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Ragnhild H. Habberstad

Cancer Induced Bone Pain

Doctoral thesis

NTNU
Norwegian University of Science and Technology
Thesis for the Degree of
Philosophiae Doctor
Faculty of Medicine and Health Sciences
Department of Clinical and Molecular Medicine

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Trondheim, January 2024

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Smertefulle skjelettmetastaser

Kreftspredning til skjelettet kan forårsake sterke smerter. Man vet i dag lite om hvorfor noen pasienter med skjelettspredning opplever mer smerte enn andre og om det finnes faktorer som kan hjelpe oss å forutsi hvilke pasienter som kommer til å få et smerteproblem. Strålebehandling gis for å redusere smerter hos pasienter med spredning til skjelettet. Den smertelindrende effekten av strålebehandling har flere mekanismer, men modulering av betennelsesreaksjon i tumor kan være bidragsytende. Ikke alle pasienter har effekt av stråling, og omtrent halvparten av pasientene opplever en signifikant smertereduksjon etter behandling. Strålebehandling kan også medføre bivirkninger, og for noen lang reisevei og sykehusinnleggelse. Vi har i dag liten mulighet til å forutsi hvilke pasienter som vil ha effekt av stråling i forkant av behandlingen, som betyr at mange pasienter får strålebehandling uten effekt.

Som del av dette Ph.d. prosjektet inngår to internasjonale, longitudinelle multisenterstudier. I studien "the European Palliative Care Cancer Symptom (EPCCS) study " utførte vi en subgruppeanalyse av 606 kreftpasienter med skjelettspredning som ble fulgt en gang i måneden i inntil 1 år. Vi undersøkte om smertekarateristika og andre kliniske variabler kunne predikere smerte og fremtidig smerteforverring. Vi fant at nåværende smerteintensitet, søvnforstyrrelser, sløvhet, mannlig kjønn og gjennombruddssmerte var assosiert med høyere smerteintensitet etter 1 måned hos pasienter med spredning til skjelettet. De samme faktorene var også assosiert med gjennomsnittlig smerte siste 24 timer. Disse faktorene bør vurderes i klinisk praksis og kan hjelpe klinikere til å identifisere pasienter som har ha nytte av tettere oppfølging eller intervensjon for å forbygge smerteforverring.

I studien "the Palliative Radiotherapy And Inflammation Study (PRAIS)" inkluderte vi 574 pasienter som fikk strålebehandling mot smertefulle metastaser til skjelettet.

Målet med studien var å undersøke om kliniske variabler og betennelsesmarkører kunne predikere effekt av strålebehandling. Dette er viktig for å minske bruken av unødvendig behandling, bivirkninger og unngå ekstra belastning hos pasienter som kan ha kortere forventet levetid. Vi fant at bedre funksjonsstatus, diagnosen bryst og prostatakreft og metastatisk bløtdelskomponent utenfor skjelettet kunne predikere bedre effekt av strålebehandlingen hos pasienten med smertefulle skjelettmetastaser. Pasienter som brukte kortikosteroider hadde dårligere effekt av strålebehandlingen. Bare 37 % av pasientene fikk engangsfraksjonert strålebehandling (8 Gy x 1) og det var ingen forskjell i stråleterapirespons sammenlignet med pasienter som fikk multifraksjonert strålebehandling over en lengre tidsperiode.

Vi undersøkte også i hvilken grad inflammasjon er viktig for stråleterapirespons hos pasienter med skjelettspredning. Ingen av betennelsesmarkørene målt før oppstart behandling kunne predikere effekt av strålebehandling, men endring i flere betennelsesmarkører 3 uker etter stråling var assosiert med stråleterapirespons. Resultatene kan indikere at inflammasjon er en viktig komponent i den smertelindrende effekten av strålebehandling, selv om disse funnene ikke kan skille pasienter med god eller dårlig forventet effekt av behandlingen før oppstart.

Navn kandidat:	Ragnhild Hansdatter Habberstad
Institutt:	Institutt for klinisk og molekylær medisin
Hovedveileder:	Professor Pål Klepstad
Biveiledere:	Professor Stein Kaasa, Førsteamanuensis Jo-Åsmund Lund og Professor Augusto Caraceni
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In the end – but above all – thank you to my husband Tobias and our two wonderful children Alma and Johannes for your love, patience, and support. We are a great team!

Ragnhild Hansdatter Habberstad

Trondheim, January 2024

List of papers

Paper 1

R. Habberstad, M. J Hjermstad, C. Brunelli, S.Kaasa, M.I Bennett, K.Pardon, P.Klepstad. *Which factors can aid clinicians to identify a risk of pain during the following month in patients with bone metastases? A longitudinal analyses.* Supportive care in cancer. 2018;08;15

Paper 2

R. Habberstad, T.C Frøseth, N. Aass, E. Bjerkeset, T. Abramova, E. Garcia-Alonso, M. Caputo, R. Rossi, J.W. Boland, C. Brunelli, J.Å. Lund, S. Kaasa, P. Klepstad. *Clinical Predictors for Analgesic Response to Radiotherapy in Patients with Painful Bone Metastases*. Journal of Pain and Symptom Management. 2021;10:62.

Paper 3

R. Habberstad, N. Aass, T.E. Mollnes, J.K Damås, C.Brunelli, R. Rossi, E. Garcia-Alonso, S.Kaasa, P. Klepstad. *Inflammatory Markers and Radiotherapy Response in Patients With Painful Bone Metastases.* Journal of Pain and Symptom Management. 2022;10:64

Summary in English

Bone metastases causing cancer induced bone pain (CIBP) is one of the most frequent reasons for pain in patients with cancer. Although pain is a common consequence of bone metastases, not all patients with bone metastases experience pain. There is limited knowledge to explain why some patients with bone metastases develop CIBP or if there are factors that could be helpful to predict pain severity in these patients. Radiotherapy (RT) can reduce CIBP. Modulation of a local inflammatory response is proposed to contribute to the analgesic effect from RT. Of patients undergoing RT for CIBP, about half of the patients experience a significant pain reduction after treatment. As RT can cause side effects, and for some patients involve a long travelling distance and admittance to hospital, it would be beneficial to avoid RT in patients with no treatment response. Little is known about which patients that are more likely to respond to RT, and many patients undergo RT without a clinical benefit.

This PhD project includes three papers from two international and longitudinal multicenter studies. In the European Palliative Care Cancer Symptom (EPCCS) study, we performed a subgroup analysis of 606 cancer patients with bone metastases that were followed approximately every month in one year or until withdrawal or death. The aim of the study was to explore if pain characteristics and other clinical factors were associated with ongoing and future pain intensity in patients with bone metastases. In paper 1 we found that patients with higher current pain intensity, sleep disturbances, drowsiness, male gender and episodic pain exacerbations had significantly higher pain intensity at the next follow-up in one month and higher average pain intensity the last 24 hours. The identified factors cannot alone be used to predict CIBP, but can identify patients with bone metastases that could benefit from more frequent follow-up or pain-relieving interventions like RT. The findings could also contribute to better understanding of CIBP towards more personalized cancer pain treatment.

In the Palliative Radiotherapy And Inflammation Study (PRAIS), we included 574 patients about to undergo palliative RT due to CIBP. We wanted to investigate if clinical variables and inflammatory markers could predict RT treatment efficacy in patients with CIBP from bone metastases.

In paper 2 we found that better performance status, breast or prostate cancer and presence of soft tissue expansion outside bone predicted RT response in patients with painful bone metastases. Patients using corticosteroids had significantly lower response rates. There was no difference in RT response in patients receiving single fraction RT compared to multiple fraction RT. A clinical index to reliably select patients that would benefit from palliative RT due to painful bone metastases could not be developed based on our findings due to a moderate discriminative ability of the model. Each of the significant variables identified in the study should be individually considered, together with other relevant factors, to decide if the patient is a suitable candidate for RT.

In paper 3 we found that none of the investigated inflammatory markers measured before the start of RT could select patients with a higher likelihood of analgesic RT response from CIBP, but the change in several inflammatory markers 3 weeks after RT was associated with RT response. A three-week change in the inflammatory makers IL-8, IP-10, eotaxin, MCP-1, G-CSF and TNF were positively associated with RT response, while a three-week change in CRP was negatively associated with RT response. These findings could indicate that inflammation is an important component to initiate an analgesic RT response in patients with painful bone metastases, although the inflammatory markers could not be used to select patients suitable for RT prior to treatment. The association between RT and change in inflammatory markers after RT could point towards inflammation as a potential future treatment target for patients with painful bone metastases.

Abbreviations

ACROP	Advisory Committee on Radiation Oncology Practice
BALP	Bone alkaline phosphatase
basic FGF	Basic fibroblast growth factor
CI	Confidence interval
CIBP	Cancer induced bone pain
BPI	Brief Pain Inventory
CNS	Central nervous system
CRP	C-reactive protein
CSS	Cancer pain prognostic scale
CT	Computer tomography
CTV	Clinical target volume
DRG	Dorsal root ganglion
DWI	Diffusion weighted imaging
ECS-CP	Edmonton Classification System for Cancer Pain
EORTC	European Organization for the Research and Treatment of Cancer
EPCCS	European Palliative Care Cancer Symptom study
EPP	Events per predictor parameter
EPV	Events per variable
ESTRO	European Society for Radiotherapy and Oncology
ESS	Edmonton Staging System
ESAS	Edmonton Symptom Assessment System
G-CSF	Granulocyte colony-stimulating factor
GEE	Generalized estimation equation
GLMM	Generalized linear mixed models
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GTV	Gross tumor volume

Gy	Gray
IASP	International Association for the Study of Pain
ICPRE	International Consensus Response Endpoints
IFNγ	Interferon gamma
IGF-1	Insulin like growth factor 1
IL	Interleukin
IMRT	Intensity-modulated radiation therapy
IP-10	Interferon gamma-induced protein-10
IQR	Interquartile range
KPS	Karnofsky Performance Status
LANSS	Leeds Assessment of Neuropathic Symptoms and Signs
LET	Linear energy transfer
MAR	Missing at random
MCAR	Missing completely at random
M-CSF	Macrophage colony-stimulating factor
MCP-1	Monocyte chemoattractant protein-1
MI	Multiple imputation
MICE	Multivariate imputation of chained equations
MIP-1β	Macrophage inflammatory protein-1 beta
MMSE	Mini-Mental State Examination
MNAR	Missing not at random
MRI	Magnetic resonance imagingN Number
NOV/CCN3/IGFBP-9: Insulin-like growth factor binding protein 9	
NRS	Numeric rating scale
NTNU	Norwegian University of Science and Technology
NTX	N-telopeptide
OMED	Oral morphine equivalent daily dose
PDGF	Platelet derived growth factor
PET	Positron emission tomography

PHQ-9	Patient Health Questionnaire 9
PRC	European Palliative Care Research Centre
PRAIS	Palliative Radiotherapy and Inflammation Study
PTHrP	Parathyroid hormone related peptide
PTV	Planning target volume
PYD	Pyridinoline
QIC	Quasi-likelihood independence model criterion
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
RANTES	Regulated on activation normal T-cell expressed and secreted
RFA	Radiofrequency ablation
RT	Radiotherapy
RQ	Research Question
s-ALP	Serum alkaline phosphatase
SINS	Spinal instability neoplastic score
SNPs	Single nucleotide polymorphisms
SPECT	Single photon emission CT
SUV_{max}	Maximum standardized uptake value
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor
VAS	Visual analogue scale
VEGF	Vascular endothelial growth factor
VMAT	Volumetric modulated arc therapy
VRS	The verbal rating scale
QST	Quantitative sensory testing
$^{89}SrCl_2$	Strontium-89 chloride
3D CRT	Conformal 3D RT planning based on CT imaging

1 Introduction

1.1 Bone metastases

1.1.1 Epidemiology

The total incidence of cancer steadily increases. In 2022 about 38 000 Norwegians were diagnosed with cancer. The prevalence of cancer in Norway now exceeds 300 000 and have increased with 100 000 patients the last 10 years.[1] Modern cancer treatment has improved cancer survival, but people also live longer with incurable and metastatic cancer disease.

Bone is a common site of metastases in cancer and responsible for a high mortality and morbidity in cancer patients.[2] At the time of diagnosis, only about 3-5% of all cancer patients presents with bone metastases; however, a significant number of patients will develop bone metastases.[3-5] There are large differences in the reported incidence of bone metastases, probably reflecting heterogenicity in study populations, differences in imaging modalities to diagnose bone metastases, and lack of initial reporting and available follow-up data in large population-based studies.[4,5] In patients diagnosed with metastatic cancer, the overall proportion of patients with bone metastases is approximately 30% across all diagnoses.[4] The risk of developing bone metastases increases with an advanced cancer stage at diagnosis and is highly dependent on the primary cancer origin.[4,6] In a metastatic cancer population, bone metastases are found in approximately 65-90% of patients with prostate cancer, 55-75% of patients with breast cancer, 20-40% of patients with lung cancer, 40% of patients with renal cancer and in only 5% of patient with gastrointestinal cancer.[2,6-9] In hematological malignancies, patients with multiple myeloma have the highest incidence of bone lesions, with at least 90% of the patients developing skeletal disease.[10,11]

The prevalence of bone metastases influences patient prognosis. For most patients presenting with bone metastases, the cancer is incurable. Bone metastases have a negative impact on survival with an overall survival from diagnosis of 6 months, including all diagnoses. The prognosis varies considerably between the different tumor diagnoses, reflecting both tumor biology and available treatment alternatives.[4] Patients with breast and prostate cancer may live for years after being diagnosed with bone metastases.[2,4,7] As a result of increased cancer incidence and survival, the number of patients living with bone metastases is expected to increase.[12] This warrants a focus on the optimal management in patients with bone metastases.

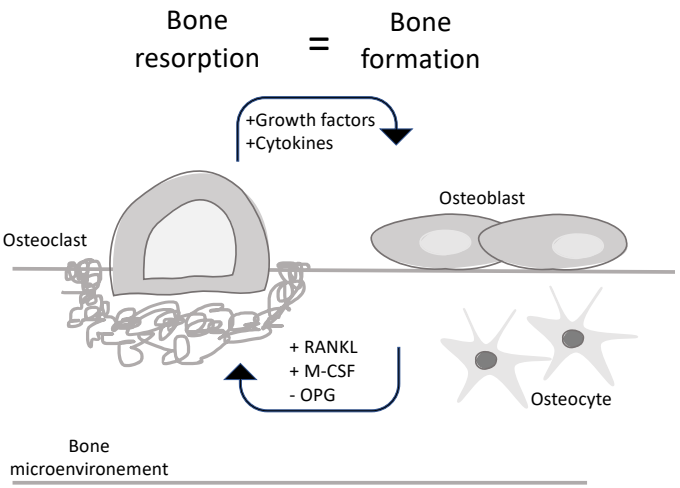
1.1.2 Pathophysiology: from healthy bone to bone metastases

Bones have a variety of important functions in the body. Bones form the skeleton, which is essential for an upright position, support of internal organs, and movement. Bones serve as storage for minerals and other substances, and the bone marrow is where hematopoiesis occurs.[13] Bone is a metabolic active tissue that is renewed several times in our lifetime to preserve its mechanical strength and function.[14] Remodeling of bone is maintained by three district cell types: osteoclasts are responsible for bone resorption, while osteoblasts produce bone matrix and differentiate to osteocytes when surrounded by bone.[14,15]

The differentiation and activation of bone cells are closely regulated by several biological pathways and crosstalk between cells.[14] Damage to the bone is sensed by osteocytes which stimulates the differentiation and activation of osteoclasts to start bone resorption. During bone resorption, several mediators, including growth factors and inflammatory molecules like cytokines, are released from the bone matrix and promote differentiation of osteoblasts to stimulate new bone formation. Cytokines are small signal molecules that regulates immune reposes at the cellular level.[16] Osteoblasts again regulate osteoclast bone destruction by secreting macrophage colony-stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) that is essential for maturation of osteoclast precursors. Osteoblasts also secrete the inhibitory osteoprotegrin (OPG) that acts as a decoy receptor and

inhibits binding of RANK-RANKL.[15,17] This tightly regulated process is important to ensure a balance between bone degradation and bone renewal and is often referred to as "the virtuous cycle" of the bone (Figure 1).[13,14] Bone remodeling is also influenced by other factors present in the bone microenvironment, hormones and factors secreted by tumor cells.[12,14,15]

Figure 1. The normal bone homeostasis; the virtuous cycle

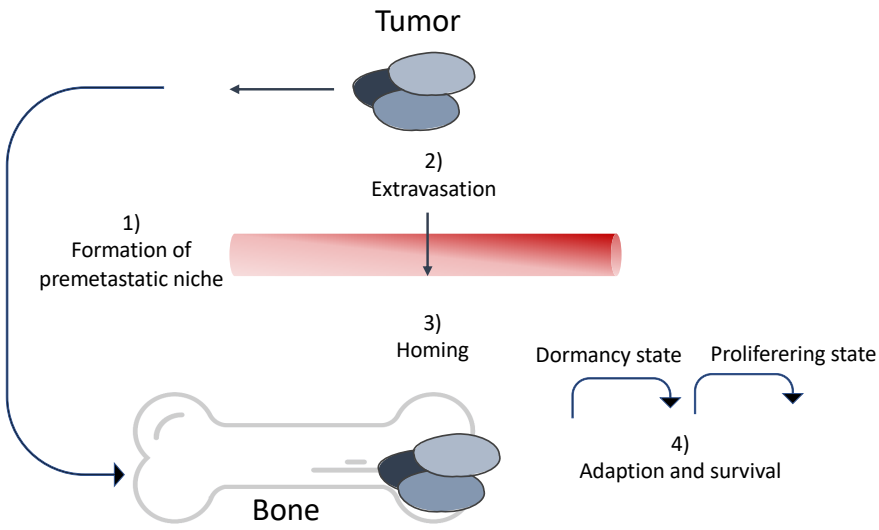


The figure illustrates that normal bone homeostasis is a tightly regulated process to ensure a balance between osteoclastic bone resorption and osteoblastic bone formation. Cytokines and growth factors are released during bone destruction and stimulates the osteoblastic bone formation. Osteoblasts secrete both receptor activator of nuclear factor kappa-B ligand (RANKL) *and* macrophage colony-stimulating factor (M-CSF) *that stimulates, and* osteoprotegrin *(OPG) that inhibits the osteoclastic bone resorption.*

The process of metastatic spread to the bone is very complex and will only be discussed briefly in this thesis. In most cases the cancer spread to bone is hematogenic. The first step to form bone metastases is believed to be the formation of a pre-metastatic niche to cause a favorable bone microenvironment for cancer cells. This process may be facilitated by factors in the normal bone marrow or factors secreted by tumor cells themselves.[12] Since Paget proposed his "seed and soil" theory in 1879, the bone microenvironment has been described as a fertile soil for malignant cells. The different ability to form a pre-metastatic niche is one explanation why

patients with certain cancer diagnoses are more likely to develop bone metastases.[18] After the establishment of the pre-metastatic niche the tumor cells extravasate through the endothelia of blood vessels to colonize the bone.[12,18] Once tumor cells have reached the bone, the bone may serve as a reservoir of resting or dormitory tumor cells or provide for proliferating tumor cells (Figure 2). Several signal molecules like inflammatory markers and growth factors are involved in the development of bone metastases and metastatic proliferation in bone.[12]

Figure 2. Metastatic spread to bone

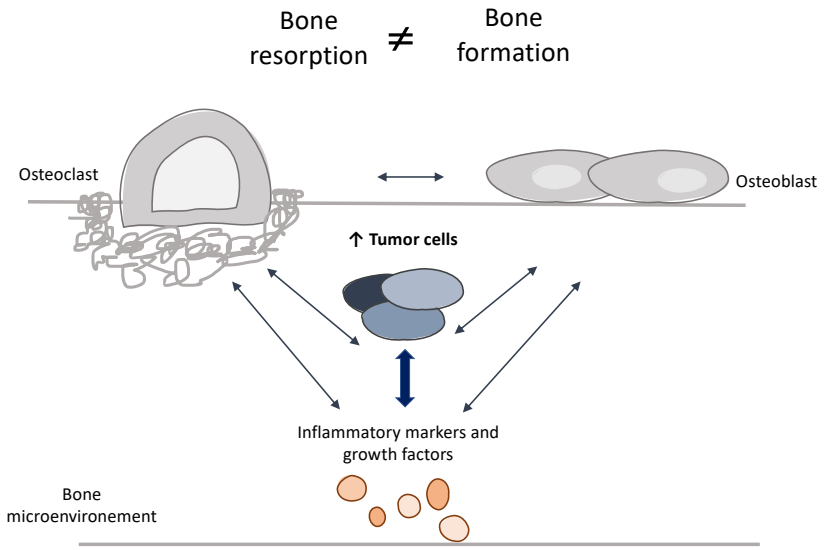


The figure presents a simplified model of the different steps in the hematogenic spread of tumor cells to bone. It is believed that the first step is formations of a premetastatic niche (1). Metastatic tumor cells extravasate into the blood stream (2) and later attracts and adhere to the bone (3). When present in bone, tumor cells must survive and adapt to the bone microenvironment (4), including either further tumor proliferation or a resting/dormant state of the tumor cells.

When tumor cells start to proliferate in the bone, the normal bone homeostasis is disrupted. Factors released by bone cells again stimulate tumor growth and a positive

feedback loop of cancer proliferation and bone destruction is initiated. This is often referred to as “the vicious cycle” of the bone (Figure 3).[14,18]

Figure 3: The pathological remodeling cycle of bone metastases: “the vicious cycle”



The figure illustrates that bone metastases cause pathological bone remodeling and that this may again stimulate an increase in tumor volume.

1.1.3 Classification of bone metastases

Bone metastases are commonly classified as osteolytic, sclerotic (osteoblastic), or mixed based on their radiological appearance, but they also represent different pathophysiological subgroups.[14] While osteolytic bone metastases are predominated by osteoclastic bone destruction, the sclerotic bone metastases are characterized by higher osteoblastic bone formation.[12,18] There are different molecular mechanisms involved in formation of sclerotic and osteolytic bone metastases, but both processes lead to weakening of the bone and a “vicious cycle” that supports tumor growth. Sclerotic bone metastases are most common in prostate cancer while other cancer

diagnoses mostly present with osteolytic bone metastases or a mixed pattern of osteolytic and sclerotic metastases.[12]

1.1.4 Diagnosis of bone metastases

The presence and distribution of bone metastases is commonly diagnosed with radiological imaging. Some patients present with pain, neurological impairment, or pathological fractures, but bone metastases may also be detected on radiological screening or follow-up scans in asymptomatic patients.[18,19] Improvement in radiological imaging modalities has led to a more precise identification of bone metastases.[5]

Plain X-ray has a low sensitivity for bone metastases (33%), and it is estimated that a 50-75% reduction in bone density is needed for a metastatic lesion to be visible in a plain x-ray investigation. As the availability of more sensitive radiological imaging techniques has increased, plain x-rays are seldom used in cancer diagnostics and follow-up.[20,21]

Computer tomography (CT) gives a more detailed image of the bony structure compared to plain X-rays.[20] The sensitivity for bone metastases is reported to be approximately 75%.[20,21] CT better visualizes both trabecular bone and the bone cortex to evaluate pathological fractures and fracture risk.[21,22] CT scans may also provide more information of the pathological process of bone metastases to characterize the lesions as osteolytic or sclerotic.[23] CT is easily accessible, it can be used for image guided tissue biopsies as well as diagnoses and evaluation of both bone metastases and other cancer sites, which reduces the number of radiological investigations needed for each patient.[21]

Magnetic resonance imaging (MRI) provides an even better precision in diagnosis of bone metastases with a sensitivity of 90-95%.[20,21] The major advance of MRI in the diagnostic setting of bone metastases is the superior presentation of soft tissue components, making MRI the gold standard in diagnosis of malignant spinal cord compression.[24] MRI is also more sensitive to the early changes in bone during a

metastatic process. With the use of different diffusion signals, MRI is sensitive enough to evaluate oncological treatment response and can be used to distinguish osteoporotic compression fractures from malignant compression of vertebras.[21] The disadvantages of MRI are the higher cost compared to CT and less availability.

Standard imaging techniques mentioned above mainly visualize the bone structure. In contrast, *nuclear medicine imaging modalities* measure the function of bone and tumor cells. Nuclear medicine imaging techniques uses the principle of radiotracers that accumulate in bone or tumor tissue and evaluate the uptake at each specific site. Traditionally, radiotracers are differentiated between those that accumulate at the site of active bone production (osteotropic radioisotopes)and those that uptake in malignant cells (oncotropic radioisotopes).[21]

Skeletal scintigraphy (also called bone scintigraphy or bone scan) is commonly used in diagnosis of bone metastases with a sensitivity above 70%.[20,21] Skeletal scintigraphy gives a great overview of the metastatic status as it images the whole skeleton, although the accuracy of each bone lesion is not as precise as in other radiological modalities. A weakness of skeletal scintigraphy is that benign bone disorders, fractures or degrative conditions may be difficult to distinguish from bone metastases. Treatment responses that result in a transient increase in bone formation may also be mistaken as metastatic progression and rapidly developing metastases with high osteolytic activity but lack of osteoblastic bone formation or very widespread disease may be difficult to detect in skeletal scintigraphy.[21] *Single Photon emission CT (SPECT)* uses the same radiotracer as in skeletal scintigraphy, but the images are obtained as CT slices which increase the precision regarding metastatic localization.[21]

Positron emission tomography (PET) has gained an important role in cancer diagnostics in recent years, especially due to the great accuracy in detecting cancer metastases. The sensitivity of bone metastases on PET depends on the primary type of cancer and pathophysiology of metastases (sclerotic, osteolytic, mixed), type of radiotracer used, and which other image modalities like CT or MRI is combined with PET.[23] The most common radiotracers in diagnosis of skeletal metastases are the oncotropic fluorine

18-fluorodeoxyglucose (18F-FDG) and the osteotropic fluorine 18–Sodium Fluoride (18F- NaF). In 18F-NaF PET-CT, both the sensitivity and specificity for bone metastases is reported to be almost 100%, with slightly lower accuracy for 18F-FDG-PET CT.[20,21]

Other investigations may also be important in the diagnosis of bone metastases.[18] To verify the diagnosis of bone metastases histologically, a biopsy is necessary. A biopsy is especially important to confirm a cancer diagnosis, but may also be useful to verify additional information like hormonal status or mutation status to tailor the optimal cancer treatment.[19,25] No laboratory tests in clinical use can confirm the presence of bone metastases, but a general blood workup is often indicated in the diagnostic evaluation. Standard laboratory tests in the evaluation of bone metastases also include a general blood count to evaluate the bone marrow function, calcium to exclude hypercalcemia, and serum alkaline phosphatase that may indicate higher bone turnover.[26] The more specific bone ALP (BALP) has a high predictive value for bone metastases.[18] Together with other biological bone-markers, BALP may support a diagnosis of bone metastases, but none of the biological markers are currently implemented or recommended for use in routine clinical practice.[12,18,19,25]

1.1.5 Distribution of bone metastases

The distribution of bone metastases often displays in a typical manner. Bone metastases most frequently affect the axial skeleton including the spine, ribs, pelvis or the end of long bones.[2,27] These sites are probably preferable to bone metastases due to a rich blood supply, presence of red bone marrow, or trabecular bone with a higher bone turnover.[18] In more than 80% of cases the bone metastases are disseminated affecting several sites which has an impact on treatment strategy and prognosis.[28] The localization of bone metastases may have implications for symptoms and complications of bone metastases.

1.1.6 Complications of bone metastases

Bone metastases lead to both increased morbidity and mortality. Bone metastases are associated with several complications that have negative impacts on quality of life.[29] With a common localization close to neurological structures, bone metastases can

cause spinal cord compression, cauda equina syndrome, or nerve root compression. Bone metastases may also infiltrate the bone marrow, leading to bone marrow failure.[11] Pathological fractures, hypercalcemia and spinal cord compression, in addition to the need for surgery or radiotherapy (RT), are often referred to as skeletal related events (SRE).[12] Early studies on bisphosphonate treatment in patients with bone metastases showed that about half of patients not treated with bone-targeting agents developed at least one SRE during a 2 year follow-up. The risk of a new SRE also increases more rapidly following the initial event, which highlights the importance of treating and preventing bone metastases.[12,29]

1.1.6.1 Pathological fractures

Pathological fractures can be the result of an imbalance in the normal bone remodeling process that weakens the load bearing capacity of the bone.[12] Typically, pathological fractures occur spontaneously during everyday activities or after minimal trauma.[30] Data on SRE summarized by Costa *et al* in 2008 showed that 52% of the patients with prostate cancer not treated with bone-targeting agents developed a pathological fracture within a 24 month follow-up.[29] More recent reviews indicate a lower incidence, but pathological fractures are still the second most common SRE.[22] The incidence of pathological fractures increases with the duration of the cancer disease and is therefore more common in diagnostic groups like breast and prostate cancer with a longer expected survival.[11] Common sites of pathological fractures are ribs, spine and extremities.[11,22] The consequence and treatment of a pathological fracture depends on the localization and distribution of metastases, but in case of fractures in long bones (e.g. femur or humerus) or in case of spinal instability, surgery is often necessary.[19,22]

1.1.6.2 Malignant spinal cord compression

Malignant spinal cord compression is considered an emergency oncological condition and is most commonly caused by spinal metastases that expand towards the medullar spine.[24] Patients frequently present with back pain and neurological symptoms, in which ataxia is more common than impaired muscle strength.[31] It is estimated that

approximately 10% of patients with spinal metastases will develop malignant spinal cord compression.[31] Rapid assessment, diagnostic workup with MRI and treatment with high corticosteroid doses and surgery or RT is essential to avoid permanent neurological damage.[11,24,31,32]

1.1.6.3 Hypercalcemia

Hypercalcemia is defined as a calcium serum concentration above the upper limit of normal, and is classified as mild, moderate, or severe according to the level measured. The incidence increases with cancer stage and is reported to affect approximately 10-30% of patients with cancer in total.[33] There are two main mechanisms for hypercalcemia in malignancy: humoral hypercalcemia with secretion of parathyroid hormone related peptide (PTHrP) to trigger RANKL expression in osteoblasts (80%), and local osteolytic hypercalcemia (20%).[34] Both conditions result in activation of osteoclasts and higher bone turnover that in turn leads to the release of calcium from bone. The kidneys, which normally contribute to a normal calcium level by secretion of excess levels of calcium, are not able to handle this “overflow” of calcium, resulting in higher serum levels. Mild hypercalcemia is often asymptomatic, but moderate and severe hypercalcemia may present with severe symptoms that are potentially life threatening.[33]

1.1.6.4 Pain

Pain is usually not defined as an SRE, but a frequent complication of bone metastases that equally contributes to reduced quality of life.[35] Bone metastases are also considered to be one of the most common causes of pain in cancer patients.[36,37] Pain in patients with bone metastases will be discussed in detail in the next section.

1.2 Cancer induced bone pain (CIBP)

1.2.1 Pain definition

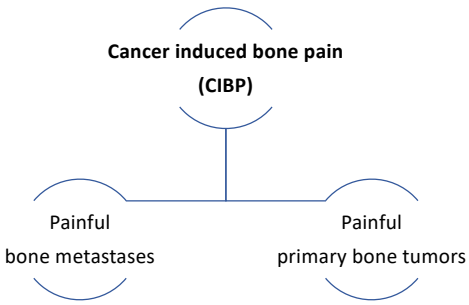
Pain is an individual experience. According to the International Association for the Study of Pain (IASP), the updated definition of pain is: "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage".[38] Pain is influenced by both biological, social and psychological factors. Traditionally pain is categorized based on pain characteristics, pathophysiology, or patient related factors.[38-40]

1.2.2 Definition of CIBP

Several terms can be used to describe cancer-related pain arising from bone.[41] In this thesis we use the term "cancer induced bone pain" (CIBP), as this is frequently used to describe the pathophysiological and clinical presentation of malignant bone pain.[42,43] CIBP can be caused by a primary tumor or metastases affecting the bone.[41] CIBP can be considered a somatic nociceptive pain, but patients may also present with elements of neuropathic pain and may have similarities with inflammatory pain conditions.[44,45]

In clinical settings CIBP most commonly refers to patients with painful bone metastases, as primary bone tumors are rare. This thesis will focus on patients with CIBP caused by bone metastases. "Painful bone metastases" is also used as a narrower term when appropriate (Figure 4).

Figure 4: Cancer induced bone pain (CIBP)



The figure illustrates that cancer induced bone pain (CIBP) can include both patients with pain caused by bone metastases and primary bone tumors.

1.2.3 Pathophysiology of CIBP

The pathophysiology of CIBP is complex and several review papers have been published in the last few years to summarize findings from pre-clinical studies.[17,43,46] Despite extensive research, the pathophysiology of CIBP is still not properly understood.

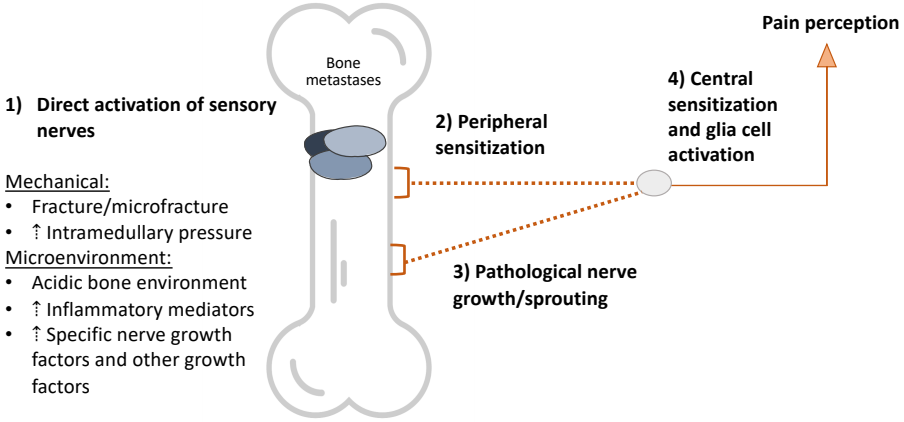
All compartments of the bone are innervated by sensory nerves. The surrounding periost and the bone marrow have a higher density of sensory nerves compared to trabecular bone.[46] The majority of sensory nerve fibers are myelinated A-delta fibers and the non-myelinated C-fibers.[14] When these sensory nerves are activated by noxious stimuli, they transmit pain signals towards the dorsal root ganglion in the spinal cord that further transfers signals to the brain to enable perception of pain.[46]

Bone cancer pain can arise from direct activation of sensory nerves in the bone but are also modulated by sensitization of neurons at the peripheral or a central level. Activation of glia cells can be important in central sensitization. CIBP may also be enhanced by pathological proliferation of sensory nerves in the affected bone.[47]

Expanding tumor cells can increase the intramedullary bone pressure, invade sensory nerves, or interfere with the normal bone strength, leading to fractures or microfractures. These mechanical factors directly activate sensory nerves and cause pain. Tumor cells can also cause alterations in the normal bone microenvironment leading to painful stimuli.[46] Low pH is one of the important biochemical noxious stimuli in CIBP. Tumor proliferation and osteoclastic bone resorption contribute to an acidic bone environment and activation of acid sensing ion channels that stimulate sensory neurones.[14,46] Further, the disrupted balance between the tumor, bone cells and immune cells result in an excessive secretion of growth factors and inflammatory mediators that are potent activators and sensitizers of sensory neurons both peripherally and centrally.[43,46] Prostaglandins, interleukin (IL)1 beta (IL-1β), monocyte chemoattractant protein (MCP)-1, tumor necrosis factor (TNF) and IL-6 are pro-inflammatory markers identified as nociceptive mediators in rodent models of CIBP.[43,48-51] The growth factors transforming growth factor beta (TGF-β) and insulin like

growth factor -1 (IGF-1) are released during bone resorption and further simulate tumor growth and the "vicious cycle" in bone metastases and may also be important pain-mediators.[14,52] Specific nerve growth factors (NGF) promote aberrant and pathological nerve innervation close to the bone that facilitate the increased neuron excitability in CIBP.[17] An overview of mechanisms of CIPB is illustrated in Figure 5.

Figure 5. Mechanisms of CIBP



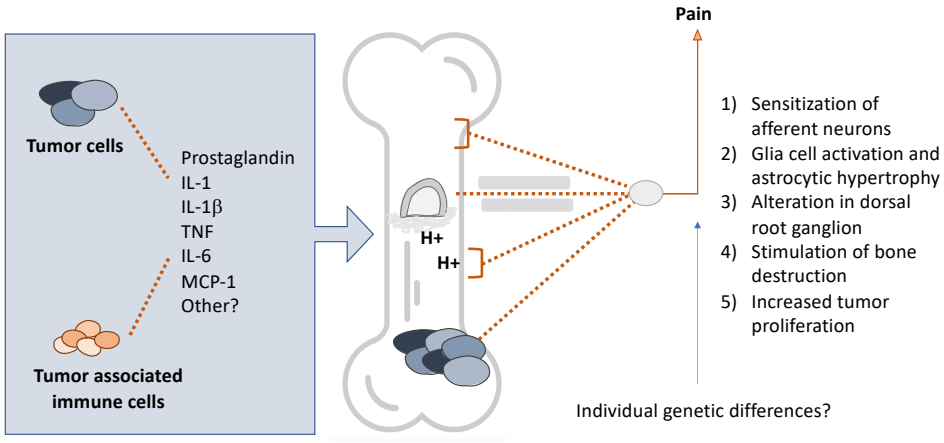
The figure schematically presents different mechanisms of CIBP. Pain can arise because of direct activation of afferent sensory nerves, caused by mechanical factors or alternations in the bone microenvironment (1). Alternations in the bone microenvironment and increase in inflammatory mediators can also promote pain by sensitization of peripheral nerves (2). Release of chemical mediators like specific nerve growth factors results in pathological growth of sensory nerves and increased sensitivity to mechanical pain stimuli (3). Pain perception may also be modulated at a central level (4).

Knowledge of biological mediators involved in CIBP is mainly based on pre-clinical animal models which typically measure allodynia, hypersensitivity, or weight distribution in different rodent models inoculated with cancer cells.[14] The subjective perception of pain is difficult to measure in animal models, and there is a lack of studies in humans to understand the importance of different biological mediators in mechanisms and potential treatment targets of CIBP.[14,17]

1.2.4 The role of inflammation in CIBP

In bone metastases, inflammatory mediators are secreted by the tumor cells and tumor associated immune cells. As briefly mentioned in the previous section, inflammation is suggested as an important factor contributing to pain in bone metastases, and several inflammatory markers have been determined to promote pain perception in animal models of CIBP.[14,43] The pro-inflammatory IL-1β is associated with hyperalgesia, and blocking of IL-1β has resulted in an anti-nociceptive response in murine models of CIBP.[50,53] TNF is also associated with nociception in murine models of CIBP, and animals with bone metastases lacking TNF receptors have less tactile hypersensitivity.[51,54] IL-6 is upregulated in the dorsal root ganglion (DRG) in rats and is demonstrated to increase sensitization of DRG neurons to facilitate CIBP.[48] MCP-1 and its receptor are also increased in the spinal cord of CIBP rats.[49] The same study demonstrated that intrathecal injection of exogenous MCP-1 stimulated mechanical allodynia while injection of an MCP-1 antibody reduced the mechanical allodynia in rats.[49] As demonstrated in animal models of CIBP, inflammatory mediators can enhance pain in CIBP by several mechanisms (Figure 6). Inflammatory mediators can sensitize primary afferent neurons and contribute to glia cell activation and astrocytic hypertrophy and proliferation in the central nervous system (CNS).[54-56] Inflammatory mediators may also stimulate tumor cell growth and contribute to increased bone destruction by activation of osteoclasts that again result in CIBP.[54,57]

Figure 6. Mechanism of inflammatory markers in CIBP



The figure illustrates that several inflammatory mediators secreted from tumor cells and tumor associated immune cells are though to promote CIBP by various mechanisms (1-5). Abbreviations: TNF: tumor necrosis factor, IL: interleukin, MCP-1: monocyte chemoattractant proteine-1.

While preclinical studies support the role of inflammation in CIBP, there is limited knowledge of the role on inflammation in studies of CIBP in humans. Clinical studies have demonstrated an association between pain and increased level of C-reactive protein (CRP), IL-6 and TNF in a general cancer population.[58-61] A study examining cytokine expression in relation to cancer pain and morphine treatment found no increase in cytokine expression in patients that described more pain, but decreased levels of eotaxin, macrophage inflammatory protein (MIP-1α and MIP1-β), IL-8 and IL-12 in patients with poor opioid treatment response.[62] There is also evidence that variations in genes coding for inflammatory cytokines are associated with pain. Genetic variations in the pro-inflammatory IL-8, IL1-ra, MCP-1 and TGF-β genes are associated with chronic non-malignant pain, and genetic differences in the IL-8 and IL-6 genes are associated with pain in lung cancer patients.[63,64] Although a few clinical studies support a relationship between inflammation and cancer pain, further clinical studies are needed to investigated the role of inflammatory mediators in CIBP in humans.

1.2.5 Clinical manifestations of CIBP

CIBP can be the first symptom of bone metastases. In most patients with bone metastases, pain intensity gradually increases several months before diagnosis.[65] CIBP may be located in all bones affected by metastases, but weight bearing bones (vertebra, pelvis, lower limbs) are the most common locations for pain.[42] Although a single location of CIPB is most common, abut 1/3 of patients will experience pain at two or more sites.[66]

Patients with CIBP often describe a combination of a constant dull aching pain and transient pain exacerbations commonly referred to as “breakthrough pain” or “episodic pain”.[42,47] Episodic pain exacerbations are common in patients with CIBP and can be spontaneous or caused by weight bearing or movement.[67,68] In a study exploring the clinical features of CIBP in 55 patients, Larid *et al* reported that 75% of the patients experienced episodic pain exacerbations. The median number of episodic pain exacerbations was 4 every 24 hours.[42] About half of the patients with episodic pain exacerbations described less than 5 minutes from awareness of pain until the pain reached a maximum level, with a duration less than 15 minutes.[42] This is relevant in regards to analgesic treatment. Oral immediate-release morphine tablets reach maximum efficacy within 60 minutes.[69] For other immediate release opioid analgesics like buccal Fentanyl, the time to reach maximum concentration is also about one hour, with a pain relieving effect reported 10-15 minutes after intake.[70,71] For intranasal fentanyl the median time to meaningful pain response after administration is reported to be approximately 11 minutes.[72] Thus, episodic pain exacerbations in CIBP can be challenging to treat with conventional analgesic medications.[42]

CIBP also has a negative impact on the individual functional status. Patients with CIBP have reduced walking ability and physical activity compared to non-cancer patients and patients with other cancer pain states.[68,73] Higher worst pain intensity and the presence of episodic pain exacerbations correlates with a greater impairment of daily activities and function in patients with CIBP.[42] Neuropathic pain that is present in

approximately 20-25% of patients with CIBP is also associated with a higher pain intensity.[74-76]

1.2.6 Assessment of pain in CIBP

Assessment of pain is important in a clinical setting in regards to symptom screening and evaluation of analgesic treatment efficacy in CIBP.[77] In clinical studies an accurate assessment of pain is important to ensure precise and comparable outcomes between studies, and the use of standardized and validated instruments for pain registrations is recommended.[78] As perception of pain is a subjective experience, pain assessment is based on patient reported outcomes.[39]

1.2.6.1 Assessment of pain intensity in CIBP

Unidimensional scales using patient reported pain intensity as an outcome of pain is commonly applied in everyday clinical practice and in clinical studies on cancer pain.[78] Examples of validated unidimensional pain measurement tools are visual analogue scales (VAS) in which pain intensity is marked in a continuous line, verbal rating scales (VRS) in which pain intensity is categorically measured in verbal scales (for example no, mild, moderate and severe pain), and numeric rating scales (NRS) in which pain intensity is measured in numeric categories typically ranging from 0 (no pain) to 10 (worst pain).[78] The validity of VRS can be limited because of fewer categories and language differences. Continuous VAS scales provide detailed information on pain intensity compared to categorical scales, but an 11-point NRS (0-10) is often recommended in pain assessment over VAS based on better compliance in clinical trials.[78] Cut-points in the 11-point NRS score are applied in some clinical trials to define pain as mild, moderate or severe. Mild pain commonly refers to an NRS of 1-4, moderate pain to NRS 5-6 and severe pain to NRS 7-10, although the reported cut-off varies.[39,79,80]

Unidimensional pain scales may also reflect different aspects of pain intensity like momentary or pain right now, average pain, least or worst pain, pain at rest, or movement and pain relief.[81] Additionally, there are often different lengths in the reporting time-periods that may impact the accuracy of the measurements.[82] Several

daily pain reports may increase reliability of the actual pain intensity, but numerous measures may introduce an unnecessary burden to the patient. On the other hand, questions addressing pain intensity within a longer time-period may again introduce potential recall issues.[78,82,83]

Self-reported worst pain last 24 hours in an 11-point NRS is recommended as a screening question for pain in cancer patients according to the 2018 ESTRO guidelines on management of pain in adult cancer patients.[77] In clinical trials the choice of pain assessment should reflect upon the study population, design, and purpose of the study.[78] It has previously been demonstrated that patients have a tendency to slightly overestimate recall pain intensity compared to momentary pain ratings, but the differences are minor.[82-84] A 24- to 48-hours, recall of average pain intensity is shown to correlate well with the average momentary pain, and longer recall assessment periods of pain intensity can be acceptable in many study settings.[82-85]

Both average and worst pain intensity is commonly reported in clinical cancer pain trials.[86-88] Worst pain is shown to better correlate with pain interreference in patients with CIBP compared to average pain, and one could therefore argue that worst pain is a more relevant assessment question than average pain in patients with CIBP.[42] Worst pain is also adapted as the preferable outcome measure for pain in the updated consensus on RT endpoints in clinical trials.[89] Some authors recommend assessing "pain intensity at rest" and "pain intensity at movement" in patients with CIBP. This is because spontaneous pain at rest and movement-related pain is to a large extent responsible for sudden or episodic pain in patients with CIBP, which is challenging to treat with traditional analgesic medications.[90] The lack of standardized assessment for CIBP is reflected by the differences in outcome measures in clinical trials.[86]

1.2.6.2 Detailed pain assessment in CIBP

A detailed pain assessment should in addition to pain intensity include information about the site of pain, timing and temporal variation of pain including factors that lead to pain exacerbation or pain relief, type of pain, radiation of pain and other associated factors.[39,77,91] There are several multidimensional pain measuring tools, like the Brief

pain inventory (BPI)[81] and McGill Short form questionnaire,[92] that are validated instruments for cancer pain assessment.[91] In patients with painful bone metastases a precise localization of pain is important to define a target site for RT. Increased pain intensity can indicate cancer progression or complications like pathological fracture, while radiating pain or signs of neurological impairment may indicate nerve root compression or spinal cord compression and can guide further diagnostics or interventions.[39]

As explained in chapter 1.2.5, transient pain exacerbations are common in patients with painful bone metastases and is important to acknowledge.[42,68,93] Different definitions and terminologies exist in the literature to describe and assess transient pain exacerbations.[39,93] A common definition of breakthrough pain was published by Portenoy et al in 1990: "Breakthrough pain is a transitory exacerbation of pain that occurs on a background of otherwise stable pain in a patient receiving chronic opioid therapy." [94] An expert Delphi survey published in 2016 suggested "episodic pain" as a broader term of transient pain exacerbations which also covers pain exacerbations in patients without pain, in patients with uncontrolled background pain, and in patients not using opioid analgesics.[95] The Alberta Breakthrough Pain Assessment tool is a detailed and validated instrument useful to evaluate the extent of transient pain exacerbations and the effect of analgesic medications in cancer patients.[96] In this assessment tool, breakthrough pain is defined as a brief "flare-up" of pain that can represent an exacerbation of baseline pain or be different to a baseline pain.[96]

Cancer pain assessment also includes an evaluation of type of pain.[39,77] Identification of type of pain is to a large extent based on clinical experience, although lots of effort have been done to better classify cancer pain and identify different pain syndromes.[39,97-99] Although bone pain is considered its own subgroup of chronic cancer pain,[41] patients with CIBP may have combined pain syndromes like neuropathic pain components or pain arising from other metastatic sites like soft tissue or visceral organs.[97,100] Identification of neuropathic pain may be especially important as these

patients may have a greater advantage of adjuvant analgesics.[69,77] Several screening tools for neuropathic pain are available and the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) score is frequently used.[39,101]

1.2.6.3 Quantitative sensory testing in CIBP

Objective measurement as a part of pain assessment is not routinely used in CIBP. Quantitative sensory testing (QST) can provide additional information about impairment of the somatosensory system. In QST, the threshold of different sensory features like pain, touch, vibration, and temperature can be measured. QST is investigated in several studies on neuropathic pain, but is not recommended in the diagnosis of neuropathic pain alone.[39,102,103] In a smaller study including patients with CIBP before and after RT, a reduction in abnormal thermal sensitivity was observed in patients with analgesic RT response.[104] The role of QST in clinical assessment of CIBP is so far limited.

1.2.6.4 Multidimensional pain assessment in CIBP

As previously mentioned, the expression of pain is multidimensional and involves social and psychosocial factors. Including these aspects in the assessment of CIBP can be useful to understand the individual pain experience.[78,105] The Edmonton Classification System for Cancer Pain (ECS-CP) was created based on the Edmonton Staging System for cancer pain (ESS), which was developed to identify a risk of a complex cancer pain management.[106-108] The ECS-CP contain five elements that are recorded by health care personnel: mechanism of pain, incident pain, psychological distress, addictive behavior, and cognitive function. These are all relevant items in assessment of CIBP as they can be important to predict a worse treatment outcome.[109] Finally, other cancer-related symptoms may also influence the expression of pain, and a validated patient reported symptom score like the Edmonton Symptom Assessment System (ESAS) is easy applicable as a screening tool for cancer related symptoms.[110]

1.2.7 Prediction of pain in patients with bone metastases

Although pain is a common symptom in patients with bone metastases, many patients have bone metastases that are not painful.[111,112] Explanations for the difference in pain

experience may be related to mechanical factors like fractures, microfractures and nerve involvement, but it is also proposed that biological, genetic or clinical features may play a role in the development and the individual perception of CIBP.[43,47,111] Today we have limited knowledge to predict pain in patients with bone metastases. Candidate biomarkers include factors involved in the pathophysiology of CIBP, but potential biomarkers are investigated to a low extent in clinical trials.[58,113,114]

Clinical features predicting cancer pain have been investigated in several trials including patients with heterogenous cancer pain etiologies, but there is little specific information about patients with CIBP.[86,87,100,105] As discussed in the previous section, the comparability between studies can be challenging due to different assessments and definitions of pain and pain control.

Several studies have used a cross-sectional study design to evaluate the association between self-reported pain intensity and clinical factors in a general cancer population. A large study including more than two thousand cancer patients with pain identified multiple clinical factors associated with higher average and worst pain intensity in univariate analyses. Psychological distress, higher opioid dose, episodic pain and sleep disturbances displayed the strongest association with pain intensity (11-point NRS last 24 hours) in multivariable models.[88] The cross-sectional association between pain intensity (worst and average pain in an 11-point NRS last week) and both episodic pain and sleep disturbances was confirmed in another large trial.[87] In addition, this study demonstrated an association between average pain and pain localization in the upper extremities, while a higher opioid dose and the use of non-steroidal anti-inflammatory drugs (NSAIDS) were associated with higher worst pain intenstiy.[87] Factors associated with higher pain intensity in cancer patients is illustrated in Figure 7.

Studies based on items from ECS-CP have demonstrated that cancer patients with younger age, neuropathic pain, episodic pain, psychological distress, addictive behavior and higher pain intensity are more likely to need longer time to achieve adequate pain control or require higher opioid doses.[40,107,115,116] A recently published cross-sectional analysis investigated characteristics from the ECS-CP in a subpopulation

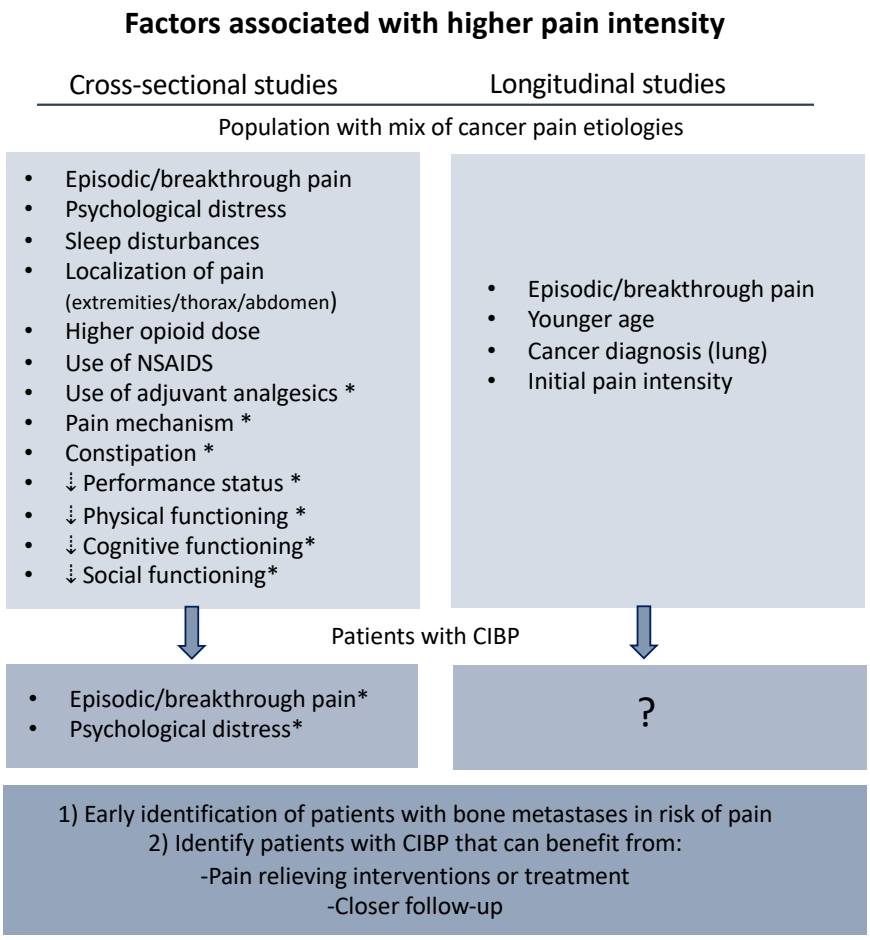
of 147 patients with bone metastases.[86] The study found that the presence of breakthrough pain was significantly associated with both average and worst pain intensity, while psychological distress was associated with a higher average pain intensity. Only univariate associations were reported.[86]

The cancer pain prognostic scale (CSS) was launched in 2002 and included worst pain intensity, emotional well-being, opioid dose, and pain characteristics (mixed pain) as predictors for an unfavorable pain outcome in cancer patients. The original study was performed on 74 patients and is, as far as we know, not validated in a larger patient population.[117]

A longitudinal study design may be advantageous to reveal factors important in predicting pain severity, as the associations can be measured at several time-points and change over time can be considered.[118] Knudsen *et al* identified initial pain intensity, breakthrough pain, lung cancer and younger age to be predictors of higher pain intensity two weeks after enrollment in a clinical study including a general cancer population.[87] A more recent study also identified episodic pain as a predictor of higher mean pain intensity over time in cancer patients. The study divided patients in different categories based on pain syndromes and patients with a combined bone pain and neuropathic pain did not have a significantly worse outcome compared to patients with only bone pain.[100]

CIBP have distinct pathophysiological and clinical features.[14,42] Factors associated with higher pain intensity in a general cancer population may not have the same relevance in a CIBP population, as illustrated in Figure 7. A robust clinical or biological model explaining the difference between patients with bone metastases with or without pain or predictors for developing higher pain intensity from bone metastases is not available.

Figure 7. Clinical factors associated with higher pain intensity in patients with cancer.



The figure presents several clinical factors that previously have been associated with higher pain intensity in a general cancer population[87,88] and in patients with cancer induced bone pain (CIBP) specifically.[86] Factors marked with an asterisk (*) have been associated with pain intensity only in univariate analyses.[86,88] The figure also illustrates the potential benefits of being able to identify risk factors for pain in the subgroup of patients with CIBP.

1.2.8 Treatment of CIBP

Therapeutically, CIBP can be a challenging condition.[66] A reason for this is the commonly disseminated disease that may affect several sites and weight bearing bones, combined with a typically short acting and intense episodic pain that often emerges before the effect of pain killers such as immediate-release acting opioids.[42,66] In a general cancer population, about 1/3 of patients are not adequately treated for pain and this untreated pain is often caused by CIBP.[77,119] Principles of treatment for CIBP include systemic anti-cancer treatment to reduce tumor load, pain killers to modify transmission of pain signals, medications to reduce the pathological bone remodeling process, or direct interventions at the affected site like RT or surgery.

1.2.8.1 Systemic anti-cancer treatment

Systemic oncological treatment includes chemotherapy, hormonal therapy, or more modern targeted therapies like tyrosine kinase inhibitors and immunotherapy. Systemic oncological treatment aims to target cancer cells to reduce tumor load, which can lead to pain relief. The type of treatment combination and its expected efficacy is highly dependent on cancer diagnosis and stage and will not be further discussed in this thesis.

1.2.8.2 Standard analgesic treatment

Standard analgesic medication is a cornerstone in CIBP treatment. Main analgesic groups include non-opioid analgesics like paracetamol and NSAIDS; weak opioids like codeine, tramadol and hydrocodone; and strong opioids like morphine, oxycodone, fentanyl, methadone, and hydromorphone.[120] Several guidelines for analgesic cancer pain treatment exist.[69,77,121] The three-step analgesic ladder for cancer pain treatment was published by the World Health Organization (WHO) in 1985, and still forms the basis of today's cancer pain treatment.[121] The principle of the ladder is to start at the lowest step and then titrate upwards until sufficient pain relief is reached. The first treatment-step is non-opioid analgesics, the second step is adding weak opioids, and the third step involves shifting to strong opioids. Adjuvant analgesics may be added to all steps in the model.[121] More recent guidelines suggest that a two-step approach

omitting the use of weak opioids is as effective.[69,77] Strong opioids can be administered orally, transdermal, subcutaneously, intramuscular, intravenously, and intrathecal and are therefore easily applicable in all stages of cancer pain treatment.[69] Common opioid side effects include gastrointestinal side effects like constipation, nausea, and vomiting, and CNS toxicities like drowsiness, hallucinations, cognitive impairment, and respiratory depression. Side effects are often a dose-limiting effect for opioids and hinders the titration to an effective analgesic dose.[77] Several opioids are also known to cause immunosuppression.[122]

1.2.8.3 Adjuvant analgesics

Adjuvant analgesics are drugs with a primary indication other than pain, that have analgesic effects in some conditions.[123] Tricyclic antidepressants and anticonvulsants are common adjuvant analgesics and recommended for use in neuropathic pain conditions.[69,77] The role of the anticonvulsant pregabalin was investigated in two placebo-controlled trials for CIPB. The first placebo-controlled study that investigated pregabalin in patients with CIBP indicated a minor treatment effect of pregabalin, but the study was terminated early due to slow recruitment.[124] A later trial did not reveal any pain-relieving effect of pregabalin in combination with RT among patients with CIBP.[125] Other adjuvant analgesics like NMDA receptor agonists (ketamine) and local anesthetics are sometimes used in complex pain situations, although formal evidence is scarse.[69,123] The analgesic effect of cannabinoids is also disputed, and cannabinoids are not routinely recommended as a part of cancer pain management.[77,123]

1.2.8.4 Corticosteroids

Corticosteroids are widely used to relieve cancer related symptoms, especially in patients with a shorter life expectancy.[126] An American survey from 2014 found that 2/3 of palliative care health care providers prescribed corticosteroids as an adjuvant analgesic to most of their patients suffering from painful bone metastases.[127] Although physicians frequently prescribe corticosteroids for CIBP, the documentation for an analgesic effect is sparse and may be more based on traditions rather than evidence based knowledge.[126,128] A recent placebo-controlled study of 47 patients with opioid

dependent general cancer pain did not indicate any analgesic effect of corticosteroids.[129] On the other hand, corticosteroids have shown to improve early pain exacerbations or pain flares after RT in several placebo-controlled studies in patients with CIPB.[128,130-134] These finding may support a role of analgesic treatment in patients with CIBP.

1.2.8.5 Bone targeting agents

Bone targeting agents in use today are bisphosphonates and denosumab that impair with osteoclastic bone resorption. Bisphosphonates induce osteoclast apoptosis while denosumab inhibits osteoclast formation.[19] These mechanisms hinder the formation of the acidic bone microenvironment which is one of the pathophysiological processes thought to be important in CIBP.[14,43] In cancer, bone targeting agents are established to prevent SREs and adjuvant to reduce recurrence and improve survival in postmenopausal patients with breast cancer.[135] The analgesic effects of bone targeting agents is debated.[77] Porta-sales *et al* published in 2017 a meta-analysis including 28 placebo-controlled studies using bone targeting agents. They concluded that the pain-relieving effects of bisphosphates and denosumab are weak, but that the bone resorption inhibitors can be useful in delaying onset of pain.[136] In this meta-analysis, a pain relieving effect was only reported in 6 of the 25 included studies on bisphosphates and the documentation on denosumab was sparse.[136] Still, some authors argue that bone-resorbing agents should have a role in pain treatment of CIBP.[137,138] According to the ESMO guidelines on bone health in cancer patients published in 2020, bone targeting agents are recommended in all patients with multiple bone metastases.[19]

1.2.8.6 Novel drug targets

Several new mechanisms of analgesic relief have been suggested based on pre-clinical animal models of CIBP, but only a few medications have been investigated in clinical trials.[139,140] A recently published phase II randomized-controlled trial investigated Saracatinib (a Src-kinase inhibitor) in patients with CIBP and found no additional analgesic effect after 4 weeks use.[141] Antihistamines, like the H1-blocker Loratadine,

reduce bone pain that may occur after injection of G-CSF, but there are no studies investigating analgesic effect in CIBP caused by bone metastases.[142,143] Another candidate pharmaceutical target for analgesic treatment in CIBP is blocking of nerve growth factor (NGF).[144] Anti-NGF is shown to reduce bone pain in pre-clinical CIBP models and in other non-malignant bone pain states.[144,145] Tranezumab, a recombinant humanized monoclonal antibody to NGF, was investigated in a phase II study on patients with CIBP. The analgesic effect was only minor and not significant compared to placebo, but a larger phase 3 study is now ongoing.[146] Other molecular targets, especially in relation to local bone remodeling, inflammation, pain signal transduction and neurotransmission are in the pipeline. A few trials are ongoing and potential novel drug targets will probably be investigated in clinical trials in the upcoming years.[46,90,139,140,145]

1.2.8.7 Surgery and minimally invasive interventions

Pain alone is seldom an indication for surgery in bone metastases, but surgery or other interventional methods can be a good treatment option under certain circumstances.[122] Pathological fractures in long bones often require surgery.[19] The surgical method depends on localization of the fracture and patient prognosis with an aim to preserve function and relieve pain. In patient with pathological fractures in long bone, diaphysis plate fixation or intramedullary nailing is often good symptomatic treatment.[19] Patients with fracture risk from bone metastases may also benefit from surgery, although surgical management of impending fractures is controversial.[22] Prophylactic surgery in patients with impending pathological fractures have shown to improve postoperative pain and function, result in better survival with less surgical complications and shorter admittance to hospital. Still, intervention should be carefully considered based on patient individual factors.[19,22] Mirels score has been developed to evaluate long bone fracture risk based on both clinical and radiological features.[147] Vertebral fractures are often handled conservatively, with surgical intervention recommended if spinal instability. Spinal instability neoplastic score (SINS) is helpful to evaluate the need for surgery.[19,22,148] If vertebral metastases cause malignant spinal

cord compression surgery can be indicated, especially in patients with longer life expectancy, less radiation sensitive tumors or progression of symptoms during or after RT, bony fragments causing compression or significant neurological impairment.[24] Surgical decompression with instrumented fusion to preserve stability is often sufficient treatment in palliative patients.[24] An alternative treatment approach for painful metastatic vertebral compression fractures is percutaneous cement argumentation like vertebroplasty or kyphoplasty.[149] Other minimally ablative procedures to relieve pain in CIBP are cryoablation and radiofrequency ablation (RFA) alone or in combination with cement argumentation for a better treatment outcome.[19,150] Treatment efficacy in the reported studies is variable and it is of note that most studies include a low number of patients, which contributes to some uncertainties regarding treatment recommendation for these interventions.[6,151] For patient with CIBP that is difficult to relieve with standard analgesics, intrathecal administration of opioids and co-analgesics can provide better pain relief.[77,151] Other pain relieving interventions for patients with refractory and localized pain include peripheral nerve blocks, neurolytic plexus or spinal blockade. Neurostimulation, like spinal cord stimulation, is another minimal invasive pain treatment strategy for CIBP with low evidence of treatment efficay.[77,151]

1.2.8.8 Radiotherapy

Radiotherapy (RT) is well-documented, efficient, inexpensive, and recommended as one of the primary treatment options for patients with CIBP.[14,77,152,153] RT treatment for CIBP will be outlined in section 1.3 of this thesis.

1.2.8.9 Radiopharmaceutical treatment

In radiopharmaceutical therapy, radionucleotides administered systemically to the patient accumulates at a tumor site and delivers radiation doses to induce tumor cell killing. A Cochrane review from 2017 found a minor analgesic short term pain relief (1-6 months) after treatment with the beta-emitting radioisotopes Strontium^{88}, Samarium^{153} and Rhenium^{188} in patients with osteoblastic metastases from different cancer origins. Hematological toxicities like thrombocytopenia and leucopenia were

common.[154] The review is now withdrawn for Cochrane due to a lack of updated knowledge. The alpha emitter Radium^{223} was later approved for use in patients with castrate-resistant prostate cancer.[155] Radium^{223} reduces the risk of skeletal related events, including the use of opioids, and improves survival in selected patients. Radium^{223} should be considered as a treatment option for CIBP in patients with castration-resistant prostate cancer and no visceral involvement.[77,156,157]

1.2.8.10 Other non-pharmacological treatments

Although the documentation is limited, some patients may gain benefits from other non-pharmacological treatments. Physical exercise may improve bone strength, results in fewer adverse events, and is potentially beneficial for patients with CIPB.[158,159] Psychological distress like anxiety and depression is also common in cancer patients and may deteriorate a pain state.[88,115] Psychological support and interventions like cognitive behavior therapy may be useful in selected patients.[111,160]

1.3 Radiotherapy in patients with painful bone metastases

1.3.1 Introduction to radiotherapy

RT in cancer treatment is based on the principle that high energy ionized radiation causes cell damage and cancer cell death. In patients with a local or locally advanced cancer disease RT can be administered with a curative intent. In some clinical settings, RT is administered together with other cancer treatments like chemotherapy or immunotherapy.[161] RT may also be provided with a palliative intent to reduce tumor load or symptoms, like patients with painful bone metastases. About half of all cancer patients receive RT throughout their disease course.[162]

1.3.2 Basic principles of radiation physics

When the ionized radiation is delivered from an external source outside the body it is called external beam RT. In contrast, brachytherapy is when the radioactive source in placed inside a body cavity. Brachytherapy is mostly used in gynecological cancers but

is also available for other cancers like prostate and gastrointestinal cancer. Radiation may also be applied as *systemic radioisotopes* like RA^{223} mentioned earlier.[163]

In clinical practice most patients receive external beam RT with high energy X-rays. X-rays are composed of mass-less photons that can be generated by electrons in a linear accelerator.[163,164] Common for photon therapy is a relatively low amount of energy transferred per unit distance, also referred to as a low linear energy transfer (LET).[164] The energy deposition is spread in a broad range that may cause irradiation effects to normal tissues surrounding the tumor.[163]

Particle radiation is an alternative source of external beam RT.[163] Electron particle therapy is typically used to treat cancers close to the body surface because of a rapid dose fall in the irradiated tissue. Proton particle therapy has an unique energy distribution curve with a deeper and sharp deposition point of energy, called the "bragg curve", that makes it easier to target the tumor and may result in less adverse effects.[165] A disadvantage is the cost and availability of protons as it requires large cyclotrons to be produced. Currently, only a minority of cancer patients are eligible for proton therapy.[165]

The radiation dose for both brachytherapy and external beam RT is measured in Gray (Gy), that is the SI unit of the absorbed dose of ionized radiation. Gy is defined as the absorption of one joule of radiation energy per kg of matter.[166]

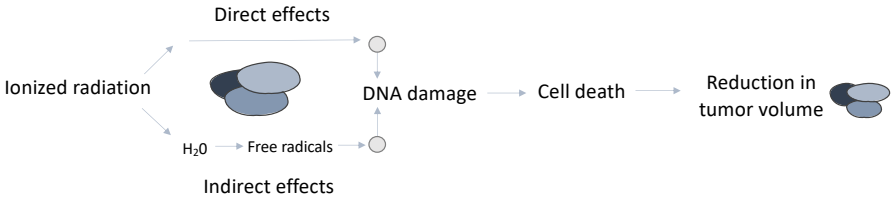
1.3.3 Basic principles of radiation biology

Ionized radiation is electronically charged particles that can deposit their energy to exposed tissues. This may harm the cells in many ways, but damage to DNA is considered the major cause of cell death due to RT.[163] Ionized radiation may cause both single and double strand DNA breaks. Double strand DNA breaks more often lead to cell death compared to single strand DNA breaks, which the cells have a better ability to repair.[163,167]

Ionized radiation can cause damage to the DNA directly or indirectly (Figure 8). The direct effect of radiation is when ionized particles directly damage DNA in the cells.

The indirect effect of ionized radiation occurs when the ionized particles interact with other molecules, usually water molecules, which leads to the production of free radicals that cause the actual DNA damage.[167]

Figure 8. Ionized radiation and biological mechanisms of tumor cell death



The figure illustrates how ionized radiation can cause damage to the DNA by direct or indirect actions. The indirect effect of ionized radiation typically occurs when ionized particles interacts with water molecules (H_2O) to produce free radicals that cause DNA damage.[167]

RT causes damage to both cancer cells and healthy tissue. One of the reasons for cancer cells being vulnerable to RT is that they are rapidly dividing and that normal repair mechanisms are often impaired. If RT is administered fractionated, as several doses given in a time-series, the healthy tissue has a better ability to regenerate between RT fractions compared to cancer cells. The biological effectiveness of RT is dependent on the type of radiation applied and its LET, RT total dose, fractionation rate and the radiosensitivity of the irradiated tissue.[164]

1.3.4 Biological RT effects inducing analgesic relief in CIBP

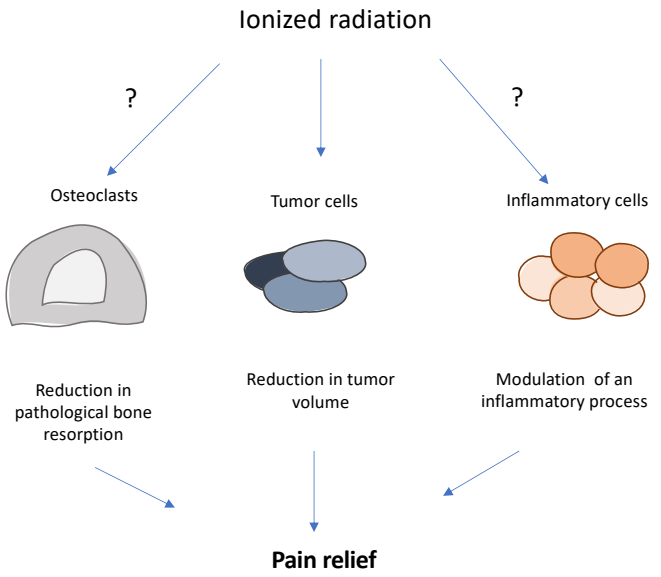
The exact mechanism for RT to induce analgesic relief in patients with painful bone metastases is not established.[168,169] Ionized radiation will cause tumor cell death resulting in shrinking of the tumor volume that leads to pain relief. Three main arguments support that the analgesic effect of RT is also caused by additional factors:

1) some patients experience a rapid pain response within days after RT that is difficult to explain by tumor reduction, 2) very low doses of RT that are unlikely to trigger tumor reduction have induced pain relief, and 3) there is little evidence that patients

with primary cancer diagnosis with a higher radiosensitivity respond better to analgesic RT for painful bone metastases.[111,169,170]

Both RT effects on osteoclasts and inflammatory cells present in the bone micromovement are suggested to induce analgesic relief from RT in patients with CIBP (Figure 9).[111,170] Inflammatory cells are to a large extent present in the bone microenvironment and several inflammatory markers are associated with CIPB.[43] In vitro studies have demonstrated that both osteoclasts and osteoclast-precursors are affected by ionized radiation and a modulation in osteoclast activity may contribute to analgesic relief after RT[170], but there is still limited clinical evidence to determine the relationship between RT efficacy and changes in inflammation.

Figure 9: RT effects proposed to contribute to pain relief after palliative RT for CIBP



Ionized radiation induces tumor cell death but could also cause alternations in the bone microenvironment and inflammatory cells leading to analgesic relief in patients with CIBP.

1.3.5 External beam RT delivery

External beam RT can be administered as a single dose at one given time-point, referred to as single fraction RT (SFRT) or as multiple doses in a time series, referred to as fractionated or multiple fraction RT (MFRT).[161] Traditionally, doses of about 2 Gy is delivered per fraction. The delivery of higher doses per fraction is often referred to as hypofractionated RT (ex. 4 Gy in 5 fractions). Palliative RT regimes are frequently hypofractionated to reduce the number of treatment days.[171] The total delivered RT dose depends on the purpose of treatment, site and radiosensitivity of the irradiated tissue. While curative RT regimes may have total doses of up to 80 Gy, palliative RT regimes typically have total doses of 35 Gy or lower.[171,172] In contrast to conventional RT mentioned above, stereotactic body radiation delivers very high doses of RT (typically 15-20 Gy) to a precise localization in one or a few fractions.[173] In the following paragraphs we will focus on conventional external beam RT, referred to as RT, which is most frequently used in general cancer treatment and in treatment of painful bone metastases.[25,174]

1.3.6 External beam RT techniques

The goal of RT in cancer treatment is to deliver therapeutical doses of RT to the cancer cells while minimizing the damage to healthy tissue.[164] Recent advances in technology have made it possible to do precise radiation dose planning based on CT scans and other radiological images. Historically, RT was delivered from one or two angles as rectangular fields based on plain X-rays. The set-up of the treatment field was typically based on bony landmarks.[25,164] This static 2D radiation delivery technique is now mostly replaced by conformal 3D RT planning based on CT imaging (3D CRT). Intensity modulated radiation therapy (IMRT) and volumetric modulated arc therapy (VMAT) makes is possible to deliver very conformal and irregularly shaped doses of ionized radiation to the tumor and spare the surrounding tissue from radiation.[25,161,163,164] Conformal RT techniques require the radiated site to be thoroughly defined. This commonly involve a specification of the gross tumor volume (GTV) with a margin to account for microscopic tumor growth to the clinical target volume (CTV) and an

additional margin to the planning target volume (PTV) to account for treatment uncertainties.[25,164]

1.3.7 Indication for palliative RT of bone metastases

Palliative treatment refers to treatment where the intention is to relieve symptoms or increase life-time rather than aiming for curative treatment.[164] The main indication for RT in patients with bone metastases is CIBP.[175] RT is also indicated to avoid or treat other complications of bone metastases like malignant spinal cord compression, cauda equina syndrome, or pathological fractures. The use of prophylactic RT to avoid pain or to provide long-time disease control in patients with oligometastatic disease is debated.[25,176]

1.3.8 Definition of analgesic RT response in patients with bone metastases

The definition of analgesic RT response in patients with bone metastases will directly influence the reported response rate after RT.[152] Variable endpoints in randomized RT trials have made it difficult to directly compare studies, and large differences in reported RT efficacy is demonstrated in systematic reviews and meta-analyses.[174,177-180] To ensure comparability and quality of clinical RT trials on patients with painful bone metastases, recommendations from an International Consensus Working Party was published in 2002, and later updated in 2012.[89,181] A patient reported NRS ranging from 0-10 is suggested to evaluate pain intensity before and after RT. In the updated consensus paper, it is recommended to evaluate worst pain at the treated site in the last 3 days.[89] All opioid analgesics should be converted into daily oral morphine equivalents before calculation of RT response. Pre-treatment assessments should be performed as close to treatment as possible and assessments of RT response is recommended after 1, 2, and 3 months.[89,181] Analgesic RT response for painful bone metastases can be divided into different response categories: 1) complete response, 2) partial response, 3) pain progression, and 4) indeterminate response.[89,181] Definitions of the endpoints of analgesic RT response are presented in Textbox 1. The overall RT response including both complete and partial RT response is commonly reported in clinical trials.[89,152] By implementing more conformity and stricter criteria for analgesic

RT response, lower response rates have been reported in randomized trials and in meta-analyses.[89,152]

Textbox 1: Definition of analgesic RT response according to international guidelines.

Complete response:	Pain score of 0 with no increase in analgesic intake.
Partial response:	Pain reduction of 2 or more at the treated site in a 0-10 NRS scale without increase in analgesic intake OR analgesic reduction of 25 % without increase in pain score.
Pain progression:	Increase in pain score of 2 or more above baseline with no increase in opioid dose or a 25 % increase or more in opioid dose with pain score equal or 1 point above baseline.
Indeterminate response:	Any response not covered by the definition complete response, partial response, and pain progression.

1.3.9 RT efficacy in painful bone metastases

The effect of RT for the treatment of painful bone metastases is well recognized and RT is recommended as standard treatment in CIBP.[77,175] A meta-analysis published in 2018 found the overall analgesic response rate in the intention-to-treat population to be approximately 60%, with complete response reported in about 1/3 of the patients.[174] In an updated meta-analysis published in January 2023, only studies reporting RT response according to the international consensus guidelines were included. This paper reports a lower RT response rate of 60% in evaluable patients and only 45% in the intention-to-treat population.[152] One explanation for a lower RT response in this meta-analysis is the stricter response definition that was not adapted in all studies included in previous reviews.[152,178,179]

1.3.10 RT regimes and analgesic RT response

Historically there have been several available RT treatment schedules to obtain analgesic relief in patients with painful bone metastases. In a review investigating the international prescribing pattern for painful bone metastases in a ten year period from 1993, the prescribed RT regimes varied from 3 Gy in 1 fraction to 60 Gy in 30 fractions.[182] Since the late 1980's several randomized controlled trials and later meta-analyses have been conducted to determine the optimal RT regime for CIBP. Most of

the studies compare the efficacy of SFRT and MFRT regimes.[152,174,177-180,183-186] The effect of stereotactic body radiation has also been investigated in several studies on patients with CIBP. [187,188] [189] In the following section the optimal dose/fraction regimes in palliative RT for CIBP will be discussed.

1.3.10.1 Uncomplicated painful bone metastases

For uncomplicated painful bone metastases with no neurological affection, fracture risk, or soft tissue expansions, the optimal dose/fractionation regime has been thoroughly investigated.[25] Large meta-analyses determined that there is no difference in analgesic outcome or adverse effects between SFRT and MFRT in patients with uncomplicated bone metastases.[152,178-180,186] SFRT is more convenient for the patients and the health care system and is considered cost-effective.[25,178,190] The most commonly administered SFRT dose in treatment of painful bone metastases is 8 Gy.[177,185] 8 Gy SFRT provides better pain relief compared to 4 Gy, but there is limited evidence to support that 8 Gy is superior to other SFRT regimes > 4 Gy.[177,185,191,192] As 8 Gy is well-established as an effective and safe SFRT treatment dose, it is now the recommended regime in treatment of painful bone metastases.[25,175,177,186] Stereotactic "high-dose" RT has not been proven beneficial over conventional RT in several clinical studies on patients with uncomplicated and painful bone metastases and is not routinely recommended.[25,193-195] SF hemi-body or wide-field RT can be considered in patients with diffuse pain from multiple metastases.[25]

1.3.10.2 Complicated bone metastases

About 1/3 of bone metastases are considered complicated.[196] The definition of complicated bone metastases varies between studies, but often includes bone metastases associated with neurological deficits like malignant spinal cord compression or cauda equina syndrome and pathological or impending pathological fractures. Some papers also define bone metastases with extraosseous soft tissue components as complicated bone metastases.[25,196] Patients with complicated bone metastases have to different degrees been excluded from clinical studies on palliative RT and the optimal dose/fractionation regime has been difficult to establish due to the

lack of relevant literature.[176] Traditionally MFRT is more frequently prescribed in complicated bone metastases, although the evidence for this practice has been limited.[196] A recommendation for RT of complicated bone metastases was published in 2022 by the European Society for Radiotherapy and Oncology (ESTRO) and Advisory Committee on Radiation Oncology Practice (ACROP).[176] In patients with malignant spinal cord compression, SFRT of 8-10 Gy is now recommended in patients not eligible for surgery, as MFRT has not been proven to provide a better outcome or survival.[176,197] In case of postoperative RT after surgery for malignant spinal cord compression, a RT regime with 30 Gy in 10 fractions is still recommended as comparable data is lacking.[176] In actual or impending pathological fractures or soft tissue expansion outside bone, there is little evidence to support the superiority of any treatment regime and 8 Gy SFRT and MFRT regimes like 20 Gy in 5 fractions or 30 Gy in 10 fractions are considered equal alternatives.[176] In patients with CIBP and a neurological pain component, a similar analgesic response rate was reported after 8 Gy SFRT and 20 Gy in 5 fractions.[198] The use of 8 Gy SFRT is therefore recommended in patients with a neurological pain component.[176]

1.3.10.3 Oligometastatic disease

Oligometastatic disease refers to patients with only a few metastatic lesions. The number of lesions defined within the term oligometastatic disease varies across studies that often include patients with 1-5 metastatic lesions.[25,199] Patients with oligometastatic skeletal disease may have an increased life expectancy compared to patients with widespread skeletal disease and several studies have been carried out to evaluate if these patients benefit from MFRT with a higher total radiation dose or stereotactic RT.[187] In a recently published prospective trial including 131 patients with oligometastatic bone metastases, no differences in analgesic response were observed in patients treated with conventional compared to stereotactic RT.[187] CIBP in an oligometastatic setting should therefore be treated with a conventional 8 Gy SFRT if the treatment goal is analgesic relief.[25] However, stereotactic RT can be beneficial for long term disease control.[25,200]

1.3.11 Time to RT response

Time to evaluation of RT response varies in different trials.[174] Only a few RT trials have reported the median time to pain relief, that varies between 1 and 4 weeks.[179] In a study by Steenland *et al*, RT response was evaluated weekly for 12 weeks after RT and the median time to pain response was 3 weeks.[201] Evaluation of RT response to consider re-irradiation should be performed at least 4 weeks after RT.[25,89]

1.3.12 Duration of RT response

Duration of pain relief from palliative RT is reported to be 24 weeks in an overall population and approximately 30 weeks for patients alive 1 year after RT.[201,202] In the following year after RT about half of the patients experienced progression of pain.[201] Time to pain progression may be dependent on cancer prognosis and diagnosis. Breast cancer patients had a median time to pain progression of 36 weeks compared to 10 weeks in lung cancer patients.[201] Duration of pain response is reported similar after SFRT and MFRT.[174]

1.3.13 Re-irradiation of painful bone metastases

Re-irradiation is considered both safe and effective in controlling pain from painful bone metastases.[203,204] An overall response in more than 60 % of evaluable patients is reported in systematic reviews from 2014 and 2023.[152,204] A randomized trial published in 2014 reported analgesic response in about 30% of patients in the intention-to-treat population.[205] Analgesic response rates are similar in MF and SF re-irradiation, but MFRT regimes were associated with more acute toxicity.[205,206] Patients with no response after the initial treatment, patients with inadequate pain relief or pain recurrence at least 4 weeks after the initial treatment should be considered for re-irradiation with an 8 Gy SFRT for uncomplicated bone metastases.[25] Patients initially treated with SFRT have higher re-treatment rates compared to patients treated with MFRT (20% vs 8 %).[174] Because time to progression of pain is shown to be equal between SFRT and MFRT,[201] it is speculated that the higher re-treatment rates may reflect that re-irradiation is more widely accepted in patients treated with initial SFRT.[174]

1.3.14 RT adverse effects

Adverse effects of palliative RT depends on the RT total dose, localization of metastases, size of the radiation field and proximity to the gastrointestinal tract and other relevant organs.[172] Acute toxicities are inconsistently reported in many RT trials.[89,180,185,207] A direct comparison of the severity and frequency of side effects between trials is difficult due to different symptom definitions and grading scales.[180,185] The most reported acute adverse effects after RT for painful bone metastases are gastrointestinal adverse effects like nausea, vomiting or diarrhea, skin reactions, tiredness or fatigue, and pain flares. Hematological toxicity and CNS adverse effects may also occur but are less frequently reported.[208] Nausea and vomiting are reported in up to 77% of patients and can be managed with antiemetics or corticosteroids.[180,209] Pain flares after RT is reported in 2-44% of patients.[210-212] Pain flares may be treated with conventional analgesics or corticosteroids.[25] Prophylactic dexamethasone may reduce the incidence of radiation-induced pain flares, but results are conflicting and corticosteroid treatment is not without risk of other complications.[131,211] There is no consensus regarding a routine use of prophylactic corticosteroids to minimize the risk of pain flares during palliative RT for painful bone metatases.[25] Although acute adverse effects seems to occur quite frequently based on results from clinical trials, the severity of side effects after palliative RT are often mild or moderate. Few or no grade 3 and 4 toxicities were reported in clinical RT trials on bone metastases.[180,181]

Late adverse effects are infrequently reported in palliative RT trials. Many patients have a limited life-expectancy and will not live long enough to experience late effects after RT.[175] The doses applied in palliative RT are often small to moderate, but one should be especially aware of repeated RT or overlapping RT fields. In spinal metastases, the risk of myelopathy after re-irradiation 6 months after the initial treatment is considered very low if the cumulative bioequivalent dose in 2 Gy fractions is under 100 Gy.[176] Pathological fractures after RT are in most studies occurring in less than 5% of the patients.[174,178] This includes vertebral compression fractures after RT for spinal metastases. Vertebral fractures are more common in patients treated with

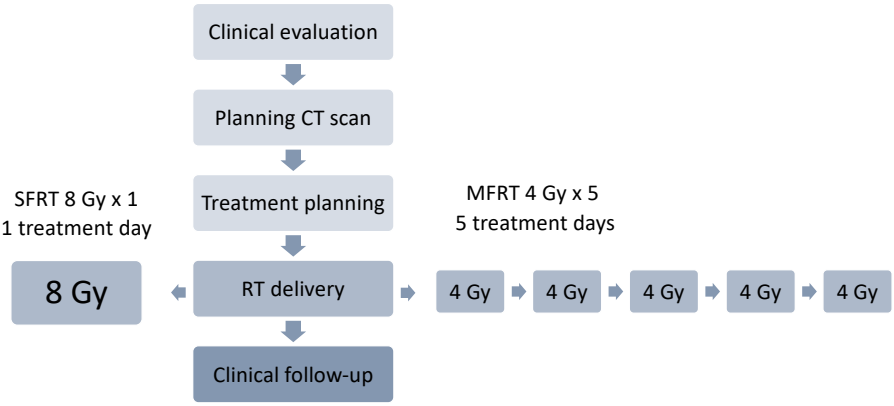
high dose RT and reported in up to 39% of patients after stereotactic RT.[213] There is no difference in the frequency of pathological fraction after SFRT and MFRT.[174] Spinal cord compression occurs in 1-6% of patients after RT, often as a sign of tumor progression or treatment failure rather than a direct toxicity after RT.[178] Malignant spinal cord compression tends to occur a bit more frequently after SFRT, but the difference is not statistically significant.[178] Hematological toxicity due to bone marrow dysfunction might be a concern after high doses and large RT fields, especially in RT against the pelvis and lumbar spine that are largely contributing to the hematopoiesis.[214] The incidence of bone marrow suppression after palliative RT for bone metastases is not easy to identify and the risk for considerable hematological toxicity is considered low.

1.3.15 Practical aspects in delivery of palliative RT for CIBP

Before the delivery of RT, a clinical evaluation should always be performed to decide if RT is appropriate for the individual patient. This includes a clinical examination, pain history and evaluation of radiological imaging.[25] Pain intensity and site of pain is important to evaluate, as pain distribution may not always correlate with findings from radiological imaging. Marking the site of pain may be helpful before the planning CT scan.[25] After the clinical evaluation, a planning CT scan is commonly performed before the physician and physics-team do the RT dose planning. It is also possible to provide RT in direct set-up without a planning CT scan. A direct set-up of one or two static fields may save time and recourses, but the dose distribution to both tumor and normal tissue is more unprecise, and conformal RT based on a planning CT scan is commonly preferred also in palliative RT.[25] After dose planning, RT is delivered as SFRT or MFRT.[174] A clinical follow-up to evaluate treatment efficacy, analgesic treatment and adverse effect should be performed by the oncologist or the patient's general practitioner.[25] Palliative RT for painful bone metastases may involve several hospital visits for each patient (Figure 10). This may result in an additional burden for patients, especially those with reduced performance status, a high symptom load, or longer travelling distances. Patients not able to receive RT as an out-patient need to be admitted to hospital. The number of patients admitted for palliative RT due to bone

metastases at St. Olavs Hospital, Trondheim, has been relatively stable in the last 10 years, with 184 patients admitted for RT due to bone metastases in 2013 and 186 patients in 2022.

Figure 10. Illustration of radiotherapy treatment and delivery in practice



The figure presents the common steps in radiotherapy (RT) planning and delivery. Single fraction RT (SFRT) is delivered in one day. Multiple fraction radiotherapy (MFRT) is administered over several days: in this figure illustrated with 4 Gray (Gy) in 5 fractions that is a common regime in palliative RT for painful bone metastases.

1.3.16 Timing of RT in painful bone metastases

There is little evidence concerning the ideal time in the disease trajectory where palliative RT for painful bone metastases should be administered. Some guidelines recommend RT as the initial treatment for painful bone metastases, while others recommend RT for inadequate pain relief after start of conventional or low dose pain killers.[25,77] RT could also potentially delay or prevent pain from bone metastases that are asymptomatic. One retrospective study reported lower incidence of pain and SRE in patients treated with RT for non-painful bone metastases, but the group of patients receiving RT was small (28 patients).[215] Another clinical trial is investigating the effect of prophylactic RT in patients with bone metastases, but results are not yet published.[216] Palliative RT for asymptomatic and uncomplicated bone metastases that

may potentially become painful is not routinely recommended.[25] With a better understanding of which patients with bone metastases that are more likely to develop pain in the future, it would be easier to select patients that could benefit from prophylactic or early intervention with RT.

1.3.17 Predictors of analgesic RT response in CIBP

Although RT is considered an effective treatment for patients with bone metastases, about half of patients treated with RT do not have a significant treatment response.[152,174] To deliver the most optimal treatment in patients with painful bone metastases, it would be helpful to know which patients that have greater or less chance of gaining analgesic relief from RT. A systematic review investigating predictors of external beam RT response in patients with CIBP was published by Gardner *et al* in 2019.[217] The group concluded that no predictors to date are reliable to select patients with higher of lower change in RT response in clinical practice. Most of the available studies investigated univariate correlation between single variables and RT response, while two retrospective and secondary analyses from the Netherlands by Westhoff *et al* (2015) and van der Velden *et al* (2017) explored multivariate correlation of clinical variables as potential predictors of analgesic RT response.[218,219] Potential and previously investigated predictors of analgesic RT response are discussed in the following section.

Age

It has been proposed that elderly patients may not benefit equally to palliative RT compared to younger patients, but results from clinical trials are not consistent.[218,220] Several studies have not discovered any difference in treatment efficacy according to age.[219,221]

Gender

No significant differences between male and female patients are observed in respect to analgesic RT response in painful bone metastases.[218,219]

Performance status

Several clinical studies have demonstrated that patients with better performance status are more likely to respond to RT compared to patients with a lower performance status.[218,219,222] A few studies report no statistical association between performance status and RT response.[221,223]

Cancer diagnosis

There are indications that primary cancer diagnosis may have an impact on treatment outcome when it comes to radiation of painful bone metastases. Studies have identified patients with breast and prostate cancer to have the highest likelihood for analgesic relief from RT, while patients with lung cancer typically have the lowest response rates.[218,223,224] One study compared the RT response in gastrointestinal cancer with other cancer origins and discovered no difference in response rates.[225] Findings might be biased by a longer life-expectancy and better performance status in patients with breast and prostate cancer.

Estimated survival

Patients with a longer survival might have a better chance of responding to RT, but estimation of survival is challenging.[201,202] Steenland *et at* published a paper in 1999 investigating the effect of SFRT and MFRT, in which patients with an estimated favorable life-time prognosis were analyzed as a sub-group. Similar response rates were reported for the favorable sub-group compared to overall response in the same diagnostic groups (breast and prostate cancer).[201] In a follow-up paper published in 2006, only 53 % of the patients in the favorable prognosis group had actually survived for more than one year forcasted.[202] Several other scores to predict survival in patients undergoing RT for painful bone metastases have been developed but have not been investigated as predictors of analgesic relief.[226-228]

Comorbidity

Comorbidity is not reported in the larger RT trials and its importance in prediction of RT response is unknown.[218,219]

Metastatic distribution

The absence of visceral metastases is identified as favorable for analgesic RT outcome.[218]

Localization of bone metastases

There is little indication that the localization of bone metastases is relevant for the RT response rate.[218,219] Two studies have compared RT response in spinal vs non spinal bone metastases and did not reveal any difference in RT response.[229,230] Patients with painful spinal metastases and spinal instability have a lower RT response rate compared to patients without spinal instability.[222,231]

Radiological features

Osteolytic and sclerotic metastases have different pathophysiological features that in theory could implicate a difference in RT response rate.[14] No studies were identified to compare RT efficacy in osteolytic and sclerotic metastases. One smaller study on patients with spinal metastases did not detect any differences in RT response rate in patients with a soft tissue component outside bone.[232]

Pain and pain treatment

Both higher baseline pain intensity and the use of opioids are associated with a positive RT outcome, but findings are inconsistent.[218,219,221,223] One trial compared RT response in patients with and without neurological pain features and found no difference in RT response rates.[74]

Physical activity and gait

Physical activity and gait did not predict the RT outcome in a secondary analysis including 60 patients with CIBP.[73]

Bone turnover markers

Bone metastases are characterized by an imbalance in the normal bone remodeling.[14] An increase in bone formation or bone destruction can be reflected in the level of bone-related markers measured in urine or blood.[12,114] Several bone turnover markers have been investigated as potential indicators in the diagnosis, disease monitoring and treatment efficacy of bone metastases. None of the bone markers are to date considered suitable in a clinical setting, partly because of measurement uncertainties.[12] Dissimilarities in the biological processes of bone metastases reflected by the level of bone turnover markers may potentially also impact RT efficacy in patients with painful bone metastases. Three available studies have investigated urinary osteoclast markers as potential predictors of analgesic RT response in CIBP and present conflicting results.[168,169,233] Two studies detected lower baseline levels in urinary Pyridinoline (PYD) and Deoxypyridinoline (DPD) in patients with treatment response after RT,[168,169] while the other study did not detect any significant difference in the levels of PYD between responders and non-responders.[233] The level of urinary N-telopeptide (NTX) was investigated in two of the studies and was not significantly associated with RT response.[168,233] Both PYD, DPD and NTX are breakdown products of type 1-Collagen and secreted in the urine as a result of bone resorption.[12] To date there is limited evidence to support the use of urinary osteoclast markers to predict the analgesic effect of palliative RT for CIBP, but several other bone turnover markers have never been investigated in this setting and could be of potential interest.[12,217]

Genetic markers

One available study presents findings to support that genetic differences may be of importance in predicting analgesic RT response in patients with painful bone metastases. Furfari *et al* discovered that several single nucleotide polymorphisms (SNPs) were associated with a positive treatment outcome after palliative RT for painful bone metastases. The SNPs relevant to analgesic treatment outcome included SNPs associated with cell signaling and adherence, DNA repair and inflammation.[234] A clinical study conducted by our research group did not detect any correlation between

opioid treatment efficacy and variants in genes related to opioid metabolism in patients with CIPB.[68]

Inflammatory markers

Inflammatory markers are of special interest regarding analgesic RT response, as inflammatory markers are related to the pathophysiology of CIBP and the modulation of a local inflammatory response that could contribute to the analgesic efficacy of RT.[111,170] In the genetic study mentioned above, SNPs related to inflammatory markers were correlated with RT response.[234] One experimental trial investigated several inflammatory markers as potential predictors of response to RT in 60 patients with CIBP.[235] The study did not identify any significant association in the baseline pre-treatment level of inflammatory cytokines and RT response in the complete sample.

Radiological imaging techniques

Different radiological techniques and modalities have been investigated in respect to the analgesic RT response in patients with CIBP. However, for many radiological techniques the numbers of included patients are low, and the findings are inconclusive.[232,236-239] The use of 18F-FDG PET-CT have been investigated as a potential predictor of RT in several studies. One study including 31 patients with CIBP found that patients with a lower maximum standardized uptake value (SUV_{max}) before treatment had better RT response compared to patients with a higher SUV_{max}.[237] These findings have not been reproduced in similar studies, although the change in SUV_{max} after treatment has been associated with analgesic relief.[238,239] MRI using diffusion weighted imaging (DWI) has also been investigated as a potential predictor of analgesic RT response in CIBP. The authors did not report findings on the use of MRI-DWI as a pre-treatment predictor of RT response.[236]

RT treatment techniques and planning target volume

Although conformal treatment techniques are now widely used in treatment planning and delivery of palliative RT, there are no clinical studies to support its superiority regarding efficacy or adverse effects.[25] One study comparing adverse effects after non-

CT planned static RT fields and conformal 3D dose planning in palliative RT for spinal or pelvic bone metastases is ongoing, but the results are still not published.[240] There is little indication that a wider RT field predicts a better overall RT response.[241] In one study including patients with pelvic bone metastases from hepatocellular carcinoma, patients treated with RT fields including the whole bone compartment had better RT response compared to RT fields only including GTV with a standard margin.[242]

Quantitative sensory testing (QST)

QST can be used to measure sensory responses of mechanical and thermal stimuli.[103] One study has published results performing QST before and after RT in 23 patients with painful bone metastases. The study did not identify any pre-treatment differences in the QST regarding an upcoming RT response in patients with CIBP, but patients who experienced an abnormal warm sensation that normalized after RT were more likely to respond to treatment.[104]

1.3.18 Which patients should be offered RT for CIBP?

RT is recommended for treatment of CIBP, but most guidelines do not discuss which patients are more likely to experience pain relief after treatment, nor in which patients RT should be avoided due to a lower likelihood of RT response.[14,19,25,77,176] As outlined in the previous section, no clinical or biomarker predictors are applicable today in clinical practice to discriminate between patients with a higher or lower chance of analgesic RT response from CIBP.[217] Selection of patients with CIBP that should be admitted for palliative RT could therefore be difficult, and questions often arise in patients with a shorter expected lifetime, reduced performance status, long travelling distance to a hospital with a RT facticity, patients in risk of adverse effect from RT, or in patients where other treatment alternatives could also induce adequate pain relief. The lack of consensus on when palliative RT should be avoided could result in an excessive use of RT in CIBP patients, causing an extra burden for the patients and the health care system. Future work is important to tailor which patients that should be offered palliative RT for CIBP.

1.4 Rationale for this thesis

Pain is a common symptom in patients with bone metastases and will, for many patients, affect both quality of life, social and physical functioning.[35,66,68] About half of all patients with advanced or metastatic cancer experience pain.[119] Several studies to improve cancer pain prediction and treatment are carried out in populations with a mix of cancer pain etiologies that may have a very different pathophysiology, disease trajectory and available treatment options.[87,88,107,115,117] It is not established if these clinical factors could be useful to identify pain in specific groups of cancer pain like CIBP. To further improve treatment of CIBP, it is important to investigate factors related to pain in patients with bone metastases as its own cohort of patients, and thereby increase the potential for better classification of patients with CIBP.[6] This may have the potential to customize pain treatment in cancer patients.

RT is a well-established treatment for patients with bone metastases causing CIBP. According to a recent meta-analysis, only 45% of treated patients had a significant analgesic RT response according to international guidelines, and more than half of patients treated with RT did not have any significant treatment effect.[152] Defining predictors to select patients suitable for palliative RT due to CIBP is important to improve management of patients with bone metastases and to avoid unnecessary treatment, adverse effects, hospital visits, travelling and potentially reduce treatment costs. There is a lack of studies originally designed to evaluate RT response in CIBP, which warrants a prospective study designed to investigate multiple factors for analgesic RT response.[217]

2 Aim of this thesis

The general aim of this project was to improve treatment of patients with CIBP by identifying clinical factors associated with pain in patients with bone metastases and by analyzing predictors for response to palliative RT. Two multinational and longitudinal studies were carried out to answer the research questions:

1) the European Palliative Care Cancer Symptom (EPCCS) study.

2) the Palliative Radiation And Inflammation Study (PRAIS).

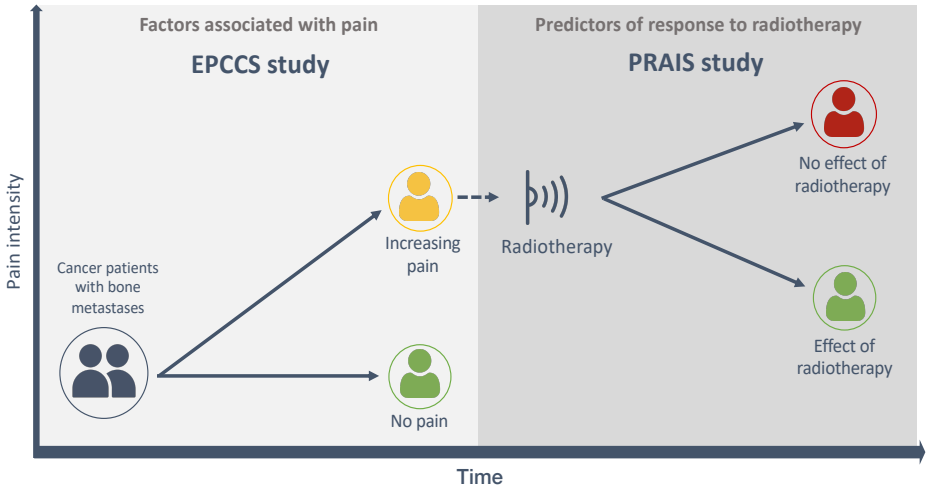
Three specific research questions (RQ) were addressed:

RQ 1. Which factors are associated with ongoing and future pain intensity in cancer patients with bone metastases?

RQ 2. What are the clinical predictors of response to RT in CIBP?

RQ 3. Are inflammatory markers associated with the analgesic response to RT in CIBP?

Figure 11. Overview of studies in the PhD project



The figure illustrates an overview of the PhD project. In the EPCCS study we investigated factors associated with ongoing and future pain intensity in cancer patients with bone metastases. In the PRAIS study we investigated predictors for analgesic response to radiotherapy (RT), aiming to select patients suitable for RT due to cancer induced bone pain (CIBP).

3 Materials and methods

3.1 Study design and study participants

This thesis is based on two international multicenter studies. The first paper in this thesis is based on the European Palliative Care Cancer Symptom (EPCCS) study, a prospective and longitudinal study conducted on patients under palliative care. The study recruited patients from 30 study centers in 12 countries between 2011 and 2013.[243] The study patients were followed approximately every month for at least 3 months or until withdrawal or death. In this paper, a sub-group analysis on patients with bone metastases from solid cancers is presented. Paper 2 and paper 3 are based on the Palliative Radiotherapy And Inflammation Study (PRAIS), a longitudinal prospective and observational study including patients referred to palliative RT because of painful bone metastases. The study recruited patients form seven oncological centers across Europe (Norway, Spain, Italy and the United Kingdom) from 2013 to 2017.[244] Baseline analysis were conducted within 1 week before RT, with follow-up at 3 and 8 weeks after the last RT fraction. Only patients with a maximum baseline pain score of 2 or more were included in analysis. An overview of inclusion and exclusion criteria and the number of patients eligible for analysis in all papers are presented in Table 1. Both studies included in this thesis were initiated and organized from the European Palliative Care Research Centre (PRC) at the Norwegian University of Science and Technology (NTNU).

Table 1. Overview of study criteria and patients eligible for analysis in this thesis

	EPCCS study	PRAIS study
Inclusion criteria	• Patients enrolled in a palliative program for advanced, incurable cancer. • Eligible for at least one follow-up assessment after inclusion. • Age ≥18 years.	• Patients about to undergo RT with palliative intent for painful bone metastases • Verified cancer diagnosis • Bone metastases verified on radiological imaging • Age ≥ 18 years
Exclusion Criteria	• Patients treated with a curative intent • Inability to comply with the study due to psychotic disorders, severe cognitive impairment, or language problems. • Imminent death • Inability to come for follow-up	• Pathological fracture in long bones • On-going RT or RT within the last 4 weeks • Not able to follow the trial procedures • Previous participation in the study
Enrolled	**1739 patients**	**574 patients**
Not Eligible	• 57: hematological cancer • 1052: no bone metastases • 24: missing baseline variables.	• 2: withdrew consent • 2: did not meet with inclusion/exclusion criteria • 1: case report form lost • 2: pathological fracture • 27: maximum pain score < 2 • 27: died before evaluation
Eligible:	**606 patients with bone metastases eligible for cross-sectional analysis** **PAPER 1**	**513 patients eligible for analysis on clinical predictors on analgesic RT response** **PAPER 2**
Inflammatory markers:	-	65: baseline inflammatory markers or RT response status missing.
Inflammatory markers:	-	**448 patients eligible for analysis of inflammatory markers at baseline** **PAPER 3**
longitudinal analysis	• 146: only one pain registration • 49: Incorrect time interval (4 weeks +/- 6 days)	• 328: follow-up blood samples for inflammatory markers not available
Eligible for longitudinal analysis:	**411 patients included in longitudinal analysis** **PAPER 1**	**120 patients included in longitudinal analysis of inflammatory markers** **PAPER 3**

3.2 Data collection and outcome measures

3.2.1 Patient demographics and oncological history

General patient characteristics including age, gender, cancer diagnosis, metastatic distribution and cancer treatment were collected in both studies. To evaluate the patient's physical state, we used the Karnofsky Performance Status (KPS).[245-247] The scale ranges from 100 (perfectly well) to 0 (dead).[247] KPS is validated in cancer patients and is shown to be an important predictor for survival.[226,228,246] In the EPCCS study a brief 4-imtem version of the Mini-Mental State (MMSE) was applied to screen for impaired cognitive function.[248] In the PRAIS study we included information on the patients comorbidity assessed by the "Charlson Comorbidity Index",[249] and detailed information on the patients RT treatment including the site of RT, RT fraction and total dose as well as the radiological presence of osteolytic or sclerotic metastases and soft tissue components outside bone.

3.2.2 Pain Assessment

Pain intensity assessed by a self-reported NRS was used in both studies. The 11-point NRS pain score ranges from 0 (no pain) to 10 (worst imaginable pain) and is based upon the Brief Pain Inventory (BPI). The BPI is validated in cancer patients and used in several similar studies.[81,250] In the EPCCS study, both average pain intensity and worst pain intensity the last 24 hours were assessed, and both are used as primary outcome in the analyses. In the PRAIS study, pain at the radiated site were assessed as "pain intensity at rest" and "pain intensity at movement" the last 24 hours. The maximum pain intensity based on these two assessments was calculated to resemble with the international consensus on endpoints in RT trials.[178,251] Pain mechanism in the EPCCS study was classified within the ECS-CP and used to identify patients with a neuropathic pain component (Appendix).[108,109] In the PRAIS study, neuropathic pain was assessed with patient reported items from The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) (range 0-5) (Appendix).[101] Transient pain exacerbations were assessed in both studies by the screening question from the Alberta Breakthrough pain questionnaire, "Have you had flare-ups of breakthrough pain the last 24 hours?" A

written description of breakthrough pain was provided: “Breakthrough pain can be defined as a brief flare-up of pain. It can be a flare-up of the usual, steady pain you always experience (your baseline pain) OR it can be a pain that is different from your baseline pain.”[96] In the EPCCS study we defined a positive answer to the screening question as “breakthrough pain”, while in the PRAIS study we decided to use the term “episodic pain” to correspond with updated consensus.[95]

3.2.3 Additional patient reported outcomes

Cancer related symptoms other than pain were assessed in the EPCCS study by two different patient reported assessment forms. Sleep disturbances and constipation the last week were assessed with the European Organization for the Research and Treatment of Cancer (EORTC) QLQ-15 pal (Appendix). This is a short form of the EORTC QLQ-C30 designed for use with palliative cancer patients.[252,253] Symptom intensities are scored as 1) not at all 2) a little 3) quite a bit 4) very much. Drowsiness, nausea, anxiety, and depression were assessed by the revised version of Edmonton Symptom Assessment System (ESAS-r) (Appendix).[110] Symptom intensity right now ranges from 0 (no symptoms) to 10 (worst possible symptoms). In the PRAIS study, the more detailed Patient Health Questionnaire-9 (PHQ-9) (0-29) was used to assess the severity of depressive symptoms (Appendix).[254]

3.2.4 Medications

In both studies, the use of opioids and non-opioid analgesics was recorded, but doses and route of administration was not available in the EPCCS study. In the PRAIS study, records of all analgesics used on regular basis and as needed were obtained. The use of corticosteroids was also recorded in the PRAIS study, but doses and route of administration were inadequately reported and could not be used in further analyses. The use of bone-targeting agents was not systematically recorded in any of the studies.

3.2.4.1 Oral morphine equivalents

For the possibility to compare the use of different opioids and to calculate analgesic RT response, all opioid analgesics used at a regular basis and as needed were summarized and converted into daily oral morphine equivalents (OMED). Relative analgesic ratios

are based on data from published literature.[69,255-257] While conversion ratios between some opioid analgesics are widely acknowledged, other conversion ratios are more controversial with inconsistent results among various clinical trials and clinical reccomendations.[69,257] Relative analgesic ratios used to calculate OMED in the PRAIS study is reported in table 2.[258-261] Nasal and sublingual fentanyl were converted to transdermal administration based on the bioavailability (transdermal 92 %, nasal 89 %, sublingual fentanyl 54 %) and later transformed from transdermal to oral morphine.[70,262,263]

Table 2: Opioid conversion table with relative analgesic ratios used to calculate daily oral morphine equivalents (OMED)

Opioids	Relative analgesic ratio
Oral codeine → Oral morphine	1 : 0.1
Oral tramadol → Oral morphine	1 : 0.1
Parenteral morphine → Oral morphine	1 : 3
Oral oxycodone → Oral morphine	1 : 1.5
Parenteral oxycodone → Oral morphine	1: 3
Oral hydromorphone → Oral morphine	1 : 5
Parenteral hydromorphone → Oral morphine	1 : 11.5
Oral Methadone → Oral morphine	1 : 5
Transdermal fentanyl → Oral morphine	1 : 100
Nasal fentanyl → Oral morphine	*
Sublingual fentanyl → Oral morphine	*
Transdermal buprenorphine → Oral morphine	1 : 75
Parenteral pethidine → Oral morphine	0.4

**nasal fentanyl and sublingual fentanyl was first converted into transdermal fentanyl based on bioavailability of the different administration forms, and later converted to oral morphine.*

3.2.5 RT response definition

Responders to RT for CIBP were defined according to recommendations from the International Consensus on palliative RT endpoints, within 8 weeks after the last RT faction.[89,181] The maximum pain intensity at the treated site was used to calculate RT response. Complete response was defined as a pain intensity of zero at the treated site on an 11-point NRS scale, with no concomitant increase in OMED. Partial response was

defined as either a) pain reduction of two or more at the treated site on the 11-point NRS scale together with no increase in OMED, or b) reduction in OMED of at least 25% from baseline without an increase in pain score at the treated site. Patients were defined as RT responders if they had either a complete or partial RT response.

3.2.6 Blood samples

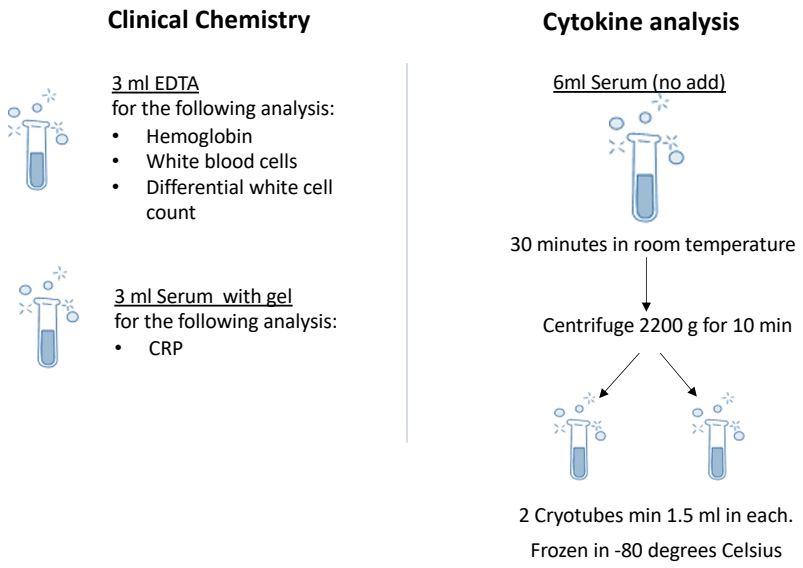
Preclinical studies indicate that several inflammatory markers are involved in pain mechanisms of CIBP and analgesic RT response in these patients.[14,43,170] Inflammatory markers were included in the PRAIS study as potential predictors of analgesic RT response. A local inflammatory process at the site of metastases may not be reflected at the same extent in blood, but as inflammatory markers in bone are difficult to measure in clinical studies, the level of inflammatory markers in circulation was applied as the best measure of inflammatory activity. The identification of relevant inflammatory markers to be analyzed in the study was based on previously published literature on clinical studies on pain and animal models of CIBP and is specified in section 1.6.3.

Clinical chemistry blood values included CRP (mg/l), total white count, total lymphocyte count and total neutrophile count (10^9/L). These blood samples were analyzed at the local hospital laboratory at each study site. A multiplex cytokine assay (Bio-Plex Pro™ Human Cytokine Plex-27 Assay, Bio-Rad Laboratories, Hercules, CA) was selected to analyze a more detailed panel of inflammatory markers in serum: interferon gamma (IFN-γ), IL-1β, IL-2, IL-4 IL-5, IL-10, IL-12p70, IL-13, IL-15, macrophage inflammatory protein 1 alpha (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).[244] All cytokine levels are reported as pg/mL.

Patient blood samples were obtained after enrollment in the study and not more than 1 week before the first RT fraction. When feasible, blood samples were also obtained at follow-up 3 and 8 weeks after the last RT fraction (+/- 2 days). Blood samples were

handled strictly and according to recommendations in published literature.[264-266] A detailed flowchart for obtaining and handling blood for cytokine analyses was developed before initiation of the study, and a shortened version of the flowchart is presented in Figure 12. Serum used for the cytokine analyses was stored in room temperature for 30 minutes before centrifuged at 2200g in 10 minutes and frozen within 1 hour. Serum was then separated into an A and B sample before stored in minus 80°C. Cytokine analyses were performed at the laboratory of Nordlandssykehuset Bodø in October 2020.

Figure 12. Flowchart for blood samples in the PRAIS study



The figure presents a simplified version of the flowchart used by all study centers during collection of blood samples in the PRAIS study. For cytokine analyses serum was collected and stored in room temperature in 30 minutes before centrifuged at 2200 g for 10 minutes and allocated into two cryotubes before freezing in -80 degrees Celsius.

3.3 Statistics

3.3.1 Sample size calculations

The EPCCS study was designed to include palliative care patients in general. For the paper included in this thesis, we present a post-hoc analysis of a subpopulation of patients with bone metastases, and no formal sample size estimation was performed.

In the PRAIS study, the sample size calculation was based on analgesic RT response as the primary outcome with up to 29 independent parameters. As usual for multivariate predictor analyses, a generalized "rule of thumb" for sample size calculation was adapted. We estimated at least 10 patients per number of parameters in the planned analyses, which resulted in a minimum sample of 290 patients. Further, we accounted for unknown interactions, missing data and patients lost to follow-up. The sample size was therefore set to 600. A validation sample including 400 patients was planned according to the study protocol. After initiation of the study, the recruitment rate was lower than expected. The steering committee therefore decided to close the study after 574 patients were enrolled and analyses were conducted without the planned validation sample.

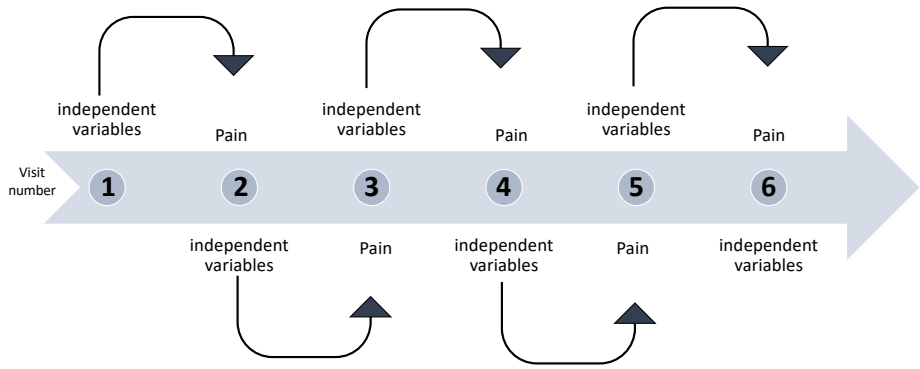
3.3.2 Statistical methods

Descriptive analysis of patient characteristics at inclusion were presented in all papers with number (N) and percentages (%) for categorical variables and mean with standard deviation (SD) or median with interquartile range (IQR) for continuous and numerical variables.

Several specific statistical methods were used in the papers included in this thesis. In the first paper we investigated factors associated with pain intensity in patients with bone metastases. Initially, a cross-sectional analysis of factors associated with pain intensity at baseline was performed using a multiple linear regression model.[267] Second, we conducted a longitudinal analysis investigating factors associated with pain intensity in the following month. Figure 13 illustrates how independent variables at one time-point were analyzed in respect to pain at the next visit in 4 weeks (+/-6 days). To account for repeated measures, we used a generalized estimation equation (GEE)

model with robust standard error and exchangeable covariance structure.[268] A maximum of 6 observations were included per patient. In the second and third paper we explored potential clinical and inflammatory predictors of analgesic RT response in patients with painful bone metastases. Multivariate logistic regression analysis were applied to investigate clinical predictors of analgesic RT response.[269] Univariate logistic regression analysis was used to explore the association between RT response and inflammatory markers measured before RT and the change in inflammatory markers from baseline to 3 and 8 weeks after RT. For baseline analysis, the models were adjusted for the significant clinical predictors identified in paper 2 to evaluate if inflammatory markers could add any meaningful power to the clinical prediction model of RT response.

Figure 13. Illustration of the longitudinal model used to investigate factors associated with pain in the next visit in one month.



The figure illustrates the analytical model used for longitudinal analysis in paper 2. The gray arrow represents time with visit number. Visits were scheduled approximately every 4 weeks and a maximum of 6 visit were included per person. The black arrows illustrate how independent variables at one time-point were investigated in respect to pain intensity at the next visit.

3.3.3 Selection of Independent variables

Selection of relevant independent or explanatory variables is important for the study outcome. For the multivariable models included in paper 1 and 2 we chose to include all potentially relevant factors in one model based on background knowledge, without doing any variable selection like forward selection or backward elimination. Additionally, age, gender, and cancer diagnosis were basic demographic parameters included in all papers.

In the first paper, 20 independent variables with a potential association with pain intensity in patients with bone metastases were selected. Symptoms and patient characteristics with a potential association with pain intensity in CIBP were based on previous literature on cancer related pain: cognitive function, performance status, neuropathic pain, breakthrough pain, anxiety, depression, and sleep disturbances.[87,88,99,107,117] Drowsiness, nausea and constipation were included as independent variables because they are known adverse effects of opioid treatment.[69] The analyses were adjusted for the use of opioid and non-opioid analgesics. Because pain intensity varied among nationalities, the analyses were also adjusted for country.

In the second paper 17 independent variables were identified as potential predictors of analgesic RT response in patients with CIBP. Most variables were selected based on results from previous RT trials: performance status, cancer diagnosis, metastatic distribution, RT fraction, radiation location, pain intensity, neuropathic pain, opioid dose and soft tissue component outside bone.[74,218,219,225,232] Depression was included as a variable because of its association with pain.[270] Comorbidity is not commonly included as a parameter in palliative RT trials but was included in these analyses as it might provide additional information on prognosis.[249] A few studies have investigated the use of bone turnover markers as predictors of analgesic RT response, but the radiological presence of osteolytic or sclerotic metastases has not been investigated as a predictor of analgesic RT response. CRP was included as a rough measure of inflammation based on previous literature, which supports that inflammation could be important in both the pathophysiology of CIBP and in the modulation of analgesic RT

response.[14,43,58] The use of corticosteroids was included as it has a previously demonstrated association with pain-flares after RT.[128,134] All analyses were adjusted for study center.[271]

In paper 3 we investigated the association between RT response and inflammatory markers. As there is limited information about specific inflammatory markers in relation to RT efficacy in CIBP, relevant markers were identified based on animal models of CIBP (IL-1β, MCP-1, TNF, IL-6), pain or poor opioid treatment response in a general cancer population (CRP, IL-6, TNF, eotaxin, MIP-1α and MIP1-β, IL-8, IL-12), and in chronic non-malignant pain (IL-6, IL-8, IL-1ra, MCP-1 and TGF-β).[14,43,50,54,58,60-64,170,272,273] A cytokine assay including 25 inflammatory markers was selected for analysis, as this cytokine kit included most of the markers that we previously defined as inflammatory markers of interest.[244] Inflammatory markers with low measurable levels in more than 20% of the patients were not included in further analyses. Inflammatory markers analyzed in paper 2 are detailed in the previous section on blood samples. Before publication of our results, one experimental study on potential inflammatory markers as predictors of analgesic RT response was published.[235] By that time our analyses were already completed, and this paper could not guide a further selection of relevant inflammatory markers.

3.3.4 Recording of variables

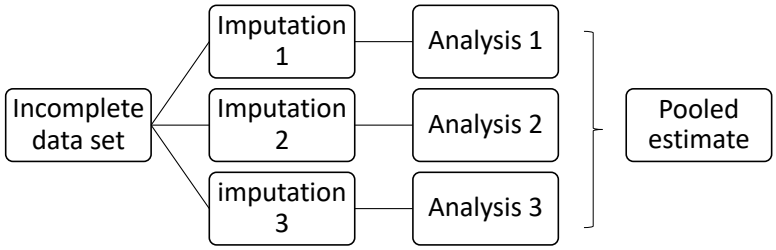
In all papers cancer diagnoses were recorded into fewer groups, as several diagnoses were rare and only included a minor number of patients. These diagnoses were defined as “other”. Breast cancer was used as reference category in the regression analysis in paper 1 because these patients had the lowest mean pain scores at baseline. Gastrointestinal cancer was used as a reference category in the clinical prediction model of RT response (Paper 2) because of the lowest RT response rate. In paper 1, variables “sleep disturbances” and “constipation” included from the EORTC QLQ-C15-PAL were converted to an 11-point numeric rating scale for similarity to ESAS symptoms.[110,252] For the longitudinal analysis a “lagged variable” for pain at the next visit was generated, and this variable was used as the dependent variable at next visit.

In paper 3 the inflammatory markers were binary logarithmic (log_2) transformed to obtain normal distribution. Five of the cytokines that were included in the analysis had a few samples (<20 %) below the lower detection limit, for statistical analysis these measures were set to 0.01 pg/mL.

3.3.5 Missing data

Different methods for handling missing data are used in the papers.[274] In paper 1 and paper 3 we did not do any imputation of missing variables and only complete case and available case analyses are presented. In paper 2 missing data were imputed.[274] Missing data were considered missing at random (MAR), meaning that the reason of missing was not considered to be dependent of the missing value but could be accounted for in other variables not missing.[275] Single imputations were performed for the missing variables on depression score (PHQ-9[254]) and the neuropathic pain score (LANSS[101]) if less than 50% of the items were missing. For the other missing variables, we used multiple imputation (MI) with multivariate imputation of chained equations (MICE).[275] MI is a complex statistical process. As a first step, multiple imputed data sets are created. Using MICE, the creation of imputed data sets is developed based on every missing variable.[275] As a second step, these imputed data sets are analyzed before an overall estimation or pooled estimate is created. A simplified illustration of MI is presented in Figure 14. Missing variables were imputed 25 times. Patients that were missing the dependent variable (unknown RT response) were deleted from analysis after the imputation process.[275] Sensitivity analyses comparing the MICE model with both complete case analysis, worst case analysis (patients with missing RT response considered non-responders) and best-case analysis (patients with missing RT response considered RT responders) were performed and presented as an appendix in paper 2 (Appendix). The complete case analysis was used to estimate the predictive probabilities, and these estimates are used as illustration in the paper.

Figure 14. Illustration of multiple imputation (MI) imputed 3 times.



The figure illustrates the process of multiple imputation (MI) imputed 3 times. First, 3 imputed datasets are created, then individually analyzed before a pooed estimate is created.

3.3.6 Goodness of fit

In paper 1, the explained variance (R^2) is reported for the cross sectional analysis and the quasi-likelihood independence model criterion (QIC) was used to select the most optimal correlation structure in the GEE model.[276] In the prediction model of RT response (paper 2), the goodness of fit was determined using concordance statistics (c-statistics).[277] In paper 3, univariate associations are reported and individually interpreted.

3.3.7 Statistical software

All analyses were performed using the statistical program STATA. For the first paper, version 14.2 was used. Analyses in the last two papers were performed using version 16 (Stata Corporation LP; College Station, TX, USA).

3.4 Ethics

Both studies were performed in accordance with the World Medical Association Declaration of Helsinki and its revisions. Patients provided an informed written consent before enrollment. The studies were approved by the Regional Committee for Medical

and Health Research Ethics (PRAIS study: 2013/1126/REK midt) (EPCCS study: 2010/2945-3/REK midt) and by the regulatory authorities at each international trial site. Both studies were additionally registered in clinical trials.gov database (the EPCCS study: NCT01362816) (the PRAIS study: NCT02107664). The PRAIS study protocol was also published.[244]

4 Results and summary of papers

4.1 Paper 1

Research question 1: *Which factors are associated with ongoing and future pain intensity in cancer patients with bone metastases?*

Six hundred six patients with bone metastases were eligible for analysis at inclusion, 541 patients (89%) and 538 patients (89%) had complete data and were included in cross-sectional analysis on average pain intensity and worst pain intensity, respectively. Baseline characteristics of patients enrolled in the study are presented in Table 3, which covers patient baseline demographics for all papers included in this thesis. The mean age was 64.3 years (SD 12.4), the mean Karnofsky performance status was 66.6 (SD 15.9) and 53% of the patients were female. The most common cancer diagnoses were breast cancer (34%), lung cancer (22%) and prostate cancer (18%). The mean pain intensity at average was 3.4 (2.7) and worst pain intensity 4.4 (3.2). Opioid analgesics were used by a majority of the included patients (68%). Non-opioid analgesics were used by 49% of the patients. Breakthrough pain was a symptom in 40% of the patients and pain was classified as neuropathic in 24% of the patients.

In the cross-sectional analysis we found that the presence of breakthrough pain, neuropathic pain, and male gender were significantly associated with both a higher average and worst pain intensity (Table 4). Breakthrough pain had the highest influence on worst pain intensity which increased with an NRS of 2.49 (95 % CI 2.00-2.97), while average pain intensity increased with an NRS of 1.45 (95 % CI 1.03-1.87) if breakthrough pain was present. Neuropathic pain had similar associations with average pain that increased by 0.89 (95 % CI 0.43-1.35) and worst pain that increased by 0.82 (95 % CI 0.29-1.36) if neuropathic pain was present. Higher age, drowsiness, nausea, anxiety and sleeping disturbances were significantly associated with higher average pain but not worst pain intensity last 24 hours (Table 4). The adjusted R^2 was 0.36 (average pain) and 0.41 (worst pain).

Table 3. Simplified table of baseline characteristics of papers included in this thesis.

	PAPER 1	N: 606	PAPER 2	N: 513	PAPER 3	N: 448
Baseline variables	**N (%)**	**Mean (SD)**	**N (%)**	**Mean (SD)**	**N (%)**	**Median (IQR)**
Age		64.3 (12.4)		66.1 (10.6)		67 (59-74)
Female gender	318 (53%)		199 (39%)		174 (39 %)	
Male gender	288 (47%)		314 (61%)		274 (61 %)	
Comorbidity index (a)				6.5 (0.92)		6 (6-7)
KPS (b)		66.6 (15.9)		72.6 (12.1)		79 (70-80)
Cognitive MMSE (c)		6.9 (1.7)				
Cancer diagnosis						
Gastrointestinal	60 (10%)		81 (16%)		68 (15 %)	
Breast	207 (34%)		103 (20%)		89 (20 %)	
Prostate	107 (18%)		133 (26%)		116 (26 %)	
Lung	133 (22%)		92 (18%)		85 (19 %)	
Urological	26 (4%)		56 (11%)		51 (11 %)	
Other/unknown	70(12%)		48 (9%)		39 (9 %)	
Oncological treatment						
Chemotherapy	244 (40%)					
Radiotherapy	53 (9%)					
Hormone therapy	145 (24%)					
RT fraction						
Multiple fraction			325 (63%)		280 (63 %)	
Single fraction <=8 Gy			188 (37%)		168 (38 %)	
Radiological appearance						
Only bone metastases			194 (38%)		168 (38 %)	
Soft tissue expansion			165 (32%)		145 (32 %)	
Osteolytic metastases			189 (37 %)		168 (38 %)	
RT in weightbearing bone			435 (85 %)		382 (85 %)	
Pain						
Average pain		3.4 (2.7)				
Worst/maximum pain		4.4 (3.2)		5.9 (2.2)		6 (4-8)
Episodic/BTP (d)	238 (40%)		313 (61%)		276 (62 %)	
Neuropathic pain (e)	143 (24%)					
Neuropathic symptom (f)				1.1 (1.2)		
Medications						
Non-opioid analgesics	293 (49%)					
Opioid analgesics	410 (68%)					
Opioid dose (g)				75.0 (143.7)		25 (5-80)
Corticosteroids			232 (45%)		194 (43 %)	
Other Symptoms						
Drowsiness (h)		3.3 (2.9)				
Nausea (h)		1.0 (2.0)				
Feel depressed (h)		2.5 (2.8)				
Anxiety (h)		2.4 (2.7)				
Sleep disturbances (i)		3.2 (3.2)				
Constipated (i)		2.8 (3.2)				
Depressive symptoms (j)				8.0 (4.8)		
Blood samples						
CRP Normal (<=5)			188 (37%)			
CRP elevated (>5)			281 (55%)			

Abbreviations and references: a) Charlson comorbidity index b) KPS: Karnofsky performance status, c) Abbreviated MMSE (maximum score 8), d) BTP: breakthrough pain, e) Neuropathic pain from Edmonton Classification System for Cancer Pain, f) neuropathic pain symptoms from LANSS, g) opioid dose in daily oral morphine equivalents, h) Edmonton Symptom Assessment System-Revised (ESAS-R), i) EORTC QLQ-C15-PAL, j) Depressive symptoms according to PHQ-9.

Table 4. Multivariate analysis of the associations with pain intensity by inclusion

	Average pain last 24h (n=541)			Worst pain last 24 h (n=538)		
Independent	**Coef**	**95% CI**	**p**	**Coef**	**95% CI**	**p**
Age	**0.02**	**0.00 to 0.04**	**0.036**	0.02	-0.01 to 0.03	0.141
Sex (female)	**-0.72**	**-1.29 to -0.16**	**0.012**	**-0.90**	**-1.55 to -0.25**	**0.007**
KPS	0.01	-0.01 to 0.02	0.257	0.01	-0.01 to 0.02	0.456
MMS [1]	-0.03	-0.15 to 0.09	0.665	0.10	-0.04 to 0.24	0.154
Cancer diagnosis: [2]						
Gastrointestinal	0.24	-0.54 to 1.01	0.548	-0.10	-1.00 to 0.80	0.821
Lung	-0.39	-1.03 to 0.25	0.231	-0.40	-1.15 to 0.35	0.278
Prostate	-0.78	-1.57 to 0.01	0.053	-0.70	-1.61 to 0.23	0.139
Kidney/Urothelial	-0.25	-1.39 to 0.88	0.659	-0.29	-1.61 to 1.03	0.663
Other origin	0.19	-0.52 to 0.91	0.596	0.13	-0.71 to 0.96	0.768
Treatment:						
Chemotherapy	0.15	-0.32 to 0.61	0.531	-0.14	-0.68 to 0.40	0.616
Radiotherapy	-0.11	-0.79 to 0.56	0.738	0.21	-0.58 to 0.99	0.607
Hormone treatment	-0.23	-0.80 to 0.34	0.424	-0.36	-1.02 to 0.31	0.294
Pain characteristics:						
Neuropathic pain [3]	**0.89**	**0.43 to 1.35**	**<0.001**	**0.82**	**0.29 to 1.36**	**0.003**
Breakthrough pain [4]	**1.45**	**1.03 to 1.87**	**<0.001**	**2.49**	**2.00 to 2.97**	**<0.001**
Other symptoms:						
Drowsiness [5]	**0.08**	**0.01 to 0.16**	**0.033**	0.08	-0.01 to 0.17	0.066
Nausea [5]	**0.14**	**0.04 to 0.24**	**0.008**	0.10	-0.02 to 0.22	0.109
Depression [5]	0.03	-0.07 to 0.13	0.569	0.08	-0.04 to 0.20	0.183
Anxiety [5]	**0.14**	**0.03 to 0.24**	**0.013**	0.11	-0.01 to 0.24	0.080
Trouble sleeping [6]	**0.09**	**0.03 to 0.16**	**0.004**	0.07	-0.00 to 0.14	0.061
Constipation [6]	0.05	-0.01 to 0.12	0.091	0.05	-0.02 to 0.13	0.150
Constant	*-0.18*	*-2.35 to 2.00*	*0.872*	*-0.48*	*-3.02 to 2.06*	*0.710*
Adjusted R^2			0.362			0.413

All analyses were adjusted for country and analgesic medications. 1) Abbreviated MMSE (maximum score 8)[248], 2) Reference category breast cancer, 3) Neuropathic pain from Edmonton Classification System for Cancer Pain[109], 4) Patient reported flare-ups of breakthrough pain last 24 h, 5) Edmonton Symptom Assessment System-Revised (ESAS-R)[110], 6) EORTC QLQ-C15-PAL[252]

Four hundred eleven patients with more than one pain assessment and registrations within the defined monthly interval were eligible for longitudinal analysis (Table 1). Available case analysis included 396 (96%) patients for association with average pain and 392 (95%) patients for association with worst pain in the upcoming month. In total, the study encompassed 1097 and 1093 observations.

We identified higher current pain intensity, drowsiness, sleeping disturbances and male gender as parameters significantly associated with a higher average and worst pain score in the next month. Breakthrough pain was associated with higher worst pain intensity. Patients with prostate cancer had lower pain intensity in the following

month. In both models, each individual variable only contributed to small changes in pain intensity. Correlation coefficients and 95% CIs are presented in Table 5.

Table 5. Longitudinal analysis on factors associated with pain intensity at the next study visit in one month.

	Average pain in last 24 h (n=396)			**Worst pain in last 24 h (n=392)**		
Independent	Coef	95% CI	p	Coef	95% CI	p
Age	0.00	-0.01 to 0.01	0.666	0.01	-0.00 to 0.02	0.130
Sex (female)	**-0.46**	**-0.91 to -0.01**	**0.045**	**-0.91**	**-1.45 to -0.36**	**0.001**
KPS	0.00	-0.01 to 0.01	0.690	0.00	-0.01 to 0.01	0.958
MMS [(1)]	-0.00	-0.08 to 0.08	0.992	0.04	-0.07 to 0.15	0.443
Cancer diagnosis: [(2)]						
Gastrointestinal	0.12	-0.46 to 0.57	0.710	0.14	-0.63 to 0.91	0.716
Lung	0.04	-0.43 to 0.52	0.861	-0.24	-0.82 to 0.34	0.419
Prostate	**-0.66**	**-1.23 to -0.09**	**0.023**	**-1.21**	**-1.92 to -0.50**	**0.001**
Kidney and urothelial	-0.54	-1.38 to 0.30	0.208	-0.90	-1.87 to 0.08	0.073
Other origin	0.05	-0.46 to 0.57	0.992	-0.05	-0.70 to 0.60	0.876
Treatment:						
Chemotherapy	0.31	-0.02 to 0.64	0.069	0.35	-0.05 to 0.75	0.085
Radiotherapy	-0.17	-0.75 to 0.40	0.556	0.06	-0.52 to 0.65	0.830
Hormone treatment	0.25	-0.12 to 0.63	0.186	-0.04	-0.48 to 0.40	0.870
Pain characteristics:						
Current pain [(3)]	**0.41**	**0.34 to 0.48**	**<0.001**	**0.34**	**0.26 to 0.42**	**<0.001**
Neuropathic pain [(4)]	-0.05	-0.35 to 0.25	0.743	0.16	-0.22 to 0.55	0.410
Breakthrough pain [(5)]	0.19	-0.11 to0.49	0.209	**0.59**	**0.20 to 0.99**	**0.003**
Other symptoms:						
Drowsiness [(6)]	**0.09**	**0.03 to 0.15**	**0.003**	**0.07**	**0.01 to 0.14**	**0.033**
Nausea [(6)]	0.06	-0.01 to 0.14	0.103	0.02	-0.06 to 0.11	0.604
Depression [(6)]	-0.01	-0.09 to 0.06	0.747	0.05	-0.04 to 0.14	0.249
Anxiety [(6)]	0.02	-0.06 to 0.11	0.589	0.03	-0.05 to 0.12	0.427
Trouble sleeping [(7)]	**0.06**	**0.02 to 0.11**	**0.010**	**0.08**	**0.02 to 0.14**	**0.006**
Constipation [(7)]	0.03	-0.02 to 0.07	0.277	0.02	-0.04 to 0.07	0.587
Constant	*0.99*	*-0.64 to 2.62*	*0.235*	*0.32*	*-1.41 to 2.05*	*0.716*

All analyses were adjusted for country and analgesic medications. (1) Abbreviated MMSE (maximum score 8)[248], (2) Reference category breast cancer, (3) Current pain: current pain intensity- in the average pain model this refers to average pain intensity last 24 hours and in the worst pain model this refers to worst pain intensity last 24 hours. (3) Neuropathic pain from Edmonton Classification System for Cancer Pain[109], (4) Patient reported flare-ups of breakthrough pain last 24 h, (5)Edmonton Symptom Assessment System-Revised (ESAS-R)[110], (6) EORTC QLQ-C15-PAL[252]

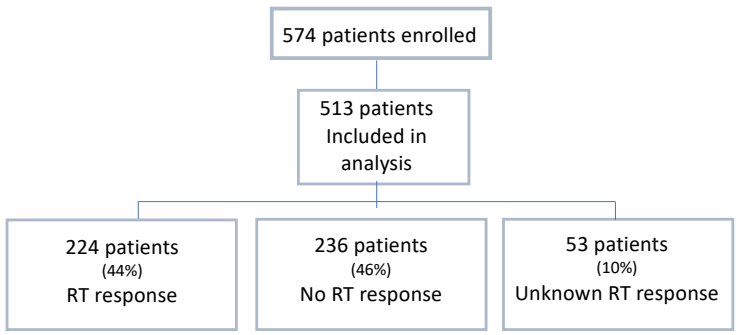
4.2 Paper 2

Research question 2: *What are the clinical predictors of response to RT in CIBP?*

In the PRAIS study 574 patients about to undergo RT due to painful bone metastases were enrolled, and 513 patients were included in the analysis (Table 1 and Figure 15). The mean age was 66.1 years (SD 10.6), and 61% of the included patients were men. The mean Karnofsky performance status was 72.6 (SD 12.1) and the most common cancer diagnoses were prostate (26%), breast (20%) and lung (18%). Most of the patients received MFRT (63%) while 37% had SFRT administered (Table 3).

Of the patients included in analysis, 224 (44%, CI 39%-48%) responded to RT within 8 weeks after the last RT fraction while 236 (46%, CI 42%-50%) did not respond to RT. Ten percent of the patients had an unknown RT response (Figure 15). Twenty-six patients had an unknown RT response because of lack of follow-up data on analgesic doses or missing information on pain intensity at the radiated site. Among study participants, 27 patients died before the first follow-up and were not included in the analysis. Sixty-seven patients died between 3 and 8 weeks after RT, and among these patients only 12% (8 patients) responded to RT.

Figure 15. Flowchart of patients included in the PRAIS study and radiotherapy response status.



The figure presents the flowchart of patients enrolled in the PRAIS study and radiotherapy (RT) response status among patients included in analyses.

The final regression model on predictors of analgesic RT response were carried out on 460 patients with a known RT response. Factors significantly associated with analgesic RT repose were better performance status (OR 1.39, CI 1.15-1.68), primary diagnosis of breast cancer (OR 2.54, CI 1.12-5.73) or prostate cancer (OR 2.83, CI 1.27-6.33) and soft tissue expansion outside bone (OR 2.00, CI 1.23-3.25). The use of corticosteroids was a negative predictor for RT response (OR 0.57, CI 0.37-0.88). RT fraction, opioid dose, initial pain intensity or CRP value before RT did not significantly impact the RT outcome. The final model had a C-statistics of 0.69 (Table 6).

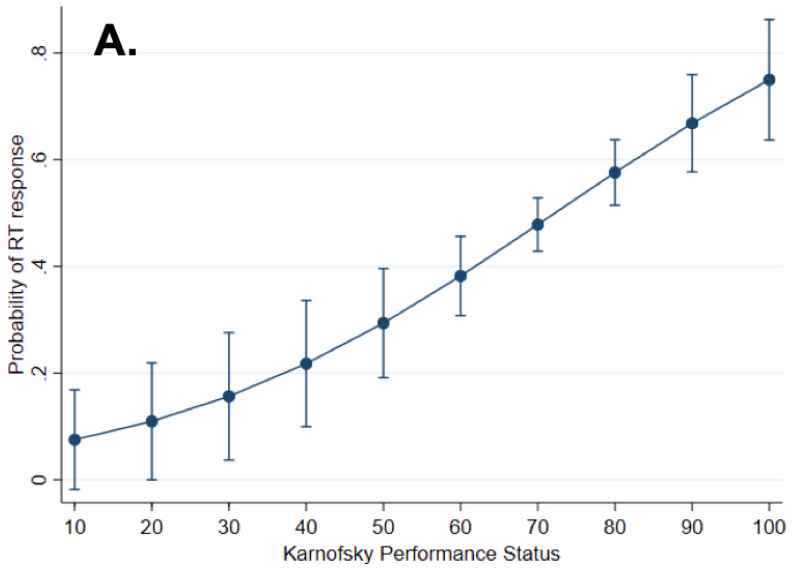
The predictive probability with a 95% CI of RT response was calculated for the significant variables based on the complete case analysis and is presented in Figure 16 and 17 for descriptive purposes. As shown, the predicted RT success rate in patients with CIBP is low in patients with reduced performance status (Figure 16) and differs according to cancer diagnosis and the presence of soft tissue expansion outside bone (Figure 17).

Table 6. Multivariate logistic regression model of predictors of RT response (n 460)

Independent variables	OR	95% CI
Age	0,99	[0.97,1.01]
Gender		
Male	1,00	[.,.]
Female	0,97	[0.54,1.75]
Charlson comorbidity index	1,12	[0.90,1.40]
Karnofsky performance status	**1,39*** **	**[1.15,1.68]**
Cancer diagnosis		
Gastrointestinal	1,00	[.,.]
Breast	**2,54***	**[1.12,5.73]**
Prostate	**2,83***	**[1.27,6.33]**
Lung	1,29	[0.61,2.71]
Urological	1,29	[0.58,2.89]
Other/unknown	1,60	[0.65,3.93]
Metastases		
Other sites than bone	1,00	[.,.]
Only bone	1,27	[0.80,2.02]
RT fraction		
Multiple fraction	1,00	[.,.]
Single fraction <=8 Gy	1,29	[0.80,2,09]
Soft tissue expansion at radiated site		
No	1,00	[.,.]
Yes	**2,00****	**[1.23,3,25]**
Not evaluable	0,68	[0.12,3.89]
Osteolytic metastases at radiated site		
No	1,00	[.,.]
Yes	1,18	[0.74,1.89]
Not evaluable	0,92	[0.34,2.47]
Radiation location in weight bearing bone		
No	1,00	[.,.]
Yes	1,24	[0.70,2.21]
Maximum pain at radiated site last 24h	1,07	[0.96,1.19]
Episodic pain		
No	1,00	[.,.]
Yes	0,91	[0.56,1.48]
Neuropathic pain symptoms (a)	0,99	[0.84,1.18]
Opioid dose (b)	1,00	[1.00,1.00]
Corticosteroids		
No	1,00	[.,.]
Yes	**0,57***	**[0.37,0.88]**
Depressive symptoms (c)	0,99	[0.94,1.04]
CRP		
Normal (<=5)	1,00	[.,.]
Elevated (>5)	0,91	[0.58,1.44]
C-statistics	**0,69**	

a) Number of self-reported symptoms of neuropathic pain according to LANSS, (b) Opioid dose in oral morphine equivalents last 24h, (c) Number of depressive symptoms according to PHQ- 9. * p<0.05, ** p<0.01, *** p<0.001

Figure 16. The predictive probabilities with 95% CI of RT response by Karnofsky performance status (A)

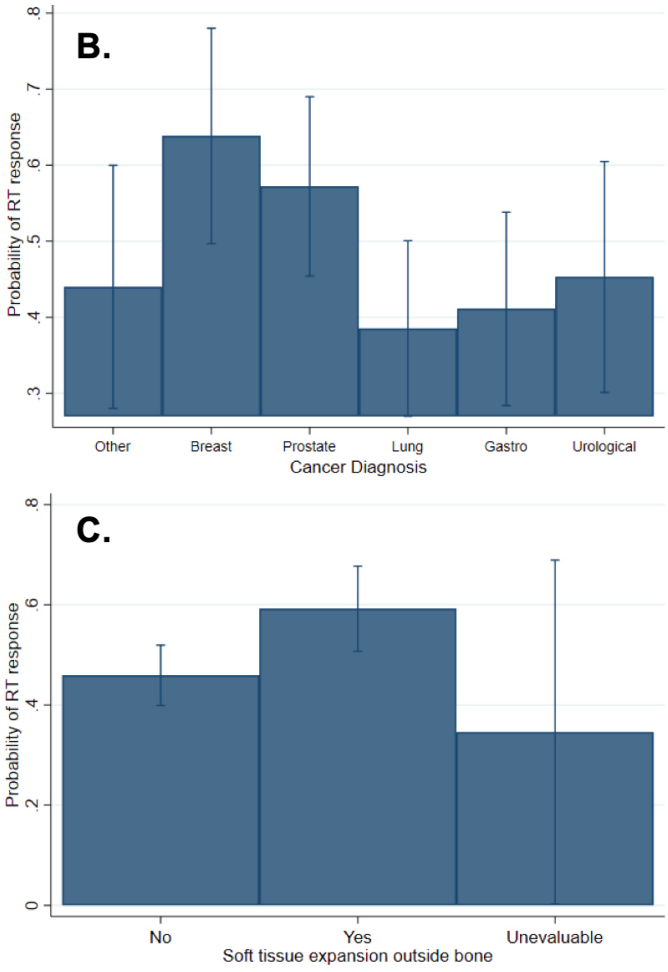


The figure illustrates how the predicted probability for analgesic relief from radiotherapy (RT) due to painful bone metastases increase with better performance status. On the X-axis Karnofsky performance status. On the Y-axis the probability of RT response. The vertical lines represent the 95% CI.

Textbox 2: Overview of the Karnofsky performance status (KPS).[247]

100	Healthy; no evidence of disease
90	Able to carry on normal activity; minor signs and symptoms of disease
80	Normal activity with effort, some signs or symptoms of disease
70	Cares for self, unable to carry on normal activity or do active work
60	Requires occasional assistance, but able to car for most of personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled: hospital admission is indicated although death is not imminent
20	Very sick: hospital admission necessary, requires supportive treatment
10	Moribund: fatal process progressing rapidly
0	dead

Figure 17. Predictive probability with 95% CI of RT response according to cancer diagnosis (B) and soft tissue component outside bone (C).



The figure illustrates how the predicted probability for analgesic relief from radiotherapy (RT) because of painful bone metastases depends on cancer diagnosis (B) and that the predicted RT response rate is higher in patients with soft tissue expansion outside bone (C). On the X-axis cancer diagnosis (B) and the presence of soft tissue expansion outside bone (C) On the Y-axis the probability of RT response. The vertical lines represent the 95% CI.

4.3 Paper 3

Research question 3: *Are inflammatory markers associated with the analgesic response to RT in CIBP?*

We included 448 patients in the analysis on inflammatory markers as potential predictors of an analgesic RT response in patients with CIBP. The patient sample is similar to paper 2, but since only a selected cohort of patients with measurable RT response status and cytokines available at baseline were included this paper, the patient demographics is also presented in Table 3. 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 more than 20% non-detectable values or low levels similar to those observed in a general population and were not analyzed further. Although the median level for some of the inflammatory markers (TNF, IL-8 and CRP) were slightly lower in RT responders compared to non-responders at baseline, no significant difference was observed in logistic regression analyses adjusted for factors that were significant in the clinical prediction model of RT response (cancer diagnosis, Karnofsky performance status, presence of soft tissue metastases and the use of corticosteroids) (Table 7).

Further we analyzed the change in inflammatory markers from baseline to 3 and 8 weeks after the last RT fraction. The number of patients with available blood samples for follow-up was 120 for inflammatory cytokines, 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 was associated with a higher likelihood of RT response. By contrast, the change in CRP (OR 0.99, 95 % CI 0.98-1.0) from baseline to three weeks was negatively associated with RT response. We observed no significant associations between RT response and change in inflammatory markers from baseline to 8 weeks after RT (Table 8). The median level of inflammatory markers during follow-up is illustrated in Figure 18.

Table 7. Inflammatory markers 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	1.05	0.84-1.33	0.653
Total Neutrophil count	0.96	0.91-1.02	0.233

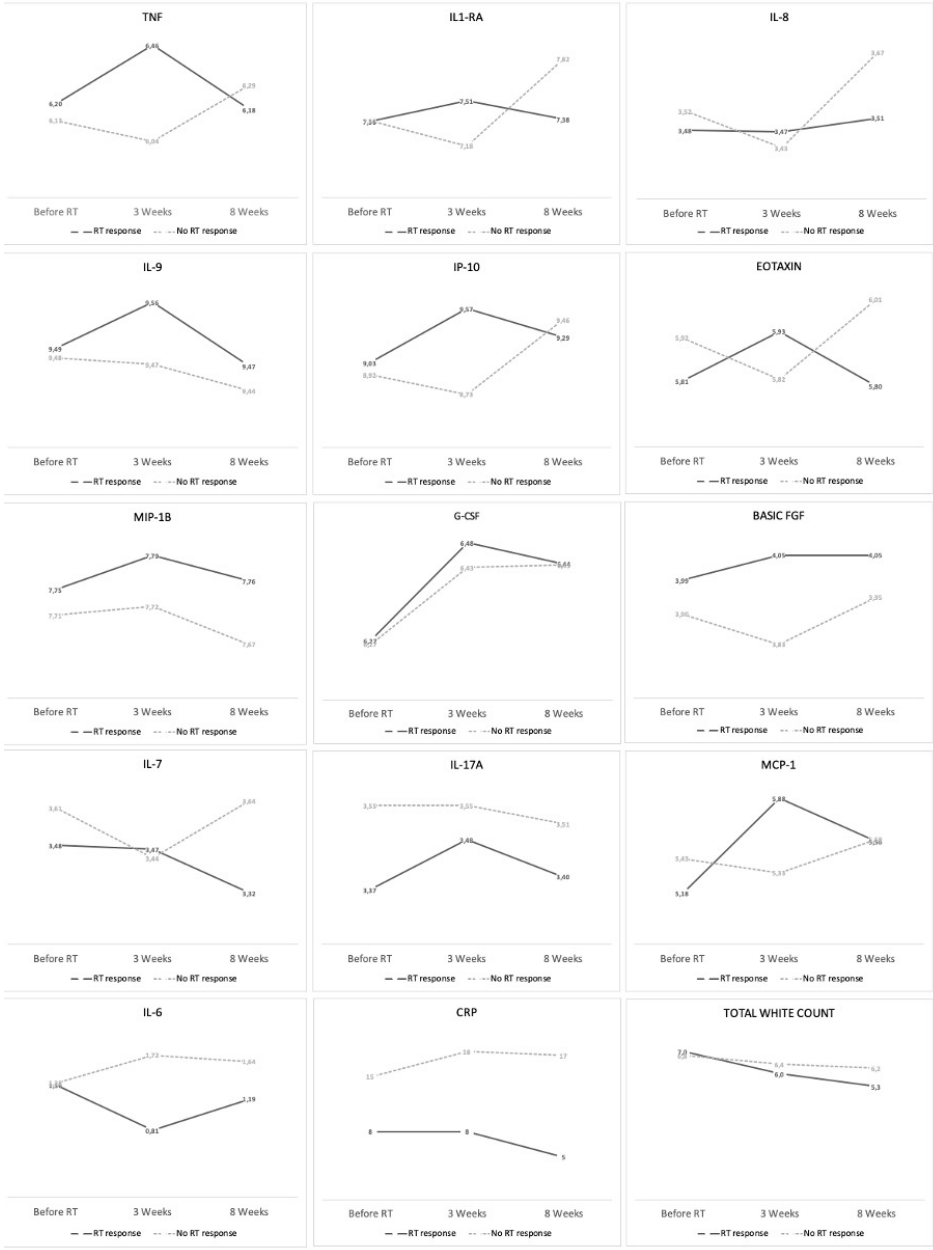
[a] *Logistic regression adjusted for clinical variables significantly associated with RT response: Cancer diagnosis, Karnofsky performance status, presence of soft tissue metastases and the use of corticosteroids.*

Table 8. Change in inflammatory markers from baseline to 3- and 8-weeks post RT and association with RT response.

		Δ 3 weeks after RT			Δ 8 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	181	1.04	0.72-1.50	0.830	1.03	0.73-1.46	0.860
Total Neutrophil	181	1.02	0.95-1.10	0.542	0.96	0.88-1.04	0.326

[a] *Univariate logistic regression. Δ = (3- and 8-weeks value of inflammatory markers) - (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.*

Figure 18. Median level of inflammatory markers in RT responders compared to non-responders.



On the X axis time after RT (before RT, 3 weeks after RT and 8 weeks after RT). On the Y axis is the median level of inflammatory biomarkers.

5 Discussion

5.1 Discussion of the main findings

Research question 1: *Which factors are associated with ongoing and future pain intensity in cancer patients with bone metastases?*

In paper 1 we observed that patients with bone metastases presenting with higher current pain intensity, drowsiness, sleeping disturbances, male gender, and breakthrough pain at one time-point had higher pain scores at the next visit in one month. These factors were also associated with a higher average pain score in the cross-sectional analysis by inclusion.

Clinicians treating patients with bone metastases should recognize that patients reporting higher pain intensity are more likely to experience more severe pain at the next visit, as demonstrated in the current study and in a general cancer population.[87,278] It is likely that this significant association is related to difficulties obtaining adequate pain relief in patients with CIBP.[14,90] Alternatively, one could speculate that pain was not recognized by the treating clinician so that pain relieving interventions or sufficient doses of analgesic medications were not initiated between visits.

Breakthrough pain and neuropathic pain are other common features in patients with CIBP that are associated with a more complex pain situation than in a general cancer population.[86,100,107,115] Our findings demonstrate the importance of identifying breakthrough pain and neuropathic pain in patients with bone metastases, as both factors were associated with higher average and worst pain intensity in the cross-sectional analysis.[278] These patients should receive individual analgesic treatment like adjuvant analgesics for neuropathic pain or adequate fast on-set acting pain killers for breakthrough pain.[69] The results are supported by findings from a recently published cross-sectional analysis, that also found breakthrough pain to be significantly

associated with average and worst pain intensity in patients with CIBP.[86] We further demonstrated that patients with breakthrough pain were more prone to report pain at the next visit and therefore could be in need for a more intensive follow-up to hinder a lack of future pain control.[278]

Opioid adverse effects may additionally impact the perception of pain. Both nausea, constipation and drowsiness are known opioid side effects that were included as independent variables in the study.[69,279] Constipation was not associated with pain intensity, while patients with more severe nausea had higher average pain intensity by inclusion. Drowsiness was associated with both higher average pain and worst pain intensity in the next visit in one month. Awareness of drowsiness as a potential side effect of opioids and other medications like sedatives is needed in clinical settings. Drowsiness may result in the use of lower opioid doses than required for optimal pain control and patients could therefore be more prone to pain during longitudinal follow-up.[278]

Several studies have also investigated pain intensity and its relation to other clinical symptoms. Like studies in a general palliative cancer population, we discovered an association between self-reported sleep disturbances and pain intensity.[87,280,281] Pain and sleep disturbance may have a mutual symptom influence. Pain can cause sleep disturbances, but sleeplessness can also cause pain exacerbation.[280] We further demonstrated that patients with a lack of sleep had higher pain intensity at the next visit in one month. Our findings emphasize that sleep disturbances should be routinely assessed in patients with bone metastases, and that treatment for sleep disturbances may potentially improve a pain situation.

Psychological distress is known to influence pain perceptions in a general cancer population, and is associated with higher average pain intensity in patients with CIBP.[86,107,115] In this cohort of patients with bone metastases, anxiety was associated with higher average pain score in the cross-sectional analysis, but neither anxiety nor depression were correlated with worst pain intensity or higher pain intensity at the next visit. Further, we demonstrated that male patients had a higher pain intensity at

baseline and at the next visit in one month. A gender difference in relation to pain intensity was not expected in the planning of the study and most similar papers report no gender difference in perception of cancer pain.[282] No relevant interactions were identified in statistical analyses, but the finding is of uncertain clinical relevance. Pain intensity was not associated with performance status or cognitive function, similar to studies of general cancer pain.[87,115]

In this study we have established several characteristics of patients with bone metastases and a lack of future pain control. Although each individual variable had a small impact on pain intensity in the longitudinal analysis, we believe that the findings could aid the clinician to provide individual tailored treatment and follow-up in patients with bone metastases. This information may also be the basis for future computer assisted clinical decision support systems where, for instance, the presence of neuropathic pain could prompt the clinicians to consider prescribing co-analgesics and schedule the next patient follow-up within an appropriate interval.[283]

Research question 2: *What are the clinical predictors of response to RT in CIBP?*

In paper 2 we identified that patients with a better performance status, primary cancer diagnosis of breast or prostate and the presence of soft tissue expansion outside bone were more likely to experience analgesic response to RT for painful bone metastases. Patients using corticosteroids had significantly lower RT response rates.

Performance status is one of the most influential clinical predictors for analgesic relief in patients with painful bone metastases both in previous and the current study.[218,219,284] Karnofsky performance status (KPS) is a simple tool for health care personnel to evaluate the patients physical state and has previously been demonstrated as important in prediction of other cancer related outcomes and survival.[226,245,246,285] In recent recommendations for RT in patients with bone metastases it is suggested to assess performance status before admittance to palliative

RT, but the role of performance status in regard to treatment response or when RT should be avoided is not further discussed.[25] In the current analysis we have demonstrated significant differences in response rates among patients with high vs low performance status: in patients with KPS of 80 or higher, about 60% of the patients responded to RT. In patients with KPS of 60 or lower only 35% of the patients responded to RT. This is illustrated in Figure 16. It is difficult to establish a defined cut-off when RT for painful bone metastases should not be recommended. Individual aspects should always be evaluated before a patient is admitted to palliative RT for painful bone metastases.[25] Still, clinical implementation of a graph, as presented in the current paper (Figure 16), could be useful for the clinicians to evaluate if RT is beneficial for the patient. The findings could also be used as a tool for shared decision making with the patients and caregivers.

We also observed that several patients died shortly after RT. Of enrolled patients, 16% (94 patients) died within the first 8 weeks after RT. In this patient population more than half of the patients had a baseline KPS of 60 or lower. RT is commonly prescribed in patients the last month of life and there is a need to identify patients with a short expected lifetime that would not have any meaningful analgesic effect from RT.[286,287] Furthermore, a "wait and see" period after RT to observe if RT is effective could also postpone other effective pain interventions. Avoiding RT in patients with low KPS status could minimize the use of RT close to death.[286]

In accordance with two previously published retrospective studies, we also confirmed that a diagnosis of breast or prostate cancer increased the chance of a benefit from palliative RT in patients with CIBP.[218,219] The reason for patients with breast and prostate cancer being more likely to respond to RT for painful bone metastases is not established. It is proposed that palliative RT induces a more pronounced inflammatory process in cancers with a higher proliferative rate and that this might have a negative impact of the pain-relieving effect of RT.[219,288] Patients with breast and prostate cancer could also be more likely to respond to RT due to a longer expected survival compared to patients with bone metastases from lung cancer or gastrointestinal

cancer.[202,226] On the other hand, RT response rates were evaluated after 8 weeks and should not be affected by long term prognosis. We observed no difference in performance status among the different diagnosis groups by enrollment in the study (data not shown). The predictive probability of RT response by cancer diagnosis is presented in Figure 17.

Previously proposed factors related to analgesic RT response like high pain intensity, absence of visceral metastases, younger age and the use of opioids was not significantly associated with RT response in our analysis.[218,219]

In addition to the previously proposed predictors of RT response we included information on tumor characteristics, inflammation, pain characteristics and depression in the PRAIS study. Among these novel variables only the presence of soft tissue expansion outside bone was significantly associated with a better RT response.[284] Soft tissue components may be more sensitive to RT compared to metastases localized in the bone matrix, have a higher inflammatory activity or edema that could more easily be targeted by RT. To our knowledge only one study has previously investigated if a soft tissue component has implications for the RT treatment outcome in patients with painful bone metastases. Mitera *et al* found no difference in RT response rate in patients with or without a soft tissue component in 33 patients with spinal bone metastases.[232] We believe that our findings are more reliable due to a larger patient material.[284]

As also observed in our study, clinicians are more likely to prescribe MFRT in patients with soft tissue components outside bone, although the evidence for this practice is not well documented in the literature.[176,196] There was no difference in analgesic RT response in patients receiving SFRT and MFRT in the complete sample. Despite numerous publications that demonstrated similar treatment efficacy after SFRT and MFRT for uncomplicated painful bone metastases, we observed that only 37% of the patients included in this study received SFRT.[174] Prescribing patterns in palliative RT for painful bone metastases vary considerably between study centers.[182,289] Results from the current study further highlight the underuse of SFRT in patients with painful bone

metastases and the difficulties to implement new treatment regimes in clinical practice.[284,289]

The RT response rate of 44% observed in paper 2 was lower than in a meta-analysis from 2018, which reported that approximately 60% of patients in the intention-to-treat population responded to RT.[174] Previous meta-analyses have reported large variations in RT response rates and RT response definitions in different studies.[174,178] A recently published meta-analysis analyzed only studies which used the international guidelines to define RT response.[152] This meta-analysis reported similar response rates as the current study (45%), and a stricter RT response criteria according to international consensus guideline is the most likely reason for the lower response rate in our study compared to older meta-analyses.[174,284]

Another interesting finding in the current analyses is that patients using corticosteroids had significantly lower RT response rate compared to patients not using corticosteroids.[284] Corticosteroids reduce inflammation and are commonly prescribed in patients with painful bone metastases.[127] Corticosteroids are also used to treat pain flares after RT in patients with CIBP.[130,134,211] One reason for corticosteroids being related to a lower RT response in patients with CIBP could be that the anti-inflammatory effect of RT is already induced by corticosteroids and that an additional effect of RT will be diminished. It could also be that a dampening of the immune response is not preferable to achieve RT response. If the use of corticosteroids has a negative effect of the RT outcome, this may lead to a change in clinical practice. Parallels can be drawn to a study investigating radionucleotide therapy Ra^{223} in combination with abiraterone and the corticosteroid prednisolone in patients with prostate cancer.[290] In this study patients in the treatment group had an unfavorable outcome and higher fracture rates compared to placebo. It has been speculated that the use of prednisolone in combination with radionucleotide therapy was the negative contributing factor.[291] Unfortunately, the type and doses of corticosteroids were not registered in our study. The impact on corticosteroids and other anti-inflammatory

drugs on RT outcome in patients with painful bone metastases needs to be further investigated.

CRP was also added to the clinical prediction model of RT as a crude measure of inflammation.[292] Although the median CRP value was higher in RT non-responders vs RT responders before RT, the pre-treatment CRP value did not predict an upcoming analgesic RT response in patients with painful bone metastases.[284] The association between a more detailed panel of inflammatory markers and analgesic RT response was investigated in paper 3.

Research question 3: *Are inflammatory markers associated with the analgesic response to RT in CIBP?*

In paper 3 we investigated the association between several inflammatory markers and analgesic RT response in patients with painful bone metastases. Results from this study and one previously published study, do not support inflammatory markers measured before RT as important predictors for RT efficacy in patients with painful bone metastases.[235,293,294]

Although we could not demonstrate inflammatory markers to improve the pre-treatment prediction of analgesic RT response in patients with painful bone metastases, we observed a significant association between RT response and the change in several pro-inflammatory markers after RT.[293] A higher change in IL-8, IP-10, eotaxin, MCP-1, G-CSF and TNF and a lower change in CRP between baseline and 3 weeks after RT were positively associated with RT response.

Higher inflammatory activity is often associated with more severe pain in cancer patients,[58,59,295,296] although an opposite trend is also demonstrated.[113] The inflammatory expression changes after RT, but there is little knowledge to support if differences in the inflammatory expression is important for the RT outcome.[60,272,297,298] Results from paper 3 support a hypothesis that modulation of the immune system

shortly after RT could be important to induce tumor cell death and potentially moderate the pathological bone remodeling process or other factors in the bone microenvironment to induce pain relief.[111,170]

There are similarities between our findings and previous findings from pre-clinical and clinical studies. Four of the inflammatory markers that were significantly associated with RT response are strong chemokines that attract white blood cells towards a chemical gradient (IL-8, IP-10, eotaxin and MCP-1).[299] The accumulation of white blood cells at a tumor site is probably important to stimulate the immune-mediated tumor response after RT.[300] G-CSF is additionally shown to stimulate the neutrophile anti-tumor activity in mouse models with cancer, which might improve RT efficacy.[301,302] MCP-1, G-CSF and TNF are also related to pain behavior in animal models of CIBP and higher TNF levels are identified in patients with cancer pain.[54,56,61,303-305]

Several of the inflammatory markers identified in paper 3 are known to stimulate the bone reportion process (eotaxin, MCP-1 and G-CSF).[305-307] It is established that an unbalance in the normal bone remodeling cycle contributes to pain mechanisms of CIBP.[14] Bone remodeling is important to restore the bone after RT and probably also contributes to analgesic RT effects. A few clinical studies have also demonstrated differences in urinary bone resorption markers and a relation to RT response, although the results are not consistent in all studies.[168,169,233]

There are only a limited number of clinical studies providing results that could be compared with findings from paper 3. A small study investigating inflammatory expression in relation to RT induced pain flares in CIBP was published in 2016.[272] Patients experiencing pain flares had lower levels of urinary IL-8 and IP-10 at 3 and 5 days after RT compared to patients that did not experience pain flares. The association between inflammatory markers and RT response was not reported.[272] There is no clear relationship between early pain flares and the final RT response,[212] still the findings are in line with results from paper 3 demonstrating that patients with no RT response had lower change in IL-8 and IP-10 from baseline to 3 weeks after treatment compared to RT responders. The role of TNF has also been investigated in relation to the

radiopharmaceutical $^{89}SrCl_2$.[294] No difference in pre-treatment level of TNF and analgesic treatment response was observed, which is similar to our results. After 4 months the TNF levels was lower in patients with treatment response, but the association of pain and inflammatory markers at an earlier time-point after RT was not reported.[294]

One explorative study has previously investigated a panel of several inflammatory markers before and after RT in a cohort of 60 patients with CIBP. Results were published by MacLeod *et al* in 2020. Unfortunately, only a few of the inflammatory markers measured in the study by MacLeod *et al* correspond with the panel of inflammatory markers analyzed in paper 3. IL-6 and G-CSF were analyzed in both papers. IL-6 was not significantly associated with RT response in any of the studies, while the change in G-CSF was only associated with RT response in our study. MacLeod *et al* additionally 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 4 weeks after RT. Although this specific inflammatory marker was not measured in our study, this is an opposite trend compared to our study demonstrating that a 3-weeks increase in several inflammatory cytokines from baseline was associated with RT response. In a subpopulation of patients with breast cancer MacLeod *et al* also observed lower level of IL-1β at baseline in responders compared to non-responders (17 patients).[235] The sample size is low and the clinical relevance of this finding is therefore limited. In our analysis, IL-1β was expressed at low levels in all patients and was not included in the final analysis.[293]

Contrary to what was found with the significantly upregulated inflammatory cytokines, we observed that a lower 3-week change in CRP from baseline was associated with RT response. The reason for this different trend for CRP compared to the other inflammatory markers is uncertain. CRP is an acute phase protein stimulated by the cytokine IL-6.[308] In our paper many patients had normal measurable levels of CRP (<=5 mg/l) that might have affected the results. Baseline CRP values were also higher in non-responding patients, although this difference was not statistically significant. High

sensitivity CRP, a more sensitive measure of CRP, might have detected smaller changes.[292]

In summary, we observed a potential role of inflammation in RT response among patients with painful bone metastases. The different pattern in inflammatory markers after RT could support the hypothesis that modulation of inflammatory activity is important to induce analgesic relief after RT.

5.2 Methodological considerations

Methodological considerations and the reliability of the measurements and results presented in this thesis are discussed in the following sections.

5.2.1 Study design

This thesis includes three papers from two observational multicenter studies with a prospective and longitudinal study design. In observational studies, the prospective study design is a strength because it excludes recall bias, which is a common problem in retrospective studies.[118] To our knowledge, we present the first paper to prospectively identify factors for upcoming pain in patients with bone metastases. Paper 2 prospectively evaluated multiple clinical predictors for analgesic RT response, and this is an important supplement to previous retrospective studies that established reliable predictors of analgesic RT response.[89,218,219]

Longitudinal data are used in all papers included in this thesis and are a strength of both the included studies. In the PRAIS study a longitudinal study design was essential to prospectively evaluate factors that could predict an upcoming RT response. Another advantage with longitudinal studies is the possibility to evaluate the individual change over time, which was utilized in analyses for paper 1 and paper 3.[118] This is in contrast to cross-sectional studies that can only provide associations between variables at one given time-point.[118] Although the longitudinal study design is mostly beneficial, these studies often have higher economical costs and are more time-consuming for the patients and the study personnel compared to cross-sectional studies.[118] Several

follow-up visits may be demanding for palliative cancer patients with bone metastases, and a number of patients are expected to die during the study period due to the natural progression of the disease.[309] Incomplete follow-up is a problem in longitudinal studies and is also addressed in studies included in this thesis. If many patients are lost to follow-up this might affect the outcome.[118] It is a shared issue in all papers included in this thesis that patients with a better performance status or less symptom burden is more likely to be represented among patients with available longitudinal data.[278,284,293] A further discussion on patients lost to follow-up is outlined in the section of missing data.

A strength of both studies included in this thesis is the multicenter design. Both the EPCCS study and the PRAIS study were organized from PRC at NTNU and included patients from different national and international study centers.[243,244] The study design made it possible to enroll a higher number of patients over a shorter period. Results from multicenter studies are also considered more generalizable compared to single center studies and increase the external validity of the results.[310] Disadvantages with multicenter trials are that differences in recruitment, data recording and analytic variations at each site may impact the study results. Lots of effort was taken to ensure quality and comparable data between study centers.[244] Still, differences in data collection between study centers could have influenced the study results; this was considered when analyzing the results.[271,311] It is also worthwhile to note that longitudinal studies also require the infrastructure and trained study-personnel to handle both patient requitement and follow-up.[118] We recognize that the study designs favored inclusion from study centers with an established research group, and that the selected patient cohort may not be representative to all hospitals. In the PRAIS study (paper 2 and paper 3) patients from a few study centers dominated the study population, which may also reduce generalizability.[310]

A validation sample of 400 patients was originally planned as a part of the PRAIS study.[244] After initiation of the study, we recognized that the recruitment rate was not as high as expected, partly because the recruitment at the international study centers

was low. The steering committee therefore closed the study after 574 patients were enrolled in the study. Analyses were performed without a validation sample as originally planned. Adding a validation sample could have increased the internal validity of the study results.[310]

5.2.2 Patients

Selection of a relevant patient population is important to ensure the external validity of the findings.[310] A strength of the studies included in this thesis is that enrollment of patients reflected a real-life clinical practice. The eligibility criteria were designed to include a relevant population and at the same time avoid exclusion of important participants (Table 1).[278,309] Both outpatients and inpatients were included in the studies and no cut-off concerning functional status or age was applied.[181] Although stricter eligibility criteria might have increased the number of participants available for longitudinal analysis, this could also have reduced the clinical relevance of the findings.[309,310]

In paper 1 we included a sub-cohort of patient with bone metastases that were enrolled in a larger study on palliative cancer patients. Only patients enrolled in a palliative program were candidates for study participation.[243] We believe that the selected patient cohort is representative for most patients with bone metastases. Still, it should be considered that patients enrolled in a palliative care program may have more severe symptoms or more widespread disease compared to patients not enrolled in a palliative care program, which could affect the generalizability of the results from paper 1.[278] Secondly, patients with pain in the included cohort of patients with bone metastases are defined as patients with CIBP, although pain from other localizations cannot be ruled out.

The selected patient population can also have implications for the precision of the study results that is important to evaluate in interpretation of an observational study.[310] In paper 1 patients were included in analyses regardless of pain intensity at baseline.[278] By including patients with no pain, the correlation between pain intensity and variables only present in patients with pain will be overestimated. On the other

hand, patients with no pain at the present time-point are also at risk for future pain, and therefore we found it important to include patients regardless of pain intensity at baseline. Additional analyses were also carried out in only patients with pain at baseline, and an association between pain intensity, neuropathic pain and episodic pain were also significant in this subpopulation (data not published).[278]

In paper 2 and paper 3 only patients with a baseline pain intensity of 2 or more were included in analyses.[284,293] This decision was made because of the difficulties to reliably evaluate RT response in accordance with international consensus in patients that had baseline pain intensity lower than 2.[181] In the updated international consensus on RT trials, it is suggested to apply an even stricter criteria for baseline pain intensity by including only patients with an NRS of 5 or more in clinical studies.[89] A higher cutoff for baseline pain intensity could possibly contribute to more precise and higher RT response rates. On the other hand, many patients admitted for palliative RT will not be represented in a patient cohort only including patients with pain intensity >=5 in an NRS, and the external validity of the findings could decrease.[310] These suggestions were therefore not adapted in the analyses included in this thesis.[284,293] The RT consensus papers also recommend a run-in period of 1-2 weeks after increase in opioids before enrollment in RT studies.[89,181] A opioid run-in period was not applied as one of the eligibility criteria in the PRAIS study.[244] If patients increased doses of opioids shortly after RT due to inadequate analgesic treatment before RT, this could have affected the RT response and resulted in a higher number of non-responders.

Patients were enrolled in the studies at different stages in their palliative disease course.[278,284,293] This can be considered as a potential limitation of the papers, but it also reflects the clinical reality. Patients with bone metastases experience pain and are admitted for RT at different time-points during a disease trajectory. Including a broad spectrum of patients is an important aspect in the prediction model of RT response. The lower response rates observed in patients with reduced performance status would not have been demonstrated if this population was not enrolled in the study.[284]

In the PRAIS study 27 patients died before the first evaluation of RT response 3 weeks after treatment.[284] These patients were not included in analyses. One could argue that patients dying within 3 weeks after treatment do not have any meaningful treatment efficacy of RT. We therefore discussed to define these patients as non-responders in the clinical prediction model of RT response.[284] On the other hand, some patients have a rapid analgesic response after RT and it was difficult to rule out a potential short term benefit.[201] To avoid inducing a bias in regard to prediction of analgesic relief from RT, patients who died before the first evaluation 3 weeks after RT were not included in analyses.

A limitation of both studies included in this thesis is that all eligible patients were not consecutively included at all study centers. This could induce a potential selection bias.

5.2.3 Recruitment

Recruitment of patients in clinical studies can be challenging, especially in a vulnerable patient population with an incurable and metastatic cancer disease as is the topic of this thesis.[309,312] Although the recruitment rates were generally good or acceptable at most study sites, the recruitment was lower than expected at the international study sites in the PRAIS study which had an impact on the final study sample.[244] Factors acknowledged as recruitment barriers in palliative research are difficulties to identify the patients, "gatekeeping" or protectiveness from health care personnel or family, denial of study participation, severity of the illness, failures in study organization and lack of recourses.[312,313] Stone *et al* also reports that the use of exclusion criteria should be carefully considered as many cancer patients will often have one or more factors that prohibits participation in ordinary clinical studies.[312] In the studies included in this thesis we deliberately chose to apply a minimum of exclusion criteria in order to study a clinically representative patient population.

In the EPCCS study used in paper 1 eligible patients were identified from palliative inpatient and the outpatient departments.[278] In the PRAIS study used for paper 2 and paper 3, eligible patients were mostly identified from RT planning charts.[284,293] While the PRAIS recruitment strategy worked well at many study centers, the identification

strategy was more challenging in the larger study centers and at sites where the study personnel were not situated at the RT department. For some patients there was also a limited time between referral to the RT department and treatment planning. Enrollment in the study and baseline blood samples were therefore difficult to obtain before RT was initiated as required in the study protocol.[284] Gatekeeping or the number of patients that did not wish to attend the studies were not formally reported, but it was not considered a common problem among the study personnel in any of the studies.[284]

The lower recruitment rate than expected at the international study centers in the PRAIS study may be related to the more complex study design and a lack of resourses.[309,313] The PRAIS study included collection of clinical data and blood samples before RT and after RT. The study procedures required trained personnel and were time-consuming. Organizational issues may also have impacted the recruitment rate. In Norway, oncologists specialize in both clinical oncology and radiation oncology, while in most other countries this is two different specialties. At the international trial sites, we experienced that it was demanding to organize a study on a palliative care population referred to RT that were administered at another department with other clinicians responsible for the treatment delivery. This might have complicated the enrollment process and resulted in fewer patients. We also recognize that the follow-up procedures, including a detailed patient case report form and blood samples, were complicated for the seriously ill patients and might have led to higher drop-out rates.[309]

5.2.4 Clinical assessment and outcome measures

Study results will depend on the variable selection and the validity of outcome measures. In both studies the independent variables were mostly selected based on previously known associations, which strengthens the potential association and relevance of the study results. A limitation in paper 1 is that the EPCCS study was planned before this analysis, and variables of interest could only be selected based on the original study.[243] In contrast, the PRAIS study analyzed in paper 2 and paper 3 was

primarily designed to investigate clinical and biomarkers predictors of RT response. Clinical variables that were considered important to evaluate RT response were all included in the study upfront and blood sample procedures were planned before recruitment in the study.[284] For patient reported outcomes, validated and formally translated instruments or screening tools were used.

In paper 1 we included both "average pain" and "worst pain" last 24 hours as dependent variables. This decision was made because of a lack of consensus regarding which measure was preferable to investigate factors associated with pain intensity in patients with bone metastases and was similar to a study in a general cancer population.[87] The primary outcome in the PRAIS study used for paper 2 and paper 3 was analgesic RT response. The outcome measure was defined based on international consensus on endpoints in RT trials, but a few adaptions were made. First, in the PRAIS study, pain at the treated site was recorded as pain at rest and pain at movement the last 24 hours.[244] An updated consensus paper was published after the planning of the PRAIS study in 2012, and recommended using worst pain at treated site last 3 days to evaluate analgesic RT response.[89] To comply with the international consensus, the maximum pain intensity was calculated based on the highest pain score of pain at rest and movement last 24 hours. This deviation from the consensus guidelines could reduce comparability to studies using worst pain intensity last 3 days as the outcome measure. However, for most patients with CIBP worst pain is likely to reflect pain at movement. The calculated maximum pain score at the treated site also correlated well the overall worst pain (pain at all sites) that was reported at baseline, but not published in the paper. The difference in reported time period of 24 hours vs 3 days is probably a minor concern as the difference in actual pain and recalled pain intensity with intervals up to one week has been shown to be minor in several studies.[84,85,314] Secondly, we decided to only report RT response as a dichotomous outcome. RT responders included both patients with complete and partial RT response, while non-responders included patients with both pain progression and indeterminate RT response according to international consensus guidelines.[89,181] Third, RT endpoint is

recommended to be assessed at 1, 2 and 3 months after RT.[89,181] In the PRAIS study, follow-up assessments were performed after 3 and 8 weeks after the last RT fraction. RT response within 8 weeks was considered the outcome measure. The minimal deviation from the standard RT consensus is not likely to have affected the outcome in the PRAIS study as the median time to analgesic RT response is 3 weeks, and it is generally accepted that re-treatment due to treatment failure can be considered 4 weeks after treatment.[25,89,201] RT response including both pain intensity and analgesic treatment is also more likely to be affected by other confounding factors 3 months after RT.[89]

Equianalgesic doses was used to calculate OMED (Table 2). For conversion between some opioid analgesics, the equianalgesic doses are not clearly defined due to a lack of relevant literature, which may represent a potential bias in calculation of RT response. Fortunately, we observed that only a minority of patients changed the type and administration form of opioids in the observational period which reduces the impact of this uncertainty. Increase in opioid doses due to pain at other than the radiated site could also affect the RT outcome. This is a common limitation in all RT trials that follow the international consensus definition of analgesic RT response that require the evaluation of both pain intensity and analgesic doses at a given time-point to calculate RT response.[89,181]

The PRAIS study was designed to evaluate predictors of analgesic RT response, but acute adverse events after RT were not reported.[284] Although palliative RT for CIBP is not commonly associated with severe adverse effects, adverse events is inconsequently reported in clinical trials and is important to account for when evaluating the clinical treatment benefit for each patient.[180,185] An accurate evaluation of acute adverse events would have required a closer interval in follow-up measures after RT, which we decided in the planning phase of the PRAIS study not to implement to reduce the additional burden for the participants.

Another limitation of both studies is the lack of detailed information on several medications. In paper 2 and 3 we had detailed information on analgesic treatment

including opioid doses and routes of administration, but in paper 1 the analgesic doses were not available. Thus, we did not have the opportunity to correct for pain intensity related to treatment efficacy or treatment failure due to inadequate opioid doses. We therefore decided to only adjust analyses for the use of opioid analgesics and non-opioid analgesics, but not include these parameters as explanatory variables in the analyses. The use of corticosteroids was included as an independent variable in the PRAIS study and a negative association between the use of corticosteroids and RT response was observed (paper 2). This finding could not be controlled for type and doses of corticosteroids as this was inadequately reported. The use of bisphosphates was not systematically recorded in any of the studies, which represents an additional confounding factor.

5.2.5 Laboratory analyses

Laboratory analyses were included in paper 2 and paper 3 of this thesis. In paper 2 only CRP was used as a potential parameter to predict the outcome of RT in patients with painful bone metastases, while we in paper 3 analyzed a detailed panel of inflammatory cytokines, CRP, and white blood cells with differential count.[284,293] CRP was included in paper 2 as we aimed to study potential demographic, clinical and clinical- chemistry predictors that are observed in current routine care without introducing the experimental analyses as done in paper 3.

CRP and white blood count were analyzed at each study site. Although all included sites used the in-hospital laboratories with validated instruments for blood sampling, it is difficult to rule out small differences in reported values. For CRP, the lower detection limit was different at the included trials sites (lower detection limit ranging from 3 to 5). For analyses we therefore chose the lowest cut-off of <=5.

A detailed set of inflammatory markers were included in analyses in paper 3.[293] As previously described, a flowchart for handling of blood samples was applied in the planning of the study to minimize the risk of measurement errors (Figure 12). Only a limited number of study personnel handled the blood samples at each site. Cytokines were analyzed in serum as recommended in the literature.[265,266] Before freezing in -80°

Celsius the blood samples were allocated in one A and B sample to avoid several freeze-thaw cycles that may affect the outcome of cytokine analyses.[265,266,315] Serum only underwent one freeze-thaw cycle before analyses in this study.[293] Shipment from each study site to the research biobank (Verdal, Norway) and further shipment to the research laboratory at Nordlanssykehuset (Bodø, Norway) for analyses was all performed by well recognized courier transport companies with established competencies for transport of frozen biological samples. Blood samples were preserved frozen during shipment, and A and B samples were transported in different batches.

A multiplex cytokine assay was used to analyze the level of inflammatory mediators. A few cytokines had measurable levels below the lower limit of detection. Although the exact value could not be measured in these cytokines, a low value is also of relevance in examining the role of inflammation in relation to the outcome. Cytokines in which <20% of the patients had measurements below the lower detection limit was therefore set to 0.01. Cytokines with low measurable levels in > 20 % of the patients were not further analyzed.

A precisely planned handling, storage, and shipment of blood samples is a strength in the PRAIS study, but two concerns in relation to the outcome of the cytokine analyses needs further discussion. First, the levels of inflammatory markers are affected by numerous factors in the body. Food intake, physical activity, acute stress, ongoing infections or inflammatory diseases are examples of factors that could impact the cytokine expression.[265,266] Several cytokines are also known to have a diurnal variation.[266] In literature it is recommended to have a standardized time-point for measurements, preferably in the morning.[265] This was not feasible in the PRAIS study, as withdrawal of blood for study samples were coordinated with routine blood samples and oncological visits to avoid extra burden for the patients; this could have affected the results. Further, the cytokine level may also be affected by the total storage time. Studies have described a considerable degrading of several cytokines (IL1-α, IL1- β, IL-10, IL-15 and IL-8) after 4 years storage in -80 degrees Celsius.[266] In the

PRAIS study patients were enrolled from December 2013 to December 2017, with a follow-up time of 1 year or until withdrawal or death. Cytokine analyses were performed in October 2020. The maximum storage time was therefore nearly 7 years. Storage time might have led to lower measurable levels that weakened the differences in cytokine expression between RT responders and non-responders.[293] There is little literature concerning long storage time of cytokines, but one study detected stable levels of TNF receptor and relative stabile values of IL-6 and CRP after 13 years storage in -80 degrees Celsius.[316] To validate the cytokine expression after long storage time, as in the current study, an internal control for multiplex cytokines assays is recommended.[266] This was not performed in the current study, but median values of baseline inflammatory markers in patients included from 2013 to 2015 were compared to patients included between 2016 and 2017. Results did not indicate a significant degradation of cytokines in patients in which cytokine samples were stored for more than 4 years before analysis (data not published).[293] To avoid a longer storage time we considered analyzing the inflammatory markers at two time-points. On the other hand, this might have led to methodological measurement uncertainties and therefore was avoided.

5.2.6 Statistical considerations

5.2.6.1 Sample Size

In experimental studies, the sample size can be calculated based on the estimated effect size, but this is more challenging in studies that investigate several variables with different effect sizes. More generalized “rules of thumb” are therefore often applied in sample size calculations for studies that explore the association between an outcome and multiple individual factors.[317] In the PRAIS study a simplified sample size calculation was performed based on 10 x the number of planned individual parameters.

In the logistic regression analysis which was used to analyze predictors of analgesic RT response as a dichotomous outcome, another commonly adapted approach is to estimate the sample size based on events per variable (EPV) or events per predictor

parameter (EPP).[318] Number of events should refer to the smallest category. It is commonly acceptable that EPP should be at least 10, while some authors argue for bringing this number to 20 or higher.[317,319] Calculation of 10 EPP in the clinical prediction model of RT response (paper 2) resulted in minimum number 230 RT responders to be included in the analysis, which is very close to the actual sample of 224 RT responders included for analysis in the paper. In the planning of the study the RT response rate was not known. To improve the sample size calculations, the number of events (RT response rate) could have been estimated based on previously published literature before initiation of the study.

Other simplified models to estimate the smallest recommended sample size for multivariable regression analysis have been proposed by Green *et al.* The formula 50 + the number of individual variables x 8 is commonly adapted.[320] Green's formula for sample size calculation estimates a minimum sample size of 210 patients in paper 1 (50 + 20 individual variables x 8). This is accomplished both in the cross-sectional and the longitudinal analyses. For single comparison Green *et al* recommend using the formula 104 + the number of individual variables.[320] Univariate analyses between inflammatory markers and RT response were investigated in paper 3. Based on Green's formula, a minimum of 105 patients should be included in the analysis. Although a formal sample size calculation was not obtained for the experimental analysis including inflammatory markers, the sample size of 448 patients included in the cross-sectional analysis and 120 patients included in the longitudinal analysis, should be sufficient based on this basic sample size assumption.

In general, based on simple sample size calculations we consider analyses presented in these papers to include an appropriate number of patients. Sample sizes in the included analyses are in general large compared to most similar studies.[61,152,235,296] More complex models for sample size calculations for multiple regression models are available and could potentially have improved the sample size calculations up-front. On the other hand, these models are more complex and difficult to implement in the

planning phase of a study as several assumptions need to be implemented in the model.[319]

5.2.6.2 Statistical methods

Both studies in this thesis included longitudinal data. Several statistical methods were evaluated to analyze the data in the different papers. In paper 1 we wanted to design a model that could guide clinicians to identify patients with a risk of pain over time in addition to a model to study factors associated with ongoing pain. First, we chose to present a cross-sectional analysis by inclusion as this could provide important information about the association with pain intensity and other variables at the given time-point.[310] An advantage with the cross-sectional baseline analysis is that the patient sample included all eligible patients with complete data, which served as a good reference for the longitudinal analysis.[118]

Further, we wished to include patient measures at several time-points. In cross-sectional regression methods, one assumes that all observations are independent. In longitudinal analysis, the same patients are measured several times, and one needs to apply a model that accounts for correlations at the individual level and the fact that some covariates change in time.[118] For these analyses we considered repeated measures ANOVA, generalized estimation equations (GEE), and generalized linear mixed models (GLMM).[268,321] Repeated measures ANOVA could not be used to analyze data in paper 1 as it does not allow to analyze time-varying covariates and is not preferable when the number of observations vary among the included patients.[322] GEE is called the marginal effect model and is designed to uncover average population effects. GLMM is called the conditional model and also uncovers individual specific effects (also called “within subject effects”). In this paper we choose to use the GEE model because our main interest was to uncover the average effects in the population.[323] Compared to the GLMM, some precision could be lost in the GEE model. A maximum number of 6 observations per person was included in the GEE analysis to ensure a balanced influence between the enrolled patients.

In paper 2 the follow-up data were used only to calculate the outcome, while the other variables were based on pre-treatment predictors of RT response and measured at baseline. A multivariate logistic regression analysis was applied because of a binary outcome (RT response: yes/no).[269]

As discussed previously, longitudinal data analysis often require the use of more advanced statistical methods.[118] In paper 3 we wanted to design a simpler statistical model to analyze longitudinal data. The change in inflammatory markers from baseline to 3 and 8 weeks was calculated and analyzed in respect to RT response within 8 weeks in logistic regression analyses.[269] This method is also performed in a similar paper.[235] As each analysis only included one variable per person, we did not need to account for repeated measurements. We recognize that applying a mixed effect model could have improved the analytic accuracy of the findings by including observations after 3 and 8 weeks in the same model.[324] Moreover, the analyses were not corrected for multiple testing.[325] If multiple variables are tested in relation to an outcome there is increased likelihood of detecting an association by chance. In this paper we decided not to correct for multiple testing because the inflammatory variables have a known biological correlation, and that correction for multiple testing could have increased the change of a false negative result (type 2 error).[326]

5.2.6.3 The GEE model: selecting the optimal correlation structure

In a longitudinal GEE model, a working correlation structure needs to be selected based on how data are organized. Tests to evaluate the best correlation structure can be a very mathematically complex process, but fortunately the GEE model is known to be robust against selecting the wrong correlating structure, especially for data with a large number of patients.[323] The most used working correlation structures are independent, exchangeable, fixed, unstructured and autoregressive. In our analyses the independent correlation structure could not be used because the correlation between time-points was not independent. We further made a model with both exchangeable, unstructured, fixed and autoregressive correlation structures. The quasi-likelihood independence model criterion (QIC) can be used to select the most

optimal working correlation structure in a GEE model.[276] We also compared the residuals in different models that identify the deviation between the estimated and observed value.[276] In the final model we decided to use an exchangeable correlation structure because it included fewer patients with high residuals and had the highest QIC value.

5.2.6.4 Variable selection in the final model

In the multivariate models included in this thesis we chose to include all potentially relevant factors in one multiple linear regression analysis, as previously described in the methods section of this thesis. While selection of variables is a common approach in multivariate regression to reduce the number of confounding variables in the final model, it also represents a potential bias as variables with a relevant correlation could be lost in the selection process. Variable selection may also influence the regression coefficients.[327] In both studies the independent variables were defined prior to analyses, and the aims were to detect relevant associations with pain intensity and predict an upcoming RT response in patients with painful bone metastases. We chose this method to avoid missing relevant correlations in the final model.[327] In paper 3 we decided only to report univariate associations between RT response and the different inflammatory markers because of the dependency between the inflammatory markers.[266]

5.2.6.5 Regression diagnostics and outputs

Regression diagnostics were performed for both the multivariate linear regression and the multivariate logistic regression models. Several potential interaction terms were individually added to the models, but no interactions were identified. In the multivariate regression analysis presented in paper 1, residuals were considered close to normally distributed. The variance inflation factor (VIF) was also analyzed, and no multicollinearity was detected. Correlation coefficients were not standardized and a direct comparison between the independent factors associated with pain intensity is therefore difficult. Categorical variables will have higher correlation coefficients than continuous and scaled parameters, which one need to be aware of when interpreting

the results. The benefit of not standardizing the correlation coefficient is that it is easier to interpret how each factor influences pain intensity per units change, as we did in this paper.

5.2.6.6 Goodness of fit

The multivariable models presented in both paper 1 and 2 have a low to moderate goodness of fit. R^2 is the variance that could be explained by factors included in the model. Adjusted R^2 corrects the R^2 estimate based on the number of variables and prediction for the null-hypothesis (no association between the given variables) to be true.[267] In paper 1 the cross-sectional models could explain 36% (average pain) and 41% (worst pain) of the variance of the outcome (adjusted R^2). Although the explained variance is not high, it is higher than in the previously discussed paper by Knudsen *et al* that analyzed factors related to pain progression in a general cancer population.[87]

In models with a dichotomous outcome like presented in paper 2 (RT response), the predicative accuracy of a multivariable model can be measured with concordance statistics (C-statistics). In paper 2 the C-statistics refers to the probability of a randomly selected patient with RT response to have a higher predictive probability of RT response compared to a randomly selected patients with no RT response.[328] The C-statistics is equal to the area under the curve. A well discriminating model is often considered to have a C-statistics of at least 0.8, while a C-statistics of <0.6 reflects a low discriminative ability.[329] In paper 2 we present a moderate discriminative ability with C-statistics of 0.69.[284] This is higher than the reported C-statistics from previously published papers that investigated multiple predictors of RT response in retrospective studies (0.56-0.63).[218,219] Still, we concluded that the discriminative ability of the model was too low to reliably design a predictive score based on our findings.[284]

5.2.6.7 Missing data

Missing data is a common issue in all clinical trials and obtaining complete data could be especially challenging in a frail palliative cancer population.[274] In general, there were few missing variables at baseline in both studies.[278,284,293] The number of missing variables increased during follow-up as expected.[330] Different patterns of missingness

were observed. For many patients missing follow-up measures were terminal and no further observations were collected. This pattern of missing data could, for example, be due to deaths or dropouts.[274] Some patients also had an intermittent pattern of missing, in which one follow-up measure could be missing, but a later observation was not missing. One should be aware that the reasons for missing could be related to increased severity of the disease or even a higher pain intensity or lack of RT response.[274]

There are three different mechanisms of missing data:[274,275]

1) Missing completely at random (MCAR): missingness is unrelated to the any of the outcomes.
2) Missing at random (MAR): missingness is not dependent of the value missing but could be related to other variables not missing.
3) Missing not at random (MNAR): missingness is also dependent on the missing variable.

In paper 1 and paper 3 we performed complete case analysis, and patients with any missing variables were not included in the analysis. This method simplifies the statistics but reduces the number of patients available for analysis. Arguments supporting the use of complete case analysis were that the sample size was high and that the number of missing variables were limited.[274] In paper 1 we found that 89% of the patients had complete data at baseline.[278] Of the 513 patients enrolled for clinical prediction of RT response, 448 patients (87%) had a known RT response and cytokine measures at baseline and could be included for analysis on inflammatory markers as predictors of RT response in paper 3.[293]

The number of patients with no missing variables during follow-up were significantly reduced from baseline in both studies, and many patients were lost to follow-up. This is a common problem in an advanced stage cancer population.[330] In paper 1 approximately 65% of the patients eligible for baseline analysis were included in the longitudinal analysis.[278] The proportion of patients available for longitudinal

observations is higher than in many other longitudinal trials on patients with a palliative cancer disease.[330] In paper 3 only 120 patients (26%) of the 448 patients included for baseline analysis had inflammatory markers measures at both 3 and 8 weeks after RT. Although the number of patients included in the longitudinal analysis of inflammatory markers is higher than in similar studies, the low proportion of patients with complete data for follow-up is a weakness in these analyses.[235,293] By not analyzing patients with incomplete data, we could have lost relevant information that could potentially lead to bias if the missing variables were not MCAR.[274] In clinical trials of palliative cancer populations it would be expected that most of the missing variables could be related to other factors not missing (MAR).[275]

In paper 2 we decided to impute missing data to avoid losing sample size and reduce selection bias in the clinical prediction model.[274,331] There were a limited number of missing variables imputed at baseline: 47 had missing CRP, 24 had missing episodic pain, 3 had missing steroids, 24 had missing neuropathic pain score (LANSS), 23 had missing depression score (PHQ-9) and 53 had missing RT response. Only 10 patients had more than two missing variables (data not published). We considered missing data to be MAR. Single imputation of the mean was performed for PHQ-9 and LANSS score. This is a simple method for imputation of missing data but will reduce the variances in the sample.[274] To minimize uncertainties with this model, patients that had more than half of the items missing were not imputed. For the other missing variables, we used multiple imputation (MI) with multivariate imputation of chained equations (MICE).[275] The method is described earlier in this thesis and improves both the variability compared to single imputation and also includes the uncertainties of the missing observation.[274] The 53 patients with a unknown RT response were not included in the final model.[284] Imputation of the main outcome is debated and we decided to drop patients with missing RT response status after imputation to ensure a reliable outcome.[275] Sensitivity analyses were performed and showed similar results as the imputed model, strengthening the validity of the results.[284]

5.2.7 Ethical considerations

Ethical concerns can be a barrier in palliative care research.[332] Health care personnel might find it unethical to include fragile patients with a high symptom load in research studies, as they present an additional burden to the patients. This can lead to gatekeeping and recruitment challenges in palliative research studies.[312] On the other hand the patients often emphasize being included in clinical studies and find it meaningful to contribute to improvement of future treatment. Patients may also benefit from study participation.[332,333] Important ethical issues in palliative care research are highlighted in the *Oxford Textbook of Palliative Medicine*: 1) benefit for future patients, 2) benefit for patients enrolled in the study, 3) the risk and burdens of patients participating in the study, 4) protecting voluntariness, and 5) ensuring decision making capacity.[334]

Results from both studies included in this thesis could benefit future patients. Results from the EPCCS study could provide knowledge to health care personnel in treatment and follow-up of patients with bone metastases. Results from the PRAIS study contributes to important knowledge in selection of patients suitable for palliative RT and the role of several inflammatory markers after RT.

Clinical research may also be beneficial to the patients enrolled in the studies. Patients included in the studies of this thesis were followed regularly by health care personnel as a part of the study procedure. Follow-up visits were undertaken at the out-patient clinic or by phone. Our impression was that the patients appreciated the additional follow-up that was provided during the study. Symptom registration by inclusion and follow-up provided additional information to the study personnel about symptoms that were not adequately managed during ordinary patient consultations or in absence of consultations. Study personnel could therefore facilitate symptom-relieving interventions or consult the treating physician during the study period to provide individual tailored treatment.[332]

In planning of the study, we aimed to minimize the burden of study participation. The number of assessment tools included in the patient case report form was thoroughly

evaluated to reduce the time each patient used to fill in the questionnaires. Follow-up was coordinated with other hospital visits when possible. When patients were not able to come to the hospital for follow-up due to long travel distance or severe illness, the follow-up visits were undertaken by phone and the patient case report forms were sent by mail. Blood samples were also obtained in the PRAIS study. Blood sampling was coordinated with routine blood samples when possible, and the amount of blood withdrawn was limited and did not induce an additional risk for anemia.

All patients received both oral and written information about the studies, presented to all the patients prior to study participation in their own language. Written consent was signed before study participation. The informed consent in both studies were written in a language and form that should be understandable for the patients. In the informed consent, voluntariness of participation and the ability to withdraw at any time-point were highlighted. Participation in the studies did not change any of the planned treatment for the patients. Most of the patients in the EPCCS and the PRAIS study were asked for participance by research personnel with no direct treatment responsibility; but sporadically, participance was asked for by health care personnel responsible for patient treatment, which could have influenced voluntariness.

The decision-making capacity of study participants was ensured in both studies by adding cognitive impairment as an exclusion criterion of the studies. Cognitive impairment was evaluated by the study team with no objective assessment required, which potentially could have led to inclusion of patients with a reduced decision-making ability.

5.3 Discussion of this thesis

In the following sections, the results of this thesis are discussed in a broader context. The general aim of the PhD project was to improve treatment of patients with CIBP by identifying clinical factors associated with pain intensity in patients with bone metastasis and by analyzing predictors for response to palliative RT.

Effective symptom management is an important part of cancer treatment. Pain is often a pronounced problem in patients with bone metastases and is, in many cases, not adequately treated.[6,42,111,112] Improved pain management is also likely to have a positive effect on quality of life as well as physical and psychosocial functioning in patients with bone metastases.[218,335]

A challenge in clinical oncology and palliative care is the difficulty to predict symptoms and outcomes during a patient trajectory.[336,337] For this reason, it can be demanding to provide the most optimal treatment or early interventions for patients at risk of symptoms. Symptomatic treatment might be initiated only after the symptoms have become severe. Patients with higher pain severity require higher opioid doses and longer time to achieve stable pain control.[116] For some patients, pain relieving interventions are also initiated too late in the disease trajectory for the patients to gain a meaningful treatment response.[286] Today, the choice of treatment and follow-up is mostly based on clinical experience, but several studies have demonstrated that clinicians have limited ability to predict cancer prognosis or the outcome of treatment like RT.[202,338] Clinicians treating patients with bone metastases are therefore in need of tools to aid their decision regarding follow-up and treatment at the individual level.[6,226] Preferably a set of predictors could guide the clinician to know which patients with bone metastases will develop pain and what the expected treatment efficacy is in each individual for different treatment options. There are to date no tools in clinical practice that are suitable to forecast the pain trajectory or analgesic treatment response in patients with painful bone metastases.

Is it possible to predict pain in patients with bone metastases?

In the first paper of this thesis, we focused on factors associated with a higher pain intensity in patients with bone metastases during longitudinal follow-up, intending to identify patients in need for special attention and patients that could benefit from pain relieving interventions like RT. We identified several clinical factors that were associated with higher pain intensity both by enrollment in the study and in the upcoming month. The presence of episodic pain exacerbations (breakthrough pain) was strongly associated with higher pain intensity by enrollment in the study and upcoming worst pain intensity, and stands out as an important factor to assess and aiming to treat to avoid further pain exacerbation in patients with bone metastases. Findings from paper 1 are important as it is the first study to prospectively evaluate factors related to pain intensity development in patients with bone metastases. Factors associated with higher pain intensity could be targeted to improve treatment of CIBP, but the presented model has limited validity for predictive purposes due to a low explained variance. The complexity of the disease and concurrent symptoms of pain may result in difficulties to establish valid predictors of pain in patients with bone metastases. Cancer pain is a cluster of pain syndromes with different pathophysiology, treatments, and clinical presentations.[77,100] We observed that several factors associated with general cancer pain were not associated with pain in patients with bone metastases and vice versa.[87,100,115,278] Findings from this study highlights the importance of further developing a model for prediction of pain in patients with bone metastases as an own cohort of patients. As for other cancer related clinical outcomes, it may be that cancer pain is also not one size fits all.

Is it possible to predict analgesic RT response in patient with CIBP?

Three large studies, two prior retrospective analyses and the prospective analysis included in this thesis, have investigated multiple clinical factors in relation to RT efficacy with an aim to provide guidelines and decision support for clinicians to select patients with a higher likelihood of analgesic RT response.[218,219,284] Although all three studies have demonstrated a few shared findings that predict analgesic RT response

(cancer diagnosis and better performance status), results are inconsistent, and models presented in all papers investigating multiple factors for analgesic RT response have a low or moderate discriminative ability.[218,219,284] It is therefore reasonable to believe that factors other than hereto investigated clinical factors are also important to predict RT efficacy. For the prediction of survival or treatment outcomes in cancer patients, reliable discriminative ability has previously been achieved by combining clinical factors with radiological or biological markers. Examples of such scores are the Mirels score to evaluate risk of pathological fractures and the Glasgow Prognostic Index to evaluate prognosis in cancer patients.[147,339] Even though in paper 2 we introduced CRP as an inflammatory marker and included several radiological features of bone metastases (osteolytic, sclerotic and soft tissue component outside bone), the discriminative ability of the model was only modestly increased compared to models previously presented, and we decided not to develop a clinical prognostic index based on the findings.[284] In the paper by Van der Velden *et al* a risk score calculation was presented despite a moderate discriminative ability of the model.[219] This score was based on cancer diagnosis, performance status and initial pain intensity. To provide a clinically useful score to select patients suitable for palliative RT, we believe that it is essential to obtain a cut-off in that reliably discriminates patients. This was not available in the model we presented in paper 2, nor in previous studies.[218,219,284] Based on these results we conclude that to date there is no clinically valid prediction tool that has a high enough validity for selecting patients that should receive or not receive RT for painful bone metastases. Still, the selection of patients that could benefit from RT can be improved by clinical implementation of single patient characteristics like performance status, cancer diagnosis and the presence of soft tissue metastases outside bone to aid the decision process.[284] These factors should be evaluated together with other treatment aspects, like RT availability and travelling distance, possible adverse effects, patient preferences, other treatment alternatives, and the possibility to achieve analgesic response from analgesic medications.

Is inflammation an important mediator of analgesic RT response?

The role of inflammation in relation to cancer and cancer treatment is a very relevant topic in oncology and a field of research in which more clinical studies are needed. It is widely acknowledged that inflammation may facilitate a metastatic process, but inflammation may also hinder cancer development.[340,341] Several novel cancer medications like immunotherapy exploit the immune system to eradicate cancer cells. A role of inflammation in relation to CIBP and analgesic relief from RT has been proposed, but the supporting literature is very limited.[111,170] In this thesis we have demonstrated in a large patient sample that a detailed panel of inflammatory markers were not important predictors of an upcoming RT response.[293] We believe that the validity of the finding is high due to the large patient sample, and that the finding should guide researchers to exploit other potential biomarkers as pre-treatment prediction of RT response.

The further observed differences in several inflammatory markers 3 weeks after RT in patients with and without analgesic RT response could support a role of inflammation to initiate a RT effect that is previously suggested in the literature.[111,170] The finding is further strengthened by results from the clinical prediction model on RT repose demonstrating that patients using corticosteroids had lower RT response rates.[284] Because of the small sample size available for longitudinal analysis and that several factors like ongoing infections, tumor load, metastatic status, and the use of medications like opioids or corticosteroids may modulate a systematic inflammatory response, it is difficult to draw absolute conclusions on the association between RT response and inflammation based our study.[293] Additionally, the local inflammatory response needed to induce analgesic relief after RT may not be reflected at the same level in serum.[342] Despite the obvious limitations of the study, we believe that result of this thesis contribute to important knowledge on the role of inflammation in patients with CIBP treated with RT.[293] Several studies are also investigating the role of immunotherapy to enhance RT effects in several cancer origins.[343] Knowledge about the inflammatory response and treatment efficacy after RT presented in this thesis

could not only be important for a population with bone metastases, but also for patients in a curative cancer setting.

6 Conclusion

Paper 1: Higher current pain intensity, sleep disturbances, drowsiness, male gender and breakthrough pain were factors associated with a higher pain intensity one month later in patients with bone metastases. The same variables were associated with ongoing average pain intensity last 24 hours. These factors may be helpful to identify patients with bone metastases that will benefit from more frequent follow-up of pain-relieving interventions like RT and contribute toward a better classification and treatment of CIBP.

Paper 2: Better performance status, breast or prostate cancer and presence of soft tissue expansion outside bone predicted analgesic RT response in patients with painful bone metastases. Patients using corticosteroids had significantly lower response rates. There was no difference in RT response in patients receiving single fraction RT compared to multiple fraction RT. The study identified clinical factors that could indicate a higher or lower chance of analgesic response for palliative RT, but a prognostic index with a reliable cut-off to recommend or not recommend RT in patients with CIBP could not be developed due to a moderate discriminative ability of the model.

Paper 3: None of the investigated inflammatory markers were reliable predictors of RT response to select patients with a higher likelihood of response prior to treatment, but a significant association between RT response and several inflammatory markers after treatment indicate that inflammation may be important to initiate an analgesic RT response in patients with painful bone metastases. A higher change in IL-8, IP-10, eotaxin, MCP-1, G-CSF and TNF and a lower change in CRP between baseline and 3 weeks after RT were positively associated with RT response. The association between RT and change in inflammatory markers after RT could point towards inflammation as a potential future treatment target.

7 Future perspectives

Papers included in this thesis have identified several areas where additional work is important to improve treatment of patients with painful bone metastases.

We believe that it is of importance to further focus on groups with similar pain etiologies and pathophysiology to investigate factors associated with pain and analgesic treatment in cancer patients. To increase the predictive value of upcoming research on patients with painful bone metastases, studies should preferably also incorporate other variables than those included in the EPCCS study, like radiological features of bone metastases and biomarkers. Factors identified as associated with pain intensity in this thesis may also be implemented in computer-based decision support systems or machine learning programs to further improve the prediction of pain in patients with CIBP.[283,344]

Prediction of analgesic RT response is challenging, and it may not be possible to design a model that could easily discriminate patients with and without an analgesic treatment response from RT. Although we could not provide a clinical prediction model based on results from the PRAIS study, we still believe that a tool to select patients with a better response rate would improve treatment of patients with CIBP. If new studies are designed to identify predictors of analgesic RT response, we believe it would be essential to further investigate radiological features of bone metastases as well as bone remodeling biomarkers that have shown promising results in a few studies.[168,169] Combing elements from radiological, clinical, and biological parameters could be promising in designing a predictive model.

SFRT is underused in patients with painful bone metastases, despite numerous studies that have shown equal analgesic efficacy after SFRT and MFRT in patients with bone metastases, and that international recommendations advocates SFRT in uncomplicated bone metastases.[186] A registry study from Norway demonstrated that the use of SFRT increased from 16% in 1997 to 41% in 2007.[345] One could expect that these numbers would steadily increase thereafter, but instead we observed that only 37% of the

patients included in the PRAIS study received SFRT.[284] It takes time to implement new knowledge in medicine, but we believe that there are also other factors related to this obvious resistance to implement SFRT in a clinical setting. One factor is probably the lack of evidence based knowledge on how to handle "complicated bone metastases". As discussed in the introduction, there has not been a clear definition of complicated bone metastases, and patients with features of complicated bone metastases are often not included in clinical trials.[152] In the PRAIS study, we included patients with soft tissue components outside bone, but not other clinical features that are often considered complicated bone metastases like spinal cord compression and pathological fractures. Although a detailed review paper on handling RT in complicated bone metastases was recently published, there are still several uncertainties regarding the optimal treatment in these patients.[176] There is a need for future randomized studies which enroll patients with complicated bone metastases to tailor more optimal treatment in patients with painful bone metastases. Establishing clear treatment recommendations for these groups would improve the individual treatment efficacy and probably also the use of SFRT for CIBP. There is also a need for more established knowledge regarding the use of stereotactic RT in patients with bone metastases, especially for patients with asymptomatic skeletal lesions and longer lifetime prognosis.[25]

An unexpected and important finding in the PRAIS study was that patients using corticosteroids had significantly lower RT response rates compared to patients not using corticosteroids.[284] Several studies have the last years investigated the use of corticosteroids to treat and prevent pain flares after RT, but none of the prior studies have investigated the use of corticosteroids and long-term analgesic effect of RT.[128,134] As corticosteroids are frequently used in patients with CIBP, it would be essential to further investigate this potential association in studies gathering detailed information of type and doses of corticosteroids and its relation to analgesic relief from RT.[127] Further research in necessary to state if corticosteroids should be abandoned to

achieve better RT outcome in patients with CIBP. This may also have implication for RT due to other conditions than CIBP.

Findings in this thesis implicates that an inflammatory response early after RT is important to achieve RT response. Several targeted therapies also exploit the immune system to target cancer cells, and immunotherapy is suggested to enhance the antitumor effect of RT.[343,346] More knowledge about the immune activation of RT may be important to establish new insights into the mechanisms of cancer treatment and combination treatment with novel anti-cancer drugs. Future studies should be longitudinal, measuring inflammatory mediators over time and include a validation cohort. It would also be interesting to investigate if there is a correlation between a local inflammatory process in the bone and systemic inflammatory markers. Clinical studies could be designed to compare systemic inflammatory markers in serum with inflammatory markers at the pain site obtained by, for example, a tissue biopsy. Alternatively, non-invasive procedures like PET could be compared with the level of systemic inflammatory mediators and pain intensity.

We also believe it is of clinical interest that the change in several inflammatory markers between baseline and 3 weeks after RT are associated with increased bone resorption, and this finding should be further investigated. Several bone biomarkers previously investigated in relation to bone metastases may be candidate predictors of analgesic RT response.[12] Detection of relevant biomarkers related to analgesic RT response could potentially contribute to development of novel drug targets.

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9 Appendix

1) Edmonton Classification System for Cancer Pain (ECS-CP)
2) The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS)
3) EORTC QLQ-15 pal
4) Edmonton Symptom Assessment System, revised version (ESAS-r)
5) Patient Health Questionnaire Depression Scale (PHQ-9)
6) Supplementary table paper 2. Sensitivity analyses.
7) Papers included in this thesis.

Edmonton Classification System for Cancer Pain

(ECS-CP)

Patient number ☐☐ ☐ ☐☐☐

Edmonton Classification System for Cancer Pain

For each of the following features, tick the response that is most appropriate, based on your clinical assessment of the patient.
If the patient does not have any pain (i.e. "No" under mechanism of pain), then no further assessment is required in relation to completion of the ECS-CP

1. Mechanism of Pain

☐ No No pain syndrome

☐ Nc Any nociceptive combination of visceral and/or bone or soft tissue pain

☐ Ne Neuropathic pain syndrome with or without any combination of nociceptive pain

☐ Nx Insufficient information to classify

2. Incident Pain

☐ Io No incident pain

☐ Ii Incident pain present

☐ Ix Insufficient information to classify

3. Psychological Distress

☐ Po No psychological distress

☐ Pp Psychological distress present

☐ Px Insufficient information to classify

4. Addictive Behavior

☐ Ao No addictive behavior

☐ Aa Addictive behavior present

☐ Ax Insufficient information to classify

5. Cognitive Function

☐ Co No impairment. Patient able to provide accurate present and past pain history unimpaired

☐ Ci Partial impairment. Sufficient impairment to affect patient's ability to provide accurate present and/or past pain history

☐ Cu Total impairment. Patient unresponsive, delirious or demented to the stage of being unable to provide any present and past pain history

☐ Cx Insufficient information to classify

The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS)

Patient number ☐☐ ☐ ☐☐☐

This pain scale can help to determine whether the nerves that are carrying your signaks are working normally or not. It is important to find this out in case different treatments are needed to control your pain.

A. PAIN QUESTIONNAIRE

- Think about how your pain has felt over the last week.
- Please say whether any of the descriptions match your pain exactly.

1) Does your pain feel like strange, unpleasant sensations in your skin? Words like prickling, tingling, pins and needles might describe these sensations.

   ☐ NO - My pain doesn't really feel like this

   ☐ YES - I get these sensations quite a lot

2) Does your pain make the skin in the painful area look different from normal? Words like mottled or looking more red or pink might describe the appearance.

   ☐ NO - My pain doesn't affect the colour of my skin

   ☐ YES - I've noticed that the pain does make my skin look different from normal

3) Does your pain make the affected skin abnomally sensitive to touch? Getting unpleasant sensations whan lightly stroking the skin, or getting pain whan wearing tight clothes might describe the abnormal sensitivity.

   ☐ NO - My pain doesn't make mye skin abnormally sensitive in that area

   ☐ YES - My skin seems abnomally sensitive to touch in that area.

4) Does your pain come on suddenly and in bursts for no apparent reason whan you're still. Words like electric shocks, jumping and bursting describe these sensations.

   ☐ NO - My pain doesn't really feel like this

   ☐ YES - I get these sensations quite a lot

5) Does your pain feel as if the skin temperature in the painful area has changed abnormally? Words like hot and burning describe these sensations.

   ☐ NO - i don't really get these sensations

   ☐ YES - I get these sensations quite a lot

EORTC QLQ-15 pal

Patient number ☐☐ ☐ ☐☐☐

EORTC QLQ-C15 PAL version 1

We are interested in some things about you and your health. Please answer all of the questions yourself by ticking the alternative that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

	Not at all	A little	Quite a bit	Very much
1. Do you have any trouble taking a short walk outside of the house?	☐	☐	☐	☐
2. Do you need to stay in bed or a chair during the day?	☐	☐	☐	☐
3. Do you need help with eating, dressing, washing yourself or using the toilet?	☐	☐	☐	☐

During the past week:

4. Were you short of breath?	☐	☐	☐	☐
5. Have you had pain?	☐	☐	☐	☐
6. Have you had trouble sleeping?	☐	☐	☐	☐
7. Have you felt weak?	☐	☐	☐	☐
8. Have you lacked appetite?	☐	☐	☐	☐
9. Have you felt nauseated?	☐	☐	☐	☐
10. Have you been constipated?	☐	☐	☐	☐
11. Were you tired?	☐	☐	☐	☐
12. Did pain interfere with your daily activities?	☐	☐	☐	☐
13. Did you feel tense?	☐	☐	☐	☐
14. Did you feel depressed?	☐	☐	☐	☐

For the following question please tick the number between 1 and 7 that best applies to you.

15. How would you rate your overall quality of life during the past week?

☐	☐	☐	☐	☐	☐	☐
1	**2**	**3**	**4**	**5**	**6**	**7**

Very poor Excellent

Edmonton Symptom Assessment System,

revised version

(ESAS-r)

Patient number ☐☐ ☐ ☐☐☐

Edmonton Symptom Assessment System (revised version) (ESAS-r)

Date completed: ☐☐.☐☐.20☐☐

Please mark the number that best describes how you feel NOW:

No pain	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible pain
No tiredness (Tiredness=lack of energy)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible tiredness
No drowsiness (Drowsiness=feeling sleepy)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible drowsiness
No nausea	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible nausea
No lack of appetite	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible lack of appetite
No shortness of breath	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible shortness of breath
No depression (Depression=feeling sad)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible depression
No anxiety (Anxiety=feeling nervous)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible anxiety
Best wellbeing (Wellbeing=how you feel overall)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible wellbeing
No_____ Other problem (for example, constipation)	☐ 0	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10	Worst possible

Patient Health Questionnaire Depression Scale

(PHQ-9)

Patient number ☐☐ ☐ ☐☐☐

PATIENT HEALTH QUESTIONNAIRE-9 (PHQ - 9)

Over the last 2 weeks, how often have you been bothered by any of the following problems?	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things	☐	☐	☐	☐
2. Feeling down, depressed, or hopeless	☐	☐	☐	☐
3. Trouble falling or staying asleep, or sleeping too much	☐	☐	☐	☐
4. Feeling tired or having little energy	☐	☐	☐	☐
5. Poor appetite or overeating	☐	☐	☐	☐
6. Feeling bad about yourself — or that you are a failure or have let yourself or your family down	☐	☐	☐	☐
7. Trouble concentrating on things, such as reading the newspaper or watching television	☐	☐	☐	☐
8. Moving or speaking so slowly that other people could have noticed. Or the opposite — being so fidgety or restless that you have been moving around a lot more than usual	☐	☐	☐	☐
9. Thoughts that you would be better off dead or of hurting yourself in some way	☐	☐	☐	☐

If you ticked any problems, how difficult have these problems made it for you to do your work, take care of things at home, or get along with other people?

Not difficult at all	**Somewhat difficult**	**Very difficult**	**Extremely difficult**
☐	☐	☐	☐

Supplementary table paper 2
Sensitivity analyses

Supplementary table paper 2: Sensitivity analyses

	Final model		Complete case analysis		Worst case analysis (a)		Best case analysis (b)	
Independent variables	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age	0.992	[0.969,1.015]	0.991	[0.966,1.017]	0.988	[0.967,1.010]	0.999	[0.979,1.020]
Female gender	0.974	[0.542,1.750]	0.931	[0.487,1.777]	0.946	[0.540,1.657]	0.928	[0.547,1.576]
Charlson comorbidity index (1)	1.119	[0.895,1.399]	1.249	[0.965,1.617]	1.142	[0.921,1.416]	1.098	[0.889,1.357]
Karnofsky performance status (2)	1.391***	[1.148,1.684]	1.540***	[1.233,1.922]	1.405***	[1.170,1.687]	1.243*	[1.043,1.481]
Cancer diagnosis								
Breast	2.535*	[1.122,5.729]	2.815*	[1.155,6.861]	2.883**	[1.315,6.322]	1.802	[0.865,3.755]
Prostate	2.830*	[1.266,6.327]	2.066	[0.880,4.854]	3.130**	[1.452,6.748]	1.977	[0.951,4.108]
Lung	1.289	[0.612,2.714]	0.887	[0.395,1.988]	1.615	[0.788,3.308]	0.890	[0.454,1.743]
Gastrointestinal	1.000	[.,.]	1.000	[.,.]	1.000	[.,.]	1.000	[.,.]
Urological	1.289	[0.576,2.886]	1.210	[0.509,2.877]	1.537	[0.706,3.348]	0.944	[0.453,1.970]
Other/unknown	1.602	[0.653,3.929]	1.141	[0.449,2.897]	1.649	[0.712,3.821]	1.408	[0.635,3.120]
Only bone metastases	1.271	[0.801,2.017]	1.514	[0.901,2.543]	1.182	[0.760,1.837]	1.231	[0.799,1.895]
RT single fraction	1.290	[0.796,2.089]	1.419	[0.846,2.380]	1.419	[0.892,2.258]	1.008	[0.642,1.582]
Soft tissue expansion at radiated site								
Yes	1.996**	[1.227,3.249]	1.855*	[1.097,3.137]	1.896**	[1.198,3.001]	1.752*	[1.120,2.740]
unevaluable	0.684	[0.120,3.891]	0.580	[0.0980,3.429]	0.779	[0.147,4.122]	0.514	[0.107,2.463]
Osteolytic metastases at radiated site								
Yes	1.179	[0.736,1.887]	1.278	[0.774,2.110]	1.141	[0.725,1.797]	1.191	[0.769,1.845]
unevaluable	0.916	[0.339,2.474]	1.060	[0.347,3.234]	0.704	[0.284,1.746]	1.204	[0.497,2.915]
Radiation to weight bearing bone	1.240	[0.696,2.207]	1.372	[0.716,2.631]	1.147	[0.660,1.994]	1.193	[0.698,2.041]
Max pain at radiated site last 24h	1.067	[0.960,1.186]	1.109	[0.989,1.245]	1.071	[0.968,1.184]	1.053	[0.955,1.162]
Episodic pain	0.908	[0.558,1.476]	0.959	[0.573,1.604]	0.948	[0.598,1.502]	0.867	[0.553,1.358]
Neuropathic pain symptoms (3)	0.993	[0.835,1.181]	1.016	[0.842,1.225]	1.029	[0.870,1.218]	0.988	[0.840,1.163]
Opioid dose (5)	0.999	[0.998,1.001]	0.999	[0.998,1.001]	0.999	[0.997,1.000]	0.999	[0.998,1.001]
Steroid use	0.573*	[0.374,0.877]	0.680	[0.424,1.091]	0.548**	[0.363,0.827]	0.650*	[0.436,0.970]
Depressive symptoms (4)	0.991	[0.943,1.042]	0.989	[0.940,1.040]	0.981	[0.935,1.029]	1.007	[0.963,1.054]
Elevated CRP (>5)	0.912	[0.578,1.439]	0.843	[0.523,1.358]	0.962	[0.621,1.492]	0.884	[0.581,1.344]
C-statistics	**0.69**		**0.70**		**0.70**		**0.65**	
Number of participants	**460**		**382**		**513**		**513**	

a) Patients with unknown RT response were defined as non-responders

b) Patients with unknown RT response were defined as responders

Paper 1

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ORIGINAL ARTICLE

Which factors can aid clinicians to identify a risk of pain during the following month in patients with bone metastases? A longitudinal analyses

Ragnhild Habberstad[1,2] · M. J. Hjermstad[3] · C. Brunelli[1,4] · S. Kaasa[1,5] · M. I. Bennett[6] · K. Pardon[7] · P. Klepstad[1,8,9]

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Abstract

Purpose Explore clinical factors associated with higher pain intensity and future pain in patients with bone metastases to identify patients who can benefit from closer follow-up or pain-modifying interventions.

Methods This is a secondary analysis of 606 patients with bone metastases included in a multicenter longitudinal study. The dependent variables were "average pain" and "worst pain" in the last 24 h (0–10 NRS). Twenty independent variables with potential association to pain intensity were selected based on previous literature. Cross-sectional analyses were performed with multiple linear regression to explore factors associated with pain intensity at baseline. Longitudinal data were analyzed with a generalized equation models to explore current factors associated with pain intensity at the next visit in 1 month.

Results Current pain intensity ($p < 0.001$), sleep disturbances (p 0.01 and 0.006), drowsiness (p 0.003 and 0.033) and male gender (p 0.045 and 0.001) were associated with higher average and worst pain intensity in 1 month. In addition, breakthrough pain was related to higher worst pain intensity (p 0.003) in 1 month. The same variables were also associated with higher average pain intensity at baseline.

Conclusion Higher current pain intensity, sleep disturbances, drowsiness, male gender, and breakthrough pain are factors associated with higher pain intensity in patients with bone metastases at the next follow-up in 1 month. These factors should be assessed in clinical practice and may aid clinicians in identifying patients that can benefit from closer follow-up or interventions to prevent lack of future pain control.

Trial registration in clinicaltrials.gov NCT01362816.

Keywords Cancer · Pain · Bone metastases · Cancer-induced bone pain · Associations

✉ Ragnhild Habberstad
Ragnhild.habberstad@gmail.com

[1] European Palliative Care Research Centre (PRC), Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology and St. Olavs hospital, Trondheim University Hospital, Trondheim, Norway

[2] Cancer Clinic, St. Olavs hospital, Trondheim University Hospital, Trondheim, Norway

[3] European Palliative Care Research Centre (PRC), Department of Oncology, Oslo University Hospital and Institute of Clinical Medicine, University of Oslo, Oslo, Norway

[4] Palliative Care, Pain Therapy and Rehabilitation Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

[5] Department of Oncology, Oslo University Hospital and University of Oslo, Oslo, Norway

[6] Academic Unit of Palliative Care, Faculty of Medicine and Health, University of Leeds, Leeds, UK

[7] End-of-life Care Research Group, Vrije Universiteit Brussel (VUB) & Ghent University, Brussels, Belgium

[8] Department of Circulation and Medical Imaging, Faculty of Medicine and Health Sciences, NTNU, Norwegian University of Science and Technology, Trondheim, Norway

[9] Department of Anaesthesiology and Intensive Care Medicine, St. Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Introduction

Pain is an important cause of reduced quality of life in cancer patients, and more than 60% of advanced cancer patients experience pain [39]. Bone metastases, which may cause cancer-induced bone pain (CIBP), are the most frequent causes of pain in cancer patients [26].

The occurrence of bone metastases is highest in patients with multiple myeloma (70–95%), breast cancer (65–75%), prostate cancer (65–75%), lung cancer (30–40%), bladder cancer (40%), and malignant melanoma (14–45%) [5, 18]. The development of bone metastases results from a close interaction between bone cells, tumor cells, and their microenvironment. Cytokines in the bone microenvironment modulate genes expressed in cancer cells and disrupt normal bone homeostasis [37]. These mechanisms, important in development of bone metastases, are essential mediators of CIBP [37]. The intensity of CIBP is related not only to the size and location of metastases but also to biological factors in the bone microenvironment, including factors that activate osteoclasts and sensitize primary afferent neurons [23, 31].

A number of preclinical studies have investigated the pathophysiological mechanisms of CIBP, but few studies have specifically described the clinical presentation of pain in patients with bone metastases [3, 5, 21, 26, 38, 40]. Laird et al. reported that 75% of patients with CIBP had breakthrough pain with usually less than 5 min from start of the pain escalation until maximum pain. The duration of a breakthrough pain episode was less than 15 min [21]. CIBP is also associated with neuropathic pain, with an incidence of approximately 25% [22]. Both breakthrough pain episodes and the presence of neuropathic pain are related to more severe pain in cancer patients [20, 21].

CIBP can be difficult to treat with analgesic medications according to the WHO pain ladder [6, 21, 41]. More specific treatment options are bone-targeting agents such as bisphosphonates and RANK ligand inhibitors, anti-cancer treatments such as chemotherapy or hormonal treatments, surgical management of pathological fractures, radioisotope treatment, or external beam radiation therapy. Physical exercise may also be beneficial in patients with CIBP [24, 42]. These interventions improve pain control in many patients but have a slow onset [16, 41]. For example, the response after external beam radiotherapy to treat painful bone metastases is approximately 60%, and the median time to response is up to 4 weeks after treatment [2, 44].

Knowledge about the clinical predictors for CIBP can contribute to early intervention to avoid or delay increased pain. Current studies are mostly cross-sectional, reporting associations between clinical and demographic variables and pain at a given time point [19]. A longitudinal analysis on the clinical factors related to pain intensity in a heterogeneous cohort of cancer pain patients found that initial pain intensity, breakthrough pain, lung cancer, and age were predictors of pain 2 weeks after the initial assessment [19]. We wanted to investigate if this model could be reproduced and further developed with a more robust study design using repeated measures in a well-defined cohort, namely, patients with bone metastases only. This group of patients will have a relatively similar pathophysiology of pain and more uniform pain treatment options. Additionally, to the best of our knowledge, there are no studies examining the associations between the clinical symptoms observed at one particular time point and the risk for increased pain needing interventions within the next weeks in patients with bone metastases. Thus, we aim to explore which clinical factors are associated with high pain intensity in patients with bone metastases and which of the current factors are associated with higher pain intensity in the following month.

Methods

Study design

This paper is based on data from the European Palliative Care Cancer Symptom study (EPCCS), a prospective longitudinal multicenter study conducted from 2011 to 2013 in 12 countries across Europe, Australia, and Canada [13].

Patients

Adult cancer patients under palliative care were included in the EPCCS study [13]. Study inclusion criteria required that patients were eligible for at least one follow-up assessment. Patients receiving curative anti-cancer treatment, those with severe cognitive or psychiatric disorders, or those who were unable to complete registrations were not included. In the present analysis, only patients with bone metastases from solid cancers were included.

Assessments

Clinical data

Patients were followed approximately once every month from baseline, for at least 3 months or until death or withdrawal. If hospitalized, health care providers completed a registration form with clinical data, and patients filled in a questionnaire on symptoms and functioning. If not hospitalized, clinical data were extracted from electronic patient records and by phoning the client if necessary, and the patient questionnaires were sent by postal mail. In the present study, the following data were used: demographics, the characteristics of the cancer disease (diagnosis, distribution of metastases, current oncological treatment), Karnofsky Performance Status (KPS) [8] for functional status, a brief 4-item version of the Mini-Mental State

Examination (MMSE) [10] for cognitive function, the Edmonton Classification System for Cancer Pain (ECS-CP) [32] for neuropathic pain, and the use of analgesic medications (non-opioid analgesics and opioids).

Symptom registration by patients

Average and worst pain intensity in the last 24 h were assessed by self-report using an 11-point numeric rating scale (NRS) anchored with 0 (no pain) to 10 (worst imaginable pain) from the Brief Pain Inventory (BPI) [4, 43]. Occurrence of breakthrough pain was self-reported using the introductory question of the Alberta Breakthrough Pain Questionnaire ("Have you had flare-ups of breakthrough pain in the last 24 hours?") [12]. Other symptom intensities were registered using the Edmonton Symptom Assessment System-Revised (ESAS-R), with patient-reported symptoms on a 0–10 numerical rating scale (NRS) with 0 = no symptoms and 10 = worst possible symptoms [1]. Further, self-reported sleep disturbances and constipation from the EORTC QLQ-C15-PAL, scored on a four-point categorical scale (not at all, a little, quite a bit, very much), were used [11].

Statistical methods

The baseline characteristics of patients with bone metastases are presented with descriptive statistics. Multivariable linear regression was used to analyze factors potentially associated with pain intensity at baseline. Factors examined were chosen based upon previous literature and clinical experience: age, sex, performance status (KPS), cognitive function (MMSE), cancer diagnoses (gastrointestinal cancer including colorectal, esophageal, gastric and pancreatic cancers, lung cancer, prostate cancer, kidney and urothelial cancer, and cancer of other origin), cancer treatment (chemotherapy, radiotherapy and hormone therapy), neuropathic pain, breakthrough pain, drowsiness, nausea, depression, anxiety, trouble sleeping and constipation [19–21, 29, 36, 38]. A generalized estimating equation (GEE) model with robust standard error and exchangeable covariance structure was applied to analyze longitudinal data on which factors were associated with higher pain intensity in patients with bone metastases at the next study visit in 1 month. The exchangeable covariance structure was chosen over unstructured and order 1 autoregressive covariance structure based on the expectancy of the data output, the quasi-likelihood independence model criterion (QIC) [35], and the distribution of residuals. The choice of an optimal covariance structure can be challenging, but the GEE model is known to be robust to misspecification of the covariance structure [45]. We created a lagged variable for pain at the next visit and used the lagged variable as the dependent variable in the GEE model. Longitudinal assessments in which the interval between the two visits was outside the range of 4 weeks (± 6 days) were excluded from the analysis. A maximum of six repeated observations per person were entered into the model to ensure a balanced influence from each patient. Complete case (list wise deletion) and available case analyses (pairwise deletion) were performed respectively in cross-sectional and longitudinal regression analyses to account for missing data. The variables "sleep disturbances" and "constipation" from the EORCT-C15 were converted to a 0–10 scale to correspond with items on the ESAS-R in all of the analyses ("not at all" 0, "a little" 3.333, "quite a bit" 6.666, "very much" 10). We did not standardize any of the parameters, and the coefficients are therefore smaller for the continuous variables than for the categorical variables. All regression models were adjusted by country and use of analgesic medications, and regression diagnostics were performed for all analyses. A potential correlation between explanatory variables was controlled by calculating their impact on standard errors with variance inflation factor. Interactions between gender and primary disease were also examined. Analyses were performed with STATA version 14.2 (Stata Corporation LP; College Station, TX, USA).

Ethical considerations

The study was registered in the clinicaltrials.gov database (NCT01362816). All patients provided written informed consent, and committees for medical research ethics in each country approved the study before initiation.

Results

The total number of patients enrolled in the EPCCS study was 1739. We excluded patients with non-solid cancers, no metastases, and metastases at sites other than bone, as well as patients missing baseline information. A total of 606 patients with bone metastases were eligible for the baseline analyses.

Four hundred eleven patients were eligible in the longitudinal analyses as 146 patients had only 1 pain registration and 49 patients had a time interval between 2 subsequent visits outside the defined monthly interval (Fig. 1).

Descriptive analyses

Sample characteristics are presented in Table 1. The most common diagnoses were breast (34%), lung (22%) and prostate (18%) cancers. There was an even distribution between genders (53% female), and the mean age of the sample was 64 years (standard deviation (SD) 12.4). The average pain score in the last 24 h at baseline was 3.4 (SD 2.7), and the worst pain in the last 24 h was 4.4 (SD 3.2) (Table 1). The distribution of pain scores (NRS 0–10) at baseline is illustrated in Fig. 2. Neuropathic pain was present in 24% of the patients, and 40% of the patients had breakthrough pain episodes.

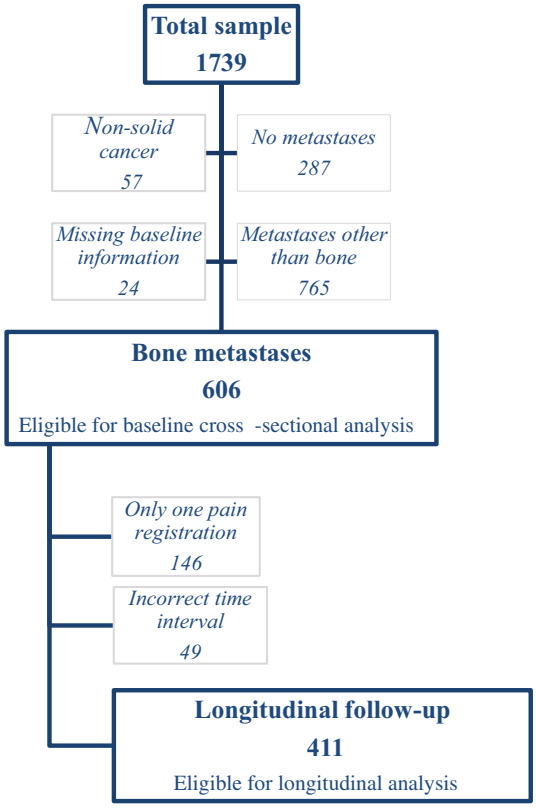

Fig. 1 Sample size

Sixty-eight percent of the patients were using opioid analgesics, and 49% were using non-opioid analgesics (Table 1).

Factors associated with pain intensity in patients with bone metastases at baseline

Twenty variables with potential associations with CIBP were entered into one multivariable model for average pain and one multivariate model for the worst pain, and both models were adjusted for country and the use of analgesic medications (Table 2). Complete case analysis included respectively 541 (89%) and 538 (89%) patients at baseline for the average and worst pain models. Breakthrough pain, neuropathic pain, and male gender were significantly associated with higher pain intensity in both the average and the worst pain model. Breakthrough pain had the strongest association with the worst pain intensity, with an increase of 2.49 (95% CI 2.00–2.97) if present. The presence of neuropathic pain influenced pain intensity in both models (increase average pain 0.89 (95% CI 0.43–1.35), worst pain 0.82 (95% CI 0.29–1.36)). Age, drowsiness, nausea, anxiety, and trouble sleeping were associated with a higher average pain intensity score but not worst pain at baseline. The explained variance (adjusted R^2) was 0.36 for the average pain model and 0.41 for the worst pain model (Table 2).

Table 1 Patient characteristics at inclusion ($N = 606$)

Characteristics	Number (%)	Mean (SD)	Missing[a]
Female sex	318 (52.5)		0
Age		64.3 (12.4)	0
Karnofsky Performance status		66.6 (15.9)	0
Abbreviated MMSE[b]		6.9 (1.7)	10
Cancer diagnosis			3
Gastrointestinal	60 (10.0)		
Lung	133 (22.1)		
Breast	207 (34.3)		
Prostate	107 (17.7)		
Kidney and urothelial	26 (4.3)		
Other origin	70(11.6)		
Oncological treatment			
Chemotherapy	244 (40.3)		0
Radiotherapy	53 (8.8)		0
Hormone therapy	145 (23.9)		0
Analgesic treatment			
Opioid analgesics	410 (68.3)		6
Non-opioid analgesics	293 (48.9)		7
Pain characteristics			
Average pain in last 24 h		3.4 (2.7)	2
Worst pain in last 24 h		4.4 (3.2)	6
Neuropathic pain[c]	143 (24.4)		19
Breakthrough pain[d]	238 (40.0)		11
Other Symptoms			
Drowsiness[e]		3.3 (2.9)	11
Nausea[e]		1.0 (2.0)	6
Feel depressed[e]		2.5 (2.8)	5
Anxiety[e]		2.4 (2.7)	5
Sleep disturbances[f]		3.2 (3.2)	4
Constipated[f]		2.8 (3.2)	7

[a] Number of patients with missing observations

[b] Abbreviated MMSE (maximum score 8) [10]

[c] Neuropathic pain from Edmonton Classifications System for Cancer Pain [33]

[d] Patient-reported flare-ups of breakthrough pain in the last 24 h

[e] Edmonton Symptom Assessment System-Revised (ESAS-r) [1]

[f] EORTC QLQ-C15-PAL [11]

Factors associated with pain intensity in one month

The same variables included in the cross-sectional analyses were applied in the longitudinal analyses, with the lagged variable for

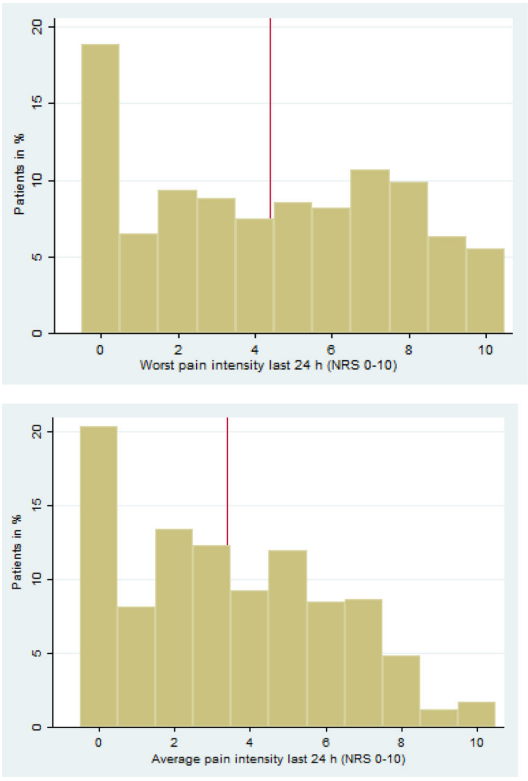

Fig. 2 Distribution of pain scores (NRS 0–10) at baseline. The vertical line represents mean pain scores

"pain the next visit" as the dependent variable. Separate models were estimated for average and worst lagged pain intensity (Table 3). Available case analysis included 396 (96%) and 392 (95%) patients for the average and worst pain models, respectively. Current pain intensity, drowsiness, trouble sleeping, and male gender were associated with more average and worst pain after 1 month. Each factor was associated with minor changes in pain intensity. Current pain had the strongest association to pain in 1 month, with a one-point increase in current pain intensity associated with a 0.41 (95% CI 0.34–0.48) increase in average pain intensity and a 0.34 (95% CI 0.26–0.42) increase in the worst pain intensity at the next visit. For the other symptoms, a one-point increase in sleep disturbances was associated with a 0.06 (95% CI 0.02–0.11) increase in average pain intensity and a 0.08 (95% CI 0.02–0.14) increase in worst pain intensity, while a one-point increase in drowsiness associated with a 0.09 (95% CI 0.03–0.15) increase in average pain intensity and a 0.07 (95% CI 0.01–0.14) increase in worst pain intensity at the next visit. Breakthrough pain at the initial time point was only significantly associated with higher worst pain intensity at the next visit, with a 0.59 (95% CI 0.20–0.99) increase in worst pain intensity. Patients with prostate cancer had a lower risk of future pain (Table 3).

Discussion

This study showed that high pain intensity, sleep disturbances, drowsiness, and male gender at the initial time point were associated with higher average and worst pain intensity at the next study visit scheduled in 1 month in patients with bone metastases. Breakthrough pain was also associated with higher worst pain intensity in 1 month. The same factors were associated with average pain intensity in the cross-sectional analyses. Although each factor in these analyses contributed to minor changes in pain intensity, they may prompt clinicians to recognize a risk for imminent lack of pain control to identify patients for closer follow-up, to consider the use of specific pain treatment modalities such as radiotherapy or other interventions like physical exercise.

A noticeable finding in the longitudinal analyses was that patients with higher pain intensity at one time point were more prone to higher pain intensity at the next visit. This association can be partly due to correlation between repeated measurements. However, the results are also supported by previous studies showing that high pain intensity itself is associated with a complex pain situation and more difficulties obtaining adequate analgesic treatment response [9]. These results are similar to results from a longitudinal study by Knudsen et al. [19] in a general cancer population, reporting that initial pain intensity was the most important factor for pain at the next consultation. Clinicians must be aware that patients who report high pain intensity are in need of special attention, as they are also more likely to present with more pain at the next study visit, regardless of the use of analgesic medication.

Similar to our study, several previous studies have demonstrated significant associations between sleep disturbances and cancer pain [7, 19, 27]. Pain can induce a lack of sleep but sleep disturbances themselves may also influence the patient's pain perception. In this study, we have further demonstrated in a longitudinal multivariate model that sleep disturbances were associated with pain in the following weeks. The present study is not designed to evaluate causality, but the longitudinal relationship between sleep disturbances and pain intensity strengthens the hypothesis that sleep disturbances also may increase the perception of pain.

Drowsiness is a known adverse effect of opioid treatment [29]. In the longitudinal analyses, we found that drowsiness was associated with both higher average and worst pain at the next visit. Adverse effects may hinder adequate titration of analgesic therapy with opioids and can explain this relationship. Similar to the other symptoms associated with cancer pain, the regression coefficients were low.

The high incidence of breakthrough pain has been used to explain some of the treatment challenges of CIBP [21, 28, 38]. The breakthrough pain incidence was 40% in this group of patients and was strongly associated with pain intensity in the cross-sectional analysis. The worst pain intensity increased by 2.49 points in patients who reported breakthrough pain, which

Table 2 Multivariate analysis of the associations with pain intensity by inclusion

	Average pain in last 24 h ($n = 541$)			Worst pain in last 24 h ($n = 538$)		
Independent	Coef	95% CI	p	Coef	95% CI	p
Constant	− 0.18	− 2.35 to 2.00	0.872	− 0.48	− 3.02 to 2.06	0.710
Age	0.02	0.00 to 0.04	0.036	0.02	− 0.01 to 0.03	0.141
Sex (female gender)	− 0.72	− 1.29 to − 0.16	0.012	− 0.90	− 1.55 to -0.25	0.007
KPS	0.01	− 0.01 to 0.02	0.257	0.01	− 0.01 to 0.02	0.456
MMS[a]	− 0.03	− 0.15 to 0.09	0.665	0.10	− 0.04 to 0.24	0.154
Cancer diagnosis:[b]						
Gastrointestinal	0.24	− 0.54 to 1.01	0.548	− 0.10	− 1.00 to 0.80	0.821
Lung	− 0.39	− 1.03 to 0.25	0.231	− 0.40	− 1.15 to 0.35	0.278
Prostate	− 0.78	− 1.57 to 0.01	0.053	− 0.70	− 1.61 to 0.23	0.139
Kidney and urothelial	− 0.25	− 1.39 to 0.88	0.659	− 0.29	− 1.61 to 1.03	0.663
Other origin	0.19	− 0.52 to 0.91	0.596	0.13	− 0.71 to 0.96	0.768
Oncological treatment:						
Chemotherapy	0.15	− 0.32 to 0.61	0.531	− 0.14	− 0.68 to 0.40	0.616
Radiotherapy	− 0.11	− 0.79 to 0.56	0.738	0.21	− 0.58 to 0.99	0.607
Hormone treatment	− 0.23	− 0.80 to 0.34	0.424	− 0.36	− 1.02 to 0.31	0.294
Pain characteristics:						
Neuropathic pain[c]	0.89	0.43 to 1.35	< 0.001	0.82	0.29 to 1.36	0.003
Breakthrough pain[d]	1.45	1.03 to 1.87	< 0.001	2.49	2.00 to 2.97	< 0.001
Other symptoms:						
Drowsiness[e]	0.08	0.01 to 0.16	0.033	0.08	− 0.01 to 0.17	0.066
Nausea[e]	0.14	0.04 to 0.24	0.008	0.10	− 0.02 to 0.22	0.109
Depression[e]	0.03	− 0.07 to 0.13	0.569	0.08	− 0.04 to 0.20	0.183
Anxiety[e]	0.14	0.03 to 0.24	0.013	0.11	− 0.01 to 0.24	0.080
Trouble sleeping [f]	0.09	0.03 to 0.16	0.004	0.07	− 0.00 to 0.14	0.061
Constipation[f]	0.05	− 0.01 to 0.12	0.091	0.05	− 0.02 to 0.13	0.150
Adjusted R-square			0.362			0.413

Analyses were adjusted for country and analgesic medications

[a] Abbreviated MMSE (maximum score 8) [10]

[b] Reference category breast cancer

[c] Neuropathic pain from Edmonton Classification System for Cancer Pain [33]

[d] Patient reported flare-ups of breakthrough pain last 24 h

[e] Edmonton Symptom Assessment System-Revised (ESAS-R) [1]

[f] EORTC QLQ-C15-PAL [11]

is consistent with findings from previous studies on cancer pain in general and CIBP [14, 19, 21]. In this study, we have further demonstrated that patients with current breakthrough pain have higher worst pain intensity at the next visit in 1 month. In the longitudinal model, the worst pain intensity increased by 0.59 if breakthrough pain was present at the previous time point. Knudsen et al. [19] reported a significant association between the presence of breakthrough pain and higher average pain score after 2 weeks in a general cancer population, but they detected no significant association with the worst pain intensity. Thus, these findings emphasize the difficulties in treating breakthrough pain episodes.

Bone metastases can involve and damage nervous tissue directly due to tumor invasion, but also by activating molecular mechanisms sensitizing primary efferent neurons [23, 25]. The pathophysiological processes of CIBP may result in neuropathic pain, and previous studies report the incidence of neuropathic pain among CIBP patients to be approximately 17–25% [17, 22]. This is consistent in our study, with 24% of patients having neuropathic pain at baseline. As in studies on general cancer pain, we found a clear association between the presence of neuropathic pain and pain intensity in patients with bone metastases in the cross-sectional analyses [14, 34]. However, in the longitudinal analyses, the presence of neuropathic pain was not associated with a future increase in pain.

In this cohort of patients with bone metastases, female patients reported lower pain intensity than men in the cross-sectional analyses, and male gender increased the risk of pain

Table 3 Longitudinal analysis on factors associated with pain intensity at the next study visit in 1 month

	Average pain in last 24 h ($n = 396$)			Worst pain in last 24 h ($n = 392$)		
Independent	Coef	95% CI	p	Coef	95% CI	p
Constant	0.99	− 0.64 to 2.62	0.235	0.32	− 1.41 to 2.05	0.716
Age	0.00	− 0.01 to 0.01	0.666	0.01	− 0.00 to 0.02	0.130
Sex (female gender)	− 0.46	− 0.91 to -0.01	0.045	− 0.91	− 1.45 to -0.36	0.001
KPS	0.00	− 0.01 to 0.01	0.690	0.00	− 0.01 to 0.01	0.958
MMS[a]	− 0.00	− 0.08 to 0.08	0.992	0.04	− 0.07 to 0.15	0.443
Cancer diagnosis:[b]						
Gastrointestinal	0.12	− 0.46 to 0.57	0.710	0.14	− 0.63 to 0.91	0.716
Lung	0.04	− 0.43 to 0.52	0.861	− 0.24	− 0.82 to 0.34	0.419
Prostate	− 0.66	− 1.23 to − 0.09	0.023	− 1.21	− 1.92 to − 0.50	0.001
Kidney and urothelial	− 0.54	− 1.38 to 0.30	0.208	− 0.90	− 1.87 to 0.08	0.073
Other origin	0.05	− 0.46 to 0.57	0.992	− 0.05	− 0.70 to 0.60	0.876
Oncological treatment:						
Chemotherapy	0.31	− 0.02 to 0.64	0.069	0.35	− 0.05 to 0.75	0.085
Radiotherapy	− 0.17	− 0.75 to 0.40	0.556	0.06	− 0.52 to 0.65	0.830
Hormone treatment	0.25	− 0.12 to 0.63	0.186	− 0.04	− 0.48 to 0.40	0.870
Pain characteristics:						
Current pain intensity	0.41	0.34 to 0.48	< 0.001	0.34	0.26 to 0.42	< 0.001
Neuropathic pain[c]	− 0.05	− 0.35 to 0.25	0.743	0.16	− 0.22 to 0.55	0.410
Breakthrough pain[d]	0.19	− 0.11 to 0.49	0.209	0.59	0.20 to 0.99	0.003
Other symptoms						
Drowsiness[e]	0.09	0.03 to 0.15	0.003	0.07	0.01 to 0.14	0.033
Nausea[e]	0.06	− 0.01 to 0.14	0.103	0.02	− 0.06 to 0.11	0.604
Depression[e]	− 0.01	− 0.09 to 0.06	0.747	0.05	− 0.04 to 0.14	0.249
Anxiety[e]	0.02	− 0.06 to 0.11	0.589	0.03	− 0.05 to 0.12	0.427
Trouble sleeping[f]	0.06	0.02 to 0.11	0.010	0.08	0.02 to 0.14	0.006
Constipation[f]	0.03	− 0.02 to 0.07	0.277	0.02	− 0.04 to 0.07	0.587

Analyses were adjusted for country and analgesic medications

[a] Abbreviated MMSE (maximum score 8) [10]

[b] Reference category breast cancer

[c] Neuropathic pain form Edmonton Classification System for Cancer Pain [33]

[d] Patient reported flare-ups of breakthrough pain last 24 h

[e] Edmonton Symptom Assessment System-Revised (ESAS-R) [1]

[f] EORTC QLQ-C15-PAL [11]

in the next visit scheduled in 1 month. Few studies have investigated differences in cancer pain between genders, and most studies report no gender differences in pain intensity [30]. To rule out a potentially different gender effect by cancer diagnosis, we tested the interactions between these two factors in all models, but none were statistically significant.

Several associations reported in other studies were not observed in this study. The assessment of psychological distress, including anxiety and depression, is included in the ECS-CP [33]. In this study, anxiety was only associated with average pain intensity at baseline, and there was no association between pain and anxiety or depression in the longitudinal model. In agreement with the longitudinal analysis on a general cancer population by Knudsen et al. [19], current pain intensity and breakthrough pain were associated with pain intensity at the next visit, but the other significant variables differed. Age and lung cancer were not associated with higher pain intensity in our model, while sleep disturbances, drowsiness, and male gender were not associated with higher pain intensity at the next visit in a general cancer population. These differences may suggest that prediction models have to be developed and validated for specific cohorts of cancer pain patients.

The potential benefit from establishing characteristics for patients with a lack of pain control is that the clinicians can be alerted to give these patients special attention. This attention may include closer follow-up or consider bone-targeting interventions, such as radiotherapy, to prevent future increases in pain. Such factors may also be included in computer-based

decision support systems [15], prompting the clinicians to address pain treatment.

Strength and limitations

Longitudinal analyses with repeated measures, as performed in the present study, increase the analytical strength of observations because the individual changes in pain and associated symptoms can be investigated. We chose to include the subgroup of patients with bone metastases only. This decision was made not only because CIBP can be classified as a unique entity of cancer pain based on pathophysiological features but also because this group of patients can receive treatment directly targeted to the bone metastases. The sample size, for a longitudinal study on palliative cancer patients, is large, and the number of missing variables is limited both in the cross-sectional and longitudinal analyses. We believe that results from this study will contribute useful information to clinicians treating patients with bone metastases with regard to (a) the symptoms and patient characteristics associated with higher pain intensity and (b) potential factors to identify patients that will develop a complex pain situation that is difficult to treat with conventional analgesics. As far as we know, this is the first study specifically addressing factors associated with higher pain intensity at the next consultation in patients with bone metastases.

We recognize that this study has some limitations. First, we included all patients with bone metastases, including those with no pain. This strategy may result in an overestimation of the correlation between pain intensity and breakthrough pain and neuropathic pain, which obviously are only present in patients with pain. However, this study analyzed patients with bone metastases in the risk for future pain, which also may arise in patients with no initial pain. Separate analyses were performed on patients with pain only and revealed the same significant associations among neuropathic pain, breakthrough pain, and pain intensity (data not shown). Second, patients with pain in the included cohort are defined as patients with CIBP, although pain due to other reasons than bone metastases can occur. Third, the selection of independent variables was limited by available variables from the original study. The use of opioids and non-opioid medications was recorded, but dosages were not registered, nor was the drug compliance. The use of bone-targeting agents such as bisphosphonates may reduce pain in patients with CIBP [16], but its use was not recorded in the present study. Only current oncological treatment was available for analyses. Oncological treatment administered before inclusion in the study or treatment initiated between two study visits could also have influenced pain intensity. Current treatment with RT is documented and reported. The use of RT was palliative in this cohort and expected to be directed against painful bone locations; however, the exact RT locations were not recorded. Potentially important variables including information about site and distribution of bone metastases, pathological fractures, or soft tissue expansion outside bone were not available. Fourth, the study did not include all eligible patients consecutively, thus introducing a risk for selection bias. Finally, patients were included in the study at different time points in their disease trajectory. On the other hand, this reflects clinical reality. There is no standardized "starting point" for pain development; thus, this has been and will remain a challenge in cancer pain studies.

In conclusion, this paper identifies higher current pain intensity, sleep disturbances, drowsiness, male gender, and breakthrough pain to be associated with future pain in patients with bone metastases. These factors should be assessed in clinical practice and may aid clinicians to identify patients with bone metastases that can benefit from closer follow-up or preventive interventions for optimal pain control. For each of the significant variables, the explained variance is low, and further research including a more detailed specter of independent variables is needed to develop predictive models for future pain in patients with bone metastases.

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Compliance with ethical standards

Conflicts of interest Kaasa S. hold stocks in EIR Solutions A/S. The other authors have no conflicts of interest.

We have collected all primary data and the journal may review the data on request.

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Paper 2

Original Article

Clinical Predictors for Analgesic Response to Radiotherapy in Patients with Painful Bone Metastases

Ragnhild Habberstad, MD, Trude Camilla S. Frøseth, MSc, Nina Aass, MD, PhD, Ellen Bjerkeset, MSc, Tatiana Abramova, MD, Elena Garcia-Alonso, MD, Mariangela Caputo, MD, Romina Rossi, MD, PhD, Jason W. Boland, MD, PhD, Cinzia Brunelli, PhD, Jo-Åsmund Lund, MD, PhD, Stein Kaasa, MD, PhD, and Pål Klepstad, MD, PhD

European Palliative Care Research Centre (PRC), Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU (R.H., T.C.S.F., P.K.), Norwegian University of Science and Technology and St. Olavs hospital, Trondheim University Hospital, Trondheim, Norway; Cancer Clinic, St. Olavs hospital, Trondheim University Hospital (R.H., T.C.S.F.), Trondheim, Norway; European Palliative Care Research Centre (PRC), Department of Oncology (N.A., S.K.), Oslo University Hospital, and Institute of Clinical Medicine, University of Oslo, Oslo, Norway; Regional Advisory Unit for Palliative Care, Department of Oncology (E.B.), Oslo University Hospital, Oslo, Norway; Dept. Oncology, Ålesund Hospital (T.A.), Møre and Romsdal Hospital Trust, Ålesund, Norway; Radiation Oncology Department Arnau de Vilanova University Hospital. IRB Lleida (E.G.-A.), España; Radiation Oncology 1, Palliative Care Pain Therapy and Rehabilitation (M.C.), Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; Palliative Care and Pain Therapy Unit (R.R.), Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS; Wolfson Palliative Care Research Centre (J.W.B.), Hull York Medical School, University of Hull, Hull, UK; Palliative Care, Pain Therapy and Rehabilitation Unit (C.B.), Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy; Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences (J.-\-Å.L.), Norwegian University of Science and Technology, Trondheim, Norway; Department of Oncology (J.-\-Å.L.), Ålesund Hospital, Møre og Romsdal Hospital Trust, Ålesund, Norway; Department of Health Sciences, Faculty of Medicine and Health Sciences (J.-\-Å.L.), NTNU Ålesund; Department of Circulation and Medical Imaging (P.K.), Norwegian University of Science and Technology (NTNU), Trondheim, Norway; Department of Anesthesiology and Intensive Care Medicine (P.K.), St Olavs Hospital, Trondheim University Hospital, Trondheim, Norway

Abstract

Background. Radiotherapy (RT) reduces pain in about 60% of patients with painful bone metastases, leaving many patients without clinical benefit. This study assesses predictors for RT effectiveness in patients with painful bone metastases.

Materials and methods. We included adult patients receiving RT for painful bone metastases in a multicenter, multinational longitudinal observational study. Pain response within 8 weeks was defined as ≥2-point decrease on a 0−10 pain score scale, without increase in analgesics; or a decrease in analgesics of ≥25% without increase in pain score. Potential predictors were related to patient demographics, RT administration, pain characteristics, tumor characteristics, depression and inflammation (C-reactive protein [CRP]). Multivariate logistic regression analysis with multiple imputation of missing data were applied to identify predictors of RT response.

Results. Of 513 eligible patients, 460 patients (90 %) were included in the regression model. 224 patients (44%, 95% confidence interval (CI) 39%−48%) responded to RT. Better Karnofsky performance status (Odds ratio (OR) 1.39, CI 1.15−1.68), breast cancer (OR 2.54, CI 1.12−5.73), prostate cancer (OR 2.83, CI 1.27−6.33) and soft tissue expansion (OR 2.00, CI 1.23−3.25) predicted RT response. Corticosteroids were a negative predictor (OR 0.57, CI 0.37−0.88). Single and multiple fraction RT had similar response. The discriminative ability of the model was moderate; C-statistic 0.69.

Conclusion. This study supports previous findings that better performance status and type of cancer diagnosis predicts analgesic RT response, and new data showing that soft tissue expansion predicts RT response and that corticosteroids is a negative

Abbreviations: RT, Radiotherapy; CRP, C-reactive protein; CT, Computerized tomography; LANSS, The Leeds Assessment of Neuropathic Symptoms and Signs; PHQ9, The Patient Health Questionnaire Depression Scale; MI, Multiple imputation; MICE, Multivariate imputation by chained equations; MAR, missing at random; CC, Complete case analysis; C-statistics, Concordance statistics

Address correspondence to: Ragnhild Habberstad, MD, European Palliative Care Research Centre (PRC), Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU). P.O.Box 8905. 7491 Trondheim, Norway, E-mail: ragnhild.habberstad@gmail.com

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Key Words
Cancer, radiotherapy, palliative, bone metastases, pain, inflammation

Key Message

This is the first prospective multicenter study to investigate predictors of RT response in patients with painful bone metastases. Performance status, cancer diagnosis and soft tissue expansions predicted RT response, while use of corticosteroids was a negative predictor. These results may be helpful in selecting patients for palliative RT.

Introduction

Pain is a frequent and feared consequence of cancer. Bone metastases are the cause of pain in in up to 45 % of patients with cancer pain.[1,2] Treatment of painful bone metastases include analgesic medications combined with anti-cancer treatment including radiotherapy (RT). RT is well-established for painful bone metastases with about 60% of patients that respond to treatment. In the non-responders other pain reliving interventions may be delayed waiting for a potential RT response that can occur weeks after treatment.[3] Many patients with bone metastases have a short life expectancy, and it is important to avoid ineffective treatments that are time-consuming and have a risk of adverse effects.

In previous trials investigating clinical predictors of RT response in patients with painful bone metastases, breast or prostate cancer and better performance status have been associated with RT response.[4,5] Higher baseline pain intensity, absence of visceral metastasis, the use of opioids and younger age may increase the likelihood of RT response, but the published results are inconsistent.[4−7] Neuropathic pain, physical activity and spinal metastases have not predicted RT response.[8−11] Depression is associated with pain in cancer patients, but as far as we know it is not previously investigated in respect to RT response.[12]

Different imaging techniques are investigated in respect to RT response in patients with painful bone metastases, but the findings are so far inconclusive.[11] Radiological scans may reveal soft tissue expansions outside bone or classify metastases as osteolytic or osteoblastic (sclerotic). A small trial on spinal bone metastases concluded with no significant difference in analgesic response rates if soft tissue expansions were present.[13] As far as we know analgesic RT response in osteolytic metastases compared to osteoblastic (sclerotic) metastases are not previously investigated based on radiological appearance, but two trials have reported increased levels of urinary osteoclast markers in patients with no RT response.[14,15]

The mechanisms of pain relief after RT is partly due to shrinkage of the tumor volume. The immediate effect of RT is also proposed to be related to inhibition of inflammatory mediators.[16] Pre-clinical studies have demonstrated the importance of inflammatory mediators in cancer induced bone pain,[17] and in one study the systemic inflammatory biomarker C-reactive protein (CRP) was associated with cancer pain intensity.[18]Only two previously published papers have investigated multiple factors of RT response,[4,5] both resulted in a predictive model with low to moderate discriminative ability. One reason for this may be that relevant predictors were not included in the models. The present study was designed to in addition to established predictors add the potential predictors radiological appearance of metastases, pain characteristics, depression and inflammation to the model in order to observe if this improves the ability to appropriately select patients for RT.[11] Thus. the aim of this prospective, multicenter study was to investigate which factors are associated with RT response in patients with painful bone metastases.

Material and Methods

Study Population

Adult patients (≥18 years) with a verified cancer diagnosis about to undergo RT with a palliative intent for painful bone metastases were included in this longitudinal observational multicenter study. RT was initiated within one week after baseline observations. Patients who received RT within the preceding 4 weeks before the study and patients with long bone pathological fractures were not included. Patients with several RT treatments were included in the study once. Patients with RT indications other than pain, such as spinal cord compression with a risk for paralysis, were not included. Enrolled patients with a worst pain score less than two at baseline were not included in analyses.[19] Patients were recruited from seven oncological centers across Europe (Norway, Italy, Spain and UK) from December 2013 to December 2017.[20] Collaborating centers in the European Palliative Care Research Centre were invited to contribute in the PRAIS study. Study information were also distributed at

international meetings and congresses prior to initiation of the study. Before the start of inclusion, the study was registered at ClinicalTrials.gov (NCT02107664).

Study Procedure and Outcome Measures

The following information were collected: age, gender, cancer diagnosis, osteolytic metastases and soft tissue expansion at each radiation site assessed by Computerized Tomography (CT) before RT (yes/no/not evaluable), RT fraction and total dose, location in weight bearing bone (yes/no), opioid dose (oral morphine equivalents last 24hours)[21] and the use of corticosteroids (yes/no). Comorbidity and performance status were assessed by Charlson Comorbidity Index (range 0-37)[22] and Karnofsky performance status (range 0−100),[23] respectively. Patient reported outcomes were: pain at rest and at movement from each irradiated site (11-point numeric rating scale); with the worst baseline pain score used in calculate RT response, episodic pain (yes/no), neuropathic symptoms assessed by The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) (range 0−5) [24] and depressive symptoms assessed by The Patient Health Questionnaire Depression Scale (PHQ9) (range 0−29).[25] Blood samples were taken before RT, and CRP was analyzed at the local laboratory in each study center. Baseline observations used in the predictive analyses of RT response were obtained within one week before the start of RT. To calculate RT response self-reported pain scores and opioid doses were obtained at 3 and 8 weeks after the last RT fraction (+/- 3 days). We aimed to consecutively include all patients admitted to RT for painful bone metastases, although this was not possible due to organizational issues in three of the including sites. If patients were unable to attend the hospital for follow-up, one of the investigators contacted the patients by phone and patients received the questionnaires by postal mail. A written guidance for data recording was distributed to all collaborating centers, and the centers could at all times contact the principal investigator. All results were manually controlled by two of the investigators (RH, TCSF) and if inconsistencies the recorded result were checked by the local investigator.

Response Definition

The primary outcome was "response to RT for painful bone metastases". Response within 8 weeks after the last RT fraction was defined as at least a 2-point decrease in the worst pain score at the irradiated site without increase in analgesic use or reduction in opioid dose of at least 25% from baseline without an increase in pain score at the irradiated site.[26] Patients with two or more radiation locations were defined as responders if they responded in at least one of the irradiated sites.

Statistical Analyses

Sample size calculation was based upon analgesic RT response as the primary dependent variable. A full statistical estimate of sample size requires knowledge of the variance-covariance matrix, which was not available at the planning stage of this study. Therefore, the widely used rule of thumb of 10 x number of variables was adopted and resulted in a need of 290 patients to be included in the study. To account for patients lost to follow up and possible unknown interactions, the number of patients was set to 600. The original protocol plan was to include a validation sample of additional 400 patients, but because of slow recruitment the analyses were performed without the planned validation sample.[20] Continuous variables are presented as means with standard deviation (SD) and categorical variables as frequencies with percentages. Potential predictors for RT response were chosen based upon previously described associations,[11] and putative clinical relationship. The 17 independent variables included in analyses are detailed in Table 3. Multivariate logistic regression analysis with multiple imputation (MI) of missing data using multivariate imputation by chained equations (MICE) were applied to identify predictors of RT response.[27] All potentially relevant variables were included in the multivariate model, without performing any variable selection in order not to lose any relevant correlation in the selection process and to obtain confidence intervals with proper coverage.[28] Missing variables were considered missing at random (MAR) and MI was chosen as it allows a considerable gain in estimates efficiency and is less biased than complete case analysis (CC) across a number of scenarios.[29] Missing variables were imputed 25 times. Patients missing the outcome variable (unknown RT response) were excluded from the analysis after imputation.[27] For the PHQ9 and LANSS score, missing items were replaced with the average value if less than half of the items were missing.[30] Imputation diagnostics were performed for all analyses. Since MI is not always better than CC for missing covariate problems,[29] sensitivity analyses (CC analysis, worst case analysis and best case analysis) were also performed to evaluate the strength of the imputed model (Supplementary 1). All regression models were adjusted by study centre in order to account for a potential centre effect [31]. Concordance statistics (C-statistics) were used to determine the goodness of fit.[32] Predictive probabilities were estimated from the complete case model for descriptive purposes. All analyses are performed using STATA v16 (Stata Corporation LP; College Station, TX, USA).

Ethics

A signed informed consent was obtained from all patients. 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

A total number of 574 patients were enrolled in the study. Sixty-one patients were not included in the analyses (Fig. 1). Complete data were available in 382 patients (74%), while 100 patients (19 %) had one missing variable and 31 patients (6%) had 2 or more missing variables.

Sixty-one percent were men and the most common cancer diagnoses were prostate (26%), breast (20%), lung (18%) and gastrointestinal (16%) cancer. The mean age was 66.1 years (SD 10.6). Multiple fractions and single fraction RT were given to 63% and 37% of patients, respectively (Table 1). The most common RT locations were spine (45%), pelvis (34%) and thorax (9%). Twenty-seven patients died within the first 3 weeks after RT and were not included in the final analysis and 67 patients died between 3 and 8 weeks after RT.

Of included patients 224 (44%, CI 39%-48%) responded to RT and 236 (46%, CI 42%-50%) did not respond to RT. Fifty-three (10%, CI 8%−13%) had an unknown RT response (Fig. 1). Among the 67 patients dying between 3 and 8 weeks after RT only 8 patients (12%) responded to RT. Baseline variables by response status are described in Table 2. Multiple imputation allowed the final regression models to be carried out on 460 patients (90% of the sample) after excluding the 53 patients with unknown RT response.

Better performance status (OR 1.39, CI 1.15−1.68), primary diagnosis of breast cancer (OR 2.54, CI 1.12−5.73) or prostate cancer (OR 2.83, CI 1.27−6.33) and soft tissue expansion outside bone (OR 2.00, CI 1.23−3.25) predicted RT response (*P*-value <0.05). The use of corticosteroids was a negative predictor for RT response (OR 0.57, CI 0.37-0.88). The discriminative ability of the model was moderate, with a C-statistic of 0.69 (Table 3, Fig. 2).

In patients with normal CRP 48 % responded to RT compared to 42% in patients with elevated CRP (Table 2), but CRP was not statistically significant in the multivariate model. There was no difference in response rates among patients receiving single compared to multiple RT fractions (Table 3). Sensitivity analyses with complete case analysis and patients with unknown RT response as worst case and best case displayed similar findings (Supplementary table 1).

Discussion

Our study shows that better performance status, primary cancer diagnosis of breast or prostate and presence of soft tissue expansion outside bone can positively predict effect of RT on bone pain in patients with painful bone metastases. The use of corticosteroids was a negative predictor for RT response.

In a systematic review, Gardner et al. identified eight studies evaluating clinical predictors for RT response.[4,5,7,9-11,33-35] Only two studies, both secondary analyses, included several potential predictors in a multivariate analysis.[4,5] Proposed factors in these two studies that could influence response to RT were breast and prostate as the primary cancer, high pain intensity, absence of visceral metastases, younger age, the use of opioids and better performance status. Both studies reported a low to moderate discriminative ability.[4,5] Based upon the findings, Gardner et al.[11] concluded

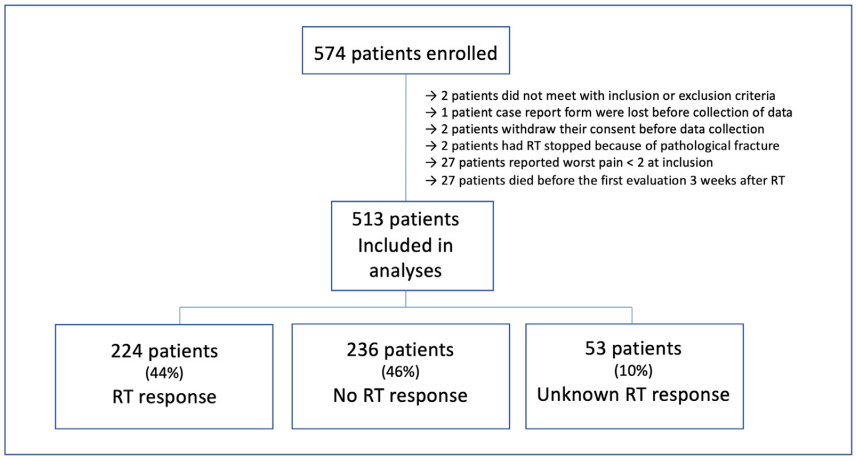

Fig. 1. Flowchart of included patients. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article

Table 1
Patient Characteristics at Baseline (N 513)

Baseline Variables	Number (%)	Mean (SD)
Age		66.1 (10.6)
Gender		
Male	314 (61%)	
Female	199 (39%)	
Charlson comorbidity index		6.5 (0.92)
Karnofsky performance status		72.6 (12.1)
Cancer diagnosis		
Gastrointestinal	81 (16%)	
Breast	103 (20%)	
Prostate	133 (26%)	
Lung	92 (18%)	
Urological	56 (11%)	
Other/unknown	48 (9%)	
Metastases		
Other sites than bone	319 (62%)	
Only bone	194 (38%)	
RT fraction		
Multiple fraction	325 (63%)	
Single fraction <=8 Gy	188 (37%)	
Soft tissue expansion at radiated site		
No	337 (66%)	
Yes	165 (32%)	
Not evaluable	11 (2%)	
Osteolytic metastases at radiated site		
No	289 (56 %)	
Yes	189 (37 %)	
Not evaluable	35 (7 %)	
Radiation location in weight bearing bone		
No	78 (15 %)	
Yes	435 (85 %)	
Maximum pain at radiated site last 24h		5.9 (2.2)
Episodic pain		
No	178 (35 %)	
Yes	313 (61%)	
Neuropathic pain symptoms (a)		1.1 (1.2)
Opioid dose (b)		75.0 (143.7)
Corticosteroids		
No	278 (54%)	
Yes	232 (45%)	
Depressive symptoms (c)		8.0 (4.8)
CRP		
Normal (<=5)	188 (37%)	
Elevated (>5)	281 (55%)	
Study center		
Lleida	19 (4 %)	
Milan	41 (8 %)	
Forli	26 (5%)	
Trondheim	212 (41%)	
Oslo	157 (31%)	
Aalesund	44 (9%)	
Hull	14 (3%)	

(a) Number of self-reported symptoms of neuropathic pain according to LANSS
(b) Opioid dose in oral morphine equivalents last 24h
(c) Number of depressive symptoms according to PHQ 9

that no clinical markers are applicable for clinical use. The lack of studies primarily designed to evaluate RT response for cancer bone pain warranted a prospective study primarily designed to identify multiple predictors for RT response.[4,5] Our study confirmed that better performance status and that a diagnosis of either breast or prostate cancer increased the chance of a benefit from palliative RT in patients with painful bone metastases.

In addition to previously proposed predictors for RT response we included information on tumor characteristics, inflammation, pain characteristics and depression. We observed that patients with soft tissue expansion outside the bone had 100% higher odds of responding to RT compared to patients without soft tissue expansion. A possible explanation for this finding is that patients with a soft tissue mass in relation to bone metastases might have more inflammation and edema causing pain and therefore are more responsive to RT. Our observation is opposite to Mitera et al.[13] who did not observe an association with soft tissue expansion and RT response. However, Mitera et al.[13] included only 33 patients all with spinal bone metastases which is a different sub-cohort compared to our sample.

Chow et al.[15] observed a higher level of urinary osteoclast markers in patients not responding to RT. Therefore, as osteolytic metastases have higher osteoclast activity, it could be expected that patients with osteolytic metastases had less RT response compared to sclerotic bone metastases. Despite this potential association, we did not observe a significant difference in RT response in respect to osteolytic versus sclerotic metastases.

It is suggested that the early pain relief from RT is due to an effect on inflammation.[16] A relationship between high level of inflammatory biomarkers and bone cancer pain intensity has been demonstrated.[17] CRP was chosen as a potential systemic inflammatory biomarker for RT response as it is associated with cancer pain and is routinely available.[18] In the multivariable model CRP did not predict RT response. CRP is a crude measure of inflammation and analyses on more specific inflammation biomarkers may reveal a relationship. Also, a RT effect on inflammation related to the bone metastases and surrounding tissue may not be reflected in a systemic inflammatory biomarker. Interestingly, patients using corticosteroids at baseline had a 57% lower odds of RT response compared to patients not using corticosteroids. Corticosteroids are known to reduce inflammation and are proposed to reduce the incidence of pain flares after RT.[36,37] One potential explanation for corticosteroids being a negative predictor for RT response is that the anti-inflammatory effect of RT is already induced by the corticosteroids which reduce the additional effect of RT. Corticosteroids are widely administered to patients with metastatic cancer disease and if has a negative impact on RT response, this could lead to a change in clinical practice. In this study a dose response relationship could not be investigated because corticosteroid doses were not available, nor was the analyses performed to evaluate the potential effect of corticosteroids as an adjunct during or after RT. Further research on the impact of corticosteroids on RT response is warranted.

Table 2
Baseline Variables by RT Response Status (N 513)

Baseline Variables	RT Response n (%)	No RT Response n (%)	Unknown RT Response n (%)
Age			
<50	22 (46 %)	20 (42 %)	6 (13 %)
51-70	125 (46 %)	127 (47 %)	21 (8 %)
>70	77 (40%)	89 (46 %)	26 (14 %)
Gender			
Male	138 (44 %)	143 (46 %)	33 (11 %)
Female	86 (43 %)	93 (47 %)	20 (10 %)
Charlson comorbidity index			
6 (only metastatic cancer disease)	145 (43%)	157 (47 %)	35 (10 %)
>6 (other comorbidities)	79 (45 %)	79 (45 %)	18 (10 %)
Karnofsky performance status			
<50	9 (25%)	18 (50%)	9 (25 %)
50−70	98 (38 %)	135 (52%)	25 (10 %)
80−100	117 (53%)	83 (38 %)	19 (9 %)
Cancer diagnosis			
Gastrointestinal	26 (32 %)	43 (53 %)	12 (15 %)
Breast	53 (51 %)	41 (40 %)	9 (9 %)
Prostate	67 (50 %)	53 (40 %)	13 (10 %)
Lung	36 (39 %)	50 (54 %)	6 (7 %)
Urological	23 (41 %)	29 (52 %)	4 (7 %)
Other/unknown	19 (40 %)	20 (42 %)	9 (19 %)
Metastases			
Other sites than bone	129 (40 %)	157 (49%)	33 (10 %)
Only bone	95 (49 %)	79 (41 %)	20 (10 %)
RT fraction			
Multiple fraction	140 (43 %)	147 (45 %)	38 (12 %)
Single fraction <=8 Gy	84 (45 %)	89 (47 %)	15 (8 %)
Soft tissue expansion at radiated site			
No	142 (42%)	162 (48 %)	33 (10 %)
Yes	79 (48 %)	67 (41 %)	19 (12 %)
Not evaluable	3 (27 %)	7 (64 %)	1 (9 %)
Osteolytic metastases at radiated site			
No	125 (43 %)	137 (47 %)	27 (9 %)
Yes	87 (46 %)	83 (44 %)	19 (10 %)
Not evaluable	12 (34 %)	16 (46 %)	7 (20 %)
Radiation location in weight bearing bone			
No	34 (44%)	37 (47 %)	7 (9 %)
Yes	190 (44 %)	199 (46%)	46 (11 %)
Maximum pain at radiated site last 24h			
2 – 4	56 (39%)	70 (49 %)	16 (11 %)
5 – 7	108 (47%)	102 (44 %)	21 (9 %)
8 t– 10	60 (43%)	64 (46%)	14 (10 %)
Episodic pain			
No	80 (45 %)	79 (44 %)	19 (11 %)
Yes	133 (42 %)	151 (48 %)	29 (9 %)
Neuropathic pain symptoms (a)			
No symptoms	93 (44 %)	98 (46 %)	21 (10 %)
One or more symptom	123 (44%)	130 (46 %)	27 (10 %)
Opioid dose (b)			
No opioids	57 (55 %)	41 (39 %)	6 (6 %)
< 60 mg	111 (46 %)	109 (45 %)	23 (9 %)
61−150 mg	33 (35 %)	51 (54 %)	11 (12 %)
>150 mg	23 (33 %)	34 (49%)	12 (17 %)
Corticosteroids			
No	138 (50 %)	117 (42 %)	23 (8 %)
Yes	86 (37 %)	117 (50 %)	29 (13 %)
Depressive symptoms (c)			
0−9	158 (48 %)	149 (45 %)	25 (8 %)
>=10	60 (38 %)	75 (48%)	22 (14 %)
CRP			
Normal (<=5)	91 (48 %)	77 (41 %)	20 (11 %)
Elevated (>5)	117 (42 %)	138 (49 %)	26 (9 %)

(a) Number of self-reported symptoms of neuropathic pain according to LANSS
(b) Opioid dose in oral morphine equivalents last 24h
(c) Number of depressive symptoms according to PHQ 9

Table 3
Multivariate Logistic Regression Model of Predictors of RT Response (N 460)

Independent variables	OR	95% CI
Age	0,99	[0.97,1.01]
Gender		
Male	1,00	[.,.]
Female	0,97	[0.54,1.75]
Charlson comorbidity index	1,12	[0.90,1.40]
Karnofsky performance status	**1,39*** **	**[1.15,1.68]**
Cancer diagnosis		
Gastrointestinal	1,00	[.,.]
Breast	**2,54***	**[1.12,5.73]**
Prostate	**2,83***	**[1.27,6.33]**
Lung	1,29	[0.61,2.71]
Urological	1,29	[0.58,2.89]
Other/unknown	1,60	[0.65,3.93]
Metastases		
Other sites than bone	1,00	[.,.]
Only bone	1,27	[0.80,2.02]
RT fraction		
Multiple fraction	1,00	[.,.]
Single fraction <=8 Gy	1,29	[0.80,2,09]
Soft tissue expansion at radiated site		
No	1,00	[.,.]
Yes	**2,00****	**[1.23,3.25]**
Not evaluable	0,68	[0.12,3.89]
Osteolytic metastases at radiated site		
No	1,00	[.,.]
Yes	1,18	[0.74,1.89]
Not evaluable	0,92	[0.34,2.47]
Radiation location in weight bearing bone		
No	1,00	[.,.]
Yes	1,24	[0.70,2.21]
Maximum pain at radiated site last 24h	1,07	[0.96,1.19]
Episodic pain		
No	1,00	[.,.]
Yes	0,91	[0.56,1.48]
Neuropathic pain symptoms (a)	0,99	[0.84,1.18]
Opioid dose (b)	1,00	[1.00,1.00]
Corticosteroids		
No	1,00	[.,.]
Yes	**0,57***	**[0.37,0.88]**
Depressive symptoms (c)	0,99	[0.94,1.04]
CRP		
Normal (<=5)	1,00	[.,.]
Elevated (>5)	0,91	[0.58,1.44]
C-statistics	**0,69**	

*** p<0.05, ** p<0.01, *** p<0.001**
(a) Number of self-reported symptoms of neuropathic pain according to LANSS
(b) Opioid dose in oral morphine equivalents last 24h
(c) Number of depressive symptoms according to PHQ 9

Several potential clinical variables did not significantly predict the response to palliative RT for bone cancer pain in the present study. This includes variables such as pain intensity,[4] age,[5] absence of visceral metastases,[5] tumor location[9,35] and neuropathic pain.[10] Furthermore, clinical factors not previously studied, such as episodic pain and depression, did not predict RT response. As expected, there was no difference in response rates among patients receiving single fraction RT compared to multiple fraction RT. Although single fraction RT is recommended for treatment of uncomplicated bone metastases and several studies have shown similar response rates,[3] only 37% of the patients included in this study received single fraction RT. This is a surprising finding given the available data and the obvious benefit for the patients with single fraction RT and can probably be explained by a lack of implementation of new evidence in clinical practice.

In our study 44 % of the patients responded to RT, which is lower than the average response rate of about 60 % in the latest systematic review by Rich et al.[3] Studies included in the latest systematic reviews reports a wide range of response rates. This probably reflects a variety in design between studies, but also differences in study populations and possible differences in radiation techniques.[3,26,38] The PRAIS study was designed to reflect a real life clinical practice. RT response were calculated according to international consensus and we included both outpatients and patients admitted to hospital. We also chose to include all patients where clinicians had evaluated the patient to be a candidate for RT due to painful bone metastases and did not apply a study specific cut-off concerning the self-reported level of pain. This might have increased the number of non-responders compared to other studies.

In our study 94 patients (16% of enrolled patients) died within 8 weeks after RT administration and this concurs with what is previously reported.[4] The response rate in patients dying between 3 and 8 weeks was only 12 %. RT given towards the end of life may not be beneficial for patients if it causes additional distress due to travelling, treatment planning and administration. RT late in the disease trajectory may be due to the known difficulties in defining a prognosis for advanced cancer patients, but it could be speculated that some patients should have been referred for palliative RT earlier.

The clinical aim of studies identifying predictors for response to palliative RT for bone cancer pain is to stratify patients to receive or not receive RT. This study demonstrates that performance status is one of the most important variables to predict RT response. We found that response rates more than doubled in patients with Karnofsky performance status >80 compared to performances status <50. Patients with a cancer diagnosis of breast or prostate cancer and patients with soft tissue expiation outside bone did also have significantly better response rates, although it is difficult to select patients for RT based on these features alone (Table 3, Fig. 2).

The discriminative ability of the model was higher than in the previously published secondary analysis by Van der Velden who presented a risk score calculation.[4] Still, we chose to not develop a specific predictive score for RT response based on the current findings. In order to be clinically useful a clinical risk score should give a certain cut-off value which reliably discriminate patients, a feature not available in previous studies or in the current study. However, we and others have identified clinical features (Fig. 2) which the clinicians should

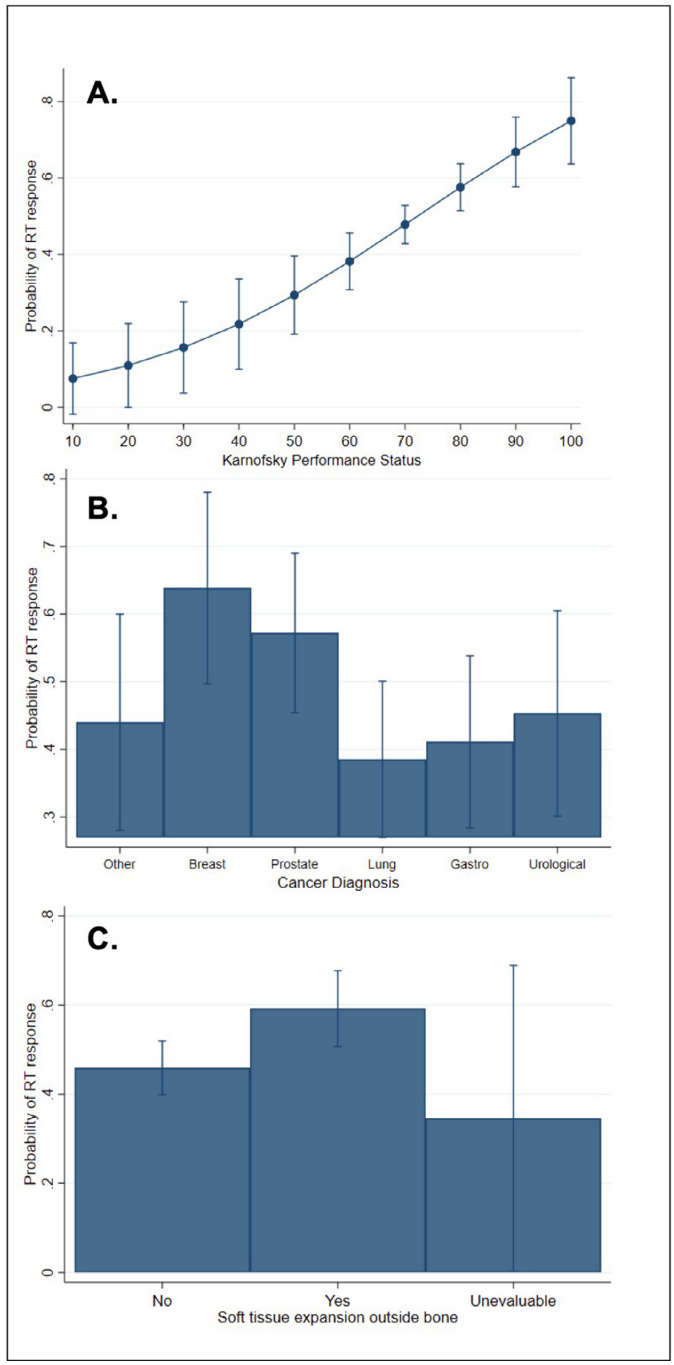

Fig. 2. Predictive probabilities based on the complete case model for A. Karnofsky performance status, B. Cancer diagnosis, C. Soft tissue expansion outside bone. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

take into consideration for RT planning together with other factors such as RT availability, patient preferences, expected adverse effects, travelling distance and to which extent pain can be controlled by analgesics.

This study has strengths and limitations. Strengths of the study are the prospective design, the large patient number, patients included from several study centers, that it was primarily designed to identify predictors of response to palliative RT, that relevant markers for tumor characteristics and inflammation were included, and that the study reflects real life clinical practice. A limitation of the study is that we have not included an analysis from a replication cohort. Second, as expected in a clinical cancer pain study several patients have one or more missing variables and some patients are lost to follow-up due to death or other causes. It is plausible that these patients have a more severe disease or higher symptom burden than patients able to complete the study procedure. However, complete-case, worst-case and best- case sensitivity analyses showed stable values in the different models. Missing variables are a shared issue in research in palliative cancer patients, and the number of missing variables in this study was similar or lower than in the previous multivariate analyses on RT predictors.[4,5] Third, we did not assess the incidence and intensity of short-term adverse effects from RT therapy. Such adverse effects are factors in a risk/benefit assessment. Forth, as in all other studies using the consensus definition for RT response the opioid dose might be increased because of pain in other sites than the irradiated one, introducing a potential bias with regard to RT effect. Finally, the participating centers may not be representative for other treating centers due to local differences in admission, treatment planning and distribution of RT. Most patients were consecutively included in the study, but in three of the participating study centers only a minor part of the treated patients was included, and there was not an even distribution of patients between the four countries.

Conclusion

In conclusion, this prospective, multicenter, clinical study showed that better performance status, breast or prostate cancer and presence of soft tissue expansion outside bone predicted RT response in patients with painful bone metastases. Inflammation measured with CRP was not a predictor for RT response, but patients using corticosteroids had significantly lower response rates.

Disclosures and Acknowledgments/Research support

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The authors have declared no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jpainsymman.2021.03.022.

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Paper 3

Original Article

Inflammatory Markers and Radiotherapy Response in Patients With Painful Bone Metastases

Ragnhild Habberstad, MD, Nina Aass, MD, PhD, Tom Eirik Mollnes, MD, PhD, Jan Kristian Damås, MD, PhD, Cinzia Brunelli, PhD, Romina Rossi, MD, PhD, Elena Garcia-Alonso, MD, Stein Kaasa, MD PhD, and Pål Klepstad, MD, PhD

European Palliative Care Research Centre (PRC), Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU (R.H., P.K.), Norwegian University of Science and Technology and St. Olavs hospital, Trondheim University Hospital, Trondheim, Norway; Cancer Clinic, St. Olavs hospital (R.H.), Trondheim University Hospital, Trondheim, Norway; European Palliative Care Research Centre (PRC), Department of Oncology (N.A., S.K.), Oslo University Hospital, and Institute of Clinical Medicine, University of Oslo, Oslo, Norway; Department of Immunology (T.E.M.), Oslo University Hospital, Oslo, Norway; Research Laboratory, Nordland Hospital (T.E.M.), Bodø, Norway; KG Jebsen Thrombosis Research and Expertise Center, Faculty of Health Sciences (T.E.M.), University of Tromsø, Tromsø, Norway; Centre of Molecular Inflammation Research, Department of Cancer Research and Molecular Medicine (T.E.M., J.K.D.), Norwegian University of Science and Technology, Trondheim, Norway; Department of Infectious Diseases, St. Olav's Hospital (J.K.D.), Trondheim, Norway; Palliative Care, Pain Therapy and Rehabilitation Unit, Fondazione IRCCS Istituto Nazionale dei Tumori (C.B.), Milano, Italy; Palliative Care Unit IRCCS- Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"-IRST-Srl, Meldola, Italy; Radiation Oncology Department Arnau de Vilanova University Hospital (E.G.A.). IRB Lleida, España; Department of Circulation and Medical Imaging (P.K.), Norwegian University of Science and Technology (NTNU) Trondheim, Norway; Department of Anesthesiology and Intensive Care Medicine, St Olavs Hospital (P.K.), Trondheim University Hospital, Trondheim, Norway

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. *© 2022 The Authors. Published by Elsevier Inc. on behalf of American Academy of Hospice and Palliative Medicine. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)*

Key Words

Cancer, bone metastases, pain, radiotherapy, inflammation

Trial registration: ClinicalTrials.gov NCT02107664.

Address correspondence to: Ragnhild Habberstad, MD, European Palliative Care Research Centre (PRC), Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology (NTNU), P.O. Box 8905, 7491 Trondheim, Norway. E-mail: ragnhild.habberstad@gmail.com

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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

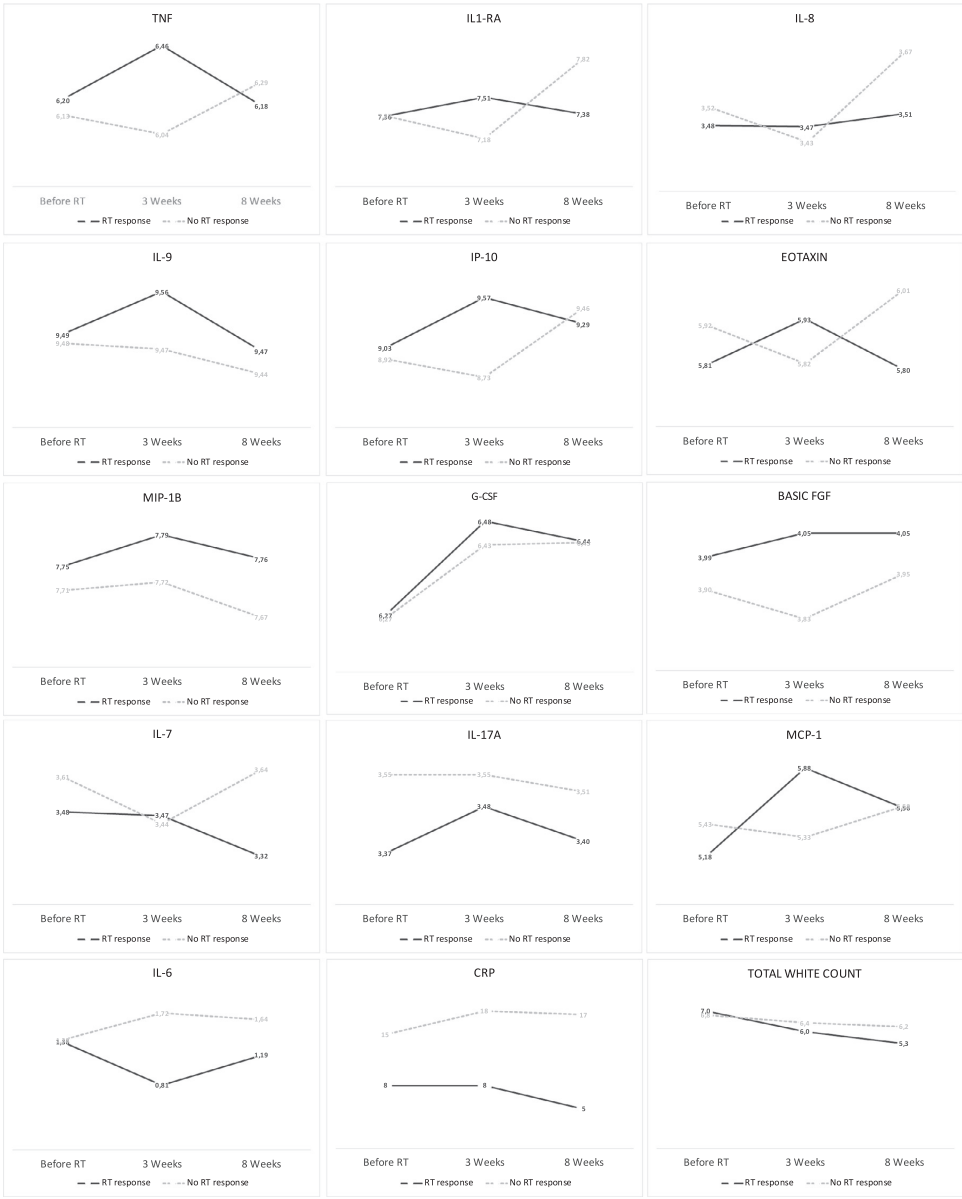

Fig. 1. Median level of inflammatory biomarkers in RT responders compared to non-responders**.** On the x axis time after RT (Before RT, three weeks and eight weeks post RT). On the y axis the median level of inflammatory biomarkers.

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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