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**REVIEW ARTICLE**

# Collagenous and non-collagenous biochemical markers of bone metastases from prostate cancer

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**Abstract**

The importance of the bone microenvironment to the pathophysiology and morbidity associated with prostate cancer bone metastasis is becoming increasingly apparent. Significant alterations take place in the microenvironment of bone, which disturb the normal coupling that exists between bone resorption and bone formation. Consequently, a better understanding of the mechanisms that interact at the molecular level will definitely result in more effective therapy for patients with this devastating complication of prostatic carcinoma. This review will discuss the diagnostic and predictive implications of various collagenous and non-collagenous bone markers, along with the novel markers of osteoclastogenesis and other matrix enzymes such as metalloproteinases and growth factors responsible for the complex biochemical mechanisms that upregulate bone resorption/formation during the development of metastasis. Further prospective studies are needed to determine whether any of these markers measured longitudinally in prostate cancer patients without bone scan evidence of skeletal disease will ultimately predict those patients who will develop bone metastases from their malignancy. Nonetheless, from the clinical point of view it is important to know that these novel markers carry the potential to provide meaningful information for daily practice by using upper normal reference values as cut-offs for identifying patients with an increased risk of developing progressive bone disease or skeletal related events. Hippokratia 2010; 14 (3): 164-169

**Key words:** Prostate cancer, bone metastases, bone turnover markers, type I collagen, RANKL, osteoprotegerin, review

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The importance of the bone microenvironment to the pathophysiology and morbidity associated with prostate cancer bone metastasis (PCBM) is becoming increasingly apparent. At the same time the identification of new biochemical pathways that control it, has allowed for the development of novel approaches aimed at preventing the devastating complications of bone metastases. Bone metabolism is characterized by two opposite activities, the formation of new bone by osteoblasts and the resorption of old bone by osteoclasts. Both normally are tightly coupled in time and space in a sequence of events that define the same remodelling unit. In the patients with PCBM, significant alterations take place in the microenvironment of bone, which disturb the normal coupling that exists between bone resorption and bone formation. At the skeletal site of metastatic invasion, the bone metabolism is dysregulated and bone resorption or bone formation, or both, is upregulated[1]. To initiate successful preventive therapy it is thus imperative that the bone metastases are detected early. This review will discuss the diagnostic and predictive implications of various collagenous and non-collagenous bone markers, along with the novel markers of osteoclastogenesis and other matrix enzymes responsible for the complex biochemical mechanisms that upregulate bone resorption/formation during the development of bone metastasis in patients suffering from prostate cancer.

## I. Traditional non-collagenous matrix proteins

Osteocalcin (OC) is a small non-collagenous matrix protein of bone that derives from new cellular synthesis and is a marker for bone formation; yet it is a specific marker of bone formation whenever formation and resorption are uncoupled. Increased serum osteocalcin reflects acceleration of osteoblastic activity and bone turnover[2]. Nonetheless, the significance of osteocalcin as a marker of PCBM is disputable mainly due to methodological heterogeneity and easy loss of the molecule's immunological activity[3,4]. Bone alkaline phosphatase (BALP) is a tetrameric protein located in the plasma membrane of osteoblasts that plays an active role in bone formation and skeletal mineralization. Yet, BALP proved particularly useful to indicate the presence of PCBM and was significant increased in such patients when compared with those without bone metastasis[5,6]. Despite its advantages in the diagnosis of PCBM, BSAP has been reported less predictive for skeletal complications or response to therapy when compared with a number of collagen-derived markers, probably due to the fact that serum levels of it also increase to balance localized or systemic increases in osteolytic activity or as an indication of bone formation to repair bone lesions that have responded to treatment[7].

**II. Byproducts of type I collagen synthesis and degradation**

Type I collagen is the most abundant collagen in many soft tissues, and accounts for more than 90% of the bone matrix[8]. Procollagen type I is the biosynthetic precursor of type I collagen. It is larger in size and contains additional aminoacidic sequences at both ends with respect to its final product[8]. During bone formation and collagen molecule synthesis, the amino-terminal and carboxy-terminal procollagen I extension peptides (PINP and PICP, respectively) are cleaved, while the central part of the molecule, is incorporated into bone matrix[1]. The first C-telopeptide carboxy-terminal assay was developed for serum and termed carboxyterminal telopeptide of type I collagen (ICTP). This assay utilizes an antibody that recognizes a unique amino acid sequence in the a1 chain of type I collagen during collagen degradation[9,10]. Serum ICTP and CTx assay results do not always agree clinically. This is because the monoclonal antibodies used for these two C-telopetide assays are different and recognize different epitopes at the carboxy terminal end of these telopeptides[11]. The second C-telopeptide assay, called Crosslaps (CTx), recognizes an 8–amino acid sequence that contains an aspartate residue[12]. Recently, assays have also been developed to measure the process of mature bone resorption via crosslinking molecules resulting from the degradation of type I collagen. Bone resorption assays in urine and serum have also been developed with antibodies raised against the N-telopeptide to helix domain (NTx)[13]. Both NTx and CTx are 8 amino acid epitopes derived from the N-telopeptide or C-telopeptide of type I collagen respectively, are cleaved by cathepsin K and the rate of their release from bone is a useful reflection of the resorbing activity of osteoclasts[14].

Collagen-derived biochemical markers have advantages because they are noninvasive, inexpensive, reflect turnover in the whole skeleton, and allow repeated evaluation. These markers have been suggested for monitoring patients with already known PCBM, to guide decision making regarding the treatment[15] and also to gauge the efficacy of treatment of metastatic bone disease[13,15]. Previous studies revealed that various bone markers such as OC, BALP, amino-terminal and carboxy-terminal propeptides and telopeptides of type I collagen are remarkably elevated in the serum of patients with PCBM and correlate with the extend of bone disease (EOD)[16,17].

**a. Bone formation markers (type I collagen propeptides)**

Serum concentrations of type I collagen propeptides (PINP and PICP) have been shown to be elevated in PCBM[16-18]. Ratios of different bone formation markers were proposed as sensitive clinical markers for judging the response to therapy of prostate cancer metastasized to bone[4,19], because the degree of appearance of each marker shows a specific pattern during three osteoblast developmental phases, including osteoblast proliferation, matrix maturation, and mineralization. Serum levels of PICP and PINP are also known to be elevated in other diseases such as bone fracture[20], osteomalacia[21], Paget's disease[22] and also in the chronic phase of hypertrophy in hypertensive patients[23]. Thus, careful assessment of their levels is required in patients who have combinations of these diseases.

Moreover, it has been reported that advanced-stage tumors which contain high-grade cancer cells tend to produce less prostate specific antigen (PSA) and actually release less PSA into the blood[24]. Thus, these propeptides may be useful for detecting bone metastases in some patients whose serum PSA levels are low. It is fair to say that patients with prostatic cancer who have high serum marker levels and low serum PSA should be carefully evaluated for metastatic spread to bone[9].

**b. Bone resorption markers (type I collagen telopeptides)**

Although osteoblastic lesions are characteristic of prostate cancer, an osteolytic component has been confirmed in several reports. Bone resorption markers such as ICTP, CTx and NTx have been noted to be higher in patients with osteoblastic disease than in patients with osteolytic disease[11,25].

Considering the first one, serum levels of ICTP were significantly higher in patients with PCBM compared with patients without bone involment or with a control group with benign prostate hyperplasia, whereas PSA was significantly higher both in patients with and without bone metastasis compared with the control group[10]. Indeed, ICTP appears to be superior to PICP for detection of bone metastasis, whereas PICP is better in terms of correlation of extend of bone disease (EOD)[9]. However, some shortcomings of serum ICTP levels in clinical application have been indicated: it is affected by the presence of metabolic bone disease, renal dysfunction, or hypercalcemia, which sometimes are found in advanced cancer patients[9,10].

Increased NTx and CTx levels showed excellent sensitivity in discriminating between patients with and without bone metastases and there is now growing evidence to suggest that these resorption markers might be of use in assessing presence and extension of skeletal disease[11,12,17]. Albeit the NTx assay is perhaps the most predictive biochemical marker of PCBM[4], both NTx and CTx typically are increased in patients with bone metastases compared with healthy controls[17], and essentially seem to provide similar information[11]. Increased NTx and CTx levels also correlate with reduced response to systemic anticancer treatments[26], or to local skeletal radiotherapy[27]. Finally, clinical studies have shown that the baseline collagen telopeptide levels in patients with only skeletal metastases may be prognostic with regard to the time to disease progression and overall survival[4,5,28].

As regards skeletal complications (SRE), for patients with bone metastases, total and bone-specific BALP, PINP, NTx, ICTP and CTx were useful tools to predict and diagnose SRE in patients with PCBM under receiv-

ing zoledronic acid therapy[25,29,30]. These analyses suggest that reducing bone turnover should have a positive effect by delaying progression of bone lesions and possibly improving survival[29-31]. An increase in bone marker levels often precedes an event by several months, thus providing another opportunity for treatment interventions[7]. In this regard, high marker levels at baseline or at any time during the course of the disease might indicate that more aggressive intervention strategies are needed to prevent skeletal morbidity.

**c. Collagen markers limitations**

Collagenous markers of bone resorption have been reported to exhibit marked biological circadian variation with a nadir during the day and in the afternoon and with a peak during the night and early morning hours[32]. Consequently, in order to obtain valid estimates of bone resorption markers, blood and urine sampling must be performed in the fasting state of individuals and within relatively narrow time frames (8–10 hours). Another source of variation for biochemical markers is the long-term variability found in an individual over days and months. Intra-individual short-term (3 days) and long-term (2 months) variation for urinary NTx has recently been found to be 13.1% and 15.6%, respectively. The corresponding numbers for serum NTx were 6.3% and 7.5%, respectively[32]. Factors that might affect these variations include dietary habits, smoking, exercise, and medication. Bisphosphonate treatment reduces the circadian variability by about 50% in magnitude[31]. Zoledronic acid in particular, causes even more pronounced decreases of bone resorption markers[29,30]. It should be noted, however, that absolute changes in marker values are often misleading if the interpretation does not take into account the respective marker's analytical and biological variability. To be clinically meaningful, changes in bone markers induced by therapeutic interventions need to exceed a predefined range of non specific variability. Changes below these marker-specific thresholds should be considered either ambiguous or non specific. Many assays for bone turnover markers, independent of the matrix used, have not achieved satisfactory standardization. Yet at present, results for most markers of bone turnover cannot be compared among laboratories without previous cross-calibration. Consequently, reference methods and international standards need to be developed[33].

Bone resorption markers do not appear to be as sensitive as standard imaging techniques such as bone scans, or other types of imaging studies for detecting bone metastases. Moreover, when there is a diagnostic suspicion of skeletal metastases, there is still a need to confirm and evidence the site of the localization by an imaging modality; especially for therapeutic decision-making it would still be reasonable to carry out diagnostic imaging. Whether the markers can be used to determine early bone involvement or micrometastases before the bone scan turns positive in high risk cancer patients still remains to be determined. Additional study on the effect of other therapeutic approaches such as irradiation, endocrine therapy and operation might be useful to clarify the significance of changes in bone metabolic markers.

**III. Markers of osteoclastogenesis**

The receptor activator of nuclear factor (NF)-kappaB ligand (RANKL) pathway has been identified as the main driving force for osteoclastogenesis and resulting bone resorption. Osteoprotegerin (OPG), a naturally occurring protein that inhibits bone resorption, is a critical regulator of osteoclastogenesis, by inhibiting stimulation of osteoclast differentiation through the RANK/RANKL interaction. It is very promising in blocking the so called "vicious cycle" between bone resorption and tumor proliferation that takes place during tumor development in bone site[34]. Indeed, high rates of RANKL/RANK/OPG driven bone resorption correlate with more aggressive, advanced, metastatic prostate carcinoma and an increased risk of skeletal morbidity, whilst many current studies have theorized that blocking osteoclast activity and differentiation via the inhibition of RANK/RANKL/OPG interaction could be a treatment option for osteolytic diseases, including malignancies that have an osteolytic component like the prostate cancer[35,36]. Recently, a fully human monoclonal antibody against RANKL (denosumab) has been developed and is currently under multiple phase III clinical trials; denosumab interferes with RANK signalling by blocking RANKL and, just like OPG, preventing RANK activation[37]. Further studies are needed to determine the antitumor effect and safety of this antibody[38].

**IV. Other enzymes of bone matrix**

One other family of enzymes that has been shown to play a role in tumor progression is the matrix metalloproteinase (MMP) family, also known as matrixins. The main physiologic function of MMPs is degradation of the extracellular matrix. In addition, MMPs are thought to promote the growth of tumor cells once they have metastasized[39]. Numerous studies have shown that the higher the MMP expression in the tumor, the more aggressive the cancer. The matrix metalloproteinases MMP-2, MMP-7 and MMP-9 are highly expressed in the tumor-bone microenvironment and their activities were closely correlated with the invasiveness of the androgen-dependent and androgen-refractory prostate cancer cells[39-41]. Recent clinical trials have been initiated on MMP-inhibitors known to down-regulate MMPs and suppress the invasive and metastatic potential in many tumor-derived cell lines[40,42]. Their usefulness as additional markers of bone metastasis remains to be better defined.

Angiogenesis is a critical process for cancer progression. Various growth factors such as the fibroblast growth factor 8 (FGF-8)[43], the angiogenesis-related vascular endothelial growth factor (VEGF)[44] and the transforming growth factor beta(1) [TGF-beta(1)][45], along with activin-A[46] and macrophage-inhibitory cytokine-1 (MIC-1)[47] (both members of the TGF-beta superfamily),

are significant prognostic factors of PCBM. Moreover, insulin-like growth factors I, II and III (IGF-1, IGF-2 and IGF-3)[48], along with macrophage colony-stimulating factor (M-CSF) and colony-stimulating factor-1 receptor (CSF-1R)[49] also predict cancer stage and prognosis. Bone-morphogenetic protein-6 (BMP-6) overexpression has been shown to increase the aggressiveness and invasiveness of prostate cancer cells[50]. Bone sialoprotein (BSP) is a secreted glycoprotein found in mineralized tissues. However, elevated BSP expression in breast and prostate primary carcinomas is directly correlated with increased bone metastases and tumor progression[51]. The endothelin (ET) axis represents another novel and exciting target in the treatment of prostate cancer. ET-1, acting primarily through the endothelin-A receptor, is integrally involved in prostate cancer progression, including cell growth, inhibition of apoptosis, angiogenesis and development of bone metastases. Biological activity of selective ET-1 antagonists in patients with prostate cancer has been shown by the suppression of biochemical markers of prostate cancer progression in bone and a delay in time to disease progression in patients with PCBM[52]. The Src (a proto-oncogene tyrosine-protein kinase) has a definite role in the cancer cell proliferation, invasion, and migration. Encouraging results have been obtained in recent preclinical and clinical studies using inhibitors of Src in prostate cancer[53]. Parathyroid hormone-related peptide (PTHrP) is a regulatory protein associated with osteoclast stimulation in bone. The high frequency of PTHrP expression in PCBM is consistent with a role in the pathogenesis of bone metastases[54]. Serum tartrate-resistant acid phosphatase (TRACP) can also be considered a useful predictor of bone metastases in prostate cancer, albeit less specific than tALP[55]. Plasma levels of urokinase-type plasminogen activator (uPA) are associated with features of biologically aggressive prostate cancer and disease progression[56]. Serum interleukin-6 (IL-6), cystatin C (an endogenous inhibitor of cysteine proteinase cathepsin K) and serum chromogranin A (sCgA), along with cell membrane antigens like the gastrin-releasing peptide receptor (GRPR), the prostate stem cell antigen (PSCA), and the prostate-specific membrane antigen (PSMA), are expressed in prostate cancer and they are all novel attractive targets for new diagnostic and therapeutic applications[57-59]. Further diagnostic, prognostic or predictive serological candidate prostate cancer biomarkers are various cytokines or chemokines, such as serum progastrin-releasing peptide[60], alpha-2 macroglobulin (alpha-2M)[61], cathepsin-D (CatD)[62], the positive nuclear factor kappa-B (NF-Kb)[63], plasma osteopontin (OPN)[64], neuron-specific enolase (NSE)[65] and even C-reactive protein (CRP)[66].

## Conclusions

In conclusion, the determination of novel bone turnover markers like byproducts of type I collagen synthesis and degradation, markers of osteoclastogenesis or matrix metalloproteinases and many other cytokines might be available for early diagnosis and follow-up of PCBM in the foreseeable future. Further prospective studies are needed to determine whether any of these markers measured longitudinally in prostate cancer patients without bone scan evidence of skeletal disease will ultimately predict those patients who later develop bone metastases from their malignancy. Nonetheless, from the clinical point of view it is important to know that these novel markers carry the potential to provide meaningful information for daily practice by using upper normal reference values as cut-offs for identifying patients with an increased risk of developing progressive bone disease or skeletal related events. Importantly also, in case of established skeletal disease, progressively or excessively high levels of such bone markers could alert for institution, re-institution, intensification or alteration of chemotherapy, hormone therapy, or bisphosphonate treatment. A better understanding of the mechanisms that interact at the molecular level will definitely result in more effective therapy for patients with this devastating complication of prostate cancer.

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