Original Article

Inflammatory Markers and Radiotherapy Response in Patients With Painful Bone Metastases

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Abstract

Context. Inflammation is proposed to influence tumor response in radiotherapy (RT). Clinical studies to investigate the relationship between inflammatory markers and RT response is warranted to understand the variable RT efficacy in patients with painful bone metastases.

Objectives. To evaluate the association between inflammatory markers and analgesic response to RT in patients with painful bone metastases.

Methods. Adult patients from 7 European study sites undergoing RT for painful bone metastases were included in this prospective and longitudinal analysis. The association between RT response and 17 inflammatory markers at baseline, as well as the association between RT response and the changes observed in inflammatory markers between baseline and three and eight weeks after RT, was analyzed with univariate regression analyses. Baseline analyses were adjusted for potential clinical predictors of RT response.

Results. None of the inflammatory markers were significantly associated with an upcoming RT response in the analysis of 448 patients with complete baseline data. In patients available for follow-up, the three-week change in TNF (P 0.017), IL-8 (P 0.028), IP-10 (P 0.032), eotaxin (P 0.043), G-CSF (P 0.033) and MCP-1 (P 0.002) were positively associated with RT response, while the three-week change in CRP (P 0.006) was negatively associated.

Conclusion. Results from this study show an association between RT response and change in pro-inflammatory mediators and indicate that inflammation may be important to achieve an analgesic RT response in patients with painful bone metastases. None of the investigated inflammatory markers were found to be pre-treatment predictors of RT response. J Pain Symptom Manage 2022;64:330–339.

Key Words

Cancer, bone metastases, pain, radiotherapy, inflammation

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Key Message

In this prospective multicenter study, we observed that inflammatory mediators can be important to initiate an analgesic RT response in patients with painful bone metastases. The investigated inflammatory markers could not predict an upcoming RT response before treatment.

Introduction

Radiotherapy (RT) is one of the primary treatment options for patients who suffer from painful bone metastases. Meta-analyses report that about 60% of patients experience a significant pain reduction from RT in painful bone metastases.[1] It would be beneficial to identify patients with a high or low probability of pain reduction, so that non-efficient RT with possible adverse effects could be avoided.[2]

When cancer cells metastases to bone, the normal bone homeostasis is disrupted.[3] Inflammatory mediators modulate both the central and peripheral transmission of pain signals.[4] Together with bone resorbing osteoclasts, the inflammatory cells promote acidosis that activate sensory nerve fibers leading to pain.[5] Inflammatory cells also stimulate osteoclastogenesis leading to higher bone turnover and weakening of the mechanical strength of the bone.[3] In murine models of cancer induced bone pain both the pro-inflammatory tumor necrosis factor (TNF) and interleukin-1β (IL-1β) was associated with hyperalgesia.[6,7] Other inflammatory mediators like monocyte chemoattractant proteine-1 (MCP-1), interleukin-6 (IL-6), macrophage inflammatory protein-1α (MIP-1α) and transforming growth factor-β (TGF-β) are also upregulated in animal models of bone metastases and probably contribute in biological pain mechanisms.[8] There is a lack of studies addressing inflammatory mediators in patients with cancer induced bone pain, however data from the general cancer population have indicated an association between pain and inflammation measured by C-reactive protein (CRP)[9–12] and IL-6.[13,14]

Pain relief after RT in patients with bone metastases is related to a reduction in tumor volume, but also to interaction with cells in the bone microenvironment including inflammatory cells.[15,16] RT is thought to trigger the immune system to target the cancer cells, but may also suppress inflammation maintaining pain.[17,18] Although RT is applied locally, effects are also observed at metastatic sites distant to the radiated field. This phenomenon is often referred to as an abscopal effect and supports that systemic immune system activation is an important effect of RT.[19] Immunomodulatory effects of RT are also demonstrated in treatment of inflammatory conditions.[20]

A putative clinical relationship between inflammation and pain response after RT increases the interest of inflammatory mediators as potential biomarkers for RT response in patients with painful bone metastases. An experimental trial investigating inflammatory cytokines in 60 patients with painful bone metastases undergoing RT was recently published.[21] This study did not reveal any significant association in pre-treatment cytokine levels and response to RT in the complete sample.[21] In 2021 we published results from a large prospective international multicenter trial that investigated clinical predictors of analgesic RT response in 460 patients with painful bone metastases.[22] As in other studies, a low discriminative ability limit the application of clinical predictors to select which patients should receive RT.[2,22] CRP was also investigated as a potential inflammatory biomarker for RT response.[22] Although CRP values were higher in the non-responding patients before treatment, this association was not significant in the multivariable model. Since CRP is a crude measure of inflammation, we suggest that a more detailed analysis of inflammatory markers is warranted. Based on previous knowledge supporting that inflammation influences cancer induced bone pain and the analgesic response after RT, our hypothesis is that a) inflammatory markers are potential predictors to select patients with a higher likelihood of RT response prior to treatment and b) the level on inflammatory markers will deviate in responders and non-responders after RT treatment. Thus, we aim to report the association between inflammatory markers and RT response in 448 patients with painful bone metastases.

Material and Methods

Study Population

Patients referred to RT caused by painful bone metastases were included in this prospective and international multicenter study from 2013 to 2017. Inclusion in the study required the patients to have a verified cancer diagnosis, radiological verified bone metastases and an age over 18 years. Patients receiving both single and multiple fraction RT were included. Exclusion criteria were pathological fractures in long bone, RT administered within the last fourweeks prior to inclusion in the study, previous participation in the study or inability to comply with trial procedures.[23] Patients with a measurable RT response status, a worst baseline pain score ≥ 2 and cytokines available at baseline were included in the analyses.

Clinical Variables and Outcome Measures

Baseline information was collected within one week prior to the start of RT, with follow-up at three and

eight weeks after the last RT fraction. Pain was reported by the patients as pain at rest and pain at movement at the radiated site last 24 hours in an 11-point numeric rating scale (0-10; 0-no pain, 10-worst imaginable pain).[24] Opioid doses and routes were obtained and converted to oral morphine equivalents last 24 hours (OMED).[25] Other baseline variables recorded were; age, gender, cancer diagnosis, metastatic distribution including site of metastases, soft tissue components at radiated site and radiologically appearance of sclerotic or osteolytic skeletal lesions, Karnofsky performance status,[26] Charlson comorbidity score,[27] and the use of corticosteroids. The worst pain score was used to assess RT response as recommended in the international consensus paper on RT trials.[28] RT response was defined according to international consensus.[29] Patients were defined as RT responders if they had at least a 2-point reduction in worst pain at the 0-10 numeric rating scale with no increase in opioid dose or a 25% reduction in opioid dose without increase in pain score.[29]

Blood Samples

Blood samples were obtained within one week before the start of RT and three and eight weeks after the last RT fraction (+/- 2 days). Clinical chemistry blood samples including CRP (mg/l), white blood cells (10^9/l) and differential count were performed at the local laboratory at each site. Serum for cytokine analyses were after the withdrawal of blood centrifuged at room temperature at 2200 g for ten minutes, frozen within one hour and stored at -80 degrees Celsius until analyses. Selection of relevant inflammatory markers was based on previously described associations with cancer induced bone pain or RT response, and the most relevant cytokine kit was selected for analyses.[6–8,15,23,30] The inflammatory cytokines (Interferon gamma [IFN-γ]), IL-1β, IL-2, IL-4 IL-5, IL-10, IL-12p70, IL-13, IL-15, MIP-1α, Granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), TNF, IL1-ra, IL-6, IL-7, IL-8, IL-9, IL-17a, interferon gamma-induced protein-10 (IP-10), eotaxin, MIP-1β, MCP-1, Granulocyte colony-stimulating factor (G-CSF), basic fibroblast growth factor (basic FGF) were analyzed in the laboratory of Nordlandssykehuset Bodø with a M*ultiplex cytokine assay* (Bio-Plex Pro™ Human Cytokine Plex-27 Assay, Bio-Rad Laboratories, Hercules, CA). All cytokine levels are reported as pg/mL and binary logarithmic ($\log_2$) transformed to obtain normal distribution. Five of the cytokines that were included in the analyses had some samples below the lower detection limit. These samples were for statistical analyses set to 0.01 pg/mL.

Statistical Analyses

The analyses were pre-planned and described in the study protocol paper.[23] Descriptive statistics are presented as median with interquartile range (IQR) or Number (N) with percentages (%). To explore if inflammatory markers could improve the prediction of RT response, logistic regression analyses were performed and adjusted for significant variables identified in the previously published clinical prediction model of RT response (Karnofsky performance status,[26] cancer diagnosis, presence of soft tissue component outside bone and the use of corticosteroids).[22] The changes in the 17 inflammatory markers from baseline to three and eight weeks after RT were calculated for patients with available follow-up data and analyzed as predictors of RT response in univariate logistic regression analyses. caused by the considerable biological dependency between the markers measured, we did not do any correction based on multiple testing. All analyses are performed using STATA v16 (Stata Corporation LP; College Station, TX).

Sample Size

Sample size was based upon prediction of RT response as the primary outcome, with 29 independent variables at baseline including the inflammatory markers analyzed in this paper. The needed number of patients was set to 290 with a consensus to enroll 600 patients to account for missing, interactions and patients lost to follow-up.[22,23] This paper presents in addition a longitudinal secondary analysis of patients with available inflammatory mediators, assessed as change from baseline to follow-up. Because sample size was determined for the analyses of baseline variables, no formal sample size calculation was performed in respect to the longitudinal analyses. The longitudinal results must therefore be carefully interpreted with respect to the risk for a type II error.

Ethics

All patients signed an informed consent before participation in the study. The study was approved by The Regional Committee for Medical and Health Research Ethics (2013/1126/REK midt) and by the regulatory authorities at each trial site.

Results

574 patients were enrolled in the study[22] but 126 patients (22 %) had missing baseline data, or a lack of RT response status. Baseline characteristics of the 448 patients included in the analysis are presented in Table 1. The median age was 67 years (IQR 59−74), 274 patients (61 %) were men, and the median Karnofsky performance status was 79 (IQR 70−80). The most common cancer diagnosis was prostate (26 %), breast (20 %) and lung (19 %). The median opioid dose in oral morphine equivalents last 24 hours was 25 mg (IQR 5−80), and the median worst pain score at the treated site was 6 (IQR 4−8). Of the included patients, 219 (49 %,

Table 1
Patient Characteristics at Baseline (N 448).

	Median (IQR[a])	N (%)
Age	67 (59−74)	
Gender		
Male		274 (61 %)
Female		174 (39 %)
Karnofsky performance status	79 (70−80)	
Charlson comorbidity Score	6 (6−7)	
Cancer diagnosis		
Prostate		116 (26 %)
Breast		89 (20 %)
Lung		85 (19 %)
Gastrointestinal		68 (15 %)
Urological		51 (11 %)
Other/unknown		39 (9 %)
Metastases		
Other sites than bone		280 (63 %)
Only bone		168 (38 %)
RT fraction		
Multiple fraction		280 (63 %)
Single fraction <=8 Gy		168 (38 %)
Soft tissue expansion at radiated site		
No		293 (65 %)
Yes		145 (32 %)
Not evaluable		10 (2 %)
Osteolytic metastases at radiated site		
No		252 (56 %)
Yes		168 (38 %)
Not evaluable		28 (6 %)
Radiation location in weight bearing bone		
No		66 (15 %)
Yes		382 (85 %)
Maximum pain at radiated site last 24h	6 (4−8)	
Episodic pain		
No		155 (35 %)
Yes		276 (62 %)
Opioid dose[b]	25 (5−80)	
Corticosteroids		
No		252 (56 %)
Yes		194 (43 %)
Study center		
Trondheim		180 (58 %)
Oslo		143 (32 %)
Milan		38 (13 %)
Aalesund		37 (8 %)
Forli		21 (5 %)
Lleida		19 (4 %)
Hull		10 (2 %)

[a]IQR = interquartile range.
[b]Oral morphine equivalents last 24 hours.

95 % CI 46 % − 56 %) responded to RT and 229 (51 %, 95 % CI 44 % − 54 %) did not respond to RT. Twelve cytokines (INF-γ, IL-1β, IL-2, IL-4 IL-5, IL-10, IL-12p70, IL-13, IL-15, MIP-1α, GM-CSF and VEGF) had non-detectable values (> 20 %) or low levels similar to population levels, and therefore not analyzed further.

Inflammatory Markers Before Treatment and Associated With RT Response

Table 2 shows the median level of the inflammatory markers in RT responders vs. non-responders before the start of RT. Patients with RT response had a slightly lower baseline level of IL-8 ($\log_2$ median 3.5 pg/mL, IQR 2.7−4.1) compared to non-responders ($\log_2$ median 3.6 pg/mL, IQR 2.9−4.3) and they had a lower CRP (median 8 mg/l, IQR 5−29) compared to non-responders (median 13 mg/l, IQR 5-40). No significant difference was observed between responders and non-responders in logistic regression analysis adjusted for clinical variables (Table 3).

Change in Inflammatory Markers After RT and the Association With RT Response

Samples from 120 patients were obtained for inflammatory cytokine measurements before RT and both three and eight weeks after the last RT fraction. The number of patients with available follow-up measures was 175 for CRP and 181 for white blood cells with differential count. The change in TNF Odds ratio (OR) 3.48, 95 % confidence interval (CI) 1.25−9.66), IL-8 (OR 1.79, 95 % CI 1.06−3.0), IP-10 (OR 1.5, 95 % CI 1.04−2.18), eotaxin (OR 2.37, 95 % CI 1.03−5.48), G-CFS (OR 1.97, 95 % CI 1.05-3.67) and MCP-1 (OR 2.08, 95 % CI 1.30−3.33) from baseline to three weeks were positively associated with RT response (Table 4). On the contrary, the change in CRP (OR 0.99, 95 % CI 0.98−1.0) from baseline to three weeks was negatively associated with RT response (Fig. 1, Table 4). There were no significant associations between RT response and change in any inflammatory markers eight weeks post RT.

Discussion

In this study we investigated the association between inflammatory markers and analgesic RT response in a large number of patients with painful bone metastases. None of the investigated inflammatory markers measured before treatment were associated with analgesic RT response, but we observed that changes in several inflammatory markers from baseline to three weeks after RT were significantly different between RT responders and non-responders. Our findings may suggest that changes in inflammation can be a part of the response to RT in patients with painful bone metastases.

The Role of Inflammatory Markers in Predicting RT Response

Inflammation has an important role in cancer, but the relationship between cancer and the immune system is complex and not fully understood.[31] Inflammatory mediators are proposed to increase pain severity,[8,10,12,32] and play an essential role in tumor response after RT.[15,17−19] However, results from this study does not support that inflammatory mediators are important pre-treatment predictors of RT response

Table 2
Median Level of Inflammatory Biomarkers Before Treatment With Comparison Between RT Responders and Non-Responders.

	RT Response			No RT Response		
	Number	Median	IQR	Number	Median	IQR
TNF	**219**	**6.2**	**(5.4−7.0)**	**229**	**6.3**	**(5.4−7.1)**
IL1-ra	219	5.7	(6.6−8.0)	229	7.6	(6.4−8.1)
IL-8	**219**	**3.5**	**(2.7−4.1)**	**229**	**3.6**	**(2.9−4.3)**
IL-9	219	8.7	(8.1−9.7)	229	8.7	(8.1−9.8)
IP-10	219	9.2	(8.4−10.0)	229	9.0	(8.2−10.3)
Eotaxin	219	5.9	(5.5−6.4)	229	6.0	(5.3−6.6)
MIP-1β	219	7.0	(6.6−8.0)	229	7.1	(6.6−8.0)
G-CSF	219	6.1	(5.4−6.7)	229	6.2	(5.6−6.8)
IL-6	219	1.3	(0.4−2.2)	229	1.3	(0.4−2.4)
IL-7	219	3.5	(2.8−3.9)	229	3.5	(2.8−4.1)
IL-17A	219	3.2	(2.8−3.7)	229	3.2	(2.7−3.7)
MCP-1	219	5.7	(4.8−6.2)	229	5.5	(4.6−6.2)
Basic FGF	219	3.8	(1.7−4.7)	229	3.8	(2.7−4.9)
CRP	**203**	**8**	**(5−29)**	**210**	**13**	**(5−40)**
Total White count	216	7.4	(5.6−9.3)	228	7.6	(5.8−10.5)
Total Lymphocyte count	211	1.4	(1.0−1.9)	220	1.3	(0.9−1.8)
Total Neutrophil count	211	5.1	(3.5−6.6)	220	5.0	(3.5−6.6)

Abbreviations: TNF = tumor necrosis factor, IL = interleukin, IP-10 = interferon gamma-induced protein-10, MIP-1β = macrophage inflammatory protein 1 beta, G-CSF = granulocyte colony-stimulating factor, MCP-1 = monocyte chemoattractant proteine-1, Basic FGF = basic fibroblast growth factor, **CRP** = c-reactive protein, IQR = interquartile range.
Statistical significance < 0,05 (Mann-Whitney U test) are marked with bold letters.

in patients with painful bone metastases. This is consistent with our previous finding that CRP did not predict RT response in the multivariable clinical model of patients with painful bone metastases.[22] Our results are also similar to an explorative study by McLeod et al. that neither found any difference in the investigated cytokines before the start of RT when analyzing samples from 60 cancer patients.[21] Our findings illustrate that clinical variables are to date better predictors for analgesic RT response in patient with painful bone metastases than the provided panel of inflammatory markers.[2,22]

Table 3
Inflammatory Biomarkers at Baseline and Association With RT Response.

	OR	95 % CI	*P*[a]
TNF-a	0.99	0.81−1.20	0.911
IL1-ra	1.02	0.97−1.06	0.436
IL-8	0.93	0.77−1.12	0.451
IL-9	1.04	0.84−1.28	0.710
IP-10	1.00	0.86−1.17	0.967
Eotaxin	0.92	0.73−1.17	0.513
MIP-1β	1.03	0.79−1.34	0.830
G-CSF	0.90	0.74−1.10	0.292
IL-6	1.08	0.97−1.19	0.143
IL-7	1.01	0.90−1.13	0.903
IL-17A	1.11	0.88−1.39	0.398
MCP-1	0.97	0.82−1.16	0.761
Basic FGF	1.01	0.96−1.05	0.724
CRP	1.00	1.00−1.01	0.878
Total White count	0.97	0.92−1.02	0.255
Total Lymphocyte count	1.05	0.84−1.33	0.653
Total Neutrophil count	0.96	0.91−1.02	0.233

[a]Logistic regression adjusted for clinical variables significantly associated with RT response: Cancer diagnosis, karnofsky performance status, presence of soft tissue metastases and the use of corticosteroids.Abbreviations: TNF = tumor necrosis factor, IL = interleukin, IP-10 = interferon gamma-induced protein-10, MIP-1β = macrophage inflammatory protein 1 beta, G-CSF = granulocyte colony-stimulating factor,MCP-1 = monocyte chemoattractant proteine-1, Basic FGF = basic fibroblast growth factor, CRP = c-reactive protein, IQR = interquartile range.

Inflammatory Markers After RT

Although we could not demonstrate inflammatory markers to improve the clinical prediction of a RT response, the pattern of inflammatory markers was different in the responding and non-responding patients after treatment. It is of interest if these findings reflect an inflammatory process which influence tumor response and analgesic relief shortly after RT in patients with painful bone metastases. With a median time to pain response of approximately 1−4 weeks after RT,[33] it could be expected that the inflammatory differences would be most prominent early after RT as observed in this study.

Noticeably, four of the six inflammatory markers with a significantly greater change after three weeks are potent chemokines (IL-8, IP-10, eotaxin, and MCP-1). Chemokines are proteins that induce chemotaxis that attracts white blood cells towards a chemical gradient.[34] Attraction and activation of white blood cells are probably fundamental to trigger an immune-mediated tumor response to RT.[18]

IL-8 (CXCL2) is a chemokine important in angiogenesis as well as inflammation by recruiting neutrophils. IL-8 can be produced by the tumor cells and circulating IL-8 is known to reflect tumor burden in cancer patients.[35] IP-10 (CXCL10) is a chemokine that in addition to recruitment of immune cells is especially important in differentiation to mature T-helper cells that plays an essential role in adaptive immune responses.[36] There are several indications that both IL-8 and IP-10 are involved in the inflammatory response

Table 4
Change in inflammatory biomarkers from baseline to three- and eight-weeks post RT and association with RT response.

			△ three Weeks After RT			△ eight Weeks After RT	
	Number	OR	95 % CI	P[a]	OR	95 % CI	P[a]
TNF	120	3.48	1.25−9.66	0.017	0.97	0.50−1.91	0.938
IL1-ra	120	1.02	0.95−1.09	0.621	1.03	0.95−1.12	0.421
IL-8	120	1.79	1.06−3.00	0.028	0.94	0.65−1.37	0.751
IL-9	120	0.97	0.44−2.14	0.949	1.26	0.55−2.88	0.585
IP-10	120	1.50	1.04−2.18	0.032	0.90	0.64−1.28	0.572
Eotaxin	120	2.37	1.03−5.48	0.043	1.19	0.64−2.21	0.589
MIP-1β	120	1.21	0.43−3.38	0.720	1.68	0.61−4.62	0.316
G-CSF	120	1.97	1.05−3.67	0.033	1.15	0.72−1.84	0.561
IL-6	120	1.05	0.89−1.24	0.569	0.94	0.79−1.11	0.464
IL-7	120	1.15	0.82−1.60	0.429	1.13	0.84−1.51	0.416
IL-17A	120	1.62	0.75−3.51	0.221	1.29	0.63−2.65	0.489
MCP-1	120	2.08	1.30−3.33	0.002	1.05	0.76−1.45	0.776
Basic FGF	120	0.92	0.81−1.04	0.177	0.94	0.85−1.03	0.192
CRP	175	0.99	0.98−1.00	0.006	0.99	0.99−1.00	0.061
Total White count	181	1.02	0.95−1.09	0.586	0.96	0.88−1.04	0.306
Total Lymphocyte count	181	1.04	0.72−1.50	0.830	1.03	0.73−1.46	0.860
Total Neutrophil count	181	1.02	0.95−1.10	0.542	0.96	0.88−1.04	0.326

[a]Univariate logistic regression. △ = (three- and eight-weeks value of inflammatory marker) - (value before the start of RT).Abbreviations: TNF = tumor necrosis factor, IL = interleukin, IP-10 = interferon gamma-induced protein-10, MIP-1β = macrophage inflammatory protein 1 beta, G-CSF = granulocyte colony-stimulating factor, MCP-1 = monocyte chemoattractant proteine-1, Basic FGF = basic fibroblast growth factor, CRP = c-reactive protein, OR = odds ratio, CI = Confidence interval.

after RT.[30,36–38] In a study of 28 patients with painful bone metastases undergoing RT, the IL-8 and IP-10 levels were lower among patients experiencing a temporary increase in pain directly after treatment.[30] This is in accordance with our results observing a significantly higher increase in both IL-8 and IP-10 from baseline to three weeks in RT responders compared to non-responding patients (Fig. 1).[39]

Interestingly, the two other significant chemokines, eotaxin and MCP-1 (CCL2), are both involved in bone remodeling and are associated with increased bone resorption.[40,41] Eotaxin attracts eosinophils, while MCP-1 mainly recruits monocytes to a site of inflammation.[42] G-CSF, that stimulates the proliferation of granulocytes and the progenitor cells from the bone marrow, does also have a role in stimulation of bone cells to promote bone resorption.[43] The process of bone remodeling is essential to restore normal bone strength and probably important to moderate pain after RT. It is therefore interesting to show that the three-week change in both eotaxin, MCP-1 and G-CSF were significantly higher in patients responding to RT.

It is also worth to notice that several of the inflammatory makers that changed after three weeks and were associated with RT response, were found to be mediators of cancer induced bone pain in previous pre-clinical studies. This supports the relevance of our findings. In rats MCP-1 is demonstrated to be a mediator of pain in bone metastases.[8,44,45] G-CSF is proposed to have direct effects on nerve fibers leading to a peripheral sensitization of pain signals promoting cancer induced bone pain,[43] and mouse models have shown that G-CSF stimulates an anti-tumor activity of neutrophils that potentiality leads to better RT outcome.[46,47]

The key inflammatory marker TNF is also associated with cancer induced bone pain in rats,[7,48] and higher levels of TNF is found in patients with cancer pain.[49] RT may induce an increase in TNF.[50,51] Fang et al investigated the level of TNF in regard to analgesic pain response in patients with painful bone metastases treated with a radiopharmaceutical (89SrCl2). They did not detect any difference in TNF levels before the start of treatment which is similar to our findings, but four months after treatment the RT responders had lower TNF values compared to non-responders. The TNF levels were also measured four weeks after RT, but an association with RT response status was not reported in the paper.[52]

In our analyses, CRP was one of the inflammatory markers with the greatest difference between RT responders and non-responders before the start of RT (Table 2). CRP is an acute phase protein and its production is stimulated by the cytokine IL-6.[53] Higher CRP levels is associated with pain in a general cancer population[9,12] and in patients treated with RT.[54] Contrary to what was found with the significantly upregulated inflammatory cytokines, we observed that the median CRP level did not increase in RT responders threeweeks after treatment (Fig. 1), and a lower three-week change from baseline was associated with RT response (Table 3). The reason for the opposite trend for CRP is difficult to explain. One reason might be that a high number of patients had normal measurable levels (<=5 mg/l) with a low variance especially in the RT responder groups. A more sensitive measure of

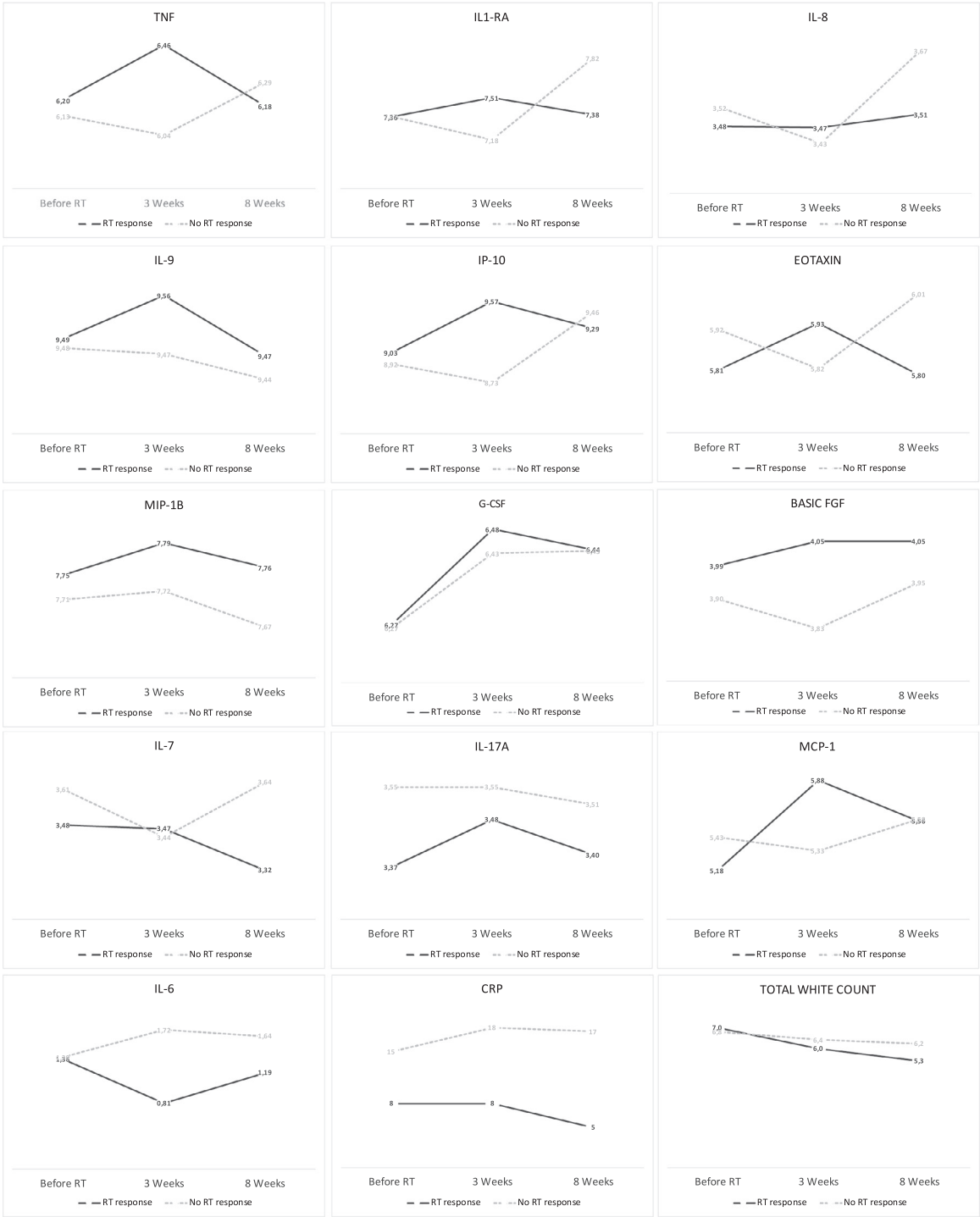

Fig. 1. Median level of inflammatory biomarkers in RT responders compared to non-responders**.** On the x axis time after RT (Before RT, three weeks and eight weeks post RT). On the y axis the median level of inflammatory biomarkers.

CRP, like high sensitivity CRP, might have detected smaller changes.

Other inflammatory markers not analyzed in this study may also be of importance in predicting RT response in patients with painful bone metastases. The explorative paper by MacLeod et al identified insulin-like growth factor binding protein 9 (NOV/CCN3/IGFBP-9) as a potential marker of RT response as it increased in non-responders and decreased in responders four weeks after RT.[21] This cytokine was not measured in our analysis. In a subgroup analysis MacLeod et al also detected lower IL-1β levels at

baseline in responders compared to non-responders in patients with breast cancer (17 of 60 patients). This finding must be interpreted carefully caused by the small sample size, but pre-clinical studies have suggested IL-1β as important in cancer induced bone pain.[8] In our analyses IL-1β was expressed at low levels in all patients and were not included in further analyses. We observed no association with IL-1α and RT treatment response, a cytokine that also act on the IL-1 receptor. MIP-1α and TGF-β are also a potential biomarkers of interest mainly based on knowledge from animal models of cancer induced bone pain.[8] Low levels of MIP-1α were also found in all patients in our study, while TGF-β were not available in the selected cytokine kit.

Summing up the results, we observed a potential role of inflammation in RT response among patients with painful bone metastases. There are similarities between our findings and previously findings from preclinical and clinical studies. The higher threeweek change in several inflammatory markers among patients with analgesic RT response strengthen the hypothesis that activation of the immune system is important to target cancer cells and induce pain relief.[15,18] However, the mechanisms involved in the interplay between inflammation and RT is still not fully understood. The role of inflammation in relation to tumor response is a field of research with a need for clinical studies. For future work we propose to focus on longitudinal studies measuring inflammatory markers over time controlling for potential confounding factors and including validation cohorts. Especially with immunotherapy emerging as a cornerstone in cancer treatment, it is important to understand the inflammatory processes and its effect on treatment outcome. RT may enhance the effect of immunotherapy and several clinical trials are initiated to investigate this treatment combination.[55]

The study has strengths and limitations. The major strength in this paper is the large patient sample compared to similar studies. Another strength of the study is that patients were included from different study sites and countries, and that the study was originally designed to evaluate inflammatory markers as potential predictors of RT response. A common limitation in clinical studies investigating inflammatory markers, is the numerous factors affecting systemic inflammation in cancer patients like tumor load, potential ongoing infections, and the use of medications such as opioids and corticosteroids, all of which may have an impact on results in this and other clinical studies. A local inflammatory process after RT may also be important although not reflected in inflammatory mediators measured in serum. Another limitation is not including a validation sample. Moreover, the analyses were not corrected for multiple testing caused by the expected dependency between variables. Finally, there were also a reduced number of patients available for blood samples at three and eight-weeks post RT. This because the patients either refused or were too sick to come to the hospital for follow-up.

Conclusion

In conclusion, findings from this study indicate that inflammatory mediators may be important to initiate an analgesic RT response in patients with painful bone metastases. None of the investigate inflammatory markers were reliable predictors of RT response to select patients with a higher likelihood of response prior to treatment. However, the association between RT and change in inflammatory markers could point towards inflammation as a potential future treatment target.

Disclosures

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