Clinical Application of Biochemical Markers of Bone Turnover

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ABSTRACT

With the ageing population in most countries, disorders of bone and mineral metabolism are becoming increasingly relevant to every day 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 have markedly grown. Together with clinical and imaging techniques, biochemical tests play an important role in the assessment and differential diagnosis of metabolic bone disease. In recent years, the isolation and characterisation of cellular and extracellular components of the skeletal matrix have resulted in the development of molecular markers that are considered to reflect either bone formation or bone resorption. These biochemical indices are non-invasive, comparatively inexpensive and, when applied and interpreted correctly, helpful tools in the diagnostic and therapeutic assessment of metabolic bone disease. This review provides an overview of the current evidence regarding the clinical use of biochemical markers of bone remodelling in bone disease, with an emphasis on osteoporosis. **(Arq Bras Endocrinol Metab 2006;50/4:603-620)**

Keywords: Remodelling; Markers; Osteoporosis; Fracture; Treatment

RESUMO

Marcadores Bioquímicos da Remodelação Óssea.
Tendo em vista o crescimento da população idosa na maioria dos países, os distúrbios do metabolismo ósseo e mineral estão tornando-se cada vez mais relevantes na prática clínica diária. Conseqüentemente, o interesse e a necessidade de medidas efetivas para serem usadas no rastreamento, diagnóstico e seguimento de tais patologias vêm crescendo acentuadamente. Além da avaliação clínica e de técnicas de imagens, os marcadores bioquímicos desempenham um importante papel na avaliação e diagnóstico das doenças ósseas metabólicas. Recentemente, a melhor caracterização dos componentes intracelulares e extracelulares da matriz óssea resultou no desenvolvimento dos marcadores moleculares, os quais refletem tanto a formação como a reabsorção óssea. Estes marcadores bioquímicos não são invasivos e comparativamente são de mais baixo custo, e quando aplicados e interpretados corretamente são instrumentos úteis no diagnóstico e tratamento das doenças ósseas metabólicas. Esta revisão abordará evidências atuais, levando em consideração o uso clínico dos marcadores bioquímicos da remodelação óssea nas doenças metabólicas ósseas, com ênfase na osteoporose. **(Arq Bras Endocrinol Metab 2006;50/4:603-620)**

Descritores: Remodelação; Marcadores; Osteoporose; Fratura; Tratamento.

BACKGROUND

BONE IS A METABOLICALLY ACTIVE tissue and undergoes continuous remodelling, a process that largely relies on the activity of osteoclasts to remove bone and of osteoblasts to form bone. Under normal conditions, bone resorption and formation are coupled to each other, and the long-term maintenance of skeletal balance is achieved through the action of systemic hormones and local mediators. In contrast, metabolic bone diseases, states of increased or decreased mobility, and therapeutic interventions are characterised by more or less pronounced imbalances in bone turnover (1,2).

With the increasing awareness of disorders of bone and mineral metabolism in clinical practice, the interest in, and the need for effective measures to be used in the screening, diagnosis and follow-up of such pathologies have markedly grown. Along with clinical and imaging techniques, laboratory tests play an integral role in the assessment and differential diagnosis of metabolic bone disease.

In recent years, the isolation and characterisation 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 [for review: (3)].

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 they are considered to reflect. For clinical purposes, therefore, markers of bone formation are distinguished from indices of bone resorption (figure 1, table 1). It should be born in mind, however, that some of these compounds may reflect, at least in part, both bone formation and resorption (e.g., urinary hydroxyproline). Also, most marker components are present in other tissues than bone and may therefore be influenced by non-skeletal processes. Thirdly, changes in bone markers are usually not disease specific, but reflect alterations in skeletal metabolism independent of the underlying cause.

This review provides an overview of the current evidence regarding the clinical use of biochemical markers of bone remodelling in bone disease, with an emphasis on osteoporosis.
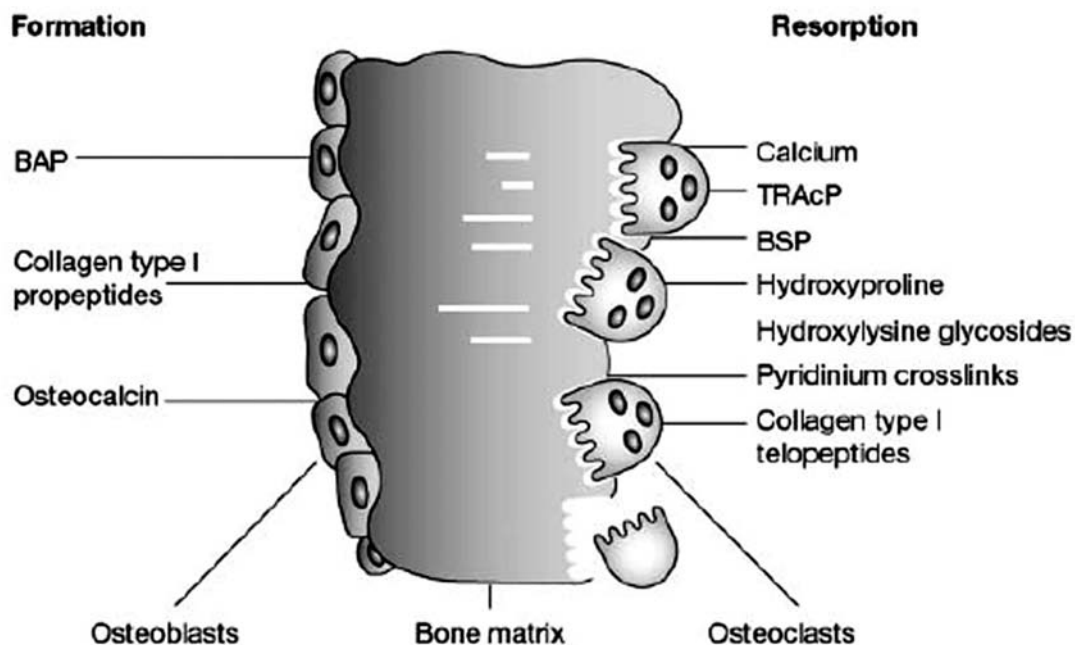

Figure 1. Schematic representation of the various bone markers currently in use.

Table 1. Markers of bone turnover

Marker	Tissue of Origin	Specimen	Analytical Method	Remarks
Markers of bone formation				
Bone-specific alkaline phosphatase (BAP)	Bone	Serum	Electrophoresis, Precipitation, IRMA, EIA	Specific product of osteoblasts. Some assays show up to 20% cross-reactivity with liver isoenzyme (LAP)
Osteocalcin (OC)	Bone, platelets	Serum	RIA, IRMA, ELISA	Specific product of osteoblasts; many immunoreactive forms in blood; some may be derived from bone resorption.
C-terminal propeptide of type I procollagen (PICP)	Bone, soft tissue, skin	Serum	RIA, ELISA	Specific product of proliferating osteoblasts and fibroblasts.
N-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.
Markers of bone resorption				
Collagen-related				
Hydroxyproline, total and dialyzable (Hyp)	Bone, cartilage, soft tissue, skin	Urine	Colorimetry HPLC	Present in all fibrillar collagens and partly collagenous proteins, including C1q and elastin. Present in newly synthesized and mature collagen, i.e. both collagen synthesis and tissue breakdown contribute to urinary Hyp.
Hydroxylysine-glycosides (HLG)	Bone, soft tissue, skin, serum complement	Urine (serum)	HPLC ELISA	Collagen HL is glycosylated to varying degrees, depending on tissue type. Glycosylgalactosyl-HL in high proportion in collagens of soft tissues, and C1q; Galyctosyl-HL high in skeletal collagens.
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.
Carboxyterminal cross-linked telopeptide of type I collagen (ICTP, CTX-MMP)	Bone, Skin	Serum	RIA	Collagen type I, with highest contribution probably from bone; may be derived from newly synthesized collagen.
Carboxyterminal 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.
Aminoterminal 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.
Collagen I alpha 1 helicoidal peptide (HELP)	All tissues containing type I collagen	Urine	ELISA	Degradation fragment derived from the helical part of type I collagen (α1 chain, AA 620-633). Correlates highly with other markers of collagen degradation, no specific advantage or difference in regards to clinical outcomes.
Non-Collagenous Proteins				
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.
Osteocalcin fragments (ufOC, U-Mid-OC, U-LongOC)	Bone	Urine	ELISA	Certain age-modified OC fragments are released during osteoclastic bone resorption and may be considered an index of bone resorption.
Osteoclast Enzymes				
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.
Cathepsins (e.g. K, L) (CathK, CathL)	K: Primarily in osteoclasts L: Macrophage, Osteoclasts	Plasma, Serum	ELISA	Cath K, a cysteine protease, plays a role in osteoclast-mediated bone matrix degradation by cleaving helical and telopeptide regions of collagen type I. Cath K & L cleave loop domain of TRACP and activate the latent enzyme. Cath L has a similar function in macrophages. Tests for measurement of Cathepsins in blood are presently under evaluation.

MENOPAUSE AND AGEING

Once somatic growth subsides, the serum and urinary concentrations of most bone markers return to a level much below those seen during normal puberty and growth. This stabilisation usually occurs during the 3rd decade and in healthy men, levels of practically all markers remain more or less unchanged until 70 years of age. After that, a slight increase is usually seen in both formation and resorption markers (4-7). In contrast, menopause is associated with a substantial acceleration in bone turnover, mirrored by a 50–100% increase in both markers of bone formation and resorption (4,5,8-14). In early postmenopausal women, this increase in bone turnover can be attenuated by oral calcium supplementation (15-19). Long-term treatment of women with oestrogen was shown to reduce resorption markers such as DPD and NTx to premenopausal levels (9,12,14,16,20-24). A prospective study covering the peri-menopausal transition in healthy women suggests that changes in bone turnover occur during the late pre-menopause with a decrease in bone formation, which only later is followed by a rise in bone resorption (25). It is now widely accepted that the accelerated rate of bone loss seen after the menopause is mainly due to an uncoupling in bone turnover and an increase in bone resorption (26,27). Studies employing specific bone markers indicate that bone turnover continues to be increased (and to be associated with bone loss) during late menopause (28-33). In some postmenopausal women (34), but particularly in the very elderly (35-37), this increase in bone turnover is often, but not always, found to be due to vitamin D and/or calcium deficiency and secondary hyperparathyroidism.

OSTEOPOROSIS

Bone turnover in osteoporosis

Osteoporosis is a heterogeneous disease. It is therefore not surprising that in untreated patients with this disorder, rates of bone turnover tend to vary over wide range. Although most cross-sectional studies show accelerated bone turnover in a certain proportion of postmenopausal osteoporotic women, there is usually broad overlap between diseased and healthy populations (11-13,38-41). In this context, it is important to bear in mind that research studies usually include highly 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 (13). However, another population-based study showed that urinary levels of NTX could discriminate between older individuals with normal hip bone density, osteopenia and osteoporosis (42). Again, this association did not hold true for men at the level of the spine.

In retrospective population-based studies, Akesson and co-workers (28,29,43,44) have demonstrated that previous fractures were associated with abnormal bone turnover. After adjustment for age and BMD, women with fractures occurring within six years prior to the study were characterised 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 (2).

Taken together, these data suggest that a long-term imbalance of bone metabolism 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 (44), these findings may provide a rationale for designing more effective intervention strategies. However, other factors such as age (see above), medication (6,18,46-51), immobilisation (32,35), thyroid function (52), co-morbidity (35) and the fracture itself (40,53,54) do influence bone metabolism and therefore need to be considered in the interpretation of biochemical data and their use in individual patients. Clearly, none of the biochemical markers of bone turnover has proven useful as a single diagnostic index of osteoporosis.

Bone turnover and bone loss

Bone mass, rates of bone loss, and the risk of osteoporotic fractures are interrelated, and both low bone mass and rapid bone loss have been shown to be independent predictors of future fracture risk (55). The rate of bone loss is determined by a number of factors, one of which appears to be the rate of bone remodelling. 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 (4,5,8-14). Similar observations have been made in ovariectomized, premenopausal women and in castrated men (56,57), indicating that the withdrawal of

endogenous sex steroid induces both high bone turnover and rapid bone loss. Conversely, markers of bone metabolism return to premenopausal levels during hormone replacement therapy (HRT) (9,12, 13,16,20-24). Other biochemical studies suggest that high rates of bone turnover may be sustained well into advanced ages (10,13,31,58,59). However, it is unclear whether this applies to all women.

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 (16,20,30,60-65). Following a small group of early postmenopausal women, Christiansen and colleagues demonstrated that the combined measurement of serum total alkaline phosphatase, osteocalcin, fasting urinary calcium, hydroxyproline or deoxypyridinoline can predict 60–70% of the variability in bone loss (60,61). These studies also showed that the correlation between baseline markers of bone turnover and the subsequent rate of postmenopausal bone loss is possibly consistent over a period of at least twelve years (55,61). Less optimistic estimates were reported by other groups using different combinations of markers (30,62). For example, a study in elderly women demonstrated that urinary NTX, serum osteocalcin and serum parathyroid hormone together explained only 43% of the variability of bone loss at the hip (30). 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 (62-65). In a retrospective study of 354 women (mean observation period: 13 years), Ross and Knowlton (65) showed a continuous relationship between the measured levels of various bone turnover markers and the risk of rapid bone loss at the calcaneus: the odds of rapid bone loss (> 2.2%/year) doubled for each standard deviation increase in serum bone specific alkaline phosphatase, serum osteocalcin, urinary free pyridinoline or deoxypyridinoline (65). In a study of 227 early postmenopausal women treated with calcium alone or HRT plus calcium, Chesnut et al. (20) and Rosen et al. (16) reported that women with high baseline rates of bone resorption were at higher risk of losing bone than women with normal turnover rates (20) (figure 2). Different results were reported by Keen et al. (66), 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 due to the high degree of variability in urinary markers of bone turnover, predicting either bone density or changes therein for an individual patient
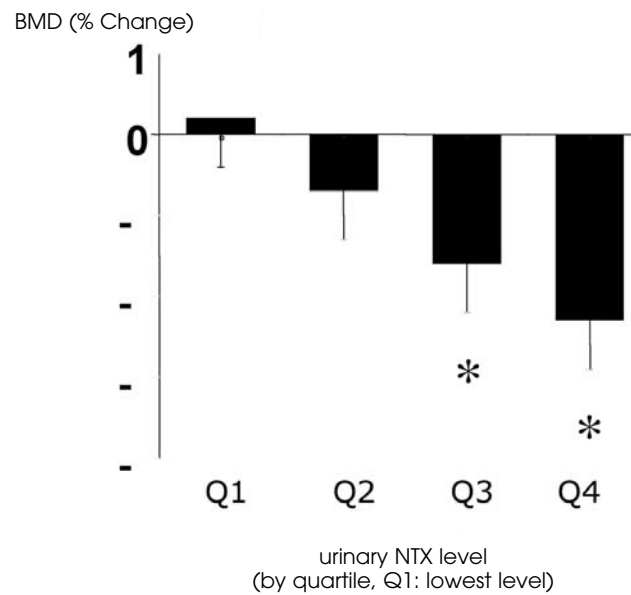
BMD (% Change)

urinary NTX level
(by quartile, Q1: lowest level)

Figure 2. Association between rates of bone resorption and bone loss in early postmenopausal women. High baseline rates of bone resorption (stratified by quartiles, Y-Axis) are associated with a higher risk of bone loss (% change in lumbar BMD after 1 year, X-Axis). 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 BAP or serum OC. (From: Rosen CJ et al. (16), with permission.)

from a single marker measurement may not be possible (62,67). Vestergaard and colleagues showed that serum OC, BSAP and hydroxyproline are poor predictors of lumbar and hip bone loss in individual perimenopausal women (68).

Taken together, there is evidence that rates of bone remodelling are associated with bone loss. However, the strength of this association seems to depend on a number of factors, such as menopausal age, skeletal site and gender. Bone remodelling markers are no substitute for individual bone mass measurements, or for a careful assessment of the patient's personal and family history.

Bone turnover and fracture risk

Bone turnover is an independent predictor of fracture risk. Earlier post-hoc analysis of data from clinical trials suggested that in untreated osteoporotic women, vertebral fracture rates increase as a direct function of either increased bone turnover or of decreased verte-

bral BMD (69). Thus, at a given level of vertebral BMD, the rate of vertebral fractures increases with the rate of bone turnover. When bone turnover is normal, however, the main determinant of vertebral fractures is vertebral BMD (69).

Using the large population-based sample of the Rotterdam study (7,983 individuals, 60% women aged 55 years and over), van Daele et al. (70) showed that women with increased urinary DPD levels had an increased risk of hip fracture. The relative risk per standard deviation increase in urinary DPD was 3.0 (95% CI 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% CI 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 lumber spine. Later analyses of the same study revealed that *low* serum osteocalcin concentrations were also associated with an increased risk of hip fracture (odds ratio: 3.1; 95% CI 1.0–9.2).

In a 5-year follow-up of the same population, Wheel et al. later showed that an increase in baseline urinary DPD above the pre-menopausal mean value was associated with an increased future risk of osteoporotic fractures (71). 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 premenopausal mean, independent of bone mineral density and disability. 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 (72). The relative fracture risks as defined by either BMD or marker measurements were similar (RR ~2) to those reported earlier by van Daele. Again, combined measurements of hip bone density and of bone resorption markers increased predictive power for hip fractures (RR 5–6). Thus, 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.

A nested case control study from the same group later suggested that levels of serum under-carboxylated osteocalcin (ucOC), but not of total osteocalcin, were predictive of future hip fractures (odds-ratio 2.0; 95% confidence interval: 1.2–3.2) (73). These data confirm and extend previous reports, which suggest that increased serum levels of ucOC are predictive of hip fractures in elderly, institutionalised women (74-76). These earlier results, however, may merely indicate an association between poor nutritional status and hip fracture risk among institutionalised 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 osteocalcin had been demonstrated earlier by Price et al. (77), and subsequent clinical studies showed that overt vitamin K deficiency may lead to a disproportionate increase in ucOC in the circulation (78,79). In addition, vitamin K_2 levels have been shown to be lower in women with osteoporotic fractures than in healthy individuals (78). Although measurement of ucOC may be useful in providing an integrated assessment of the factors that are responsible for the gamma-carboxylation of osteocalcin, such as vitamins K and D, the underlying biochemical mechanisms by which ucOC could be associated with impaired bone metabolism are, as yet, unknown.

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 (43). Thus, increased rates of bone resorption or decreased rates of bone formation seem to be associated with future osteoporotic fractures.

Recently, Meier and colleagues demonstrated in a case-cohort control study of 151 elderly men followed prospectively over 6.3 years that accelerated bone resorption was associated with increased risk of osteoporotic fracture, independent of BMD. Combining measurements of BMD and bone turnover improved fracture prediction in elderly men (80) (figure 3).

Prospective data from the Australian FREE study of 1,112 frail elderly men and women indicate that high bone turnover is also an independent predictor of all cause mortality. This association appeared to be mainly manifested in deaths from cardiovascular causes (33) (figure 4).

In summary, data from several independent and large prospective studies indicate that in both post-menopausal women and healthy men, increased rates of bone resorption are associated with an increased risk of vertebral and non-vertebral fractures, independent of BMD, age and disability. In the future, markers of bone turnover, in combination with other risk factors for osteoporotic fracture, may be used to define fracture risk and intervention thresholds.

Pre-treatment bone turnover and therapeutic effect

From both a theoretical and clinical point of view, it is conceivable that intervention strategies may differ between patients with accelerated, normal or even
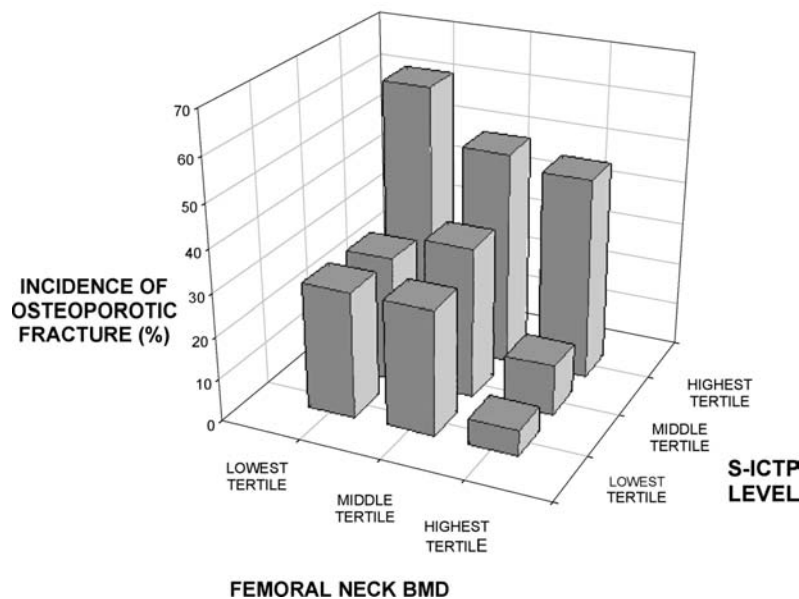
Figure 3. Fracture risk and bone turnover in men. Incidence of any osteoporotic fracture according to serum ICTP (S-ICTP) levels and femoral neck BMD. Case-cohort control study of 151 older men from the Dubbo Epidemiological Study (DOES) followed prospectively over 6.3 years. (From: Meier et al. 2004 (80), with permission.)
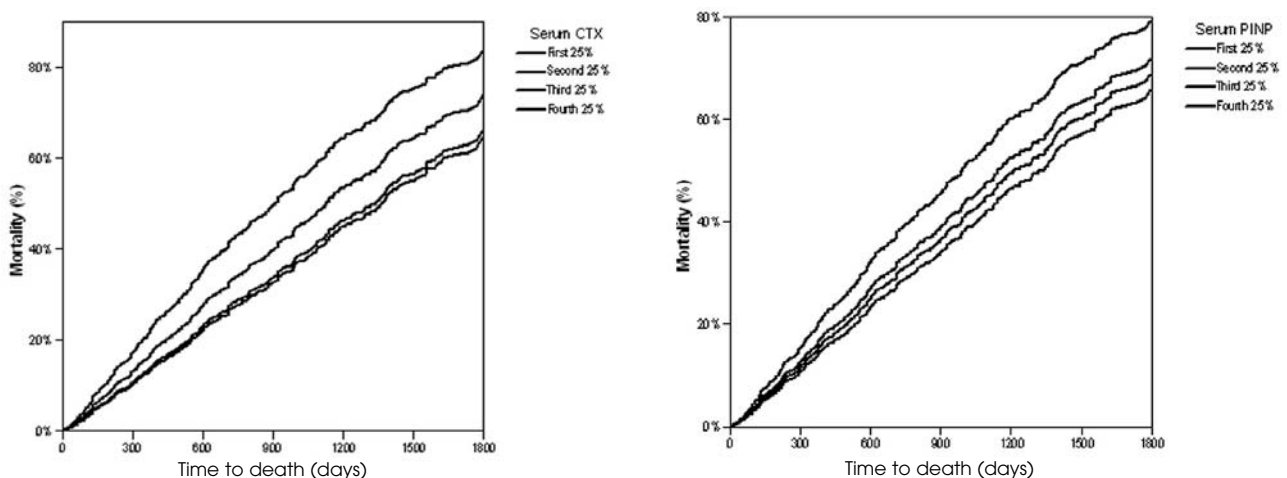

Figure 4. High bone turnover is associated with all-cause mortality in the frail elderly. In a prospective analysis of 1,112 frail persons (79% female, mean age 86 years) recruited into the Australian FREE-Study, high bone turnover was an independent predictor of all cause mortality. This association appeared to be mainly manifested in deaths from cardiovascular causes. Upper panel: Age- and sex-adjusted mortality curves by serum CTX-I, a bone resorption marker. Lower panel: Age- and sex-adjusted mortality curves by serum PINP, a bone formation marker. (From: Sambrook et al. 2006 (33), with permission.)

abnormally low bone turnover at the time of diagnosis; hence, 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. So, are pre-treatment bone marker measurements helpful in guiding the selection of therapy for individual patients? Some studies (81-83) have shown that in osteoporotic patients treated with sub-

cutaneous 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 (84), 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 (31).

Chesnut et al. (20) and Rosen et al. (16) 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 (16) (figure 2). This observation is in agreement with the hypothesis that the rate of bone turnover influences the likelihood of vertebral fractures only if accelerated (69). In contrast, Stevenson et al. (85) 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 (85).

Post-hoc analyses 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 (i.e. with normal or accelerated bone resorption) (86) (figure 5). 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. 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 point of view (86).

A similar post-hoc analysis of the Fracture Intervention Trial (FIT), examining the influence of pre-treatment bone turnover on the anti-fracture efficacy of daily alendronate in postmenopausal women found that the non-spine fracture efficacy of alendronate was significantly greater among both and osteoporotic and non-osteoporotic women with higher baseline levels of the bone formation marker PINP. However, no such association was observed for vertebral fractures, and changes in both serum bone alkaline phosphatase and CTX-I were not associated with fracture outcomes at any site (87) (figure 6).

Taken together, it remains unclear whether there is a clinically relevant relationship between bone turnover at baseline and the response to anti-resorptive treatment. Drugs even of the same class may differ in this respect.

Bone turnover markers and therapeutic monitoring

Bisphosphonates, raloxifene, denosumab, strontium ranelate, oestrogens, calcium, calcitonin and teriparatide all improve bone mineral density (BMD) to varying degrees. In contrast, the effects of these anti-osteoporotic drugs on bone turnover differ greatly: bisphosphonates (figure 7), oestrogens, denosumab (figure 8), calcitonin, raloxifene tend to reduce bone resorption and bone formation in a dose dependent manner (88-96). Strontium ranelate, in contrast, has only subtle effects on bone turnover, showing a slight reduc-
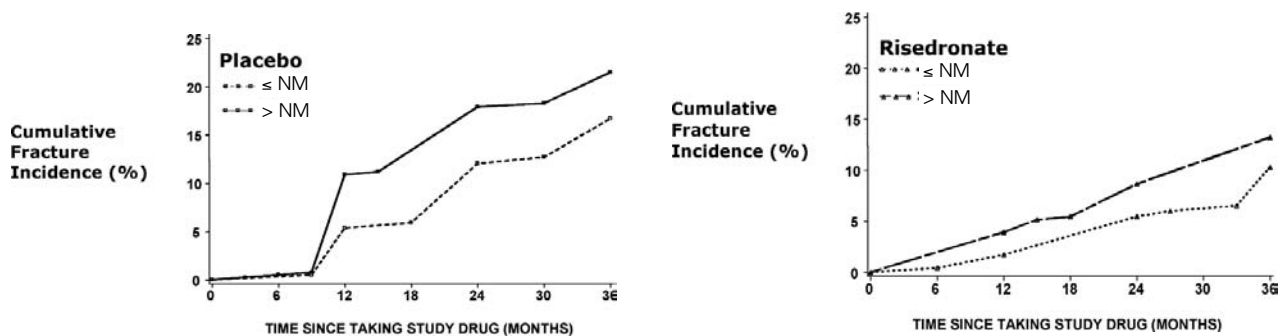

Figure 5. Pre-treatment bone turnover and change of non-vertebral fracture risk in response to risedronate treatment. Post-hoc analysis of a subset of the risedronate phase III clinical programs using urinary deoxypyridinoline as an index of pre-treatment bone resorption (PBR). A total of 1593 women with postmenopausal osteoporosis were pooled, in similar proportions, from the risedronate multinational and North American VERT, and from the risedronate HIP trials. Patients from treatment and placebo groups were stratified by the deoxypyridinoline premenopausal normative median. The figures show the cumulative incidence of new vertebral fractures by treatment group. The dotted lines represent patients with a PBR rate below the normative mean (< NM), while the solid lines represent those with a PBR above the normative mean (> NM). (From: Seibel et al. (86), with permission.)
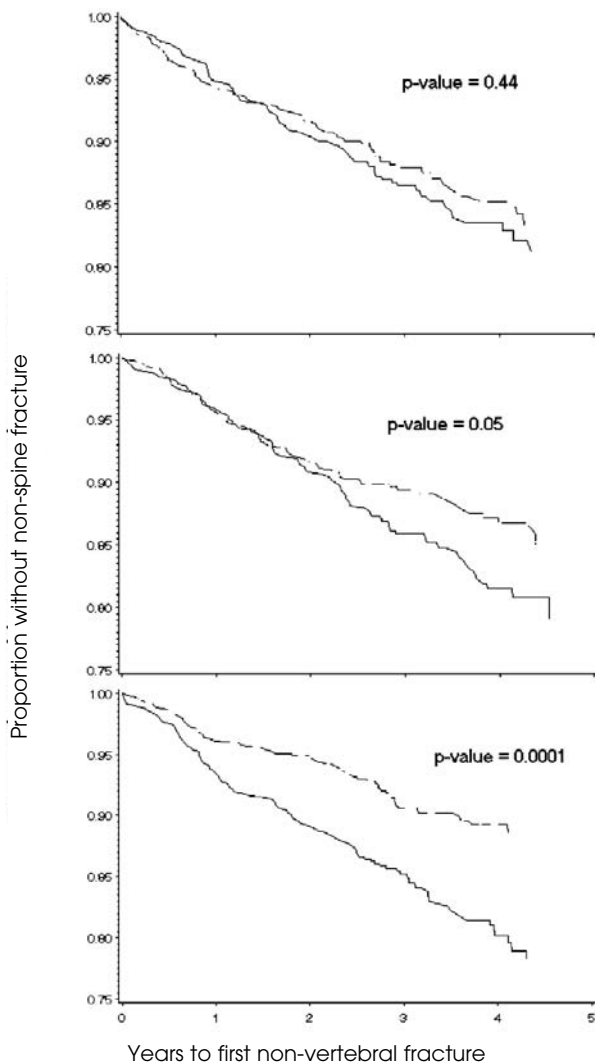
Figure 6. Pre-treatment bone turnover and change of non-vertebral fracture risk in response to alendronate treatment (Fracture Intervention Trial). Osteoporotic women treated with alendronate are represented by dotted lines, whereas the placebo group is denoted by solid lines. The analysis was performed by tertile of pre-treatment serum PINP levels. P values refer to comparisons between alendronate versus placebo groups within each tertile. No relationships were found for CTX-I as a bone resorption marker, or for vertebral fractures with any of the markers used in this study. (From: Bauer et al. (87), with permission.)

tion in bone resorption markers and a mild increase in bone formation markers (97) (figure 9). Finally, teriparatide strongly increases both bone formation and, after a certain gap period, bone resorption markers (98-102) (figure 10).

Most bisphosphonates, raloxifene, strontium ranelate, oestrogens and teriparatide have also been shown to reduce the risk of osteoporotic fractures.

However, the observed reduction in fracture risk is only partly explained by the documented changes in BMD (103-106), with the reduction in fracture risk being much greater than predicted from improvements in BMD only. Hence, it has been estimated that changes in BMD explain only 4% to 28% of the reduction in vertebral fracture risk attributed to antiresorptive treatments (107-109). It is therefore likely that changes in other determinants of bone strength, including the rate of bone turnover and its changes during antiresorptive therapy, may be better predictors anti-fracture efficacy. 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 (69), raloxifene (94,95,111), risedronate (112), alendronate (92) and ibandronate (110).

The relationship between 6 and 12 months changes in bone turnover markers and vertebral fracture risk after 3 years of raloxifene treatment in postmenopausal women clearly favours bone turnover markers as the better predictor of outcome (111). 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 3 years of 0.69 (CI 0.54–0.88; p= 0.003). Similarly, for a decrease of 5.91 μg/L in serum bone alkaline phosphatase the odds ratio was 0.75 (CI 0.62–0.92; p= 0.005). Importantly, these relationships remained after adjustment for baseline vertebral fracture status and BMD. Two subsequent analyses including postmenopausal women with osteoporosis from the same cohort (MORE trial) extended and confirmed these results showing that both, 1-year percentage changes in serum PINP (95) and OC (94) are able to predict the reduction in vertebral fracture risk after 3 years of treatment.

Two studies have investigated the change in bone turnover markers and fracture risk in bisphosphonate treated postmenopausal women (92,112). Post-hoc analyses of data from the VERT studies including postmenopausal women with at least one vertebral fracture demonstrated that reductions in urinary CTX-I (by 60%) and NTX-I (by 51%) at 3-6 months of risedronate treatment were significantly associated with the reduction in vertebral and non-vertebral fracture risk after 3 years (112). The change in bone resorption markers explained 50–60% of the risedronate-related fracture risk reduction for both, vertebral and non-vertebral fractures. Bauer et al. (92) reported that in alendronate-treated women, greater reductions in bone turnover were associated with fewer osteoporotic fractures. In their study, each SD
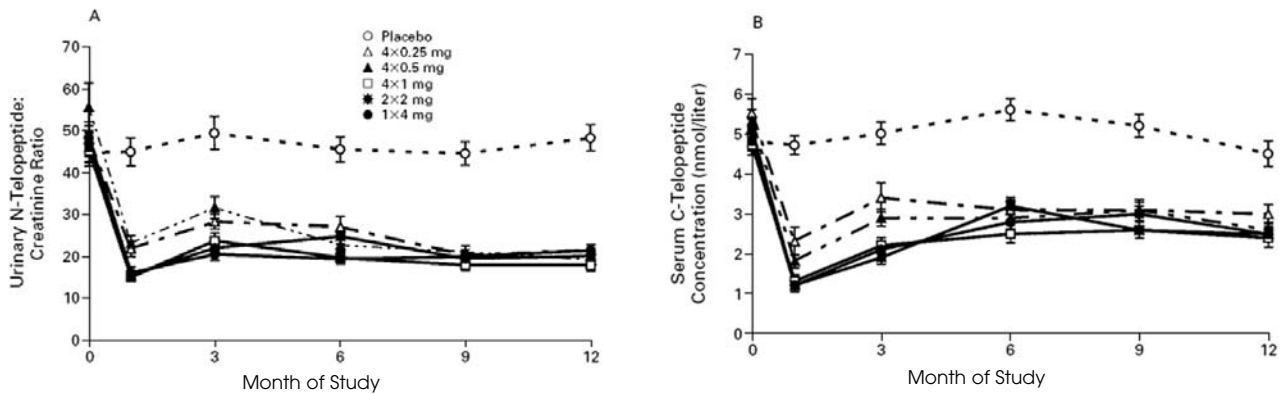
Figure 7. Change in markers of bone turnover following treatment with intravenous zoledronate. Patients were injected with varying doses of zoledronate as shown in the legend. Of note, a single dose of 4 mg zoledronate resulted in a suppression of bone turnover markers for up to 12 months, without any signs of recovery at that time point. Newer data suggest that this suppression may last up to 18 months. (From: Reid et al. (90), with permission.)
S-TAP: serum total alkaline phosphatase, U-DPD: urinary deoxypyridinoline, S-Ca: serum calcium.
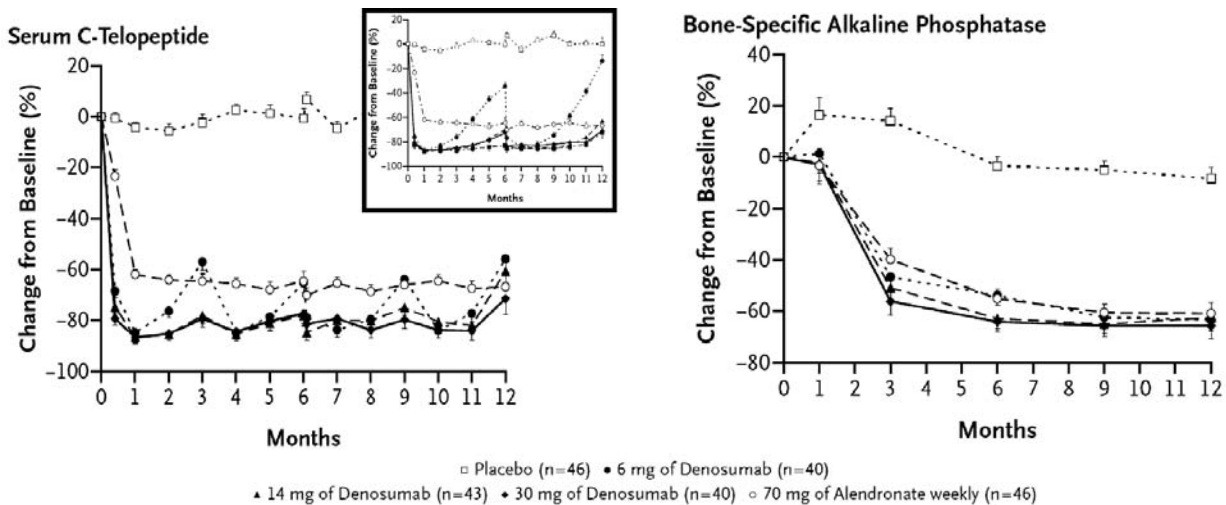p versus baseline: *< 0.05, *** < 0.001.

Figure 8. Change in markers of bone turnover following a 3-monthly dosing of sc. Denosumab. Patients received 3-monthly sc. injection of varying doses of denosumab (AMG 162), as shown in the legend. The large panels show the percentage change from baseline in serum levels of C-telopeptide (upper panel) and in bone-specific alkaline phosphatase (lower panel). The small insert shows the percentage change from baseline in serum levels of C-telopeptide (CTX-I) in patients treated with 6-monthly injections of denosumab. In the group of patients treated with 14 mg of denosumab, a pronounced rise in CTX-I levels can be seen before the next dose is administered. A similar, but much smaller rise in CTX-I levels can be seen with the 3-monthly 6 mg dose (large panel). The formation marker bone-specific alkaline phosphatase decreases with a delay, but exhibits no major dose-time dependent changes. (From: McClung et al. 2006 (88), with permission.)
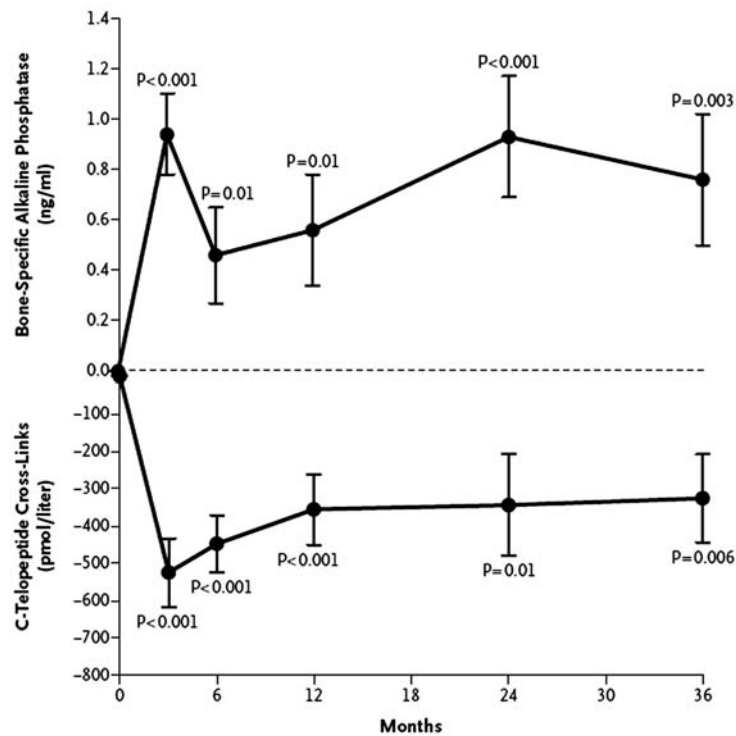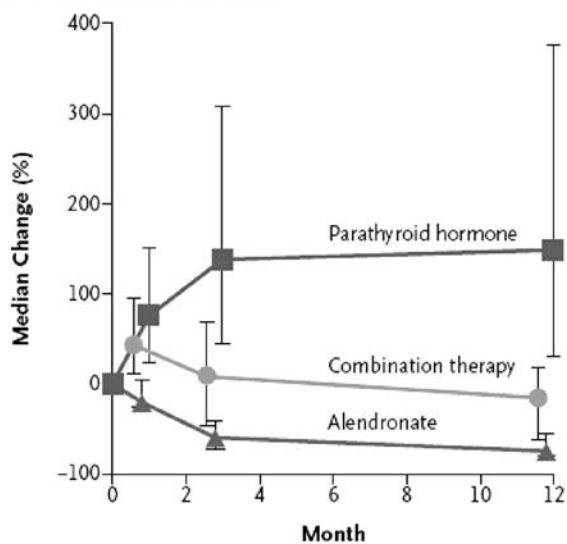
Figure 9. Change in markers of bone turnover during treatment with oral strontium ranelate. The graph shows the differences in biochemical markers between the two treatment groups over time. For each marker (upper panel: bone alkaline phosphatase; lower paner: CTX-I), and time point, mean (± SE) values of the placebo group were subtracted from the mean (± SE) values of the strontium ranelate group. Absolute changes in marker levels were small: max. change in bone alkaline phosphatase: +2.5 ng/mL in the treated group at 24 months; max. change in CTX-I: -400 pmol/L in the treated group at 6 months, and +600 pmol/L in the placebo group at 24 months. (From: Meunier et al. 2004 (97), with permission.)
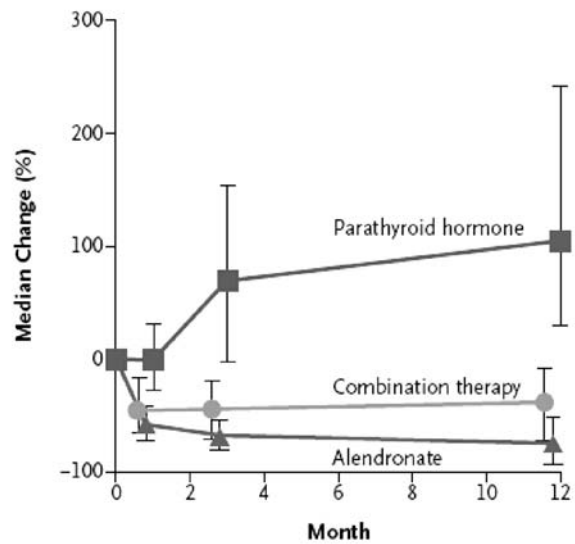
Figure 10. Change in markers of bone turnover during therapy with daily s.c. teriparatide and/or alendronate. Patients received teriparatide, alendronate or a combination of both agents. Teriparatide results in an increase of both bone formation and resorption markers, with an earlier response in bone formation markers. As expected, alendronate leads to a suppression of these indices. The net result of combined treatment with teriparatide and alendronate was a suppression of bone turnover. (From Black et al. 2004 (98), with permission.)

reduction in the change in serum BALP at 1 year was associated with fewer spine (odds ratio 0.74; CI: 0.63, 0.87), non-spine (relative hazard [RH] 0.89; CI: 0.78, 1.00) and hip fractures (RH 0.61; CI: 0.46, 0.78). Furthermore, alendronate-treated women with at least a 30% reduction in serum BALP had a lower risk of non-spine (RH 0.72; CI: 0.55, 0.92) and hip fractures (RH 0.26; CI: 0.08, 0.83) relative to those with reductions < 30%. Again, this effect was at least as strong as anti-fracture effect observed with 1-year change in BMD (92).

In summary, changes in bone turnover during raloxifene and bisphosphonate therapy seem to be related to subsequent fracture risk with a far greater effect on fracture reduction as what has been attributed to treatment-induced changes in BMD. These data suggest that biochemical markers of bone turnover 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 to a specific antiresorptive treatment. Whether changes in bone turnover during treatment with agents such as strontium ranelate, denosumab or teriparatide predict fracture outcomes is presently not clear.

Monitoring patient compliance using bone markers

Long-term compliance with treatment for osteoporosis is usually poor (113). Several studies reported that up to 50% of postmenopausal women were not adherent to their treatment after one to 5 years of HRT (114-118). A major cause of non-compliance were unwanted side-effects or fear of side-effects, inconvenience caused by medication, and high drug costs (119,120). Hence, monitoring patients on antiresorptive medication is an eminent part of patient management in order to improve adherence and persistence to therapy, and ultimately treatment effectiveness.

Biochemical markers of bone turnover have been advocated to facilitate follow-up of patients receiving antiresorptive treatments for osteoporosis. As bone turnover markers, in particular indices of bone resorption, decrease rapidly after initiation of treatment within 3–6 months, they might represent useful surrogate markers for monitoring patient compliance. Only few data, however, are available to support this theoretical approach. Using a decision analysis model, Chapurlat et al. (121) compared two strategies of follow-up: a) treatment of a woman without specific monitoring, and b) treatment of this woman with measurement of a serum marker of bone resorp-

tion after 3 months of treatment, with change of treatment if response to treatment as assessed by this marker was not satisfactory. It has been suggested that the approach of monitoring osteoporotic women with measurements of bone markers early during treatment course may increase effectiveness of treatment with greater quality adjusted life years than no follow-up. In another study of 75 postmenopausal women treated with raloxifene, Clowes et al. (122) examined whether monitoring (nurse-monitoring or marker-monitoring) enhances adherence and persistence with antiresorptive therapy, and whether presenting information on the biochemical response to therapy provided additional benefit. Survival analyses showed that in the group being monitored, cumulative adherence to therapy increased by 57% compared with no monitoring; also, there was a trend for the monitored group to persist with therapy for longer periods of time. However, presentation of results of effects on NTX-I levels did not improve compliance to therapy compared with nurse-monitoring alone. Nevertheless, results from the IMPACT study in postmenopausal women on risedronate have shown that a reinforcement message based on bone marker response influences persistence with long-term treatment. In patients in whom a verbal feedback on the change of urinary NTX-I was provided, one-year persistence was higher than in non-reinforced subjects. Interestingly, the message given to patients with a bone turnover marker response considered "good" was associated with significant improvement in persistence, whereas the information given to those with a poor resorption marker response led to a lower persistence (123). Another large study investigating patient compliance using measurements of urinary CTX-I is under way and should give further evidence whether monitoring osteoporosis treatment using bone turnover markers should be encouraged in clinical practice (124).

OTHER CONDITIONS

An abundance of experimental and clinical studies have demonstrated that markers of bone formation and resorption are useful 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 (57,125), hyperparathyroidism (126,127), Paget's disease (128), physical exercise (129), immobilisation (32,130), alcoholism (131), smoking (132), vitamin D deficiency (33,35,37,133), chronic inflammatory
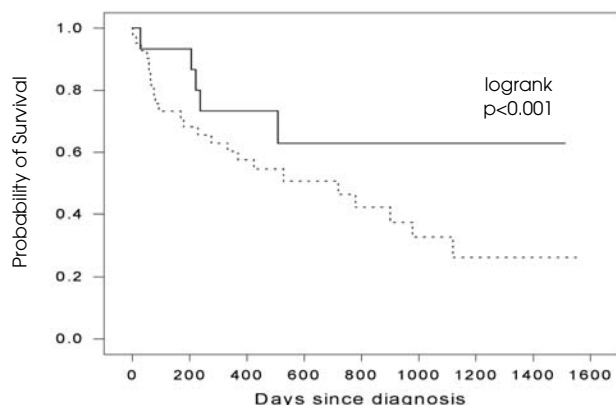
Figure 11. Probability of survival according to serum BSP values in patients with multiple myeloma. Patients were stratified by serum BSP concentrations at baseline (solid line: serum BSP < 21 ng/mL, dashed line: serum BSP > 21 ng/mL). In a multivariate multivariate Cox proportional analysis, serum BSP (p= 0.012) and monoclonal protein (p= 0.028) were the only predictors of survival in this patient group. From: Woitge et al. 2001 (145), with permission.)

bowel disease (134,135), chronic starvation (136), thyroid disorders (52,137) as well as the pharmacological effects of glucocorticosteroids (48,139,140), androgens (6,7,141), gonadotropin-releasing hormone agonists (142), warfarin (143), growth hormone or insulin-like growth factors (144). Bone turnover markers may be useful in the diagnosis and management of certain of the above conditions, but in most cases has not been rigorously examined.

The situation is somewhat different in cancer: as bone metastases profoundly perturb normal bone remodelling, biochemical markers of bone turnover have been shown to reflect these tumour-induced changes in bone remodelling and may therefore be useful in the diagnosis, follow-up and prognosis of patients with malignant (bone) disease (145,146) (figure 11). Most markers of bone turnover, particularly those of bone resorption, are elevated in patients with established bone metastases [recently reviewed in (147)]. While this may indicate a role of bone markers as diagnostic tools in cancer patients, available evidence does not provide any final conclusions as to the accuracy and validity of the presently used markers in the early diagnosis of bone metastases.

Markers of bone resorption respond promptly and profoundly to bisphosphonate and anti-neoplastic therapy, and this response appears to be associated with a favourable clinical outcome in patients with bone metastases (148-149). Recent evidence indicates that the aim of bisphosphonate therapy should be to normalize increased rates of bone remodelling (150,151). However, it remains unknown whether the use of bone markers in the routine clinical setting has any defined beneficial effects on overall outcome in cancer patients. In particular, no study has addressed the question whether patients with bone metastases should be treated according to their rate of bone turnover, and what the treatment goals are in this respect. While it is unlikely that bone turnover markers have sufficient diagnostic or prognostic value to be used in isolation, the combination of these markers with other diagnostic techniques may be the way forward to improve the clinical assessment of patients with bone seeking cancers.

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.

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