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Bone Turnover Markers for Diagnosis of postmenopausal osteoporosis – A review

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ABSTRACT

Postmenopausal osteoporosis is an age related skeletal metabolic disorder that affects most elderly women worldwide owing to the loss of estrogen activity, increase in bone turnover rate and circulating level of bone turnover markers. Although osteocalcin, bone specific alkaline phosphatase, and pro-collagen type I N- and C-terminal pro-peptides are considered as the most sensitive markers of bone formation however, they suffer some limitations. Consequently, periostin, sclerostin and dickkopf-1 protein (Dkk-1) are emerging diagnosis markers of bone pathologies and monitoring therapeutic interventions and management of postmenopausal osteoporosis. These markers serve as effective tools for diagnosis of bone disorders, predict reduction in fracture risk, reflect metabolic effect of drugs during bone remodeling and could be used to establish the lowest dose that elicit the required change in bone mineral density. Biological variability from different sources including controllable and uncontrollable factors could serve as the major limitations of these established markers of bone formation (BFMs). This review, therefore focus on the therapeutic applications of selected bone turnover markers for early diagnosis of postmenopausal osteoporosis.

Keywords: Postmenopausal osteoporosis, bone, turnover markers, therapeutic applications.

1.0 INTRODUCTION

Osteoporosis is a major health problem worldwide. It is a skeletal metabolic disorder characterized by low bone mineral density, progressive deterioration of bone tissue micro-structure leading to increased bone fragility and fracture (Rachner *et al.*, 2011). The loss of estrogen during menopause leads to increase in bone turnover rate (Mada *et al.*, 2018). The increased bone in turnover markers (BTMs) at menopause state is associated with an imbalance in bone resorption markers (BRM) and bone formation markers (BFM) in favor of bone resorption (Mada *et al.*, 2017; Delmas *et al.*, 2000). Bone turnover is the total volume of bone that is both resorbed and formed over a period of time. Bone turnover markers are bone tissue proteins or their fragments, or enzymes released from bone cells which are widely used for monitoring the

therapeutic effects of potential anti-resorptive or osteo-anabolic agents (Bhattacharyya, 2008). Markers of bone formation are either osteoblastic enzymes or by-products of active osteoblasts expressed during the various phases of bone development and are measured in serum or plasma. The most specific and sensitive established markers of bone formation includes bone specific alkaline phosphatase (BALP), osteocalcin (OC), and the pro-collagen type I N-terminal pro-peptide (PINP). These markers reflect the rate of synthesis of the main constituent of bone tissue and different aspects of osteoblast function. BALP is secreted by the outer surface of osteoblasts into the extracellular fluid during the maturation of the newly synthesized osteoid matrix. While osteocalcin is one of the most abundant non-collagenous proteins in the bone matrix synthesized by osteoblasts during bone

formation (Lee *et al.*, 2000). However a substantial proportion of osteocalcin finds its way into the extracellular compartment where it can be measured. PINP is a precursor of type I collagen, an important component of bone matrix secreted by osteoblasts during bone formation. An expert panel convened by the International Osteoporosis Foundation (IOF), the International Federation of Clinical Chemistry (IFCC), and Laboratory Medicine recommended the systematic use of serum PINP as a reference marker of bone formation in clinical studies (Vasikaran *et al.*, 2011). The most studied of the emerging markers of bone formation is periostin which is expressed mainly by periosteal osteoblasts and osteocytes (Merle *et al.*, 2014). Sclerostin is also a novel marker found to serve as an important regulator of osteoblastic bone formation (Sharifi *et al.*, 2015). It is a glycoprotein mainly secreted by osteocytes. Dickkopf-1 protein (DKK-1) is an endogenous soluble inhibitor of Wnt signaling pathway. Both sclerostin and DKK-1 are detected in serum and plasma and are found to act through inhibition of the Wnt signaling pathway (Baron *et al.*, 2013). Strontium ranelate slightly increases bone-ALP

2.0 CONVENTIONAL BONE FORMATION MARKERS

Changes in biochemical markers of bone are usually not disease specific but reflect alterations in skeletal metabolism independent of the underlying cause. Bone formation markers (BFMs) are direct or indirect products of active osteoblasts (bone forming cells) expressed during various phases of their development. These markers reflect the rate of synthesis of the main constituent of bone tissue and different aspects of osteoblast function. The most specific and sensitive markers of bone formation are; serum bone-alkaline phosphatase (BALP), serum total

2.1 Alkaline Phosphatase

Alkaline phosphatase (ALP) is a ubiquitous enzyme serving as one of the major regulators of bone matrix mineralization (Reddi *et al.*, 2016). During maturation of newly synthesized osteoid followed by mineralization, ALP is secreted by the outer surface of osteoblasts into the extracellular fluid and can be measured in serum. The total

level and slightly lowers serum CTX-I in the first week of treatment (Meunier *et al.*, 2004). It has been shown that humanized sclerostin monoclonal antibody induces a rapid and dose-dependent increase in serum levels of bone formation markers followed by a rapid, but transient, decrease in serum CTX-I in healthy subjects (Padhi *et al.*, 2011). Potent bone formation-stimulating drugs i.e. teriparatide (recombinant human PTH (1–34) or PTH (1–84) increase bone formation rapidly (Glover *et al.*, 2009). Although the established markers of bone formation can be synthesized in other connective tissues and are unable to be distinguished between the metabolic activities of different skeletal compartments, diseases and treatments may have differential effects on them. Therefore biological variability arising from a number of sources affects their overall pre-analytical variability hence, the development of new bone formation markers that reflect the remodeling of a particular bone compartment are therefore been suggested. This review therefore focus on the therapeutic applications of selected bone turnover markers for early diagnosis of postmenopausal osteoporosis.

osteocalcin (OC), and the pro-collagen type I N-terminal pro-peptide (PINP). Serum levels of these bone formation markers reflect the intensity of bone formation and are correlated positively with histo-morphometric parameters of bone formation. The independent contribution of different risk factors for fracture has been quantified (Kanis *et al.*, 2008), permitting the calculation of absolute risk with the FRAX® tool. Since the availability of FRAX, treatment decisions in the management of osteoporosis are increasingly being based on the assessment of a patient's probability of sustaining bone fragility and fracture (Berry *et al.*, 2010, Lippuner *et al.*, 2010).

ALP serum pool consists of several dimeric isoforms originating from various tissues such as liver, bone, intestine, spleen, kidney, and placenta. A healthy adults with normal liver function derive approximately 50% of its total serum ALP activity from the liver where as 50% arises from the bone (Delmas *et al.*, 2000). Many techniques have been developed to differentiate between the two main isoforms of circulating ALP. These include; heat

denaturation, electrophoresis, precipitation, selective inhibition, and more recently immunoassays which allow the quantitation of either enzyme mass or activity. This enzyme catalyzes the cleavage of organic phosphates and

pyrophosphate (inhibitor of mineralization) hence, provides inorganic orthophosphate which serve as a substrate for the synthesis of hydroxyapatite (Orimo, 2010).

2.2 Osteocalcin

Osteocalcin (OC), also known as bone gamma-carboxyglutamic acid-containing protein, is a small protein composed of 49 amino acids and synthesized by mature osteoblasts, odontoblasts and hypertrophic chondrocytes (Kuo & Chen, 2017). It is the most abundant non-collagenous protein in bone comprised about 2% of total protein in the human body. OC play key role in metabolic regulation, bone mineralization and calcium ion homeostasis (Lee *et al.*, 2007). The level of OC in the serum is highly correlated with

increased BMD during treatment with osteo-anabolic drugs against osteoporosis. Serum OC has been considered a specific biomarker of osteoblast function for evaluation of bone formation rate in osteoporosis. In most studies, osteocalcin has been demonstrated as an important biomarker for investigating the efficiency of the drug on bone formation. Serum OC level might be a useful biomarker for the assessment of osteoporosis and prediction of fracture risk in elderly persons especially women.

2.3 Pro-collagen type I N- and C-pro-peptides (PINP and PICP)

During bone formation, osteoblasts secretes the precursor of Type I collagen, pro-collagen molecules. Type I collagen is an important component of bone matrix and it was discovered that bone has a faster metabolism than any other type I collagen-containing tissues. The extension peptides at each end of the pro-collagen molecule; pro-collagen type I N-pro-peptide (PINP) and pro-collagen type I C-pro-peptide (PICP) are cleaved by enzymes during bone matrix formation and released into the circulation in a stoichiometric fashion. These pro-peptides are considered

quantitative measures of the newly formed type-I collagen and may be assayed by specific, polyclonal based immuno-assays. An expert panel convened by the International Osteoporosis Foundation (IOF), the International Federation of Clinical Chemistry (IFCC), and Laboratory Medicine has performed a comprehensive review of the available data and has recommended the systematic use of serum PINP as a reference marker of bone formation in clinical studies. Based on this assessment, PINP is to be measured by standardized assays in intervention and observational studies in order to assess their clinical performance (Vasikaran *et al.*, 2011).

3.0 EMERGING MARKERS OF BONE FORMATION

The development of new BFM's that reflect the remodeling of a particular bone compartment has been suggested and novel markers of bone formation has enriched the spectrum of analytes

used in the assessment of bone pathologies and monitoring of therapeutic interventions critical for the management of postmenopausal osteoporosis.

3.1 Periostin

The most studied among the novel markers of bone metabolism is periostin (POSTN) which is expressed by collagen-rich connective tissues such

as heart valves, aorta, periodontal ligament (Merle and Garner, 2012), and mainly by periosteal osteoblasts and osteocytes, although osteoclasts may also express low level (Merle *et al.*, 2014). At the protein level it comprises the signal sequence,

a cysteine-rich Emilin (EMI) domain, 4 repeated and conserved Fasciclin-1 (FAS-1) domains, and a C-terminal hydrophilic and a variable domain. At least five different human isoforms are generated through alternative splicing of the C-terminal domain, gamma-carboxylation and dimerization (Merle & Garnero, 2012). Periostin contain binding sites for extra cellular matrix proteins, such as fibronectin, type I and type V collagens, and cell surface receptor integrin through its four central fasciclin1 (FAS-1) domains and N-terminal EMI-domain hence promote and serve as a key regulator of collagen cross-linking through proteolytic activation of lysyl oxidase. This enzyme activation is as a result of its interactions with bone morphogenetic protein-1 (Maruhashi *et al.*, 2010). Periostin deficient mice were found to develop periodontitis and osteoporosis with lower BMD, altered cancellous and cortical bone microarchitecture, decreased bone strength and showed disorganized collagen matrix without

significant differences in osteoclast and osteoblast number per bone surface (Bonnet *et al.*, 2009). The relative risk for fragility fracture among women in the highest quartile of periostin concentrations independent of age, BMD and prevalent fracture was 1.88 (1.1-3.2), while in a French cohort of post-menopausal women the relative risk was 7.2 for women with both low hip BMD (t-score < -2.5) and high periostin (highest quartile). (Rousseau *et al.*, 2014). A possible explanation for these counterintuitive results is the positive effects of periostin on bone formation and bone strength. Periostin may therefore serve as a candidate marker of periosteal metabolism (Garnero, 2017). Due to its secretory status, this protein can be detected in the peripheral blood and several ELISAs and further studies with assays specific to a bone isoform may be useful in elucidating its possible clinical utility in the management of bone disease (Bonnet *et al.*, 2017).

3.2 Sclerostin

Sclerostin, a glycoprotein produce exclusively in the skeleton by the *SOST* gene and secreted mainly by osteocytes. In both men and women, its level increases with age by an average of 4.6 and 2.4 folds respectively and cleared from the body by the kidney. Its renal excretion increases with declining kidney function (Cejka *et al.*, 2014; Modder *et al.*, 2011). Sclerosteosis and Van-Buchem diseases are characterized by increase in bone mass from transcriptional attenuation or deletion of the *SOST* gene. This protein serve as an important regulator of osteoblastic bone formation as it binds to low-density lipoprotein receptor-related proteins-5 and 6 on osteoblasts with high affinity to inhibit the canonical Wnt/ β -catenin signaling pathway thereby, decrease bone formation (Sharifi *et al.*, 2015; Van-Bezooijen *et al.*, 2004). Also it inhibits terminal differentiation of osteoblast and promotes their apoptosis (Papapoulos, 2011). This protein is an independent predictor of bone loss in patients with chronic kidney disease on dialysis independent of serum PTH concentration (Malluche *et al.*, 2014). High concentrations have surprisingly been found in dialysis patients with higher bone volume and density and it is not clear whether sclerostin has a

true protective effect or if these high values occur as a secondary phenomenon (Cejka *et al.*, 2012). Circulating sclerostin is correlated with bone marrow plasma values, suggesting that serum level is likely to reflect local bone production (Drake *et al.*, 2010). Serum sclerostin is associated with vascular calcification in postmenopausal women (Hampson *et al.*, 2013). A study in 1235 premenopausal women and 568 postmenopausal women showed a linear increase of sclerostin with age (Ardawi *et al.*, 2011). These data suggest that increase in sclerostin production by osteocytes may be involved in the age-related impairment of bone formation. Moreover, sclerostin was negatively correlated with total and free estradiol and with intact parathyroid hormone in postmenopausal women (Mirza *et al.*, 2010). In another study conducted to assess osteoporotic fracture, elderly women with the highest sclerostin level were found to have significantly higher areal and volumetric bone mineral density of 9.4% to 15.3% pQCT parameters of bone strength, and larger bones than women with the lowest sclerostin level (Thorson *et al.*, 2013). Furthermore, a study of Afro-Caribbean subjects showed circulating sclerostin is genetically determined and three independent SNPs accounting for part of this effect were identified

(Kuipers *et al.*, 2014). With the availability of a new anti-sclerostin agent, there was increase interest in this analyte and its exclusive production by the skeleton has made it an attractive target for therapy, however its measurement was quite difficult and the available tests in the market have

been shown to provide conflicting results sometimes (Durosier *et al.*, 2013). Thus, robust analytical methods are required to provide true measurements suitable for clinical interpretation.

3.3 Dickkopf-1 Protein

Dickkopf (DKK-1), an inhibitor of the Wnt signalling pathway secreted by osteocytes, osteoblasts, and non-osseous cells and plays an important role in osteoblastogenesis. DKK-1 is not exclusively expressed in bone but also in other organs. Different changes of circulating DKK-1 level was observed in various cancer entities without evidence of a direct osseous involvement (Feldmann *et al.*, 2011). Both sclerostin and DKK-1 are stimulated by TNF- α in an inflammatory response and are detected in serum or plasma (Garnero *et al.*, 2014; Wendling & Claudepierre, 2013) and are found to act through their inhibition of the Wnt signaling pathway as negative regulators of osteoblast development and activity (Baron *et al.*, 2013). Their relationship to the actual observed bone mass and other circulating

bone turnover markers has often been found contrary to their assumed regulatory effect on osteoblasts (Gatti *et al.*, 2014). Therefore, the influence of biological factors (age, menopausal, hormonal status and renal function) on the concentration of both analytes in serum or plasma (Modder *et al.*, 2011; Dovjak *et al.*, 2014), and the molecular processes in the bone is yet to be fully understood (Rachner *et al.*, 2014, Sapir-koren and Livshits, 2014). All these uncertainties require a particular caution in interpreting the concentrations of both circulating DKK-1 and sclerostin. However, it is worth evaluating them especially the prognostic, predictive, and monitoring potential of both markers in future studies under the condition that the analytical issues will be resolved.

4.0 THERAPEUTIC APPLICATIONS OF BONE TURNOVER MARKERS

Anabolic agents serve as the most promising therapy for osteoporosis as they do not increase bone resorption and can uncouple bone remodeling. Majorly, BFM are very useful for profiling the mode of action of a given compound or drug, such as the early phase of bone formation called “anabolic window” induced by osteoanabolic drugs. BMD increases rapidly during this period as bone resorption is reduced whereas bone formation remains at the baseline level (Lindsay *et al.*, 2007). These markers reflect the metabolic effect of drugs on bone formation during bone remodeling, hence help to establish the lowest dose which induces the required change in the level of the BFM with a given medication, predict treatment-related increases in BMD and reduction in fracture risk. Osteocalcin and alkaline phosphatase are effective tools for the diagnosis of osteoporosis severity and development (Mada *et al.*, 2017). It has been shown that humanized sclerostin monoclonal antibody induces a rapid

and dose-dependent increase in serum level of bone formation markers followed by a rapid but transient decrease in serum CTX-I in healthy subjects (Padhi *et al.*, 2011). The increase in PINP is most rapid and followed by a slower increase in serum level of OC and bone-ALP. Strontium ranelate slightly increases bone-ALP and slightly lowers serum CTX-I in the first weeks of treatment (Meunier *et al.*, 2004). Thereafter, both remain stable, which suggests dissociation between resorption and formation. However, data on the effect of strontium on bone formation are not consistent. For instance, PINP level decreases and there is no evidence of the dual effect of strontium in bone histomorphometry (Recker *et al.*, 2009). On the other hand, teriparatide is a genetically engineered 1–34 or 1–84 fragments of human parathyroid hormone (Table 1) and the first effective anabolic agent to undergo successful trial. This drug stimulates osteoblasts, with a subsequent stimulation of osteoclasts leading to a major increase in BFM especially PINP level followed by a smaller increase in BRM (Glover *et al.*, 2009). In the early phase of treatment, bone

formation increase mainly on the endocortical and trabecular surfaces, whereas bone resorption still remains low. Increase in PINP induced by teriparatide occurs 3 days after the beginning of treatment and is followed by an increase in other BTMs (Glover *et al.*, 2009). Romosozumab, a novel sclerostin-antibody therapy currently undergoing clinical trial, induces increase in BFM and a decrease in BRM resulting in an optimum effect on bone mass and strength. This gives an indication that osteoblast and osteoclast activity may be uncoupled due to the action of this drug

(Cosman *et al.*, 2016). Romosozumab treatment in clinical trials in postmenopausal women with osteoporosis has been shown to be associated with a lower risk of vertebral fracture than placebo in the FRAME study (Saag *et al.*, 2017) and a lower risk of all fractures than alendronate in the ARCH study (Bauer *et al.*, 2012). However, there is a need to further investigate a higher rate of cardiovascular adverse events associated with its use compared to alendronate before licensing for routine use.

Table 1: Effect of osteoporosis therapy on the levels of some bone turnover markers

Drug/Therapy	Bone Marker(s)	Activity/Level	First Author(s) (Reference)
Humanized sclerostin monoclonal antibody	CTX-1	Increase	Padhi <i>et al.</i> , 2011
Strontium ranelate	PINP, OC, BALP	Decrease	Meunier <i>et al.</i> , 2004
	BALP	Increase	
	CTX-1	Decrease	
Teriparatide	PINP	Increase	Glover <i>et al.</i> , 2009; Jilka., 2007
Abaloparatide	PINP	Increase	Glover <i>et al.</i> , 2009
Alendronate	ALP	Increase	Eastell <i>et al.</i> , 2006
Residronate	NTX-1, CTX-1	Decrease	Eastell <i>et al.</i> , 2006

Footnotes: ALP: Alkaline phosphatase, OC: Osteocalcin, CTX 1: C-telopeptide cross link, NTX-1: N-telopeptide crosslink, PINP: Procollagen type I N-terminal propeptide

5.0 SOME LIMITATIONS OF BONE TURNOVER MARKERS

However, the established markers of bone formation discussed have some limitations. For example, they are synthesized in other connective tissues and are unable to be distinguish between the metabolic activities of the different skeletal compartments, although treatments may have differential effects on them. For instance, type I collagen is the most abundant protein in the bone matrix, however this protein and its pro-peptides (PINP and PICP) are not specific for bone. BFM are all protein-based markers and reflect mainly the function of osteoblast and not the activity of osteocytes, despite the pivotal role they play in the maintenance of skeletal integrity (Garnero, 2014). Despite the fact that the level of osteocalcin correlates with the rate of bone formation, the peptide is rapidly degraded in serum therefore; both intact peptides and fragments of various sizes

coexist in the circulation (Reddi *et al.*, 2019). Biological variability arising from a number of sources affects the overall pre-analytical potential of these markers.

5.1 Controllable Factors

The variations arising from these markers are categorized into controllable and uncontrollable factors. The factors which can be modified e.g. menstrual or exercise effects, circadian variability etc. are grouped under the controllable factors. Sub-acute exercise can cause increased in bone formation markers and a decrease in bone resorption markers (Thorsen *et al.*, 1996), making it necessary to enquire about regular exercise and asking patients to refrain from exercise at least 24hrs before sample collection. There was a marked increase in markers of bone turnover at menopause (Khosla *et al.*, 1997). BFM are 10-15% higher in the luteal phase than in the

follicular period of the female menstrual cycle with OC and BALP reaching maximal level in the mid-luteal phase and PICP in the early luteal phase. This amplitude of rhythm is considerably greater for bone resorption markers (mainly serum CTX-I) than bone formation markers and to reduce its effect on clinical interpretation, it is essential to control the timing of sample collection (Schlemmer & Hassager, 1999). It is therefore important that biological samples be collected in standardized conditions (preferably in the fasting state in the morning) for all the subjects participating in a clinical study/practice.

5.2 Uncontrollable factors

Uncontrollable factors are those that cannot be easily modified and they include pregnancy and lactation, gender, age, disease, drugs, or recent fracture etc. A considerable burden is placed on the maternal skeleton of a pregnant or lactating mother to provide calcium for the growing fetus or infant. Markers of bone resorption and formation are elevated during the first month of lactation. Level of markers tends to be higher in young men but lower in elderly men compared to postmenopausal women (Delmas *et al.*, 2000). Biochemical markers are significantly higher in children than adults particularly in the first year of life and at puberty. In liver and kidney diseases, levels of bone markers may reflect extra skeletal production and/or impaired metabolism. Levels of markers of bone turnover can also be affected by other drugs prescribed for unrelated conditions e.g. corticosteroid treatment significantly reduces serum osteocalcin levels but has less impact on other markers of bone turnover. During the first weeks of fracture healing, markers of bone resorption and formation increase by 20-50% and remain elevated for at least six months and possibly for one year.

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5.3 Other factors

In addition, other factors preferentially influence various markers of bone formation. For instance, inhaled glucocorticoids decreases OC level but do not have a consistent effect on other BFM's (Richy *et al.*, 2003). Alcohol abuse is associated with lower bone formation, whereas smoking is rather associated with higher bone resorption (Szulc *et al.*, 2002). Although bone specific alkaline phosphatase is highly sensitive for determination of bone turnover rate (Mada *et al.*, 2020) however, the currently available assays still have 15–20% cross-reactivity with the liver isoenzyme. Approximately one third of serum osteocalcin is intact, one third consists of the mid-molecule fragment 1-43 and one third is smaller fragments; the presence of multiple isoforms of OC in serum and the differences between assays in detection of these isoforms therefore limit its clinical usefulness. Type I collagen is also a component of several soft tissues; thus, there is a possible contribution from sources other than bone.

CONCLUSION

This review recapitulates the conventional and emerging markers of bone formation as well as their therapeutic applications for early diagnosis of postmenopausal osteoporosis. Considering the limitations of the established conventional markers of bone formation, emerging markers such as periostin, sclerostin, DKK-1 etc. may serve as candidate markers to enrich the spectrum of analytes used in the prediction of fracture risk, monitoring therapeutic intervention, and establish dose of potential osteoanabolic agents required to elicit changes in bone mineral density in patients suffering from bone metabolic disorders. Although a number of studies have shown alterations in the level of bone formation markers with regards to use of some anti-osteoporotic drugs, further investigations are required towards that direction.

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