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REVIEW

# Towards optimising the provision of laboratory services for bone turnover markers

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**Summary**

Bone turnover markers (BTMs) are either secreted by osteoblasts during bone formation or released by degradation of the collagen matrix of bone during bone resorption, and may be measured in blood or urine to provide an estimate of the rate of bone remodelling. Increased bone remodelling rate is often associated with bone loss which can result in osteoporosis; however, lack of data preclude the inclusion of BTMs in fracture risk algorithms. The changes in BTMs following therapy for osteoporosis may be useful for monitoring. Serum procollagen type I amino-terminal propeptide (s-PINP) and serum carboxy-terminal cross-linking telopeptide of type I collagen (s-βCTX) have been designated as reference standard markers of bone formation and resorption respectively in osteoporosis; further research is needed for their routine use in osteoporosis. BTMs are useful in diagnosing and monitoring Paget's disease of bone and other bone diseases associated with abnormal bone formation and/or resorption. Standardised patient preparation is required to mitigate the effect of biological variation, and appropriate sample handling and storage are important to minimise sample degradation. Significant inter-method differences exist for BTMs, and harmonisation of methods for the reference BTMs is being pursued. This will help develop universally accepted decision limits and treatment goals. Australian consensus reference intervals have been developed for some methods for s-PINP and s-βCTX.

*Key words:* Amino-terminal cross-linking telopeptide of type I collagen, bone formation, bone remodelling, bone resorption, carboxy-terminal cross-linking telopeptide of type I collagen, deoxypyridinoline, osteocalcin, osteoporosis, Paget's disease of bone, procollagen type I amino-terminal propeptide, tartrate-resistant acid phosphatase.

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## NORMAL BONE PHYSIOLOGY AND BONE REMODELLING

Once growth is complete, the adult bone may stop growing, but remains a dynamic organ throughout life, continually renewed by the process of bone remodelling which occurs in discrete areas on bone surface. The functional anatomy of this process has been defined as the bone remodelling unit (BRU). BRUs provide a mechanism for repair of micro-damage and for adaptation to stresses, as well as for the maintenance of calcium homeostasis.[1] Cortical or compact bone makes up approximately 80% of the skeleton, and is found especially in the shafts of long bones. Trabecular or cancellous bone is more porous and makes up most of the vertebral bodies. Due to its relatively large surface area, trabecular bone accounts for the majority of bone remodelling activity. The bone remodelling cycle starts with attraction of osteoclast precursors to a particular bone surface area to be remodelled, where they fuse to form multinucleated osteoclasts, which resorb a pocket of bone. This is followed by the reversal phase where pre-osteoblasts migrate to the newly resorbed site and differentiate into osteoblasts and form new bone (osteoid, the amount of new bone being equal to that resorbed). Following mineralisation with calcium, phosphate and carbonate, arranged predominantly as hydroxyapatite crystals, the bone surface returns to its quiescent state. In the process, osteoblasts undergo transformation into osteocytes and become embedded in the osteoid or die by apoptosis.

Bone remodelling is influenced by mechanical strain, local and systemic hormones, growth factors and cytokines. The key molecules secreted by osteoblasts and stromal cells that regulate osteoclasts are macrophage colony stimulating factor (M-CSF), receptor activator of nuclear factor κB ligand [RANKL, tumour necrosis factor (TNF) ligand superfamily member 11], and its decoy receptor osteoprotegerin (OPG, TNF receptor superfamily member 11B).[2,3] RANKL interacts directly with its receptor RANK on the surface of osteoclasts and their precursors to activate them.[2,3] Similarly, osteoblast differentiation and activation is promoted by ligands of the Wnt signalling pathway, and inhibited by Wnt pathway inhibitors such as sclerostin.[4]

## COMMON SUBTYPES OF BONE DISEASE

Bone formation and resorption are 'coupled' and, in health, the net cumulative effect of all the bone remodelling cycles in the adult skeleton is the maintenance of bone volume. Disturbances of coupling where formation does not equal resorption can lead to net bone loss, and over time, osteoporosis. Conversely, excessive bone formation can lead to osteosclerosis, such as is seen in Paget's disease of bone,[5] or in relation to osteoblastic bony metastases from prostate or breast cancer. The majority of cancer-related bone diseases are osteolytic in nature and result in net loss of bone, leading to osteoporosis when this is generalised, or lytic lesions when the lesions are discrete.[6] Multiple myeloma is an example of malignant bone disease leading to both lytic lesions as well as generalised bone loss.[7] The generalised increase in bone

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resorption in multiple myeloma is not associated with an increase in bone formation; i.e., there is uncoupling of resorption and formation in this condition.

Osteoporosis is defined as a disease characterised by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk.[8] The World Health Organization (WHO) diagnostic criterion for osteoporosis is a bone mineral density (BMD) measurement equal to or more than 2.5 standard deviations (SD) below the young reference mean (T-score $\leq -2.5$ SD).[9] The majority of cases are found among post-menopausal women where oestrogen deficiency is implicated. Bone loss is also related to ageing and the prevalence increases with age in both women and men. In addition, osteoporosis may be associated with medications such as high dose glucocorticoids, and several diseases, such as rheumatoid arthritis, hypogonadism (in males), hyperparathyroidism and hyperthyroidism, conditions associated with malabsorption of calcium and/or vitamin D and malignant bone disease such as multiple myeloma. Paget's disease is a focal disorder of increased local bone remodelling which results in expansion and structural weakness of bone leading to pain, deformity and increased fracture risk, and is more common in older people.[5] The osteogenesis imperfectas are a group of inherited diseases characterised by defective connective tissue and decreased bone formation due to a deficiency of type-I collagen caused by mutations in the *COL1A1* and *COL1A2* genes and resulting in growth abnormalities and/or brittle bones.[10]

Severe vitamin D deficiency leads to lack of mineralisation of bone and in children causes rickets, characterised by soft bones and skeletal deformities.[11] The adult form of the disease is osteomalacia and is generally associated with serum 25-hydroxy-vitamin D $<12.5$ nmol/L.[12] Osteoid calcification is impaired and both bone resorption and formation are increased. Mild to moderate forms of vitamin D deficiency (serum 25-hydroxy-vitamin D levels of 25–50 nmol/L and 12.5–25 nmol/L respectively), which are much more common in developed countries, are associated with secondary hyperparathyroidism and increased bone loss, which can result in osteoporosis.[12] Primary hyperparathyroidism is also associated with osteoporosis due to increased bone resorption and consequent bone loss.[13]

Renal osteodystrophy, which is associated with chronic kidney disease, may be characterised by one or more of the following:[14]

1. Osteitis fibrosa cystica, in which bone turnover is increased due to secondary hyperparathyroidism.
2. Adynamic bone disease, in which bone turnover is low, mostly due to excessive suppression of parathyroid hormone secretion, although aluminium toxicity was implicated in the past.
3. Osteomalacia.
4. Mixed uraemic osteodystrophy, a mixture of both high and low bone turnover together with marrow fibrosis and increased unmineralised osteoid.

Further discussion of renal osteodystrophy is beyond the scope of this review.

## OVERVIEW OF BTMs AND THE THEORY AND EVIDENCE FOR THEIR CLINICAL UTILITY

BTMs are classified as bone formation markers or bone resorption markers. Their concentration in blood and in urine may reflect bone remodelling rate, although the accuracy with which they reflect bone turnover may be influenced by their tissue specificity for bone and a number of physiological and pathological factors, some of which may be disease-specific. All BTMs to some extent reflect both bone formation and resorption since bone formation and resorption are 'coupled' in most cases.

Bone formation markers are peptides or enzymes produced by osteoblasts during the different phases of bone formation. Osteoblasts synthesise type 1 procollagen molecules during the early stages of formation of bone matrix. The amino- and carboxy-terminal propeptides (PINP and PICP) of the procollagen molecules are cleaved off when collagen is laid down, and released into blood where their concentrations reflect bone formation rate.[15–18] Bone-specific alkaline phosphatase (BALP) is an enzyme produced by osteoblasts and its concentration in blood reflects osteoblast activity.[19,20] Osteocalcin is a non-collagenous bone matrix protein, the concentration of which in blood also reflects osteoblast activity.[21]

Bone resorption markers are degradation products of bone collagen such as the amino-terminal and carboxy-terminal cross-linking telopeptides of type I collagen (NTX and CTX) and deoxypyridinoline (DPD), or enzymes secreted by osteoclasts such as tartrate-resistant acid phosphatase (TRACP5b).[22–28] Whilst the former reflect type 1 collagen breakdown, mainly bone resorption rate, TRACP5b reflects osteoclast numbers.

Other molecules such as the inflammation marker highly sensitive C-reactive protein (hs-CRP) might be useful to predict risk of future fracture,[29] but are not considered markers of bone turnover, nor are they currently utilised in clinical practice, and will not be further discussed here.

## BTMs IN CURRENT USE

Although several BTMs have been studied for their utility in the investigation and management of bone diseases, the formation markers that are used in practice are s-PINP, s-BALP and osteocalcin. The resorption markers that are in current use are s-βCTX, urine NTX (u-NTX) and u-DPD. Currently available commercial immunoassays for s-BALP, s-PINP and osteocalcin as well as for s-βCTX, u-NTX and u-DPD are adequately bone-specific; however, their clinical utility in terms of diagnostic efficiency and utility for monitoring disease progress and response to treatment may vary between different bone diseases.

## DISEASE-SPECIFIC ISSUES FOR BTMs

BTMs have been studied in metabolic and metastatic bone diseases and their ability to reflect bone turnover or activity in different disease states has been empirically determined. Recommendations for their clinical use for diagnosis, prognostication, monitoring disease progression and response to treatment have been based on the findings of these studies.[30]

Osteoporosis is a silent disease, with fracture being the clinically important outcome. Whilst the diagnosis of osteoporosis is based on BMD measurement, the latter is just one of many risk factors for fracture; other important independent risk factors for fracture include age, sex, a history of prior fractures, family history of fracture, body mass index, height, ethnicity, smoking, alcohol use, glucocorticoid use, rheumatoid arthritis and other diseases which lead to bone loss as well as a propensity to fall. The contributions of these risk factors to fracture risk have been quantified and the absolute fracture