PERSPECTIVES

Biochemical Markers of Bone Turnover and Osteoporosis Management

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Abstract

Osteoporosis is defined as a systemic disease characterized by low bone mass and microarchitectural deterioration of bone tissue, both of which are related to abnormalities of bone turnover. Bone mass can be assessed by measuring bone mineral density (BMD) using dual X-ray absorptiometry (DXA) and there is a large body of evidence suggesting that low BMD is an important determinant of fracture risk. However, BMD measurement is not the only determinant of fracture risk. Increased bone resorption as evaluated by specific biochemical markers has been shown to be associated with an increased risk of hip, spine and non-vertebral fractures independently of BMD. The combination of bone mass measurement and the assessment of bone turnover by biochemical markers is thus helpful in the assessment of osteoporotic fracture risk. Repeated measurement of biochemical markers during treatment appears to improve the management of osteoporotic patients. The decrease in bone turnover markers during antiresorptive treatment is inversely related to the subsequent increase in BMD. Furthermore, several studies have shown that short-term reductions in bone turnover were associated with a reduction in vertebral and/or non-vertebral fracture risk in women treated with antiresorptive agents. Preliminary data also suggest that serial bone marker measurements could be useful in identifying skeletal responders to anabolic therapy with PTH. *BoneKEy.* 2007 July;4(7):191-203.
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Osteoporosis is defined as a systemic disease characterized by low bone mass and microarchitectural deterioration of bone tissue, both of which are related to abnormalities of bone turnover. Bone mass can be assessed by measuring bone mineral density (BMD) using dual X-ray absorptiometry (DXA) and there is a large body of evidence suggesting that low BMD is an important determinant of fracture risk (1-3). There is a consistent doubling of fracture risk for each standard deviation reduction in BMD, irrespective of fracture type and BMD measurement site. However, there is no threshold below which fracture risk increases, indicating that there is rather a gradient of risk of fracture with decreasing BMD. The risk prediction has been found across various age ranges but the gradient of risk decreases with advanced age (1). BMD is not the only determinant that contributes to bone strength and fracture risk. Bone fragility is also dependent on morphology, architecture, and bone remodeling, as well as on qualitative

properties of bone matrix (4;5). Much effort has been devoted to the identification of predictors of osteoporotic fracture risk. In the treatment-naive patient, age, gender, comorbidity, disability, calcium intake, low body mass index, low bone mass and prevalent fractures are the most common and powerful predictors of osteoporotic fracture (5;6). More recently, it has become evident that accelerated bone turnover is also associated with a higher risk of osteoporotic fracture, independently of age, disability and bone mass.

In recent years, considerable progress has been made in the isolation and characterization of cellular and extracellular components of the skeletal matrix, which, in turn, has facilitated the development of biochemical markers that reflect specifically either bone formation or bone resorption (7;8). These biochemical indices have greatly enriched the spectrum of analyses 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. Biochemical markers of bone turnover can be divided into two groups: markers of resorption and markers of formation (Table 1). The principal, specific markers of bone formation, measured in serum by immunoassays, are bone alkaline phosphatase (BAP), osteocalcin (OC), and the procollagen type I N-terminal propeptides (PINP). Markers of bone resorption include pyridinium crosslinks (pyridinoline and deoxypyridinoline) and their associated peptides (telopeptides), which are released during collagen breakdown, as well as tartrate resistant acid phosphatase (TRACP).

Bone formation	Bone resorption

- Bone alkaline phosphatase (BAP)
 	
- Osteocalcin (OC)
 	
- Type I collagen extension propeptides (PICP, PINP)
	- Pyridinium crosslinks
	  - Pyridinoline (PYD)
	  - Deoxypyridinoline (DPD)
	- Crosslinking telopeptides of type I collagen
	  - C-terminal (CTX) – N-terminal (NTX)
	  - CTX generated by MMP*
	- Tartrate resistent acid phosphatase (TRAP)
	- Galactosyl-hydroxylysine
	- Urinary OC-fragments (mid-region)
	- Hydroxyproline

Table 1: Biochemical markers of bone turnover.
*MMP: matrix metalloproteinases

While total pyridinium crosslinks are measured by HPLC, immunoassays for pyridinoline (PYD) and deoxypyridinoline (DPD) in urine and for C-terminal and N-terminal type I collagen peptides (CTX and NTX, respectively) have become available more recently. It has been demonstrated that type I collagen molecules can undergo racemization and isomerization at the aspartic acid residue within the C-telopeptide (9-11). As a consequence of these spontaneous, non-enzymatic post-translational modifications, the CTX molecule may occur in one of four different forms: as a native linear peptide (αL); as the isomerized beta form (βL); as the racemized linear peptide (αD); and finally as the isomerized and racemized beta form (βD). The processes of racemization and isomerization have been attributed to protein aging (9-11). Measurement of the relative amounts of newly synthesized (αL) and age-

modified CTX may possibly provide information about the age of resorbed bone and the metabolic activity of bone. The first assay to be developed for the measurement of collagen telopeptides in serum was a radioimmunoassay (RIA) for the C-telopeptide cross-link domain generated by metalloproteinase (MMP) treatment (12). The assay appears to be more sensitive in the assessment of pathological bone resorption (*i.e.*, in multiple myeloma or metastatic bone disease) than of the changes occurring in postmenopausal osteoporosis (13).

Recently, immunoassays have been developed that preferentially detect the TRACP isoenzyme 5b, which is predominantly expressed by osteoclasts (14;15). The TRACP5b isoenzyme is thought to represent mainly osteoclast

number and activity and not directly the rate of collagen breakdown. More recently, the significance of OC fragments in urine as a marker of bone resorption has been investigated and clinical studies suggest that the quantification of specific OC fragments

in urine may provide an index of bone resorption (16;17). Many of these assays have been adapted to an automated platform with increased precision and superior accuracy, providing a more widespread availability of these markers.

Study	Age (years)	Fx	RR-BMD (95% CI)	Marker	RR-Marker (95% CI)	Ref.
EPIDOS	>75	hip	2.8 (1.6-5.1)	u-CTx fDPD	2.2 (1.3-3.6) 1.9 (1.1-3.2)	29
OFELY	64 (mean)	all	2.8 (1.4-5.6)	u-CTx s-CTx α-L/β-L CTX	2.3 (1.3-4.1) 2.1 (1.1-3.6) 1.8 (1.03-3.1)	30
HOS	69 (mean)	all	1.6 (1.2-2.2)	u-CTx	1.6 (1.2-2.0)	31
Rotterdam	>75	all	1.3 (0.6-2.7)	u-DPD	1.9 (1.2-3.8)	32
Malmö	>75	all	2.2 (1.5-3.1)	TRACP u-OC	2.2 (1.2-4.2) 2.1 (1.1-4.1)	16

Table 2: Relationship between increases in bone resorption rate and fracture risk.
(Fx: fracture; BMD: bone mineral density; CTx: C-terminal telopeptide; fDPD: free deoxypyridinoline; TRACP: tartrate resistant acid phosphatase; OC: osteocalcin; u: urinary; s: serum).

Biochemical Markers and the Rate of Bone Loss

There is evidence indicating that bone turnover increases rapidly after menopause and that this increase in bone turnover persists long after menopause, up to 40 years (18;19). In general, bone loss at the spine in the immediate menopausal period is approximately 1% per year, but as many as one-third of postmenopausal women lose bone at a rate exceeding 1% per year. Cross-sectional data suggest that a sustained increase in bone turnover is associated with faster and greater bone loss. It has been more difficult to assess this relationship in longitudinal studies as the amount of bone loss is often in the same order of magnitude as the precision error of BMD measurement (20;21). A strong correlation between the rate of turnover and the rate of bone loss has only been demonstrated in a study measuring BMD at a precise site, i.e., at the radius, but not with measurements at the spine and hip (20;22). However there is some evidence indicating that biochemical markers can detect "rapid

losers" and can predict those women most likely to respond to antiresorptive therapy, i.e., hormone replacement therapy (23-25).

At the present time, a single measurement of a biochemical marker cannot predict the absolute rate of bone loss in a single individual. However increased bone turnover markers can be regarded as a risk factor for rapid bone loss.

Biochemical Markers and Fracture Risk

As mentioned above, there is a strong association between BMD and the risk of hip, spine and forearm fractures. However, up to half of patients with incident fractures have baseline BMD, as assessed by DXA, that is above the diagnostic threshold of osteoporosis, defined as a T-score of -2.5 standard deviations or more below the average value of young healthy women (WHO definition of osteoporosis) (26-28). Thus, improvement in the identification of patients at risk for fracture is needed. Furthermore, there is increasing evidence that the decision to use pharmacological

intervention for the prevention of fractures should be based on fracture probability, rather than only on the presence of osteoporosis as defined by BMD (5;6). A potential clinical application of biochemical indices of skeletal metabolism is in the assessment of fracture risk. Findings from prospective studies indicate an association between osteoporotic fractures and indices of bone turnover, independent of BMD, in women at menopause and in elderly women, and also more recently in men (Table 2 and Figure 1) (16;29-34).
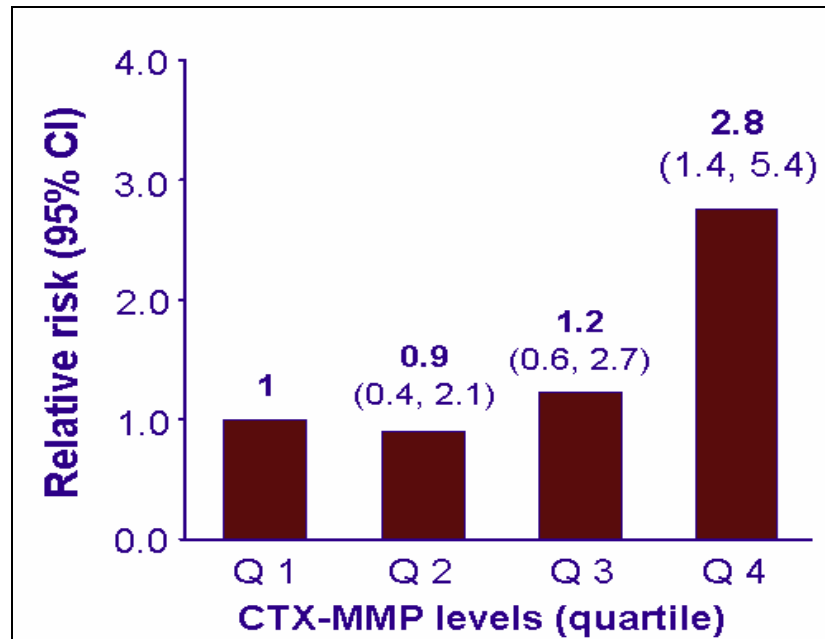
Figure 1. **Serum levels of CTX-MMP and the risk of subsequent fracture in elderly men (RR, 95% CI)**. The reference group consisted of men with marker serum concentrations in the lowest quartile (Q1) (33).

The results for the relationship between bone formation markers and fracture risk are conflicting. In the French cohort study of elderly women (EPIDOS), no significant relationship between OC or BAP and fracture risk could be demonstrated during a 2 year follow-up, whereas in younger, healthy postmenopausal women (OFELY and HOS), a significant association between increased BAP serum levels and the risk of vertebral, as well as non-vertebral, fractures was found (29-27). In the OFELY study, a reassessment was performed after a median of 10 years follow-up and a significant positive association was found between baseline levels of serum OC, BAP and PINP and the risk of fracture (28;35). In contrast, others did not find a significant relationship between a bone formation marker and the risk of fracture that occurred in the following 20 years (36).

More concordant results were obtained with markers of bone resorption. In five prospective studies in postmenopausal women (EPIDOS, Rotterdam, OFELY, HOS, and Malmö), a significant relationship between baseline levels of urinary or serum CTX, urinary free DPD, or serum TRAP5b and fracture risk could be demonstrated (16;18;29-32) (Figure 2 and Table 2). An increase of these markers above the premenopausal range was associated, after adjustment for BMD, with a twofold increase in risk for hip, vertebral and non-vertebral fractures, over a follow-up period of 1.8 to 5 years. In a subset of the OFELY cohort, an increased ratio of α-L/β-L CTX was associated with an increased fracture risk independently of BMD and of the bone turnover rate (37).

These results suggest that a combined approach, using BMD and indices of bone turnover, could improve fracture prediction in

postmenopausal women. In fact, by calculating the absolute risk, such as the 10-year probability of fracture based on 2 prospective studies (EPIDOS and OFELY), it was found that combining clinical risk factors (*i.e.*, previous fractures), BMD and bone turnover results in a 10-year probability

of hip fracture that was about 70-100% higher than that associated with low BMD alone (38) (Figure 3). In elderly men, high bone resorption was also associated with an increased risk of osteoporotic fracture (33). Thus there is evidence that postmenopausal
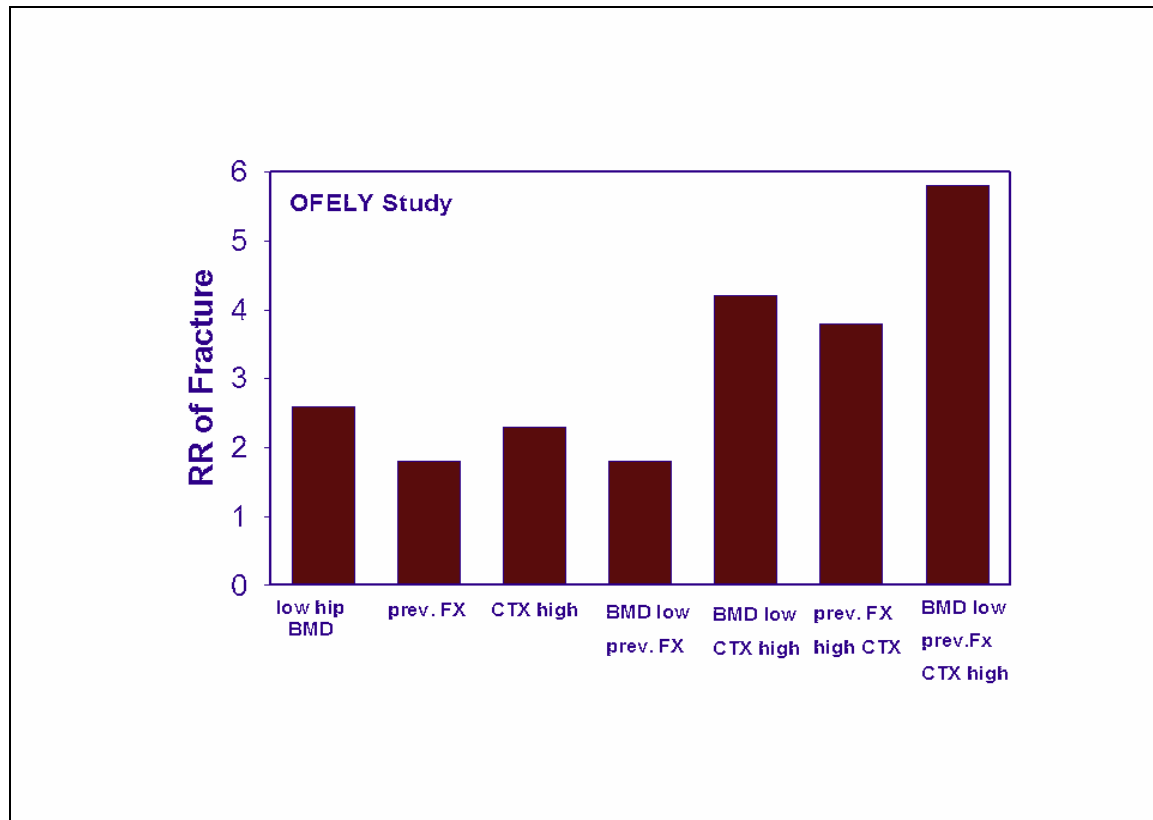
Figure 2: **Combination of different independent predictors to identify women with the highest risk of fracture.** BMD: bone mineral density; CTX: C-terminal telopeptide; Fx: fracture (30).

women with low BMD and high bone turnover are at high risk for osteoporotic fractures. The same appears to be true for elderly men.

Biochemical Markers and Monitoring Treatment

Another domain for the clinical use of biochemical bone markers is the monitoring of osteoporosis therapy. This application includes keeping an eye on both therapeutic efficacy (*i.e.*, the prediction of the therapeutic response regarding changes in BMD and reduction in fracture risk) and patient compliance. The ultimate goal in treating patients with osteoporosis is to

reduce their fracture risk. However, the short-term incidence of osteoporotic fractures is low, and the absence of fractures during treatment does not necessarily mean that the treatment is effective. Thus, monitoring the effect of treatment by fracture incidence alone is inadequate for evaluating the effects of therapy. Consequently, serial measurements of changes in BMD as a surrogate marker of therapeutic efficacy are currently the standard approach to monitor osteoporosis therapy. However, changes in BMD occur slowly and therapeutic effects are usually not detectable before 1-2 or more years of treatment (25;39-42). In contrast, biochemical markers of bone

turnover change much faster than BMD in response to therapeutic interventions (25). In several prospective intervention trials with antiresorptive agents, it has been shown that a rapid decrease in bone resorption markers has already occurred at 2-4 weeks and is reaching a plateau after 3-6 months of treatment (25;43). The decrease in bone formation markers, reflecting the physiological coupling of bone formation to bone resorption, is delayed and reaches a plateau after 6-12 months. The magnitude of the decrease varies according to the antiresorptive potency of the medication. On bisphosphonate treatment, urinary CTX and NTX decreased by about 40-70%, and total DPD by about 50% (43;44). As compared to bisphosphonates, raloxifene induces smaller decreases in bone resorption markers, of about 30–40% for urinary CTX and 20–30% for bone formation markers (45). Calcium and vitamin D supplements induce small but significant decreases in bone resorption markers of about 10–20% (46). The decrease in bone turnover markers during
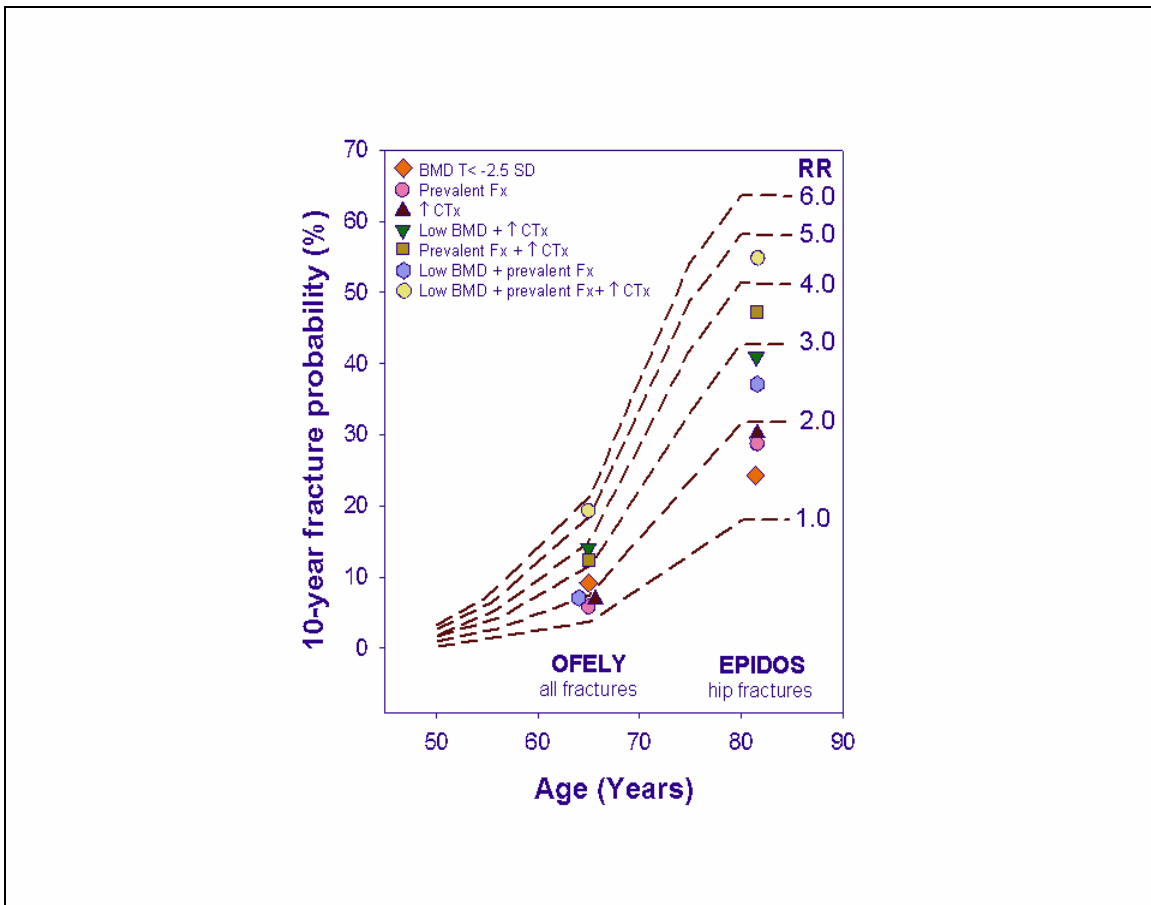
Figure 3. **Combination of clinical risk factors, bone mineral density and bone turnover measurements to identify women with the highest risk ten-year probability of hip fracture according to age and relative risk.** The symbols show the effect of risk factors on fracture probability derived from women aged 65 years (OFELY study) and 80 years (EPIDOS study). (Low BMD= t-score $\leq$ -2.5; high CTX= value > upper limit of premenopausal reference range) (38).

antiresorptive treatment is inversely related to the subsequent increase in BMD, predominantly at the lumbar spine (47). Several studies in postmenopausal women treated with antiresorptive agents (HRT, raloxifene, bisphosphonates) have indicated that the degree of short-term reduction in bone turnover markers (after 3-6 months) correlates with the observed long-term

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increase in BMD after 1-3 years of treatment (23;24;43;47-50).

Although several randomized trials have found that antiresorptive agents improve BMD and reduce the risk of fractures, recent studies have shown that the observed reduction in fracture risk is only partially explained by the documented changes in BMD. The reduction in risk was greater than predicted from improvements in BMD, and it has been estimated that changes in BMD explain only 4% to 28% of the reduction in vertebral fracture risk attributed to antiresorptive treatment (39;51-55). Therefore, it is possible that changes in other determinants of bone strength, including the rate of bone turnover and its changes during antiresorptive therapy, may be more predictive of anti-fracture efficacy than changes in BMD. In fact, several studies confirmed that short-term reductions in bone turnover were associated with a reduction in vertebral and/or non-vertebral fracture risk in women treated with HRT, raloxifene, risedronate, and alendronate (34;44;52;56;57). Recently, studies have been published investigating changes in bone turnover markers and fracture risk in bisphosphonate-treated postmenopausal women. It was found that reductions in markers of bone resorption were significantly associated with reductions in vertebral and non-vertebral fracture risk after 3 years. Urinary C-terminal telopeptides (CTX) were reduced by 60% and N-terminal telopeptides (NTX) by 51% at 3-6 months of risedronate treatment (44). The change in bone resorption markers explained more than 50% of the risedronate-related fracture risk reduction for both vertebral and non-vertebral fractures. Interestingly, there appeared to be a threshold level for the decrease in bone resorption markers (*e.g.*, 55-60% as measured by CTX, and 35-40% as measured by NTX) below which there was no further increase in therapeutic benefit.

Recently, potent bone formation stimulating therapy with peptides from the parathyroid hormone family have become available (58;59). In contrast to antiresorptive agents, PTH administered intermittently in low doses increases bone remodeling, stimulating bone formation preferentially over bone resorption and thus resulting in a net gain of bone. Teriparatide (PTH 1–34) and full-length PTH (PTH 1–84) have been shown to increase BMD. Teriparatide reduces spine and non-vertebral fractures, an effect that is sustained for up to 30 months after the withdrawal of treatment. The intact hormone (1-84 amino acids) showed similar results on spine fractures. Increases in bone turnover markers indicate a skeletal response to PTH and there is evidence that early measurements of bone formation markers (OC, BAP and particularly PINP) correlate positively with the subsequent BMD response (60-63). These data suggest that serial bone marker measurements could become useful in identifying skeletal responders to anabolic therapy with PTH.

There are several reports that women with higher levels of pre-treatment bone turnover markers have greater increases in BMD on antiresorptive therapy (43;64;65). It is therefore tempting to conclude that patients with increased bone turnover will have greater benefits from antiresorptive treatment, and patients with low bone turnover should be treated with an anabolic agent. In a *post hoc* analysis from the fracture intervention trial (FIT), it was found that in osteoporotic women with low BMD at the non-spine, but not at the spine, antifracture efficacy of alendronate was greater in those with higher pre-treatment levels of PINP (66). A similar, but not significant, trend was observed for bone ALP. In contrast, the efficacy of risedronate in reducing incident vertebral fractures in postmenopausal women with osteoporosis was independent of pre-treatment bone resorption marker levels (urinary DPD) (67). A recent analysis of the Fracture Prevention Trial has shown that the antifracture efficacy of teriparatide was also independent of pre-treatment bone turnover (68).

Long-term adherence to and persistence with any therapy is very poor and is not specific to the disease, disease severity, or treatment. As with other diseases, poor compliance and persistence is a concern in osteoporosis, due to its negative impact on

fracture risk and healthcare costs (69;70). Adherence to bisphosphonate therapy is associated with significantly greater fracture risk reduction (70). However, the probability of continued treatment with a bisphosphonate after 1 year is only 50% (71). Published data suggest that monitoring visits during antiresorptive drug treatment may enhance adherence, and there is preliminary evidence that monitoring with a bone turnover marker during antiresorptive therapy may also improve adherence (72-74). In a *post-hoc* analysis, patients monitored with bone turnover markers and a nurse visit, and given a positive message regarding their response to antiresorptive therapy, were 92% more likely to adhere to therapy compared with usual care, and 18% more likely to adhere to therapy than patients monitored with a nurse visit alone (72). The type of message patients were given from the bone resorption marker results impacted their subsequent persistence with therapy: a positive message was associated with improved persistence, while a neutral message had no effect and a negative message reduced persistence (72;74). Monitoring anti-osteoporotic treatment using bone markers may thus be useful to improve persistence of treatment and thus its effectiveness.

Conflict of Interest: The author reports that no conflict of interest exists.

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