REVIEW

The role of markers of bone remodeling in multiple myeloma

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Summary Osteolytic bone disease is a frequent complication of multiple myeloma, resulting in skeletal complications that are a significant cause of morbidity and mortality. A characteristic feature of myeloma bone disease is that the lesions rarely heal and bone scans are often negative in myeloma patients who have extensive lytic lesions, offering very little in the follow-up of bone disease. X-rays are also of limited value in monitoring bone destruction during anti-myeloma or anti-resorptive treatment. Biochemical markers of bone turnover, such as N- and C-terminal cross-linking telopeptide of type I collagen (NTX, CTX/ICTP, respectively), and newer ones such as the tartrate resistant acid phosphatase isoform 5b, provide information on bone dynamics that in turn may reflect disease activity in bone. Several studies have shown bone markers to be elevated in myeloma patients and reflect the extent of bone disease, while in some of them bone resorption markers correlate with survival. These markers may also be helpful in identifying those patients likely to respond to bisphosphonate treatment, and monitoring the effectiveness of bisphosphonate therapy in the management of myeloma bone disease. This review attempts to summarize the existing data for the role of markers of bone remodeling in assessing the extent of bone destruction in myeloma and monitoring bone turnover during specific anti-myeloma treatment. We also discuss some novel markers that may be of particular interest in the near future.

KEYWORDS Myeloma; Bone markers; N-terminal cross-linking telopeptide of type I collagen (NTX); C-terminal cross-linking telopeptide of type I collagen generated by MMPs (ICTP, CTX-MMP); Pyridinoline; Deoxypyridinoline; Bone-specific alkaline phosphatase; Osteocalcin; Tartrate resistant acid phosphatase isoform 5b; Bone disease

Introduction

Multiple Myeloma (MM) is a clonal plasma cell disorder characterized by bone destruction, immunodeficiency, and renal impairment. Nearly 3500 people in the UK are diagnosed with MM each year. This accounts for 10% of blood cancers and 1% of all cancers. More than 55% of patients who present with MM are aged 60 or older, while less than 3% of MM occurs in patients younger than 40 years; median survival ranges between 3.5 and 5 years. A cure in MM appears to be achievable only by allogeneic transplantation, which is not the treatment of...
choice for the majority of patients due to high rate of transplant-related mortality. A major clinical feature of MM is the presence of osteolytic bone disease and generalized osteoporosis, which can lead to severe bone pain, pathologic fractures, spinal cord compression, and hypercalcemia. Over 85% of myeloma patients have bone disease. It thus represents a major cause of morbidity and mortality. Progression of skeletal disease is often not affected by chemotherapy even in responding patients. The mechanisms of bone destruction appear to be related to increased osteoclastic bone resorption, which is not accompanied by a comparable increase in bone formation. Thus, a characteristic feature of myeloma bone disease is that the lesions rarely heal even when the patients are in complete remission. This finding is in keeping with the observation that bone scans are often negative in myeloma patients who have extensive lytic lesions, and offer very little in the follow-up of bone disease in these patients. Furthermore, a recent study has shown that sequential measurement of bone mineral density (BMD) using DEXA-scans produced heterogeneous local BMD changes and the available data do not support routine use of sequential DEXA-scans in MM. The bone disease in MM is usually assessed by X-rays of the skeleton. X-rays are useful in the diagnosis of osteolytic lesions, but do not give any dynamic information on the ongoing bone resorption. In contrast, biochemical markers of bone metabolism specifically reflect bone resorption or bone formation rates and are strongly affected by the processes active in myeloma bone disease. Therefore, biochemical markers of bone remodeling are used more often nowadays in an attempt to improve monitoring of bone destruction in MM.

In addition, strategies that target osteoclast activation and proliferation represent an important approach to the management of myeloma bone disease. Bisphosphonates consist of a heterogeneous group of agents that affect bone metabolism and regulation of calcium homeostasis, mainly through an inhibitory effect on osteoclasts. Several studies have demonstrated their efficacy in myeloma bone disease and therefore they are included in all therapeutic regimens for stage II/III myeloma patients. Biochemical markers of bone resorption or formation have also been used to follow up myeloma bone disease during bisphosphonates administration. This review attempts to summarize the existing data for the role of markers of bone remodeling in assessing the extent of bone destruction in myeloma and monitoring bone turnover during specific anti-myeloma chemotherapy or bisphosphonates administration. We also discuss novel markers that may be of particular interest in the near future.

Pathogenesis of bone disease in multiple myeloma

Although the mechanisms responsible for the development of myeloma bone disease currently remain unclear, several studies have begun to shed new light on this process. Histomorphometric studies have demonstrated that myeloma bone destruction is related to increased osteoclastic activity, which is not accompanied by a comparable increase in osteoblast formation. This uncoupling of resorption and formation leads to rapid bone loss. A number of cytokines and growth factors that are produced either by myeloma cells or by stromal cells, due to interactions between them, have been implicated in the increase in osteoclast formation and activity in MM. The adherence of myeloma cells to bone marrow stromal cells results in enhanced production of cytokines, such as interleukin-6 (IL-6), interleukin 1-β (IL-1β), interleukin 11 (IL-11), tumor necrosis factors α and β (TNFα, TNFβ), basic fibroblast growth factor (bFGF), macrophage-colony stimulating factor (M-CSF), which stimulate osteoclast formation. IL-6 also acts as a survival factor for myeloma cells. Furthermore, stromal cells also produce receptor activator of nuclear factor κ-B ligand (RANKL), a member of the tumor necrosis factor (TNF) gene family. Following activation of the cellular receptor RANK on osteoclasts by its ligand, RANKL, differentiation, proliferation, and survival of osteoclasts is enhanced, osteoclast fusion and activation is promoted, and osteoclastic apoptosis is suppressed, leading to a dramatic increase in the number and activity of osteoclasts. In addition, production of osteoprotegerin (OPG), a soluble decoy receptor of RANKL produced by marrow stromal cells, is suppressed through the above interactions and has been found to be reduced in patients with MM. The mechanisms through which OPG levels are decreased have not been clearly defined yet, but a study by Standal et al. has shown that OPG is bound, internalized, and degraded by the myeloma cells through CD138. The ratio RANKL/OPG is reversed in myeloma, leading to osteoclast activation and bone destruction. Several reports have suggested that myeloma cells also produce RANKL and that the expression of RANKL by human myeloma cells mediates osteoclast formation in vitro and correlates with bone destruc-
tion in vivo. However, other investigators have not been able to detect RANKL expression on myeloma cells. The amount of RANKL produced by myeloma cells is rather small to stimulate osteoclast formation by itself and it may be sufficient only to prevent osteoclast apoptosis. Several cytokines produced by both myeloma and stromal cells, such as IL-3, IL-6, IL-11 exert their effect through RANKL/RANK/OPG pathway, inducing osteoclastogenesis.

Myeloma cells also produce large amounts of macrophage inflammatory protein-1α (MIP-1α), which is a low molecular weight chemokine that belongs to the RANTES family, and activates the osteoclasts directly. MIP-1α levels are elevated in both bone marrow plasma and serum of patients with active MM and correlate with the presence of lytic lesions. High concentrations of MIP-1α were mainly found in patients with advanced and active disease. Furthermore, MIP-1α has also been shown to increase RANKL expression on bone marrow stromal cells, thus inducing further bone destruction. The major role of both RANK/RANKL and MIP-1α pathways in myeloma bone disease has been established in murine models. The inhibition of RANKL and MIP-1α activity results in markedly decreased bone destruction and a significant reduction in tumor burden.

As mentioned above, in MM the increased bone formation is accompanied by suppression in osteoblast function. Osteoblasts in active myeloma are functionally exhausted and promptly undergo apoptosis in the presence of myeloma cells from patients with severe bone disease. A recent study by Tian et al. reported that myeloma cells produce dickkopf 1 (DKK1) protein, an inhibitor of Wnt signaling pathway, which is crucial for osteoblast differentiation. In this study, marrow plasma from patients with MM that contained >12 ng/ml of DKK1 inhibited osteoblast differentiation. Furthermore, gene expression levels of DKK1 correlated with the extent of bone disease. The presence of a soluble factor produced by myeloma cells that suppresses osteoblast differentiation is a very important finding, which, however, does not entirely explain why myeloma bone lesions do not heal when the patients are in complete remission. There may possibly be a more long-lasting change in the marrow microenvironment that results in an inability of osteoblast precursors to differentiate, even in the absence of myeloma cells. However, this hypothesis remains to be proven.

Fig. 1 summarizes the currently available data on osteoclast activation in MM reflecting the role of myeloma microenvironment in the development of myeloma-related bone disease.

### Markers of bone remodeling

Throughout life bone undergoes continuous remodeling with removal of old bone and replacement with new bone. Bone turnover is always initiated by osteoclasts eroding a mineralized surface. This process is followed by the recruitment of successive teams of osteoblasts to the outer edge of the erosion cavity that secretes new bone matrix and gradually fills in the resorption cavity. Resorption of old bone and formation of new bone are balanced under normal conditions. In MM, there is a pronounced imbalance in these processes: increased activation of osteoclasts and suppression of osteoblast function. Over the past two decades, the isolation and characterization of cellular and extracellular components of the skeletal matrix have resulted in the development of biochemical markers that specifically reflect either bone formation or bone resorption. Most of the traditional and new markers of bone resorption measure the collagen degradation products from osteoclast activity and include urinary hydroxyproline, hydroxylysine glycosides, total or free pyridinoline cross-links, and cross-linked N- or C-telopeptides. A novel marker, serum tartrate resistant acid phosphatase isoform type 5b (TRACP-5b) which is an enzyme secreted by activated osteoclasts and bone sialoprotein (BSP), a non-collagenous protein have been demonstrated to reflect bone resorptive processes. The formation markers are direct or indirect products of active osteoblasts that enter into the circulation (Fig. 2). These include serum bone-specific alkaline phosphatase, osteocalcin and type-I procollagen peptides. All the above markers are depicted in Tables 1 and 2. Fasting urinary calcium, used by some investigators to measure bone resorption, is not discussed here as it has been found to be of limited value in MM.

Biochemical markers of bone remodeling are noninvasive, comparatively inexpensive, and, when applied and interpreted correctly, helpful tools in the assessment of bone diseases. However, factors that affect the markers’ levels, including circadian rhythmicity, diet, age, gender, renal function and drugs, should be clearly defined and appropriately adjusted whenever possible. Another important issue is that biochemical indices reflect the total bone turnover and give
little information about the function of individual groups of osteoclasts. All these issues are discussed below.

**Biochemistry**

**Resorption markers**

**Hydroxyproline and hydroxylysine**

Hydroxyproline (Hyp) is formed in the cell from the posttranslational hydroxylation of proline. Hyp is the predominant amino acid of collagens, comprising about 13% of these proteins. Hydroxylysine (Hyl) is another amino acid essentially unique to collagenous proteins. Bone is the primary store of collagen in the body, but both Hyp and Hyl are present in essentially all tissues and all genetic types of collagen. The Hyp released during collagen degradation is primarily metabolized in the liver and subsequently excreted in the urine. However, only about 10% of Hyp-containing products from...
<table>
<thead>
<tr>
<th>Marker</th>
<th>Abbreviation</th>
<th>Tissue of origin</th>
<th>Analytical method</th>
<th>Analytical specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyproline</td>
<td>Hyp</td>
<td>All tissues and all genetic types of collagen</td>
<td>Colorimetric, assay, HPLC</td>
<td>Urine</td>
</tr>
<tr>
<td>Hydroxylysine</td>
<td>Hyl</td>
<td>All tissues and all genetic types of collagen</td>
<td>Reversed-phase HPLC</td>
<td>Urine</td>
</tr>
<tr>
<td>Galactosyl–hydroxylysine</td>
<td>Gal–Hyl</td>
<td>Both Gal–Hyl and Glc–Gal–Hyl appears to be specific for bone collagen degradation</td>
<td>Reversed-phase HPLC</td>
<td>Urine</td>
</tr>
<tr>
<td>Glucosyl–galactosyl–hydroxylysine</td>
<td>Glc–Gal–Hyl</td>
<td>Bone, cartilage, tendon, blood vessels</td>
<td>HPLC, ELISA</td>
<td>Urine</td>
</tr>
<tr>
<td>Deoxypyridinoline</td>
<td>PYD</td>
<td>Bone, dentin</td>
<td>RIA</td>
<td>Urine (free DPD can be also measured in serum or plasma)</td>
</tr>
<tr>
<td>N-terminal cross-linking telopeptide of type-I collagen</td>
<td>NTX</td>
<td>All tissues containing type-I collagen</td>
<td>ELISA, RIA</td>
<td>Urine, serum</td>
</tr>
<tr>
<td>C-terminal cross-linking telopeptide of type-I collagen</td>
<td>CTX</td>
<td>All tissues containing type-I collagen</td>
<td>ELISA, RIA</td>
<td>Urine, serum (β-form only)</td>
</tr>
<tr>
<td>C-terminal cross-linking telopeptide of type-I collagen generated by MMPs</td>
<td>CTX-MMP or ICTP</td>
<td>All tissues containing type-I collagen</td>
<td>RIA</td>
<td>Serum</td>
</tr>
<tr>
<td>Tartrate resistant acid phosphatase isoform 5b</td>
<td>TRACP-5b</td>
<td>Bone (osteoclasts)</td>
<td>Colorimetric RIA, ELISA</td>
<td>Serum, plasma</td>
</tr>
<tr>
<td>Bone sialoprotein</td>
<td>BSP</td>
<td>Bone, dentin, hypertrophic cartilage, cancer cells</td>
<td>RIA, ELISA</td>
<td>Serum</td>
</tr>
</tbody>
</table>

* According to the Bone Marker Nomenclature by the Committee of Scientific Advisors of the International Osteoporosis Foundation.115
Collagen breakdown are excreted in the urine. The small pool of urinary Hyp originates from the N-propeptide of type I collagen. Normal ingestion of gelatin or collagen-rich foods such as meat can increase the level of urinary Hyp, and the urinary peptides containing Hyp from endogenous collagen breakdown are indistinguishable from the dietary peptides. This is a major disadvantage for the use of this marker as a specific index of bone resorption.

Hyl is glycosylated and two glycosides are formed, galactosyl–hydroxylysine (Gal–Hyl) and glucosyl–galactosyl–hydroxylysine (Glc–Gal–Hyl), which also appear in the urine. While Hyl and its glycosides are less abundant than Hyp in bone collagen, certain properties make Hyl theoretically a better marker of bone turnover than urinary Hyp. Gal–Hyl is not metabolized and not influenced by dietary factors. In normal urine, 80% of the total Hyl is in the form of Hyl, 10% is free and unglycosylated, and the remainder is peptide-bound, which suggests that free Hyl is largely metabolized and not excreted. However, in addition to all structural collagens, both Hyp and Hyl are also found in certain serum proteins, such as the C1q component of complement. This disadvantage, in combination with the effect of age and the circadian rhythm (both have their peak excretion after midnight), make them less specific indices of bone resorption and therefore they have been largely replaced by newer markers.

**Pyridinoline and deoxypyridinoline cross-links of type I collagen**

In the last decade, collagen cross-links have evolved as promising markers of bone resorption. Pyridinoline (PYD) and deoxypyridinoline (DPD) are formed by the enzymatic action of lysyl oxidase on lysine and Hyl. Newly deposited collagen fibrils in the extracellular matrix are stabilized by cross-links formed by the action of lysyl oxidase on lysine and Hyl residues in telopeptide domains of the collagen molecules. The resulting aldehydes condense with Hyl or lysyl residues on adjacent collagen molecules to form divalent cross-links, which can mature by further condensation with telopeptide aldehydes to the trivalent structures DPD and PYD. PYD and DPD act as mature cross-links in type I collagen of all major connective tissues. These include type I collagen of bone, dentin, tendon, vascular walls, muscle, intestine, etc. Fig. 3 depicts the basic cross-linking of collagen fibrils that construct type I collagen, the protein comprising most of the organic matrix of the bone. Each of the fibrils contains aminoterminal and carboxyterminal ends that are termed N-telopeptides (NTX) and C-telopeptide (CTX), respectively. In type I collagen, these ends are each linked to a helical portion of a nearby molecule by a PYD or DPD cross-link. The pyridinoline cross-links occur essentially at two intermolecular sites in the collagen fibril: two amino-telopeptides are linked to a helical site at or near residue 930 and two carboxytelopeptides to helical residue 87. DPD is derived from two hydroxylysines and one lysine residue, while PYD is derived from three hydroxylysine residues. In all tissues, PYD predominates, while DPD is the minor component. The products of collagen degradation by osteoclasts include NTX and CTX fragments of various sizes, still attached to helical portions of a nearby molecule by a pyridinium

**Table 2** Markers of bone formation.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Abbreviation</th>
<th>Tissue of origin</th>
<th>Analytical method</th>
<th>Analytical specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin (or bone gla-protein)</td>
<td>OC</td>
<td>Bone, platelets</td>
<td>RIA, ELISA, IRMA</td>
<td>Serum</td>
</tr>
<tr>
<td>Bone alkaline phosphatase</td>
<td>Bone ALP</td>
<td>Bone</td>
<td>ELISA, IRMA, colorimetric assay</td>
<td>Serum</td>
</tr>
<tr>
<td>Procollagen type-I N-propeptide</td>
<td>PINP</td>
<td>Bone, soft tissue, skin</td>
<td>RIA, ELISA</td>
<td>Serum</td>
</tr>
<tr>
<td>Procollagen type-I C-propeptide</td>
<td>PICP</td>
<td>Bone, soft tissue, skin</td>
<td>RIA, ELISA</td>
<td>Serum</td>
</tr>
</tbody>
</table>

*According to the Bone Marker Nomenclature by the Committee of Scientific Advisors of the International Osteoporosis Foundation.*

![Figure 3](image.png)
cross-link, that insert into the circulation. With additional degradation in the liver and kidney the fragments are finally broken down to their constituent amino acids, and the pyridiniums, PYD and DPD. Although it is possible that soft tissues contribute to the normal excretion of DPD and PYD, bone represents the major reservoir of total collagen in the body and turns over faster than most major connective tissues. In contrast to Hyp and Hyl, the measurement of urine DPD and PYD is not influenced by the degradation of newly synthesized collagen fibrils or by dietary collagen intake. The pyridinolines are present in the diet, but unlike Hyp, they are not absorbed. Furthermore, unlike Hyp, the pyridinoline amino acids are fully excreted with no known pathway of metabolic degradation.

Amino- and carboxy-terminal cross-linking telopeptide of type I collagen
During collagen type I degradation by osteoclasts, N- and C-terminal peptide fragments (NTX and CTX, respectively) are released into the circulation. These fragments represent a spectrum of proteins having different sizes, as shown in Fig. 4. The majority of these is relatively small and passes through the glomerulus into the urine. NTX are regarded to be specific for bone tissue breakdown as other tissues comprised of type I collagen, e.g., skin, are not actively metabolized by osteoclasts, and, therefore, different types of fragments are formed during breakdown of non-skeletal tissues. An ELISA method has been developed to recognize a discrete pool of NTX isolated from urine. The monoclonal antibody recognizes the α2 chain N-telopeptide fragment. This fragment contains the pyridinium cross-links, but this assay does not recognize the PYD and DPD per se. This implies bone specificity since the pyridinoline cross-link in bone primarily involves the α2 chain whereas in other tissues the α1 chain predominates. NTX, contains the cross-linked α2 N-telopeptide sequence, QYDGKGVG, which is a product of osteoclastic proteolysis and in which lysine (K) is embodied in a trivalent cross-linkage. Collagen must be broken down to small cross-linked peptides that contain this exact sequence before the antibody can bind to the NTX antigen. This suggests that the NTX peptide is a direct product of osteoclastic proteolysis, does not require further metabolism in the liver or kidney for generation, and is rapidly cleared by the kidney.

Other assays have also been developed for the measurement of epitopes associated with the C-terminal telopeptide of type I collagen (α-CTX, β-CTX, ICTP) in serum and urine. Due to bone specificity and their unique characteristics NTX, ICTP, and CTX have almost totally replaced the use of older resorption indices in the diagnostic assessment of bone diseases.

Tartrate resistant acid phosphatase isoform type 5b
Tartrate resistant acid phosphatase isoform type 5b (TRACP-5b) is a novel marker of bone resorption which has been in use over the last 3–4 years with very encouraging results. TRACP is produced by both osteoclasts and activated macrophages and subsequently is secreted into the circulation. Two forms of TRACP circulate in human serum, macrophage-derived TRACP-5a and osteoclast-derived TRACP-5b. The only structural difference between TRACP-5a and 5b is that the former contains sialic acid residue(s) that are not found in TRACP-5b. In human serum, TRACP-5b circulates in a large complex that contains α2-microglobulin and calcium. Osteoclasts secrete TRACP-5b into the blood circulation as a catalytically active enzyme that is inactivated and degraded to fragments in the circulation. Thus, all catalytically active TRACP-5b molecules measured in the serum are freshly liberated from the osteoclasts, providing a sensitive resorptive index.

Formation markers

Osteocalcin
Osteocalcin is one of the most abundant non-collagenous bone proteins, produced by osteoblasts, odontoblasts, and hypertrophic chondrocytes. Most
of the circulating osteocalcin is a product of osteoblast activity and thus considered a marker of bone formation. It is a small protein of 49 amino acids and in most species contains three residues (at 17, 21, and 24) of γ-carboxy glutamic acid, a calcium-binding amino acid. This vitamin K-dependent post-translational modification of newly synthesized proteins results in γ-carboxylation of specific glutamate residues. The reaction is comparable to the activation of vitamin K-dependent blood coagulation factors and is inhibited by warfarin. The human osteoblast produces an 11-kDa molecule consisting of a 23-residue hydrophobic signal peptide, a 26-residue propeptide, and the 49-residue mature protein. The pro-region contains a γ-carboxylation recognition site homologous to corresponding regions in the vitamin K-dependent clotting factors. After the hydrophobic regions cleaved by a signal peptidase, pro-osteocalcin is γ-carboxylated. Subsequently, the propeptide is removed and the mature protein is secreted. In serum, osteocalcin is degraded so that both the intact peptide and fragments coexist in the circulation. Therefore, assays that evaluate both intact osteocalcin and fragments are more accurate for the measurement of serum osteocalcin.

A fraction of newly synthesized osteocalcin is secreted into the circulation, while during bone resorption osteocalcin is also degraded. Thus there is some question whether osteocalcin should be considered an indicator of osteoblast activity or a marker of bone matrix metabolism or turnover.

The human osteocalcin gene is located at the distal long arm of chromosome 1. Various promoter elements contribute to basal expression and osteoblast specificity. The gene is further modulated by vitamin D and glucocorticoid response elements. Although osteocalcin is present in significant amounts in bone, dentin, and calcified cartilage, it has recently also been found in osteosarcomas, prostate, ovarian, lung and brain cancer. Osteocalcin function has not clearly defined yet. However, it is assumed that much of the newly synthesized protein is incorporated into the bone matrix binding calcium. Serum levels of osteocalcin are significantly influenced by gender, age, and renal function.

**Bone-specific alkaline phosphatase**

Alkaline phosphatase (ALP) is a ubiquitously expressed, cell-membrane enzyme. ALP belongs to the category of molecules that are localized to cell membranes through a COOH-terminal glycanscaffolding domain anchor, providing a basis for understanding the generation of different isoforms observed in plasma. Isoforms produced by differential cleavage or preservation of the glycan–phosphatidylinositol anchor originate from different tissues, such as liver, bone, intestine, spleen, kidney, and placenta. Liver and bone (bALP) isoforms account for almost 95% of the total ALP activity in the serum. Bone ALP is produced by osteoblasts and has been demonstrated in matrix vesicles deposited as ”buds” derived from the cell membrane. These deposits seem to play an important role in bone formation.

Bone ALP is produced in extremely high amounts during bone formation phase of bone turnover, and is, therefore, an excellent indicator of total bone formation activity.

**Type I procollagen propeptides**

Collagen type I, a 300-kDa protein, makes up 90% of the organic bone matrix and is synthesized by osteoblasts in the form of procollagen, having a molecular weight (MW) of 450-kDa. Extracellular processing of procollagen before fiber assembly includes cleavage of the N- and C-terminal extension propeptides, that are termed procollagen type I N- and C-propeptide (PINP, and PICP, respectively), having a MW of 35 and 100 kDa, respectively. PINP is cleared via the scavenger endothelial system in the liver, and PICP via the mannose receptors on liver endothelial cells. Because these peptides are generated in a stoichiometric 1:1 ratio with newly formed collagen molecules, their levels in serum are considered an index of collagen synthesis and thus of bone formation. Serum levels of the PICP have been demonstrated to correlate with histomorphometric measures of bone formation, and hormone replacement or bisphosphonate therapy leads to a reduction in the circulating concentration of this marker. Most recent studies, however, suggest that the PINP has a greater diagnostic validity than PICP.

**Markers of bone remodeling in myeloma bone disease**

**Comparison with normal individuals and correlations with the extent of myeloma bone destruction**

Markers of both resorption and formation have been used in an attempt to better evaluate the extent of bone disease in multiple myeloma and assess clinical correlations. Table 3 summarizes the available data for the use of markers of bone
Table 3  Studies describing the levels of markers of bone remodeling in MM patients and correlation with clinical data.

<table>
<thead>
<tr>
<th>Authors–year</th>
<th>Number of patients</th>
<th>Parameter studied</th>
<th>Comparison with controls</th>
<th>Correlation with extent of bone disease</th>
<th>Correlation with survival</th>
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<tr>
<td>Nawawi et al., 1996&lt;sup&gt;85&lt;/sup&gt;</td>
<td>17</td>
<td>DPD, TRACP, OC, bALP, PICP</td>
<td>↑&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Abildgaard et al., 1997&lt;sup&gt;86&lt;/sup&gt;</td>
<td>109</td>
<td>ICTP, OC, bALP, PICP, PIIINP</td>
<td>↑&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Withold et al., 1998&lt;sup&gt;87&lt;/sup&gt;</td>
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<td>DPD, PYD, bALP, PICP</td>
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<td>Carlson et al., 1999&lt;sup&gt;88&lt;/sup&gt;</td>
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<td>Woitge et al., 2001&lt;sup&gt;103&lt;/sup&gt;</td>
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<td>OC, bALP</td>
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<td>Corso et al., 2001&lt;sup&gt;91&lt;/sup&gt;</td>
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<td>Jakob et al., 2002&lt;sup&gt;92b&lt;/sup&gt;</td>
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<td>Alexandrakis et al., 2002&lt;sup&gt;93&lt;/sup&gt;</td>
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remodeling in MM. Markers of bone resorption are increased in the urine or serum of patients with MM compared to healthy individuals. Both PYD and DPD, and NTX in the urine and ICTP in the serum are elevated in patients with MM compared to controls, and correlate with the extent of bone disease. Our group has shown that TRACP-5b serum levels also correlate with the extent of bone disease in newly diagnosed myeloma patients. NTX urinary levels have also been found to be increased in myeloma including those patients in plateau phase of myeloma, while PYD, DPD and NTX were elevated in myeloma patients before autologous transplantation. A histomorphometric study in bone marrow biopsies of 16 myeloma patients has shown that urinary NTX levels have shown the strongest positive correlation with the dynamic histomorphometric indices of bone resorption, followed by serum ICTP and urine DPD, while urine PYD did not correlate with the histomorphometric findings. Moreover, a recent comparison between these four markers (PYD, DPD, NTX, and ICTP) has revealed that serum ICTP and urine NTX reflected the extent of myeloma bone disease more accurately and could also predict early progression of the bone disease after standard chemotherapy. A further analysis of the same group has confirmed that both ICTP and NTX have predictive value for myeloma bone destruction and disease progression. Furthermore, serum ICTP levels were significantly elevated in myeloma patients with abnormal bone magnetic resonance imaging (MRI) scans compared with myeloma patients with normal MRI findings. In this study by Jakob et al., the sensitivity of ICTP for depiction of MRI abnormalities was 79%, while the positive and negative predictive values were 85% and 84%, respectively. NTX urinary levels also correlated with the overall score of skeletal involvement as measured by Tc-99m-MIBI scintigraphy, and bone marrow infiltration by plasma cells. These results suggest that serum ICTP and urinary NTX correlate significantly with the extent of myeloma bone disease and we believe that the measurement of these values is feasible in the context of any therapeutic clinical trials.

Markers of bone formation have been used in several studies but the results have been variable. In other studies, osteocalcin and bALP were elevated in myeloma patients compared with controls, while in others they were either reduced or within normal limits. In the study of Fonseca et al., which includes the largest number of myeloma patients (313 patients) serum levels of bALP correlated significantly with bone pain, lesions and fractures even if the mean values were not different from control values; osteocalcin levels were lower in myeloma patients than in controls but they showed no correlation with the extent of bone disease. Our data, confirmed that OC levels were decreased in myeloma patients but we found that they were associated with the extent of bone disease, while bALP levels were not.

<table>
<thead>
<tr>
<th>Authors–year</th>
<th>Number of patients</th>
<th>Parameter studied</th>
<th>Comparison with controls (symbol refers to MM patients)</th>
<th>Correlation with extent of bone disease</th>
<th>Correlation with survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abildgaard et al., 2003</td>
<td>34</td>
<td>OC, bALP</td>
<td>↑^a</td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>PYD, DPD, ICTP, NTX</td>
<td>↑^a</td>
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<td>NA</td>
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<tr>
<td>Terpos et al., 2003</td>
<td>121</td>
<td>NTX, TRACP-5b, OC, bALP, sRANKL/OPG</td>
<td>↑^a</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>OC, bALP</td>
<td>↓^a</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

NS, non significant; NA, not assessed.
^a Statistically significant (p<0.05).
^b MM compared with MGUS patients.
differences among the different studies may be due to different study population and the different phase of bone turnover in each population. PICP values do not predict the extent of myeloma bone disease. As shown in Table 3, we believe that markers of bone formation may be of some value but may not necessarily reflect the extent of myeloma bone destruction.

Correlations with disease activity and survival

There are several studies in which markers of bone remodeling have shown strong correlation with the stage of myeloma. Serum levels of ICTP and urinary values of NTX were higher in myeloma stage II/III than stage I disease. Abnormal high DPD urinary levels also correlated with advanced myeloma stage. Our group has also reported in 121 newly diagnosed myeloma patients, that stage of the disease correlated with both TRACP-5b and NTX (p<0.0001, and p=0.014, respectively), showing a borderline correlation with OC (p=0.046) but no correlation with bALP (p=0.73). Fig. 5 depicts the differences in NTX urinary levels among myeloma patients at different disease stages, patients with MGUS and normal individuals reported by our group. The association of osteocalcin with stage III disease has also been shown in the study of Woitge et al., while two other studies showed no correlation of either osteocalcin or bALP with stage or other clinical parameters. Markers of both formation and resorption also correlated with markers of disease activity, such as β2-microglobulin and IL-6, in several studies.

However, it is clear that markers of bone resorption and in particular, ICTP correlates with survival in myeloma. Fonseca et al. have shown that the median survival was 4.1 and 3.5 years for patients with low and high ICTP levels, respectively (p=0.02). Furthermore, Jakob et al. have shown that ICTP is a prognostic factor for overall survival (p<0.03), while urinary NTX showed borderline significance (p=0.05). Turesson et al. has shown in an unselected series of 394 myeloma patients that ICTP correlated with survival in the univariate analysis; something that was not confirmed in the multivariate analysis. We had the same results in terms of association of urinary NTX levels with survival; univariate analysis showed a predictive value of NTX, but this was not confirmed by the multivariate analysis. Recently, Abildgaard et al. using sequential measurements of both ICTP and NTX showed that both markers predict progression of myeloma. No marker of bone formation has been shown to correlate with survival.

![Figure 5](image.png)

**Figure 5** NTX urinary levels in MGUS patients, myeloma patients and normal controls.
Bone markers for monitoring bisphosphonates or anti-myeloma treatment

Biochemical markers of bone turnover have been used in patients with MM in order to monitor bisphosphonate treatment and identify who will benefit the most from bisphosphonate therapy. Both ICTP and NTX have shown a dramatic decrease after clodronate, pamidronate or zoledronic acid administration, in myeloma patients, confirming the strong anti-resorptive activity of these agents that consist part of anti-myeloma therapy in patients with bone lesions.87,90,97,106–108 Elomaa et al.106 have shown that clodronate administration resulted in a significant decrease of PINP and ICTP in 244 myeloma patients compared to the control group; furthermore, high baseline ICTP, PINP and ALP levels indicated a poor prognosis. Our group has shown that pamidronate in combination with chemotherapy reduces urine NTX levels significantly, compared with myeloma patients who receive chemotherapy.

Figure 6  Pamidronate (90 mg per month) in combination with chemotherapy (group I) results in a dramatic decrease of urinary NTX levels compared to controls (group II in (a)) and patients receiving a combination of ibandronate (4 mg per month) and chemotherapy (group II in (b)). Pamidronate (group I) was also more effective than ibandronate (group II) in reducing serum TRACP-5b levels in MM patients (c).90,108
alone. Fig. 6(a) depicts this difference between the two groups of patients. Furthermore, in addition to the inhibition of osteoclastic activity, pamidronate in combination with interferon-\(\alpha\) was shown to induce bone formation in patients with myeloma in the plateau phase. In this point it is reasonable to note that bALP and/or OC rises after successful treatment of MM in those patients with bone disease as a sign of repair. Zoledronic acid, which is a potent inhibitor of osteoclastic activity has also shown a reduction in NTX levels at a dose of 4 mg per month in myeloma patients, while we reported that pamidronate at a dose of 90 mg could reduce both NTX and TRACP-5b levels more effectively than 4 mg of ibandronate (Fig. 6(b) and (c)). In addition, bone remodeling markers seem to be normalized after high dose chemotherapy with autologous stem cell support (Fig. 7). However, we believe that bisphosphonate treatment has to be continued post ASCT even if all markers of bone remodeling have been normalized due to the possible antimyeloma effect of bisphosphonates.

A recent study has shown that high levels of ICTP and NTX correlated with an increased risk for early progression of bone lesions during standard melphalan–prednisolone treatment in myeloma. This study suggests that ICTP and NTX are clinically useful for identifying patients with increased risk of early progression of bone disease and therefore of disease progression.

**Future developments**

**RANKL/Osteoprotegerin**

RANKL and OPG play a crucial role in the development of myeloma bone disease (Fig. 1). It has been demonstrated that patients with advanced prostate cancer have significantly higher serum OPG levels than patients with early stage disease. In another study, significant differences in OPG serum levels were found between prostate cancer patients with bone metastases compared with healthy controls, patients with non-metastasized cancer, and patients with benign hyperplasia. Even when compared with NTX serum levels, OPG was found to be of higher sensitivity in detecting bone metastases than NTX. However, serum levels of soluble RANKL have no correlation with bone disease in prostate cancer.

OPG levels have been found to be reduced in myeloma patients, while the ratio of sRANKL/OPG...
is increased.\textsuperscript{17–19} RANKL/OPG ratio has also reported to be increased in patients with cancer and severe osteolysis.\textsuperscript{24} Our group has shown that the ratio of sRANKL/OPG correlates with the extent of bone disease in myeloma, and with markers of bone resorption, such as NTX and TRACP-5b (Fig. 8).\textsuperscript{19} Recently, the results of a phase I study using a recombinant osteoprotegerin construct in patients with multiple myeloma or breast cancer-related bone metastases were published. A single dose of AMGN-0007, a recombinant osteoprotegerin construct, suppressed bone resorption as indicated by a rapid, sustained, and profound decrease of urinary NTX levels, and the role of OPG as a therapeutic tool in this area will be clarified in future clinical trials.\textsuperscript{113}

Other potential markers reflecting bone destruction in MM

Bone sialoprotein (BSP) is a phosphorylated 70–80 kDA glycoprotein that accounts for 5–10\% of the non-collagenous bone matrix. The protein is a major synthetic product of active osteoclasts and odontoblasts. In bone remodeling BSP is involved in the adhesion of bone resorbing cells to the extracellular bone matrix.\textsuperscript{114} BSP levels were associated with skeletal involvement and tumor cell burden and survival in MM patients.\textsuperscript{46} The quantification of serum
BSP may be a non-invasive method for the diagnosis and follow-up, and may improve the prognostic value of conventional staging in myeloma.

Finally, the novel protein DKK1, a WNT pathway inhibitor, has been linked to the function of osteoblasts in myeloma. Immunohistochemical analysis of bone marrow-biopsy specimens showed that myeloma cells contained detectable DKK1. Elevated DKK1 levels in bone marrow plasma and peripheral blood from patients with multiple myeloma correlated with the gene-expression and peripheral blood from patients with multiple myeloma. Barlogie B, Shaughnessy J, Tricot G, et al. Treatment of multiple myeloma. Blood 2004;103:20–32.

Conclusions

Biochemical markers of bone remodeling are potentially useful tools for the evaluation of the extent of bone disease and the follow-up of anti-resorptive treatment in patients with multiple myeloma. In patients with established bone lytic lesions, most bone markers are abnormal, indicating that these parameters faithfully reflect changes in bone metabolism associated with the malignant process. However, ICTP and NTX seem to be better markers in reflecting both, the severity of bone destruction and the efficacy of bisphosphonate treatment in myeloma. TRACP-5b also seems to be a useful marker but needs further evaluation. Although it is unlikely that a single marker of bone remodeling has sufficient diagnostic or prognostic value in myeloma bone disease, the combination of these markers with other laboratory tests and imaging techniques is likely to improve the clinical assessment of patients with myeloma.

There is a correlation between serum ICTP and urinary NTX and an increased risk of progressive bone disease, and consequently to myeloma progression. Patients with smoldering myeloma and myeloma patients with normal radiographs are subgroups in which the assays might be particularly useful. Elevated levels of these resorption markers may support a decision to initiate anti-myeloma therapy in patients with smoldering myeloma or of starting bisphosphonate treatment in patients without lytic bone disease. In the present era where new anti resorptive agents are in development (more potent bisphosphonates, osteoprotegerin, Rank-Fc, etc.) these assays might be of particular value. However, further trials are needed to establish the predictive value of these markers before introducing them into routine use.

References


