Review Article

Bone Metabolic Markers in Bisphosphonate Therapy for Skeletal Metastases in Patients with Breast Cancer

Mitsuru Koizumi*[1], Shunji Takahashi*[2], and Etsuro Ogata*[3]

*Departments of *[1]Nuclear Medicine, *[2]Medical Oncology, and *[3]Internal Medicine, Cancer Institute Hospital, Japan.*

The use of bisphosphonates for skeletal metastasis of breast cancer is now well established. Although clinical judgement for treating skeletal metastasis is based on symptoms and imaging studies, accurate or quantitative means are few. Various bone metabolic markers have been developed and these were evaluated in patients with metastasis to bone. Bone metabolic markers, especially resorption markers, have been shown to be a good tool for the monitoring the response to therapy for skeletal metastasis. This is also true for bisphosphonate treatment for skeletal metastasis. Bone metabolic markers are produced by different mechanisms. There are some different classes of resorption markers; tartrate-resistant acid phosphatase (TRAP) is secreted by osteoclast, N- and C-terminal cross-linking telopeptide of type I collagen (NTx and CTx) are the degradation the products of type I collagen, mainly produced by cathepsin K, and pyridinoline cross-linked carboxyl-terminal telopeptides of type I collagen (ICTP) is also a degradation product of type I collagen, by matrix metalloproteases. Even though bone resorption markers are a good tool to monitor response to bisphosphonate therapy, there remains the question of which class of bone resorption markers is best suited to the task.

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Key words: Bone metabolic marker, Skeletal metastasis, Bisphosphonate

The breast is one of the most common sites of malignancy in women. The number of patients is now growing in Japan. In 1999, about 9,000 women (8% of female cancer deaths) died of breast cancer. Bone is the most common site of metastasis in patients with breast cancer. Post-mortem studies indicate that metastasis to bone was present in 47-85% of women who die of breast cancer[1]. The spread of breast cancer to bone can cause significant morbidity in terms of pain and decreased activity. Pathologic fractures can be devastating, as in decreased mobility from femoral fractures or in spinal cord compromise from vertebral body collapse. Because metastatic breast cancer is essentially not curable with current treatments, quality of life is emphasized. Reducing morbidity from bony metastasis is important for patients' quality of life.

Bisphosphonates have been shown in many studies to reduce skeletal complications associated with breast cancer[2-6]. In most studies, bisphosphonates have been shown to improve quality of life, but not to improve survival[2,5]. The guidelines of the American Society of Clinical Oncology state that bisphosphonates are recommended in patients with metastatic breast cancer with evidence of lytic destruction of bone on imaging and who are currently receiving systemic hormonal therapy or chemotherapy[7].

Diagnosis of skeletal metastasis commonly relies on imaging studies. There is no doubt that bone scan is very effective for diagnosis, because it is very sensitive and covers the whole body[8,9]. There are many publications regarding bone scan use and diagnosis of skeletal metastasis in patients with malignancies[8-10]. It should be empha-

Reprint requests to Mitsuru Koizumi, Department of Nuclear Medicine, Cancer Institute Hospital, 1-37-1, Kami-ikebukuro, Toshima-ku, Tokyo 170-8455, Japan.
E-mail: mitsuru@jfcr.or.jp

Abbreviations:
Al-p, Alkaline phosphatase; BAl-p, Bone specific alkaline phosphatase; BGP, Bone gla protein also called osteocalcin; BSP, Bone sialoprotein; Cat K, Cathepsin K; CTx, C-terminal cross-linking telopeptide of type I collagen; DPD, Deoxy-pyridinoline; fDPD, Free deoxy-pyridinoline; ICTP, Pyridinoline cross-linked carboxyl-terminal telopeptides of type I collagen; MMP, Matrix metalloprotease; MRI, Magnetic resonance imaging; NTx, N-terminal cross-linking telopeptide of type I collagen; OC, Osteocalcin; PICP, Carboxy-terminal propeptide of type I procollagen; PINP, amino-terminal propeptide of type I procollagen; PYP, Pyridinolone; TRAP, Tartrate resistant acid phosphatase; TRAP 5b, Tartrate resistant acid phosphatase 5b

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Table 1. Bone Resorption Markers

Type I collagen degradates
Pyridinium cross link
Urine pyridinoline (PYP), deoxy-pyridinoline (DPD); HPLC method
Urine free deoxy-pyridinoline (fDPD)
Pyridinium cross link collagen peptide fragment
Cathepsin K digestion
Urine and serum C terminal telopeptide (CTx, Crosslaps)
Urine and serum N terminal telopeptide (NTx, Osteomark)
MMPs digestion
serum C terminal telopeptide (ICTP)
Enzyme secreted from osteoclasts
Tartrate resistant acid phosphatase 5b (TRAP 5b)

sized that the accumulation of radiopharmaceuticals in bone depends on the activity of bone formation, so that some bone metastatic foci that are predominantly osteolytic may not be visualized on bone scan. In vertebral lesions, magnetic resonance imaging (MRI) is superior to bone scan in terms of sensitivity[11]. However, MRI cannot cover the whole body, and the sensitivity is not good for rib lesions.

Even though current imaging methods have been improved, it is difficult to assess the effect of various therapies on metastasis to bone exactly or quantitatively. The accuracy of bone scan is affected by the flare phenomenon[12, 13]. The bone scan flare phenomenon refers to the increased intensity of radiotracer uptake in metastatic bone lesions and/or the appearance of new lesions which occurs shortly after the commencement of therapy in patients who ultimately respond to the therapy. The healing process of lytic skeletal metastasis begins with a sclerotic rim or increased bone formation in the periphery of the lesion, which results in flare-up on bone scan. Changes on X-ray are evident at around 6 months after the start of therapy, which delays acquisition of critical therapeutic information. Bone metabolic markers, once proven accurate and effective, will become the tools of choice to monitor skeletal metastasis.

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. However, none of these markers is specific for bone and all are unreliable for detecting skeletal metastasis. Recently, newly characterized biochemical markers of bone metabolism have been applied to detect and monitor various bone diseases[14]. These metabolic markers are candidates for developing screening methods to diagnose and assess skeletal metastasis.

Table 2. Bone Formation Markers

Type-I procollagen propeptide — proliferation
C-terminal propeptide fragment (PICP)
N-terminal propeptide fragment (PINP)
Alkaline phosphatase — matrix formation
Total alkaline phosphatase (Al-p)
Bone alkaline phosphatase (BAl-p)
Osteocalcin, bone gla protein (BGP) — mineralization
C-terminal fragment
Intact
Bone sialoprotein (BSP) — mineralization

In this article, current research and the application of bone metabolic markers in clinics to assess metastasis to bone, especially their use for monitoring response to bisphosphonate therapy are described.

Bone Metabolic Markers

Tables 1 and 2 list the clinically useful bone metabolic markers currently in use[14, 15]. They are divided into 2 classes, resorption markers (Table 1) and formation markers (Table 2).

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 osteoclasts. Indeed, the results of immunohistochemical studies 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[16]. 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 deoxypyridinoline (DPD), pyridinoline cross-linked carboxyl-terminal telopeptides of type I collagen (ICTP), C-terminal cross-linking telopeptide of type I collagen (CTx), and N-terminal cross-linking telopeptide 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 on the second morning urination are usually recommended). Food intake also influences CTx levels.

Two major classes of proteases, matrix metalloproteases (MMPs) and cysteine proteases, are believed to degrade the bone matrix[17]. MMPs are zinc-containing endopeptidases that are active at neutral pH. Several MMPs have been identified in isolated osteoclasts or bone tissue, including gelatinase B (MMP 9), membrane-type (MT)-MMP (MMP 14), collagenase 1 (MMP 1), gelatinase A (MMP 2), stromelysin (MMP 3), and collagenase 3 (MMP 13).

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[18]. 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 the 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[19]. *In vitro* studies show that treating bone with cathepsin K, but not with MMPs, produces NTx[20] and that treating with cathepsin K destroys the antigenic portion of ICTP[21]. 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 MMP 9[22]. MMP 2 or MMP 13 produces ICTP[22]. 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).

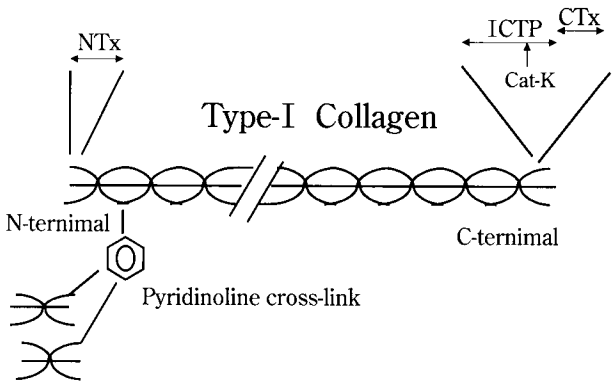

Fig 1. Cross-linked N- and C-telopeptides of type I collagen. The pyridinoline cross-links occur essentially at two intermolecular sites in collagen fibrils. Type I collagen, comprised of two $\alpha 1$ and one $\alpha 2$ chains, is a triple helix except at the telopeptides which contain the cross-linking 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.

TRAP is another bone resorption marker that is produced by osteoclasts[15]. 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, with 5a containing a sialic acid moiety 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

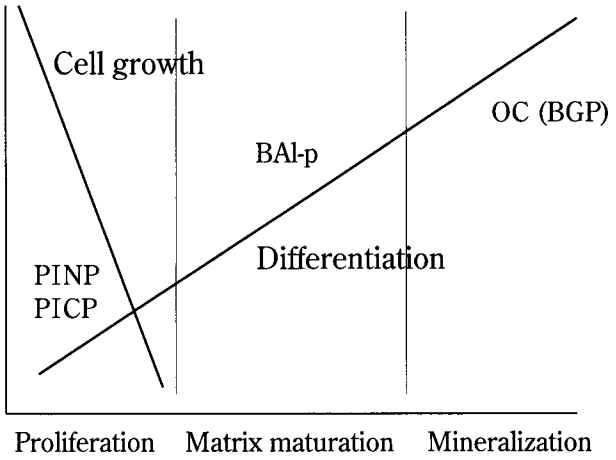

Fig 2. Schematic illustration of the osteoblast developmental sequence is shown. Three principal periods of osteoblast developmental sequence are designated as proliferation, matrix maturation and mineralization. At completion of proliferation genes associated with extracellular matrix development and maturation are upgraded, and genes associated extracellular matrix mineralization start to increase.

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[15].

2) Bone Formation

Osteoblast-mediated bone formation can be divided into three phases: proliferation, matrix maturation, and mineralization[23]. This process is shown schematically in Fig 2. There are many bone formation markers, each specific to one of these phases. The amino-terminal propeptide of type-I procollagen (PINP) 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[24]. 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.

One other marker of note is bone sialoprotein (BSP), another class of protein that appears rather late in the osteoblast development sequence[25]. BSP is important for both mineralization and the regulation of cell-cell interaction.

Diagnosis of Skeletal Metastasis Using Metabolic Markers

Many reports discuss the use of bone metabolic markers to discriminate between breast cancer patients with metastasis to bone and those patients without. 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 for detecting metastasis to bone[26-30]. However, the clinical usefulness of bone metabolic markers in diagnosing skeletal metastasis has not yet been established because most physicians still use highly sensitive imaging techniques for diagnosis. Also, the levels of bone metabolic markers change with many naturally occurring physiologic conditions in addition to skeletal metastasis, for example, menopause. ICTP changes minimally during menopause, whereas NTx, CTx, and other metabolic markers change significantly during menopause[30, 31]. 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 choices for detecting 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 with serial measurements of patients at high-risk of developing metastasis. Therefore, detection techniques for bone metabolic markers must keep a proper signal (change produced by metastasis to bone) to noise (change produced by processes other than metastasis to bone) ratio so that the effectiveness of screening and treatment can be assessed accurately. Recently, the enzymatic processes that produce type-I collagen degradation have come to light. These results indicate type-I collagen degradation products not produced by cathepsin K, such as ICTP, might be the

key to solve this problem.

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[25]. Although Diel *et al.* reported that high levels of BSP are a significant prognostic indicator for the development of skeletal metastasis, this observation has not yet been confirmed.

Monitoring of Skeletal Metastasis

Physicians who use the UICC criteria[32] 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 metastasis to bone, however, none of these have proven to be an ideal method of monitoring response for several reasons[33]. Because techniques to measure bone metabolic markers are non-invasive and do not expose the patients to radiation, bone metabolic markers are expected to be a better tool for monitoring the treatment response of metastatic tumors to bone.

1) Conventional Systemic Therapies (Chemotherapy and Hormone Therapy)

Because of their accuracy and safety, bone metabolic markers have the most potential to allow monitoring of the therapeutic response of tumors that metastasize to bone. Many authors have reported the usefulness of bone metabolic markers for monitoring. ICTP is a good serum metabolic marker for monitoring the response of breast cancer tumors that metastasize to bone[34]; NTx is currently the best urinary marker among urinary Ca, urinary hydroxyproline and CA15-3[35]. Another study evaluated the efficacy 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[36]. ICTP was useful for discriminating between progression of disease and other conditions, even in patients whose bone scan exhibited the flare phenomenon. BAl-p was not a good marker to monitor bone response because of a transient elevation in patients with bone scan flare[35].

In conventional chemo- and hormone therapy, bone metabolic markers, especially bone resorption markers, add useful information. However, the question of 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 to monitor tumors of different tissue origins.

2) 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[36]. They are also able to reduce skeletal complications in patients with breast cancer[2-5]. While results of several studies suggested that bisphosphonates retard or prevent the formation of new skeletal metastasis, other studies failed to confirm the result[4, 37, 38]. Therefore, the 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[33, 39-44]. 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%, and PYP falls by 20%-30%. Free DPD and ICTP falls by 10-20%[30]. There is controversy over which bone resorption marker is suitable to monitor the therapeutic response to bisphosphonate treatment of tumors that metastasize to bone. It is also critical to note that bisphosphonates act on all bones, regardless of whether they harbor metastatic tumors. However, what physicians need to know is the effect on metastatic bone only. Additionally, bone formation marker levels decrease gradually but the degree is small[43, 44]. Therefore, bone formation markers are considered not useful for monitoring response to bisphosphonates. According to the American Society of Clinical Oncology Guidelines, the use of biochemical markers to monitor bisphosphonate response is not suggested for routine care. Bone resorption markers are currently under investigation. These markers were used in clinical trials of bisphosphonates, and showed good correlation with the response of bone metastases[43-45].

Conclusions

Bisphosphonates are used for skeletal metastasis of breast cancer. Although clinical judgement

to determine treatment for metastasis to bone is based on symptoms and imaging studies, accurate or quantitative assessments are lacking. Bone metabolic markers, especially those that assess resorption, have been shown to be a good tool for monitoring the impact of bisphosphonate therapy on metastasis to bone. There are some different classes of resorption markers; TRAP is secreted from osteoclasts, NTx and CTx are the degradation products of type I collagen mainly by cathepsin K, and ICTP is a degradation product of type I collagen by MMPs. Even though bone resorption markers are good tools for monitoring response to bisphosphonate therapy, there is still the question of which class of bone resorption markers is best suited to this purpose.

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