# Markers of Bone Metabolism

A.N. Sontakke
Principal,
MIMER Medical College,
Talegaon Dabhade,
Pune 410507

ABSTRACT

Bone turnover markers are protein originating from activity of osteoblasts and osteoclasts or fragments released during the formation and degradation of Type I collagen. Biochemical markers of bone resorption and formation are being intensively studied. Measurement of several of there molecules in serum and urine provide a valuable indication of ongoing homeostatic bone processes. Markers currently used in clinical practices are serum CTx, BAP and PINP. These markers are dynamic and reflect the resorption and formation rates. Current evidence suggests that the markers may be useful in conditions such as osteoporosis for monitoring the response to antiresorptive therapy (ART). Effective ART is associated with a bone marker reduction of 20 to 80% of the basal levels. As opposed to this other diagnostic modalities like dual energy X-ray absorptiometry (DEXA) need 1 to 3 years for observing the response to ART. In conjuction with BMD the biochemical markers can identify individuals at risk of osteoporosis. The limitations of biochemical markers as diagnostic tools can be minimised by measurement of several indices at a time as well as serial measurement of these indices.

**Introduction**

The skeletal system is a dynamic organ, with bone having two independent roles: provision of support and maintenance of mineral homeostasis. Both functions are successfully achieved by continuous bone remodelling. Products of bone remodelling process are termed 'bone turnover markers'. They are proteins originating from activity of osteoblasts and osteoclasts or fragments released during the formation and degradation of type I collagen. Disturbance in the balance and nature of bone formation and resorption produce common bone diseases.

**Bone structure**

Bone consists of 3 major types of cells-osteoblasts, osteoclasts and osteocytes.

**1. Osteoblasts**

i.  Found along the surface of both cortical and trabecular bone and are specialised fibroblasts

ii. They secrete and calcify a specific bone matrix that consists of collagen and non-collagen proteins.

iii. They have receptors for a large number of hormones. Stimulation by PTH, calcitriol, growth hormone and estrogens causes osteoblasts to produce insulin like growth factor-I (IGF-I) which has a significant role in local bone regulation and modelling.

**2. Osteocytes**

i.  As osteoblasts become embedded in bone matrix, they differentiate into mature osteocytes.

ii. They synthesize small amounts of matrix continuously to maintain bone integrity and under exceptional circumstances are able to resorb bone (osteolysis).

iii. They also are a part of the system that senses mechanical force – in response they produce nitric oxide and prostaglandins that mediate bone's response to load.

**3. Osteoclasts**

i. Osteoclast formation requires interaction with cells of the osteoblastic lineage. They are related to monocyte/macrophage cells and macrophage colony stimulating factor(MCSF)

is required to initiate osteoclast differentiation. Many hormones and local factors also can act on osteoblasts to promote osteoclastogenesis. E.g. Calcitriol, PTH,TNF,$PGE_2$or inhibit

the same eg. IL4 and interferon.

ii. Osteoclasts resorb the bone by dissolving minerals and degrading the matrix with the help of hydrolases and proteases – TRAP 5b, Cathepsin K. The locally acidic pH of 4.5 decalcifies the tissues exposing the organic matrix to degradation.

Excessive osteoclasticresorption occurs in osteoporosis, Paget's disease, hyperparathyroidism and inflammatory bone loss. It is less in osteopetrosis. Only a small portion of bone is cellular.Calcified matrix predominates. This matrix is primarily composed of type I collagen fibres,a glycosaminoglycan – containing ground substance and non-collagenous proteins.

A. Type I collagen: It is the major collagen produced by osteoblasts.

B. Non collagen, calcium binding proteins are critical for regulating mineralisation and strengthening of the collagen backbone. These include -

i. Osteocalcin (OC) and matrix gla protein. These delay mineralisation and allow bone matrix to mature.

ii. Bone sialoprotein and osteoportin bind both calcium and collagen and may play a role in adherence of osteoclast to the bone surface.

iii. Recently two products have been identified that appear to be final common pathway in coordinating osteoblastic and osteoclastic activity. The first, receptor activator of nuclear factor – kB (RANK) ligand binds to a receptor on osteoclast progenitor cell and increases osteoclast differentiation and activity. The second, osteoprotegerin (OPG), serves as a decoy receptor of RANK ligand. When OPG binds to RANK ligand, the osteoclast stimulation activity is prevented. The relative ratio of the two molecules determines the bone turn over.

A. Spindle shaped hydroxyapatite crystals are present in the ground substance and are aligned on and within the collagen fibres.

Collagen is deposited in the lamellar fashion and is strengthened by multiple crosslinks, both within andbetween the triple helical collagen molecules. These crosslinks are'pyridinolines'.They are resistant to degradation and are released during bone resorption as free or peptide bound forms that can be measured in serum and urine. Glycosaminoglycans play a major role in calcification and fixation of hydroxyapatite crystals to collagen fibres. Approximately one fourth of the amino

acids present in collagen consist of proline or hydroxyproline, neither of which is present to any great extent in other tissues. When collagen is metabolised, hydroxyproline containing oligopeptides are excreted in urine and the amount present correlates with the amount of bone turnover. The minerals of bone consist mostly of crystals of calcium and phosphate arranged amorphously or as hydroxyapatite. A wide range of other elements including sodium, magnesium, copper, zinc, lead and fluoride may be present.

**Bone mass**

About 45% of the adult skeleton is built and enlarged during adolescence. The concept of peak bone mass has become crucial to an understanding of osteoporosis especially postmenopausal osteoporosis. Peak bone mass is determined by several factors including genetics, nutrition, mechanics and environment. Genetic effect on adult female bone mass may be mediated largely through effects on bone formation rather than resorption. A strongpositive relationship exists between current and past calcium intake and peak bone mass achieved. Higher calcium intake during adolescence theoretically may optimize within genetic limits, peak bone mass. Physical activity, use of osteogenic oral contraceptives and dietary calcium intake exert a positive effect on bone mass in young adult women. Bone strength is determined by bone density and bone quality. Bone density is expressed as grams of mineral per area of volume. Bone Mineral Density(BMD) accounts for approximately 70% of bone strength. Bone quality refers to architecture, turnover, damage accumulation and mineralization.

**Bone function**

Under normal circumstances disturbances like increased resorption, osteoporosis & hypercalcemia are not seen because, in bone remodelling that occurs throughout the body, bone formation and bone resorption are "coupled" resulting in equal amount of bone formation and bone resorption. Most bone diseases result from alterations in coupling that are new or that occur secondary to hormonal imbalance.

**Bone changes**

Growth of the skeleton and changes in the bone shape are produced by modelling. Inherent to bone physiology is the physiological coupling of the process of bone formation and resorption called 'remodelling'. At any time approximately 10% of bone mass participates in bone remodelling. In infancy and adolescence there is predominance of bone formation and hence increase in bone mass. In young adults bone formation balances bone resorption. In old age bone

resorption is increased andpredisposes the older individuals to decreased bone mass. The fact that most of the skeleton consists of remodelled bone led to the concept of bone structural units (BSU) or remodelling units responsible for bone remodelling cycle.

**Bone remodelling cycle**

**i)** Activation: There is recruitment of osteoclast precursor cells which are then converted to osteoclast.

**ii)** Resorption: Mature osteoclast release proteolytic enzymes that digest collagen matrix. The length of this phase is regulated by apoptosis of osteoclasts.

**iii)** Reversal: Pro-osteoblasts are attracted from mesenchymal stem cells in the bone matrix.

**iv)** Formation: Mature osteoblasts synthesize the bone matrix, mainly typeIcollagen and regulate the mineralization of newly formed matrix.

**v)** Resting Phase: A phase of quiescence follows the formation of new bone and the cycle repeats.
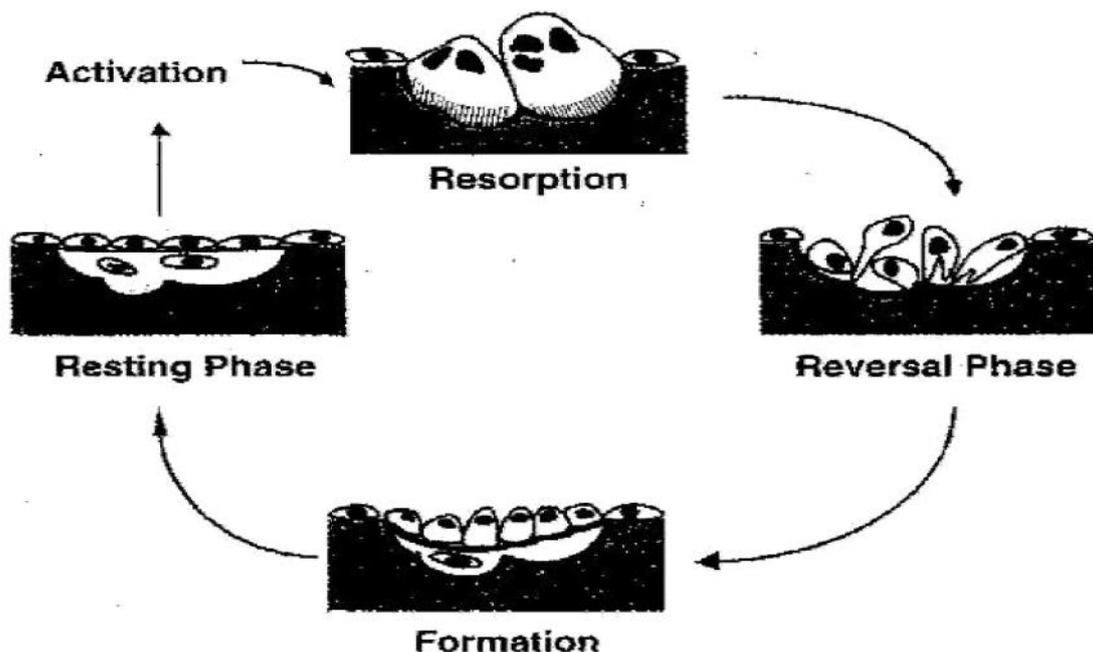


Bone Remodeling: The bone remodeling sequence includes activation, reversal, formation and resting phases.

In healthy adults, bone resorption and bone formation are tightly coupled, so the bone formation in new BSUs equals the amount of bone resorbed. Pathological remodelling can result in bone

loss in several ways:

i)      Excessive resorption depth.

ii)     Bone thinning.

iii)    Progressive decrease in wall thickness. i.e. Ageing, Glucocorticoid therapy.

**Biochemical Markers of Bone Turnover**

Bone turnover markers are a 'plethora' of proteins originating from activity of osteoblasts and osteoclasts or fragments released during the formation and degradation of Type I collagen. Measurement of several of these molecules in serum and urine provide a valuable indication of ongoing homeostatic bone processes. An ageing population, overall, is accelerating the need for determining the bone markers for monitoring bone status.

In contrast to the focal assessment of disease by bone histology, regional assessment of bone mass or radiography, biochemical markers provide integral assessment of the underlying pathology. Biochemical markers of bone resorption and formation are being intensively studied and clinical assays developed. Increased rates of bone loss are ordinarily accompanied by increase in both resorption and formation. Under physiological conditions a balanced interaction is maintained by processes of bone formation, resorption and mineralisation.

A)   **Bone Formation Markers –**

These are proteins produced by the osteoblasts responsiblefor formation of bone matrix, especially Type I collagen they are:

a)   Serum osteocalcin (OC)

b)    Serum alkaline phosphatase(ALP), bone specific ALP

c)    Serum procollagen I extension peptides PINP,PICP

a)   Serum osteocalcin (OC)–It is the most abundant non- collagenous protein in bone. It is exclusively synthesized by osteoblasts. Clinically, serum osteocalcin concentration is elevated in bone disease characterised by increased osteoblastic activity such as Paget's disease, renal osteodyprophy and osteitis fibrosa. It is a more sensitive index of post-menopausal increase in bone turnover then serum alkaline phosphatase (ALP) or Urinary hydroxyproline (UHP). Limitation of serum osteocalcin measurement is the instability of the compound after collection. It has half-life of 5 minutes.

b)   Serum Alkaline phosphatase (ALP) and Bone specific ALP(BAP)–In healthy individuals the serum total ALP is mainly contributed by bone & liver fractions. Far more common than decreased concentration are diseases associated with increased serum ALP concentration. Significantly high osteoblastic activity is seen in osteoblastic sarcoma, rickets, Paget's disease acromegaly, hyperparathyroidism, osteoporosis and osteomalacia. Increase is also seen in focal disturbances of bone metabolism most commonly due to fractures. Bone specific ALP can be used as a confirmatory marker in the above bone disorders. In addition to increased serum ALP, of current markers, total and bone ALP, provide the highest clinical sensitivity and specificity in diagnosis and monitoring of Paget's disease.

c)   Serum procollagen I extension peptides - Type I collagen is a part of bone matrix. It is formed into a triple helix inside the osteoblasts. Then the procollagen is secreted into the matrix, where enzymes cleave off the amino terminal peptide (PINP) and the carboxy terminal peptide (PICP) which can be measured in the blood.The concentrations of PINP, PICP in the circulation are thought to reflect the rate of bone formation. As type I collagen is also a major matrix of several other tissue, propeptides are not as sensitive and specific markers of bone formation as OC or BAP.

**B)   Bone Resorption Markers –**

These markers include:

a)Urine hydroxyproline (UHP)

b)   Urine pyridinoline (PYD), Urine deoxypyridinoline(DPD)
c)   Type I peptide collagen telopeptides-
i.    N terminal telopeptides to helix in urine and serum (NTx)
ii.   C terminal telopeptides- II in urine and serum (CTx)
d)   Serum tartarate resistant acid phosphatase, hydroxylysine and its glycosides

The bone resorption markers have been showed to correlate well with bone resorption rates in normal persons compared with patients suffering from osteoporosis, oestrogen treated postmenopausal osteoporosis, Paget's diseases and hyperparathyroidism. The higher the level of bone turn over, the more bone resorption increased compared to bone formation.

a) Urine Hydroxyproline (UHP) – Collagen which is present predominantly in bone and skin is the sole source of amino acid hydroxypoline. Its excretion rises in increased bone turn over. Determination of UHP, however, is a nonspecific test because sources of hydroxyproline included bone, diet, connective tissue, serum proteins and degradation of propetides from collagen biosynthesis.

b) Urine Pyridinoline (PYD) and Deoxypyridinoline (DPD) – These are small, cyclic amino structures linking peptide chains of collagen molecules. These are released upon degradation of mature collagen from skeletal tissue. Their

urinary concentration reflects the rate of collagen degradation. These are among the best available specific biomarkers for bone resorption. DPD is a sensitive and specific marker of bone resorption because:

i) It is formed during collagen maturation, not biosynthesis and originates only as a breakdown product of mature matrix.

ii) It is not metabolised before excretion in urine.

iii) Bone is the major sources of DPD.

iv) It does not appear to be absorbed from diet.

Increased levels of DPD and PYD are reported in post-menopausal women osteoporosis, Paget's diseases metastatic bone disease, primary and secondary hyperparathyroidism, and hyperthyroidism. Due to diurnal variation, early morning or 24hours sample of urine is recommended for estimation.

c) Type I Collagen Telopeptides(NTx&CTx) – These are molecules which are released from the proteins that link the collagen bundles in bone. These fragments are liberated into the circulation as a result of the breakdown of collagen with in bones and they are excreted unchanged in urine. Very high levels are seen in adolescents. Peak is seen at 14 years and then there is gradual decline to adult values. CTx, a C-Telopeptide is used for monitoring antiresorptive therapy (ART) in osteopenics. NTx, amino terminal fragment is used as a first baseline test to start ART and again 3-6 months later. Due to diurnal variation the telepeptides are best measured in early morning sample or 24hours urine sample. A reduction of 40% to 50% in these levels over a period of 8-12 weeks suggests a good response to therapy.

d) Serum Tartarate Resistant Acid Phosphatase 5(b) (TRAP 5b): During bone reporption, osteoclasts produce and secrete a glycoprotein i.e. TRAP 5b. This enzyme helps in process of bone resorption by chiselling the bone matrix. High levels of TRAP 5b are usually associated with active bone remodelling. Elevated serum TRAP 5b concentrations are seen in Paget's diseases, Haemodialysis, metastasis, multiple myeloma, post-menopausal women and hyperparathyroidism. Measurement of serum TRAP 5b as well as intact OC may be clinically relevant assays for estimation of bone metabolic status in haemodialysis patients. TRAP 5b is measured usually by immunological or RIA method.

### C) Mineral Status

a) Calcium & Phosphorus :

Almost all the body's calcium (99%) resides in the bone. The reminder resides in the body fluids and serves a crucial role in multitude of physiological process. In circulation, calcium exits in three forms: 45% of total serum calcium is the biologically active ionised or 'free' calcium, 45% is protein bound and 10% is complexedto anions (phosphates, lactate, and citrates). In the skeleton calcium is complexed with phosphorus to form calcium hydroxyapatite crystals. This compound has high tensile strength, suitable for weight bearing. Bone contains about 80 to 85% of total body phosphorus. Approximately 9% is in muscle and the remainder is present in viscera and ECF. The intracellular concentration of phosphorus (phosphates and organic phosphates) is greater than extracellular levels. Inorganic phosphate is required for energy metabolism, nucleic acid synthesis, bone mineralisation and cell signalling. The activity of cell surface sodium-phosphate co transporters, regulated by PTH & calcitriol mediates uptake of inorganic phosphorus from extra cellular environment.

b) Magnesium :

Most of the total body magnesium (50-60%) is concentrated in bone tissue as an integral component of the hydroxyapatite lattice (30-40%) and as and exchangeable fraction (15-20%) adsorbed to apatite and in equilibrium with ECF. Changes in total body magnesium are reflected largely by changes in skeletal magnesium and to lesser extent in serum magnesium concentration. All though acute lowering of serum magnesium appears to increase serum PTH concentration, chronic magnesium deficiency results in hypoparathyroidism and secondarily hypocalcaemia, because of the concomitant decrease in calcium release from bone. The concentration of calcium, phosphate, and magnesium in plasma are dependent on the net effect of bone mineral deposition and resorption, intestinal absorption, and renal excretion. PTH and 1, 25- dihydroxy vitamin D are principal hormones regulating these three processes. Significant research progress has increased our understanding of bone and mineral metabolism and the pathophysiology of associated disorders. At the same time, improvements in technology have allowed laboratories to expand their role from measuring total calcium, phosphate and magnesium to measuring other analytes such as 'free' calcium, intact PTH, vitamin D metabolites and calcitonin.

**D) Bone Turnover Markers in clinical practice –**

Current evidence suggests that the markers may be useful in some patients with conditions such as osteoporosis for monitoring the response to antiresorptive therapy. IV/Oral bisphosphonates lead to decrease in bone resorption markers within days and weeks.Their decrease is followed by a decline in bone formation markers. A decline up to 65% of the baseline bone turn over markers may be expected after potent antiresorptive therapy with bisphosphonatesor denosumab. Effective ART is one in which there is significant reduction in resorption markers with in few weeks, normally reaching plateau at 3 to 6 months. Bone formation markers respond more slowly and plateau at 6 to 12 months. Effective ART is associated with a bone marker reduction of 20 to 80% of the basal levels. As opposed to this, dual energy X-ray absorpsiometry (DEXA) needs 1 to 3 years for observing the response to ART.

### Applications of Biochemical Markers

1. Selection of patients for therapy.
2. Monitoring the effects of therapy – current use.
3.  Adjust dosage when appropriate.
4. Determine consequences of discontinuing therapy.
5. Prediction of bone loss by identifying 'fast and slow' losers of bone mass.

   Studies by Christiansen and his group in Denmark have suggested that a combination of markers of resorption and formation early in postmenopausal years can identify fast and slow losers of bone mass.
6. Diagnosis and monitoring of metastatic bone disease.
7. In conjunction with BMD the biochemical markers can identify individuals at risk of osteoporosis.

 Markers currently used in clinical practices are serum CTx, BAP and PINP. These markers are dynamic and reflect the resorption and formations rates. However other analytes such as total ALP, PTH and 25 hydroxyvitaminD should also be considered.

Techniques and reference intervals for the bone turnover markers of clinical use:

| Sr.No. | Marker | Method | Reference Interval | |
|---|---|---|---|---|
| | | | Premenopausal Female | Male |
| A | Bone formation Marker | | | |
| 1 | **Serum OC** ng/ml( g/L) | IRMA | 7.2 - 27.9 | 11.3 – 35.4 |
| 2a | **Serum Total ALP** U./L | Spectrophotometric | 42 - 98 | 92 – 128 |
| 2b | **BAP** ng/ml ( g/L) U/L | i)IRMA ii)immunoabsorption | 5 – 20 11.6 – 29.6 | 5 – 20 15 – 43.3 |
| 3a | **PINP** | Immunoassay | Not validated | |
| 3b | **PICP** | Immunoassay | Not validated | |
| B | Bone Resorption Markers | | | |
| | **Urinary** | | | |
| 1 | **UHP** mmol/molcreatinine | HPLC, photometric | 16 – 31 | 16 – 31 |
| 2 | **DPD** µmol/molcreatinine | ELISA, HPLC, ICMA, Fluorimetry | 3.0 – 7.4 | 2.3 – 5.4 |
| 3 | **PYD** | ELISA | Not validated | Not validated |
| 4 | **NTx** | ELISA, HPLC, | 5 - 65 | 3 - 63 |

| | | | | |
|---|---|---|---|---|
| | nmolBCE/mmolcreatinine<br><br>(BCE- BoneCollagne Equivalents) | Fluorimetry | | |
| 5 | **CTx**<br>mg/mol/creatinine | ELISA, HPLC,<br>Fluorimetry | 0 - 476 | 0 - 505 |

| | **Serum** | | | |
|---|---|---|---|---|
| 1 | **NTx**<br>nmolBCE/L | ELISA,ICMA | 6.2 - 19 | 5.4 – 24.2 |
| 2 | **CTx**<br>ng/L | ELISA electroche-<br>miluminiscence | < 574 | < 574 |
| 3 | **TRAP 5b**<br>U/L | Enzyme, inhibition,<br>immunoassay | 1.5 – 4.5 | 1.5 – 4.5 |

**Limitations for the use of biochemical markers as diagnostic tools**

1. Preanalytical and analytical variables decrease the usefulness of the bone marker assessments as their concentrations are affected by diurnal variations, age, sex, diet, exercise and methodology. The long term, within individual variability of urine markers is generally high (15 to 60%) than that of serum markers (5 to 10%).
2. Prediction of fracture risk is not very prospective though
    i)    Increased BAP and decreased OC concentrations are comparatively predictive.
    ii)   Increase in NTx and CTx suggest increased risk of fracture independent of BMD.
3. Lack of validation as screening tests in routine clinical practice.
4. Lack of standardisation of analytical methods and common reference intervals

especially in paediatric age group.

Measurement of several indices at a time, as well as serial measurements may help to overcome some of the limitations. Most recently, methods have been developed for measurement of osteoprotegerin and RANK ligand proteins synthesized by the osteoblasts. Additional studies are required to determine the roles of above proteins as markers of bone turnover

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