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REVIEW ARTICLE

Biochemical Markers of Bone Remodeling in Osteoporosis - Current Concepts

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ABSTRACT

Biochemical markers of bone remodeling have been developed over the past 20 years, which are more specific for bone tissue than the conventional ones. They have been widely used in clinical research and in the clinical trials of new therapies as secondary endpoints of treatment efficacy. Most of the interest has been devoted to their use in postmenopausal osteoporosis, a condition which is characterized by the subtle modification of bone metabolism that cannot readily be detected by conventional markers of bone turnover. Biochemical markers that reflect remodeling and can be measured in blood or urine include resorption markers (eg: pyridinoline, deoxypyridinoline, collagen cross links) and formation markers (eg: alkaline phosphatase, osteocalcin).

The new bone remodeling markers have been found to be more sensitive in

1) Monitoring bone loss
2) To see the antiresorptive treatment efficacy
3) To predict fracture risk.

Key Words: Biochemical markers of bone remodeling, N-telopeptide, Osteoporosis, Osteocalcin, Pyridinoline.

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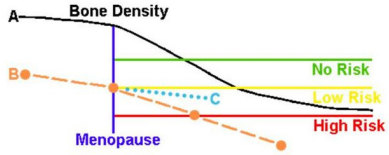
Introduction

Bones are in a dynamic metabolic state throughout life. They are continuously resorbed and formed in a finely regulated process known as remodeling. Through childhood and early adulthood, their formation exceeds resorption so that bone density increases and then plateaus until the age of 30-40 years. After that, resorption exceeds formation and bone density decreases through the rest of life, which in turn may lead to osteoporosis [1]. Osteoporosis is a condition which is characterized by enhanced bone fragility, leading to pain, increase in fracture risk and skeletal deformity as the skeleton is unable to sustain ordinary stress. It has been estimated that about 30% of elderly women and 20% of elderly men have osteoporosis. This silently progressing metabolic bone disease is widely prevalent in India and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women [2]. In India, about 60 million adults have osteoporosis and approximately 2-3 million cases are being added annually [3]. Hip fractures occur at a relatively earlier age in Indians as compared to western people. It is possible that a dietary deficiency of calcium, beginning early in life, leads to a low peak bone mass and consequently leads to osteoporosis at an earlier age. Malabsorption of calcium due to the deficiency of vitamin D also contributes towards osteoporosis [2].

The process of bone remodeling from resorption to matrix synthesis to mineralization normally takes about 8 months--a slow but constant process. Bones in older persons just aren't as efficient as bones in younger persons at maintaining themselves, since there is decreased activity of osteoblasts and the decreased production of growth factors and bone matrix. [Table/Fig 1] illustrates changes in bone density with aging in women.



A-Normal bone density before Menopause. B-Low bone density before Menopause. C-Slowed bone loss following intervention.

(Table/Fig 1) Changes in Bone mineral density with aging in women [4].

The normal curve (A) steepens following menopause, but even by old age, the risk for fracture is still low. A woman who begins with diminished bone density (B) even before menopause is at great risk, particularly with a more accelerated rate of bone loss. Interventions such as postmenopausal oestrogen (with progesterone) therapy, the use of drugs such as the non hormonal compound Alendronate that diminishes osteoclast activity and the use of diet and exercise regimens can help to slow bone loss (C), but will not stop bone loss completely or restore prior bone density [4].

Osteoporosis can be classified as primary or secondary. Primary osteoporosis is simply the form which is seen in older persons and women who are past menopause, in which bone loss is accelerated over that predicted for age and sex. Secondary osteoporosis results from a variety of identifiable conditions that may include metabolic bone disease such as hyperparathyroidism, neoplasia as with multiple myeloma or metastatic carcinoma, malnutrition, drug therapy as with corticosteroids, prolonged immobilization and weightlessness with space travel.

Aetiology: The risk factors for osteoporosis include: Female sex, age > 70 years, Caucasian or Asian race, early onset of menopause, longer postmenopausal interval, inactivity, especially lack of weight bearing exercise. Modifiable risk factors that may potentiate osteoporosis include: Smoking, alcohol abuse, excessive caffeine consumption, lack of dietary calcium and lack of sunlight exposure (to generate endogenous vitamin D) [4].

Diagnosis: The diagnosis of osteoporosis is made by three methods:

1. Bone mineral density measurement by Dual energy X-ray absorptiometry (DXA)
2. Laboratory biochemical markers
3. Bone biopsy with pathological assessment

Of these three, the best method is radiographical bone mineral density measurement (BMD). A variety of techniques are available including single-photon absorptiometry, dual-photon absorptiometry, quantitative computed tomography, dual energy x-ray absorptiometry and ultrasonography. Most often, site specific measurements are performed. The most common sites which are analyzed are those with the greatest risk for fracture: hip, wrist and vertebrae [4].

Clinical Value Of Biochemical Markers

Metabolites of bone remodeling in serum and urine can serve as markers for monitoring bone loss, bone reformation and the effectiveness of therapy in patients with osteoporosis. More than 50 yrs ago, Fuller Albright, the father of metabolic bone diseases, noted that postmenopausal women were losing excessive amounts of calcium in their urine. He correctly deduced that the resulting negative calcium balance led to osteoporotic fractures. He is credited with introducing the use of biochemical markers into the clinical arena[5]. Bone turnover markers now appear promising for defining the skeletal status of postmenopausal women. Bone diseases like osteoporosis have a high prevalence in adults; so the clinical challenge is to identify individual patients with high turnover

and to monitor interventions to slow bone loss and prevent complications. This will eventually help in improving the quality of life.

Three current indications for using bone markers in clinical practice are:

1) To monitor bone loss in the premenopausal or postmenopausal period
2) To monitor skeletal response to treatment.
3) To predict fracture risk [5].

Classification and Nomenclature of bone turnover markers

Bone turnover markers are broadly classified as markers of bone formation and markers of bone resorption. [Table/Fig 2] Classification of bone turnover markers [6].

(Table/Fig 2) Classification of bone turnover markers [6]

Markers of bone formation	Abbreviation	Sample used for analysis
1) Total alkaline phosphatase	Total ALP	S
2) Bone- specific alkaline phosphatase	Bone-ALP	S
3) Osteocalcin	OC	S
4) Undercarboxylated osteocalcin	ucOC	S
5) Procollagen-I extension peptides		
i) N- Propeptide	PINP	S
ii) C- Propeptide	PICP	S
Markers of bone resorption	**Abbreviation**	**Sample used for analysis**
1) Hydroxyproline	Hyp	U
2) Hydroxylysine	Hyl	U
3) Galactosyl hydroxylysine	Gal-Hyl	U
4) Glucosyl-Galactosyl hydroxylsine	Glc-Gal-Hyl	U
5) Pyridinoline	PYD	U
(Total, Free, peptide-bound)		
	DPD	U
6) Deoxypyridinoline (free,total)		
7) Type I collagen telopeptides	NTX	S/U
i) N-telopetide		
(N-terminal cross linking telopeptide)	CTX	S/U
ii) C – telopeptide		
(C- terminal cross linking telopeptide)	CTX-MMP	U
iii) C – telopeptide generated by		
matrix metallo proteinases	BSP	S
8) Bone Sialoprotein	ACP	S
	TRACP	S
9) Acid Phophatase		
10) Tartarate resistant acid phosphatase		
(5b isoform Osteoclasts)		

Deabbreviations: S-Serum, U-Urine

Bone – specific proteins (eg, osteocalcin, bone specific alkaline phosphatase, procollagen – I extension peptide) are synthesized by mature osteoblasts and find their way from the skeleton into the circulation. These peptides can be measured by sensitive radioimmunoassay or by enzyme linked immunosorbent assays. Similarly, osteoclasts induce bone degradation, releasing skeletal specific matrix products into the interstitium. These products enter the circulation and often clear the kidney without being metabolised. These fragments include collagen cross-links, which are small amino acids that bridge collagen fibrils and add support to the tertiary collagen structure. Cross-links are added during the final stages of collagen synthesis and are the first segments to be hydrolysed by proteases during bone resorption.

Depending on the exact site of cleavage, the cross-links found in urine include free pyridinoline and deoxypyridinoline, N-telopeptides and C-telopeptides. Cross-links can also be found in low concentration in the serum, and several assays have recently been developed to measure serum N-telopeptides and C-telopeptides [5].

Measuring Bone Formation

1) Alkaline phosphatase (ALP) is a ubiquitous enzyme that plays an important role in osteoid formation and mineralization. Bone specific ALP can distinguish the osteoporotic ones from normal postmenopausal women. This is measured by heat denaturation, electrophoresis, lectin precipitation, selective inhibition and more recently, immunoassays [6].

2) Osteocalcin (bone Gla-protein/OC) is a small (49 amino acids) hydroxyapatite-binding protein synthesized by osteoblasts, odontoblasts and to a lesser extent by hypertrophic chondrocytes. It is a vitamin K dependent protein. It contains three gamma-carboxyglutamic acid (Gla) residues which are responsible for the calcium binding properties of the protein. OC is involved in bone remodeling via a negative feedback mechanism. It is predominantly synthesized by the osteoblasts and is incorporated into the extracellular matrix of bone. Serum OC is considered to be a specific marker of osteoblast function, as its levels correlate with bone formation rates. However, this peptide is rapidly degraded in serum and both intact peptides and OC fragments of various sizes coexist in the circulation. Assays that measure both the intact molecule and the large N-mid fragment of OC (1-43) appear to be more stable and reproducible [6]. Measurement of decarboxylated osteocalcin levels have been shown to be a good predictor of

hip fracture in elderly women [7]. Serial measurements of OC have been shown to be an excellent marker to assess the long term effects of antiresorptive therapy [1].

3) Procollagen I Extension Peptides (PICP) (PINP): Collagen is synthesized as procollagen containing peptide extensions in both C and N terminal ends. These are cleared from the rest of the molecule before its incorporation into the collagen fibrils. Byproducts of type 1 collagen synthesis are the amino- and carboxy-terminal procollagen 1 extension peptides (PINP and PICP). PINP is an elongated protein of 35 kDa. PICP is a globular protein of 1000 kDa and contains disulfide bonds [8]. Procollagen peptides are produced in equimolar ratios to collagen and are then released into the circulation [1]. Both propeptides may be measured by specific polyclonal based immunoassays [6]. A cut off level of >45.0µg/L for PINP, as measured by Orion diagnostica RIA assay had a diagnostic sensitivity of 83% and a specificity of 64% for identifying women with decreased BMD, with an overall diagnostic efficiency of 73% [9].

Measuring Bone Resorption

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

1) Hydroxyproline**:** Hydroxyproline is mainly found in collagens, comprising about 13% of the amino acid content of these proteins. About 90% of the hydroxyproline released by the breakdown of collagen in the tissues, especially during bone resorption, is degraded to the free amino acid form that readily passes through the glomerulus. It is eventually completely oxidized and catabolised in the liver to form urea and carbon dioxide. The remaining 10% is released as small poly peptide chains that are excreted in urine without any further metabolism. Since half of the human collagen resides in bone, excretion of hydroxyproline in urine is regarded as a marker of bone resorption [10],[11].

Urinary hydroxyproline(OHPr) is thus considered as an index of bone resorption and a major determinant of bone status. Monitoring bone status through the urinary excretion of OHPr could serve as a surveillance measure in the early intervention against excessive bone loss. There is therefore, the need to establish normal acceptable ranges for the urinary excretion of OHPr in various communities beyond which individuals will be at a risk of excessive bone loss and may consequently be predisposed to fractures [12].

Hydroxylysine glycosides are integral parts of bone collagen and occur in two forms:- Glycosyl –galactosyl-hydroxylysine (Glc-Gal-Hyl) and galactosyl-hydroxylysine (Gal-hyl). Both components are released into the circulation during collagen degradation and may be measured in urine by HPLC[6]. Gal-hyl appears to be specific for bone collagen degradation. It is a more sensitive marker than hydroxyproline, since it is not metabolised further, not reutilised by the body nor is affected by diet[13]. It's major disadvantage is the lack of immunoassay format [6].

2) Pyridinoline And Deoxypyridinoline: The hydroxypyridinium cross links of collagen, pyridinoline (PYD) and deoxypyridinoline(DPD) are formed during the extracellular maturation of fibrillar collagens and are released upon the degradation of mature collagens [6]. Post translational modification of lysine and hydroxylysine produces the nonreducible pyridinium cross-links. Both PYD and DPD are released from bones in a ratio of approximately 3:1. DPD is relatively specific for bone. PYD is also found in articular cartilage and in soft tissues. Approximately 60% of the cross links released during resorption are bound to protein, with the remaining 40% being free. PYD and DPD can be measured in urine by HPLC or chemiluminescence immunoassay [8],[14]. Total, free or protein bound PYD and DPD can be measured as bone resorption markers. Urinary PYD and DPD levels increase to 50%-100% with menopause and return to premenopausal levels with hormone replacement therapy (HRT)[13].

3) Cross-Linked Telopeptides**:** In the process of bone resorption, the amino- and carboxy-terminal fragments of collagen are released, with cross-links attached. These fragments with attached cross-links are called telopeptides. N-telopeptides (NTX) and C-telopeptides (CTX/crosslaps) are excreted in urine [8]. Since more than 90% of the organic matrix of bone consists of type I collagen, measuring its degradation products in urine makes crosslaps a potential specific marker of bone resorption [1]. Several studies have shown that NTXs and CTXs are reportedly specific and sensitive for the resorption of bone tissues, because nonskeletal type I collagen- containing tissues are not actively degraded by osteoclasts and therefore, different fragments are formed in the degradation of other tissues which are comprised of type I collagen [10],[15]. The overall diagnostic efficiency of a single NTX measurement for identifying women with low BMD was 89% [9].

A pronounced and significant increase (47-142%) in crosslaps at menopause indicates that it is a very sensitive marker of metabolic bone changes taking place at menopause. Crosslaps has a specificity of 80% and a sensitivity of more than 70%. It can thus be used as a potentially useful screening parameter in the risk assessment of postmenopausal osteoporosis and Paget's disease. Crosslaps values decreases substantially in response to replacement therapies, thus suggesting its usefulness in monitoring treatment efficacy [1]. CTX and NTX can be measured by specific immunoassays [15],[16].

4) Bone Sialoprotein (BSP): It accounts for 5-10% of the non-collagenous matrix of bone. The protein has been shown to be a major synthetic product of active osteoblasts and odontoblasts. BSP may play an important role in cell-matrix adhesion processes and in the supramolecular organization of the extracellular matrix of mineralized tissues. It is measured by immunoassay [6].

5) Tartrate-Resistant Acid Phosphatase: (TRACP) exists in two sub-iso forms named 5a and 5b, of which only TRACP-5b has been shown to be characteristic for osteoclasts [6].

6) Vitamin D**:** It plays a critical role in the maintenance of strong bones and teeth and in promoting calcium uptake fromm diet. Vitamin D deficiency is an unrecognized epidemic in the middle aged and the old population. The risk factors that put people at vitamin D related health problems are - post menopause, improper diet, lack of sun exposure, high cholesterol, advancing age, high blood pressure, smoking, diabetes and corticosteroid drug use.

Vitamin D insufficiency is associated with secondary hyperparathyroidism, which is further amplified by inadequate calcium intake. Serum 25 hydroxy vitamin D [25(OH)D] is the most reliable indicator of vitamin D levels of an individual. When there are low 25(OH)D levels, the effective calcium absorption from the gut is reduced. This leads to increased mobilisation of minerals and matrix from bone and hence, increased risk of fracture, especially in postmenopausal women and elderly patients [15] . Given the many variables that can affect serum 25(OH)D levels and the positive outcome effects of treatment, it is reasonable to conclude that physicians should evaluate 25(OH)D levels for patients at risk for osteoporosis and hypovitaminosis [1].

Several prospective studies have noted that turnover markers in the elderly increase during the winter months. This increase which isattributed to a decline in serum levels of 25(OH)D caused by reduced exposure to sunlight, leads to secondary hyperparathyroidism, which then causes an increase in bone turnover [5].

One of the studies suggests that bone resorption markers are more efficient than bone formation markers in the diagnosis of postmenopausal osteoporosis. Urinary DPD/creatinine ratio has the highest diagnostic value [18].

Monitoring Bone Loss

In general, women lose about 1% of their spinal

bone density per year during and after menopause. However, nearly 35% of women lose bone at a faster rate during the late perimenopausal period. Biochemical markers can detect women who are considered "rapid losers" (i.e., those who lose 3% to 5% of bone per year) [5] .

Biochemical markers provide a more representative index of the overall skeletal bone loss than would be obtained by measuring the rates of changes in bone mineral density (BMD) at specific skeletal sites containing different ratios of cancellous to cortical component with different metabolic rates [6]. Measurements of specific biochemical markers of bone turnover (urinary N-telopeptide crosslinks, free pyridinoline, total deoxypyridinoline, hydroxyproline, serum osteocalcin, bone-specific ALP) are correlated with longitudinal bone loss in elderly women (>65 yrs). These markers may help identify women who are at a greatest risk for bone loss, who would benefit most from therapeutic Interventions [19]**.** The results of several studies of bone loss at the forearm, support the view that 80% of patients having increased biochemical markers in the early postmenopausal years are confirmed 2-12 years later as 'fast bone losers', based on BMD measurements [6]. For the screening of bone turnover in women at menopause and for the assessment of the levels of bone turnover in elderly women with vertebral osteoporosis, serum osteocalcin and urinary PYD and DPD appear to be the markers which are used so far [20],[21].

Monitoring The Effects Of Therapy:

Follow-up bone mineral density measurements (BMD) using T score (T Score < -2.5 indicates osteoporosis) by Dual-energy X-ray absorptiometry is the "gold standard" for assessing the effects of treatment on bone mass. Treatment related changes in BMD occur very slowly. This fact, coupled with the precision of BMD technologies, suggests that BMD cannot be reliable until at least two years. In contrast, changes in bone turnover markers can be anticipated after 3 months of therapy. Therefore, bone turnover markers may be assessed at diagnosis to provide a baseline, followed by repeat assay at 3 months to determine the response to therapy[5],[6].

Since the current therapy for osteoporosis centers on inhibiting bone turnover with agents such as calcium, calcitonin, oestrogens or bisphosphonates, the measurement of baseline and follow-up biochemical markers of turnover could be particularly useful.

Review of studies in healthy, early postmenopausal women showed that the percent change in N-telopeptides from the baseline to 6 months, was the strongest predictor of subsequent spinal bone mineral density. Similarly, bone turnover marker declined by 50% to 100% in postmenopausal women who were treated with HRT for at least 6months.On treatment with bisphosphonate, urinary markers of resportion declined by at least 50% within 3 months of therapy [5]. Women on active treatment with alendronate / HRT/ combination therapy, with the greatest decrease in turnover markers (NTX, Bone ALP,OC) at 6 months, had the greatest increase in spine and hip bone density at 3 years [21]. Urinary NTX and serum OC provide the greatest sensitivity and specificity for change in bone density, with either calcium supplementation or hormone replacement therapy [23]. Greater short term changes in turnover with parathyroid hormone therapy (especially bone formation-PINP) are associated with greater 1-year increases in spine and hip BMD among postmenopausal osteoporotic women [24]. Bjarnason and Christiansen measured U-CTX and BMD levels after 3 years following HRT. The maximum decrease in CTX levels occurred at 6 months, which was significantly correlated to a 3 year bone mass response [25]. Treatment with nasal calcitonin also decreased markers. Even bone formation markers decreased after 3 months of treatment with bisphosphonates (alendronate sodium Fosamax) [6].

Hence, short term changes in biochemical markers of bone turnover have been suggested as predictors of long term response in bone mass during antiresorptive therapy. The bone markers predicted a change in spine BMD to be greater

than 0%, with a high positive predictive value and specificity [26].

[Table/Fig 3] Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive values (NPV) of bone turnover markers in monitoring the effects of therapy.

(Table/Fig 3) Sensitivity, Specificity, Positive predictive value (PPV) and Negative predictive values (NPV) of bone turnover markers in monitoring the effects of therapy.

			At these cutoff values		
	NPV	Cutoff value for best performance (Sensitivity + specificity) (Change from baseline	Sensitivity	Specificity	PPV
U-CTX	64 %	-29%	66%	80%	82 %
U-NTX	70 %	-45%	76%	75%	80 %
Total-OC(ELISA)	64 %	-13%	70%	71%	77 %
Total-OC (RIA)	71 %	-15%	83%	55%	71 %

Tabulated from Ravn P etal [26]
In the Danish cohort of the Early Postmenopausal Intervention Cohort (EPIC) Study (n = 1609) of oral alendronate (ALN) for prevention of postmenopausal osteoporosis, bone markers (urine C-telopeptides of type I collagen (uCTX), urine N-telopeptide cross-links of type I collagen (uNTX), serum total osteocalcin measured by ELISA [total OC (ELISA)], and serum total osteocalcin measured by RIA [total OC (RIA)]) were measured at 6-month intervals. ROC curves were used to analyse the ability of the bone markers to predict a change in spine BMD greater than 0%.

Predicting Fracture Risk

Bone resorption markers and fracture risk are consistent. Riis et al reported that within 3 years of menopause, women classified as 'fast bone losers' had a 2 fold higher risk of sustaining vertebral and peripheral fracture during a 15 year follow up than women who were classified as 'normal'or 'slow'losers [6].

Women with both a low BMD and a fast rate of bone loss after menopause had a higher risk of subsequently sustaining fracture, than women with only one of the two risk factors. Concordant results have been obtained in four prospective studies (EPIDOS, Rotterdam, OFELY and the Hawaii Osteoporosis Study), thus indicating that increased levels of bone resorption markers are associated with increased risk of hip, vertebral and non-hip and non-vertebral fracture over follow up periods ranging from 1.8 – 5years. Increased levels of bone resorption markers and ucOC have been shown to predict the risk of fracture independently of the level of BMD [6].

The practical outcome of such a strategy is that the number of women who need to be treated to avoid one hip fracture is significantly reduced, which could result in a more cost-effective approach of treatment strategy. In the OFELY study, those women with both low hip BMD and high S-CTX had a probability of fracture over 5 years of 55% i.e., higher than the probability of fracture associated with low BMD alone (39%) or with high CTX alone (25%) [27].Combination of bone mineral density (BMD) and bone turnover markers to predict the risk of fractures in postmenopousal women: the OFELY Study [27] [Table/Fig 4].

(Table/Fig 4) Combination of bone mineral density (BMD) and bone turnover markers to predict the risk of fractures in postmenopausal women: the OFELY study.

	Odds Ratio (95%CI)	Likelihood Ratio	Probability of fracture over 5 years
All women(N=435)			12.6%
Low femoral neck BMD (T Score < -2.5)	2.8(1.4-5.6)	2.80	39%
High S-CTX (T Score > 2)	2.1(1.2-3.8)	1.70	25%
High U-free DPD (T Score > 2)	1.8(1.0-3.4)	1.68	24%
Low BMD + high CTX	3.8(1.9-7.3)	3.70	54%
Low BMD+High free DPD	2.1(0.7-6.2)	3.04	45%

Limitations of Biochemical Markers**:** Biochemical markers of bone turnover can differ in response to specific osteoporosis therapies and may even vary in the same patient. For example, bisphosphonates increase the urinary excretion of peptide-bound collagen products without significantly changing free cross-link excretion. On the other hand, oestrogen suppresses both free and peptide bound cross links to the same degree. The inherent problem of the biological variation for markers like non uniform rates of bone turnover, time of the day and season, remains a major concern. In general, serum markers of bone formation vary by less than 10% within a given patient. However, measurements of urinary markers can differ by as much as 30% in one person, even on the same day. Changes due to seasonal effects, like greater bone resorption during winter than summer months, must be considered. However, most patients have a 50% or greater decline in bone resorption markers during treatment with calcitonin, oestrogen, or the bisphosphonates [5].

Recent Developments

Since 1994, much interest is shown in identifying the genes which are involved in the

regulation of bone mass- Vitamin D receptor (VDR) gene, parathyroid hormone and its receptor, oestrogen receptor gene and collagen type I receptor gene [1].

Conclusions

These current biochemical markers allow clinicians to evaluate the risk of bone loss and to provide insight into the response to therapy. As technology improves, tests for these markers will become more reliable and more widely available. Efforts to reduce costs will almost certainly enhance utilization. As the familiarity with the value of biochemical markers grows, the use of these tests will no doubt expand.

Recommendations for research [6]

1 Normal values should be established for all bone markers in large samples (150-200 women) of healthy premenopausal women who are 30-45 years old, with normal BMD at the spine and hip as measured by DXA. Potential differences in normal values across geographical areas and races should be searched. The vitamin D status [serum 25(OH)D levels] of the population should be studied and further remedial measures have to be undertaken.

2 Quality control programs of bone marker measurements should be established and widely implemented, as already done for other biological tests in clinical chemistry.

3 The association between bone markers and the probability of fractures should be explored in large clinical trials.

4 Cut off values should be established for defining responders and non-responders using the same therapeutic regimens.

5 The ability to monitor treatment with bone markers to improve compliance and treatment efficacy should be tested prospectively.

Clearly, the onus is now on the manufacturers of assays to optimize their in-house quality control and to demonstrate to the research and ultimately, the clinical community that they have reached acceptable standards with reagents and reagent sets in which the assay methods have been optimized. Clinical chemists will have to demonstrate that their application of these methods meets acceptable proficiency standards. While this is going on, the previously skeptical clinician will need to critically review the published research concerning what biochemical markers of bone turnover can and cannot do when measured with proper standardization, accuracy, and precision. We will all need to keep open minds as this quality improvement is progressing and will be in a position to put bone turnover markers into practice just as soon as this final crucial piece of the puzzle has been solved to everyone's satisfaction! [28].

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