Role of biochemical markers in assessment of osteoporosis

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Background

Methods of evaluation in osteoporosis

Osteoporosis is one of the major problems facing the aging population. The two most important variables in evaluating patients with osteoporosis, either from the standpoint of research studies or in their clinical management, are the prevailing level of bone mineral density (BMD) and the level of bone turnover.

It has been possible to measure the BMD of sites of the skeleton subject to fracture for almost 20 years. However, in the past, bone turnover could be assessed only by combined calcium balance and isotope kinetic studies (which are time consuming and enormously expensive) or by tetracycline based histomorphometry (which is invasive, expensive, averages out changes in bone density over a period of 10 to 14 days, and measures bone formation much better than bone resorption). Thus, the more recent availability of biochemical markers for bone turnover represented a huge methodological advance. These measurements are non-invasive, relatively inexpensive, generally available, can measure changes in bone turnover over short intervals of time, can be assessed repetitively, and measure bone formation and bone resorption equally well. Moreover, measurements of bone densitometry and bone biochemical markers provide complimentary information in the assessment of osteoporosis.

Bone biochemical markers

A variety of biochemical markers are now available, either clinically or as research procedures, which can reliably measure the activity of bone formation and bone resorption (Table 1). The most useful of the current markers are serum osteocalcin and bone alkaline phosphatase for measurement of bone formation and the urinary pyridinium cross-links for measurement of bone resorption. These are discussed below. The reader is referred to more comprehensive reviews (Delmas 1991 and Delmas 1995) for information on the other bone markers.

For most disorders, including osteoporosis, bone formation can be adequately assessed by measurement of serum osteocalcin (OC) or the serum bone alkaline phosphatase isoenzyme (BAP). OC is a non-collagenous protein produced by osteoblasts and odontoblasts and is highly specific for bone. BAP is also synthesized by osteoblasts and plays a role in bone mineralization. Both can be measured conveniently and with great specificity by immunoassays. However, because bone formation is a multistep process, these measurements do not always provide identical information and can, indeed, at times give values that differ not only in magnitude, but also in direction (Duda et al. 1988).

Older, less sensitive, markers for bone resorption have been largely replaced by measurement of urinary pyridinium cross-links—pyridinoline (Pyd) and deoxypyridinoline (Dpd)—as discussed elsewhere in

Table 1. Listing of biochemical markers for bone turnover

Formation
Serum
Bone alkaline phosphatase*
Osteocalcin*
Type I procollagen (C-terminal)
Type I procollagen (N-terminal)
Resorption
Serum
Tartrate resistant acid phosphatase
ICTP
Urine
Hydroxyproline
Hydroxylysine glycosides
Deoxypyridinoline*
Pyridinoline*
*Most useful markers

this symposium. These are highly specific for bone resorption and are not affected by the diet. Originally, these measurements required time-consuming and expensive analysis employing high performance liquid chromatography (HPLC) although, more recently, ELISAs have been developed with specificity for the urinary free cross-links (Pyrilinks) (Seyedin et al. 1993), the urinary N-telopeptide to helix cross-links (Osteomark) (Hanson et al. 1992) the urine C-telopeptide-2 cross-link (Crosslaps), and the serum C-telopeptide-1 to helix cross-link (ICTP) (Risteli 1993). These assays have high sensitivity and specificity for bone resorption. However, as with the bone formation markers, there is a divergence of results among assays in certain situations. This suggests that they may be measuring different components of the resorbed collagen.

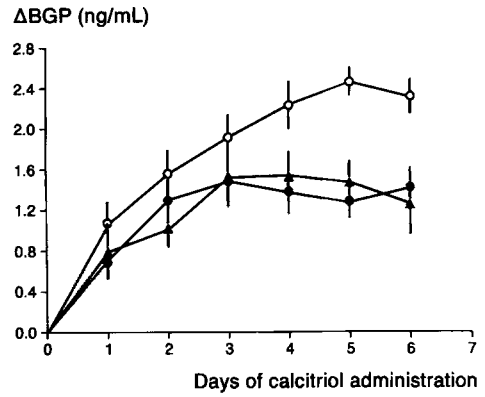

Figure 1. Incremental change in serum osteocalcin (also called BGP) (mean (SE) over baseline during 1,25(OH)2D administration in premenopausal normal women (solid triangles), in postmenopausal normal women (solid circles), and in postmenopausal osteoporotic women (open circles). From Duda et al. (1988).

Use in assessing pathophysiology of osteoporosis

Bone biochemical markers were initially used as research procedures and this still is their main use. Below we give examples of studies in which measurement of bone turnover has been critically important in developing new concepts about the characteristics of bone turnover in osteoporosis.

Postmenopausal (Type I) osteoporosis

This form of the disease is characterized by vertebral crush fractures and by Colles' fractures of the distal forearm (Riggs and Melton 1983). In the 1940s, postmenopausal osteoporosis was defined as a disease of decreased bone formation due to estrogen deficiency. This continued to be the prevailing belief until the 1960s when radiocalcium kinetic studies demonstrated that bone formation rates were normal in postmenopausal osteoporosis. However, subsequent bone biopsy studies using histomorphometry still suggested that bone formation usually was impaired (Parfitt et al. 1980).

Measurements using bone biochemical markers have resolved this issue decisively. First, using measurements of urinary pyridinium cross-links, Uebelhart et al. (1991) demonstrated in 60 perimenopausal women that bone resorption increases after menopause by up to 80%. In more elderly patients with postmenopausal osteoporosis and vertebral fractures, Delmas et al. (1983b) found small, but significant, increases in serum OC. Eastell et al. (1993) reported that women with postmenopausal osteoporosis had 40% higher levels of urinary Dpd than age-matched postmenopausal women ($P<0.001$) whereas serum OC was only 11% higher ($P<0.01$). Finally, Duda et al. (1987) used the novel approach of stimulating osteocalcin production in normal and osteoporotic women by short-term administration of 1,25-dihydroxyvitamin D, thereby assessing the reserve capacity of osteoblasts to produce OC. As shown in Figure 1, the osteoporotic women showed a significantly increased serum OC level during stimulation, suggesting an enhanced capacity for bone formation.

In the aggregate, these findings clearly show that bone turnover is increased by estrogen deficiency and that it is increased even more in osteoporotic women. Moreover, in postmenopausal osteoporosis, bone resorption is increased more than bone formation, and this uncoupling is responsible for their accelerated bone loss as compared with premenopausal or postmenopausal women.

Age-related (Type II) osteoporosis

This form of osteoporosis occurs throughout the aging population of men and women and is characterized by hip fracture and other related fractures (Riggs and Melton 1983). Until the advent of bone biochemical markers, it was felt that bone loss associated with aging was caused by a decrease in bone formation (Schenk and Merz 1969). However, Delmas et al. (1983a) and Duda et al. (1988) demonstrated highly significant increases of over 50% for serum OC and 77% for serum BAP (Figure 2). These increases correlated with and probably were related to the age-related increase in serum parathyroid hormone. Moreover, Eastell et al. (1992) has recently shown that urinary pyridinium cross-links almost doubled

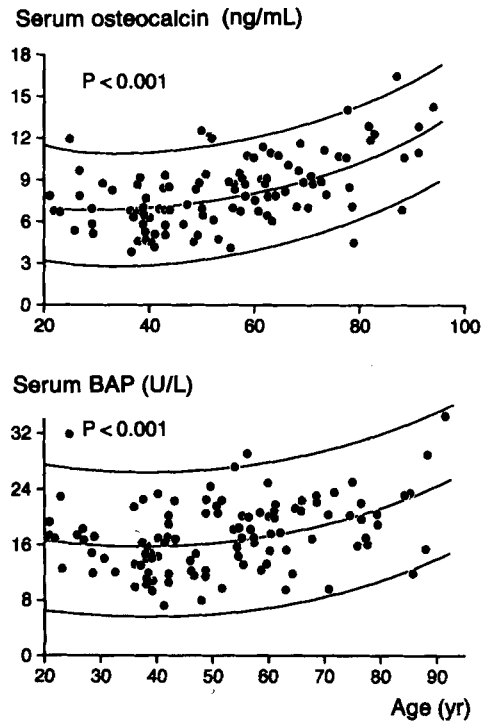

Figure 2. Increases in markers for bone formation with aging in an age-stratified sample of 109 normal women. From Duda et al. (1988).

between young adulthood and old age in women.

These findings indicate that the major cause of age-related bone loss is an increase in bone turnover, probably caused by secondary hyperparathyroidism resulting from age-related decreases in calcium absorption. This does not exclude the possibility of a decrease in osteoblast function at the cellular level as has been suggested by measurement of thickness of trabecular packets in bone biopsy samples (Lips et al. 1978). Such a defect would lead to an uncoupling of formation to resorption and, in the presence of increased bone turnover, would enhance bone loss.

Clinical utility

Biochemical markers hold great potential for the clinical management of individual osteoporotic patients, as discussed below.

Assessment of high turnover state

In assessing women with or at risk for osteoporosis, it is important to know whether bone turnover is increased for several reasons. First, high turnover is a good predictor for increased bone loss. Second, high turnover leads to perforation of trabecular plates and loss of structural elements and appears to be an independent predictor of fractures (Riggs 1993). Third, as discussed below, patients with high turnover are likely to respond best to antiresorptive therapy.

Table 2. Relative ability of various biochemical markers of bone turnover to discriminate between normal premenopausal women and late postmenopausal women. From Garnero et al. 1994

Excellent	Good	Poor
Serum	*Serum*	*Serum*
BAP	OC	Type I procollagen C-extension peptide
Urine	*Urine*	ICTP
HPLC D-Pyr	HPLC Pyr	
NTX		
F-Pyr		

It is not as yet clear which biochemical marker, or combination of markers, most efficiently predicts increased bone turnover. However, a large amount of new information was provided recently by the study of Garnero et al. (1994). These workers compared results of a panel of markers in 46 normal premenopausal women and in 85 women who were more than 5 years postmenopausal and had decreased values for BMD (Table 2). They found that all formation markers, except for serum COOH-terminal propeptide of type I procollagen, and all resorption markers, except for serum ICTP, were significantly increased above normal in the latepostmenopausal women.

Assessment of risk for osteoporosis

Because of the magnitude of the problem of osteoporosis, the only cost effective approach is prevention. Prospective studies have shown that for each standard deviation (~15%) decrease in BMD in postmenopausal women, there is a doubling of future fracture risk (Riggs and Melton in press). Similar studies are not available for bone biochemical markers. However, Hansen et al. (1991) reported that baseline measurements of the radius BMD plus several simple measurements including serum total alkaline phosphatase and urine calcium/creatinine ratio were highly predictive (r=0.9 with SEE of 9%) of the radius BMD 12 years later. Presumably, the newer and more discriminant biochemical markers would be even more effective in predicting future BMD. This is supported by the recent data of Melton et al. (in press) who demonstrated in a random sample of women from Rochester, Minnesota that lower values for BMD was associated with higher values of urinary pyridinium cross-links. What now is needed is a prospective

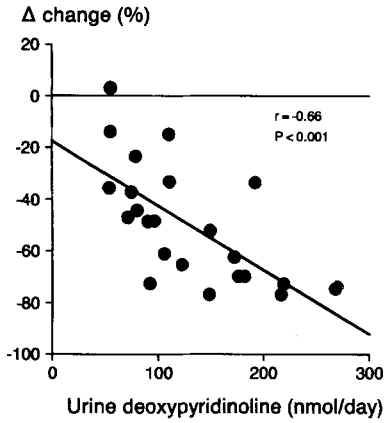

Figure 3. Effect of 12 months' treatment with transdermal estrogen on urinary excretion of deoxypyridinoline in 24 women with postmenopausal osteoporosis. Note that the decrease in values during treatment is highly correlated with the pretreatment value. From Lufkin et al. (1992).

study demonstrating that the ability of baseline measurements of BMD to predict future fractures can be enhanced measuring bone turnover using biochemical markers. It is highly likely that such an effect will be demonstrated.

Selection of patients for antiresorptive therapy

Patients with osteoporosis and low bone mass have a range of bone turnover levels. Civitelli et al. (1988) assessed bone turnover in postmenopausal osteoporotic women by skeletal isotope uptake prior to initiation of calcitonin therapy: they found that those women with high turnover showed a 22% increase in spinal BMD whereas those with normal or low turnover had no change. Similarly, Lufkin et al. (1992) found that the decrease in urinary pyridinium cross-links following estrogen replacement therapy correlated significantly with pretreatment levels (Figure 3). Thus, particularly in postmenopausal women with moderate decreases in BMD, a high value would be an indication to begin estrogen or bisphosphonate therapy whereas a normal level would support the use of calcium alone.

Monitoring therapeutic response

The clinician needs to know whether the patient is responding to therapy so that changes in dosage or even in the type of therapy can be made. This determination is best made by assessing the rate of bone loss with serial bone densitometry measurements. However, because the decreases in BMD in untreated patients with osteoporosis are relatively small (1–3% per year) compared with total BMD, an interval of up to two years is required to make this assessment. In contrast, a reduction in urinary pyridinium cross-links by up to 50% occurs with successful antiresorptive therapy so this determination can be made much earlier. However, the intraindividual variability of the resorption markers is relatively large. Blumsohn et al. (1994) reported that the critical difference, i.e. the degree that two measurements must differ to be significant, ranged from 20% to 50% for a variety of different assays for pyridinium cross-links. Thus, more than two measurements may be necessary to assess early treatment results.

Recent studies indicate that there may be differences in assay performance with the type of treatment. Garnero et al. (1994) evaluated the effect of treatment with the bisphosphonate, alendronate, on subsequent changes in BMD in 85 women. They found that results obtained with assays that measure the urinary total or the protein-related pyridinium cross-links decreased by 30% to 65% whereas those for the urinary free pyridinoline cross-links did not change significantly. In contrast, the decrease in values measured with assays for the urinary free pyridinium cross-links after estrogen replacement therapy in postmenopausal women was almost as great as those measured with assays for urinary total cross-links (Fledelius et al. 1994). The explanation for these differences at present is unclear and may be related to differences in the fractions of bone collagen resorbed with the two types of therapy. In any event, assays for free pyridinium cross-links should not be employed to monitor bisphosphonate therapy.

Conclusion

Assessment of bone turnover can now be made accurately and conveniently using bone biochemical markers. These have been extraordinarily useful for research studies and will become increasingly important in the near future in the clinical management of the individual patient with osteoporosis.

Acknowledgements

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