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Bone metabolic markers as gauges of metastasis to bone: a review

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Currently, imaging techniques are the leading methods used to diagnose of metastasis to bone. However, these techniques are expensive, expose patients to toxic and radioactive compounds, and monitor response to treatment poorly; these drawbacks have prompted the search for alternative screening methods. Therefore, bone metabolic markers have been evaluated as possible methods to diagnose and monitor the development and progression of metastatic bone disease. Although bone metabolic markers are often grouped as either resorption or formation markers, studies have revealed that each marker has its own biologic meaning and clinical relevance. Recent milestones in the use of bone metabolic markers as screening methods for metastatic bone disease and as evaluation methods for treatment response are shown in the following lists.

1. Bone metabolic marker measurements provide insight into mechanisms of metastasis to bone.
2. Although promising data have been reported, bone metabolic markers are not yet considered to be reliable screening methods for metastasis to bone.
3. Bone metabolic markers are reliable indicators of response to both conventional and bisphosphonate therapies.
4. Preliminary results indicate bone metabolic markers might be an independent prognostic factor in patients whose tumors metastasize to bone.
5. New or refined assays for bone metabolic markers are expected to improve the sensitivity and specificity of bone metabolic marker use in diagnosing and monitoring metastasis to bone.

Key words: bone metabolic markers, metastatic bone disease, diagnosis of bone metastasis, monitoring bone response

INTRODUCTION

BONE is the third most common site of tumor metastasis ranking after lung and liver.[1] Breast, prostate, lung, and thyroid cancers as well as multiple myeloma are tumors that most frequently metastasize to bone. Because increased patient survival and recovery depend on the early detection and treatment of metastatic tumors, physician are always looking for the best diagnostic techniques to provide their patients with the best treatment options possible. To aid physician in this task, the authors of this article discuss the most recent developments in research on bone metabolic markers and their clinical application to detect metastasis to bone.

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Current Techniques to Diagnose Metastasis to Bone

Diagnosis of bone metastasis commonly relies on imaging techniques of which bone scan plays the major role, because of its high sensitivity and the ability to examine whole body.[2] There are many reports and guidelines that describe bone scan results can be used to diagnose metastasis to bone in patients with malignancies.[2–4] The key points for the diagnosis of metastasis to bone on bone scan are summarized in Table 1. As the table shows there are several characteristic patterns in the appearance of bone scans that aid the diagnosis of metastasis to bone.

Another use of imaging techniques is to assess the efficacy of anti-metastatic treatment. However, even though the accuracy of current imaging methods have improved since their introduction, using these techniques

Table 1 Identification of malignant metastatic tumors by accumulation of bone scan

1. Tumors that metastasize to bone grow in the bone marrow space and therefore show tracer uptake parallel to the axis of bone. In contrast, traumatic bone shows tracer uptake at orthogonal or oblique angles to the bone axis. This is typically seen in the ribs.
2. Tumors that metastasize to bone develop inside the bone itself, but degenerative bone changes develop along the joint space.
3. Metastatic bone tumors often show an abnormal uptake called a "doughnut" lesion. These lesions result of tumor replace in the central part and reactive bone formation is prominent in the peripheral part. The resulting picture is increased tracer uptake in the rim and cold or decreased tracer uptake in the center of the lesion, which mimics "doughnut." In contrast, tracer uptake by benign bone lesions shows high uptake in the center of the lesion, and gradually decreases to the peripherally of the lesion. Osteoblastic skeletal metastasis typically caused by prostate cancer is the exception.
4. Skeletal metastasis often appears asymmetric or scattered.
5. The sites of skeletal metastasis are most often in the axial skeleton, metastasis to the appendicular skeleton is rare. This is typically seen in diffuse bone metastasis. Although the appendicular metastasis is rare, this occurs in patients with squamous cell lung cancer or renal cell cancer.

to assess treatment for metastasis to bone quantitatively remains difficult. One reason is that the accuracy of bone scan is affected by the flare phenomenon.[5,6] The bone scan flare phenomenon refers to the increased intensity of radiotracer uptake by metastatic bone lesions, the appearance of new lesions, or both that occur shortly after commencement of therapy in patients whose metastatic tumors ultimately respond to the therapy. Although this effect usually subsides within 6 to 9 months after the start of therapy, this effect unfortunately delays gathering critical information on treatment efficacy.

Although bone scan is considered the most powerful technique for the initial diagnosis of bone metastasis, its efficacy and high cost prevented its wide spread use for the routine screening of metastasis to bone.[7,8] It should be mentioned that the accumulation of radiopharmaceuticals of bone scan relies on the activity of bone formation. Therefore, bone metastatic foci that are predominant of osteolytic may not be visualized by bone scanning. Consequently, less expensive, less toxic, and more accurate methods to detect and assess metastasis to bone have been urgently sought by physician and patients alike. This led researchers to assess the suitability of biologic bone markers as a diagnostic and prognostic technique.

Rationale Behind Using Bone Metabolic Markers

There are three clinical types of bone metastasis: osteolytic, osteoblastic, and mixed. Each reflects the tumor effect on bone physiology differently. Uncoupling, or imbalance of bone formation and resorption, in the bone remodeling process occurs during osteolytic bone metastasis. Bone resorption increases but fails to stimulate adequate bone formation. The consequent calcium or mineral loss from bone results in destruction of bone trabecullae. Typically, this is seen in patients with renal cell carcinoma, multiple myeloma, thyroid cancer and most of lung cancer. In contrast, osteoblastic metastasis promotes positive uncoupling of bone formation and resorption. Although bone formation predominates, bone resorption also increases. Osteoblastic metastasis typically accompanies prostate cancer, but also occurs in other cancers such as gastric and breast cancers, and carcinoid tumors. However, most patients with bone metastasis of breast or gastric cancer show mixed or lytic patterns, relatively few patients show strictly osteoblastic metastasis to bone. The mixed appearance reflects the coexistence of both osteolytic and osteoblastic processes, and is usually seen in patients with breast cancer. For most other kinds of cancer, almost all metastases to bone are the mixed type with each patients exhibiting different ratios of osteolytic and osteoblastic components. Finally an additional type of bone metastasis is the intertrabecullar type. Although this is included in histopathologic classification, is difficult to diagnose clinically.[9] Consequently, this type is not usually included in common clinical use.[10]

Another purpose to study bone metabolic markers in metastasis to bone is to clarify and measure the disease process. A new strategy for treating and preventing bone metastasis using bisphosphonate compounds is in development. These compounds work by interfering with osteoclast activity. Consequently, accurate understanding and measurement of the process of bone remodeling is needed to use this new treatment strategy to its full potential.

Until recently, the only available metabolic markers to measure bone turnover were serum alkaline phosphatase for bone formation and urinary calcium and hydroxyproline for bone resorption.[7] However, none of these markers is specific for bone and all are unreliable for detecting metastasis to bone. Recently, newly characterized biochemical markers of bone metabolism have been applied to detect and monitor various bone diseases.[11] These metabolic markers are good candidates for developing screening methods to diagnose and measure metastasis to bone. Recently, clinical data on the prognostic value of bone metabolic markers have been reported.[12] These data indicate that patients with high levels of bone metabolic markers had a poor prognosis. However, these data must be critically examined with the proper use of statistical methods.

Bone Metabolic Markers

Tables 2 and 3 list the clinically useful bone metabolic markers currently in use.[11,13] They are divided into 2

Table 2 Bone resorption markers

Type-I collagen degradates
1. Pyridinium cross-links
   - Urine pyridinoline (PYP), deoxy-pyridinoline (DPD); HPLC method
   - Urine free deoxypyridinoline (fDPD)
2. Pyridinium cross-linked collagen peptide fragment
   - Digested by Cathepsin K
     - C-terminal telopeptide (CTx, Crosslaps)
     - N-terminal telopeptide (NTx, Osteomark)
   - Digested by MMPs
     - serum C-terminal telopeptide (ICTP)
3. Galactosyl hydroxylysine (GHYL)
4. Hydroxyproline

Non-collagenous protein in mineral component
- Bone sialoprotein (BSP)

Enzymes secreted from osteoclasts
1. Tartrate-resistant acid phosphatase 5b (TRAP 5b)

Table 3 Bone formation markers

1. Type-I procollagen propeptide; proliferation
   - C-terminal propeptide fragment (PICP)
   - N-terminal propeptide fragment (PINP)
2. Alkaline phosphatase; matrix maturation
   - Total alkaline phosphatase (Al-p)
   - Bone alkaline phosphatase (BAl-p)
3. Osteocalcin, bone gla protein (BGP); mineralization
   - C-terminal fragment
   - mid portion
   - intact

Abbreviations

BAl-p; bone specific alkaline phosphatase
BGP; osteocalcin, also known as bone gla protein
BSP; bone sialoprotein
CA 15-3; cancer antigen 15-3 specific to breast cancer
CTx; cross-linked C-telopeptides of type I collagen
DPD; deoxy-pyridinoline
ICTP; pyridinoline cross-linked carboxyl-terminal telopeptides of type I collagen
MMPs; matrix metalloproteinase
NTx; cross-linked N-telopeptides of type I collagen
PICP; carboxyl-terminal propeptide of type I procollagen
PINP; amino-terminal propeptide of type I procollagen
PYP; pyridinoline
ROC; receiver operating characteristics
TRAP 5b; tartrate resistant acid phosphatase type 5b
UICC; international union against cancer

classes: resorption markers (Table 2) and formation markers (Table 3).

1. Bone resorption

The osteoid matrix consists principally of collagen (90%), other smaller proteins, and proteoglycans. The main structural protein of bone is type-I collagen. Consequently, most available bone resorption markers are based on degradation products of type-I collagen. The bone resorption that occurs at the site of bone metastasis is thought to be mediated by osteoclast. Indeed, results of immunohistochemistry using antibody against tartrate-resistant acid phosphatase (TRAP) show a layer of osteoclasts between the bone matrices and tumor cells in tissue samples taken from both nude mice and humans.[14] Therefore, the measurement of bone resorption markers, both collagen degradation products and osteoclast-secreted proteins such as TRAP, is thought to reflect the bone resorption process produced by bone metastasis.

Type-I collagen cross-links such as deoxy-pyridinoline (DPD), pyridinoline cross-linked carboxy-terminal telopeptide (ICTP), cross-linked C-telopeptides of type-I collagen (CTx), and cross-linked N-telopeptides of type-I collagen (NTx) are the best choice of resorption markers for clinical use. Type-I collagen cross-links are sensitive and specific to bone. DPD can be measured in urine samples, and ICTP in serum samples. NTx and CTx can be measured both in urine and serum. However, the circadian change and deviation in some marker concentrations are large. Therefore, analysis of sampling time and deviations is critical to provide accurate clinical information (urine samples from the second morning urination are usually recommended). Food intake also influences CTx level.

Two major classes of proteases, matrix metalloproteinases (MMPs) and cysteine proteases, are believed to degrade the bone matrix.[15] MMPs are zinc-containing endopeptidases that are active at neutral pH. Several MMPs have been identified in isolated osteoclasts or bone tissue, including gelaninase B (MMP9), membrane-type (MT)-MMP (MMP14), collagenase 1 (MMP1), gelatinase A (MMP2), stromelysin (MMP3), and collagenase 3 (MMP13). It is thought that ICTP is made by MMPs (MMP2 or MMP13).

In contrast, cathepsins are members of the papain superfamily of cysteine proteases. Cathepsins work optimally at low pH and degrade acid-soluble type-I collagen. A newly described member of this class, cathepsin K, is a prominent and critical mediator of osteoclastic bone resorption.[16] Cathepsin K is abundantly expressed by osteoclasts, specifically at the cell surface adjacent to bone. Inhibition of cathepsin K activity inhibits osteoclast-mediated bone resorption *in vitro* and *in vivo*. Mutation in cathepsin K gene leads to impaired bone resorption. One manifestation of this condition is a broad fringe of demineralized matrix. In a patient with cathepsin K gene deficiency, most resorption markers such as NTx, CTx and DPD are not elevated, however, ICTP is elevated.[17] *In vitro* studies show that treating bone with cathepsin K, but not with MMPs produce NTx[18] and that treating with cathepsin K destroys its antigenic part of

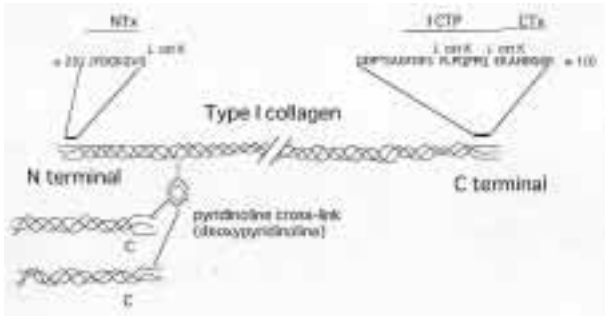

Fig. 1 Cross-linked N- and C-telopeptides of type-I collagen. The pyridinoline cross-links occur at two intermolecular sites in collagen fibrils. Type-I collagen, comprising two α1 chain and one α2 chain, is a triple helix except at the telopeptides which contain the cross-linking sites. Cathepsin K (cat K) cleaves type-I collagen at several sites, some sites are shown. The epitopes in the N- and C-telopeptides detected by three bone markers (NTx, CTx, and ICTP) are indicated.

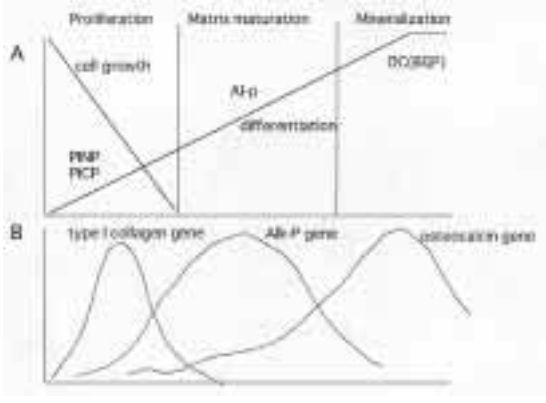

Fig. 2 Schematic illustrations of cell growth and differentiation in osteoblast developmental sequence (A), and temporal cell growth and differentiation related gene expression (B). The 3 principal periods of osteoblast developmental sequence are designated proliferation, matrix maturation, and mineralization. When the proliferation phase ends, expression of genes associated with extracellular matrix development and maturation increase quickly, and expression of genes associated extracellular matrix mineralization start to increase slowly. Representative genes for each phase are the type-I collagen gene for proliferation, the alkaline phosphatase gene for matrix maturation, and the osteocalcin gene for mineralization.

ICTP.[19] These findings indicate that NTx is produced by cathepsin K and that ICTP is not produced by cathepsin K. The results of recent studies suggest that CTx and NTx are produced by cathepsin K or MMP9.[20] MMP2 or MMP13 produces ICTP.[20] Cathepsin K plays the main role in physiologic bone resorption, and NTx and CTx are the products of this process. Therefore, NTx and CTx are thought to be indicators of healthy bone resorption processes, while ICTP is thought to be an indicator of pathologic bone resorption processes (Fig. 1).

TRAP is another bone resorption marker that is produced by osteoclasts.[13] TRAP was first discovered in leukocyte extracts of patients with hairy cell leukemia. It was named type 5 acid phosphatase according to its fast electrophoretic mobility. Later, this band 5 acid phosphatase was found in serum from healthy subjects, and it could be separated into two distinct bands; 5a and 5b. The 2 isoforms are almost identical, but they have a different carbohydrate content, 5a containing sialic acid that not found in 5b. Further studies suggest that TRAP 5b is derived from osteoclasts and 5a from other tissues.

Although several enzymatic assays for TRAP have been developed, these are not specific for bone because serum contains TRAP enzymes from erythrocytes and platelets and because bilirubin interferes with spectrophotometric detection. Several immunoassays have also been developed. An immunoassay that measures serum TRAP 5b has been published recently, and the results are promising.[13] Other immunoassays only measure total TRAP.

One other marker of note is bone sialoprotein (BSP), non-collagenous protein isolated from the mineral compartment of bone.[21] Clinical data suggest that its serum level mainly reflects process related to bone resorption.

2. Bone formation

Osteoblast-mediated bone formation can be divided into three phases: proliferation, matrix maturation, and mineralization.[22] This process shown schematically in Figure 2. There are many bone formation markers, each specific to one of these phases. The carboxy-terminal propeptide of type-I procollagen (PICP) is a marker of early bone formation and generally appears during osteoblast proliferation. Bone specific alkaline phosphatase (BAl-p) is a marker of the middle stage of bone formation and appears during the matrix maturation phase. Osteocalcin, also known as bone gla protein (BGP), is a marker of late bone formation and appears during the mineralization phase. The role of BGP may be related to the regulation of bone formation. Evidence for this hypothesis comes from experiments conducted with mice carrying BGP knockout mutations that eliminate BGP gene expression. Results from these experiments show that mice lacking BGP activity show excessive mineralization.[23] Although all these markers are all bone formation markers, the difference in the expression of these formation markers may be important when assessing the mechanisms of bone formation during metastasis to bone or when discrepancies arise during analysis with bone formation markers.

Diagnosis of Bone Metastasis Using Metabolic Markers

Measurement of bone metabolic markers to assess bone metastasis has been reported for several malignant diseases. Several groups have used bone metabolic markers to study the development of breast cancer and prostate cancer metastases to bone. These studies found bone

resorption markers are generally superior to bone formation markers in patients with most metastatic bone diseases except for prostate cancer. The range of sensitivity of bone resorption markers, which is the ability to detect metastasis to bone, has been reported to be 50% to 80%.[10] The sensitivity of markers changes with the cut-off level, the kind of primary disease and bone metastatic burden.[24] For example, the sensitivity of bone metabolic markers is not very high in patients with a single lesion or a small number of lesions. At present, there is only one report by Diel et al. that clearly shows the clinical value of bone metabolic markers in predicting metastasis to bone.[21] Although Diel et al. reported that high levels of BSP are a significant prognostic indicator for the development of bone metastasis,[21] this observation has not yet been confirmed.

1. Breast cancer

Many reports discuss the use of bone metabolic markers to discriminate between breast cancer patients with metastasis to bone and those patients without metastasis to bone. Levels of both resorption and formation markers are elevated significantly in patients with metastasis to bone associated with breast cancer. The results of several studies indicate that levels of bone resorption markers, especially Type-I collagen cross-links (DPD, ICTP, NTx and CTx), are promising indicators in detecting metastasis to bone.[10,24–27] However, the clinical usefulness of bone metabolic markers in diagnosing bone metastasis has not yet been established because most physician still use high sensitive imaging techniques for diagnosis. Also, the levels of bone metabolic markers change with many naturally occurring physiologic conditions besides bone metastasis, for example menopause. ICTP changes minimally during menopause, whereas NTx, CTx, and other metabolic markers change significantly during menopause.[27,28] Because the age of women affected by breast cancer ranges from pre-menopausal to post-menopausal, markers that change during menopause might not be good choice for the detection and monitoring metastasis to bone. Furthermore, chemotherapy and hormone therapies often change the menstruation status of breast cancer patients, creating a false menopausal effect. Additionally, increases in bone metabolic marker levels during menopause might cause problems in the serial measurement of patient at high-risk for developing metastasis. Therefore, detection techniques for bone metabolic markers must keep a proper signal (change produced by bone metastasis) to noise (change produced by processes other than bone metastasis) ratio so that the effectiveness of screening and treatment can be assessed accurately. Recently, the enzymatic processes that produce type-I collagen degrade have come to light. These results indicate type-I collagen degrade not produced by cathepsin K, such as ICTP, might be the key to solve this problem.

2. Prostate cancer

Prostate cancer tumors that metastasize to bone are usually osteoblastic. Not only bone formation markers[29,30] but also bone resorption markers[31–34] are elevated in patients with prostate cancer that metastasizes to bone. Even though metastatic tumors that arise from prostate cancer appear as osteosclerotic on X-rays, increased bone resorption also takes place, and the osteosclerotic appearance of X rays reflects the imbalance of bone formation and resorption. As bone metastasis progresses, PICP, PINP, and BAl-p levels increase but the osteocalcin also known as bone gla protein (BGP) levels do not.[29] This observation implies that BGP might be a regulatory signal protein for bone formation. As mentioned in a previous section, mice with mutations that knock out BGP gene showed increased bone density without changes in osteoclast activity.[24] Therefore, the presence of BGP is thought to be necessary to prevent excess bone formation. From a clinical standpoint, the larger the gap between BAl-p and BGP level is, the worse the bone lesion is.[35]

3. Other cancers

Bone resorption marker levels are elevated in patients with multiple myeloma, but bone formation marker levels are not.[36] Both bone resorption and formation marker levels are elevated in patients with skeletal metastasis of lung cancer, but bone resorption marker levels are much more elevated than the formation markers, and the sensitivity of bone resorption markers in determining metastasis to bone is very high.[37] Bone resorption markers might aid the interpretation of bone scan results.

Monitoring of Bone Metastasis

Physicians who use the UICC criteria[38] experience trouble in monitoring the therapeutic response of tumors that metastasize to bone. This is because bone lesions are evaluable but non-measurable lesions as the UICC criteria define. Relatively new imaging techniques, such as bone scan, CT, and MRI are also used to monitor the therapeutic response of bone metastasis, however, none of these have proven to be an ideal method of monitoring response for several reasons.[39] Because techniques to measure bone metabolic markers are non-invasive and do not exposed the patients to radiation, bone metabolic markers are expected to be a better tool for monitoring the response of metastatic tumors on bone to treatment.

1. Conventional systemic therapies (Chemotherapy and hormone therapy)

Because of accuracy and safety, bone metabolic markers hold the greatest promise as a means of monitoring the therapeutic response of tumors that metastasize to bone. Many authors report the usefulness of bone metabolic markers in monitoring. ICTP is a good serum metabolic marker for monitoring the response of breast cancer tumors that metastasize to bone[40]; NTx is currently the

best urinary marker among urinary Ca, urinary hydroxyproline and CA15-3.[39] Another study evaluated the ef-ficacy of bone scan, ICTP, BAl-p and CA 15-3 in monitoring the response of breast cancer patients with metastases to bone who were receiving combination chemotherapy.[41] ICTP was useful in discriminating between progression of disease and other conditions, even in patients whose bone scan exhibited flare phenomenon. BAl-p was not a good marker to monitor bone response because of a transient elevation in patients with bone scan flare.[41]

In patients with prostate cancer, DPD, PICP, PINP and ICTP are good markers to monitor the therapeutic response of tumors that had metastasis to bone.[30,33,34,41,42] Although the usefulness of bone metabolic markers to monitor prostate cancer that metastasize to bone is not yet established, one formation marker (PINP) showed the best ROC curve.[34]

In conventional chemotherapy and hormone therapy, bone metabolic markers, especially bone resorption markers, add useful information in monitoring of tumors that metastasize to bone. However, the question which resorption marker is best suited for monitoring is still unanswered. As the results discussed in this section indicate different markers or marker panels might be used for to monitor tumors of different tissue origins.

2. New therapy (Bisphosphonates)

Bisphosphonates are important new compounds in the management of tumors that metastasize to bone. Bisphosphonates became the treatment of choice for hypercalcemia associated with malignancy because they specifically inhibit bone resorption.[43] They are also able to reduce the skeletal complications in multiple myeloma[44] or breast cancer.[45] While results of several studies suggested that bisphosphonates retard or prevent the formation of new skeletal metastasis, other studies failed to confirm the result.[46] Therefore, question of whether bisphosphonates can prevent skeletal metastasis remains unanswered.

Nonetheless, for the existing therapeutic use of bisphosphonates, bone metabolic markers are used to monitor the response to therapy. Many authors have reported the following changes in bone metabolic marker levels during bisphosphonate treatment.[39,47–53] Bone resorption markers drop to a nadir 3–7 days after intravenous bisphosphonate administration. NTx and CTx levels show 80–90% reductions, DPD falls by 40–50%, PYP fell by 20–30%. Free DPD and ICTP falls by 10–20%.[29] There is controversy over which bone resorption marker is suitable to monitor the therapeutic response of tumors that metastasize to bone to bisphosphonate treatment. It is also critical to note that bisphosphonates act on all bones, regardless whether they carry metastatic tumors or not. However, what physicians need to know is the effect on metastatic bone only. Additionally to note, bone formation marker levels decrease gradually but the degree was small.[52,53] Therefore, bone formation markers are considered not to be useful to monitor the bisphosphonate response.

Prognostic Indicators in Patients with Bone Metastasis

Research is beginning to suggest that bone metabolic markers are independent prognostic indicators of survival in patients with metastasis to bone. In multiple myeloma, ICTP is a good marker for therapeutic response.[33] They stated that the prognosis is poor when the ICTP level is high at the time skeletal metastasis is diagnosed. Similar results were also reported in breast cancer patients with metastasis to bone.[12]

CONCLUSIONS

Recent milestones of bone metabolic markers in metastatic bone disease can be summarized as follows:

1. Bone metabolic marker measurements provide insight into mechanisms of metastasis to bone.
2. Although promising data have been reported, bone metabolic markers are not yet considered to be reliable screening methods for metastasis to bone.
3. Bone metabolic markers are reliable indicators of response to both conventional and bisphosphonate therapies.
4. Preliminary results indicate bone metabolic markers might be an independent prognostic factor in patients whose tumors metastasize to bone.
5. New or refined assays for bone metabolic markers are expected to improve the sensitivity and specificity of bone metabolic marker use in diagnosing and monitoring metastasis to bone.

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