The utility of bone remodeling markers in the diagnosis, evolution and treatment response evaluation in bone metastases

Mihai-Lazăr Mioč1, Horia George Hărăuşă2, Andrei Dan Bălănescu1, Pompiliu Horăţiu Petrescu2, Mihaela Iacob3, Radu Prejbeanu2

1) Department of Orthopedics and Traumatology, Emergency County Hospital, Timisoara, Romania
2) Department of Orthopedics and Traumatology, “Victor Babeș” University of Medicine and Pharmacy, Timisoara, Romania
3) Department of Pathology, Emergency County Hospital, Timisoara, Romania

Abstract
After the lungs and liver, the bone is the third most common site for metastatic disease, appearing frequently in breast and prostate malignancies. These pathological bone events that occur during the evolution of the metastatic disease are usually the onset of osteolysis and they lower the patient’s life quality, and are sometimes the cause of death due to the required treatments (surgery, radiotherapy). Due to the nature of the bone remodeling process, the markers that control bone resorption are the main early indicators of bone malignancy. These markers can be found in excess quantities of 50–150% in patients with bone metastases. Analyzing these indicators in conjunction with traditional tumoral markers such as the prostate specific antigen (PSA) and the type I collagen cross-linked telopeptide (ICTP) can often increase the sensibility of the investigation and the chances of diagnosing bone metastases. The studies that were carried out in order to research this area of knowledge have had good and expected results. Most of the efforts are now channeled into developing a better therapeutic strategy that would allow for the early diagnosis and treatment of the pathological bone events. Until these markers can be used as standard investigation methods in all of our patients, some controlled studies must be carried out in order to statistically prove these results, which are purely observational.

Keywords: bone remodeling markers, bone metastases, bone resorption, osteolysis, tumoral markers.

Introduction
After the lungs and liver, the bone is the third most common site for metastatic disease, appearing frequently in breast and prostate malignancies [1]. The metastatic rate of occurrence has increased significantly over the past years as a direct result of the development of new treatment paths in oncology, and the rise in life expectancy. The increased morbidity that these metastases deliver to the patient is caused by pathological skeletal events such as bone pain, hypercalcemia and pathological fractures. These events that occur during the evolution of the metastatic disease are usually the onset of osteolysis (Figures 1 and 2) and they lower the patient’s life quality, and are sometimes the cause of death due to the required treatments (surgery, radiotherapy). Bone metastatic complication rates of over 60% were reported by Lipton et al. [2] on a group of 751 women with breast malignancy.

New treatment paths such as bisphosphonates have opened up better therapeutic possibilities in the treatment of bone metastases. Their strong inhibitor effect over the osteoclasts allows for the suppression of bone resorption, the main mechanisms that decreases structural integrity and increases the chance of pathological fracture occurrence. The efficacy of this treatment has been shown by Ross et al., especially when administered in an early progression stage [3]. Furthermore, in vitro studies show us that bisphosphonates may have a direct action on the tumoral cell by inducing apoptosis and inhibiting tumoral cell adhesion to the bone tissue [3]. The introduction of such treatment ways, thus, led to the necessity of developing a more sensible and specific diagnostic method that could follow-up on the patient’s response to the treatment itself. The main attribute of the diagnosis method is that it has to be able to detect the bone metastatic disease in its incipient stages.

Radiology methods, especially positron emission tomography–computed tomography (PET–CT) and bone scintigraphy, follow the same basic principle – the tumoral tissue has high affinity towards radionuclide uptake. These two investigations have high sensibility, but they do not posses the required specificity that would allow for an early diagnosis. Furthermore, in the early stages of bone metastatic disease, the tissue may not even have a high enough affinity towards radioactive isotopes, or it may not present any morphological modifications that could be seen on CT or magnetic resonance imaging (MRI).

Bone turnover markers. Diagnostic utility
During the metastatic bone disease, the natural homeostasis of the tissue is disrupted and thus a great unbalance is created between the osteoclasts’ and osteoblasts’ activity. The mechanism that stands at the start of this process is a vicious cycle (Figure 3) that is based on the tumoral cell production of proresorptive factors such
as the parathyroid hormone-related protein (PTHrP) – a protein from the parathormone group, who’s direct action in osteoblasts is the increase of the receptor activator of nuclear factor kappa-B ligand (RANKL) expression and the reduction of osteoprotegerin (OPG – an inhibition factor of osteoclastic genesis) expression. This results in the growth of osteoclast differentiation and activity. The osteoclasts synthesize and release growth factors such as the transforming growth factor-beta (TGF-β), which additionally stimulates tumoral growth and the production of PTHrP. This entire self-sustained mechanism leads to an exponential tumoral growth [4], thus explaining the aggressiveness of bone metastases.

Taking into consideration the above-mentioned facts, the benefits of turnover markers must be mentioned. First, due to the nature of the bone remodeling process, the markers that control bone resorption are the main early indicators of bone malignancy. These markers can be found in excess quantities of 50–150% in patients with bone metastases. The prognostic value and life expectancy of the patient can also be followed with the aid of these markers, a fast-pacing bone resorption being translated into a low prognostic value, high chances of pathological bone event occurrence (pathological bone fracture) and a weak therapeutic response. The effects of bisphosphonate treatment can also be evaluated with the help of marker values, their decrease being often associated with less pain and less chances of a pathological bone fracture occurrence. Disease progression during treatment is indicated by stationary or grown marker values.

![Figure 1](image1)

**Figure 1** – Bone metastasis caused by a low differentiated adenocarcinoma with solid and tubular areas. The osteolytic aspect of the tumor can be noticed, which are presented with remaining, unconnected, lytic bone tissue. Its’ margins are eroded and it is surrounded by the tumoral expansion, with a few noticeable osteoclasts. Hematoxylin–Eosin (HE) staining, ×100.

![Figure 2](image2)

**Figure 2** – Osteolytic bone metastases of ductal adenocarcinoma, with a ductal growth pattern, organized in nests and tubes, arising from the mammary gland: (a) Osteolytic bone is in the center with nests of malignant cells in the left and adipocytes in the right; (b) The tumoral mass is organized in tubes and nests, infiltrating the stroma; (c) Large, round cells with pleomorphic characters and a variation in nuclear size, with present nucleoli and frequent mitotic figures; (d) Chronic inflammatory cells and desmoplastic stroma, which may obscure tumor cells. HE staining, ×100.
The utility of bone remodeling markers in the diagnosis, evolution and treatment response evaluation... 

Figure 2 (continued) – Osteolytic bone metastases of ductal adenocarcinoma, with a ductal growth pattern, organized in nests and tubes, arising from the mammary gland: (e) Nests of malignant cells surrounding the remaining bone tissue; (f) Hyperemic vessels and two types of cells – malignant and fusiform. HE staining, ×100.

Figure 3 – Tumoral osteolytic mechanism, presented as a vicious cycle. PTHrP: Parathyroid hormone-related protein; OPG: Osteoprotegerin; RANKL: Receptor activator of nuclear factor kappa-B ligand; TGF-β: Transforming growth factor-beta.

Analyzing these indicators in conjunction with traditional tumor markers such as the prostate specific antigen (PSA) and the type I collagen cross-linked telopeptide (ICTP) can often increase the sensibility of the investigation and the chances of diagnosing bone metastases [5, 6]. Out of all the markers that represent bone formation, the ones found often in bone metastases are the total alkaline phosphatase (TAP) and the bone alkaline phosphatase (BAP) [7, 8]. Elevated seric BAP levels are correlated with osteoblastic differentiation and activity, being translated into tumoral tissue formation or healthy bone formation in lytic tumors [9, 10]. Nevertheless, there are bone markers such as bone sialoprotein (BSP), osteocalcin (OC) and hydroxyproline (OHP), which can be elevated both in bone formation or bone resorption [11].

Specific turnover markers that show bone tissue formation are presented in Table 1. These are direct or indirect products of osteoblastic activity. Bone alkaline phosphatase is a fraction of total alkaline phosphatase that can signal hepatic injury or bone metabolic disease when found in elevated levels. Osteocalcin is an osteoblast-induced protein, which can be found in large quantities in tissues like bone, dentin and malignancies such as osteosarcoma, breast, pulmonary, ovarian and prostatic carcinomas [12]. Since OC is an osteoblast product but it can also be found in the bone matrix (and thus can be released during bone resorption), it can be a marker that has elevated levels in both bone turnover phases. Pro-collagen type I pro-peptides (PICP, PINP) make up for 80–90% of the bone matrix, and they are specific products of osteoblastic activity. Because of their generation mechanism (1:1 ratio with collagen molecules), their seric level is considered a factor of collagen synthesis and bone formation. All these markers can be identified and measured with the aid of specific lab works such as radioimmunoassay (RIA) and immunoenzymatic methods (enzyme-linked immunosorbent assay – ELISA, enzyme immunoassay – EIA).

Table 1 – Bone turnover markers that can be found in the bone formation process

<table>
<thead>
<tr>
<th>Marker name</th>
<th>Specific properties</th>
<th>Tissue of origin</th>
<th>Lab works used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procollagen type I carboxy-terminal propeptide (PICP)</td>
<td>Specific product of osteoblast and fibroblast proliferation</td>
<td>Bone, skin, soft tissue</td>
<td>ELISA, RIA</td>
</tr>
<tr>
<td>Procollagen type I amino-terminal propeptide (PINP)</td>
<td>Specific product of osteoblast and fibroblast proliferation. Partially found in extracellular bone matrix</td>
<td>Bone, skin, soft tissue</td>
<td>ELISA, RIA</td>
</tr>
<tr>
<td>Bone alkaline phosphatase (BAP)</td>
<td>Specific product of osteoblasts</td>
<td>Bone</td>
<td>Precipitation electrophoresis, IRMA, EIA</td>
</tr>
<tr>
<td>Osteocalcin (OC)</td>
<td>Specific product of osteoblasts. Part of it can result from bone resorption</td>
<td>Bone, dentin, platelets</td>
<td>RIA, IRMA, ELISA</td>
</tr>
</tbody>
</table>

ELISA: Enzyme-linked immunosorbent assay; RIA: Radioimmunoassay; IRMA: Immunoradiometric assay; EIA: Enzyme immunoassay.

Bone resorption specific markers (Table 2) are represented by the products resulted from type I collagen degradation, osteoclast specific enzymes such as tartrate-resistant acid phosphatase (TRAcP) and bone sialoprotein.
Bone turnover markers. Prognostic and treatment efficacy evaluation utility

The prognostic utility of bone turnover markers has become a very controversial and studied phenomenon in the oncological research field lately. Specialists have run many studies that analyze the prognostic capability of these indicators, be it alone or in conjunction with other well-known tumoral markers [17]. The purpose of these studies has been to create an easy and precise method of evaluating anti-resorptive treatment in patients with bone metastases. Therefore, new patterns of treatment response investigations have been developed, with great impact on therapy modulation and personalization in accordance with current needs. Usually, urinary and blood levels of the bone resorption markers have been noticed to drop heavily after bisphosphonate treatment initiation. These drops usually occur within a couple of days and they are an indicator of a good response to the treatment, though the real indicator of treatment response appears after a few weeks when the bone formation markers’ levels begin to rise [21, 22].

The level of seric osteocalcin (sOC) proved to be a good signal when bisphosphonate therapy is initiated on bone metastases and even sub-standard values were encountered after the start of the treatment [23]. This drop in sOC levels is believed to concur with osteoblastic activity and bone tissular repair [24]. The decrease of sOC and seric alkaline phosphatase (sALP) levels has proved to be a direct consequence of pamidronate treatment in prostatic malignancy [25] and ibandronate treatment in breast cancer [26]. Treating multiple myeloma (MM) patients with osteoprotegerin or bisphosphonates lowers the levels of DPD, PYD, NTX-I and CTX-I (all being bone resorptive markers) [27, 28]. Lipton et al. showed that monitoring the levels of urinary NTX-I is a sensitive method of evaluating anti-resorptive treatment in patients with bone metastases [29].

Recently, some of these bone turnover markers (BTM) were assessed in a study that was meant to verify their degree of association with cortical porosity, cortical thickness and the incidence of pathological bone fractures.

Table 2 – Bone turnover markers that can be found in the bone resorption process

<table>
<thead>
<tr>
<th>Marker name</th>
<th>Specific properties</th>
<th>Tissue of origin</th>
<th>Lab works used</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Collagenic markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline (OHP)</td>
<td>Present in all fibrillar collagen as well as in immature collagen</td>
<td>Bone, cartilage</td>
<td>HPLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>skin, soft tissue</td>
<td></td>
</tr>
<tr>
<td>Pyridinol (PYD) and deoxypyridinol (DPD)</td>
<td>Collagenic products found in large amounts in cartilage (PYD) and bone (PYD and DPD), Not present in skin, present only in mature collagen</td>
<td>Bone, cartilage</td>
<td>HPLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tendon, dentin</td>
<td>ELISA</td>
</tr>
<tr>
<td>Type I collagen cross-linked telopeptides (ICTP, CTX-I, NTX-I)</td>
<td>Degradation products of type I collagen with the highest bone components.</td>
<td>All type I collagen</td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>tissue</td>
<td>RIA</td>
</tr>
<tr>
<td><strong>Non-collagenic markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone sialoprotein (BSP)</td>
<td>Extracellular bone matrix glycoprotein associated with osteoclastic activity</td>
<td>Bone, dentin,</td>
<td>RIA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hypertrophic cartilage</td>
<td>RIA</td>
</tr>
<tr>
<td><strong>Osteoclast specific enzymatic markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase (TRAcP)</td>
<td>Isoenzyme found inside the osteoclasts’ membrane and resorptive space secretions</td>
<td>Bone, blood</td>
<td>ELISA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RIA</td>
</tr>
<tr>
<td>K and L cathepsin</td>
<td>Proteases found in osteoclasts and macrophages with utility in bone matrix degradation</td>
<td>Osteoclasts, macrophages</td>
<td>ELISA</td>
</tr>
</tbody>
</table>

HPLC: High-performance liquid chromatography; ELISA: Enzyme-linked immunosorbent assay; RIA: Radioimmunoassay.
The study was carried out in Norway, on a group of 211 postmenopausal women that participated in the Tromsø 4 study. Shigded et al. measured the femoral neck areal bone mineral density (FN aBMD) using dual-energy X-ray absorptiometry (DXA), cortical thickness using high-resolution peripheral quantitative computed tomography (HR-pQCT), as well as other parameters such as serum PINP and serum CTX. They found increased values of BTM in fracture cases compared to the control group \((p<0.001)\), a positive correlation of PINP and CTX in association with bone architecture measurements [30]. In conclusion, they found a tight association of increased levels of BTM and high cortical porosity, thinner cortices and higher odds for non-vertebral fractures.

A new line of treatment regarding patients with MM may reside in something completely different than bisphosphonates. This line of research was initiated due to the multiple complications of bisphosphonate treatment (osteonecrosis of the jaw, renal toxicity, no effect on restoring bone formation) [31, 32]. A mechanism that would improve bone remodeling while also inhibiting bone resorption and having less adverse effects is represented by proteasome inhibition [33]. This type of treatment represented by bortezomib (1st generation) and carfilzomib (2nd generation) is already an approved drug for patients with MM and its effectiveness has been demonstrated in many clinical and preclinical studies [34–36]. After doing a literature review, Zangari & Suva concluded that proteasome inhibitors can influence bone remodeling in MM patients substantially, as a class, and that the new generation drugs (carfilzomib) have an even greater potency of taking the bone tissue from a catabolic to an anabolic state, when compared to bortezomib [37].

\section*{Conclusions}

The treatment of bone malignancies and metastases especially, is currently a surgical one. With time, we believe that there will be a shift in therapy development that will leave the surgical treatment only for extremely advanced cases. Most of the efforts are now channeled into developing a better therapeutic strategy that would allow for the early diagnosis and treatment of the pathological bone events. Bone turnover markers represent a novelty in the research field of bone mineral density, bone pathological events and bone malignancies. The studies that were carried out to expand this area of knowledge have had good and expected results that can benefit bone metastatic diagnosis, evaluation of the disease stage and treatment response (bisphosphonates or chemotherapy) with the aid of bone turnover markers. However, the biggest impact regarding the diagnosis comes from correlating different markers (tumoral or bone turn-over) with certain imaging techniques. This wide variety of diagnostic and outcome anticipation methods can be of great help when it comes to managing these complex patients. Until these markers can be used as standard investigation methods in all our patients, some controlled studies must be carried out in order to statistically prove these results, which are purely observational.

\section*{Conflict of interests}

The authors declare that they have no conflict of interests.

\begin{thebibliography}{10}


\bibitem{4} Mundy GR, Yoneda T, Hiraga T. Preclinical studies with zoledronic acid and other bisphosphonates: impact on the bone microenvironment. Semin Oncol, 2011, 29(2 Suppl 6): 35–44.


\end{thebibliography}


**Corresponding author**
Pompiliu Horăţiu Petrescu, Assistant Professor, MD, PhD, Department of Orthopedics and Traumatology, "Victor Babeș" University of Medicine and Pharmacy, 2 Eftimie Murgu Square, 300041 Timișoara, Romania; Phone +40722–239 771, e-mail: lalusha87@yahoo.com

**Received:** November 9, 2015

**Accepted:** December 4, 2016