



Biochemical markers of bone remodeling

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Bone is a metabolically active tissue and undergoes continuous remodeling, a process that largely relies on the activity of osteoblasts (bone formation) and osteoclasts (bone resorption). Under normal conditions, bone formation and bone resorption are coupled to each other, and the long term maintenance of skeletal balance is achieved through the action of systemic hormones and local mediators (see also article by Rodan and Raisz in this volume). In contrast, metabolic bone diseases, states of increased or decreased mobility, and therapeutic interventions are characterized by more or less pronounced imbalances in bone turnover.

Because of the aging population in most countries, disorders of bone and mineral metabolism are becoming increasingly relevant to everyday clinical practice. Consequently, the interest in, and the need for effective measures to be used in the screening, diagnosis, and follow-up of such pathologies has markedly grown. Together with clinical and imaging techniques, laboratory tests play an important role in the assessment and differential diagnosis of metabolic bone disease.

In recent years, the isolation and characterization of cellular and extracellular components of the skeletal matrix have resulted in the development of biochemical markers that specifically reflect either bone formation or bone resorption. These biochemical indices have greatly enriched the spectrum of analytes used in the assessment of skeletal pathologies. They are non-invasive, comparatively inexpensive and, when applied and interpreted correctly, helpful tools in the diagnostic and therapeutic assessment of metabolic bone disease.

Although the various serum and urinary markers of bone turnover include both cellular derived enzymes and non-enzymatic peptides, they are usually classified according to the metabolic process that they are considered to reflect. Therefore, for clinical purposes markers of bone formation are distinguished from indices of bone resorption (Table 1 and Table 2).

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Table 1
Biochemical markers of bone formation

Marker (abbreviation)	Tissue of origin	Analytical		Remarks/specificity
		Specimen	Method	
Total alkaline phosphatase (total ALP)	Bone, liver, intestine, kidney, placenta	Serum	Colorimetric	Attached to the extracellular surface of cell membranes; may play a role in matrix mineralization. Specific for bone formation only in the absence of liver or biliary disease.
Bone-specific alkaline phosphatase (bone ALP, BALP)	Bone	Serum	Colorimetric, electrophoretic, precipitation, IRMA, EIA	Attached to the extracellular surface of cell membranes; may play a role in matrix mineralization. Specific product of osteoblasts. Carbohydrate side-chain differences confer bone specificity. Some assays show up to 20% cross-reactivity with liver isoenzyme.
Osteocalcin (OC)	Bone, platelets	Serum	RIA, ELISA	Contains calcium-binding amino acid, gamma-carboxyglutamic acid (Gla) which facilitates interaction with hydroxyapatite. Specific product of osteoblasts; many immunoreactive forms in blood; some may be derived from bone resorption.
Carboxy-terminal propeptide of type I procollagen (PICP)	Bone, soft tissue, skin	Serum	RIA, ELISA	Specific product of proliferating osteoblasts and fibroblasts.
Amino-terminal propeptide of type I procollagen (PINP)	Bone, soft tissue, skin	Serum	RIA, ELISA	Specific product of proliferating osteoblast and fibroblasts; partly incorporated into bone extracellular matrix.

However, some of these compounds may reflect, at least in part, both bone formation and resorption (eg, urinary hydroxyproline). Most marker components are also present in other tissues than bone and may therefore be influenced by non-skeletal processes. Changes in bone markers are usually not disease specific, but reflect alterations in skeletal metabolism independent of the underlying cause.

Following a brief, non-referenced overview on the basic biochemistry of bone markers (for a detailed review, see [1]), this article reviews the current scientific evidence regarding the use of molecular markers of bone remodeling in osteoporosis.

Basic biochemistry

Markers of bone formation

Bone specific alkaline phosphatase (BALP) is one of several isoenzymes of the alkaline phosphatase (ALP) family. The entire family is encoded by four gene loci, three tissue-specific genes (intestinal, placental, and germ-cell ALP) and one tissue-non-specific gene (bone, liver, kidney, placenta, and other tissues). Although the non-specific ALPs are the products of a single gene, the isoenzymes present in tissues such as bone, liver, or kidney vary greatly because of variations in their carbohydrate side-chains. These post-translational modifications are exploited to distinguish the various ALP-isoforms from each other, employing methods such as gel electrophoresis, heat denaturation, chemical inhibition, or binding through specific monoclonal antibodies.

The liver isoenzyme and BALP each contribute approximately 50% of the total ALP activity measured in adult serum. Today, monoclonal immunoassays are the most commonly used and probably most specific method to measure BALP levels in serum. Although serum ALP levels vary greatly between individuals, intra-individual variability over time is comparatively low and BALP levels are usually not influenced by food intake.

Osteocalcin (OC) is a small protein synthesized by mature osteoblasts, odontoblasts, and hypertrophic chondrocytes. It is characterized by the presence of three residues of the calcium-binding amino acid, gamma-carboxyglutamic acid (Gla). Serum OC is considered a sensitive and specific marker of osteoblastic activity. OC is measured by immunochemical techniques, with considerable inconsistency in results between various assays (because of protein fragmentation). OC serum levels follow a circadian rhythm with high values in the early morning, but are usually not influenced by food intake. The protein is cleared through the kidney with a plasma half-life of about 20 minutes in humans.

The amino- and carboxy-terminal procollagen propeptides of type I collagen (PINP, PICP) are cleaved by specific extracellular endoproteinases from newly translated collagen type I polypeptide. As these extension peptides are generated in a stoichiometric relationship with collagen biosynthesis, they are considered to reflect the collagenous phase of bone formation. However, because type I collagen is also a component of several soft tissues (fibrocartilage, tendon, skin, gingiva, intestine, heart valve, and large vessels) there is a potential contribution to circulating procollagens

Table 2
Biochemical markers of bone resorption

Marker (abbreviation)	Tissue of origin	Analytical		Remarks/specificity
		Specimen	Method	
Hydroxyproline, total and dialyzable (Hyp)	Bone, cartilage, soft tissue, skin	Urine	Colorimetry HPLC	Present in all fibrillar collagens and partly collagenous proteins, including Clq and elastin. Present in newly synthesized and mature collagen, i.e. both collagen synthesis and tissue breakdown contribute to urinary hydroxyproline.
Pyridinoline (PYD)	Bone, cartilage, tendon, blood vessels	Urine Serum	HPLC ELISA	Collagens, with highest concentrations in cartilage and bone; absent from skin; present in mature collagen only.
Deoxypyridinoline (DPD)	Bone, dentin	Urine Serum	HPLC ELISA	Collagens, with highest concentration in bone; absent from cartilage or skin; present in mature collagen only.
Carboxy terminal cross-linked telopeptide of type I collagen (CTX-MMP)	Bone, skin	Serum	RIA	Collagen type I, with highest contribution probably from bone; may be derived from newly synthesized collagen.
Amino-terminal cross-linked telopeptide of type I collagen (NTX-I)	All tissues containing type I collagen	Urine Serum	ELISA CLIA, RIA	Collagen type I, with highest contribution from bone.
Carboxy terminal cross-linked telopeptide of type I collagen (CTX-I)	All tissues containing type I collagen	Urine (α -/ β) Serum (β only)	ELISA RIA	Collagen type I, with highest contribution probably from bone. Isomerization of aspartyl to β -aspartyl occurs with ageing of collagen molecule.
Hydroxylysine- glycosides	Bone, soft tissue, skin, serum complement	Urine	HPLC	Hydroxylysine in collagen is glycosylated to varying degrees, depending on tissue type. Glycosylgalactosyl- OHLys in high proportion in collagens of soft tissues, and Clq; Galactosyl-OHLys in high proportion in skeletal collagens.
Bone sialoprotein (BSP)	Bone, dentin, hypertrophic cartilage	Serum	RIA ELISA	Acidic, phosphorylated glycoprotein, synthesized by osteoblasts and osteoclastic- like cells, laid down in bone extracellular matrix. Appears to be associated with osteoclast function.

Table 2 (continued)

Marker (abbreviation)	Tissue of origin	Analytical		Remarks/specificity
		Specimen	Method	
Tartrate-resistant acid phosphatase (TRACP)	Bone, blood	Plasma Serum	Colorimetry RIA, ELISA	Six isoenzymes found in human tissues (osteoclasts, platelets, erythrocytes). Band 5b predominant in bone (osteoclasts). Enzyme identified in both the ruffled border of the osteoclast membrane and the secretions in the resorptive space.

from soft tissue synthesis of type I collagen. Both PICP and PINP demonstrate a circadian rhythm with peak values in the early morning, and are usually not influenced by food intake. Serum levels are measured by type and site specific immunoassays.

Markers of bone resorption

Hydroxyproline (Hyp) is an amino acid present in all collagen types and tissues. It is released following the enzymatic breakdown of collagen, but only 10% of the total circulating Hyp pool is excreted in the urine. The remainder is reabsorbed, further metabolized, or reused for collagen synthesis. Urinary Hyp levels reflect both collagen synthesis and breakdown of all body collagens and because of this low degree of specificity, its measurement (usually colorimetric methods or high performance liquid chromatography, HPLC) has been largely replaced by other, more specific bone resorption markers. Since Hyp is absorbed through the GI tract, the intake of collagen-rich foods needs to be restricted for at least 24 hours before sample collection.

Galactosyl hydroxylysine (GHL) and glucosyl-galactosyl-hydroxylysine (GGHL) are both synthesized during procollagen synthesis. While GHL is predominantly found in bone collagen, GGHL is more prevalent in skin collagen. Both compounds are essentially not metabolized in the body and contributions from dietary sources are less pronounced than for hydroxyproline. GHL can be measured in the urine by HPLC after a dansylation step. Although this assay shows good correlation with other resorption markers, the lack of a direct immunoassay for GHL has limited its wider clinical application.

The pyridinium crosslinks pyridinoline (PYD) and Deoxypyridinoline (DPD) are the main crosslinks in skeletal tissues, but act as stabilizers of mature crosslinks in type I, II and III collagens of all major connective tissues (bone, dentin, ligaments, tendons, vascular walls, muscle, and

intestine) except skin. While PYD predominates in most tissues, DPD is most abundant in bone and therefore is considered the more bone-specific marker. The pyridinium crosslinks provide distinct advantages over urinary Hyp as they are not influenced by dietary intake and unaffected by the degradation of newly synthesized collagen. Furthermore, they are not further metabolized or reused in collagen biosynthesis. Both PYD and DPD can be measured by HPLC or direct immunoassay.

The term “crosslinked telopeptides” refers to the measurement of collagen degradation products associated with the crosslink regions in type I collagen. The immunoreactive epitopes are located on peptide fragments derived from the N-terminal (NTX-I) or C-terminal (CTX-I and ICTP) telopeptides of the collagen type I molecule. The NTX-I and CTX-I epitopes can be measured in both serum and in urine, while the ICTP assay allows serum measurements only. Serum levels of CTX-I are reported to be strongly influenced by food intake, so that samples must be taken in the fasting state. This disadvantage does not apply to the NTX-I or ICTP assays.

Tartrate-resistant Acid Phosphatase 5b (TRACP 5b) is synthesized and secreted by osteoclasts during active bone resorption. The enzyme belongs to a large family of isoenzymes (Types 0–5), but like TRACP 5a is resistant to tartrate inhibition. TRACP 5b activity in serum reflects bone resorption rates, and more recently it has been possible to measure the isoenzyme by very specific immunoassays. Serum TRACP 5b levels are not influenced by food intake.

Bone sialoprotein (BSP) is abundant non-collagenous phosphoproteins in bone. BSP is synthesized by osteoblasts and certain osteoclastic cell lines and has important functions in bone mineralization processes and in integrin-mediated cell-matrix interactions. Recently developed assays for immunoreactive BSP in human serum showed increased levels in patients with metabolic and malignant bone disease, and suggested that circulating BSP primarily reflects processes associated with bone resorption. Serum BSP levels are not influenced by food intake.

Variability of bone marker measurements

The meaningful interpretation of laboratory results should always include the consideration of potential sources of non-specific variability. In general, non-specific variability comprises both pre-analytical (ie, mostly subject related; CV_{PA}) and analytical (ie, mostly assay related; CV_A) factors. Total variability is considered the sum of pre-analytical and analytical variation and defined as $CV_T^2 = CV_{PA}^2 + CV_A^2$. The ideal marker and assay is characterized by (1) excellent analytical performance (i.e. high precision and accuracy), and (2) minimal and predictable pre-analytical variability. Unfortunately, no method in clinical chemistry meets all of these criteria. However, most of the available assays for biochemical markers of bone turnover are characterized by substantial analytic and pre-analytic

Box 1. Sources of preanalytical (biological, subject related) variability

Age

- Puberty
- Growth
- Menopausal transition
- Menopausal status

Gender

Ethnicity

Recent fractures (up to 1 year)

Pregnancy

Lactation

Drugs

- Anti-resorptives
(Hormone replacement therapy, bisphosphonates, and SERMs)
- Glucocorticosteroids
- Anticonvulsants
- Warfarin (?)
- GnRH agonists
- Oral Contraception

Disease

- Metabolic bone diseases
- Diabetes
- Thyroid disease
- Renal impairment
(GFR < 20 mL/min/1.73 m²)
- Liver disease
- Rheumatoid arthritis
- Osteoarthritis

Bedrest/immobility/remobilization

Diet

Exercise

Temporal variability

- Diurnal (circadian)
- Menstrual
- Seasonal

(particularly biological) variability. The relevant preanalytic factors affecting marker variability are summarized in Box 1.

The choice of sample, the mode of sample collection, the preparation of the patient, and the correct processing and storage of specimens all influence

the measurement. Therefore, special care needs to be taken of these technical issues, as they are modifiable and controllable. For the purpose of practical use, some technical aspects of variability are discussed in greater detail.

Thermodegradation and photolysis

Some bone marker components are sensitive to ambient conditions such as temperature or UV radiation. Thermodegradation should always be a concern with assays directed against the intact OC (1–49) molecule. Rapid enzymatic cleavage of the peptide into smaller fragments will lead to significant signal losses if the serum sample is kept at room temperature for more than 1 hour. Adding protease inhibitors will delay, but by no means prevent this process [2]. The same is true for the older assays measuring plasma TRACP activity [3,4]. Pyridinium crosslinks in aqueous solutions are unstable when subjected to intensive UV irradiation [5–7]. The effect increases with rising pH [6] and has been greater for free than for total PYD. Urinary NTX and CTX are not affected by UV light exposure [5].

Timing and mode of sample collection

For convenience, measurement of bone markers in urine is usually performed either in first or second morning voids (FMV, SMV), or in 2-hour collections. In each case, values need to be corrected for urinary creatinine, which introduces additional pre-analytic and analytic variability. Creatinine output has been reported to be fairly constant with time (variations within 10%) and to correlate with lean body mass [8], but there are also reports suggesting that the correction for creatinine in a urine spot sample could be misleading. Alternatively, the excretion rate of the marker may be determined in a 24-hour urine collection. However, these collections are subject to inevitable inaccuracies caused by collection errors. With most markers, similar results are obtained from either 24-hour, 2-hour, spot urine, FMV, or SMV collections.

It is long known that bone turnover and consequently bone markers show significant diurnal variations, with highest values in the early morning hours and lowest values during the afternoon and evening [9]. Most studies report daily amplitudes of 15% to 30% [10–16], although more pronounced diurnal changes have been communicated for CTX (up to 200% [16]). The slope of diurnal changes is steepest during the morning hours, which is usually the time when urine samples are collected. This is true for both urinary and serum markers. Controlling the timing of sample collection is therefore a “bare necessity” for all types of markers.

In addition, the effects of diet and food intake need to be considered with certain markers. For example, the ingestion of hydroxyproline-rich foods, such as meat or gelatin will markedly affect measurements of OHP in urine [17]. It is therefore necessary to instruct patients to keep a collagen-free diet for at least 24 hours before collecting their urine for OHP measurements. In

contrast, urinary and serum DPD, NTX, and CTX are unaffected by collagen ingestion.

Unlike most other bone markers, serum CTX values are influenced by food intake, and samples for this marker need to be taken in the fasting state.

Variation between laboratories

Markers of bone turnover are now offered by a number of commercial laboratories and in some countries are widely used among practicing physicians. A recent trial among laboratories in Europe showed marked variability of most commercialized test kits, with inter-laboratory coefficients of variation up to 40%. Results obtained from identical blood and urine samples using the same assay and the same method differed up to 5.6-fold between laboratories [18]. It therefore seems that results from different laboratories cannot be readily compared to each other, even if the same method and sample has been used. Immunoassays for bone turnover markers should be included in routine proficiency testing programs.

The concept of least significant change

Numerous biologic factors affect bone turnover and therefore bone marker levels (see Box 1). As a rule, markers showing large changes in response to disease processes or interventions also show substantial degrees of biologic variability. In the clinical setting, variability of bone markers should be of particular concern when it comes to serial measurements, for example during therapeutic monitoring. Often, a moderate reduction in a bone resorption marker is believed to be the effect of anti-resorptive treatment, when it really should be attributed to non-specific variability [1]. However, a true (“significant”) response in either (bone mineral density) BMD or bone turnover can only be assumed, when within a single individual the change in signal is greater than the imprecision of the measurement. This change is called the least significant change (LSC). The LSC can be defined for various levels of confidence (eg, 80% or 95%) and depends on the short- and long-term within subject variability (CV) of a given marker. The CV of bone formation markers is lower than that of most bone resorption markers, and so is their LSC. Therefore, for formation markers, a change greater than 25% should under regular circumstances be considered significant, while for most bone resorption markers (serum and urine) the LSC is around 60% to 80%.

The pronounced variability and heterogeneity of bone markers makes it difficult to determine precise thresholds or cut-offs for practical use in individual patients. Employing receiver operating characteristics analyses or logistic regression models, attempts have been made to define marker-specific cut-offs at 3 to 6 months into treatment predicting the response in BMD after 2 years of therapy [19–22]. However, all of these analyses have been retrospective in nature, and so far, none of these cut-off values has been tested in prospective studies (using fracture as an endpoint).

Effects of menopause and aging on markers of bone remodeling

Once somatic growth subsides, the serum and urinary concentrations of most bone markers return to a level somewhat lower than seen during normal puberty and growth. This stabilization usually occurs during the third decade and in healthy men, levels of practically all markers remain more or less unchanged until age 70. After that, a slight increase is usually seen in formation markers such as serum BALP or OC, and most resorption markers [23–26]. In contrast, normal menopause is associated with a substantial acceleration in bone turnover, which is mirrored by a 50% to 100% increase in both bone formation and bone resorption markers [10,23,26–34]. In early postmenopausal women, this increase in bone turnover may be attenuated by calcium supplementation [35,36]. Long-term treatment of women with estrogen was shown to reduce resorption markers such as DPD and NTX to premenopause levels and to correct secondary hyperparathyroidism [37,38,122]. A recent prospective study covering the perimenopause transition in healthy women suggests that changes in bone turnover start during late premenopause with a decrease in bone formation, which only later is followed by a rise in bone resorption [39,40]. It is now widely accepted that the accelerated rate of bone loss seen after menopause is mainly caused by uncoupling in bone turnover and an increase in bone resorption. Studies employing specific bone markers indicate that bone turnover continues to increase (and to be associated with bone loss) even during late menopause [41]. In some postmenopausal women [42], but particularly in the very elderly [43], this increase in bone turnover is often, but not always result in vitamin D or calcium deficiency and secondary hyperparathyroidism.

Biochemical markers of bone turnover in osteoporosis

The past decade witnessed the development of a number of highly sensitive and specific biochemic indices of bone metabolism. These new bone markers have been successfully applied to the characterization of normal and abnormal bone remodeling in animal and human studies. An abundance of experimental, pre-clinical and clinical studies have demonstrated that markers of bone formation and resorption are valid tools in the assessment of the skeletal response to a great variety of influences. For example, markers of bone turnover may reflect changes in bone metabolism induced by oophorectomy [44–46], physical exercise [47], immobilization [48], alcoholism [49], smoking [50], vitamin D deficiency [51], chronic inflammatory bowel disease [52,53], chronic starving [54], hyperthyroidism [55] as well as the pharmacologic effects of glucocorticosteroids [56–58], androgens [59], parathyroid hormone [60,61], gonadotropin-releasing hormone agonists [62], warfarin [63], and growth hormone or insulin-like growth factors [64]. Although the above-mentioned studies represent only a

small selection of the available literature, they all demonstrate that markers of bone turnover are extremely helpful tools in evaluating the physiology and pathophysiology of bone metabolism, and in elucidating the pathogenesis of bone disease.

Using the databases of large epidemiologic and pharmaceutical trials, some markers of bone turnover were found to be predictors of bone loss and osteoporosis fracture risk. In general, groups of patients with accelerated bone turnover have been shown to not only lose bone at a faster rate, but also to carry a significantly higher risk of vertebral and non-vertebral fractures. Therefore, in larger study cohorts certain biochemical markers seem to identify groups at increased fracture risk.

Other trials have suggested that bone markers may be used to predict or monitor the therapeutic response in, or the compliance of individual patients. According to these studies, the measurement of bone turnover may not only be useful in therapeutic decision making but may also help to reduce costs by identifying individuals not responding to treatment. However, the use of bone markers in individual subjects (as opposed to large study cohorts) is not without difficulty.

Clearly, none of the biochemical markers of bone turnover has proven useful as a single diagnostic index of osteoporosis. As with bone density measurements, a broad overlap between healthy and diseased populations is observed and the measurement only provides an estimate of statistic risk rather than being a disease qualifying marker [26].

Biochemical markers of bone remodeling in osteoporosis bone disease

Osteoporosis is a heterogeneous disease. It is therefore not surprising that in untreated patients with either overt postmenopausal or age-related osteoporosis, rates of bone turnover tend to vary over a wide range. Although most cross-section studies show accelerated bone turnover in a certain proportion of postmenopausal osteoporotic women, there is usually broad overlap between diseased and healthy populations [26,65–72]. In this context, it is important to bear in mind that research studies usually include high selective patient populations, which may not always represent the population seen in the typical clinical setting. Using a population-based data set, and therefore avoiding this selection bias, we have previously shown that none of the major biochemical markers of bone turnover provide sufficient diagnostic information to be useful in the screening for vertebral osteopenia or osteoporosis [26]. However, another population-based study showed that urinary levels of NTX could discriminate between older individuals with normal hip bone density (T-score ≤ -1 SD), osteopenia (T-score > -1.0 and < -2.5 SD) and osteoporosis (T-score ≥ -2.5 SD) [73]. Again, this association did not hold true for men at the level of the spine.

In retrospective population-based studies, Akesson and co-workers [74–77] have demonstrated that previous fractures were associated with abnormal bone turnover. After adjustment for age and BMD, women with fractures occurring within six years before the study were characterized by lower serum levels of OC and PICP, but normal rates of bone resorption. In another investigation, the same authors found decreased serum levels of OC, and elevated urinary concentrations of collagen crosslinks in elderly women at the time of admission for a newly sustained hip fracture [74]. A recent cross-section study from Japan suggests that age, IGF-I and urinary levels of DPD are determinants of BMD at the lumbar spine, while lumbar spine BMD, lean body mass, IGF-I and albumin are predictors of vertebral fractures in postmenopausal women [78].

Taken together, these data suggest that a long-term imbalance of bone metabolism, namely decreased bone formation and normal or increased bone resorption, may lead to increased fragility. Together with the fact that high bone turnover may be sustained for long periods and bone loss may increase with age [79], these findings may provide a rationale for designing more effective intervention strategies. However, other factors such as age, medication, immobilization and the fracture itself [80,81] do influence bone metabolism and therefore need to be considered in the interpretation of biochemical data and their use in individual patients.

Prediction of bone loss

Bone mass, rates of bone loss, and the risk of osteoporosis fractures are interrelated, and both low bone mass and rapid bone loss have been shown to be independent predictors of future fracture risk [66,82]. The rate of bone loss is determined by a number of factors, one of which appears to be the rate of bone remodeling. Earlier observations demonstrated that bone formation and bone resorption increase shortly after natural menopause, a phase that in most women is also associated with significantly accelerated bone loss. Similar observations have been made in ovariectomized, premenopausal women and in castrated men [46,83], indicating that the withdrawal of endogenous sex steroid induces both high bone turnover and rapid bone loss. Conversely, markers of bone metabolism return to premenopause levels when HRT is implemented [72,84]. Newer biochemical studies suggest that high rates of bone turnover may be sustained well into advanced ages [26,41,85–87]. However, it is unclear whether this applies to all women (or men).

A number of cross-section studies in early and late-postmenopausal women have shown that bone mass is negatively correlated with bone resorption and formation rates [26,41,73,87–91]. While highest correlations were observed between resorption markers and total BMD, associations were usually lower when calculated for individual sites of measurements (ie, spine, hip, and radius). This difference is because biochemical markers of

bone turnover reflect metabolic changes of the entire skeleton and not of specific skeletal sites. In most studies, the inverse correlation between bone metabolism and bone loss increases with the time after menopause. Therefore, in women more than 20 years after menopause, up to 52% of the variance in BMD could be explained by changes in bone turnover. These relationships are less pronounced, but still present in early postmenopausal women, but seem absent in premenopausal women [41]. Newer studies suggest that biochemical markers of bone turnover may be associated with specific life style factors that affect bone health [8].

Most longitudinal studies support the notion that individuals with high rates of bone turnover lose bone at a faster rate than subjects with normal or low bone turnover [92–100]. Following a small group of early postmenopausal women, Christiansen and his colleagues demonstrated that the combined measurement of serum total alkaline phosphatase, OC, fasting urinary calcium, Hyp or DPD can predict 60% to 70% of the variability in bone loss [93,94,101]. These studies also showed that the correlation between baseline markers of bone turnover and the subsequent rate of postmenopause bone loss is possibly consistent over a period of at least 12 years [82,94]. Similar but less optimistic estimates were reported by other groups using different combinations of markers [95,96]. For example, Dresner-Pollak et al [95] showed that in elderly women, urinary NTX, serum OC and serum parathyroid hormone together explained 43% of the variability of bone loss at the total hip. Using single marker measurements instead of combinations, similar results were reported for a number of markers of both bone formation and resorption, and in a variety of skeletal sites (lumbar, hip, and radius). Again, markers of bone resorption seemed to be stronger predictors of future bone loss than markers of bone formation, and correlations were stronger in elderly than in younger women [96–98, 100]. In a retrospective study of 354 women (mean observation period: 13 years), Ross and Knowlton [100] showed a continuous relationship between the measured levels of various bone markers and the risk of rapid bone loss at the calcaneus. Thus, the odds of rapid bone loss (>2.2% per year) doubled for each standard deviation (SD) increase in serum bone specific alkaline phosphatase, serum OC, urinary free PYD or DPD [100]. In a study of 227 early postmenopausal women treated with either calcium alone or HRT plus calcium, Chesnut et al [92] and Rosen et al [99] showed that untreated women with high baseline rates of bone resorption were at higher risk of losing bone than women with normal turnover rates. The authors calculated that a woman with high baseline values of urinary NTX (>67 units) had a 17.3 times higher risk of bone loss if not treated with HRT [92] Fig. 1).

Somewhat contrasting results were reported by Keen et al [102], who in a four-year prospective study were unable to detect any correlation between rates of bone turnover and changes in lumbar or hip BMD. Other groups argue that because of the high degree of variability in urinary markers of

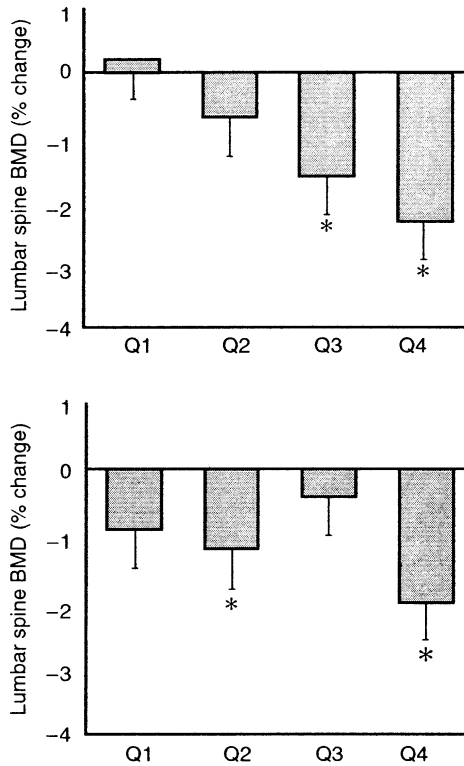


Fig. 1. Association between bone resorption rates and bone loss during the early menopause. Urinary NTX (*upper panel*), urinary free DPD (*lower panel*). In this prospective study of early postmenopausal women, baseline rates of bone resorption (stratified by quartiles Q1–Q4) were associated with the risk of bone loss (% change in lumbar BMD after 1 year). When compared to women on hormone replacement therapy, the relative risk (RR) of bone loss in untreated women in the highest quartile (Q4) of baseline urinary NTX (>67 BCE) was 17.3 (CI: 2.5–119). The RR (CI) in the three lower quartiles were Q1, 1.4 (0.8–2.5); Q2, 2.5 (1.0–6.1); and Q3, 3.8 (1.6–9.1); demonstrating a relatively flat gradient of risk. The association between bone loss and turnover seems to apply only to a small proportion of the entire population studied, and was not as clear in other markers of bone turnover such as free DPD, serum BALP or serum OC. * = $P < 0.05$ (From Rosen CJ, Chesnut III CH, Mallinik NJ. The predictive value of biochemical markers of bone turnover for bone mineral density in early postmenopausal women treated with hormone replacement or calcium supplementation. *J Clin Endocrinol Metab* 1997;82:1904–10; with permission).

bone turnover, predicting either bone density or changes therein for an individual patient from a single marker measurement may not be possible [96,103]. Vestergaard and colleagues [40] in a more recent study showed that serum OC, BALP and hydroxyproline are poor predictors of lumbar and hip bone loss in individual perimenopausal women.

Taken together, there is viable evidence that rates of bone remodeling are predictive of future bone loss. However, the strength of this association

seems to depend on a number of factors, such as menopause age, skeletal site, and gender. It is unclear whether markers of bone turnover are able to identify “fast bone losers” on an individual basis (ie, in the clinical context). At present, bone-remodeling markers are certainly unfit to substitute for individual bone mass measurement, or for a careful assessment of the patient’s personal and family history. The question remains open, whether or not the combined use of BMD and marker measurements will become the standard in individual risk assessment.

Prediction of fracture risk

Bone mass is not the only determinant of skeletal fractures. Other determinants of skeletal micro-architecture not necessarily reflected in bone mass measurements, such as trabecular connectivity or the number of bone remodeling sites, also contribute to bone strength. Therefore, bone turnover may be an independent predictor of fracture risk [104]. Analysis of data from several clinical trials suggests that in placebo-treated osteoporotic women, vertebral fracture rates increase as a direct function of either increased bone turnover or of decreased vertebral BMD [105]. Therefore, at a given level of vertebral BMD the rate of vertebral fractures increases with the rate of bone turnover. However, when bone turnover is normal the main determinant of vertebral fractures is vertebral BMD [105].

Using the large population-based sample of the Rotterdam study (7983 individuals, 60% women aged 55 years and over), Van Daele et al [106] showed that women with increased urinary DPD levels had an increased risk of hip fracture. The relative risk per SD increase in urinary DPD was 3.0 (95% confidence interval 1.3–8.6). Interestingly, part of this association appeared to be related to disability at baseline. However, when the data were corrected for disability, a relative risk of 1.9 (95% confidence interval: 0.6–5.6) remained. This number is very similar to the increase in fracture risk calculated for 1 SD decrease in BMD at the lumbar spine. Later analyses of the same study revealed that low serum OC concentrations were also associated with an increased risk of hip fracture (odds ratio: 3.1; 95% confidence interval: 1.0–9.2).

In a 5-year follow-up of the same population, Wheel et al [107] later showed that an increase in urinary DPD above the pre-menopause mean value was associated with a greatly increased risk of osteoporosis fractures. In this nested case-control study, the authors included all independent living women suffering from an incident non-vertebral fracture between 1991 and 1996 ($n = 207$). Their baseline bone resorption rate was then compared with that of a random selection of 220 age-matched women without fractures. All types of non-vertebral fractures, but especially fractures of the hip (OR 5–6) and the upper humerus (OR 3–5) were associated with urinary levels of DPD above the premenopause mean, independent of BMD and disability. Interestingly, the increase in risk was discontinuous, once the threshold of

the premenopause mean value was passed. Also, fracture risk increased dramatically when elevated rates of bone resorption were combined with low BMD.

Similar results have been published for the French EPIDOS study [108]. The relative fracture risks as defined by either BMD or marker measurements were similar (RR \sim 2, ie, close to what was reported earlier by van Daele). Interestingly, in elderly women, the relative risk of hip fracture seems to be highest in individuals with both low hip BMD and high rates of bone resorption Fig. 2).

A nested case control study from the same group later suggested that levels of serum under-carboxylated osteocalcin (ucOC), but not of total OC, were predictive of future hip fractures (odds-ratio 2.0; 95% confidence interval: 1.2–3.2) [109]. Although serum levels of ucOC showed a significant negative correlation with BMD, adjustment for BMD did not change the odds ratios significantly. Again, when BMD (lowest quartile) and serum ucOC (highest quartile) were considered together, the odds ratio increased to 5.5 (confidence interval: 2.7–11.2). These data supplement and extend previous reports, which suggest that increased serum levels of ucOC are predictive of hip fractures in elderly, institutionalized women [91,110,111]. However, these earlier results may merely indicate an association between

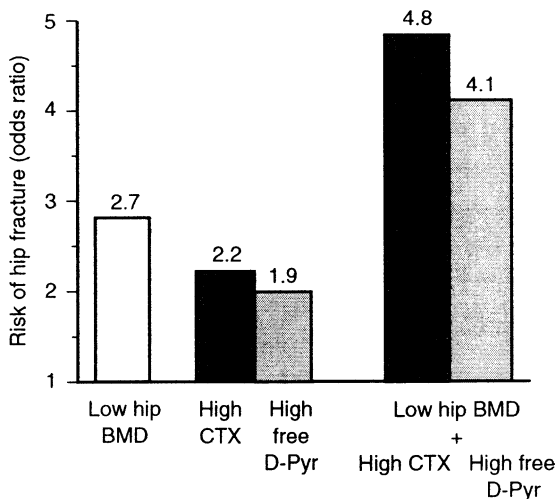


Fig. 2. Relationship between bone resorption rates, bone mineral density and risk of hip fracture in elderly women. In this study of elderly women (mean age 82.5 years), low hip bone mineral density and increased bone resorption rates are independent predictors hip fracture risk. The highest fracture risk is calculated in women with a combination of low femoral neck BMD and high bone resorption. CTX, urinary carboxyterminal collagen type I telopeptide, D-Pyr, urinary deoxypyridinoline. (From Garnero P, Hausherr E, Chapuy MC, et al. Markers of bone resorption predict hip fractures in elderly women. The EPIDOS study. *J Bone Miner Res* 1996b;11:1531–8; with permission).

poor nutritional status and hip fracture risk among institutionalized subjects, and not a general biological mechanism possibly relevant to a more representative sampling of the population. The significance of vitamin K deficiency to the under-carboxylation of OC had been demonstrated earlier by Price et al [112], and subsequent clinical studies showed that overt vitamin K deficiency may lead to a disproportionate increase in ucOC in the circulation [113,114]. Additionally, vitamin K₂ levels have been shown to be lower in women with osteoporosis fractures than in healthy individuals [113]. Although measurement of ucOC may be useful in providing an integrated assessment of the factors that are responsible for the gamma-carboxylation of OC, such as vitamins K and D, the underlying biochemical mechanisms by which ucOC could be associated with impaired bone metabolism are unknown. Analysis of OC in bone samples from a relatively small number of patients with osteoporosis has shown negligible differences in the proportion of ucOC compared with bone specimens from healthy controls [115]. The lower affinity of the under-carboxylated protein for bone mineral may also serve to diminish its relative importance in influencing bone metabolism. Alternatively, OC-deficient, transgenic mice show enhanced bone formation [116], suggesting an inhibitory function of OC on osteoblast activity.

In another prospective study from Sweden, low serum levels of both the carboxyterminal propeptide and telopeptide of type I collagen were associated with an increased risk of hip fracture, independent of age and BMD [76]. Therefore, increased rates of bone resorption or decreased rates of bone formation seem to be associated with future osteoporotic fractures [117].

In summary, data from several independent and large prospective studies indicate that in postmenopausal women increased rates of bone resorption are associated with an increased risk of vertebral and non-vertebral fractures, independent of BMD, age, and disability. Measurements of markers such as ucOC may provide additional information, (eg, on nutritional status). All these associations were shown in large groups of postmenopausal women, and it remains to be shown whether they hold true on a patient to patient basis and across genders. However, it can be anticipated that in the future, markers of bone turnover will play a role both in fracture risk assessment and in the definition of intervention thresholds. To achieve this goal, markers of bone turnover will most likely be used in combination with other risk factors for osteoporosis fracture.

Bone markers and therapy

Osteoporosis is a heterogeneous disease, and so is its treatment. Ideally, treatment of patients with osteoporosis should be individualized, taking into account factors such as age, sex, bone mass and mineral density, life style, disability, mobility, co-morbidity, and life-expectancy. In addition, the rate

of bone remodeling may be an important determinant of therapeutic efficacy and response.

The role of pre-treatment bone turnover

From a theoretic point of view, it is conceivable that intervention strategies may differ between patients with accelerated, normal or even abnormally low bone turnover at the time of diagnosis. It was therefore hypothesized that a patient presenting with high rates of bone resorption may benefit from anti-resorptive therapy, whereas in an individual with low bone turnover, a stimulator of bone formation may yield better long-term results. The question was therefore brought forward whether pre-treatment bone marker measurements may be helpful in guiding the selection of therapy for individual patients. Some studies [118–120] have shown that in osteoporosis patients treated with subcutaneous calcitonin, increases in lumbar (but not necessarily in hip) BMD were significantly greater in individuals with high than with normal or low baseline rates of bone turnover. Similar results were later reported for short term Alendronate treatment [121], although one report (with an equally small number of subjects) suggests that changes in BMD during treatment with Alendronate are independent of pre-therapeutic bone turnover rates [41].

Riggs et al [105] demonstrated that depending on the baseline rate of bone turnover, both an increase in vertebral BMD and a decrease in bone turnover are equally effective in reducing vertebral fractures in women with osteoporosis. Therefore, in patients with high levels of bone turnover, estrogen treatment and normalization of bone metabolism resulted in a decrease in vertebral fractures, independent of changes in vertebral BMD. In contrast, when bone turnover was normal, changes in lumbar BMD remained the main determinant of therapeutic efficacy. In women treated with non-toxic levels of fluorides, lumbar BMD and fracture rates were inversely related to each other. Therefore, it was concluded that bone forming agents, such as fluorides, act by directly increasing BMD and vertebral strength, whereas inhibitors of bone resorption prevent fractures mainly through the reduction of bone turnover. These results not only support the concept that, under certain conditions, bone turnover is a predictor of fracture risk, but also provide a rationale for the use of bone markers in the selection of therapy and in the prediction of therapeutic response.

Prospective studies in early postmenopausal women seem to substantiate this concept. Chesnut et al [92] and Rosen et al [99] demonstrated in 227 women treated with either calcium alone or a combination of HRT plus calcium, that individuals within the highest quartile for baseline measures of bone turnover also experienced the greatest gain in BMD after six and twelve months of treatment with HRT and calcium. In this study, baseline urinary NTX and serum OC showed the highest predictive values for a change in spinal BMD after one year of either HRT or calcium. In reverse, those women showing a gain in BMD after one year of HRT had

significantly higher baseline rates of bone resorption (as determined by urinary NTX) than non-responders or subjects losing bone during HRT [99]. This observation is in agreement with the hypothesis that the rate of bone turnover influences the likelihood of vertebral fractures only if accelerated [105].

In contrast, Stevenson et al [123] in a three-year prospective study on the effect of HRT on spine and hip BMD were unable to distinguish between responders and non-responders by means of either baseline or follow-up measures of bone turnover. Both groups showed the same pre-treatment values of bone formation and resorption, and the change in bone markers in response to HRT was identical in the affected and unaffected groups [123].

A recent analysis of the Risedronate clinical phase III programs show that the reduction in fracture risk during one and three years of Risedronate treatment is similar in patients with baseline urinary DPD below or above the premenopausal median (ie, with normal or accelerated bone resorption). However, the number of patients needed to treat (NNT) to avoid one fracture during one and three years of treatment with Risedronate is significantly lower in patients with elevated baseline bone turnover as compared to patients with low baseline bone turnover [124]. Thus, although the reduction in overall fracture risk seems to occur independent of baseline bone turnover, patient stratification by pre-treatment bone resorption rates seems to make some sense from a pharmaco-economic standpoint.

Taken together, there is moderate evidence favoring a relationship between bone turnover at baseline and the response to anti-resorptive treatment. When treated with calcitonin, HRT or bisphosphonates, subjects with accelerated pre-treatment bone turnover appear to gain BMD at a faster rate than patients with normal or low bone turnover. For HRT, this relationship holds true only for patients with marked accelerated rates of bone turnover. Preliminary data suggest that Risedronate treatment reduces fracture risk independent of baseline bone turnover.

The role of bone turnover markers in therapeutic monitoring

Therapeutic (and disease) monitoring is believed to be the major domain for the clinical use of biochemical bone markers. These applications include the prediction of therapeutic response during (not before) treatment, the monitoring of therapeutic efficacy, and possibly, the monitoring of patient compliance. Treatment of osteoporosis aims at a reduction in the number fractures. However, because of the comparatively low short-term incidence of osteoporosis fractures, monitoring the effects of treatment can be difficult. Consecutive measurements of BMD as surrogate marker of treatment effects are the standard approach to monitor treatment of osteoporosis patients. However, changes in bone mass or mineral density occur slowly, and a therapeutic effect is usually not detectable before several years of treatment. This is partly because of the slow dynamics of bone remodeling, and a disadvantageous short-term signal to noise ratio (SNR)

of most BMD measurements. Within a year of treatment, comparatively small changes in bone density (eg, 2% to 4%) are contrasted by relatively high precision errors (eg, 1% to 3%). This relatively high SNR renders BMD measurements unfit for short term, (ie, early assessments of therapeutic effects). Biochemical bone markers also exhibit a high degree of imprecision, which is mainly attributable to their significant biologic variability. However, as far as short-term changes are concerned, their SNR compares somewhat favorably to BMD measurements. Bone markers usually react rapidly to therapeutic interventions. Significant changes, that is changes exceeding the respective marker's "background noise" are mostly seen within 6 to 12 weeks of therapy.

Therefore, treatment with subcutaneous calcitonin [125–127], subcutaneous osteoprotegerin [128] or intravenous bisphosphonates [41,129,130] leads to a significant reduction in resorption marker levels as early as 12 to 72 hours after the first injection. Although intermittent bisphosphonate treatment (eg, with cyclic oral etidronate or intravenous ibandronate) results in a rapid decrease of bone resorption markers, this change tends to be sustained for only a short time and bone resorption markers often return to sub-baseline levels within a few weeks [131,132]. Interestingly, and so far unexplained, a single dose of 1 or 4 mg of intravenous Zoledronate causes a significant reduction in bone turnover that last over a period of more than 12 months [133] Fig. 3). Treatment with estrogen [38,75,134–137], testosterone [138], selective estrogen receptor modulators [139–143], or oral bisphosphonates [144–148] usually results in a 50 to 100% decrease in markers of bone turnover within three to four months of treatment. With most anti-resorptive treatments, the reduction in bone resorption markers is generally followed by a decrease in bone formation markers—a result of the normal coupling of bone formation to resorption processes. If therapy is continued, bone turnover markers usually plateau on a lower level of activity within 6 to 12 months. Once therapy is discontinued, most resorption markers tend to rise to baseline levels within a few weeks or months. This pattern is again mirrored by a parallel but somewhat delayed change in bone formation markers [136].

During short-term Raloxifene treatment in elderly men, changes in urinary NTX were restricted to a subset of subjects with very low serum estradiol levels at baseline, indicating that only men with significant estradiol deficiency may benefit from SERMs [149].

The effects of statins on bone turnover markers are unclear, as the few specific reports on this issue are ambiguous [150–152]. Dehydroepiandrosterone, given in physiologic dose and over a short period of time either to healthy men [153] or to women with adrenal insufficiency [154] appears to have no effect on bone turnover.

Does a change in bone turnover early during treatment predict therapeutic outcome at a later point in time? More specific, Does the degree of reduction in bone resorption markers after three to six months of anti-resorptive

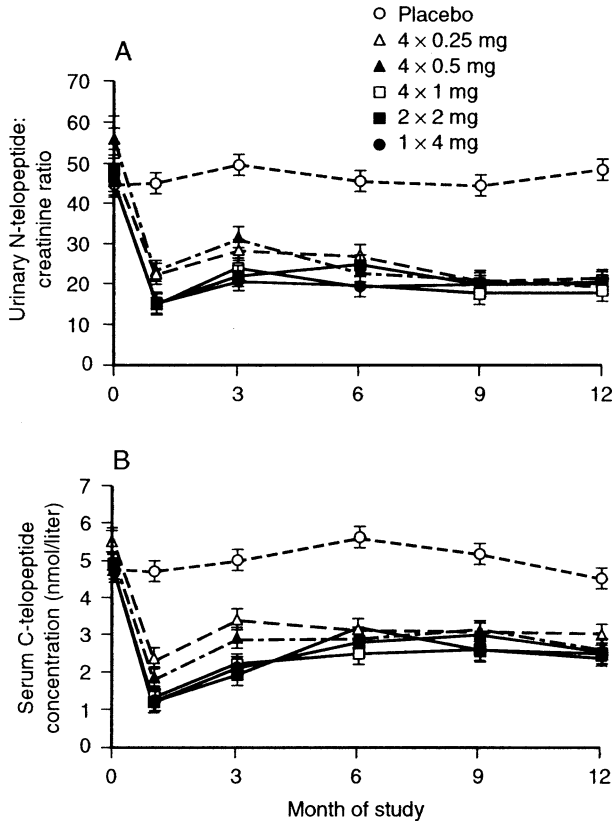


Fig. 3. Change in bone resorption markers induced by intravenous Zoledronate. Urinary NTX (upper panel), serum CTX (lower panel). Mean change (\pm SE) in bone resorption markers following intravenous administration of various doses of Zoledronate as compared to placebo (dashed line, open circles). Changes in bone markers were significantly different from placebo from months 1 onwards. (From Reid IR, Brown JP, Burckhardt P, et al. Intravenous zoledronic acid in postmenopausal women with low bone mineral density. *N Engl J Med* 2002;346:653–61; with permission).

treatment correlate with the increase in BMD after one to three years of treatment? A large number of studies [92,99,136,155] seem to indicate that patients with the most pronounced short term change in bone resorption markers during HRT exhibit the highest gain in BMD after one or two years of treatment. Likewise, patients with no change in BMD (non-responders) often showed little or no change in bone markers. Similar results have been published for bisphosphonate treatment [20–22,68,147,156]. However, the power of this association seems variable and often depends on whether or not placebo treated patients were included in the analysis. In contrast to some optimistic studies reporting strong correlation between changes in markers of bone turnover, spinal, hip, and wrist BMD [155] other authors have observed only weak [84] or even no [157] such associations.

A recent report indicates that the 6 and 12 months change in bone turnover markers was predictive for the reduction in fracture risk after 3 years of raloxifene treatment in postmenopausal women [158]. In this study, a decrease of 9.3 pg/mL in serum OC after one year of raloxifene treatment was associated with an odds ratio for new vertebral fractures after three years of 0.69 (CI 0.54–0.88, $P = 0.003$). This relationship remained after adjusting for baseline vertebral fracture status and BMD (Fig. 4). These results are interesting as raloxifene induces only 2% to 3% increase of BMD

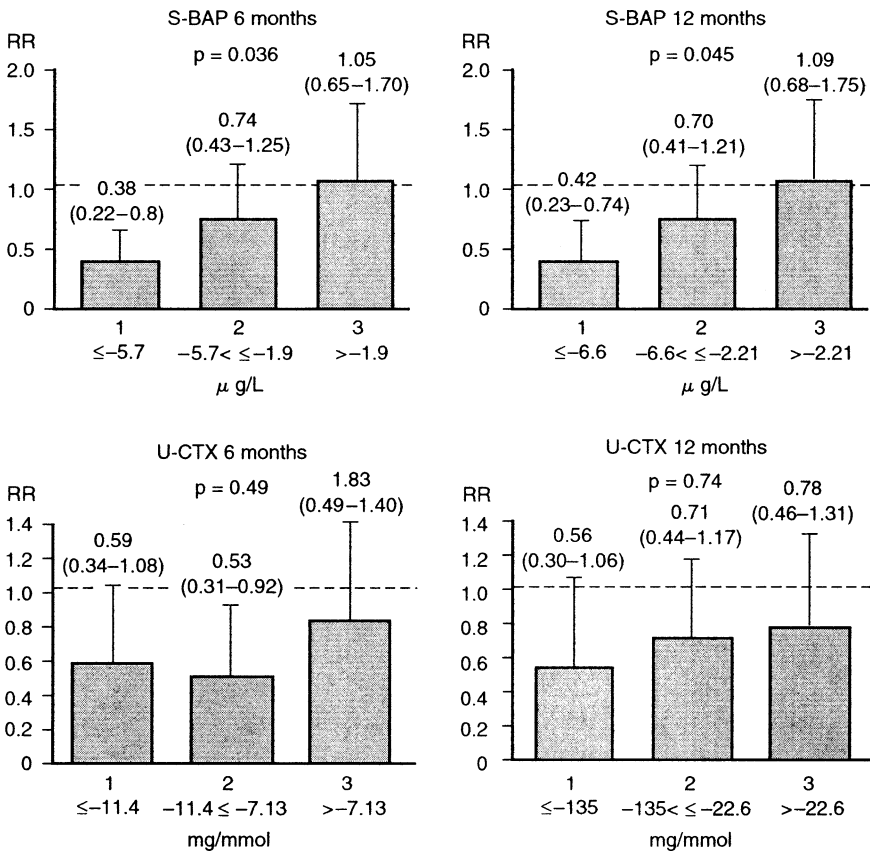


Fig. 4. Relationship between change in bone formation markers and fracture risk reduction during Raloxifene treatment. In a subgroup analysis ($n = 2622$) of the MORE study, only patients with a pronounced (tertile 1) suppression in serum bone alkaline phosphatase activity at 6 and 12 months of treatment showed a significant reduction in fracture risk at 3 years of treatment. Similar associations were seen for serum osteocalcin, but not for urinary CTX. The change in BMD was not related to fracture risk. (From Bjarnason NH, Sarkar S, Duong T, et al. Six and twelve month changes in bone turnover are related to reduction in vertebral fracture risk during 3 years of raloxifene treatment in postmenopausal osteoporosis. *Osteoporos Int* 2001b;12:922–30; with permission).

(all sites), but a 30% to 50% reduction in vertebral fracture rates. It is therefore conceivable that for certain therapies, changes in bone markers may be more predictive of anti-fracture efficacy than changes in BMD. Focussing on fracture outcome rather than change in BMD as primary endpoint, similar results have recently been reported for treatment with oral Risedronate [159]. However, one should remember that both BMD and bone markers will always remain surrogate endpoints that in an individual patient may or may not be adequate to assess therapeutic response.

Taken together, these data suggest that biochemical markers of bone metabolism are useful tools to evaluate therapeutic effects after a relatively short period of time, and that serial measurements of bone markers may help to decide whether or not a patient responds (or adheres) to a specific treatment regimen. In the case of non-responsiveness or non-compliance, bone marker measurements have the potential to save medication-related costs that otherwise would accumulate over a period of several years. However, it should be noted that presently, none of these concepts has been proven in controlled prospective trials, and the clinical use of these markers, in monitoring individual patients has not been sufficiently addressed. Consequently, no scientific or clinical consensus exists as to the use of bone markers in the follow-up of patients with osteoporosis.

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