decreased within the observation period. Twelve patients (31.6%) showed an increase in one; 6 patients (15.8%) in 2 or 3 of the 5 candidate cytokines. In contrast, we observed a decrease in all 5 cytokines in 20 patients (52.6%).

The 2 unresponsive patients showed inconsistent cytokine level changes (data not shown or analyzed).

Correlation Between Cytokine Levels and the Secondary Variables Pain Entities, Gender, NRS, or Location of Primary Tumor

Only one significant correlation was detected at TP0 between OPG and pain entities ($r = -0.334$; $P = 0.045$). OPG serum levels increased more in patients with nociceptive pain compared to patients with visceral pain ($P = 0.028$) or bone pain ($P = 0.019$). No further correlations were observed (data not presented). Using multiple regression analysis, we identified IL-4, IL-7, IFN-γand OPG as significant predictors for gender ($P < 0.05$), albeit undefined which gender is favored.

Table 2. *Patient demographic characteristics*.

Patient No.	Age	Gender	Location of Primary Tumor	Tumor Stage	Histology Grading	Site of Metastasis	Classification of Pain
P01	59	F	Breast, CUP, Oropharynx	X	2	Bone, Pelvic mass, LN	Multiple
P03	57	F	Uterus	NA	2	Lung, soft tissues	nociceptive
P04	66	F	Pancreas	NA	2	Liver	visceral
P05	49	M	Gall bladder	X	3	LN	nociceptive
P06	43	M	CRC	2	3	Liver	nociceptive
P07	57	F	Pancreas	X	NA	Lung, LN	nociceptive
P08	77	F	Pancreas	4	2	LN, pleura, small intestine	visceral
P09	64	M	Kindey	1	2	Bone	bone
P10	51	M	Lung	NA	3	Bone	bone
P12	79	F	CRC	3	2	Liver, pelvic mass	nociceptive, neuropathic
P13	44	F	Breast	X	3	Liver, none, brain	bone
P14	61	F	CRC	4	3	Liver, lung	nociceptive
P15	58	F	Breast	X	3	Lung, LN	neuropathic
P16	48	F	Breast	X	3	Liver, lung, LN, brain	nociceptive
P17	49	F	Breast	X	2	Cerebellum li	nociceptive
P18	56	M	Stomach	3	3	Liver, LN, pleura	visceral
P19	68	M	Prostate	3	3	LN	bone
P20	57	F	Breast	NA	NA	Pleura	bone
P21	72	F	Klatskin	NA	NA	Liver, pleura	visceral
P24	55	M	Prostate	NA	NA	Bone	neuropathic
P25	46	M	CRC	4	2	Liver, bone	nociceptive
P26	72	M	Pleura	X	NA	Liver	visceral
P27	78	M	Urothelium	3	2	LN	neuropathic
P29	87	F	Tongue	3	2	NA	multiple
P30	69	F	Ovar	NA	NA	Liver, pleura	visceral
P31	67	M	Prostate	X	3	Pleura	visceral
P32	52	M	Ceacum-Ca	4	NA	Liver, pleura	visceral
P34	69	M	Prostate	4	3	Bone	neuropathic
P35	60	M	CUP, Pancreas	X	NA	LN	visceral
P36	57	F	Pancreas	X	3	LN	visceral
P37	72	M	Pancreas	NA	2	Liver, pleura	visceral
P38	70	M	Lung	2	3	Bone	multiple
P39	64	M	CRC, Bladder	3	3	Liver, LN, pleura	neuropathic
P40	88	F	Mamma	CST	NA	LN, pleura	bone
P41	70	F	Mamma	T1C(M)	2	Liver, bone	bone
P42	68	F	Bone	IA	NA	bone	bone
P43	69	F	CRC	3	3	Liver, lung, LN, pleura	bone
P44	77	F	Breast, Pancreas	NA	2	Liver	visceral

M, male; F, female; NA, not available; LN, lymph nodes; CRC, colorectal cancer; CUP, cancer of unknown primary

Table 3. *Serum level concentrations of 19 cytokines of cancer patients and healthy controls including P-values for comparison within and between groups.*

Cytokines	Serum Concentration [pg/mL][a]			*P* Values [b]	
	Healthy Controls (TP 0, n=20)	Patients (TP 0, n=38)	Patients (TP 2, n=38)	Patients vs. Healthy Controls (both at TP 0)	Patients TP0 vs. TP 2
IL-1a	2.6 (0-47.7)	0.1 (0-153.7)	0.1 (0-958.1)	n.s.	n.s.
IL-1b	0 (0-1479)	1.4 (0-2693.8)	1.6 (0-3390.2)	n.s.	n.s.
IL-2	0 (0-82.3)	2.85 (0-370.3)	12.6 (0-331.4)	0.008	n.s.
IL-4	0 (0-0)	2.6 (0-4709.9)	3.7 (0-4517.3)	<0.001	n.s.
IL-5	0 (0-0)	3.3 (0-6298.6)	3.4 (0-4888.9)	<0.001	n.s.
IL-6	0 (0-0)	6.65 (0-118.9)	8.1 (0-97.6)	0.001	n.s.
IL-7	0 (0-123.1)	2.5 (0-63.1)	63.5 (0-6950.9)	0.005	0.045
IL-8	0 (0-211.9)	84.65 (0-8653.7)	0.2 (0-49.8)	<0.001	n.s.
IL-10	0 (0-0)	7.5 (0-183.9)	6.7 (0-162.2)	<0.001	n.s.
IL-12	0 (0-32.7)	0 (0-1594.9)	0 (0-1646.1)	0.035	n.s.
IL-13	0 (0-31.6)	27.45 (0-195.5)	11.2 (0-229)	<0.001	n.s.
IL-18	267.1 (67.9-476.8)	834.6 (38.5-25427)	775.2 (148-10996.1)	<0.001	0.016
TNF-α	0 (0-32)	1.15 (0-2896.1)	0.6 (0-2596.3)	0.009	n.s.
TNF-β	0 (0-542.1)	0 (0-2973.4)	0 (0-5174.8)	n.s.	n.s.
IFN-γ	0 (0-47.5)	0 (0-1894.6)	1.5 (0-1719.4)	0.030	n.s.
MCP-1	437.75 (215.9-722.3)	1027.4 (353.9-7639)	886 (357.3-5746.7)	<0.001	<0.001
MIP-1α	434.6 (2-2263.4)	195.45 (0-171820)	159 (0-167090.1)	n.s.	n.s.
MIP-1β	26.1 (14.7-118.4)	47.9 (8.3-1205.4)	37(10-1146.9)	0.019	<0.001
OPG	41.7 (0-371.8)	136.1 (42.4-633.3)	112.4 (38.4-560.8)	<0.001	<0.001

a Cytokine concentrations in pg/ml, depicted is median (min-max).
b *P* values < 0.05 were considered significant.

Discussion

This is the first prospective, controlled study describing direct associations of cytokine serum concentration changes and a clinically highly relevant reduction in pain intensity within 3 hours after induction of morphine analgesia. Only one recent prospective and uncontrolled study by Makimura et al (11) reported a correlation between different cytokines and those responsive or unresponsive to morphine analgesia on day 8 of morphine treatment.

Of 19 analyzed cytokines in our study, 5 cytokines (IL-7, IL-18, MCP-1, MIP-1β and OPG) turned out to significantly correlate with a pain reduction of at least NRS 3 (Figs. 3a, 3b, Table 3). To exclude that these changes happened by chance or may have occurred due to circadian rhythms, we analyzed a healthy, pain-free control population. All 5 candidate biomarkers showed no intra-individual changes in healthy individuals within the observed period of time. Based on the findings of this study, we expect a strong correlation between the cytokines IL-7, IL-18, MCP-1, MIP1-β, and OPG and a pain relief of NRS ≥ 3 in at least patients with exacerbated cancer-related pain. These findings may also apply for non-cancer-related pain, encouraging further investigations. It is noteworthy that serum concentrations of cytokines did not decrease in all patients; 47.4% of the patients showed an increase of one, 2 or 3 of the above-mentioned cytokines (Figs. 3c-g). This could have been due to confounding by concomitant diseases and/or medication. Despite strong efforts, we were not able to identify obvious confounders common in these patients.

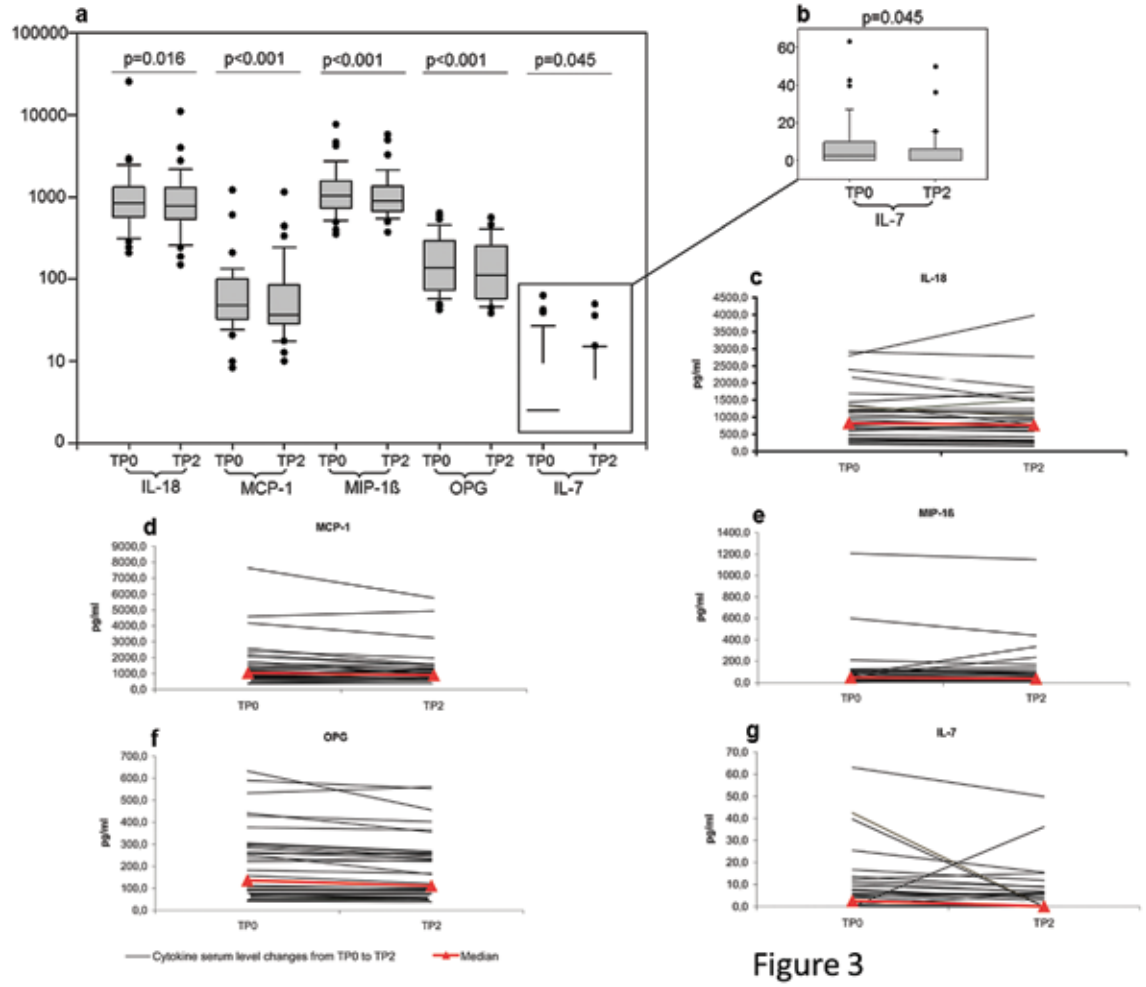

Fig. 3. *Effect of morphine analgesia on cytokine serum concentration changes. Comparison of serum concentrations of the final patient population (n = 38) before initiation of morphine treatment (TP 0) and after reaching target pain relief (NRS reduction of ≥ 3; TP 2).*
a) Cytokine levels for IL-18, MCP-1, MIP-1β and OPG are transformed logarithmically for better visual presentation of the data. The box plots represent median and interquartile ranges plus outliers (nonparametric Wilcoxon Test). P values < 0.05 were considered significant.
b) Boxplot for IL-7 is scaled linear to enable visualization.
c) Serum concentration changes of IL-18
d) Serum concentration changes of MCP-1
e) Serum concentration changes of MIP-1β
f) Serum concentration changes of OPG
g) Serum concentration changes of IL-7
Each black line represents a single patient (n = 38); the thick red line, the median concentration.

Additionally, 15 of the analyzed cytokines had significantly elevated levels at TP0 in the patients with cancer pain compared to the pain-free control group, which is in line with previous reports in the literature (3,4,16,17). At TP2 the same cytokines were significantly elevated except for MIP-1β. This could be easily explained by the strong serum level decrease of MIP-1β after a pain reduction of NRS 3 similar to serum levels observed in healthy, pain-free individuals. In the secondary analysis IL-4, IL-7, IFN-γ, and OPG appeared to account for the ability to predict gender, even if it was undefined which gender was favored due to the lack of enough power of the data. Although there are several studies evaluating the presence of a gender difference of immune response in association with different diseases (18-22), there is only one study reporting a gender difference in cytokine levels in patients with cancer (23). Furthermore, we did not found any significant differences in cytokine levels associated with pain entity or tumor type due to our small sample size, although our patients collectively had several tumor and pain entities.

Four of our 5 identified biomarkers have been previously associated with the development of pain in both human studies and animal models. Verri et al (24-26) demonstrated a novel nociceptive pathway triggered by IL-18 which is mediated by endothelin acting on ETB receptors independent from endogenous release of prostaglandin in mice. Miyoshi et al (27) showed an increase in IL-18 and IL-18R expression in cells of the nervous system after nerve injury in the spinal nerve ligation model. Suppression of IL-18 in animal models suggested a novel therapeutic approach in inflammatory diseases like rheumatoid arthritis, Crohn's disease, and psoriasis (28-31). Sun et al (32) reported a direct interaction of MCP-1 with nociceptive sensory neurons in rats. Another longitudinal study reported a correlation with increasing levels of IL-8 and MCP-1 and pain severity in patients with fibromyalgia (33). It has also been shown that intrathecal administration of MCP-1 leads to neuropathic pain-like behavior in rats, while an MCP-1 neutralizing antibody reduced neuropathic pain (34). Cuellar et al (35) observed higher concentrations of IL-6, MCP-1, MIP-1β, and IFN-g in intraarticular lavage samples from painful human knees compared to nonpainful knees. Makimura et al (11) reported a decrease of MIP-1β and MIP-1α concentrations during morphine treatment.

In contrast to our study, where only 2 patients showed a resistance to morphine treatment, more than 25% of the patients were classified as unresponsive in the study by Makimura et al (11). Their resistance group was rather inaccurately defined as the requirement of > 30 mg of morphine and persistent pain of NRS ≥ 6 without any specification relating the time frame of treatment before classification. However, our definition of unresponsive included patients with a dose of more than 30 mg morphine, possibly explaining our low rate of those unresponsive. Another weakness of the study from Makimura et al (11) is that the authors did not present any control groups. Furthermore, they did not provide any test for normal distribution, even when they analyzed their data using parametric tests, although cytokine serum concentrations are usually not normally distributed. Nevertheless, in our study we also observed a trend for MIP-1α reduction, which was no longer significant after a Bonferroni correction. However, for MIP-1β we can confirm the possible potential of a pharmaco-dynamic biomarker.

A potential involvement on pain mechanisms was also stated for OPG (36). In contrast to previous studies where OPG treatment reduced pain-related behavior in mice and eliminated cancer-induced bone destruction (37,38), we found a decrease of OPG serum levels in correlation with pain reduction. However, the study from Luger et al (31) was performed on bone cancer-related pain, where it is suggested that osteoclast activity is directly involved in generation of bone-cancer pain. Another study revealed that high levels of OPG could be predictive of lack of analgesic response to pamidronate, a bisphosphonate with anti-bone-resorption activity often administered to reduce pain in patients with bone metastases (39). Our finding that OPG serum levels were significantly elevated in patients with nociceptive pain compared to other pain entities, including bone pain, indicates that there exist other, so far unknown, mechanisms for OPG and the generation, maintenance, and/or reduction of different pain entities.

It is well known that IL-7 is part of the regulation of T-cells (40,41). {an article from 9 years ago is not recent} IL-7 induces an expression of cytokines, including MCP-1 and MIP1-ß, in peripheral blood mononuclear cells in patients with unstable angina (42). Even if there is no obvious reason for IL-7 to induce pain, it may be possible that IL-7 is acting via MCP-1 and MIP1-ß.

Even if a direct interaction between the immune system and pain is well known, it is hard to determine whether a decrease of pro-inflammatory cytokines results in pain reduction or vice versa. We cannot exclude

that blocking pain with opioids or other painkillers results in a reduction of cytokine production. On the one hand, opioids are able to activate T-cells (43), but on the other hand there is also evidence that they have apoptotic effects on macrophages (44). Other studies have demonstrated a direct down-regulation of MIP-1ß in leukocytes, astrocytes, and astroglial cells during morphine treatment in vitro (45-47). A couple of studies dealing with regulation of cytokine and cytokine receptor expression by agonists for the 3 major opioid receptor types were reviewed by Finley at al (48). They propose that activation of the kappa opioid receptor induces an anti-inflammatory response through the down-regulation of cytokines.

Nevertheless, it is worth mentioning that our study is exploratory by nature and has several limitations. We evaluated multiple cytokines in a modest sample size, leading to the possibility of false positives despite a Bonferroni correction. The fact that therapies including steroid or immune therapy, chemotherapy, or radiotherapy were excluded from the study made it difficult to recruit a larger population. Another obstacle for inclusion was the existence of breakthrough pain during sufficient pain therapy in many patients. Furthermore, the design of our study did not allow a distinction between cancer and non-cancer-related pain, or opioid therapy versus nonopioid therapy. Additional studies are needed to obtain more statistical power, to elucidate the apparent interpatient heterogenity of cytokine concentration changes, to determine the sensitivity and specificity of our observations, to determine whether significant associations are consistent and causative of pain reduction or opioid treatment, and to test for any correlations between cytokine level changes and different pain entities. Further studies to confirm our findings depend particularly on a precise definition of the study population and control groups.

Summing up our results, we suggest that IL-7, IL-18, MCP-1, MIP1-β and OPG may serve as biomarkers for pain perception and/or pain reduction, therefore forming the basics for development of objective diagnostic tools for pain assessment. Moreover, these biomarkers could serve as potential new targets for painkiller development. This study provides the first evidence of a significant correlation between pain reduction in patients with cancer and at least 5 cytokines. From a clinical perspective, it would be of great advantage having special biomarkers to assess pain intensities, perception, and relief in an objective manner based on biochemical findings.

Conclusion

Since pain is a very subjective symptom, its assessment is prone to bias. So far, there are no objectively measurable biomarkers available to examine pain perception, and/or treatment response. The identified cytokines from this study could serve as such biomarkers for objective pain assessment and monitoring a treatment's outcome. Especially, critically ill or patients with a mental handicap are often unable to effectively self-report their pain at all, making it difficult to assess and manage pain sufficiently. For this reason identification of biomarkers assessing pain using the biological/biochemical way is of great interest, not only for patients suffering from pain, but also for caregivers to manage this pain.

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