Editor's note:
"Palliative Radiotherapy Column" features articles emphasizing the critical role of radiotherapy in palliative care. Chairs to the columns are Dr. Edward L. W. Chow from Odette Cancer Centre, Sunnybrook Health Sciences Centre in Toronto and Dr. Stephen Lutz from Blanchard Valley Regional Cancer Center in Findlay, gathering a group of promising researchers in the field to make it an excellent column. The column includes original research manuscripts and timely review articles and perspectives relating to palliative radiotherapy, as well as editorials and commentaries on recently published trials and studies.

Palliative Radiotherapy Column (Original Article)

Urinary cytokines/chemokines pattern in patients with painful bone metastases undergoing external beam radiotherapy experiencing pain flare

Ahmad Bushehri[1], Edward Chow[1], Liying Zhang[1], Azar Azad[2], Sherlyn Vuong[1], Mark Pasetka[3], Michelle Zhou[3], Amanda Hird[1], Kristopher Dennis[4], Rachel McDonald[1], Carlo DeAngelis[3,5]

[1]Department of Radiation Oncology, Odette Cancer Centre, Sunnybrook Health Sciences Centre, [2]Department of Laboratory Medicine and Pathobiology, [3]Department of Pharmacy, Odette Cancer Centre, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, ON, Canada; [4]Division of Radiation Oncology, University of Ottawa, Ottawa Hospital Research Institute, Ottawa, Canada; [5]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, Canada

Contributions: (I) Conception and design: C DeAngelis, E Chow; (II) Administrative support: S Vuong, R McDonald; (III) Provision of study materials or patients: E Chow; (IV) Collection and assembly of data: A Bushehri, E Chow, S Vuong, M Zhou, A Hird, K Dennis; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. Edward Chow, MBBS, MSc, PhD, FRCPC. Department of Radiation Oncology, Odette Cancer Centre, Sunnybrook Health Sciences Centre, 2075 Bayview Avenue, Toronto, Ontario, Canada. Email: edward.chow@sunnybrook.ca.

Background: External beam radiotherapy (EBRT) is a mainstay for treatment of painful bone metastases. Transient worsening of pain ("pain flare") occurs in 40% of patients. We investigated the pathophysiology of pain flare through assessment of changes in urinary cytokines/chemokines in patients receiving EBRT for painful bone metastases.

Methods: Urine samples were collected from patients receiving a single 8 Gy fraction for painful bone metastases preparation, day 1 or 2 and on an additional day between days 3 to 5 post radiation. Patients completed a standardized pain and analgesic use diary daily for 10 days following radiation. Patients were deemed to have pain flare if they had a two-point increase from baseline worst pain on 0–10 scale and no decrease in analgesic intake or a 25% increase in analgesic intake with no decrease in worst pain. The Millipore Milliplex 42-Plex Cyto-kine/Chemokine Kit™ was used to measure urinary levels of a panel of cytokines/chemokines.

Results: Forty-six patients consented to the study of which 28 were evaluable (complete urine and diary data), and 83/84 urine samples were available for analysis. Pain flare was experienced by 11 patients (39%). The following cytokines/chemokines were detectable in at least 50% of the patients: EGF, fractalkine, GRO, IL-4, IL-8, interferon gamma induced protein 10 (IP-10), MCP-1, macrophage derived chemokine (MDC), PDGF-AA, sIL-2Ra, TGF-Alpha, VEGF. Comparing patients with or without pain flare EGF, fractalkine, GRO, IL-8, IP-10, MCP-1, MDC, sIL-2Ra, and TGF-alpha increased following radiation in both groups. Patients with pain flare have significant lower levels on IL-8, IP-10, and MDC over time. No specific time

trend was noticed.

Conclusions: Patients who experience pain flare appear to have a different pattern in urinary cytokine/chemokine levels than patients without pain flare. A larger study is required to confirm the possible role of cytokines/chemokines in predisposition to and/or the cause of pain flare following radiation to painful bone metastases.

Keywords: Bone metastases; external beam radiotherapy (EBRT); pain flare; inflammatory markers; urinary cytokines/chemokines

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Introduction

Bone metastases are a common occurrence in patients with metastatic cancer. Cancer induced bone pain (CIBP) is a complex phenomenon mediated by the combined effects of locally produced cytokines, local primary afferent nerve fibers and the central nervous system (1). Pain is experienced by 50% to 75% of patients with bone metastases (1). Recent evidence-based guidelines reaffirm external beam radiotherapy (EBRT) as the mainstay for treatment of painful bone metastases (2). The overall pain response rate is approximately 60% (3). Worsening of pain ("pain flare") within 10 days of radiotherapy to bone metastases occurs in up to 40% of patients (4). The pathophysiology of pain flare is poorly understood (5). We postulated that inflammatory mediators play a role in pain flare.

The inflammatory markers are often measured in serum, but can also be measured in urine samples, which is preferable for our palliative patient population. This has been demonstrated in several studies using enzyme-linked immunosorbent assay (ELISA) for analysis of these cytokines (6,7). A recent study by Sirera et al demonstrated no statistically significant difference in levels of IL-6 and TNFα measured in serum and urine (6). We investigated the pathophysiology of pain flare through assessment of changes in urinary cytokines/chemokines in patients receiving EBRT for painful bone metastases to better understand the mechanism of pain flare.

Methods

Eligible cancer patients with bone metastases treated with single fraction EBRT at Odettte Cancer Centre (OCC) were approached. Inclusion criteria included pathological diagnosis of cancer, radiological evidence of bone metastases (e.g., bone scan, plain X-ray, CT or MRI scans) and treatment with a single 8 Gy fraction. Patients were able to understand English and complete the interview/diary, as well as willing to complete the daily diary on pain score and analgesic intake before, daily during radiation, and 10 days following the completion of radiation treatment. Patients were to give a written consent to enrollment. Exclusion criteria included pathological fracture at the irradiated site, spinal cord or cauda equina compression, patients taking any form of steroids within a week of the start of radiation, planned steroid during the radiation and then 10 days following the completion of radiotherapy, planned surgery, start or change of bisphosphonates and/or systemic therapy during the radiation and the 10 days following the completion of radiotherapy.

Ethics approval was obtained from the Research Ethics Board at Sunnybrook Health Sciences Centre. Urine samples were collected from patients receiving a single 8 Gy radiotherapy for painful bone metastases at baseline (day of EBRT), day 1 or 2 and on an additional day between days 3 to 5 post radiation (*Figure 1*).

Patients completed a standardized pain and analgesic use diary daily for 10 days following the day of radiation. Completing the diary could be done independently or alternatively through the research assistant who could contact the patient daily by telephone to collect the data.

Patients were deemed to have pain flare if they had a two-point increase from baseline pain on 0–10 scale and no decrease in analgesic intake or a 25% increase in analgesic intake measured by daily oral morphine equivalent (OME) with no decrease in pain (8). In addition the pain score and analgesic intake had to return back to baseline levels after the flare. Pain flare was distinguished from progression of pain by returning of the worst pain score and analgesic

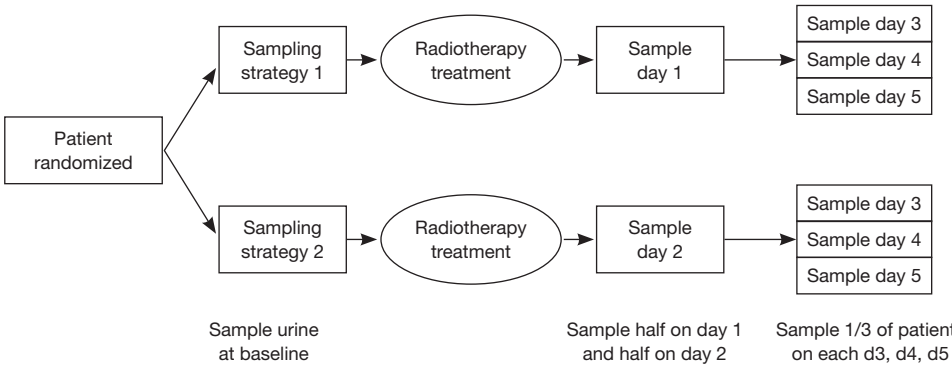

Figure 1 Procedures.

intake to baseline level within the 10 days follow up period.

Given the underlying medical condition for palliative patients, we decided to use the least invasive method to measure cytokines/chemokines. The Millipore Milliplex 42-Plex Cyto-kine/Chemokine Kit™ was used to measure urinary levels of a panel of cytokines/chemokines. Cytokine levels were corrected according to urine creatinine level. In each urine sample we measured EGF, eotaxin, FGF-2, Flt-3 ligand, fractalkine, G-CSF, GM-CSF, GRO, INFα2, INFγ, IL-1ra, IL-1α, IL-1β, IL-2, sIL-2Rα, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17A, interferon gamma induced protein 10 (IP-10), MCP-1, MCP-3, macrophage derived chemokine (MDC), MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, sCD40L, TGFα, TNFα, TNFβ and VEGF as well as markers of bone turnover (N-telopeptides).

Patients were randomized in blocks of three to one of the following groups of urine sample collection time:

(I) Group A—sample on day 1 and day 3;
(II) Group B—sample on day 2 and day 3;
(III) Group C—sample on day 1 and day 4;
(IV) Group D—sample on day 2 and day 4;
(V) Group E—sample on day 1 and day 5;
(VI) Group F—sample on day 2 and day 5.

Statistical analysis

Using a random number generator, we randomly allocated patients in a two-step process first to either sampling strategy 1 or 2 and then to 1 of 3 urine collection strategies for the second urine sample to limit the number of urine samples the patients had to provide and at the same time having urine samples from the group of patients covering baseline through to 5 days after treatment. At the time of study planning there was no data to indicate if and when a cytokine may increase in amount in the urine so we wanted to cover up to 5 days post radiation for the population.

Demographics were summarized in all patients and in patients with pain flare, using mean, standard deviation (SD), median, interquartiles (Q1, Q3), and range for continuous variables; and proportions for categorical variables. Wilcoxon Rank-sum nonparametric test was applied for comparing each cytokine levels between pain flare and non-pain flare at baseline and day 1–5, respectively. To search for significant time trends for each cytokine variable from baseline to day 5 and investigate if pain flare patients had different time trends compared to non-pain flare patients, general linear regression analysis was conducted on each cytokine variable. To normalize the cytokine distribution, natural log-transformation was applied for all cytokine variables. The outcomes were corrected cytokine levels (log scale), the independent variables including time (from baseline to day 5), binary variable of pain flare (1= yes, 0= no), and the interaction term between post treatment day and pain flare. Heat maps were created for 42 cytokine variables in all patients and in patients with or without pain flare. Box plots of percent change from baseline were generated for EGF, IL-8, IP-10, and MDC in all patients and in patients with or without pain flare. All analyses were conducted using Statistical Analysis Software (SAS version 9.4 for Windows), and P value <0.05 was considered statistically significant.

Results

From January 2010 to May 2012, 46 patients consented for the study. Of those, we had 28 patients with complete data. The most common reason for exclusion was incomplete data

Table 1 Demographics in all patients (N=28) and patients with pain flare (N=11)

Characters	All patients (N=28)	Patients with pain flare (N=11)
Age (years)	28	11
Mean ± SD	68.3±12.2	68.4±12.7
Median (Q1–Q3)	68 [59–78]	68 [65–78]
Range	46–88	46–88
Gender		
Female	8 (28.57%)	2 (18.18%)
Male	20 (71.43%)	9 (81.82%)
Primary cancer site		
Prostate	12 (42.86%)	4 (36.36%)
Lung	8 (28.57%)	3 (27.27%)
Breast	5 (17.86%)	2 (18.18%)
Colorectal	2 (7.14%)	1 (9.09%)
Other	1 (3.57%)	1 (9.09%)
Pain score at baseline	28	11
Mean ± SD	6.1±1.5	7.3±1.3
Median (Q1–Q3)	6 [5–8]	8 [6–8]
Range	4–10	6–10
Pain score at day 1–2	28	11
Mean ± SD	6.7±2.5	9.2±1.3
Median (Q1–Q3)	6 [5–10]	10 [8–10]
Range	2–10	6–10
Pain score at day 3–5	28	11
Mean ± SD	6.6±2.6	9.5±0.8
Median (Q1–Q3)	6 [5–10]	10 [9–10]
Range	2–10	8–10
OMED at baseline	28	11
Mean ± SD	8.6±3.9	11.3±4.3
Median (Q1–Q3)	8 [6–10]	10 [8–14]
Range	4–18	4–18
OMED at day 1–2	28	11
Mean ± SD	10.0±5.7	15.3±5.1
Median (Q1–Q3)	8 [6–14]	16 [12–20]
Range	2–22	6–22
OMED at day 3–5	28	11
Mean ± SD	10.6±6.6	17.3±5.2
Median (Q1–Q3)	8 [6–18]	20 [14–20]
Range	2–22	6–22
Radiation site		
Extremities	8 (28.57%)	4 (36.36%)
Spine	8 (28.57%)	3 (27.27%)
Pelvis	7 (25.00%)	3 (27.27%)
Rib	4 (14.29%)	1 (9.09%)
Spine/extremities	1 (3.57%)	0 (0.00%)
Chemotherapy		
No	14 (50.00%)	5 (45.45%)
Yes	14 (50.00%)	6 (54.55%)
Hormone therapy		
No	18 (64.29%)	9 (81.82%)
Yes	10 (35.71%)	2 (18.18%)
Bisphosphonates		
No	23 (82.14%)	9 (81.82%)
Yes	5 (17.86%)	2 (18.18%)

OMED, oral morphine equivalent (mg/day); SD, standard deviation.

in the pain diary. Other reasons included the deteriorating health or death of the patients for which samples were not included in the study. There were no statistically significant differences between enrolled and excluded patients in terms of gender, age, primary cancer site, baseline pain score, baseline analgesic consumtion or previous systemic therapy. None of the patients recruited took non steroidal anti-inflammatory agents during the study period.

Table 1 summarises the demographics for 28 patients enrolled. The median age of enrolled patients was 68 years. There were 20 males (71%) and 8 females (29%). The most common primary cancer site was prostate (43%) followed by lung (29%) and breast (19%). Out of the 28 patients enrolled, 11 (39%) developed pain flare (*Table 1*). Nine were males (82%) and 2 were females (18%). For this group, prostate was the most common primary cancer site (36%), followed by lung (27%) and breast (18%).

All available urine samples for analyzing 42 cytokine variables at baseline, and days 1–5 were used. Each cytokine variable was compared between patients with pain flare and patients without pain flare at baseline and day 1–5, respectively (*Table 2*). *Figure 2* shows that the

Table 2 Comparisons of cytokine levels between patients with and without pain flare at day 0 to 5 (n=11 *vs.* 17) using Wilcoxon rank-sum test

Cytokine variable	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
EGF	0.3694	0.0902	0.1436	0.3329	0.0801	0.6514
Eotaxin	0.5074	0.3898	0.0579	0.7461	0.3490	0.0114
FGF-2	0.3221	0.9815	0.0886	0.6492	0.4005	0.5604
Fit-3 ligand	0.5971	0.0345	0.3021	0.0449	0.4865	0.1350
Fractalkine	0.1128	0.9445	0.8667	0.8465	0.3747	0.9484
G-CSF	0.5970	0.8343	0.2206	0.9999	0.4006	0.1346
GM-CSF	0.7057	0.9999	0.3023	0.6503	0.1305	0.3319
GRO	0.0021	0.9445	0.2213	0.3319	0.5173	0.3324
IFNalpha2	0.4653	0.9444	0.5808	0.9999	0.5805	0.1350
IFN-gamma	0.9799	0.7272	0.2206	0.9999	0.7914	0.1346
IL-1alpha	0.6686	0.7272	0.1303	0.6488	0.4006	0.1346
IL-1beta	0.9999	0.7272	0.2206	0.9999	0.7186	0.1346
IL-1ra	0.0031	0.1703	0.0977	0.0115	0.3492	0.0113
IL-2	0.9799	0.7272	0.2206	0.9999	0.7914	0.1346
IL-3	0.9999	0.7272	0.2206	0.9999	0.7914	0.1346
IL-4	0.7058	0.0273	0.2598	0.6514	0.2400	0.0081
Il-5	0.6443	0.7272	0.2206	0.9999	0.7914	0.1346
IL-6	0.8470	0.4711	0.4008	0.7458	0.9426	0.0440
IL-7	0.7947	0.7272	0.2206	0.6492	0.5167	0.1350
IL-8	<0.0001	0.0666	0.4571	0.9484	0.0885	0.1372
IL-9	0.5073	0.7272	0.1565	0.8454	0.7914	0.1346
IL-10	0.9531	0.7272	0.2206	0.9999	0.7186	0.1346
IL-12-p40	0.7947	0.8707	0.3018	0.9999	0.7914	0.1350
IL-12-p70	0.8733	0.7272	0.2206	0.9999	0.7914	0.1346
IL-13	0.9999	0.7272	0.2206	0.9999	0.7914	0.1346
IL-15	0.4653	0.7272	0.5168	0.9999	0.6482	0.6492
IL-17	0.6686	0.7272	0.2206	0.9999	0.7914	0.1346
IP-10	0.0086	0.4164	0.9426	0.8461	0.4011	0.6510
MCP-1	0.7819	0.2365	0.0582	0.9999	0.7553	0.0612
MCP-3	0.5514	0.3178	0.5171	0.8461	0.2803	0.6514
MDC	0.0781	0.5613	0.7189	0.4762	0.3493	0.7458
MIP-1alpha	0.8601	0.7273	0.3018	0.8456	0.6141	0.0442
MIP-1beta	0.2504	0.6588	0.3495	0.8456	0.9045	0.0449
PDGF-AA	0.1091	0.5616	0.9427	0.1372	0.7919	0.1368
PDGF-AB-BB	0.3516	0.6586	0.0579	0.4747	0.9426	0.0601
RANTES	0.1612	0.1853	0.1078	0.3313	0.6484	0.3303
sCD40L	0.5739	0.7273	0.1303	0.9999	0.9999	0.1350
sIL-2Ra	0.3784	0.4165	0.1310	0.9999	0.7920	0.0454

Table 2 (*continued*)

Table 2 (*continued*)

Cytokine variable	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
TGF-alpha	0.4255	0.4714	0.8291	0.3319	0.9426	0.9999
TNFalpha	0.7689	0.7272	0.2206	0.9999	0.7914	0.1346
TNF-beta	0.7689	0.7272	0.2206	0.9999	0.7914	0.1346
VEGF	0.1613	0.9815	0.3026	0.8465	0.9427	0.1376

IP-10, interferon gamma induced protein 10; MDC, macrophage derived chemokine.

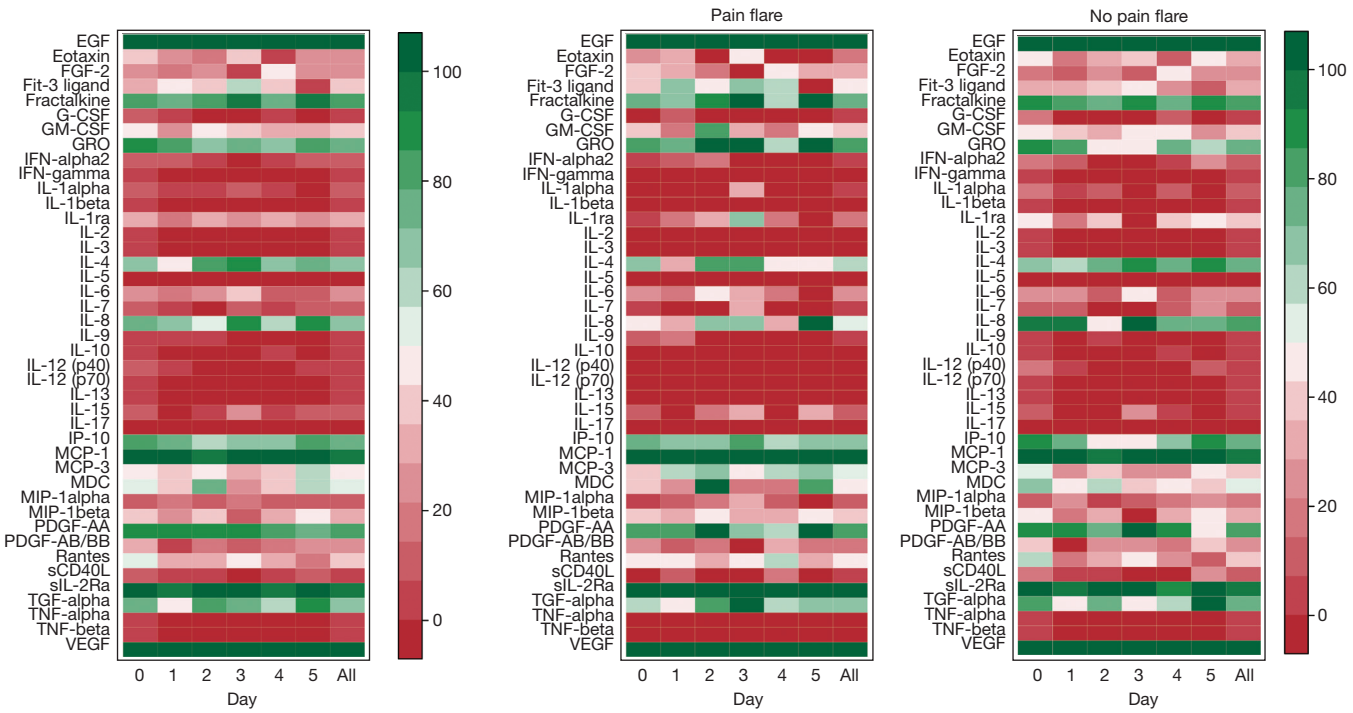

Figure 2 Heat maps showing the proportion of patients with cytokines/chemokines above (green) or below (red) the limit of detection of the assay.

levels of cytokines/chemokines detectable in the urine varied significantly in all patients and in patients with or without pain flare, respectively. The following cytokines/chemokines were detectable in at least 50% of the patients: EGF, fractalkine, GRO, IL-4, IL-8, IP-10, MCP-1, MDC, PDGF-AA, sIL-2Ra, TGF-alpha, VEGF.

Comparing patients with or without pain flare EGF, fractalkine, GRO, IL-8, IP-10, MCP-1, MDC, sIL-2Ra, and TGF-alpha increased following radiation in both groups. *Figures 3* and *4* show boxplot examples of changes in urine selected cytokines/chemokines post radiation in patients with or without pain flare. Pain flare patients had significant lower IL-8 levels (P=0.0007), IP-10 levels (P=0.046), and MDC levels (P=0.03) compared to non-pain flare patients. There was no significant time trends of these cytokines in patients with or without pain flare. For other cytokine variables, there was no significant time trends in all patients, or by pain flare. In general, all cytokine levels were stable over time.

Discussion

Short course radiotherapy can be used for palliating painful bone metastases. The exact mechanism of its palliative benefit is still unclear (9). Pain flare is a common side effect following palliative radiotherapy (10). This pain flare

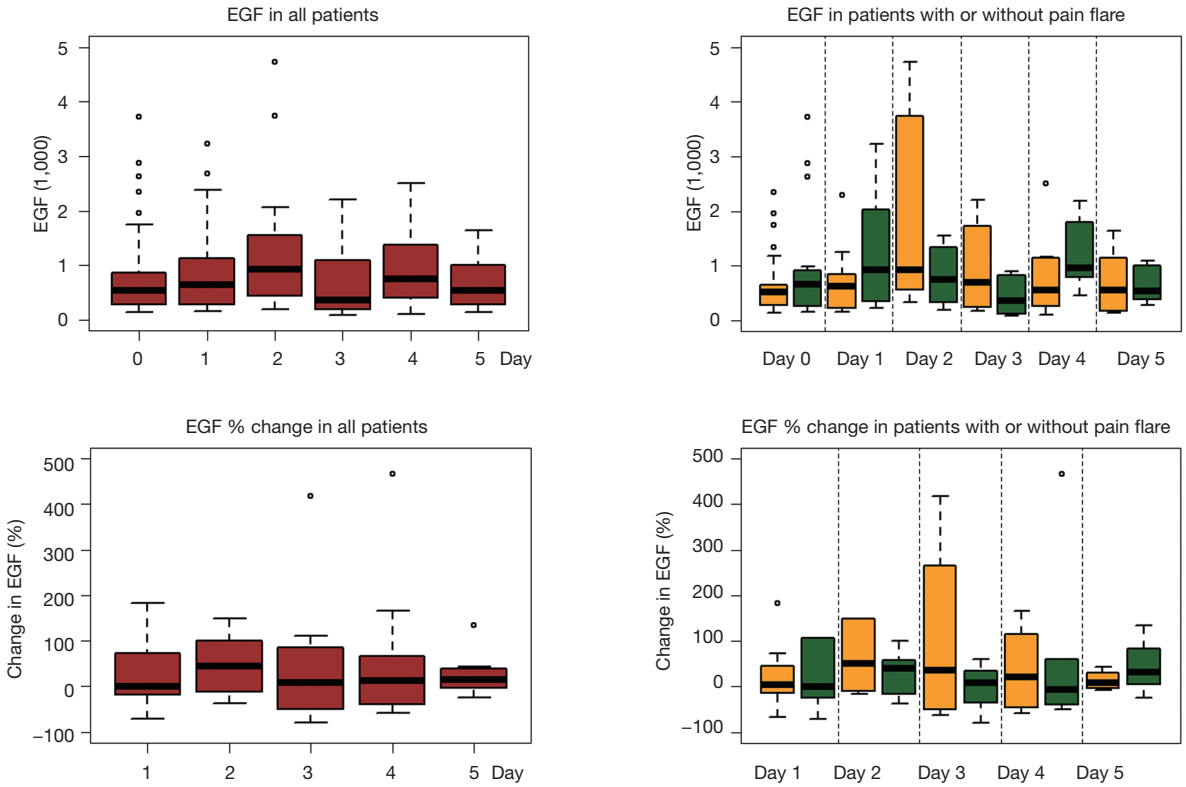

Figure 3 Corrected level (top) and percent change (bottom) in EGF in urine for all patients (red), patients with pain flare (green), and patients without pain flare (yellow)—EGF (epidermal growth factor) is a growth factor that stimulates cell growth, proliferation, and differentiation by binding to its receptor EGFR.

episode can be worrisome and debilitating for patients. Loblaw *et al.* compared a single 8 Gy fraction to 20 Gy in 5 fractions for the treatment of painful bone metastases. Their study concluded that patients receiving single fraction radiotherapy may be at a higher risk of pain flare (11). A single dose of dexamethasone can decrease the incidence of pain flare during the first 2 days immediately after palliative radiotherapy for bone metastases (12). They found in their phase II study that the incidence of pain flare dropped to 22% with the use of dexamethasone. The mechanism behind this phenomenon is still unkown but may be related to suppressing inflammatory cytokines.

Cytokines play a significant role in pain initiation and maintenance. Cytokines may be either pro or anti inflammatory, and an imbalance between the two is thought to contribute to pain flare (13). IL-8 (neutrophil chemotactic factor) cytokine is produced by macrophages and other cell types including epithelial cells. It acts as a signal attracting neutrophils to sites of inflammation (14). IP-10 cytokine is expressed by macrophages, neutrophils and epithelial cells (15). It has neutrophil chemo-attractant properties. It is involved in the processes of angiogenesis, inflammation, wound healing, and tumorigenesis. MDC cytokine recruits monocytes, memory T cells, and dendritic cells to sites of tissue injury, infection, and inflammation (16).

In conclusion, we demonstrated measurable changes in urinary cytokine/chemokine levels following radiation for painful bone metastases. Patients who experienced pain flare appear to have a different pattern in urinary cytokine/chemokine levels than patients without pain flare. To our knowledge, this is the first study that looked at the pathophysiology of pain flare through assessment of changes in urinary cytokines/chemokines in patients receiving EBRT for painful bone metastases to better understand the mechanism of pain flare. Our study was limited by small sample size. Further research is required to confirm the possible role of cytokines/chemokines in predisposing to and/or the cause of pain flare following radiation to painful bone metastases.

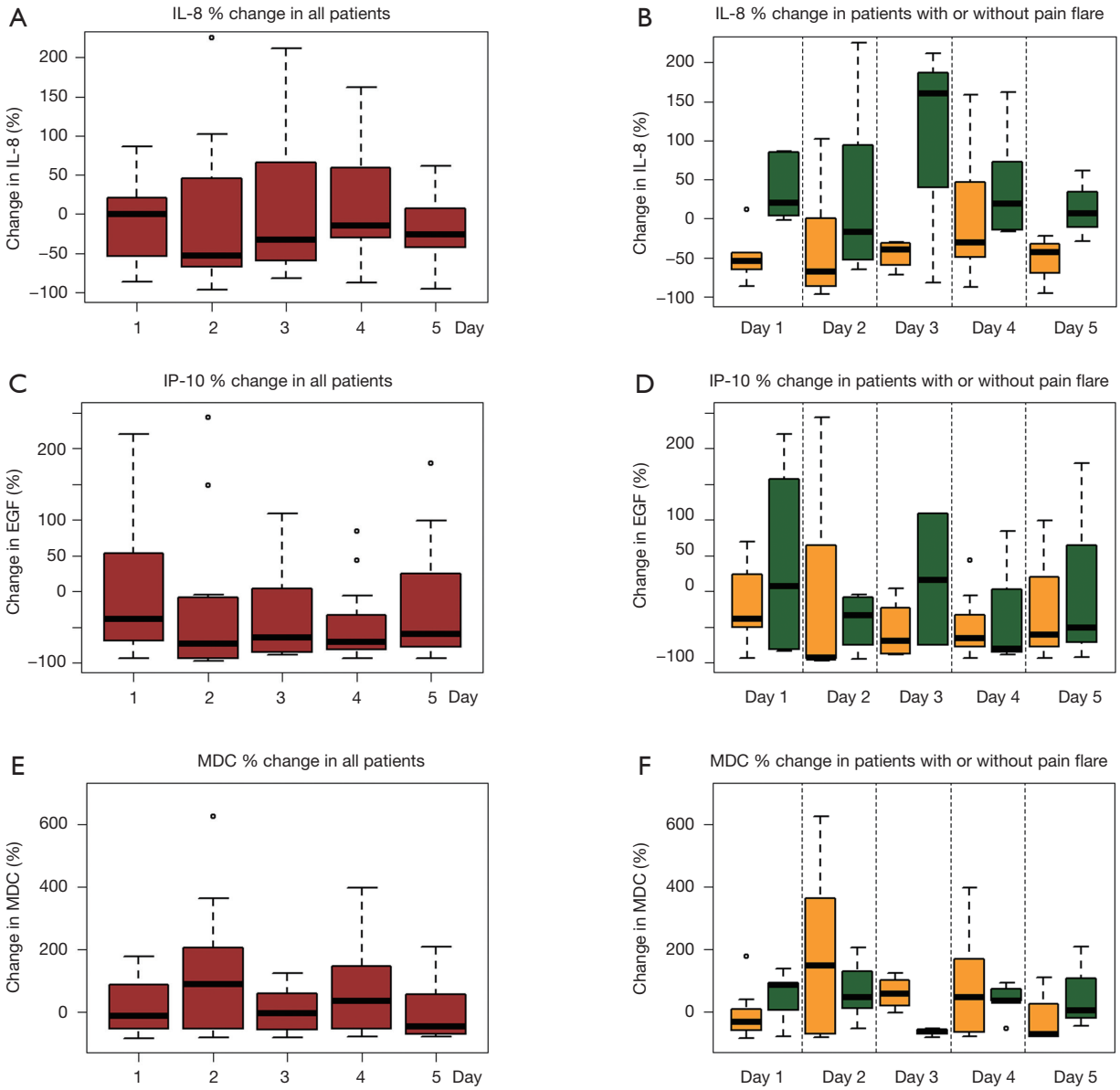

Figure 4 Percent change in IL-8 (A,B), IP-10 (C,D), and MDC (E,F) in urine for all patients (red), patients with pain flare (green), and patients without pain flare (yellow).

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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