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The Use of Biochemical Markers of Bone Turnover in Osteoporosis

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Introduction

Biochemical markers of bone turnover have been developed over the past 20 years that are more specific for bone tissue than conventional ones. As a result, several studies have shown that these new markers are more sensitive than conventional ones for detecting abnormalities of bone turnover rate. They have been widely used in clinical research and in clinical trials of new therapies as secondary endpoints of treatment efficacy. Most of the interest has been devoted to their use in postmenopausal osteoporosis, a condition characterized by subtle modifications of bone metabolism that cannot readily be detected by conventional markers of bone turnover. However, their clinical use in the management of the individual patient has not been clearly defined and is a matter of debate.

Because of the crucial importance of clarifying this issue, the Committee of Scientific Advisors of the International Osteoporosis Foundation commissioned an expert committee to summarize the available data and to make recommendations. The following paper includes:

- A synthesis of the literature divided into five section summaries, based on five resource documents included in this issue of *Osteoporosis International*. For detailed information, the reader is invited to refer to these resource documents
- Recommendations for nomenclature and abbreviations, for clinical use and for future research of biochemical markers of bone turnover.

Biochemical, Technical and Analytical Aspects

The development of new markers of bone metabolism has greatly enriched the spectrum of serum and urine analytes used in the assessment of skeletal pathologies. For clinical purposes, markers of bone formation are distinguished from markers of bone resorption. It should be borne in mind, however, that some of these markers may reflect, at least to a certain degree, both bone formation and bone resorption. Furthermore, most if not all of these markers are present in tissues other than bone and may therefore be influenced by nonskeletal processes as well. Thirdly, changes in biochemical markers of bone turnover are usually not disease-specific, but reflect alterations in skeletal metabolism independently of the underlying cause.

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Bone Formation Markers

Bone formation markers are direct or indirect products of active osteoblasts expressed during different phases of osteoblast development and reflecting different aspects of osteoblast function and bone formation. All markers of bone formation are measured in serum or plasma.

Alkaline phosphatase (ALP) is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. The total ALP serum pool consists of several dimeric isoforms which originate from various tissues such as liver, bone, intestine, spleen, kidney and placenta. In adults with normal liver function, approximately 50% of the total ALP activity in serum is derived from the liver, whereas 50% arises from bone [1]. Many techniques have been developed to differentiate between the two main isoforms of circulating ALP, including heat denaturation, electrophoresis, precipitation, selective inhibition and, more recently, immunoassays. The last allow the quantitation of either enzyme activity or enzyme mass. However, even these assays show some cross-reacivity between bone and liver ALP (15–20%), and in subjects with high liver ALP the results of bone ALP measurements may be artificially high. From a clinical perspective, however, detection of the bone-specific ALP (bone ALP) isoenzyme is increasingly preferred because of its higher specificity.

Osteocalcin (OC) is a small, hydroxyapatite-binding protein syntheized by osteoblasts, odontoblasts and to a lesser extent by hypertrophic chondrocytes. It contains three gamma-carboxyglutamic acid (Gla) residues, which are responsible for the calcium binding properties of the protein. The precise function of OC has yet to be determined, but recent studies suggest that OC is involved in bone remodeling via a negative feedback mechanism. Serum OC is considered as a specific marker of osteoblast function, as its levels correlate with bone formation rates. However, the peptide is rapidly degraded in serum and both intact peptides and OC fragments of various sizes coexist in the circulation [2]. The resulting heterogeneity of OC fragments in serum results in limitations in the clinical application of this a priori specific marker. In practice, different immunoassays have routinely yielded such varying results that values in one assay cannot readily be compared with those obtained with another assay [3]. Assays that measure both the intact molecule and the large N-mid fragment of OC appear to be more stable and reproducible.

Procollagen type I propeptides (PINP) are derived from collagen type I, in which they form amino- (N-) and carboxy- (C-) terminal extension peptides. Since both the carboxy- and the amino-terminal propeptides of type I collagen (PICP, PINP) are generated in a stoichiometric fashion, the propeptides are considered quantitative measures of newly formed type I collagen. Both propeptides may be measured by specific, polyclonal-based immunoassays. Moderate correlations between serum PICP levels and the rate of bone formation have been reported [4]. Measurement of the trimer of PINP appears to be a more sensitive marker of bone formation rate in osteoporosis.

Bone Resorption Markers

Most biochemical markers of bone resorption are degradation products of bone collagen, but noncollagenous proteins such as bone sialoprotein or tartrate-resistant acid phosphatase are being investigated.

Hydroxyproline (Hyp) constitutes 12–14% of the total amino acid content of mature collagens, but only 10% of Hyp released during bone resorption reaches the urine in free or peptide-bound forms. Urinary Hyp has long served as the only marker of bone resorption, despite the fact that significant amounts of urinary Hyp are derived from the degradation of newly synthesized collagens, from collagens of tissues other than bone, and from the diet. Today, Hyp is considered a nonspecific index of collagen turnover and has been largely replaced by more specific techniques. The hydroxylysine-glycosides are integral parts of bone collagen and occur in two forms: glycosyl-galactosyl-hydroxylysine (Glc-Gal-Hyl) and galactosyl-hydroxylysine (Gal-Hyl). Both components are released into the circulation during collagen degradation and may be measured in urine by high-performance liquid chromatography (HPLC). The ratio of the two glycosides may allow for the recognition of tissue specificity. Although the hydroxylysines have potential as markers of bone resorption, their major disadvantage is presently the absence of a convenient immunoassay format.

The hydroxypyridinium crosslinks of collagen, pyridinoline (PYD) and deoxypyridinoline (DPD), are formed during the extracellular maturation of fibrillar collagens and are released upon the degradation of mature collagens. The measurement of PYD and DPD is not influenced by the degradation of newly synthesized collagens and independent of dietary sources. In addition, the two components show a high specificity for skeletal tissues. While PYD is found in cartilage, bone, ligaments and vessels, DPD is found in bone and dentin only.

Both crosslink components may be measured by a reverse-phase ion-paired HPLC technique. In urine PYD and DPD are present both as free moieties (about 40%) and peptide-bound (about 60%). In addition, The free (non-peptide-bound) forms can be detected by direct immunoassays (free DPD, ‘Pyrilinks-D’) [5]. Sensitive immunoassays are available for the measurement of type I collagen telopeptides in urine (U) and serum (S). Currently, these include a serum radioimmunoassay (RIA) for the carboxy-terminal type I collagen telopeptide generated by matrix metalloproteases (CTX-MMP, also called ‘ICTP’) in serum [6], several immunoassays involving a synthetic octapeptide from the C telopeptide of type I collagen containing the crosslinking site (CTX-I, ‘Crosslaps’) [7] and an

enzyme-linked immunosorbent assay (ELISA) for the crosslinked N-terminal telopeptide of type I collagen (NTX-I, 'Osteomark') [8]. The pyridinium crosslinks and the collagen telopeptides involving the crosslinking site are currently considered the best indices for the assessment of bone resorption [9]. Their urinary levels need to be corrected by creatinine excretion.

Bone sialoprotein (BSP) accounts for 5–10% of the non-collageneous matrix of bone. The protein has been shown to be a major synthetic product of active osteoblasts and odontoblasts. BSP may play an important role in cell–matrix adhesion processes and in the supramolecular organization of the extracellular matrix of mineralized tissues. Immunoassays have been developed for the measurement of the immunoreactive form of BSP in serum. Based upon clinical data and the rapid reduction of serum BSP levels following intravenous bisphosphonate treatment, it is assumed that serum BSP reflects processes mainly related to bone resorption, but data are lacking to assess the utility of this new marker in osteoporosis [10].

Tartrate-resistant acid phosphatase (TRACP) exists in two sub-isoforms named 5a and 5b, of which only TRACP-5b has been shown to be characteristic for osteoclasts [11]. Recently, immunoassays for TRACP-5b have been described and preliminary clinical results indicate that this marker may be useful to assess osteoclast activity.

Like all chemical analytes, markers of bone turnover have their specific technical and analytical limitations. As pointed out before, some markers are sensitive to thermodegradation, to UV radiation, to hemolysis and other ambient influences. In order to obtain meaningful results, sample handling should be strictly standardized to keep the components stable and to provide reproducible conditions for their measurement. Furthermore, the various assays used for the measurement of biochemical bone markers need to be standardized and included in routine proficiency testing programs [12].

Preanalytical Variability

Clinical interpretation of biochemical markers of bone turnover must take into account the preanalytical variability of these markers. Numerous sources of biological variability contribute to preanalytical variability and they can be broadly divided into two categories: (1) uncontrollable factors such as age, gender, menopausal status, disease or recent fracture, which can be accounted for in the interpretation of levels of bone markers by using appropriate reference ranges or by making suitable individual adjustments to a given reference range; and (2) controllable factors such as circadian, menstrual or exercise effects, which can be minimized by standardizing the timing and conditions under which samples are taken. However, despite being able to identify and minimize some sources of preanalytical variability there will remain some endogenous day-to-day variability that cannot be reduced. The preanalytical variability is larger than the analytical variability. For example, the preanalytical variability estimates for bone ALP, OC, DPD and NTX in one study were 3%, 4%, 4% and 10% and the analytical variability estimates were 9%, 7%, 9% and 24%, respectively [13].

Uncontrollable Sources of Biological Variability

Age and Renal Function. Biochemical markers are significantly higher in children than adults, particularly in the first year of life and at puberty when they increase to levels 2–10 times the levels found in adults. After mid-puberty levels decrease toward adult levels; however, they probably do not reach a nadir until the fourth decade or later. In men the majority of markers do not change with age in subsequent years [14]. In women there is a marked increase in markers of bone turnover at the menopause [15]. It is debatable whether there are any changes in bone turnover in the perimenopausal period; however, once markers have increased at the menopause they remain elevated and generally do not change with age. In the very elderly gradual renal impairment may lead to an increase in osteocalcin and in other markers metabolized and/or excreted by the kidney (pyridinoline crosslinks and related peptides); care should be taken in the interpretation of these bone markers when the creatinine clearance decreases below 30 ml/min.

Gender. Markers levels tend to be higher in young men in the third and fourth decades than in young women, but in older men the levels tend to be lower than in postmenopausal women.

Ethnicity. Comparative studies of black and white populations have found that in children and young adults markers of bone resorption are somewhat lower in black subjects than in white subjects. OC, but not bone ALP, may be lower (20%) in black subjects. However, in women this difference may not be apparent until the menopause.

Fractures. During the first 4 weeks of fracture healing markers of bone resorption and formation increase by 20–50% and remain elevated for at least 6 months and possibly for 1 year [16]. It is important to establish whether the subject has had a fracture of any kind in the year preceding a measurement and also to be aware that an asymptomatic vertebral fracture will also cause an increase in markers.

Pregnancy and Lactation. Pregnancy and lactation place a considerable burden on the maternal skeleton to provide calcium for the growing fetus and infant. The greatest demand from the fetus for calcium comes in the

third trimester, but an anticipatory mechanism results in a gradual increase in bone resorption from the sixteenth week of pregnancy onward, followed by an increase in bone formation [17]. Contrary to this overall pattern, serum levels of OC appear to decrease and may even be undetectable during pregnancy. It has been suggested that this is due to placental clearance of OC. However, it may also be due in part to the type of assay used and to the increase in renal function during pregnancy. A small increase in OC may be seen in the third trimester or after delivery. At term, markers of bone resorption such as NTX-I are increased by 200% and markers of bone formation such as PINP by 60% compared with prepregnancy levels. After delivery, urinary NTX-I and CTX-I decrease but there may be a continued increase in the less bone-specific markers such as PYD, possibly due to the involution of the uterus. During the first months of lactation, markers of bone resorption and formation are elevated, in some studies up to twice the level in aged-matched nonlactating controls. However, once lactation stops markers of bone turnover return to premenopausal levels.

Drugs. Antiresorptive treatments for osteoporosis and other metabolic bone diseases such as hormone replacement, selective estrogen receptor modulators and bisphosphonates, rapidly reduce markers of bone turnover by up to 70%. Other drugs prescribed for unrelated conditions can affect markers of bone turnover. Corticosteroid treatment significantly reduces serum OC levels but has less impact on other markers of bone turnover, although markers of bone resorption may be elevated. Anticonvulsant therapy and GnRH agonist treatment both result in significant increases in markers of bone turnover. In contrast thiazide diuretics decrease bone turnover.

Diseases. Changes in markers of bone turnover are found not only in metabolic bone diseases but in other conditions. In some metabolic bone diseases the changes in markers of bone turnover may not be concordant. An example is Paget's disease of bone where there is large increase in total and bone ALP but only a small increase in OC [18]. In nonskeletal diseases such as liver or kidney disease, levels of bone markers may reflect extraskeletal production and/or impaired metabolism.

Oral Contraception. The effect of oral contraception on bone turnover appears to be age-dependent. In women in the third decade results of studies on the effect of oral contraception give inconsistent results. In contrast, significant decreases of between 15% and 30% in specific markers of bone resorption and bone formation have been reported in women aged 35–49 years [19].

Immobility. Bed rest results in a very rapid increase in markers of bone resorption [20]. Urinary excretion of PYD and DPD are significantly increased after only 2 days and by 40% after a week. Markers of bone formation change little or remain unchanged during bed rest or immobility. In elderly, partially immobile subjects, the increase in urinary HYP is related to the degree of immobility. Once remobilization occurs resorption markers gradually return to initial levels, although paradoxically PICP may increase.

Controllable Sources of Biological Variability

Circadian. Circadian variability has more impact on markers of bone turnover than most other sources of variability [21]. Most markers of bone turnover are increased at night, reaching a peak between 0200 and 0800 hours, after which they decrease rapidly and reach a nadir between 1300 and 2300 hours. The amplitude of the rhythm is considerably greater for resorption markers than for formation markers. Serum level of CTX-I at the nocturnal peak may be twice that at the nadir. Due to the rate of decrease in these markers in the morning, the difference between a measurement of U-NTX at 0700 and 1500 hours could be as much as 50%, similar to the mean response of NTX to HRT; this variation is equivalent to a CV of 10%. Serum PICP and OC are increased by 20% at night compared with their nadir in the early afternoon. Bone ALP has a somewhat different circadian rhythm with a peak between 1100 and 1400 hours and possibly another peak at 2330 hours. The nocturnal peak in urinary DPD excretion may be greater and extend into the morning in postmenopausal women with osteoporosis. Calcium supplementation taken at night and bisphosphonate treatment can both suppress the circadian rhythm of markers of bone resorption. Fasting also greatly diminishes the rhythm of urinary and serum CTX-I, in particular the rapid decrease during the morning [22]. To reduce the effect of circadian rhythms on the clinical interpretation of markers of bone turnover it is essential that the timing of sample collection is tightly controlled.

Menstrual. The changes in markers of bone turnover across the menstrual cycle are small. Indeed, some studies have failed to identify any changes at all [23]. Markers of bone formation are 10–15% higher in the luteal phase than in the follicular period, with OC and bone ALP reaching maximal levels in the mid-luteal phase and PICP reaching maximal levels in the early luteal phase. The reported patterns of changes in resorption markers across the menstrual cycle are inconsistent. The amplitude of the changes is between 15% and 30%. These changes are so small that the effect of the menstrual cycle on levels of bone turnover may be regarded as insignificant.

Seasonal. Seasonal variation in markers of bone turnover is not a universal finding. It has been suggested that overall seasonal changes may be low, accounting for up to 12% of the variability of the markers [24], but some studies suggest that differences between summer and

winter may be greater. OC is elevated in the winter and spring whereas bone ALP shows an inverse rhythm and is decreased during the winter and spring. PICP does not appear to have a significant seasonal rhythm. Most markers of bone resorption are elevated during the winter although one study showed that urinary PYD excretion was elevated during the summer. The impact of seasonal changes of bone turnover may be important when monitoring the short-term response to treatment.

Exercise. Exercise may affect the variability of markers of bone turnover in two ways: the effect of persistent exercise and the acute effect of a bout of exercise within a day of the sample collection. In trained endurance athletes PICP and ICTP are 18–20% lower than in age-matched sedentary controls but other formation markers are unchanged. Sub-acute exercise results in an increase in bone formation markers and a decrease in bone resorption markers. In most studies the acute effect of exercise is to increase markers of collagen formation and degradation by 15–40%. These increases persist for 24 h and possibly for as long as 72 h [25]. It is therefore important to enquire about regular exercise and ask the subject to refrain from exercise for at least 24 h before samples are collected.

Diet. Serum and urinary levels of most markers of bone turnover are unaffected by diet, with the exception of HYP, a nonspecific marker of bone resorption. Before samples are collected for HYP measurements, subjects must have an overnight fast. The other markers of collagen degradation, PYD and DPD, have been shown to be unaffected by normal dietary collagen (gelatine) intake. Specific dietary restrictions are therefore only applicable to HYP measurements.

Reference Ranges. Each laboratory should establish its own reference ranges. Age, gender, menopausal status and race all affect levels of markers of bone turnover. Therefore separate reference ranges should be established for men, premenopausal women and postmenopausal women. Because markers are still elevated in the third decade the male and premenopausal reference ranges should only include subjects over the age of 30 years. Standardized time and conditions for sample collection must also be defined for each reference range.

Long-Term Intra-individual Variability of Biochemical Markers. The intra-individual reproducibility of bone markers remains a challenge, if treatment decisions have to be taken based on a single measurement. There are some differences between bone marker variability figures reported by the various studies which probably result from differences in the populations studied, the number of subjects, assay features, length of the study period or sample collection. Nevertheless, in general intra-individual variability expressed as coefficient of variation (CV%) is lower for serum bone formation markers than for urinary resorption markers. For example, in a cohort of 259 healthy untreated postmenopausal women aged 51–89 years who had four sequential measurements over 3 years, the within-patient CV was 12% for serum OC, 14% for bone ALP and 24% for urinary CTX [26]. The intra-patient variability of bone markers can be improved in different ways. The technical features, especially of the antibodies used, are critical. For example, the long-term precision error of serum OC measured seven times over 18 months in untreated postmenopausal women was reduced from 28% to 12% by using an assay that measures both the intact and N-Mid fragment instead of using a conventional RIA recognizing mainly the intact molecule. Measuring a marker in serum rather than in urine results in better reproducibility as the variable ionic strength of urine samples and the need to correct for creatinine excretion may introduce some variability into the results. Variability of serum CTX over 12 months in 44 postmenopausal women (three samples) was recently reported to be 13%, i.e., about 2-fold lower than that of urinary CTX measurements [27]. In another 2 month study of 150 untreated postmenopausal women, the intra-patient CV% was of 7.2% for serum NTX and 14.2% for urinary NTX [28]. An extensive review of the within-subject variability of bone markers is included in the paper by Hannon and Eastell in this issue. As discussed below, intra-patient CV% can be used to calculate the least significant change of bone markers under treatment and to identify individual responders.

Prediction of Bone Loss in Postmenopausal Women

Biochemical markers reflect the whole-body rates of bone resorption and bone formation and are likely to reflect changes in the number of bone remodeling sites [29]. Therefore, they may provide a more representative index of the overall skeletal bone loss than would be obtained by measuring the rates of change in bone mineral density (BMD) at specific skeletal sites containing different ratios of cancellous to cortical component with different metabolic rates.

Estrogen deficiency after spontaneous as well as after artificial menopause results in an increase in bone remodeling. A sustained increase in the bone turnover induces a faster bone loss and therefore an increased risk of osteoporosis. The increase in markers of bone resorption (of 50–150%) is rapid and precedes by a few months the increase (of 50–100%) in markers of bone formation [30–34]. During the first years after ovariectomy, the ratio between the markers of bone resorption and bone formation indicates an imbalance in bone remodeling, with an inappropriately high rate of bone resorption compared with formation [32]. This imbalance remains even in late postmenopausal women [30–33]. Thus, the negative correlation between BMD and the bone turnover becomes much stronger with advancing age, as documented in population-based

studies [31,32,35]. However, measurement of bone marker, even when combined with anthropometric measures, offers little practical information for estimating BMD level in individual women and cannot be used as a surrogate measure to predict bone mass and therefore to diagnose osteoporosis.

Relationships between the biochemical markers of bone turnover and the rate of bone loss in women after menopause have been investigated in prospective studies that avoid several confounding factors. However, these studies are limited by (i) the precision error of repeated measurements of BMD in a single individual, which is of the same order of magnitude as rate of bone loss over 2–4 years, i.e., 3–4%; (ii) the precision error of repeated measurements of the markers; (iii) by differential rates of bone loss between various skeletal sites; and (iv) because it is not clear whether bone loss at the various sites is consistent over time. A variable production of sex hormone precursors and individual response to estrogen deficiency is one of the possible causes for an increased inter-individual as well as long-term variability of the bone loss. Therefore, in this review, the association of the biochemical markers with the bone loss is considered separately at the different skeletal sites.

Association of Biochemical Markers with Bone Loss at the Forearm

Consistent associations have been found in prospective studies between bone markers and bone loss rate at the distal forearm. In some studies, the relationship between the markers and the rate of bone loss appears to be continuous [36,37], with greater probability of rapid bone loss with increasing levels of the markers. However, in some studies [38] estimated rates of bone loss were not stable over time, making it difficult to identify long-term 'fast-losers'. The best markers (OC, PINP, U-CTX, U-DPD) contributed 16–27% to the variance in 1-year percentage change of the forearm bone mineral content [30,33,34,37,39,40]. Women with marker values 2 SDs greater than the mean had a 75–80% probability of rapid bone loss compared with women with values 2 SDs below the mean, who had a 20–25% probability of rapid loss [30,37]. Recently [40], in a large population-based prospective cohort of 305 women aged 50–88 years (mean 64 years), 1–38 years postmenopausal, the baseline levels of a panel of specific and sensitive biochemical bone markers were found to be highly correlated ($p<0.001$) with the rate of change of forearm BMD assessed by four measurements over a 4-year period using dual-energy X-ray absorptiometry (DXA). In 51 untreated women within 5 years of menopause who had the highest rate of bone loss, the predictive value of bone markers was increased, with correlation coefficients reaching -0.53 for S-OC and -0.47 for S-CTX. Corrections of the observed correlation coefficients by errors on bone loss and bone marker estimations resulted in an increase in the r values to -0.91 for S-OC and -0.79 for S-CTX. Women with levels of bone markers at baseline 2 SD above the mean of premenopausal women had a rate of forearm bone loss that was 2- to 6-fold higher than in women with a low turnover ($p=0.01$–0.0001), according to the marker. In a logistic regression model, the odds ratio of fast bone loss, defined as the rate of bone loss in the upper tertile of the population, was increased by 1.8- to 3.2-fold for the levels of biochemical markers in the high turnover group compared with the levels within the premenopausal range; however, the value for identifying individual fast bone losers was limited. A strong association was also observed in a retrospective study between biochemical markers and bone loss measured at the calcaneus.

Association of Biochemical Markers with Bone Loss at the Lumbar Spine

Several studies have shown a deceleration or even a cessation of bone loss at the lumbar spine with advancing age – an unexpected finding that is probably related to the high prevalence of spinal osteoarthritis in the elderly. This might explain why only a slight though significant association was found in some [41] but not other [35,42] prospective studies between the rate of bone loss at the lumbar spine and some baseline biochemical markers of bone turnover. With the exception of a period of several months after estrogen withdrawal, a single marker accounted for no more than 10% of the variance of BMD change. Adding the single most robust resorption marker to age and BMI increased R^2 to no more than 19% of the variance [35]. The maximum available information obtained by a panel of the markers explained 40% of the variance in the BMD change. In a study of 117 early postmenopausal women treated for 1-year with calcium (500 mg daily), women in the highest quartile of U-NTX values had a significantly greater decrease in the spine BMD than subjects in the lowest quartile of NTX values [43].

Association of Biochemical Markers with Bone Loss at the Hip

Bone loss from the femoral neck is approximately linear across life in postmenopausal women, although some studies have shown an apparent acceleration of bone loss with age and season. In one retrospective study, associations have been reported between the rate of bone loss at the hip and some markers (U-NTX, U-DPD), that contributed to about 27% of the variance of bone loss at the hip [44], whereas other studies have demonstrated more modest correlations [35,45] or failed to find a significant association [39,41,42,46]. From the available data, it is not evident whether there are subsets of the fast and slow losers of bone from the hip.

Conclusion

The current evidence indicates that in postmenopausal women, biochemical markers of bone turnover are associated with bone loss measured at the forearm, calcaneus and hip, with a progressively greater risk of rapid bone loss with increasing levels of markers. The results of several studies of bone loss at the forearm support the view that 80% of patients having increased biochemical markers in the early postmenopausal years are confirmed 2–12 years later as 'fast bone losers' (bone loss >3%/year) based on BMD measurements. An increase above the upper normal limit in serum or urinary markers of bone resorption suggests that the patient is losing bone, in contrast to normal or low values of markers of bone resorption and of serum OC. However, adequate thresholds are lacking and the current data do not indicate that markers can predict the rate of bone loss at the spine and hip over a 3-year period in an individual with sufficient accuracy to be used in clinical practice. Combinations of demographic and biochemical variables predict some (30–40%) of the variance of bone loss rates at these skeletal sites in untreated postmenopausal women.

Prediction of Fracture Risk

The major consequence of osteoporosis is an increase in the risk of fracture. Several prospective studies have shown that a 1 SD decrease in BMD measured by DXA is associated with an approximately 2-fold increase in the relative risk of fracture including the hip, spine and forearm. In this context the question arises as to what extent bone markers could add to BMD in order to improve the assessment of fracture risk. Relationships between biochemical markers of bone turnover and fracture risk have been investigated, first in retrospective studies comparing bone marker levels in patients with osteoporotic fractures and in controls and more recently in prospective studies in which biochemical markers were measured before the occurrence of fractures.

Association Between Markers of Bone Turnover and Fracture Risk in Retrospective Studies

Several retrospective studies have compared bone marker levels in patients with osteoporotic fractures and in controls. When samples are taken within 48 h following the fracture event – which can easily be registered for hip fracture in elderly women – a 20–30% decreased level of serum OC was consistently reported in hip fracture cases compared with apparently healthy age-matched controls. Thus in patients with fracture bone formation may be decreased, although this finding has been consistently found only for serum OC [47,48]. For bone resorption, studies using the most specific markers, i.e., urinary PYD crosslinks, suggest that hip and other fractures cases are associated with increased bone resorption [47,49]. For example, Akesson et al. [47] in a large case–control analysis including 174 patients who had sustained a hip fracture within 22 h before assessment found a significant 36% and 40% increase in total urinary PYD (U-total PYD) and urinary total DPD (U-total DPD), respectively. However, when biochemical markers are measured within the few hours after hip fracture one cannot exclude the possibility that part of these changes of bone turnover may be related to acute changes in body fluid and hormonal levels related to the trauma. If bone turnover is measured later after the fracture, it may be difficult to determine whether differences in bone turnover levels are related to the underlying rate of bone turnover leading to fracture, or to changes in bone turnover occurring after the fracture. Relating baseline bone turnover levels to the subsequent risk of osteoporotic fractures is the valid methodology to assess their clinical utility.

Association Between Markers of Bone Turnover and Fracture Risk in Prospective Studies

Markers of Bone Formation. Prospective studies relating levels of bone formation markers to risk of fracture have yielded somewhat conflicting results. Indeed either a decrease, no difference or an increase [50–53] in bone formation markers has been reported to be associated with increased fracture risk. The difference between studies may be related to the type of fracture or the population studied, but more probably to the duration of follow-up. In the EPIDOS study with a follow-up of 2 years, no significant association between either OC or bone ALP and hip fracture risk was observed [51]. In contrast in the OFELY study including a large population of healthy postmenopausal women followed prospectively for 5 years, increased bone ALP was associated with increased fracture risk, independently of the level of BMD [52]. Because increased levels of these bone formation markers are associated with significantly greater rate of bone loss in postmenopausal women, if the increased risk of fracture is mediated in part through a more rapid rate of bone loss, a follow-up of several years may be necessary to detect it. In summary, whether bone formation marker levels are related to fracture risk remains unclear.

Markers of Bone Resorption. In contrast to bone formation markers, data on the relationship between bone resorption markers and fracture risk are consistent. Riis et al. [54] reported that women within 3 years of menopause women classified as 'fast bone losers' had a 2-fold higher risk of sustaining vertebral and peripheral fractures during a 15-year follow-up than women classified as 'normal' or 'slow' losers. Interestingly, a low BMD and a high rate of bone loss at the radius predispose to the same extent to fractures with an odds

ratio of about 2. Women with both a low BMD and a fast rate of bone loss after the menopause had a higher risk of subsequently sustaining fractures than women with only one of the two risk factors. Concordant results have been obtained in four prospective studies (EPIDOS, Rotterdam, OFELY and the Hawaii Osteoporosis Study), indicating that increased levels of bone resorption markers are associated with increased risk of hip, vertebral and non-hip and non-vertebral fractures over follow-up periods ranging from 1.8 to 5 years [50–53,55]. This predictive value is consistently in the order of a 2-fold increase in the risk of fracture for levels above the upper limit of the premenopausal range. Both increased levels of S-CTX [52,55] and of U-CTX C-terminal crosslinking telopeptide of type I collagen and free deoxypyridinoline (U-f-DPD) [50,52,55] have been shown to be associated with a higher risk of hip, vertebral and other nonvertebral fractures. Increased bone resorption is associated with increased risk of fracture only for values above a threshold, suggesting that bone resorption rate becomes deleterious for bone strength only when it exceeds the normal physiologic range. As bone resorption rate predicts fracture independently of BMD, these data suggest that increased bone resorption can lead to increased skeletal fragility by two factors. First, a prolonged increase in bone turnover will lead after several years to a lower BMD, which is a major determinant of reduced bone strength. Second, increased bone resorption above the upper limit of the normal range may induce microarchitectural deterioration of bone tissue such as perforation of trabeculae, a major component of bone strength.

Undercarboxylated Osteocalcin. OC contains three residues of γ-carboxyglutamic acid (Gla), a vitamin-K-dependent amino acid. It was postulated that impaired γ-carboxylation of OC could be an index of both vitamin D and vitamin K deficiency in elderly populations. In two prospective studies performed in a cohort of elderly institutionalized women followed for 3 years [56,57] and in a population of healthy elderly women (EPIDOS study) [58], levels of serum undercarboxylated OC (ucOC) over the premenopausal range were associated with a 2- to 3-fold increase in the risk of hip fracture. Like markers of bone resorption, the prediction was still significant after adjusting for hip BMD.

Clinical Use of Bone Markers in the Assessment of Fracture Risk

Increased levels of bone resorption markers and of ucOC have been shown to predict the risk of fracture independently of the level of BMD. Thus, combination of these two diagnostic tests could be useful to improve the identification of women at high risk for fracture. Using the database of the EPIDOS study, it was shown that combining a bone resorption marker (or ucOC) and hip BMD measurement can detect women at very high risk of fracture. Indeed women with both low hip BMD (according to the WHO definition of osteoporosis) and high bone resorption had a 4- to 5-fold higher risk compared with the general population [51]. This has been confirmed for vertebral, nonvertebral and non-hip fractures in two other cohorts of postmenopausal women [52,59]. By using such a combination the specificity of hip fracture prediction is increased without a loss of sensitivity [60]. The practical outcome of such a strategy is that the number of women who need to be treated to avoid one hip fracture is significantly reduced, which could result in a more cost-effective approach of treatment strategy. In the OFELY study, those women with both low hip BMD (T-score $\leqslant -2.5$) and high S-CTX had a probability of fracture over 5 years of 55%, i.e., higher than the probability of fracture associated with low BMD alone (39%) or high CTX alone (25%)

Table 1. Combination of bone mineral density (BMD) and bone turnover markers to predict the risk of fractures in postmenopausal women: the OFELY study

	Odds ratio (95% CI)	Likelihood ratio	Probability of fracture over 5 years
All women	–	–	12.6%
Low femoral neck BMD (T-score $\leqslant -2.5$)	2.8 (1.4–5.6)	2.80	39%
High S-CTX (T-score $\geqslant 2$)	2.1 (1.2–3.8)	1.70	25%
High U-free DPD (T-score $\geqslant 2$)	1.8 (1.0–3.4)	1.68	24%
Low BMD + high CTX	3.8 (1.9–7.3)	3.70	54%
Low BMD + high free DPD	2.1 (0.7–6.2)	3.04	45%

Modified from Garnero et al. [52].
Four hundred and thirty-five healthy untreated postmenopausal women (mean age 64 years, range 50–89 years) were followed prospectively for an average of 5 years. During this follow-up period, 58 incident fractures (21 vertebral, 37 peripheral fractures) occurred in 55 women. The table shows the odds -ratio (adjusted for age, prevalent fractures and physical activity), the likelihood ratio of fracture and the 5-year probability of fractures associated with low BMD, high bone resorption assessed by serum C-terminal crosslinking telopeptide of type I collagen (S-CTX) or urinary free deoxypyridinoline (U-free DPD) and the combination of BMD and bone resorption. The T-scores of BMD, S-CTX and free DPD were calculated from the mean and standard deviation of premenopausal women from the same cohort.

(Table 1). These probabilities of fracture should then be compared with the treatment intervention threshold. Because of economic constraints in health care, it appears that effective (but somewhat expensive) treatments that have shown a marked reduction in incident fractures should be targeted to those women who are at higher risk. Clearly, bone markers are not surrogates for BMD measurements, but instead the two diagnostic tools could be combined to improve the risk assessment in an individual, when BMD measurement alone is not sufficient to assess the risk of fracture. In that strategy, bone markers can be used as risk factors of skeletal fragility independent of BMD, in the same way as history of fractures and low body weight are used.

In summary, large prospective studies have shown that increased bone turnover – more consistently markers of bone resorption – is associated with increased vertebral and nonvertebral fractures independently of BMD on a group basis. The upper limit of the premenopausal range appears to be an adequate cutoff. The main issue that remains to be explored is the practical use of these markers in identifying individual women at risk of osteoporotic fractures, i.e., to determine which postmenopausal women could benefit from these measurements. The place of biochemical markers of bone resorption in the assessment of fracture risk is likely to be in combination with other important risk factors including low BMD, personal and maternal history of fracture and low body weight.

Monitoring Treatment of Osteoporosis with Antiresorptive Drugs

Similar to most chronic diseases, monitoring the efficacy of treatment of osteoporosis is a challenge. The goal of treatment is to reduce the occurrence of fragility fractures, but their incidence is low, and the absence of events during the first year(s) of therapy does not imply necessarily that treatment is effective. Measurement of BMD by DXA is a surrogate marker of treatment efficacy that has been widely used in clinical trials. Its use in the monitoring of treatment efficacy in the individual patient, however, has not been validated. Given a short-term precision error of 1–1.5% of BMD measurement at the spine and hip, the individual change must be greater than 3–5% to be seen as significant. With potent bisphosphonates such as alendronate or risedronate, repeating BMD measurement 2 years after initiating therapy will show whether a patient is responding to therapy, i.e., has a significant increase in BMD, at least at the lumbar spine which is the most responsive site. With treatments such as raloxifene or nasal calcitonin that induce much smaller increases in BMD, DXA is not appropriate to monitor therapy, and with any treatment DXA does not identify all responders within the first year of therapy. Failure to respond may be due to noncompliance (probably the most important single factor), to poor intestinal absorption of drug (i.e., bisphosphonates), to other factors contributing to bone loss, or to other unidentified factors. Monitoring treatment of osteoporosis with bone markers may have the added advantage of improving compliance, although this needs to be proven. We will review the evidence suggesting that markers of bone turnover may be used for monitoring antiresorption therapy and discuss their clinical utility in the management of the individual patient. Given the paucity of data, we will not review studies looking at bone marker changes under bone-forming agents.

Effects of Antiresorptive Therapy on Bone Markers

Estrogen deficiency induces a rapid and sustained increase in skeletal remodeling that is reflected by a 50–100% mean increase in formation and resorption markers. Hormone replacement therapy (HRT) induces a rapid decrease in bone resorption markers that can be seen as early as 2 weeks with a plateau reached within 3–6 months. The decrease in bone formation markers under HRT is delayed, reflecting the physiologic coupling of formation to resorption and a plateau is usually achieved within 6–12 months [34]. The magnitude of the decrease in bone markers depends both on the sensitivity of the marker and on the dose of estrogen, but in most studies using an adequate dose of estrogen bone markers fall within ± 1 SD of the premenopausal mean normal value [34,61,62]. The plateau is maintained as long as HRT is continued (Fig. 1). Resorption markers rise toward untreated values within a few weeks after HRT cessation, and formation markers rise within a few months [63].

Oral daily treatment of osteoporotic patients with bisphosphonates (alendronate, clodronate, ibandronate, pamidronate, risedronate) induces changes in bone markers that follow a pattern comparable to that with

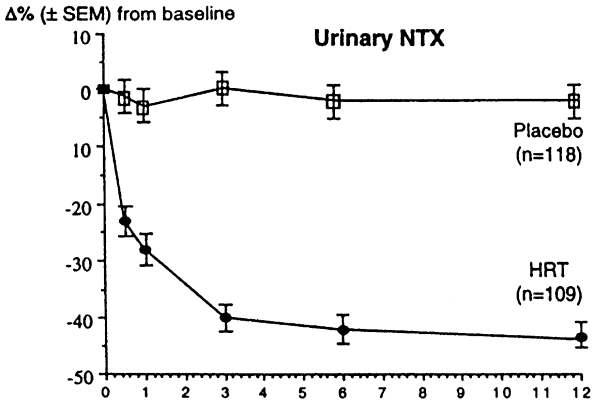

Fig. 1. Effect of HRT on bone resorption in early postmenopausal women. The figure represents the percentage change from baseline of urinary N-telopeptide of type I collagen (NTX) for HRT (0.625 mg conjugated equine estrogen) and placebo groups. In the placebo group, the sustained increased NTX levels during the 1-year study period was associated with bone loss. HRT decreased NTX within 2 weeks of treatment and levels reached a plateau after 3 months. This rapid decrease in NTX was associated with an increase in spinal BMD. Adapted from Chesnut et al. [62], reproduced with permission.

HRT. These changes have been extensively studied with alendronate treatment. Alendronate induces a dose-dependent decrease in bone turnover markers with levels around 20% of baseline values (i.e., 80% suppression) at the daily dose of 10 mg for the most sensitive markers of resorption (i.e., U-NTX and U-CTX) with stable values throughout treatment [64]. Intermittent bisphosphonates, either cyclical oral etidronate and risedronate, or intravenous ibandronate and pamidronate, produce a different pattern of bone marker changes, with a rapid decrease in resorption markers followed within a few weeks by a slow increase that usually does not reach the baseline value at the time of the second course of bisphosphonate [65,66]. This cyclical pattern of change depends on the potency and dose of the bisphosphonate.

Oral daily raloxifene produces a sustained decrease in bone turnover of smaller magnitude than most HRT regimens, with a 30–40% reduction in U-CTX and a 20–30% reduction in bone formation markers [67]. The reduction in bone turnover is even smaller with nasal calcitonin.

In summary, most effective antiresorptive treatments induce a decrease in bone turnover that reaches a plateau within a few weeks or months, depending on the potency and route of administration of the drug and on the marker. These early changes might be used, as discussed below, as a surrogate marker for treatment efficacy.

Prediction of BMD Changes by Bone Markers Under Antiresorptive Therapy

It has been suggested that baseline bone turnover is a determinant of BMD response, i.e., that patients with high-turnover osteoporosis show a higher increase in BMD than patients with low-turnover osteoporosis. Patients with high bone turnover have a significantly greater increase in spinal BMD with injectable or nasal calcitonin than those with low turnover [68]. A similar trend has been observed in patients treated with HRT and with alendronate [69]. There is, however, a large overlap in the BMD response between the two groups, so that baseline bone turnover does not appear to be a useful parameter to predict the individual response to therapy. In contrast, the decrease in bone turnover markers under antiresorptive therapy, usually expressed as a percentage of the initial value, is strongly correlated

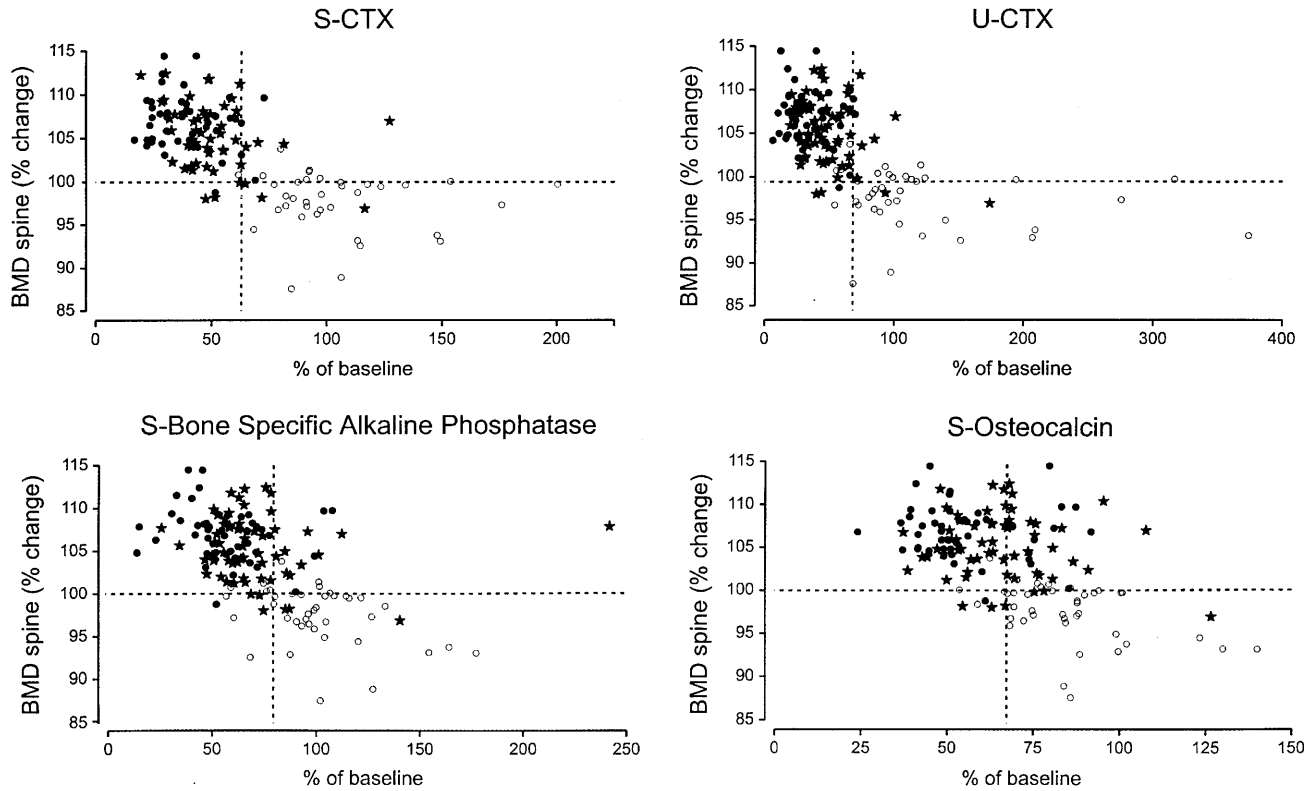

Fig. 2. Serum and urinary CTX, serum OC and serum bone ALP at 6 months of treatment expressed as a percentage of baseline, versus spinal BMD change at 3 years. Patients were treated daily with 2 mg estradiol (*filled circles*), 1 mg estradiol (*stars*) or placebo (*open circles*). The optimum cut-offs of bone markers, i.e., the best trade-off between sensitivity and specificity, were derived from ROC analyses and were used to estimate the accuracy of the marker changes after 6 months of treatment to predict the long-term changes of BMD at the spine. For example, a −37% cutoff for serum CTX provided a sensitivity of 86.9%, a specificity of 88.6%, a positive predictive value of 95.6% and a negative predictive value of 70.5%. From Bjarnason and Christiansen [71]; reproduced with permission.

with the increase in BMD. Several studies of HRT in the past 10 years [34,43,63,70,71], with one exception [35], have shown that the short-term (3–6 months) decrease in bone turnover markers is significantly correlated with the long-term (1–2 years) increase in BMD at the spine and radius. A marked decrease in markers is associated with a subsequent positive BMD response, while nonresponders show little or no changes in bone markers, suggesting that bone markers, especially new sensitive and specific ones, can be used to monitor HRT. Similarly, studies with alendronate suggest that the magnitude of the short-term decrease in bone turnover is correlated with the magnitude of the increase of BMD [64,72–74], especially when placebo-treated patients are included in the analysis. Few studies, however, have addressed the clinical use of these markers, i.e., how they should be used in the monitoring of the individual patient.

For the clinician, the primary concern is the identification of nonresponders, i.e., of patients who will fail to demonstrate a significant increase in BMD after 2 years of treatment. A BMD response has been defined either as a positive BMD change or as a positive change greater than the precision error in a single individual, also called the least significant change. Several methods have been suggested to identify responders and nonresponders according to the bone marker response to therapy. One approach is to consider the least significant change of a bone marker (based on the short-term or long-term within-subject variability), regardless of the BMD response [13]. Another approach is to search for the minimum marker change associated with a positive BMD response, as previously defined. The optimal threshold of bone marker change can be defined using receiver operating characteristics analysis, or by using logistic regression models [70–73]. The percentage change and/or the absolute value of the marker under treatment can be used [74], and cut-off values can be obtained with a prespecified sensitivity or specificity [70]. These retrospective analyses of several clinical trials using HRT or alendronate suggest that, for a given marker of resorption or formation, a cut-off value under treatment can be defined that provides adequate predictive value of the subsequent 2–3 year BMD response in a single patient. Figure 2 shows the results from one of these studies. For resorption markers, the decrease is usually largest with U-CTX and U-NTX, and slightly less with S-CTX. The decrease in U-free DPD is consistent under HRT, not under bisphosphonates. In terms of formation markers, the decrease is of similar magnitude for serum OC, bone ALP and probably for PINP. For a given marker, the decrease with alendronate treatment is more pronounced than with HRT, leading to cut-off values, expressed as the percentage change from baseline, that are approximately 20% lower for alendronate than for HRT. If the goal is to identify responders with a high specificity (i.e., 90%, with $\leqslant$10% false positive cases), a low threshold (i.e., a large decrease in the marker) should be chosen. Conversely, a higher threshold, corresponding to a smaller decrease in the markers should be chosen to identify most responders (i.e., with a high sensitivity). The same approach can be applied to the identification of nonresponders. When the decrease in a bone marker is equivocal, a third measurement 3 months later is likely to correctly identify 50% of misclassified patients [70]. The recommended cut-offs listed below are derived from the available data [64,70–74]. These cut-off values should be tested in other cohorts using the same therapeutic regimens in order to strengthen their clinical utility.

Prediction of Fracture Risk under Antiresorptive Therapy by Bone Markers

The value of BMD changes to predict the risk of fracture with treatment is debated, especially because treatments, such as raloxifene, can induce a 30–50% reduction in vertebral fracture rate despite a small 2–3% increase of BMD at all skeletal sites. Thus, BMD changes may not be an adequate surrogate endpoint to analyze the ability of bone markers to predict fracture risk. Unfortunately, there have been few attempts to correlate bone marker changes with fracture risk. In a retrospective analysis of a small placebo-controlled trial of HRT, Riggs et al. [75] suggested that changes in bone turnover (assessed by histomorphometry) predict change in vertebral fracture risk as well as change in BMD in osteoporotic women. More recently, it was found that the short-term changes in serum OC with raloxifene treatment were associated with the subsequent risk of vertebral fractures in a large subgroup of osteoporotic women enrolled in the MORE study, while changes in BMD were not predictive [76]. Clearly, such analyses should be performed in current and recently completed large clinical trials performed in postmenopausal women with osteoporosis treated with bisphosphonates, HRT or SERMs. Ultimately, recommended cut-off values of bone marker changes with treatment should be based on prospective studies with incident fractures as an endpoint.

In summary, changes in new markers of formation and resorption during treatment with HRT, bisphosphonates and raloxifene have been adequately documented in many clinical trials. The fact that they decrease rapidly and reach a drug- and dose-dependent plateau within a few months suggests that they could be used to predict the longer-term response to therapy. Statistical models have recently been developed, indicating that the percentage decrease in some bone markers after 3–6 months of HRT or alendronate can be used to predict the 2-year response in BMD with adequate sensitivity and specificity. These studies provide cut-offs values to predict responders and nonresponders to therapy that should be tested in other cohorts. Importantly, the same approach should be applied to large trials with incident fracture as an endpoint.

Recommendation for Bone Marker Nomenclature and Abbreviations [77,78]

Marker	Abbreviation	Comments about what the assay measures
Formation markers		
Osteocalcin		
Osteocalcin (or bone gla-protein)	OC	
Undercarboxylated osteocalcin	OC	
Total osteocalcin	total OC	Intact + N-mid fragment
Intact osteocalcin	OC [1–49]	
N-mid fragment of osteocalcin	OC [1–43]	
Alkaline phosphatase		
Total alkaline phosphatase	total ALP	Bone + liver + other sources
Bone alkaline phosphatase	bone ALP	
Type I collagen propeptides		Also called extension peptides of type I collagen
Procollagen type I N propeptide	PINP	Refers to the trimer
Monomer of Procollagen type I N propeptide	mon PINP	
Intact procollagen type I N propeptide	intact PINP	
Total procollagen type I N propeptide	total PINP	Monomer + trimer
Procollagen type I C propeptide	PICP	
Resorption markers		
Hydroxyproline	Hyp	Total (i.e., free + peptide-bound) urinary excretion unless otherwise specified
Hydroxylysine	Hyl	
Galactosyl hydroxylysine	Gal-Hyl	Urinary excretion of free moieties unless otherwise specified
Glucosyl galactosyl hydroxylysine	Glc-Gal-Hyl	
Pyridinoline	PYD	Can be qualified by total, free moieties or peptide-bound (pb), and in serum (S) and urine (U)
Deoxypyridinoline	DPD	
Type I collagen telopeptides		
N-terminal crosslinking telopeptide of type I collagen	NTX-I	In publications concerning bone, the I can be omitted
C-terminal crosslinking telopeptide of type I collagen	CTX-I	β (beta) isomerized unless otherwise specified. In publications concerning bone, the I can be omitted
C-terminal crosslinking telopeptide of type I collagen generated by MMPs	CTX-MMP	Also called ICTP
Bone sialoprotein	BSP	Formerly BSP-2; gene name is IBSP = integrin binding sialoprotein
Acid phosphatase	ACP	
Tartrate-resistant acid phosphatase	TRACP	Includes two isoforms: type 5a (platelets and other sources) and type 5b (osteoclasts)
Prefix		
Serum	S	
Urine	U	
Immunoreactive	i	
Peptide-bound	pb	
Non-isomerized (alpha) aspartic acid	α	
Isomerized (beta) aspartic acid	β	

(*Continued*)

Recommendations for the Use of Bone Markers in Postmenopausal Osteoporosis

Monitoring of Antiresorptive Agents

a) Which marker to choose preferentially and when to measure?

- Type of marker
  - Bone resorption: U-NTX, or U-CTX or S-CTX for monitoring bisphosphonate therapy; the same markers or free U-DPD for monitoring HRT
  - Bone formation: Bone ALP, OC, PINP
  - Use one marker or one resorption and one formation marker
- Timing of sample:
  - Serum: morning (before 0900 hours) after an overnight fast
  - Urine: either first or second morning void, with creatinine correction, after an overnight fast
- Intervals of measurement:
  - Resorption markers: before starting treatment, and 3 or 6 months after treatment has been initiated
  - Formation markers: before starting treatment and 6 months after treatment has been initiated
  - More than one measurement before starting treatment will reduce the variability of the measurement (not mandatory)

b) Which cut-off to use?

- Ideally cut-off values should be based on fracture probability, but data are not yet available. Currently cut-offs are based on BMD changes during treatment with HRT and alendronate. These cut-offs are consistent with least significant changes of bone markers (see Appendix)
- For a given marker, the decrease with alendronate treatment is more pronounced than with HRT. Thus, the lowest values of ranges of the following cut-offs apply to alendronate, the upper values to HRT.
- For a 90% specificity to predict a positive BMD response (+3%), cut-offs, expressed as a percentage decrease from baseline, are:
  - −45% to −65% for U-NTX and U-CTX
  - −35% to −55% for S-CTX
  - −20% to −30% for total or free U-DPD
  - −20% to −40% for OC and bone ALP
- For a 90% sensitivity, cut-offs are higher by approximately 20%, i.e., −25% to −45% for U-NTX and U-CTX
- In case of an equivocal change in bone markers, a third measurement should be performed 3 months later

Prediction of Fragility Fractures

- High levels of bone resorption markers (above the premenopausal range, i.e., mean +2 SD, $T \geqslant 2$) are associated with an approximately 2-fold increased risk of osteoporotic fractures
- Resorption markers can be used in the assessment of fracture risk in selected patients in whom BMD and clinical risk factors are not sufficient to take a treatment decision
- In patients with osteoporosis , a very high level of bone turnover markers ($T \geqslant +3$) is suggestive of other metabolic bone disease, including malignancy
- Normal values are reference values established in healthy premenopausal women 30–45 years of age

Prediction of Bone Loss

- Currently, bone markers cannot be recommended for the prediction of spontaneous bone loss
- It is not clear whether the lack of reliability of markers to predict bone loss in untreated individuals is related to the precision error of markers, to the precision error of DXA to assess individual rates of bone loss, or to both

Recommendations for Research

- Normal values should be established for all bone markers in large samples (150–200 women) of healthy premenopausal women, 30–45 years old, with normal BMD at the spine and hip measured by DXA; potential differences in normal values across geographic areas and races should be searched.
- Available data were obtained in research centers with bone markers measured under controlled conditions. Quality control programs of bone marker measurements should be established and widely implemented, as already done for other biological tests in clinical chemistry.
- The association between baseline bone marker levels and the subsequent rate of bone loss measured by DXA at various skeletal sites over the long term (>5 years, ideally 10 years) should be further explored.
- The association between bone markers (baseline values, 3–6 month percentage decrease and absolute value under treatment) and the probability of fractures should be explored in large clinical trials of anti-osteoporotic drugs.
- Cut-offs of markers established for defining responders and nonresponders should be validated in other cohorts using the same therapeutic regimens.
- The ability to monitor treatment with bone markers to improve compliance and treatment efficacy should be tested prospectively.

Appendix

Response to treatment based on BMD/fractures	Bone marker response to treatment	
	+	–
Responders	A	B
Nonresponders	C	D

A, true positives; C, false positives; D, true negatives; B, false negatives.

Sensitivity = A/A+B
= proportion of true responders correctly identified by the bone marker

Specificity = D/C+D
= proportion of true nonresponders correctly identified by the bone marker

Positive predictive value = A/A+C

Negative predictive value = D/B+D

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