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PRACTICAL CONSIDERATIONS FOR THE CLINICAL APPLICATION OF BONE TURNOVER MARKERS IN OSTEOPOROSIS

Samuel D. Vasikaran[1], Masakazu Miura[2], Richard Pikner[3,4,5], Harjit P. Bhattoa[6], Etienne Cavalier[7] on behalf of the IOF-IFCC Joint Committee on Bone Metabolism (C-BM)

[1] *PathWest Laboratory Medicine, Fiona Stanley Hospital, Murdoch, WA, Australia*
[2] *Faculty of Pharmaceutical Sciences, Hokuriku University/Hokuriku University Healthy Aging Research Group, 3 Ho Kanagawa-machi, Kanazawa City, Ishikawa 9201181, Japan*
[3] *Department of Clinical Biochemistry and Bone Metabolism, Klatovska Hospital, Klatovy, Czech Republic*
[4] *Department of Clinical Biochemistry and Haematology, Faculty of Medicine Pilsen, Charles University Prague, Pilsen, Czech Republic*
[5] *Faculty of Health Care Studies, University of West Bohemia, Pilsen, Czech Republic*
[6] *Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[7] *Department of Clinical Chemistry, University of Liege, CHU de Liege, Domaine du Sart-Tilman, 4000 Liege, Belgium*

✉ *Samuel D. Vasikaran samuel.vasikaran@health.wa.gov.au*

KEYWORDS

PROCOLLAGEN TYPE I *N*-PROPEPTIDE (PINP) · C-TERMINAL TELOPEPTIDE OF TYPE I COLLAGEN (β-CTX) · BONE-SPECIFIC ISOENZYME OF ALKALINE PHOSPHATASE (B-ALP) · TARTRATE-RESISTANT ACID PHOSPHATASE 5b (TRACP-5b) · BONE TURNOVER MARKERS · OSTEOPOROSIS

ABSTRACT

Bone turnover markers (BTMs) are released during the bone remodelling cycle and are measurable in blood or urine, reflecting bone remodelling rate. They have been useful in elucidating the pharmacodynamics and effectiveness of osteoporosis medication in clinical trials and are increasingly used in routine clinical management of osteoporosis, especially for monitoring therapy, in addition to their use in other metabolic bone disease such as Paget's disease of bone and osteomalacia. Serum β isomerised C-terminal telopeptide of type I collagen and pro-collagen I N-terminal propeptide have been designated as reference BTMs for use in osteoporosis. In addition, bone-specific isoenzyme of alkaline phosphatase (B-ALP) secreted by osteoblasts and tartrate-resistant acid phosphatase 5b (TRACP-5b) secreted by osteoclasts are also found to be specific markers of bone formation and resorption, respectively. The concentrations of the latter enzymes in blood measured by immunoassay provide reliable measures of bone turnover even in the presence of renal failure. B-ALP is recommended for use in the assessment of renal bone disease of chronic kidney disease, and TRACP-5b shows promise as a marker of bone resorption in that condition. BTMs in blood do not suffer from biological variation to the same extent as the older BTMs that were measured in urine. Appropriate

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patient preparation and sample handling are important in obtaining accurate measures of BTMs for clinical use. Reference change values and treatment targets have been determined for the reference BTMs for their use in monitoring osteoporosis treatment. Further ongoing studies will enhance their clinical applications.

Introduction

Bone turnover markers (BTMs) are released during the bone remodelling cycle and are measurable in blood or urine. BTMs are either products of osteoblasts secreted during bone formation (bone formation markers), or products of osteoclasts or bone degradation products released during bone resorption (bone resorption markers) [1]. β isomerised C-terminal telopeptide of type I collagen (β-CTX) and pro-collagen I N-terminal propeptide (PINP) have been designated as reference BTMs for use in osteoporosis [2] (Table 1). A detailed discussion of the rationale for the designation of β-CTX and PINP as the reference BTMs in osteoporosis is beyond the scope of this article but has been published previously [2]. Practical considerations for the clinical application of β-CTX and PINP in osteoporosis will be addressed in this article. Alkaline phosphatase in blood is a collection of enzymes, which have also been measured for several decades as a marker of liver 'function' or disease. However, since bone is the source of roughly half of the alkaline phosphatase activity in adult blood, total alkaline phosphatase activity may reflect bone diseases which are associated with major changes in bone remodelling such as Paget's disease and osteomalacia [3, 4]. Immunoassays for bone-specific alkaline phosphatase (B-ALP) became available subsequently and have better utility for detecting and following up more subtle changes in bone turnover. Alkaline phosphatase and B-ALP have been extensively reviewed elsewhere [5] and will not be discussed further in this article.

Table 1. *Reference BTMs for osteoporosis, and BTMs least affected by renal failure*

	Formation markers	Resorption markers
Reference BTMs in osteoporosis	PINP	β-CTX
BTMs least affected by renal failure	B-ALP	TRACP-5b

Tartrate-resistant acid phosphatase 5b (TRACP-5b) is an isozyme of acid phosphatase (ACP). There are five types of ACP isozymes (ACP 1–5), which can be separated by polyacrylamide gel electrophoresis [6]. There are two isoforms of TRACP-5; TRACP-5a and TRACP-5b, differentiated by post-translational modifications that occur in their respective cells of origin. TRACP-5b is derived from osteoclasts, whereas TRACP-5a is derived from other sources, such as macrophages [7]. The functions of TRACP-5b are not well known. It has been suggested that TRACP-5b, when released into the bone resorption lacuna, helps osteoclast migration, and that it has a role in degrading bone matrix type I collagen [8, 9].

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Osteocalcin in blood, is specific to bone, being synthesised almost exclusively by osteoblasts, and showed promise initially as a marker of bone formation [10], but has been largely superseded for investigation of bone disorders by the more modern BTMs such as PINP, β-CTX, B-ALP and TRACP which are specific to bone and are less beset by analytical problems. Recent interest in the role of osteocalcin in energy metabolism should be mentioned here, but further discussion of this function is outside the scope of this article [11].

Metabolism

Type I collagen is the main form of collagen found in bone, and is synthesised by osteoblasts in the form of a larger precursor, type I procollagen. These precursor molecules are characterised by propeptide domains at both ends, namely the amino terminal propeptide (PINP) and the carboxy terminal propeptide (PICP) [12, 13]. During secretion, the propeptide molecules are cleaved off by enzyme action and enter the circulation from where they are removed and metabolised in the liver by its endothelial cells [14]. Type I collagen is found to some extent in other non-skeletal tissues such as skin, but since their mass is lower and turnover slower than that of bone, they add insignificantly to the circulating propeptide pool except possibly in pathological states where their metabolism is increased [12].

β-CTX and N-telopeptide of type I collagen (NTX) are found in the telopeptide region of the type I collagen molecule, and are released into the circulation when collagen is degraded during bone resorption, and excreted in urine [12]. β-CTX is mostly measured in blood, and NTX in urine. TRACP-5b is secreted by osteoclasts as an active enzyme, and is released into the circulation. TRACP-5b becomes inactivated when it loses its iron content and is degraded into fragments that are cleared by the liver [15]. Less than 10% of the circulating TRACP-5b is present in the intact enzymatically active form [16]. Serum TRACP-5b concentration is known to reflect bone resorption; however, it must be noted that it reflects osteoclast number rather than osteoclast activity [17]. TRACP-5b is cleared by the liver, and its concentration in blood is not influenced significantly by chronic kidney disease (CKD), food intake or diurnal variation [18].

Role of BTMs

BTMs concentrations in blood or urine are thought to reflect bone remodelling rate and hence they have been used to research metabolic bone diseases including Paget's disease of bone, osteoporosis and osteomalacia as well as metabolic bone disease of chronic kidney disease (CKDMBD) in the last several decades. BTMs are also used in clinical practice, in conjunction with other diagnostic modalities, especially imaging studies, for the diagnosis and or monitoring of metabolic bone diseases [3, 19, 20]. BTMs are not useful for diagnosis of osteoporosis and are currently not included in fracture

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risk assessment, but are still useful in initial assessment of patients with osteoporosis to identify presence of secondary causes for osteoporosis, and are largely used for monitoring of therapy [2, 21]. The role of BTMs in the clinical management of osteoporosis will be briefly discussed further down, but detailed guidelines are available elsewhere [21]. BTMs are useful for both the diagnosis and in the monitoring of treatment of Paget's disease of bone, osteomalacia and CKD-MBD [3, 19, 20].

Biological Variation of BTM

Knowledge of intra-individual (day-to-day) variation (CV_I) of BTMs is important for their appropriate use in monitoring bone disease. The statement that BTMs are bedevilled by large intra-individual variation has become accepted as fact [1], but we argue that this is not the whole picture and the effect of biological variation on BTMs should be treated in a nuanced way. One of the important determinants of the CV_I of all measurands (analytes) is the sample matrix; urine analytes suffer from large day-to-day variation. The measurands albumin and calcium can be used as examples (Table 2). Serum albumin has a very low CV_I as opposed to urine albumin. The latter necessitates measurement of three consecutive urine samples for the diagnosis of microalbuminuria [22], whereas a single serum albumin measurement gives a very precise measure of the serum concentration. Similarly, serum calcium has very low CV_I as opposed to urine calcium (Table2).

Historically, urine markers such as hydroxyproline, hydroxylysine, pyridinoline and deoxypyridinoline were the commonly used BTMs and were found to be non-specific to bone and/or suffer from large intra-individual variations which, as mentioned above, are inherent to urine analytes [23, 24]. The belief that all BTMs suffer from large CV_I has likely originated from these observations. Urine NTX was found to be more specific for bone but is still affected by large day-to-day variation and is the last of the commonly used urine markers for bone resorption. The modern BTMs are measured in blood, and exhibit significantly lower dayto-day variations than the urine markers and are relatively more specific to bone than the historical markers (Table 3). The serum BTMs have considerably less C V_I even compared to, for example, serum parathyroid hormone (PTH) (Table 3).

The European Biological Variation Study (EuBIVAS), using most up-to-date quality criteria for biological variation studies have recently published extensive data of biological variations in BTMs [25]. Table 4 describes analytical and intra-individual variation data for serum β-CTX and PINP from that study.

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Table 2 *Intra individual variations [within-subject (CV_I) biological variation (BV)] of calcium and albumin in serum and urine. Source https:// www. westg ard. com/ bioda tabas e1. htm*

Serum or urine	Measurand	CV_I %
Serum	Albumin	3.2
Serum	Calcium	2.1
Urine	Albumin, concentration, first morning	36.0
Urine	Calcium, concentration, 24 h	27.5

Table 3 *Intra individual variations [within-subject (CV_I) biological variation (BV)] of BTMs and PTH*

Serum or urine	Measurand	CV_I
Serum	Procollagen type I *N*-propeptide (PINP)	7.4
	C-terminal telopeptide type I collagen (β-CTX)	10.9
	Osteocalcin	6.4
	Alkaline phosphatase, bone (B-ALP)	6.2
	Acid phosphatase tartrate-resistant (TRACP)	8.0
Urine	Deoxypyridinoline/creatinine, first morning	13.8
	Hydroxyproline/minute—excretion rate, first morning	36.1
	N-telopeptide type I collagen concentration	15.5
Serum	Parathyroid hormone (PTH)	25.9

Based on https://www.westg ard.com/biodatabase1.htm

Table 4 *Intra individual variation [within-subject (CV_1) biological variation (BV)] estimates for the reference BTMs for osteoporosis, PINP and ß-CTX, with 95% confidence interval (CI), based on Cavalier et al. [25]*

Measurand	Mean value (95% CI)	CV_A % (95% CI)a	CV_I % (95% CI)
PINP, µg/L	63.7 (62.3–65.0)	3.7 (3.6–3.9)	8.8 (8.4–9.3)
β-CTX, ng/L	514.3 (499.5–529.1)	5.0 (4.8–5.3)	15.1 (14.4–16.0)

[a] Analytical variation (CV_A) estimates were based on CV-ANOVA of duplicate analysis of all study samples

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Pre-analytical Considerations

Biological variation has been addressed above. BTMs, like all measurands in blood, show circadian variation that is specifically addressed in a separate paper in this issue and will be only briefly addressed here.

The effect of food and circadian variation on β-CTX concentration in blood is clinically significant unlike their effect on serum PINP. A number of steps for patient preparation and sample handling have been recommended in order to mitigate such effects on day-to-day variation of BTMs [26], and these are summarised below.

Venesection should be performed for β-CTX measurement in the morning between 7.30 and 10.00 am after an overnight fast. These steps are not necessary for PINP, but if both markers are measured, the above steps should be followed [26].

Whilst both serum and EDTA plasma are acceptable for the measurement of PINP and β-CTX, the latter is unstable in serum and if the blood sample cannot be spun and serum separated immediately from cells, EDTA plasma is preferred for β-CTX [26].

Samples should be frozen at ≤ − 20 °C until analysis, but if latter is delayed for > 3 months for β-CTX or > 6 months for PINP then samples should be stored at ≤ − 70 °C [26].

TRACP-5b can be measured in serum or plasma. The anticoagulants for plasma differ depending on the assay manufacturer, and the package insert should be referred to for determining the appropriate anticoagulant to use. Samples can be stored for at least 2 days at room temperature, 3 days in the refrigerator at 4 °C and frozen up to 1 month at − 20 °C and several years at − 70 °C. However, refreezing samples after thawing is not recommended since TRACP-5b rapidly loses activity [18].

Analytical Considerations

PINP ASSAYS

PINP in serum is measured by immunoassay; details of commercial assays have been published elsewhere [27] and the commonly used assays are summarised here. The antibodies used in immunoassays for PINP recognise the high molecular weight trimeric intact PINP molecule; some assays in addition also cross-react with the low-molecular weight monomeric fragments. The latter assays are identified as 'total PINP' assays whilst the former are termed 'intact PINP assays'. The three commonly used commercially available assays for serum PINP are listed in Table 5 and their analytic characteristics summarised in Table 6.

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β-CTX ASSAYS

As mentioned above, β-CTX is best measured in EDTA plasma due to better stability; details of commercial immunoassays have been published elsewhere [27] and the commonly used assays are summarised below. All commercial assays for β-CTX detect a glu-lys-ala-his-asp-gly-gly-arg (EKAHD-β-GGR) octapeptide sequence in the C-terminal telopeptide region of the α1 chain of the collagen molecule with the use of the CrossLaps® antibody.

There are three commonly used assays available for the measurement of β-CTX in blood (Table 7). A manual ELISA [CrossLaps®] is FDA approved for use as bone resorption marker, and two automated immunoassays have been developed, by Roche Diagnostics and IDS, using the CrossLaps antibody. Table 8 summarises characteristics of the commonly used commercial assays for β-CTX in blood.

Table 5 *Commonly used commercial immunoassays available for serum PINP measurement (based on Bhattoa et al. [27])*

Vendor	Methodology	Measurand	Analytics
Cobas, Roche Diagnostics, Germany	Electrochemiluminescence immunoassay	Total PINP	Automated
Aidian (Orion Diagnostica), Finland	Radioimmunoassay	Intact PINP	Manual
iSYS, Immunodiagnostics Systems (IDS), UK	Chemiluminescence immunoassay	Intact PINP	Automated

Table 6 *Analytical characteristics of commercial assays for serum PINP (based on Bhattoa et al. [27])*

PINP assay	Measuring range (μg/L)	Limit of detection (μg/L)	Intra-assay CV (%)	Inter-assay CV (%)
Cobas, Roche Diagnostics, Germany	5–1200	5.0	1.4–2.3	2.1–4.5
Aidian (Orion Diagnostica), Finland	5–250	2.3	2.3–3.5	2.7–6.1
iSYS, Immunodiagnostics Systems (IDS), UK	2–230	2.0	2.6–3.0	4.2–5.3

Table 7 *Commonly used commercial assays for β-CTX in blood (based on Bhattoa et al. [27])*

Vendor	Methodology	Measurand	Analytics
IDS, UK	Enzyme-linked immunosorbent assay	β-CTX	Manual
IDS, UK	Chemiluminescence immunoassay	β-CTX	Automated
Roche Diagnostics, Germany	Electrochemiluminescence immunoassay	β-CTX	Automated

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TRACP-5b Assays

The active TRACP-5b enzyme, inactive TRACP-5b fragments and the TRACP-5a enzyme are found in blood. To accurately evaluate bone resorption, only active TRACP5b should be measured, and not "total" TRACP-5b (active TRACP-5b and inactive fragments), nor TRACP-5a. In the past, TRACP activity was measured with kinetic assays that were unable to distinguish TRACP-5a activity from TRACP-5b activity. Although the assays were subsequently improved, they still could not distinguish between the active and non-active forms, and thus lacked specificity. However, more recently, two immunoenzymatic assays with high specificity for the active TRACP-5b form have been developed and are commercially available [16, 28–31]; (1) Nittobo Medical (Tokyo, Japan) ELISA and (2) IDS assay available as an ELISA (Boldon, UK) or automated on the iSYS system. The Nittobo assay is a fragment absorbed immunocaptured enzymatic assay that uses two monoclonal antibodies (anti-TRACP-5b (active) and anti-TRACP-5b fragment (inactive) antibodies), which enable highly specific TRACP5b measurements without cross-reactivity with the TRACP5a derived from macrophages. In contrast, the TRACP-5b assays from IDS involve the capture of both TRACP-5 forms by a biotinylated monoclonal antibody. Both assays use a substrate at an optimal pH for TRACP-5b, and are specific for TRACP-5b.

Reference Values

Reference intervals for BTMs are required for the interpretation of results obtained in patients and should ideally be established for each population group. Several studies have been published from different regions of Europe, USA and other parts of the world, and we have detailed them in a previous publication [32]. A large Danish study of both sexes and all adult ages, including 450 healthy premenopausal women not taking the oral contraceptive pill, has been published since then [33], and the reference intervals for premenopausal women based on that study are summarised in Table 9. The two automated assays give similar results for serum PINP in subjects with normal renal function and reference intervals are interchangeable. However, there are significant differences in values obtained by the PINP (Aiden-Orion®) RIA method and the two automated assays, with the RIA for PINP showing a proportionate bias compared to each of the two automated methods. Hence, method specific reference intervals should be used for PINP values obtained by the RIA method. For β-CTX, the reference intervals for the two automated methods cannot be used interchangeably as there is a significant proportional bias between the two methods.

The reference intervals of TRACP-5b have mainly been reported from Japan [34, 35]. The Japan Osteoporosis Society has issued Guidelines for the Use of Bone Turnover Markers in the Diagnosis and Treatment of Osteoporosis (2018 Edition) [36]. According to the guidelines, the reference intervals of TRACP-5b are 1.70–5.90 U/L in healthy men, 1.20–4.20 U/L in premenopausal women and 2.50–7.60 U/L in postmenopausal women. The reference interval for men provided in the guideline is not age

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specified; however, when interpreting individual patient results, values widely outside of the reference interval for healthy men should trigger a search for a secondary cause. Similarly, for women, at initial assessment, the appropriate reference interval used would be based on menopausal status. Harmonisation of TRACP-5b assays is desirable and is planned as more than one commercial assays are available.

Table 8 *Analytical characteristics of commercial assays for β-CTX in blood (based on Bhattoa et al. [27])*

β-CTX assay	Measuring range	Limit of detection (ng/L)	Intra-assay CV (%)	Inter-assay CV (%)
CrossLaps®, IDS, UK	20–3380 ng/mL	20	< 2.5	2.2–5.5
CrossLaps®, iSYS, IDS, UK	50–6000 ng/L	20	2.7–3.7	2.5–5.2
B-CrossLaps, Cobas, Roche Diagnostics, Germany	10–6000 ng/L	10	1.2–4.1	< 5.7

Table 9 *Reference intervals for PINP and β-CTX in premenopausal women > 30 years age for the two automated assays derived from 450 women by Jorgensen et al. [33]*

Manufacturer	PINP (µg/L)	β-CTX (ng/L)
IDS	18–87	70–920
Roche	19–92	137–643

Clinical Applications in Osteoporosis

In untreated and newly diagnosed osteoporosis patients, BTM values do not contribute independently to fracture risk calculations, and most patients with uncomplicated osteoporosis would have BTM values within reference intervals albeit in the upper half of the interval [21, 37]. Hence, BTMs are not included in fracture risk calculators such as FRAX. However, measurement of BTMs at initial assessment of osteoporosis patients may be of use for two reasons: firstly, a very high BTM value (three standard deviations above mean, which is a SD above the upper reference limit) may indicate the presence of a secondary cause for the osteoporosis [21]. Secondly, a baseline BTM may be useful to compare post treatment values with if required, to confirm efficacy of treatment and adherence [37].

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Role of BTMs in Monitoring Osteoporosis Treatment

A significant change in BTM concentration following initiation of treatment for osteoporosis reflects effectiveness of treatment [37, 38]. Whilst parenteral therapy almost always elicits a significant response in BTMs, effectiveness of oral therapy is dependent on adherence to and persistence with therapy by the patient and absorption of the medication in the gut, neither of which can always be reliable. In fact, adherence and persistence with long-term medication is notoriously unreliable. Hence, monitoring with BTMs has been promoted as a useful tool to confirm adherence and effectiveness of long-term oral therapies for osteoporosis [38]. BTMs may be measured 3–6 months post initiation of oral bisphosphonates. A change in BTM values is considered significant if it exceeds the reference change value (RCV) defined as the smallest difference between sequential laboratory results which is associated with a true change. RCV is calculated as = $Z \times \sqrt{(2 \times [\ CV_I^2 + \ CV_A^2)]}$ where Z is the z-value that is associated with a desired probability of a true uni- or bi-directional change [39]. This equation is abbreviated to a coefficient of 2.77 for calculation of a bidirectional RCV with a 95% probability using a z-value of 1.96. Where the direction of change is known as with osteoporosis therapy, a z-value of 1.65 is the appropriate number to apply [39]. Cavalier et al. have calculated RCV for β-CTX and PINP in blood in the EuBIVAS study based on their intra-individual variation estimates (Table 10) [25]. These values are very similar to the RCV data of 18% and 30% for PINP and β-CTX, respectively, calculated by Tan et al. at the probability level of 95% for significant unidirectional change using the TRIO study biological variation data [39, 40]. Note that RCV is calculated using data derived from observed average biological variation and the calculated RCV is independent of the absolute value. In practice, both CV_I and CV_A expressed as percentages may be lower at higher concentrations and higher at lower concentrations. Absolute RCVs of 100 ng/L for β-CTX and 10 µg/L for PINP have been proposed for oral bisphosphonate therapy, using the TRIO study data [37]. Following initiation of treatment with anabolic agent such as teriparatide, an increase in serum PINP of 10 µg/L at 1–3 months signifies evidence of response to treatment [41].

In addition to detecting a significant change in BTMs following initiation of treatment, optimum treatment effect is reflected by the BTMs attaining treatment targets. The median of the premenopausal reference interval is commonly used as treatment target for anti-resorptive therapy in osteoporosis [32, 37, 38]. These values are method dependent due to the inter-assay differences described above both for PINP and for β-CTX, and ideally should be determined for the population of interest and the assay used [32]. These targets would be approximately around 35 µg/L for PINP and around 300 ng/L for β-CTX, the exact value being dependant on the assay used and the population of interest [32].

Generally, a review of the need for continuation of treatment for osteoporosis is undertaken after 5 years of oral alendronate therapy or three annual infusions of zoledronic acid, and again after a period of cessation of such therapy ("drug holiday"). There is a lack of good evidence for the utility of BTMs to assess fracture risk and the need for continuation of or restarting therapy in such situations.

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Nevertheless, BTMs are sometimes used in practice to determine if bisphosphonate effect is persisting after a period of cessation of therapy (drug holiday) in order to help decide to restart therapy when BTMs rise above the treatment target [42].

Monitoring BTMs following cessation of long-term denosumab therapy (> 2.5 years) may be useful to guide management of such patients to mitigate the associated increase in vertebral fracture risk. A recent European Calcified Tissue Society guideline recommended measurement of BTMs after 3 and 6 months of bisphosphonate therapy following denosumab discontinuation and, if stable, every 6 months to ensure that they remain within the lower half of the premenopausal reference interval [43].

Serum TRACP-5b is recommended for clinical use by the Japan Osteoporosis Society [36]. Its utility has been reported in treatments with bisphosphonates, denosumab and romosozumab [44, 45]. TRACP-5b has also been used in different predictive models, such as for bone mineral density for up to 2 years following zoledronate administration [46, 47], and for hypocalcemia following denosumab administration in haemodialysis patients with osteoporosis [48].

Table 10 *Reference change values (RCV) for a decrease in BTM following antiresorptive therapy based on the biological variation (BV) estimates as reported in Table 4, based on Cavalier et al. [25]*

Measurand	RCV (%)
PINP	− 19.9
β-CTX	− 30.8

Influence of CKD on BTMs

Patients with CKD stages G3a to G5D have an increased fracture risk from development of CKD-MBD [19], characterised by disturbances in mineral and bone metabolism and abnormal bone histology. CKD-MBD may encompass abnormalities in bone turnover (low, normal or high), bone mineralization [normal or abnormal (osteomalacia)] and bone volume (low, normal or high) [49]. Studies of BTMs in CKD-MBD have demonstrated an association of BTMs with bone turnover although BTMs by themselves were not sufficient to diagnose low, normal and high bone turnover [50–52]. B-ALP, together with PTH are the bone markers currently recommended by KDIGO for monitoring CKDBMD. Nizet et al. have addressed B-ALP in CKD-MBD in detail in a recent publication [5] and hence it will not be discussed here. CKD-MBD is associated also with extraskeletal calcification. Calcium phosphate metabolism regulators such as FGF23, 1,25 dihydroxyvitamin D, PTH 1–84, Klotho, as well as sclerostin and DKK-1 significantly contribute to endothelial-to-mesenchymal transition (EndMT) leading to vascular calcification and cardiovascular disease [52]

β-CTX accumulates in blood progressively with decreasing renal function in CKD patients, and therefore its clinical value is limited in such patients [53, 54]. The monomeric PINP isoform also accumulates in

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the blood in CKD patients. Total PINP assay, which measures both intact and monomeric forms of PINP, therefore, has limited value in patients with glomerular filtration rate below 45–30 mL/ min/1.73 m^2 [55, 56]. Intact assays that recognise only the trimeric form of the PINP molecule may be used in CKD patients [55, 56]. TRACP-5b seems to be the only resorption marker that is not influenced by decline in kidney function or dialysis in patients with CKD [51, 57–61]. The Japan Osteoporosis Society recommends using the TRACP-5b level for monitoring bone turnover in patients with CKD, which include renal transplant patients and haemodialysed patients [36]. Suzuki et al. reported that the serum TRACP-5b can reflect the efficacy of denosumab treatment for osteoporosis in patients with CKD [62].

Risk Stratification for Osteonecrosis of the Jaw (ONJ) and Atypical Femoral Fracture (AFF)

Long-term treatments with potent antiresorptive agents such as potent bisphosphonates have been thought to be potentially associated with ONJ and AFF, which have been suggested to be causally related to suppression of bone turnover resulting in compromised ability to heal micro damage to bone structure [63, 64]. However, direct aetiological relationship has not been established between long-term use of antiresorptive agents and ONJ or AFF [21]. The use of BTMs, and in particular β-CTX to identify patients at risk of ONJ based on a low serum β-CTX (< 150 ng/L) has been proposed by some researchers [65], but does not have robust evidence to support it, and is not recommended [63]. BTMs are not uniformly suppressed in patients with AFF and further research is required to elucidate any possible application of BTM in risk stratifying potential candidates for ONJ or AFF [63, 64]

Summary and Conclusion

Serum PINP and β-CTX are useful for monitoring oral therapy in osteoporosis. Further studies for their application in managing offset of drug action after cessation of antiresorptive therapies with bisphosphonates and denosumab would be useful. Large-scale fracture risk prediction studies of PINP and β-CTX in various untreated population groups to assess how they interact with established risk factors used in risk calculators such as FRAX may help to include BTMs in such algorithms.

B-ALP and TRACP-5b are least affected by renal failure and may be of potential use in assessment for osteoporosis in patients with CKD and monitoring such patients when treated. Studies of utility of TRACP-5b and B-ALP in fracture risk assessment as well as monitoring therapy and assessing offset of treatment effect in osteoporosis patients with CKD stages 3a-5D is warranted.

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From an analytical point of view, standardisation or harmonisation of commercial assays for BTMs is important for collation of data from different studies and uniform application of decision limits and treatment targets in clinical guidelines. IOF-IFCC C-BM is pursuing these activities.

Declarations

DISCLOSURES EC is a consultant for Diasorin, IDS, Fujirebio, Menarini and Nittobo. MM is a consulatant for Beckmann-Coulter Japan and Nittobo Medical. RP is a member of advisory board for Amgen Czech Republic and has received a speaker honorarium from Amgen, Takeda, Roche, DiaSorin, Abbott and Beckmann-Coulter. HB and SV have no conflicts to declare.

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