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SPECIAL REPORT

Guidelines for the use of bone metabolic markers in the diagnosis and treatment of osteoporosis (2012 edition)

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Abstract Recently the clinical application of bone metabolic markers has achieved significant progress and the measurements of these indices give us a better understanding of the pathogenesis of osteoporosis. Bone metabolic markers were adapted to select drug treatment for osteoporosis and to evaluate drug efficacy. Therefore, the proper application and assessment of bone metabolic markers in clinical practice is very important. To achieve these aims, the committee on the guidelines for the use of biochemical markers of bone turnover in osteoporosis authorized by the Japan Osteoporosis Society has summarized recent progress in bone markers and proposed the proper utilization of bone markers. Although the use of bone metabolic markers now has an important role in the daily management of osteoporosis, their use in Japan is still insufficient because of insurance coverage limitations. Since the Japan Osteoporosis Society first created the 2001 guidelines, new bone metabolic markers have been

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introduced into clinical practice. The availability of new osteoporosis treatments that promote bone formation has changed the clinical application of bone metabolic markers in current practice. Therefore, revisions to the current clinical practice are needed which led to the proposal to create these new 2012 guidelines.

Keywords Guideline · Bone metabolic marker · Osteoporosis

Introduction

Current definition and concepts in osteoporosis

Since osteoporotic fractures and the associated medical costs are a serious concern in an aging society, considerable effort has been made to prevent fractures [1]. In 2000, at the National Institutes of Health (NIH) consensus conference in the United States, osteoporosis was defined as 'a skeletal disorder in a person who already has compromised bone strength, thus increasing the risk of bone fracture' [2]. Bone strength is determined by integrating bone mass and bone quality. The measurement of bone mineral density (BMD) using dual-energy X-ray absorptiometry (DXA) is extraordinarily important for the diagnosis and monitoring of osteoporosis [3]. BMD measurements are widely used to diagnose osteoporosis in accordance with diagnostic criteria around the world [4]. Since low BMD is known to be an independent risk factor for future fractures, BMD measurement has been adapted as a predictive factor for fractures in the calculation of the 10-year fracture probability in the WHO fracture risk assessment tool (FRAX®) [5]. However, in terms of the judgment on the treatment efficacy on an individual level, the clinical significance of BMD measurement is still controversial [6].

Bone quality, which is another constitutional factor of bone strength, is characterized by the following components—bone microarchitecture, bone turnover rate, microdamage accumulation, degree of calcification, and properties of bone matrix proteins including collagen and other bone-specific proteins [7, 8]. Among them, bone turnover rate and the properties of bone matrix proteins can be assessed at every clinical site by the measurement of bone metabolic markers and bone matrix markers [9] in serum and urine. Recently the clinical application of bone metabolic markers has achieved significant progress and the measurements of these indices give us a better understanding of the pathogenesis of osteoporosis. Furthermore, some of the bone metabolic markers predict future fracture risk. The bone metabolic markers were adapted to select drug treatment for osteoporosis and to evaluate drug efficacy. Therefore, the proper application and assessment of bone metabolic markers in clinical practice is very important. To achieve these aims, the committee on the guidelines for the use of biochemical markers of bone turnover in osteoporosis authorized by the Japan Osteoporosis Society has summarized recent progress in bone markers and proposed the proper utilization of bone markers.

If the progression of osteoporosis is prevented with effective treatment, quality of life (QOL) will be maintained in osteoporosis patients, and the costs of medical care for fractures are thought to be reduced. Therefore, early diagnosis of osteoporosis, effective treatment in patients who already have osteoporosis, more accurate treatment monitoring, and evaluation of fracture risk are important. Currently, bone morphological parameters based on bone biopsy are evaluated to meet these requirements. The findings can serve as markers to ascertain bone dynamics, including degree and rate of bone calcification, extent and degree of bone resorption, and degree and rate of bone formation. In addition, bone biopsy is an essential means to evaluate bone architecture. However, bone biopsy is an invasive test and is therefore not performed repeatedly in general clinical settings. Moreover, the results only reflect localized bone changes at the bone tissue sample site and may be unsuitable for evaluation of systemic bone changes.

Recently, quantification of BMD as the main method to diagnose osteoporosis and measurement accuracy has dramatically improved. However, essential clinical parameters of osteoporosis include more dynamic markers such as bone metabolic markers. Bone metabolism undergoes daily dynamic changes, and even with the same BMD, the metabolic state differs and the pathologic significance also differs. Therefore, to use BMD measurement as a dynamic marker, one must wait for an observation period of 6 months to 1 year before remeasurement, whereas bone metabolic markers accurately reflect the state of bone metabolism at the point in time of the measurement.

Bone metabolic markers can also be used as a guide to selecting pharmacotherapy. When there is doubt about choosing a drug, the use of bone metabolic markers can enable a more appropriate selection. Furthermore, to evaluate the effects of drug therapy on disease improvement, assessing the state of bone metabolism at the time of diagnosis is recommended whenever possible (Fig. 1). However, if a decision is made to select treatment with little influence on bone metabolism, then measuring bone metabolic markers to monitor drug treatment effects has little clinical significance.

Since the mechanism of bone remodeling has come to be better understood, novel bone metabolic assays to measure the products of collagen metabolism have also been developed. These measurements are now available in

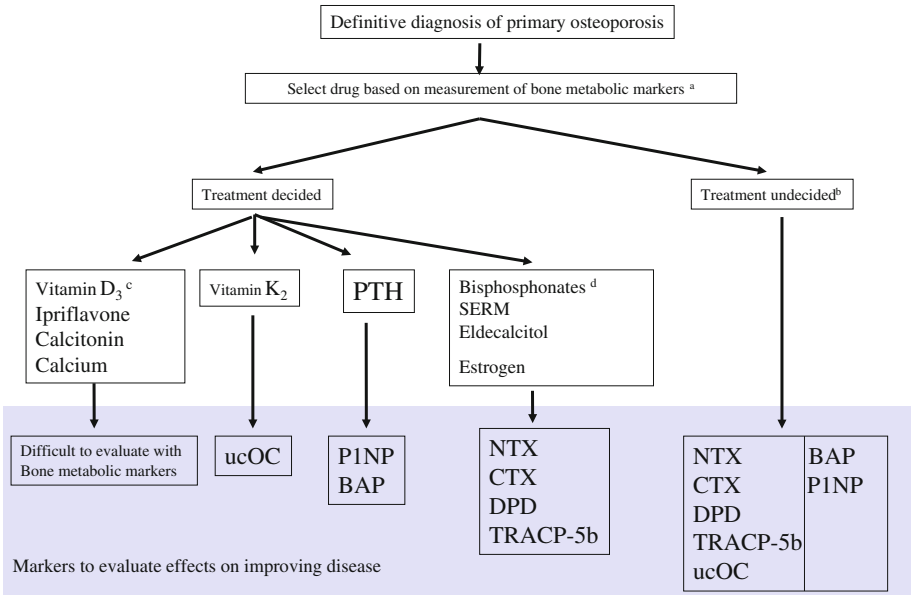

Fig. 1 Measurement of bone turnover markers in drug treatment of osteoporosis. This figure is taken from page 28 of the 2011 Osteoporosis Prevention and Treatment Guidelines (in Japanese). *a* In patients taking bisphosphonates, measure after stopping drug for at least 6 months, and in patients taking other osteoporosis drugs, measure after stopping drug for at least 1 month. *b* Measure one type each of a resorption marker and formation marker. *c* Excluding eldecalcitol. *d* In patients expected to be on long-term bisphosphonate therapy, measure bone resorption markers and BAP or P1NP

addition to those with a high sensitivity and specificity for the enzyme activity of osteoblasts and osteoclasts. Thus, bone metabolic markers have attained a position as a tool to clinically evaluate bone turnover. Other than bone metabolic markers, there are no other means to clinically evaluate bone turnover. Bone metabolic markers have become indispensable clinical test parameters in the management of osteoporosis and their use continues to expand. Guidelines for the use of bone metabolic markers in osteoporosis (2012 edition) are a revision of the 2004 edition [10] and subsequent new National Health Insurance (NHI) coverage of bone metabolic markers.

Changes in the diagnosis and treatment of osteoporosis

Together with significant changes in the disease concept of osteoporosis, new technology continues to be incorporated into clinical diagnosis and treatment of osteoporosis. With the introduction of DXA to measure BMD, more precise diagnostic criteria have been established [11]. The measurement of bone metabolic markers, approved by NHI in routine clinical practice in the field of osteoporosis, has allowed (1) estimation of bone turnover state at the time of measurement, (2) prediction of the rate of BMD change in near future, (3) assessment of the effect of drug treatment, and (4) evaluation of bone quality [10].

In addition, with the introduction into clinical practice of various bone antiresorptive drugs which can prevent fractures based on scientific evidence, the incidence of fractures due to osteoporosis has decreased according to epidemiologic studies [12].

In the future, with the goal of ideal treatment to increase bone mass, the risk of fracture or osteoporosis will be evaluated from the bone loss to decide whether to initiate drug treatment, and strategies will be sought to maintain or increase QOL in osteoporosis and assess fracture risk in lifestyle-related diseases. In other words, there will be relentless efforts towards establishing a comprehensive system to manage osteoporosis.

Change in views about the significance of measuring bone metabolic markers

The significance of measuring bone metabolic markers was originally considered important as a surrogate marker for BMD change rates, but now its significance as a means to evaluate bone quality [13] and to assess the future risk of fracture has been emphasized [14–16]. In addition, because the newly available antiresorptive drugs markedly inhibit bone metabolic markers, the measurement of bone metabolic markers is a useful means to assess drug efficacy [17, 18].

Although the use of bone metabolic markers now has an important role in the daily management of osteoporosis, their use in Japan is still insufficient because of insurance coverage limitations [19]. Since the Japan Osteoporosis Society first created the 2001 guidelines, new bone metabolic markers have been introduced into clinical practice. The availability of new osteoporosis treatments that promote bone formation has changed the clinical application of bone metabolic markers in current practice. Therefore, the necessity to revise the current clinical practice led to the proposal to create these new 2012 guidelines.

Changes in guidelines

If we look back at the process of creating the guidelines to date, from the 2001 edition [20] to the 2002 edition [21], there was a strong awareness of the relationship between bone metabolic markers and changes in BMD which was reflected in their actual use. On the other hand, in the 2004 guidelines [10], there was a marked shift regarding what is described below. Based on the terms 'bone resorption markers' and 'bone formation markers' it was conceived that changes in BMD might be determined by changes in the ratio of these two types of bone metabolic markers, which significantly reflect different aspects of bone. However, although demonstrated in relatively younger persons, it could not be confirmed in older persons and osteoporosis patients. At that point, it was recognized that the clinical significance of bone metabolic markers in osteoporosis patients needed to be re-evaluated. In other words, measurements of BMD and bone metabolic markers in osteoporosis management (each related to bone strength) were a means of observing two different aspects of bone. As stated by the NIH consensus conference [2], these two factors are also independent bone strength parameters. Conversely, the phenomenon of a discrepancy between changes in BMD and bone turnover with drug treatment is characteristic of the clinical picture of osteoporosis.

The fundamentals of clinical significance of bone metabolic markers

Since proper treatment of osteoporosis may be expected to reduce osteoporosis-related medical cost, the early diagnosis of osteoporosis and the precise understanding of bone dynamism in osteoporosis are important in terms of fracture prevention. Bone formation and resorption play a key role in maintaining bone mass volume and bone quality. Bone mineral content or density is increased by bone formation process regulated by osteoblasts, and decreased by bone resorption process regulated by osteoclasts. These two different cell activities are coupled and balanced by cross-talk between these two cellular processes in normal conditions. A few decades ago, bone morphometrical analysis of bone specimens was the only method to evaluate bone dynamism. Measured BMD is a powerful predictor of future fracture; however, the evaluation of individual values of BMD obtained during close observation of a patient, remains considerably controversial [6, 22]. Furthermore, areal densitometrical analysis gives us limited information about bone strength; in fact, this index does not provide bone material composition and structural design [23]. Therefore, the BMD value is not a complete surrogate to estimate bone strength. In addition to the areal mineral density, we need to know the cellular mechanisms responsible for bone modeling and remodeling which are mediated by osteoblasts and osteoclasts [24].

Bone modeling and remodeling change the size of bone and internal architecture by the deposition or removal of bone from the surface of bones. Bone strength therefore depends highly on the bone remodeling activity in reverse U-shape [7]. Bone remodeling activity also affects bone mineral apposition rate. Bone mineral accumulation consists of two metabolic processes—firstly primary calcification occurs mediated by osteoblasts followed by secondary calcification induced by non-cellular processes in each bone multicellular unit. Since this entire calcification process on bone takes approximately 3 months, the excess bone remodeling speed interferes with the complete mineralization process and the subsequent bone resorption may reduce BMD [24]. Bone remodeling strongly influences bone material properties including nature and amount of collagen as well as other bone-specific proteins such as bone sialoprotein, osteopontin or osteocalcin. Among them, the role of collagen metabolism and osteocalcin on bone strength has been well documented. Collagen cross-linking is a major post-translational modification and plays an important role in the biological and biochemical features of bone [25]. The proposed determinants of bone strength at the material level are the degree of mineralization of basic structure units, microdamage accumulation, and collagen cross-link formation; these are regulated by cellular activities and tissue turnover [23]. There are two types of collagen cross-link formation—one is enzymatic and the other is non-enzymatic one. Enzymatic cross-links are formed by lysyl hydroxylase- and lysyl oxidase-mediated processes [26]. On the other hand, non-enzymatic cross-links are produced by time-dependent glycation processes such as advanced glycation end-products. Impaired enzymatic cross-links and/or an increase in non-enzymatic cross-links in bone collagen are both determinants of impaired bone mechanical properties in aging, osteoporosis and diabetes mellitus [25]. The enzymatic synthesis of collagen cross-linkings is highly regulated by $1,25(OH)_2$ vitamin D_3 through expression of lysyl hydroxylase and lysyl oxidase. Therefore, vitamin D deficiency in bone may deteriorate bone strength [27]. In addition to vitamin D deficiency, homocysteine has been reported to be a negative regulator of enzymatic collagen cross-links via a reduction in gene expression and enzymatic activity of lysyl oxidase [28, 29]. Furthermore, recent progress in the risk analysis for fracture has revealed that mild elevation of plasma homocysteine is an independent predictor for future fracture [30, 31].

Osteocalcin is a bone-specific protein produced by osteoblasts. Osteocalcin receives subsequent post-translational modification on Glu residues to γ-carboxy glutamic

acid (Gla) in its molecule by vitamin K-dependent carboxylase. Secreted Gla containing osteocalcin binds to hydroxyapatite crystals in bone and bound Gla osteocalcin may be stabilized by hydroxyapatite crystals [32]. Since γ-carboxylation of osteocalcin depends highly on vitamin K nutrition, vitamin K deficiency produces under-carboxylated osteocalcin (ucOC), which has less ability to bind hydroxyapatite. Therefore, the serum level of ucOC is a sensitive marker for vitamin K deficiency in bone [33]. Although the exact mechanism is still obscure, many reports indicated that ucOC is an independent predictor for osteoporotic fracture [34, 35].

Basic principles in guideline development

These guidelines are proposed based on the following three basic principles:

- To provide a conceptual introduction about the significance of measuring bone metabolic markers in patients with osteoporosis;
- To revise the 2004 guidelines with a focus on bone metabolic markers for which assay methods have changed since creation of the 2004 guidelines, or which are newly covered by insurance; and to propose reference values as specific numerical values; and
- The proposed reference values are equally applicable to all Japanese persons.

Osteoporosis and bone metabolic marker assay methods

Deoxypyridinoline (DPD), a hydroxypyridinium cross-link, is formed during the extracellular maturation of fibrillar collagen and is released during mature collagen degradation. Measured values of DPD are not affected by the degradation of collagen after being newly synthesized, are not influenced by meals, and are thus highly specific for bone tissue. In urine, DPD is present as a free form (about 40 %) and a peptide-bound form (about 60 %) [9]. A highly sensitive immunoassay to measure type I collagen cross-linked telopeptide has been developed. Assay kits for both urinary type I collagen cross-linked N-telopeptide (uNTX) and type I collagen cross-linked C-telopeptide (uCTX) are commercially available [9]. The free form of DPD and collagen telopeptides containing NTX and CTX cross-linked sites have now been confirmed as useful clinical parameters to evaluate bone resorption, and simple immunoassays have been available since the 1990s (Table 1).

In Japan, clinical trials have been conducted in patients with osteoporosis, bone and calcium metabolic disorders, and metastatic bone disease; much clinical data have been accumulated on type I collagen cross-linked peptides and related measurements using immunoassays. As a result, in December 1999, the use of DPD and NTX as bone metabolic markers for osteoporosis was first approved for reimbursement by health insurance plans in Japan. These measurements are performed using the Osteolinks® DPD and Osteomark® NTx kits [10]. Both are enzyme-linked immunosorbent assay (ELISA) kits using urine samples. Four years later (2003), NHI also started to cover the measurement of urinary CTX (uCTX) using FRELISA® β CrossLaps® [36].

Thus, the measurement of urinary free DPD and telopeptide is becoming widespread in clinical practice. NTX and CTX can also be measured in blood. Measurements of serum NTX (sNTX) using the Osteomark® NTx serum kit, and blood (serum/plasma) CTX (sCTX) using the FRELISA® β CrossLaps® N kit was approved in 2003 [10]. In addition, bone tartrate-resistant acid phosphatase-5b (TRACP-5b), an isozyme of the osteoclast enzyme tartrate-resistant acid phosphatase, can be measured in blood (serum/plasma) using Osteolinks® TRAP-5b, which was approved in 2008 [37].

Bone formation markers are substances directly or indirectly produced by osteoblasts at each stage of osteoblast differentiation. They reflect various aspects of osteoblast function and bone formation, and most are measured in the blood. One of these, alkaline phosphatase (ALP), is an enzyme that plays an important role in osteoid formation and mineralization. The serum pool of total ALP consists of several isozymes from various tissues, including the liver, bone, intestine, spleen, kidneys, and placenta. In adults with normal liver function, about 50 % of total ALP activity in serum is from the liver, and 50 % is from bone [9]. Immunoassay of bone alkaline phosphatase (BAP) is widely performed for disorders of abnormal bone metabolism; the assay is similar to that used to measure bone formation markers. BAP immunoassays for abnormal bone metabolism including osteoporosis can be used clinically [38] using two assay kits— Osteolinks® BAP [enzyme immunoassay (EIA)] [10] and Access Ostase® [chemiluminescence enzyme immunoassay (CLEIA)] [38]. Type I procollagen-N-propeptide (P1NP), which is a metabolic product released when type I collagen (synthesized and secreted by osteoblasts) is cleaved by peptidase, can also be measured. Measurement using the Procollagen Intact P1NP kit was approved in 2010 [39].

Osteocalcin is well known as a bone-specific non-collagen protein secreted from osteoblasts. Insufficient γ-carboxylation and the glutamic acid type of osteocalcin, which is a bone matrix marker, is called ucOC and can be measured. Measurement using the Picolumi® ucOC kit was approved in 2007 [33].

Table 1 Bone turnover markers used in the diagnosis and treatment of osteoporosis

Marker	Abbreviation	Sample	Assay method	Comments
Bone formation markers				
Osteocalcin	OC	Serum	IRMA·ECLIA	IRMA: intact OC: not approved
				ECLIA: N-Mid OC: not approved
Bone alkaline phosphatase	BAP	Serum	EIA·CLEIA	
Type 1 procollagen-N-propeptide	P1NP[a]	Serum	RIA·ECLIA	RIA (intact P1NP)
				ECLIA (total P1NP): not approved
Bone resorption markers				
Pyridinoline	PYD	Urine	HPLC	Not approved
Deoxypyridinoline	DPD	Urine	HPLC·EIA·CLEIA	HPLC: not approved
Type 1 collagen cross-linked N-telopeptide	NTX	Serum/urine	EIA·CLEIA	CLEIA (urine): not approved
Type 1 collagen cross-linked C-telopeptide	CTX	Serum/plasma/urine	EIA·ECLIA	ECLIA (serum): not approved/in development
Tartrate-resistant acid phosphatase 5b	TRACP-5b	Serum/plasma	EIA	
Bone matrix-related markers				
Undercarboxylated osteocalcin	ucOC	Serum	ECLIA	
Pentosidine[a]	–	Plasma/urine	HPLC·EIA	HPLC: not approved
				EIA: not approved for the evaluation of bone/in development, but it is applied to evaluate renal function
Homocysteine[a]	HCY	Plasma/urine	HPLC·enzymatic·CLIA	HPLC·enzymatic·CLIA: not approved
				Applied to diagnose homocystinuria

Enzymatic: compatible with general purpose autoanalyzers widely used for clinical laboratory tests

Homocysteine: denotes total homocysteine (protein-bound form + free oxidized form + free reduced form). By HPLC, covered by National Health Insurance (homocystinuria, folate/vitamin B_{12} deficiency): National Health Insurance points 320

IRMA immunoradiometric assay, *ECLIA* electrochemiluminescent immunoassay, *EIA* enzyme immunoassay, *CLEIA* chemiluminescent enzyme immunoassay, *RIA* radio immunoassay, *HPLC* high-performance liquid chromatography, *CLIA* chemiluminescent immunoassay

[a] A promising bone quality marker if evidence for bone mass loss and bone fracture risk is further accumulated

Thus, various bone metabolic markers can be measured in osteoporosis management; however, there are some restrictions on their use for measurements under health insurance coverage in Japan. In osteoporosis, the primary purpose of measuring bone metabolic markers is to evaluate the state of bone metabolism in patients clinically diagnosed with osteoporosis in order to select drug treatment and assess treatment effects. Bone resorption markers, which reflect this state, are approved for measurement when starting treatment and once within 6 months after starting treatment to evaluate treatment effects.

Evaluation by measurement of bone metabolic markers

Now that fractures due to osteoporosis may be predicted, three types of evaluations are necessary in osteoporosis management. The first evaluation that should be performed is to assess the risk of bone fracture in each individual patient. Based on this, a decision is made whether to initiate drug therapy. The second evaluation is to select the most appropriate drug, and the third is the evaluation of treatment effects.

Evaluation of fracture risk should include BMD, history of previous fracture, bone metabolic markers, age, and the risk of falling. FRAX® is also used as a standard to evaluate fracture risk and determine the need for drug therapy. Bone metabolic marker values are useful as parameters to assess fracture risk [40]. In selecting drug therapy, evaluation of nutritional disorders and evaluation of bone turnover are important factors. In particular, evaluation of the therapeutic effect of bone antiresorptive drugs, changes in BMD and bone metabolic markers, the occurrence of new fractures, and changes in QOL are important factors to assess treatment effects. At each stage of osteoporosis treatment, measurement of bone metabolic markers provides an important basis for evaluation. Measurement of BMD is also important, but the measurement methods are limited and various (non-uniform) measurement sites and methods are also a major drawback. In contrast, values of bone metabolic markers can easily be obtained at any institution. Bone metabolic markers, as compared to BMD, fractures, and QOL, show earlier changes and, characteristically, the degree of change may be remarkable. Furthermore, an early decrease in bone resorption marker values during treatment reflects a reduction in long-term fracture risk [41, 42].

In other words, appropriate evaluation of changes in bone metabolic markers at the earliest stage provides a basis for deciding whether to continue treatment. Increased BMD alone has recently been shown to under-estimate the reduction in fracture risk with bone antiresorptive therapy [43]. Even in a setting where BMD can be measured, the measurement of bone metabolic markers has been established as an essential tool to supplement BMD measurement. However, when assessing treatment effects, bone metabolic markers are significant for both bone antiresorptive and bone formation-promoting parathyroid hormone (PTH) drugs, particularly teriparatide (daily subcutaneous injection). Measured values of bone metabolic markers, irrespective of BMD and history of previous fracture, are an independent predictor of new fractures [40]. This serves as a basis for using bone antiresorptive drugs with higher antiresorptive effects in patients with elevated values. Bone metabolic markers show relatively large changes in response to treatment with bone antiresorptive drugs. Showing patients the changes in these values may increase treatment compliance; this is also an advantage of using bone metabolic markers [44].

Appropriate use of bone metabolic markers in the diagnosis and treatment of osteoporosis

Specimen collection and handling

Bone metabolic marker values in individual patients are known to have diurnal variations [9]. Therefore, early morning fasting urine and blood samples are recommended. However, TRACP-5b, BAP, P1NP and ucOC levels are not affected by food intake, so collection of fasting samples is not necessary. For measurement of urinary DPD, uNTX, and uCTX, values should be corrected for creatinine using early morning first- or second-voided urine samples [9].

When measuring bone metabolic markers to evaluate bone metabolism for the purpose of initiating drug therapy, if other drugs that affect bone and calcium metabolism are discontinued for at least 1 month previously, they will have little influence on bone metabolic marker values. However, the effects of bisphosphonates may last for at least 3 months. For patients who are already on drug therapy, bone metabolism should be assessed while the current medication is being continued.

When repeated measurements are performed on the same patient, some bone metabolic markers may have intra-day or inter-day variations. Therefore, samples should be collected and handled consistently (i.e., same time of day).

Recently, a high prevalence of chronic kidney disease (CKD) has been increasingly recognized in elderly patients, particularly women [45], in whom osteoporosis often co-exists. Among various bone metabolic markers in serum, some markers accumulate in serum due to impairment of urinary excretion by renal dysfunction, while others do not (Table 2) [46]. Since urinary bone metabolic

Table 2 Influence of renal function on bone turnover markers

Marker	Effect of renal dysfunction
Bone formation markers	
OC	(+)
BAP	(−)
P1NP	(−)
Bone resorption markers	
PYD	(+)
DPD	(+)
NTX	(+)
CTX	(+)
TRACP-5b	(−)
Bone matrix-related marker	
ucOC	(+)

Decreased renal function: ≥Stage 3 CKD: (+) is affected by the marker, (−) is not affected by the marker

OC osteocalcin, *BAP* bone alkaline phosphatase, *P1NP* Type 1 procollagen-N-propeptide, *PYD* pyridinoline, *DPD* deoxypyridinoline, *NTX* Type 1 collagen cross-linked N-telopeptide, *CTX* Type 1 collagen cross-linked C-telopeptide, *TRACP-5b* tartrate-resistant acid phosphatase 5b, *ucOC* undercarboxylated osteocalcin

markers are excreted into urine by the kidney, they should be affected by renal dysfunction. Moreover, urinary levels of bone metabolic markers are corrected for urinary creatinine. Age-related decline in activities of daily living and in muscle mass can also decrease serum creatinine levels and thus urinary creatinine excretion [47]. Therefore, when bone metabolic state is estimated using the marker dependent on renal function, one should be careful to interpret the data taking into account the possible apparent effect of renal dysfunction, independent of bone metabolic state. Moreover, long-term treatment is usually required in the clinical practice of osteoporosis, and these age-related issues should be kept in mind when interpreting the values.

Therefore, the measurement of bone metabolic markers independent of renal dysfunction allows one to assess the bone metabolic state precisely without being affected by age-related issues which may result in false interpretation.

Reference values and abnormal values [42, 48–52]

In osteoporosis, the degree of bone formation and resorption, as evaluated by bone metabolic markers which reflect the underlying condition, may not be in agreement. In many cases, the degree of bone resorption is more prominent than the degree of bone formation. Therefore, prior to treatment of patients with a definitive osteoporosis diagnosis, the status of bone metabolism can be more clearly ascertained by simultaneous measurement of both bone formation and resorption markers. Reference values for bone metabolic markers are within the range of mean ±1.96 SD of the values established in healthy premenopausal women (Table 3). When bone metabolic marker values are high (exceeding reference values stratified by gender and menopause), metastatic bone tumors, other bone metabolism disorders, or calcium metabolism abnormalities may be present which warrant further examination (Table 4).

Evaluation of bone loss and fracture risk using bone metabolic markers

An increase in systemic bone turnover reflected by high bone metabolic marker values is associated with future bone loss independent of bone mass and other osteoporosis risk factors. This does not apply, however, when the high values are due to increases in localized bone resorption due to fracture or arthritis. Values of bone formation markers above the upper reference range limits, and values of bone resorption markers >1.0 SD above the mean in healthy premenopausal women, indicate a high future risk of bone loss [9, 10]. However, in osteoporosis patients who already have a reduction in bone mass, bone metabolic marker values have not been shown to be predictive of future bone mass changes [9].

In a prospective epidemiologic study, high bone metabolic marker values were reported to be related to an increase in fracture risk (vertebral and femoral neck fractures) associated with osteoporosis. In cases where bone resorption markers show values above the upper reference range limits (>1.96 SD above the mean in healthy premenopausal women), a high future fracture risk has also been reported [53]; however, sufficient consensus has not been achieved to date.

Selection of drug treatment using bone metabolic markers

Bone metabolic markers, particularly measured values of the bone resorption markers DPD, NTX, CTX, and TRACP-5b, serve as a basis for selecting drug therapy. Drugs with bone antiresorptive effects, including bisphosphonates, selective estrogen receptor modulators (SERMs), estrogen, and activated vitamin D_3 (particularly, eldecalcitol) are recommended for patients with elevated values above the upper reference range limits. However, drug selection should be based on a comprehensive assessment, including BMD, history of previous fractures, bone metabolic marker values, patient background factors, symp-

Table 3 Bone turnover marker reference values and established conditions

Type of marker (assay method)	Reference values	Established conditions (women)
Bone formation markers		
BAP (CLEIA)[a]	2.9–14.5 μg/L	Premenopausal
BAP (EIA)[b]	7.9–29.0 U/L	30–44 years
P1NP[c]	17.1–64.7 μg/L	30–44 years
Bone resorption markers		
DPD[b]	2.8–7.6 nmol/mmol Cr	30–44 years
sNTX[b]	7.5–16.5 nmol BCE/L	40–44 years
uNTX[b]	9.3–54.3 nmol BCE/mmol Cr	30–44 years
sCTX[c]	0.100–0.653 ng/mL	30–44 years
uCTX[b]	40.3–301.4 μg/mmol Cr	30–44 years
TRACP-5b[a]	120–420 mU/dL	Young adult mean (YAM 30–44 years)
Bone matrix marker		
ucOC[a]	3.94 ng/mL (not established as reference value)	Upper limit in women ≤44 years
	4.5 ng/mL	Cut-off value for the determination of vitamin K insufficiency (more frequent use in clinical setting)
	5.5 ng/mL	Cut-off value for the risk of fracture

Reference values of bone metabolic markers are within the range of the mean ± 1.96 SD, as established in healthy premenopausal women

Established condition shows the age range for which data was collected

BAP bone alkaline phosphatase, *BCE* bone collagen equivalents, *CLEIA* chemiluminescent enzyme immunoassay, *EIA* enzyme immunoassay, *P1NP* Type 1 procollagen-N-propeptide, *DPD* deoxypyridinoline, *sNTX* and *uNTX* serum and urinary (respectively) Type 1 collagen cross-linked N-telopeptide, *sCTX* and *uCTX* serum and urinary (respectively) Type 1 collagen cross-linked C-telopeptide, *TRACP-5b* tartrate-resistant acid phosphatase 5b, *ucOC* under-carboxylated osteocalcin

[a] Described in kit manufacturer's package insert or manufacturer's in-house data

[b] Described in 2004 guidelines

[c] Article being prepared for submission

Table 4 Bone turnover marker values to consider prompt search for serious bone disease such as metastatic bone tumors or bone/calcium metabolic disorders other than osteoporosis

Type of marker (assay method/sample)	Men	Premenopausal women	Postmenopausal women	Units
Bone formation markers				
BAP (CLEIA)[a]	>20.9	>14.5	>22.6	μg/L
BAP (EIA)[b]	>44.0	>29.0	>75.7	U/L
P1NP[c]	>66.8	>64.7	>79.1	μg/L
Bone resorption markers				
DPD[b]	>5.6	>7.6	>13.1	nmol/mmol Cr
sNTX[b]	>17.7	>16.5	>24.0	nmol BCE/L
uNTX[b]	>66.2	>54.3	>89.0	nmol BCE/mmol Cr
sCTX[c]	>0.845	>0.653	>1.030	ng/mL
uCTX[a]	>299.0	>301.4	>508.5	μg/mmol Cr
TRACP-5b[a]	>590	>420	>760	mU/dL

As a bone metabolic marker in metastatic bone tumors, there is a type I collagen-C-telopeptide (1CTP) assay

With elevated values of bone metabolic markers (≥mean ± 1.96 SD), bone diseases such as metastatic bone tumors, or bone/calcium metabolic disorders such as hyperparathyroidism or hyperthyroidism, should be suspected

Be careful of differences in cut-off values among facilities

BAP bone alkaline phosphatase, *CLEIA* chemiluminescent enzyme immunoassay, *EIA* enzyme immunoassay, *P1NP* Type 1 procollagen-N-propeptide, *DPD* deoxypyridinoline, *sNTX* and *uNTX* serum and urinary (respectively) Type 1 collagen cross-linked N-telopeptide, *sCTX* and *uCTX* serum and urinary (respectively) Type 1 collagen cross-linked C-telopeptide, *TRACP-5b* tartrate-resistant acid phosphatase 5b, *Cr* creatinine, *BCE* bone collagen equivalent

[a] Partially revised from the kit manufacturer's package insert or manufacturer's in-house data

[b] Described in the 2004 guidelines

[c] Described in manufacturer's in-house data and article in preparation for submission

toms, complications, drug contraindications, and previous treatment history. The bone matrix marker, ucOC, reflects vitamin K deficiency, so this information is useful when selecting vitamin K_2 drugs and as an adjunct when evaluating their efficacy (Figs. 2, 3).

Evaluation of drug treatment effects in osteoporosis using bone metabolic markers

Combination of evaluable bone metabolic markers and therapeutic drugs

Using only baseline values of bone metabolic markers it is difficult to predict drug treatment effectiveness. Drug treatment effectiveness can be monitored by repeating the measurement at a given interval after the start of treatment to evaluate changes from baseline values. With drug treatment, only significant changes from baseline values in bone metabolic markers indicate that bone metabolism has changed and the treatment has been effective. In individual patients, the effectiveness of bisphosphonates, SERMs, or estrogen treatment can be assessed using DPD, NTX, CTX, TRACP-5b, BAP, or P1NP. The effectiveness of activated vitamin D_3 (particularly, eldecalcitol) can be assessed using NTX or BAP. The effectiveness of PTH drugs (daily subcutaneous injection) is assessed using P1NP. For other drugs, evaluation by measurement of these bone metabolic markers is not easy. In addition, in treatment using bisphosphonates such as alendronate that have amino groups, changes in urinary free DPD, compared to telopeptides, are known to be smaller [9, 15] (Fig. 4).

One criterion for evaluating treatment effectiveness is whether a change has exceeded the minimum significant change (MSC). The MSC is defined as twice the inter-day variation in the morning in premenopausal women (Table 5). Despite measurement at uniform sample collection times, if no significant changes in bone metabolic markers with drug treatment are observed, patient treatment compliance should first be confirmed. The possibility of another underlying disease causing secondary osteoporosis must also be considered (Table 6). With bisphosphonate therapy, it is also important to check that the time interval between drug administration and meals is sufficient so that there are no problems with drug absorption. If there is no problem with treatment compliance, then the

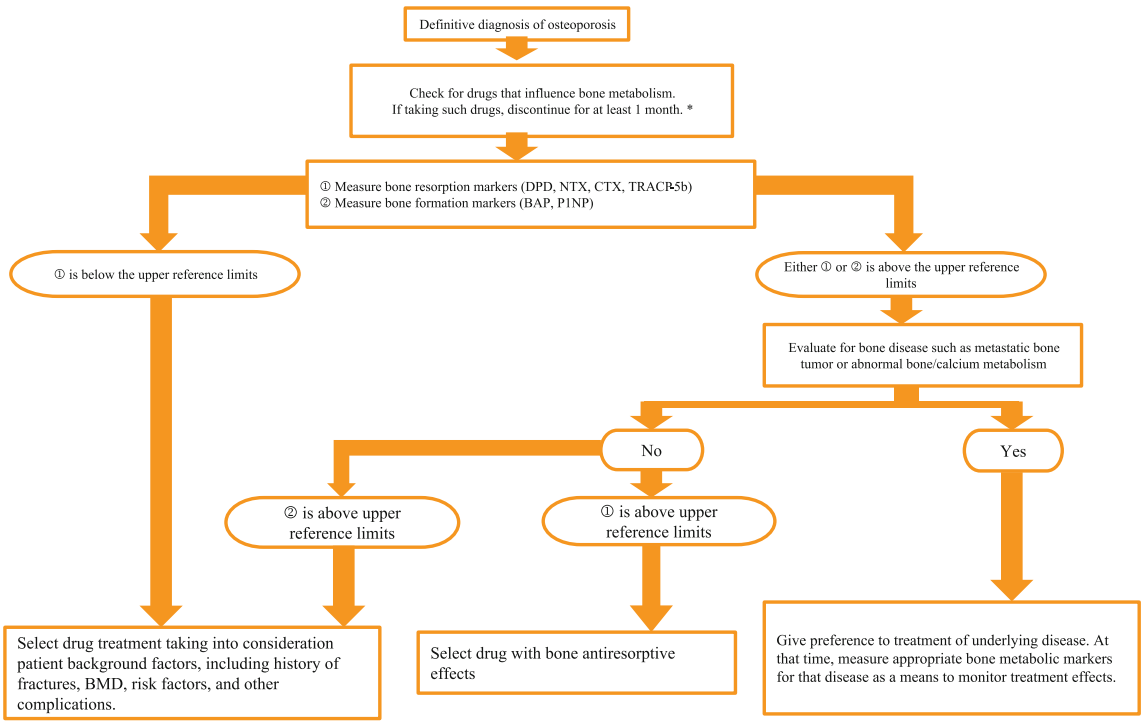

Fig. 2 Measurement of bone resorption markers and bone formation markers when selecting drug treatment for osteoporosis. *Asterisk* for bisphosphonates after stopping for at least 3 months. Bisphosphonates (etidronate disodium, alendronate sodium hydrate, risedronate sodium hydrate, minodronic acid hydrate), SERMs (raloxifene, bazedoxifene), estrogens (estradiol, estriol), calcitonin (elcatonin, salmon calcitonin), and activated vitamin D_3 (eldecalcitol) drugs are known to have bone antiresorptive effects

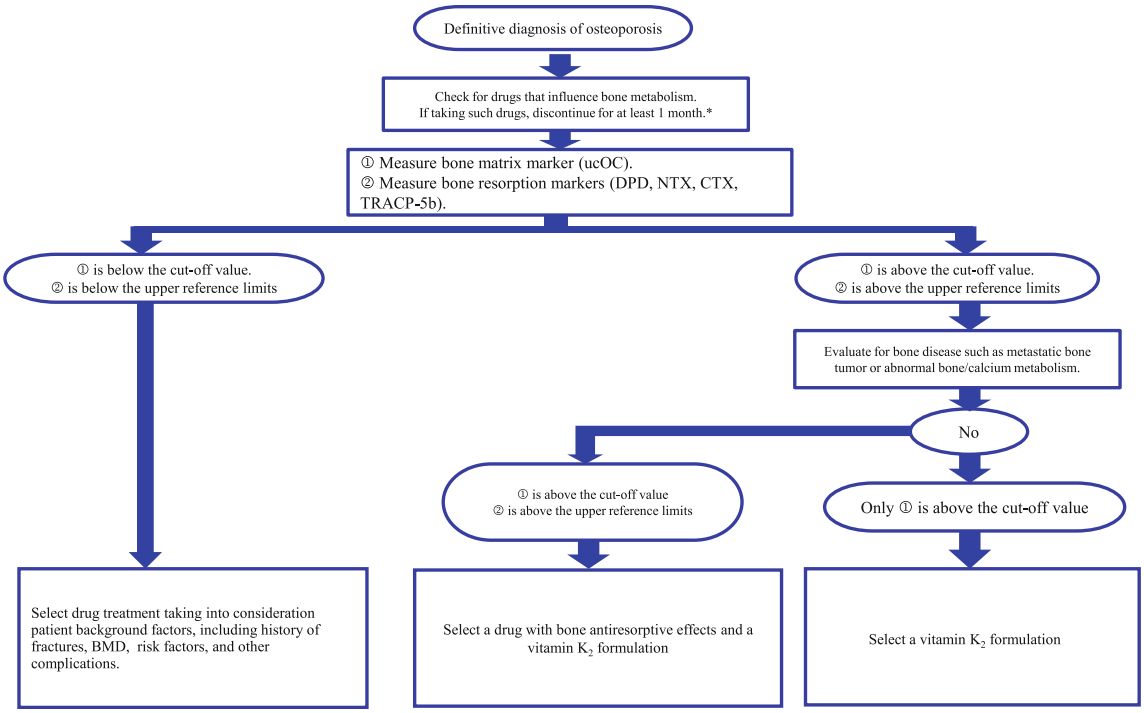

Fig. 3 Measurement of ucOC and bone resorption markers when selecting drug treatment in osteoporosis. *Asterisk* for bisphosphonates after stopping for at least 3 months

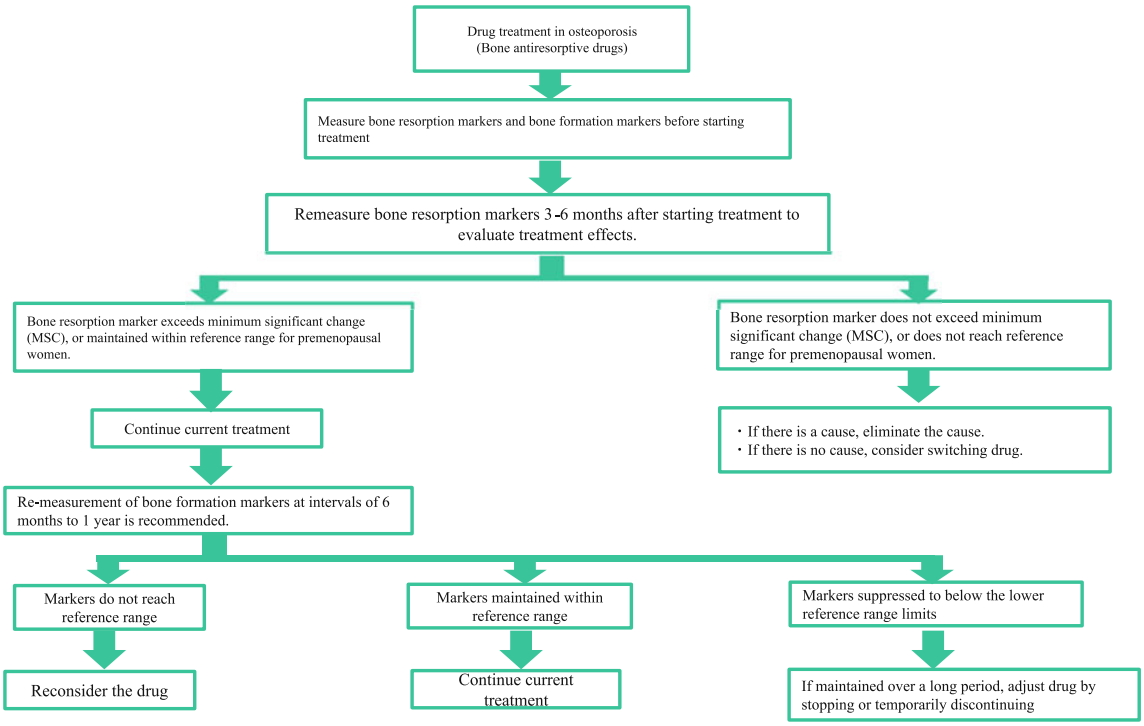

Fig. 4 Evaluation of therapeutic effects of bone antiresorptive drugs using bone resorption markers. Please refer to Table 6

response to drug treatment is inadequate and an increase in dose or switch to another drug is indicated. It should also be kept in mind that depending on the drug administered, there are some drugs for which significant changes in DPD, NTX, CTX, TRACP-5b, BAP, or P1NP are not readily apparent.

Table 5 Minimum significant changes (MSC) in bone turnover markers approved for osteoporosis

Type of marker	Assay method	Units	MSC (%)[a] (twice the mean day-to-day variation)	Reference (%)[b]
Bone formation markers				
BAP	CLEIA	μg/L	9.0	–
BAP	EIA	U/L	–	23.1[c]
P1NP	RIA	μg/L	12.1	–
Bone resorption markers				
DPD[c]	EIA	nmol/mmol Cr	23.5	29.6[c]
sNTX	EIA	Nmol BCE/L	16.3	14.2[c]
uNTX	EIA	nmol BCE/mmol Cr	27.3	35.0 [c]
sCTX	EIA	ng/mL	23.2	–
uCTX	EIA	μg/mmol Cr	23.5	51.1[c]
TRACP-5b	EIA	mU/dL	12.4	16.2[d]
Bone matrix-related marker				
ucOC	ECLIA	ng/mL	32.2	–

BAP bone alkaline phosphatase, *CLEIA* chemiluminescent enzyme immunoassay, *EIA* enzyme immunoassay, *P1NP* Type 1 procollagen-N-propeptide, *RIA* radio immunoassay, *DPD* deoxypyridinoline, *Cr* creatinine, *sNTX* and *uNTX* serum and urinary (respectively) Type 1 collagen cross-linked N-telopeptide, *BCE* bone collagen equivalent, *sCTX* and *uCTX* serum and urinary (respectively) Type 1 collagen cross-linked C-telopeptide, *TRACP-5b* tartrate-resistant acid phosphatase 5b, *ucOC* undercarboxylated osteocalcin

[a] MSC values calculated as twice the day-to-day variations, as requested by committee [basis for establishment: in 10 volunteer premenopausal women, blood and urine samples were collected 5 times during 14 days. These samples were deep-frozen stored until measurement, and measured as batches at a laboratory center (SRL Inc.)]

[b] MSC values are excerpts from the 2004 guidelines and kit package inserts

[c] Described in 2004 guidelines

[d] Described in kit manufacturer's package insert

Table 6 Possible causes for the variation within MSC value in osteoporosis under drug treatment

1. Causes related to various variations
The samples before and after the treatment should be collected at the same time because of the diurnal variation
Measurement errors over a long period of time (e.g., seasonal variation, change in patient status)
Measurement interval is too short
Change in the laboratory performance measurement or change the laboratory site
2. Low compliance of drug and instructions
Inadequate timing with meals (bisphosphonates)
Insufficient medication (low compliance)
3. Current drug for osteoporosis has no effect on bone markers

Appropriate times to measure bone metabolic markers in evaluating treatment effectiveness

The bone resorption markers DPD, NTX, CTX, and TRACP-5b should be measured twice, when treatment is started and 3–6 months after starting treatment, and the percent change should be calculated. With administration of bone antiresorptive drugs, changes in the bone formation markers BAP and P1NP are slightly delayed. For this reason, they should be measured twice—when treatment is started and again at 6 months—and the percent changes should be calculated.

After treatment with bone formation-promoting PTH drugs (recombinant, daily subcutaneous injection), changes in P1NP compared to BAP are more prominent among the bone formation markers. These should be measured twice—when treatment is started and 1–3 months after starting treatment—and the amount/percent of change should be calculated [54, 55]. However, for PTH drugs (weekly subcutaneous injection of teriparatide acetate) administered once a week for 18 months, the bone formation marker osteocalcin (OC) tends to be high throughout the drug administration period, whereas P1NP tends to be high until 3 months, and low from 6 months

onwards. In addition, the bone resorption markers DPD and uNTX are reported to be low after starting treatment, so this should also be considered [56].

Displaying the measurement results

The results of bone metabolic marker measurements can be displayed in two ways for easier interpretation of the changes. The percent changes in response to treatment are calculated and plotted as changes from baseline values [57]. The graph may also include threshold values, which indicate the MSC [57]. In addition, the absolute values can be shown together with reference values obtained from premenopausal women. If the data are displayed in this manner, it is easier to explain to patients.

Future issues

This guideline presents the data, as completely as possible, for current NHI-approved bone formation markers (BAP, P1NP), bone resorption markers (DPD, sNTX/uNTX, sCTX/uCTX, TRACP-5b), and a bone matrix marker (ucOC). Drug therapy for which effectiveness has been evaluated is limited to drugs that have been approved in Japan. The proposals in this guideline (based on examined outcomes) assume primary osteoporosis, and in particular, post-menopausal osteoporosis. Accordingly, whether these can be expanded to apply to secondary osteoporosis due to underlying or drug-induced disease is an issue for further investigation.

Meanwhile, bone metabolic marker changes using *T* scores and scoring, fracture risk, and bone loss (categorical data 2 %/3 years) were each examined. No significant relationship between fracture risk and bone metabolic markers was observed. Similarly, based on the examined categorical data, no relationship to the prediction of bone loss rates was observed. No significance was found in scoring of markers for bone loss prediction. With respect to the evaluation of bone metabolic markers using *T* scores, further studies are needed in a larger number of patients, including evaluation by fracture site for each drug, and evaluation of the relationship between percent decrease in markers and fracture reduction.

In examining the issues leading to these guideline proposals, the measurement of bone metabolic markers was performed at a limited number of laboratory test centers. However, in clinical practice, because bone metabolic markers are measured by multiple laboratory test centers, differences and variations among facilities performing measurements should be recognized and kept in mind. For initially approved bone resorption markers, reagent manufacturers voluntarily perform reagent control studies, and efforts to reduce differences among facilities continue in the direction of further improvement. Some issues that must be resolved in the future include how to differentiate and effectively use bone formation markers and bone resorption markers; establishing optimal levels of bone metabolic markers, not only for assessment of effects; and applying these markers in men and in secondary osteoporosis.

These proposed guidelines for the appropriate use of bone metabolic markers take into consideration current health insurance regulations in Japan. However, in order to achieve a more appropriate use of bone metabolic markers it is now recognized that periodic repeated measurement for monitoring after treatment is also effective. In addition, with bone antiresorptive drugs, particularly bisphosphonates containing amino groups, excessive inhibition of bone metabolism has often been observed and may also be a problem. Keeping target values (optimal levels of absolute values) of bone turnover (based on bone metabolic markers) within the physiologic range of reference values in premenopausal women is also considered important in maintaining bone strength [7, 13]. This issue should also be investigated in Japan by further accumulation of clinical data.

Conflict of interest All authors declare that they have no conflict of interest.

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