
Introduction

Current definition and concept of osteoporosis

A definition of osteoporosis was agreed upon at the 1993 consensus conference held in Hong Kong. It states that osteoporosis is “characterized by low bone mass and the microarchitectural deterioration of bony tissue, with a consequent increase in bone fragility and susceptibility to fracture.” This definition had been internationally used without revision until recently, when the definition was significantly changed at a National Institutes of Health (NIH) consensus conference in 2000 [1].

According to the consensus statement, osteoporosis is defined as “a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture.” Bone strength is determined by integrating bone mineral density (BMD) and bone quality. BMD is expressed as grams of mineral per area or volume, and, currently, BMD is defined by the individual peak bone density and the resorption rate from the peak. Bone quality is determined by characteristics of the bone matrix, such as microarchitecture, bone turnover, microdamage accumulation, the degree of calcification, and collagen [2,3]. Currently, it is thought that bone quality may not be clinically assessed by measures other than the determination of bone metabolism with biochemical markers of bone turnover.

The change in definition may be the result of more recent findings [4], one that demonstrates bone fractures routinely occur despite patients having modest BMD levels, and another that has shown no significant reduction in the risk of a fracture occurring in patients taking one of the two standard medications, one that significantly increases BMD and the other that moderately increases it.

Changes in treatment for osteoporosis

Responding to such major changes in the concept of osteoporosis, new medical technologies have been adopted in...
Develop a basic understanding of bone turnover markers

The proposed values should treat equally all the data

Propose distinct reference values for bone turnover

The future management of osteoporosis in Japan will not
be restricted to its treatment, but will develop to include the
identification of an ideal peak bone density, and include
risk assessment for the development of osteoporosis or in-
creased fractures relative to the degree of bone loss, along
with strategies to promote quality of life (QOL) for those
suffering with osteoporosis. Thus, concerted efforts towards
the establishment of a comprehensive clinical system for
osteoporosis will be required.

Changes in the implications of measuring bone
turnover markers

The measurement of bone turnover markers was initially
considered as a surrogate for BMD; however, this idea has
changed and this measurement is now used to assess bone
quality [7] and risk of future fractures [8–10]. Because the
newly introduced bone antiresorptive drugs significantly
suppress bone turnover markers, their measurement has
been an effective method to evaluate the efficacy of the
drugs [11,12].

While the routine measurement of bone turnover mark-
ers in clinical practice has been increasingly considered im-
portant in Japan, the data they generate have not been
adequately used [13]. The main reason for this problem is
thought to be the lack of detailed clinical guidelines for
measuring bone turnover markers in the Japanese popula-
tion. Such guidelines exist in other countries [14–16]. There-
fore, the Japan Osteoporosis Society established a set of
guidelines on the use of biochemical markers of bone
turnover in osteoporosis, for the first time, in 2001. Since then
we have been seeking to encourage the use of bone turn-
over markers in clinical practice and in clinical applications,
such as for identifying drugs that treat osteoporosis. To
this end, we have recently outlined proposals for the 2004
guidelines.

Changes in guidelines

We reviewed the issues involved in creating the past guide-
lines; in particular, we focused on those topics related to the
changes in bone turnover markers and BMD, as identified
in the guidelines of 2001 [17] and 2002 [18]. From this effort,
a major amendment was made to the updated version of
the guidelines. It emphasizes a need for the reassessment of
the clinical implications of bone turnover markers in
osteoporosis patients. In the past, “bone resorption mark-
ers” and “bone formation markers,” led one to assume that
the two different bone turnover markers reflected different
aspects of BMD. This terminology led some to believe that
a change in the ratio between the two would indicate a
change in BMD. However, this could only be demonstrated
in the younger population, but not in the older population
and/or in patients with osteoporosis (as we will describe
later). The measurements of bone turnover markers and
BMD in the diagnosis of osteoporosis are, therefore, two
distinct characteristics of bone strength.

It was also noted in a statement from the NIH consensus
conference [1], that these two factors are independent
indices of bone strength and are not necessarily related to
each other, at least in osteoporosis. In other words, observa-
tions of either altered bone turnover or altered BMD by
medications may illustrate the clinical characteristics of
osteoporosis.

Principles of the proposed guidelines

The proposed guidelines are based on the following three
principles:

- Develop a basic understanding of bone turnover markers
  and their implications in osteoporosis
- Propose distinct reference values for bone turnover
  markers in osteoporosis, those expected to be approved
  currently by Japanese health insurance regulators, as well
  as those expected to be approved in the future
- The proposed values should treat equally all the data
  collected from the Japanese population, and the impor-
tance of each marker should be determined, based on
previously obtained data, as quickly as possible. Additionally,
data collected from other populations should be
analyzed for comparison.

Measurement of bone turnover markers and
osteoporosis (Table 1)

Deoxypyridinoline (DPD), a hydroxyypyridinium crosslink
of collagen, is formed during the extracellular maturation of
fibrillar collagens, and is released in the degradation of
mature collagens. The measurement of DPD is not influ-
ceved by the degradation of newly generated collagens, or
by dietary intake, and shows a high specificity for skeletal
tissues. DPD is present as a free moiety (about 40%) and as
a peptide-bound moiety (about 60%) in urine. Highly sensi-
tive immunoassays have been developed for the measure-
ment of type I collagen crosslinked telopeptide in urine.
Simple kits to measure type I collagen crosslinked N-
telopeptide (NTX) and type I collagen crosslinked C-
telopeptide (CTX) were developed in the 1990s, and are
sold in other countries. Currently, free collagen telopeptide
(DPD, NTX, and CTX) has been identified as the best
measurement for the assessment of bone resorption.

Urinary levels of these bone turnover markers need to
be corrected by creatinine excretion to accurately assess
bone resorption. Measurements of type I collagen cross-
Markers of bone turnover used for the diagnosis of osteoporosis (2004)

**Table 1.**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Sample</th>
<th>Method</th>
<th>Points allotted for medical reimbursement (for osteoporosis)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bone resorption markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deoxypyridinoline (DPD)*</td>
<td>Urine</td>
<td>ELISA</td>
<td>190</td>
</tr>
<tr>
<td>Type I collagen crosslinked N-telopeptide (NTX)*</td>
<td>Urine</td>
<td>ELISA</td>
<td>180</td>
</tr>
<tr>
<td>Type I collagen crosslinked N-telopeptide (NTX)*</td>
<td>Serum</td>
<td>ELISA</td>
<td>180</td>
</tr>
<tr>
<td>Type I collagen crosslinked C-telopeptide (CTX)*</td>
<td>Urine</td>
<td>ELISA</td>
<td>190</td>
</tr>
<tr>
<td>Type I collagen crosslinked C-telopeptide (CTX)*</td>
<td>Serum</td>
<td>ELISA, ECLIA</td>
<td>—</td>
</tr>
<tr>
<td>Tartrate-resistant acid phosphatase (TRAP)</td>
<td>Serum</td>
<td>Enzymatic activity, ELISA etc</td>
<td>—</td>
</tr>
<tr>
<td><strong>Bone formation markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone alkaline phosphatase (BAP)*</td>
<td>Serum</td>
<td>EIA, IRMA</td>
<td>190</td>
</tr>
<tr>
<td>Osteocalcin (OC)</td>
<td>Serum</td>
<td>IRMA, etc</td>
<td>—</td>
</tr>
<tr>
<td>Type I procollagen-N-propeptide (PINP)</td>
<td>Serum</td>
<td>RIA, ECLIA</td>
<td>—</td>
</tr>
<tr>
<td>Type I procollagen-N-propeptide (PICP)</td>
<td>Serum</td>
<td>RIA</td>
<td>—</td>
</tr>
</tbody>
</table>

RIA, radioimmunoassay; ELISA, enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; IRMA, immunoradiometric assay; ECLIA, electrochemiluminescent immunoassay

*Approved method for the diagnosis of osteoporosis

Usefulness of measuring bone turnover markers

Currently, fractures resulting from osteoporosis can be effectively prevented. Three areas are required to evaluate the proper treatment of osteoporosis. The first is an individual assessment of the risk of a fracture occurring for each patient, the second is the proper selection of medication to administer to the patient, and the third is the assessment of the therapeutic effects.

Criteria for the assessment of fracture risk include BMD, existing fractures, bone turnover markers, age, and risk of falling. Criteria for the proper selection of medication include the evaluation of various nutritional disorders and the level of bone metabolism. Also, changes of BMD and bone turnover markers, new fractures, and changes in QOL are important parameters to assess the therapeutic effects of...
bone antiresorptive drugs. Thus, bone turnover markers give us a critical measure for the assessment of each stage of treatment of osteoporosis.

While analysis of BMD is an important measure, the methods to determine it are limited, and the protocols are varied. Alternatively, measurements of bone turnover markers can be ordered from a number of test centers and may ultimately provide more consistent results. Bone turnover markers change earlier and to a larger extent compared to changes in BMD, risk of fracture, and decline in QOL. It has been reported that a decrease in bone turnover markers in the early stage of treatment may reflect a reduction in the long-term risk of fracture [19,20].

Therefore, a proper assessment of changes in bone turnover markers may provide the earliest indication of whether to continue a particular treatment. Recently, it has been reported that an increase in BMD may explain little of the reduction in fracture risk with antiresorptive therapy [21]. Therefore, the measurement of bone turnover markers has been recognized as an essential tool, even when BMD can be measured. As a result, the argument for measuring bone turnover markers to evaluate the therapeutic effects of bone antiresorptive medications can be justified. However, there is not yet sufficient evidence for medications that have other mechanisms of action.

The measurement of bone turnover markers is an independent factor for predicting a new fracture, which is separate from BMD or the presence of a current fracture. This gives a basis for administering bone antiresorptive drugs that are effective for reducing fracture risk to patients with high levels of bone turnover markers. Test results have shown that bone turnover markers change considerably after the administration of bone antiresorptive drugs. Therefore, the use of bone antiresorptive drugs may provide another benefit; informing patients of these changes may improve their compliance and reduce the number who prematurely drop out of therapy [22].

Reference and abnormal ranges [18,23]

Regarding a patient’s pathological status, the degrees of bone formation and bone resorption assessed by bone turnover markers sometimes do not agree in the patient with osteoporosis. In many patients, the degree of bone resorption outweighs that of bone formation. Therefore, both bone formation markers and bone resorption markers should be measured simultaneously for patients diagnosed with osteoporosis, prior to therapeutic intervention [24].

The reference range for each bone turnover marker has been set within the mean established for healthy premenopausal women (mean ± 1.96 SD; Table 2). When high levels of bone turnover markers (i.e. exceeding each reference range for sex and menopausal status) are exhibited, the presence of metastatic bone tumor, metabolic bone disease, or abnormal calcium metabolism should be considered (Table 3).

In addition, it should be noted that abnormal bone turnover marker values may indicate that a fracture has developed within the previous 3 months, resulting in accelerated local bone metabolism [25].

Bone loss and fracture risks assessed by bone turnover markers

Accelerated bone metabolism, indicated by high levels of bone turnover markers, could imply potential future bone loss, which is separate from the risk factors for reduced bone mass and osteoporosis. High levels of bone formation markers (over the high end of the reference ranges) and of bone resorption markers (≥mean + 1.0 SD; the mean established for healthy premenopausal women) may indicate a high risk of bone loss. At present, predicting changes of future bone mass with bone turnover markers has not been verified for patients with osteoporosis, because their bone mass has been already compromised.

In addition, in epidemiologic studies, it has been reported that high levels of bone turnover markers are related to an increased risk of fracture associated with osteoporosis (e.g., vertebral fracture and femoral neck fracture). It is considered that high levels of bone resorption markers (over the high end of the reference ranges, i.e., mean + 1.96 SD; the mean established for healthy premenopausal women) indicate a high fracture risk in the future (Table 4).
Drug selection assessed by bone turnover markers (Fig. 1)

The measurement of bone turnover markers, particularly DPD, NTX, and CTX, is an important piece of information that is needed to choose the appropriate drug therapy. In the selection of medications for patients with high bone turnover marker levels (over the high end of the reference ranges), bone antiresorptive drugs, such as bisphosphonates, raloxifene (a selective estrogen receptor modulator: SERM), estrogen, and active forms of vitamin D₃ may be recommended. In addition, patient background, symptoms, complications, drug allergies/contraindications, and treatment history should be considered, along with the level of bone turnover markers.

Therapeutic effects of anti-osteoporosis drugs assessed by bone turnover markers

Because the therapeutic effects of drugs cannot be predicted from the baseline measurement of bone turnover markers only, the monitoring of changes in the levels of the markers over time is recommended. A therapeutic effect of a drug can be accepted only when a significant change from the baseline level of the bone turnover markers occurs during the course of treatment. The therapeutic effects of bisphosphonate, SERM, and hormone (estrogen) replacement therapies in each patient can be assessed by measuring DPD, NTX, CTX, BAP, or PINP [12,26,27]. It is difficult to assess the therapeutic effects of other osteoporosis-related drugs by measuring bone turnover markers. It is known that changes in urinary free DPD in patients receiving therapy with bisphosphonates containing a certain amino group (e.g., alendronate) are smaller compared to changes in those receiving bisphosphonates containing a certain telopeptide [11,28].

The therapeutic effects of anti-osteoporosis drugs should be assessed in terms of minimum significant change (MSC).
The MSC shows twice the percentage change of the day-to-day variability of the early morning level in postmenopausal women (Table 5).

When no significant change is found in the levels of a bone turnover marker, despite the same test conditions being used (e.g., same collection time of samples), drug compliance should be checked first. Other possible complications, causing secondary osteoporosis, should also be considered (Table 6). For bisphosphonate therapy, it needs to be confirmed that there has been a sufficient interval between drug intake and a meal to avoid any problems in absorption. If there has been no problem in drug compliance, it can be assumed that the response to the drug may be insufficient, and increasing the dosage, or the administration of an alternative medication, should be considered.

For drugs that cause little significant change in DPD, NTX, CTX, BAP, and PINP levels, the measurement of other kinds of bone turnover markers should be considered (e.g., it is known that undercarboxylated osteocalcin (ucOC) will decrease in subjects treated with vitamin K2).

Timing of bone turnover marker measurement

The bone resorption markers DPD, NTX, and CTX should be measured at the initiation of therapy, and then again 3 to 6 months after the initiation of therapy to calculate the percent change. Because changes in the levels of bone formation markers (BAP and PINP) will appear later than the changes in bone resorption markers, it is recommended that levels be measured at the initiation of therapy and 6 months thereafter. When the fluctuation in bone resorption markers levels is large, it would be preferable to measure the levels on two or more occasions before the initiation of therapy, and use the average level as a baseline. When the level is unclear, it may be necessary to repeat the measurement after 3 months to confirm the result, although these tests are not approved for reimbursement by health insurance regulators in Japan.

Presentation of data [29]

There are two approaches to illustrate the test results of bone turnover markers to help interpret their changes. (1) Calculate the percent change in levels of bone turnover markers that responded to the treatment, and plot each result as a change from the baseline level. In order to effectively explain results to patients, a threshold, as a minimum significant change (MSC), can be included in the chart. Alternatively, (2) the absolute value of bone marker measurements can be presented together with the baseline

### Table 5. Minimum significant change: MSC of approved bone turnover markers as indicators of osteoporosis

<table>
<thead>
<tr>
<th>Marker</th>
<th>Method</th>
<th>Unit</th>
<th>MSC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum BAP</td>
<td>EIA</td>
<td>U/l</td>
<td>23.1</td>
</tr>
<tr>
<td>Urine DPD</td>
<td>ELISA</td>
<td>nmol/mmol·Cr</td>
<td>29.6</td>
</tr>
<tr>
<td>Urine CTX</td>
<td>ELISA</td>
<td>µg/mmol·Cr</td>
<td>51.1</td>
</tr>
<tr>
<td>Urine NTX</td>
<td>ELISA</td>
<td>nmolBCE/mmol·Cr</td>
<td>35.0</td>
</tr>
<tr>
<td>Serum NTX</td>
<td>ELISA</td>
<td>nmolBCE/l</td>
<td>14.2</td>
</tr>
</tbody>
</table>

MSC shows twice the percentage change of the day-to-day variability of the early morning level in postmenopausal women

The confidence of MSC is equal to less than 8% risk

Note: there is interlaboratory variability for each level

### Table 6. Reasons for the lack of no significant change in the levels of bone turnover markers being induced by the therapeutic effects of anti-osteoporosis drugs

1. Reasons related to population and measurement methods
   - Different times of measurement before and after therapy commenced
   - Long-term error caused by seasonal variance, patient’s status, etc.
   - Too short an interval between measurements
   - Change of laboratory carrying out the measurement

2. Incorrect or insufficient drug compliance
   - Insufficient interval between drug intake and a meal (bisphosphonate)
   - Insufficient drug compliance

3. The drugs may cause little significant change in bone turnover

4. Other possible complications causing secondary osteoporosis

5. Recent fracture

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**Fig. 1.** Diagnosis of osteoporosis and selection of drugs by measuring markers of bone turnover. *Bisphosphonate therapy requires at least a 6-month washout time; **bisphosphonate, selective estrogen receptor modulators (SERMs; e.g., raloxifene), estrogen, calcitonin, and ipriflavone are known as anti-bone resorptive drugs. DPD, deoxypyridinoline; NTX, type I collagen crosslinked N-telopeptide; CTX, type I collagen crosslinked C-telopeptide; BAP, bone alkaline phosphatase.**
value obtained from premenopausal women. Some examples showing the data from measurements of urinary NTX for the assessment of therapeutic effects during bone antiresorptive therapy are demonstrated in Fig. 2.

Further discussion

In these guidelines, we have discussed the bone turnover markers (serum BAP, urinary DPD, urinary CTX, and serum and urinary NTX) that have been approved by health insurance regulators in Japan, and we have included all other bone turnover markers that are expected to be approved in the future. The drugs discussed here are only those that are currently available in Japan. The proposals for these guidelines are based on data assuming primary osteoporosis; in particular, postmenopausal osteoporosis. Therefore, extended application to secondary osteoporosis induced by various diseases or drugs should be considered for future discussions.

All the measurements of bone turnover markers used for these proposed guidelines have been performed at a limited number of test centers. However, because measurements of bone turnover markers will be performed in a larger number of test centers, special attention will be required to identify differences in the procedures and variability of findings among the test centers [30,31]. Regarding the bone resorption markers that have previously been approved for testing, the standard protocols for their use have been improving since the manufacturers of the reagents have worked to reduce differences among test centers. Further differentiation of bone formation markers from bone resorption markers, and how to effectively utilize the markers. Additionally, we will need to identify and describe the optimum levels of bone turnover markers, and how to apply them to male subjects and/or patients with secondary osteoporosis.

We have proposed these guidelines for the use of biochemical markers of bone turnover, taking into consideration the current health insurance regulations in Japan. However, it has been recognized that repeated monitoring of biochemical markers of bone turnover after the initiation of therapy significantly improves the physician’s ability to follow the progress of the disease. This would be particularly useful in patients receiving bone antiresorptive drugs, particularly bisphosphonates, which have been shown to inhibit bone metabolism. It has also been reported that maintaining target values (optimal levels) of bone metabolism within the reference ranges for premenopausal women may be necessary to maintain bone strength [2,7]. In order to fully understand these issues, further clinical studies will be required.

References