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Bone markers in multiple myeloma

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ABSTRACT

Bone disease, a hallmark of multiple myeloma occurs in the majority of the patients, is associated with bone pain, fractures, hypercalcemia and has major impacts on quality of life. Myeloma is characterized by a unique form of bone disease with osteolytic bone destruction that is not followed by reactive bone formation, resulting in extensive lytic lesions. This review will focus on the pathophysiology of osteoclast activation and osteoblast inhibition in multiple myeloma and on biochemical markers of bone turnover. Since osteolytic lesions do not rapidly heal in myeloma, X-rays cannot reflect the activity of bone disease during antimyeloma treatment. Activity in bone turnover does not parallel changes in monoclonal protein levels. Thus, there is a need for biochemical markers reflecting disease activity in bone. The utility, prognostic implications and limitations of classical and novel markers of bone remodeling (e.g. ICTP, NTx, TRACP-5b, osteoprotegerin, sRANKL) will be discussed in this overview.

1. Background

For myeloma patients, osteolytic bone destruction is a major clinical problem, which negatively affects their quality of life.[1] About 75% of the myeloma patients have skeletal involvement with bone pain, lytic lesions, diffuse osteoporosis or pathologic fractures at the time of diagnosis and almost all patients develop bone manifestations in the later clinical course.[2] Most common osteolytic lesions include the central skeleton, the skull and the femur, while in approximately 15% of patients, diffuse osteopenia is the only bone manifestation. The standard diagnostic procedure for the detection of skeletal involvement is conventional radiography. Since histomorphometric studies have shown that abnormal bone degradation can exist in the absence of osteolytic lesions in skeletal radiography,[3] the diagnostic sensitivity of conventional X-ray appears to be low in early myeloma. Magnetic resonance imaging (MRI) was established as a non-invasive technique, which can recognize bone abnormalities in multiple myeloma patients with greater sensitivity than conventional radiography or bone densitometry.[4,5] In addition to imaging techniques, new biochemical parameters have been evaluated for monitoring the present bone resorption activity in multiple myeloma.

The basic principle of increased bone resorption in multiple myeloma is an uncoupling of the normal bone remodeling

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with enhanced osteolytic resorption and decreased bone formation. By mechanisms discussed in the following section, myeloma cells both stimulate osteoclast activity and suppress osteoblast function. The increase in number and activity of osteoclasts further promote myeloma progression directly by cell-cell interactions and indirectly by cytokines released from the bone matrix during enhanced bone resorption, thus maintaining a vicious circle between bone destruction and tumour cell survival.

2. Myeloma cells increase osteoclast activity

A consistent histological finding in myeloma bone disease is enhanced osteoclast accumulation and bone resorption adjacent to myeloma cells, while osteoclasts are not increased in bone not invaded by myeloma.[6] *In vitro* co-cultures of purified preosteoclasts and primary human myeloma cells show that myeloma cells induce the differentiation of progenitors into mature osteoclasts, which in turn support the survival of plasma cells.[7] Therefore, it has been suggested that osteoclasts are stimulated by local osteoclast activating factors (OAFs) which are produced by myeloma cells or cells of the bone microenvironment.[8] Several factors have been found to be overproduced in myeloma and were discussed as potential osteoclast promoting factors. Lymphotoxin was one of the first OAFs implicated in myeloma bone disease.[9] However, using sensitive ELISA assays, it was undetectable in freshly isolated bone marrow plasma from patients with advanced disease.[10] TNF-α was another factor studied because of its osteoclast stimulating capacity.[11] Although TNF-α protein [12] and mRNA[13] were detected in myeloma, recent studies could not find a correlation between TNF-α levels and bone disease in myeloma patients.[10] IL-1β has been described as another potential stimulator of osteoclast formation[14] and was found in supernatants of isolated myeloma cell cultures.[12] However, IL-1β protein was not detectable in myeloma cells or in bone marrow plasma from patients with myeloma bone disease.[10,13] Moreover, neutralizing IL-1β antibodies could not inhibit the activation of osteoclasts.[15] Several *in vitro* and *in vivo* studies suggested that IL-6 can stimulate osteoclast formation and induce bone resorption.[16] IL-6 is secreted by stromal and myeloma cells and acts as an important growth and survival factor for myeloma cells.[17] While some experiments showed a correlation between IL-6 gene expression and bone disease,[18] other groups were unable to find a correlation between IL-6 protein levels and bone resorption.[10,13] Moreover, blocking antibodies against IL-6 could not inhibit the osteoclastogenic effects of secreted factors by myeloma cells.[19] IL-11 is an IL-6 like molecule that is produced by myeloma cells.[20] Using cultured mouse calvarial bones, Ahlen suggested that IL-11 can stimulate osteoclast resorption.[21] IL-3 was recently introduced as a new OAF in myeloma. Increased levels of IL-3 mRNA and protein were found in primary myeloma cells and bone marrow plasma.[22] However, there is no clear-cut correlation between protein levels of these factors and severity of bone resorption, thus these factors can not be regarded as main inducers of osteoclast activation in multiple myeloma. Recently, three major groups of factors have been identified as main osteoclast inducers in multiple myeloma: the receptor activator of NF-κB ligand (RANKL), the chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β, and stromal derived factor-1α (SDF-1α).

2.1. *Myeloma cells lead to an imbalance in the RANKL/OPG system*

RANKL (synonym: tumour necrosis factor-related activation induced cytokine, TRANCE) has been characterized as the key mediator of osteoclast differentiation and activation. RANKL is a member of the tumour necrosis factor (TNF) superfamily[23] and is produced mainly by osteoblastic lineage cells and stromal cells. RANKL exists as a cell membrane bound isoform, a secondary soluble variant that is cleaved from the cellular form by metalloproteases and TNF-α converting enzyme (TACE)[24,25] and a primary secreted isoform.[26] The cellular receptor for RANKL, RANK, is expressed by osteoclast precursors and mature osteoclasts. RANKL induces differentiation, formation, fusion and survival of preosteoclasts.[27] Moreover, it has direct effects on mature osteoclasts causing actin ring formation, cytoskeletal rearrangements that precede bone resorption, and activating mature osteoclasts to resorb bone.[28]

OPG acts as a decoy receptor antagonist for RANKL.[29] It is secreted mainly by osteoblastic lineage and stromal cells.[30] A balanced RANKL/ OPG ratio is essential for a normal bone turnover. In animal models, unbalanced expression of these cytokines led to extreme skeletal phenotypes, e.g. severe osteopetrosis in RANKL knockout mice[31] or osteopenia in OPG deficient mice.[32] In humans, an abnormal RANKL/OPG ratio is found both in benign and malignant bone disease.[33–35] Several studies investigated the role of the RANKL/RANK/OPG system in myeloma bone disease.[36,37] It could be shown that myeloma cells induce the RANKL expression by stromal cells[36,38,39] and endothelial cells[40] within the bone microenvironment through direct cell to cell contact. Moreover, animal models[41] as well as studies in humans could show a direct RANKL expression of myeloma cells themselves on protein level[42,43] and by RT-PCR.[44–46] Interactions between plasma and stromal cells lead to increased RANKL expression in both cell types.[47] In a study evaluating the clinical impact of RANKL expression, normal plasma cells from controls showed no or only a weak expression of RANKL, whereas surface RANKL could be detected on bone marrow plasma cells from all patients with multiple myeloma. Myeloma cells from patients with lytic bone lesions showed a significantly higher level of surface RANKL expression compared to myeloma cells from patients without osteolyses.[48]

In addition to the effects on RANKL, myeloma cells decrease the OPG availability within the bone microenvironment. They lead to a reduced OPG secretion by osteoblasts and stromal cells.[36,38] Moreover, myeloma cells produce and shed syndecan-1 (CD 138), a transmembrane proteoglycan that binds to the heparin-binding domain of OPG and mediates its internalization and lysosomal degradation.[49] The combination of these effects results in an increased RANKL/OPG ratio in the bone marrow microenvironment that favours the formation and activation of osteoclasts. The resulting enhanced bone resorption releases various cytokines and growth factors (e.g. TGF-β, IL-6) from the extracellular bone matrix that further stimulate myeloma proliferation, thus

maintaining a vicious circle between osteoclasts and myeloma cells (Fig. 1). The binding of RANKL to its transmembrane receptor RANK activates signalling cascades, including the NF-κB-pathway. Inhibition of proteasome, a treatment used in multiple myeloma, was shown to inhibit the NF-κB-pathway in osteoclasts and reduce osteoclast differentiation and activity.[50,51] Direct RANKL blockade has been evaluated in animal models[41,52] and first clinical trials[53] showed not only inhibition of development of osteolytic bone lesions, but as well a decreased tumour burden.

2.2. *Myeloma cells produces MIP-1α and MIP-1β*

MIP-1α belongs to the RANTES (regulated on activation normal T cell expressed and secreted) family of chemokines and was known as chemoattractant and activator of phagocytes.[54] Recent studies showed that in addition, MIP-1α is chemotactic for osteoclast precursors,[55] induces late stage of differentiation on human osteoclast progenitors[56] and promotes osteoclast formation in bone marrow cultures.[10,57] Both MIP-1α and MIP-1β are produced and secreted by myeloma cells.[10,56] The levels of MIP-1α and MIP-1β correlated with the severity of myeloma bone disease or the ability of myeloma cells to enhance osteoclastic bone resorption by several authors.[10,56,58,59] In preclinical experiments, antibodies against MIP-1α and MIP-1β or their receptor CCR5[56] as well as transfection of myeloma cells with an antisense construct to MIP-1α[60] could block enhanced bone resorption. Studies on the action of MIP-1α and MIP-1β suggested that their effects on osteoclasts are dependent on the RANKL pathway. MIP-1α and MIP-1β enhance the RANKL expression in stromal cells.[56] In a murine model of myeloma, injection of recombinant MIP-1α produced a strong increase in osteoclast formation in normal mice, but not in RANK-/- animals.[61]

In addition to these effects, MIP-1α can directly act on myeloma cells, since they express the receptor CCR5.[56] Several studies showed that MIP-1α promotes growth, survival and migration of myeloma cells.[62] It could be shown that MIP-1α- induced signalling involved activation of the phosphatidylinositol 3-kinase (PI3-K)/AKT and mitogen-activated protein kinase (MAPK) signalling pathway in myeloma cells leading to increased proliferation and protection against apoptosis.[62]

In animal models, inhibition of MIP-1α reduced myeloma cell homing, tumour growth and the development of osteolytic lesions.[60,61] Moreover, MIP-1α inhibits the proliferation of CD34+ cells and thereby impairs haematopoiesis.[63] Although yet not clinically used, targeting MIP-1α could provide an additional approach in the treatment of myeloma bone disease.

2.3. *Myeloma cells express SDF-1α*

SDF-1α is a chemokine expressed by bone vascular endothelial and marrow stromal cells. SDF-1α binds to its receptor CXCR4, which is expressed on osteoclast precursors, thereby inducing chemotaxis, matrix metalloproteinase-9 (MMP-9) activity, and collagen transmigration.[64] Recently, it was shown that myeloma cells produce SDF-1α protein.[65] The plasma levels of SDF-1α were elevated in myeloma patients as compared to controls and positively correlated with the presence of bone lesions on radiology. *In vitro,* SDF-1α increased osteoclast motility and bone-resorbing activity.[65] This was associated with an overexpression of osteoclast activation–related genes, including RANKL, RANK, TRAP, MMP-9, CA-II, and Cathepsin K. In this model, osteoclast activation mediated by myeloma cells could be reduced using the CXCR4-specific inhibitor 4F-Benzoyl-TE14011 (T140).[65] These finding implicate that SDF-1α is an important factor in the pathogenesis of myeloma bone disease.

3. Myeloma cells suppress osteoblast function

While most studies on myeloma bone disease were focused on osteoclast activation, the influence of myeloma cells on osteoblasts is yet not well characterized. In contrast to bone metastases in other malignancies, multiple myeloma causes bone destruction without a sufficient osteoblastic reaction.

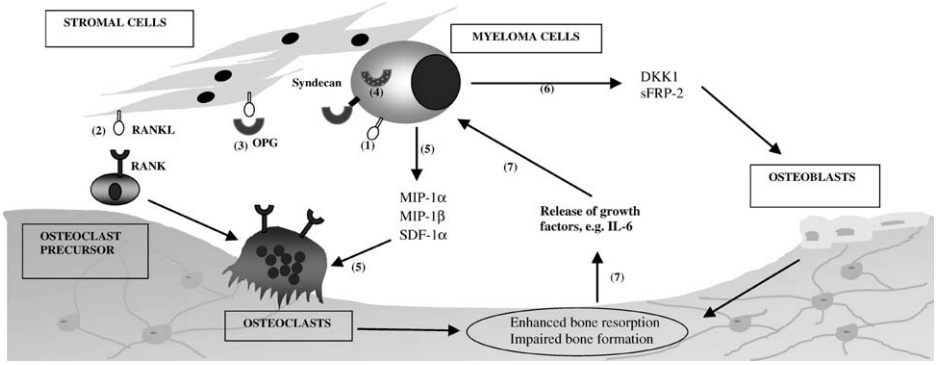

Fig. 1 – Pathophysiology of myeloma bone disease. Myeloma cells produce RANKL (1) and cause bone marrow-residing stromal cells to over-express RANKL (2). OPG is the soluble RANKL antagonist (3). Myeloma cells inhibit OPG production by stromal cells and bind circulating OPG by Syndecan-1, thus facilitating its internalization and degradation (4). The ensuing enhanced RANKL-to-OPG ratio promotes osteoclast formation. In addition, myeloma cells express MIP-1α, MIP-1β and SDF-1α, which recruit osteoclast precursors and enhance osteoclast activity (5). On the other hand, myeloma cells secrete DKK-1 and sFRP-2, which inhibit osteoblast differentiation and function (6). The resulting enhanced bone resorption releases various cytokines and growth factors, which in turn promote myeloma cells proliferation and survival (7).

Histomorphometric analysis of bone biopsies from patients with overt myeloma showed a reduced number and activity of osteoblasts on bone surfaces adjacent to myeloma cells.[66,67] Moreover, *in vitro* studies revealed that osteoblast growth and function are inhibited when co-cultured with myeloma cells or in medium conditioned by myeloma cells, suggesting that this effect is due to soluble osteoblast inhibiting factors.[68,69]

3.1. *Effects on the Wnt pathway*

The canonical Wingless-type (Wnt) pathway has been demonstrated to be a major signalling pathway in osteoblasts. Wnt glycoproteins bind to the Wnt receptor and its coreceptors LRP5/LRP6 and lead to a stabilization of β-catenin. This results in its cytoplasmic accumulation, translocation into the nucleus and stimulation of expression of osteoblastic target genes.[70,71] In the absence of a Wnt signal, β-catenin is phosphorylated and degraded by the proteasome. Extracellular Wnt antagonists prevent the binding of Wnt glycoproteins to their receptors and can be divided into two functional classes.[72] Members of the secreted frizzled-related protein (sFRP) class, for example sFRP-2 and sFRP-3 (synonym FrzB), are known to bind to Wnt proteins, whereas members of the DKK family bind to the LRP5/LRP6 component of the Wnt receptor complex. Both result in a suppression of Wnt-signalling and a reduced osteoblast function.

Using gene-expression profiles of myeloma patients, Tian found an overexpression of the *DKK-1* gene in multiple myeloma patients with focal bone lesions.[73] Moreover, DKK-1 protein could be detected in myeloma cells and elevated levels of DKK-1 in peripheral blood and bone marrow plasma from patients with osteolytic lesions. *In vitro,* recombinant human DKK-1 or bone marrow plasma with high DKK-1 levels inhibited osteoblast function. This effect was neutralized by a polyclonal anti-DKK-1 antibody.

In addition, myeloma cell lines and primary myeloma cells from patients with bone lesions have been shown to produce the soluble Wnt inhibitor sFRP-2 and thereby suppress the mineralisation and alkaline phosphatase activity in osteoblasts. Immunodepletion of sFRP-2 significantly restored mineralized nodule formation *in vitro*.[74]

3.2. *Other effects of myeloma cells on osteoblasts*

Other mechanisms add to the effect of myeloma cells on osteoblasts. Myeloma cells inhibit osteoblast formation via cell-to-cell contact by suppressing the activity of Runx2/Cbfa1, another critical osteoblast transcription factor in pre-osteoblastic cells.[75] In addition, they are able to induce apoptosis in osteoblasts. Silvestris found a significantly increased expression of Fas ligand (Fas-L) and tumour-necrosis-factor-related apoptosis inducing ligand (TRAIL) in myeloma cells and an overexpression of Fas and death receptor (DR) 4/5 by osteoblastic lineage cells obtained from patients with extensive osteolytic lesions.[76] In that study, osteoblasts from patients with active myeloma were functionally exhausted and promptly underwent apoptosis in the presence of myeloma cells. Moreover, osteoblasts upregulate intercellular adhesion molecule-1 (ICAM-1) and monocyte chemotactic protein-1 (MCP-1) when co-cultured with myeloma cells, thus promoting adhesion between both cell types and triggering pro-apoptotic signals.[77] Furthermore, TNF-α and IL-1β, cytokines that are overexpressed in myeloma, have been demonstrated to increase apoptosis in osteoblasts[78,79] and may contribute to the inhibitory effects of myeloma on osteoblasts. IL-3, a factor produced by myeloma cells, has been shown to inhibit osteoblast differentiation.[80]

Additional effects of myeloma cells on osteoblasts include an upregulation of IL-6 secretion through cell-to-cell contact[81] and a downregulation of OPG mRNA by osteoblastic lineage cells.[36] Further research on the interaction between myeloma cells and osteoblasts is needed in order to understand the mechanism of osteoblast inhibition and to identify possible therapeutic targets in the treatment of myeloma bone disease.

4. Urinary and serum markers of bone destruction

Most markers of bone resorption measure collagen degradation products. Fasting urinary calcium and hydroxyproline were used in the past, but are now regarded as obsolete due to their low sensitivity and specificity.[82] During collagen type I degradation by osteoclasts, N- and C-terminal peptide fragments of collagen I (NTx and CTX, respectively) are released into the circulation. The majority of these is relatively small and passes through the glomerulus into the urine. The degradation products of type I collagen, pyridinoline (PYD), deoxypyridinoline (Dpd) and amino-terminal collagen type-I telopeptide (Ntx) can be measured in urine and carboxy-terminal telopeptide of type-I collagen (ICTP) in serum. The cleavage points of the ICTP molecule are mainly affected by proteases which are activated under pathological conditions.[83] Serum markers are more reproducible than urine markers. These parameters have been reported to have diagnostic and prognostic relevance in some types of metabolic or malignant bone disease, such as in solid tumours with bone metastases.[84] In multiple myeloma, urinary Dpd levels were reported to be significantly elevated in myeloma patients compared to a control population.[85] Deoxypyridinoline is more specific for bone than pyridinoline (PYD). A histomorphometric study in bone marrow biopsies of myeloma patients showed that urinary Ntx levels and serum ICTP, but not urine PYD correlated with the histomorphometric findings.[86] ICTP serum levels have been reported to be significantly elevated in myeloma patients compared to control individuals.[87] In a study with 75 patients with multiple myeloma or monoclonal gammopathy of undetermined significance, serum ICTP and urinary Dpd levels increased parallel to the stage of the disease and differed significantly between MGUS, myeloma stage I and myeloma in stages II-III according to Durie and Salmon ($P < 0.001$ for ICTP and $P = 0.03$ for Dpd).[88] ICTP and Dpd were significantly elevated in patients with multiple myeloma in stage I compared to individuals with MGUS, while no significant difference was found for NTx. In this first study comparing the prognostic relevance of ICTP, NTx and Dpd in multiple myeloma patients, ICTP was found to be a prognostic factor for overall survival in the Kaplan-Meier analysis, while urinary Ntx showed borderline

significance and Dpd had no prognostic value. Furthermore, Jakob and colleagues evaluated serum ICTP levels in untreated patients with multiple myeloma, who had no skeletal abnormalities in conventional radiographs and correlated these data to MRI findings of the spine.[89] Serum ICTP was significantly elevated in patients with abnormal bone MRI compared to those patients with normal MRI findings. The sensitivity of ICTP for depiction of MRI abnormalities was 79%, the positive and negative predictive value was 85% and 84%, respectively. Compared to ICTP the parameters of disease activity, β2-microglobulin and C-reactive protein had a much lower sensitivity for abnormal MRI (29% and 64%, respectively). This study showed that in myeloma patients without osteolytic lesions in conventional radiography, abnormal skeletal MRI is accompanied by an increase in serum levels of ICTP. ICTP can be used as an inexpensive parameter to identify myeloma patients with normal skeletal survey who have a high probability of skeletal involvement and deserve more accurate diagnostic evaluation using MRI.

Another study compared the biological sensitivity and clinical usefulness of ICTP in serum and urinary PYD, DPD and Ntx.[90] ICTP remained more sensitive than the urinary assays when patients with impaired renal function were excluded from analysis. High levels of ICTP and urinary Ntx correlated with an increased risk for early progression of bone lesions during standard melphalan–prednisolone treatment. In a sequential analysis of biochemical markers of bone resorption, Abilgaard found that serum ICTP and urinary NTx were predictive of progressive bone events.[91] In Cox analysis, ICTP showed the highest predictive value, but should be replaced with NTx in patients with nephropathy. In a recent study in patients receiving zoledronic acid or pamidronate, myeloma patients with high and moderate Ntx levels had 2-fold increases in their risk of skeletal complications and disease progression compared with patients with low Ntx levels. High Ntx levels were associated with a 4- to 6-fold increased risk of death on study, and moderate Ntx levels a 2- to 4-fold increased risk of skeletal events compared with low Ntx levels.[92]

Table 1 – Serum and urinary markers of myeloma bone disease

Bone markers in myeloma	Specimen	Molecular mechanism	Clinical relevance
Dpd Deoxypyridinoline	Urine	Degradation product of type I collagen, bone resorption marker	Correlation with MM stage[88] Correlation with extent of bone disease[87]
Ntx Amino-terminal collagen type-I telopeptide	Urine	Degradation product of type I collagen, bone resorption marker	Correlation with histomorphology[86] Correlation with extent of bone disease[90] Predictive for bone events[91]
ICTP Carboxy-terminal telopeptide of type-I collagen	Serum	Degradation product of type I collagen, bone resorption marker	Correlation with MM stage[88] Correlation with extent of bone disease[90] Correlation with MRI abnormalities[89] Prognostic factor for overall survival[88] Predictive for bone events[91]
TRACP-5b *Tartrate-resistant acid phosphatase isoform-5b*	Serum	Produced by osteoclasts	Reflects osteoclast activity Correlation with extent of bone disease[93]
sRANKL *Soluble Receptor activator of NF-κB ligand*	Serum	Stimulator of osteoclast differentiation and activation, enhanced in MM	RANKL is elevated in bone marrow environment in myeloma bone disease, measurement in serum experimental, correlation of serum sRANKL/OPG with extent of bone disease was reported in one publication,[106] confirmatory data needed
OPG *Osteoprotegerin*	Serum	RANKL antagonist	decreased in bone marrow environment in myeloma bone disease, measurement in serum cannot be recommended for clinical purposes
OC *Osteocalcin*	Serum	Non-collagenous protein produced by osteoblasts	Marker of bone formation Reduced in MM as compared to MGUS[94] Correlation with bone disease[106]
bAP *Bone-specific alkaline phosphatase*	Serum	Produced by osteoblasts during bone formation phase of bone turnover	Marker of bone formation Reduced in MM[90,94]
PICP *Carboxy-terminal propeptide of type I collagen*	Serum	Type I Procollagen propeptide	Marker of bone formation
PINP *N-terminal telopeptide of type I collagen*	Serum	Type I Procollagen propeptide	Marker of bone formation

Recently, tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) was evaluated as a new marker reflecting osteoclast activity in myeloma.[93] TRACP-5b levels were found to be associated with the radiographically assessed severity of bone disease.

In summary, serum ICTP and urinary Ntx are the most sensitive tools for estimating the increased bone resorption in multiple myeloma and are clinically useful for identifying patients with increased risk of progression of bone disease (Table 1). Today, we have only bisphosphonates as antiresorptive drugs, thus the clinical usefulness of bone markers may be limited due to the lack of alternative therapies targeting osteoclast activity. In future, there is hope that a number of different treatment strategies will be available for myeloma bone disease and there will be need to guide the treatment using biological markers of bone remodeling.

5. Serum markers of bone formation

Osteocalcin and bone-specific alkaline phosphatase (BAP) are the most widely used parameters of osteoblast activity, carboxy-terminal propeptide of type I collagen (PICP) and N-terminal telopeptide of type I collagen (PINP) are used less frequently. In contrast to bone metastases in other malignancies, myeloma causes bone destruction without a propositional osteoblastic reaction. The impairment of osteoblast function is reflected by reduced osteocalcin and BAP levels in patients with multiple myeloma in comparison to MGUS patients or controls.[94] Fonseca also reported a significant decrease of osteocalcin levels in patients with multiple myeloma in comparison to healthy controls.[95]

Bisphosphonates are currently used as antiresorptive drugs in myeloma bone disease and target osteoclasts, but not osteoblasts. Myeloma treatment which leads to a disease remission is usually not accompanied by an increase of osteoblast markers or bone mineral density.[96,97] Thus there is a need for treatment strategies to improve bone formation in multiple myeloma.

6. Novel serum markers associated with myeloma bone disease

As discussed in detail above, myeloma bone disease is a disease of the bone marrow microenvironment and a result of the interaction of myeloma cells with stromal cells, endothelial cells, osteoclast precursors, mature osteoclasts and osteoblasts. These interactions contain cell-cell interactions and paracrine interactions. Myeloma bone disease is not a systemic (humoral) disease regulated hormonally. Therefore it is not necessary, that cytokines and ligands specifically regulated in the bone marrow microenvironment and involved in myeloma bone disease must be associated with valid changes of the serum levels of these cytokines. For example, although it is clear that RANKL and MIP-1α levels are elevated and osteoprotegerin levels are decreased in the bone marrow in multiple myeloma, it is not clear whether the serum levels of these molecules can be considered as valid surrogates of bone marrow levels. The authors suggest that serum levels of these cytokines should be considered with caution and given the limited and conflicting data, serum cytokine levels should not be regarded as a valid reflection of the bone marrow microenvironment.

6.1. Serum OPG levels

An increased RANKL/OPG ratio in the bone marrow microenvironment favours the activation of osteoclasts. Seidel reported lower median OPG in serum in patients with myeloma (7.4 ng/mL) than in healthy age- and sex-matched controls (9.0 ng/mL), with a large overlap between two groups.[98] The median OPG level in patients lacking osteolytic lesions was higher than in patients with osteolytic disease. There was no correlation between serum OPG levels and clinical stage or survival.[98] Lipton reported that OPG values did not differ significantly by age, but serum levels were significantly higher than levels in plasma.[99] Patients with myeloma had lower serum levels than controls. In other studies, pretreatment OPG serum levels were found to be increased in myeloma patients in comparison to healthy controls.[100–102] Strikingly different values of serum OPG were reported in healthy controls from studies on cancer patients. Different antibody-based ELISAs have been used in these studies; however, it is worth noticing that quite discrepant results have been obtained by different groups when using presumably the same matched antibody pair provided by the same manufacturer.[103,104]

These conflicting results seem to be related to following facts: OPG is produced by various skeletal and extra-skeletal tissues[105]; there is no bone-specific fraction of OPG in contrast to other skeletal markers such as alkaline phosphatase; and most OPG assays measure both free and RANKL-bound OPG and do not distinguish between these two fractions.[103] Thus, serum OPG levels should be interpreted with caution and there is no clear evidence that serum OPG reflects the availability of OPG in the bone marrow environment.

6.2. Serum soluble RANKL (sRANKL) levels

This test measures free RANKL not bound to OPG. Terpos reported elevated serum levels of sRANKL in patients with multiple myeloma.[106] The sRANKL/OPG ratio in serum was also increased and correlated with osteolytic lesions and survival. No confirmatory data have been published so far. The sRANKL concentrations in serum were remarkably lower in the hand of other groups. Some data showed no significant difference in sRANKL levels between multiple myeloma patients with or without osteolytic lesions, others reported even a reduced sRANKL/OPG ratio in serum in myeloma patients.[107] Since the serum levels of sRANKL were lower than the detection limit of the ELISA in a relevant portion of patients, the same company now provides a novel kit termed total RANKL. This test is told to measure both free and OPG-bound RANKL. The clinical relevance of the measured sRANKL or total RANKL levels in serum is not clear at this time.

6.3. Serum levels of other factors

MIP-1α was also reported to be elevated in the peripheral blood of patients with multiple myeloma.[108] Confirmatory data are needed for serum levels of some novel players in

myeloma bone disease to establish whether serum concentrations reflect the availability of these molecules in the bone marrow environment and the clinical utility of these novel serum markers.

Conflict of interest statement

None declared.

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