

# Research progress on bone turnover biochemical markers and their application in clinical diagnosis

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**Abstract:** Bone turnover is the key mechanism for maintaining the dynamic balance between bone tissue formation and resorption. As biological molecules released during this process, biochemical markers of bone turnover (BTMs) reflect the metabolic state of bone tissue. They play a crucial role in elucidating the mechanisms of bone formation and remodeling, diagnosing osteoporosis (OP), evaluating therapeutic responses in metabolic bone diseases, and monitoring disease progression. In recent years, the discovery of new markers, advances in detection techniques, and the expansion of clinical applications have made BTMs increasingly prominent in the management of OP patients, establishing them as effective tools in comprehensive treatment strategies. This review summarizes the latest research advances in BTMs, exploring the biological basis, detection techniques, and the clinical application potential and challenges of both bone formation and resorption markers. Our comprehensive analysis indicates that BTMs play an increasingly important role in the diagnosis and management of OP and other bone metabolic diseases. However, certain limitations must be addressed to fully realize their potential in precision medicine.

**Abbreviations:** OP = osteoporosis, BTMs = bone turnover markers, BALP = bone-specific alkaline phosphatase, OC = osteocalcin, PINP = procollagen type I N-propeptide, PICP = procollagen type I C-propeptide, HOP = hydroxyproline, TRACP-5b = tartrate-resistant acid phosphatase 5b, PYD = pyridinoline, DPD = deoxypyridinoline, CTX = C-terminal telopeptides of type I collagen, NTX = N-terminal telopeptides of type I collagen.

**Keywords:** bone formation, bone metabolism, bone resorption, bone turnover markers, osteoporosis

## 1. Introduction

Bone remodeling is a fundamental process for maintaining skeletal health and biological function, involving the coupled actions of bone formation and resorption. Bone turnover markers (BTMs) are biological molecules released into the blood or urine during bone metabolism, serving as crucial biochemical indicators for assessing bone metabolic activity. BTMs are primarily categorized into markers of bone formation and bone resorption. Formation markers, such as bone-specific alkaline phosphatase

(BALP), osteocalcin (OC), and procollagen type I propeptides (PINP [procollagen type I N-terminal propeptide], PICP [procollagen type I carboxy-terminal propeptide]), reflect the synthesis of new bone tissue. Conversely, resorption markers, including hydroxyproline (HOP), tartrate-resistant acid phosphatase 5b (TRACP-5b), and collagen telopeptides (CTX [C-terminal telopeptide of type I collagen], NTX [N-terminal telopeptide of type I collagen]), indicate the degradation of bone tissue. The measurement of BTMs provides clinicians with a non-invasive, dynamic monitoring tool, aiding in the diagnosis of osteoporosis (OP), prediction of fracture risk, and monitoring of response to antiosteoporotic therapy. Therefore, in-depth research and application of BTMs are of paramount importance for improving skeletal health and the quality of life for patients with OP.

## 2. Basic concepts and classification of BTMs

### 2.1. Historical development of BTMs

The application of BTMs in scientific research and clinical practice spans over 2 decades. The earliest marker, total alkaline phosphatase (T-ALP), emerged in the 1920s. Although not bone-specific, it has been widely used in diagnosing metabolic bone diseases and monitoring treatment efficacy.<sup>[1]</sup> As research into conditions with more subtle changes in bone turnover deepened, more specific markers were developed. In the 1960s, the assay for HOP was introduced, but its utility was limited by dietary influences on its

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concentration.<sup>[2]</sup> The 1980s saw the introduction of OC as a bone formation-specific marker and the use of pyridinium cross-links (PYD [pyridinoline], DPD [deoxypyridinoline]), which were less affected by diet.<sup>[3]</sup> By the 1990s, the clinical adoption of immunoassays allowed for the widespread measurement of markers such as PINP, PICP, BALP, TRACP-5b, CTX, and NTX.<sup>[3–5]</sup> Technological advancements, including chemiluminescence and electrochemiluminescence, have progressively replaced traditional radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) techniques, leading to automated, high-throughput platforms.<sup>[6]</sup> The following sections will delve into the biological basis and clinical applications of these key BTMs.

## 2.2. Bone formation markers

**2.2.1. Serum BALP.** Alkaline phosphatase comprises a group of homodimeric glycoprotein isoenzymes distributed in various human tissues, particularly the liver, bone, intestine, and placenta.<sup>[7]</sup> In healthy adults, approximately 50% of total serum alkaline phosphatase activity originates from the liver and 50% from bone. In children and adolescents, due to skeletal growth, the bone-specific isoenzyme predominates (up to 90%).<sup>[8]</sup> BALP is produced by osteoblasts and its secretion directly reflects osteoblast activity, making it a reliable indicator of bone formation.<sup>[9]</sup> Immunoassays using specific monoclonal antibodies provide excellent sensitivity and sufficient specificity for measuring BALP, proving effective in detecting subtle changes in bone turnover. These assays are quantitative, rapid, robust, and reproducible. Notably, BALP shows no significant circadian variation and is unaffected by food intake, so fasting is not required for sampling. Serum samples for BALP are stable for up to 7 days at room temperature, 14 days at 4°C, and 3 months at –20°C. However, these assays lack absolute specificity, with all reported methods exhibiting some cross-reactivity with the liver isoenzyme (7%–18%). Therefore, elevated BALP levels in patients with known liver disease must be interpreted with caution.<sup>[10–12]</sup> Since BALP is cleared from circulation by the liver and not excreted renally, its concentration is not directly influenced by renal function. This makes it particularly suitable for assessing OP and monitoring treatment in patients with chronic kidney disease (CKD).<sup>[13]</sup>

**2.2.2. Serum OC.** OC, also known as bone Gla protein, is the most abundant noncollagenous protein in bone. It is a 49-amino acid peptide produced primarily by osteoblasts.<sup>[14]</sup> A portion of newly synthesized OC is incorporated into the bone matrix, while the remainder is released into the bloodstream, where it is ultimately cleared by the kidneys.<sup>[15]</sup> Serum OC levels are considered a specific marker of osteoblast activity and reflect bone formation.<sup>[16]</sup> However, because OC is incorporated into the matrix and released during resorption, its levels can also be influenced by bone turnover overall. The application of methods like ELISA and radioimmunoassay has improved the accuracy and sensitivity of serum

OC detection. Currently, various commercially available OC immunoassays exist, including automated chemiluminescent and electrochemiluminescent platforms. Like other BTMs, OC exhibits significant diurnal variation. While its concentration is unaffected by food intake, it is susceptible to degradation from freeze–thaw cycles and hemolysis. Therefore, consistent sampling timing and strict sample handling protocols are crucial for accurate results.<sup>[17,18]</sup> Samples are best processed within 4 h of collection and kept near 4°C.<sup>[19]</sup> While OC is a valuable marker, its interpretation requires caution in conditions affecting bone turnover or renal function. Its utility as an independent predictive tool requires further research, and standardization of assays across laboratories is needed for widespread clinical use.

**2.2.3. Serum PINP and PICP.** PINP and PICP are byproducts of type I collagen synthesis, the most abundant collagen in bone.<sup>[20,21]</sup> During extracellular bone formation, type I procollagen is secreted, and its PINP and PICP are enzymatically cleaved and released into the circulation. Their concentrations thus directly reflect the rate of new type I collagen synthesis *in vivo*.<sup>[20,22–24]</sup> While other tissues like skin and tendons also produce type I collagen, their contribution to the circulating propeptide pool is minimal compared with bone due to its higher turnover rate.<sup>[25,26]</sup> PINP and PICP are both considered sensitive markers of bone formation.

PINP is often preferred and is the reference marker recommended by the International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). PINP exists in two forms: a trimeric form (intact PINP) cleared by the liver, and a monomeric form cleared by the kidneys. Total PINP assays measure both. In patients with renal impairment, monomeric PINP can accumulate, leading to elevated levels that may not solely reflect bone formation.<sup>[27–29]</sup> PICP metabolism is also influenced by hormonal regulation, particularly by thyroid hormones and insulin-like growth factor I (IGF-I).<sup>[29]</sup> Levels of both markers can be affected by factors such as age, sex, fractures, and conditions like liver fibrosis.<sup>[30–32]</sup> Measurement of PINP and PICP is typically performed using automated chemiluminescent immunoassays.<sup>[33]</sup> Clinically, PINP is the preferred marker for monitoring OP treatment, evaluating other metabolic bone diseases, and serving as a pharmacodynamic biomarker in drug development.

## 2.3. Bone resorption markers

**2.3.1. Hydroxyproline.** HOP is an amino acid formed from the posttranslational hydroxylation of proline during collagen synthesis. It is abundant in collagen, and approximately 90% of the HOP released during bone collagen degradation is excreted in urine, leading to its historical use as a marker of bone resorption.<sup>[34]</sup> However, HOP lacks specificity for bone, as it is also released from the degradation of newly synthesized collagen in soft

tissues like skin and from dietary sources. Consequently, urinary HOP has been largely replaced by more specific and sensitive techniques.<sup>[35]</sup>

**2.3.2. Serum TRACP-5b.** TRACP exists in two isoforms: 5a and 5b. TRACP-5a is derived from macrophages and dendritic cells, while TRACP-5b is specifically generated by osteoclasts.<sup>[36–38]</sup> Secreted into the circulation during bone resorption, TRACP-5b is involved in the degradation of the bone matrix. As it is specifically expressed by osteoclasts, it serves as a highly specific marker of osteoclast number and, by extension, bone resorption activity.<sup>[39–41]</sup> TRACP-5b is typically measured in serum or plasma using enzyme immunoassays or ELISA. To accurately assess bone resorption, assays must specifically measure active TRACP-5b, distinguishing it from inactive fragments and TRACP-5a. The Nittobo assay, for example, is a highly specific fragment-absorbed immunocapture enzyme assay using two monoclonal antibodies to achieve this.<sup>[42–45]</sup> A key advantage of TRACP-5b is its lack of significant diurnal variation and independence from food intake, allowing for convenient sampling. Furthermore, as it is cleared by the liver, its concentration is not affected by renal function, making it another valuable marker for patients with CKD.<sup>[41,46]</sup> Sample stability is good under various storage conditions, although repeated freeze–thaw cycles should be avoided.<sup>[41,47]</sup> While TRACP-5b reflects osteoclast number, it is important to note that it reflects cell number rather than the activity of individual osteoclasts.<sup>[48]</sup> Nevertheless, its high tissue specificity makes it a valuable tool for evaluating and monitoring bone resorptive activity, particularly in conditions like OP and in patients with renal impairment.

**2.3.3. PYD and DPD.** PYD and DPD are mature collagen cross-links that stabilize the collagen molecules within the bone matrix. They are formed during the extracellular maturation of collagen and are released upon its degradation. PYD is found in cartilage and bone, while DPD is more specific to bone.<sup>[49]</sup> Their release into the bloodstream and excretion in urine directly reflect bone resorption.<sup>[50]</sup> Historically, urinary PYD and DPD were measured by high-performance liquid chromatography after acid hydrolysis, a complex and time-consuming process.<sup>[51]</sup> While they are more bone-specific than HOP, these assays have largely been replaced by automated assays for more convenient markers like CTX and NTX.

**2.3.4. Serum CTX and serum NTX.** CTX and NTX are degradation products released from type I collagen during osteoclastic bone resorption. Enzymes like cathepsin K digest the collagen, releasing small fragments from the cross-linking region, such as CTX and NTX, which enter the circulation and are eventually excreted in urine.<sup>[52]</sup> Their levels in blood or urine directly reflect the rate of bone resorption. CTX is typically measured in plasma or serum and is the IOF/IFCC reference marker for bone resorption.<sup>[53]</sup> It exhibits significant diurnal variation and is affected by food intake; therefore, fasting morning samples are mandatory.<sup>[54]</sup> NTX is often measured in

urine and expressed as a ratio to creatinine to correct for dilution.<sup>[55]</sup> Serum NTX assays exist but are less commonly used due to a smaller dynamic response.<sup>[55]</sup> In clinical practice, CTX and NTX are primarily used to monitor the efficacy of antiresorptive therapy in OP patients, where a significant decrease confirms adherence and response. They can also aid in evaluating other conditions associated with high bone turnover, such as Paget disease or bone metastases.<sup>[53]</sup>

## 2.4. Emerging and novel BTMs

While established BTMs like PINP and CTX are valuable clinical tools, ongoing research has identified several novel markers that may provide additional insights into bone metabolism. These emerging markers often reflect specific pathways or regulatory mechanisms within the bone remodeling process.

**2.4.1. Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL) and osteoprotegerin (OPG).** The RANK (receptor activator of nuclear factor- $\kappa$ B)/RANKL/OPG system is a fundamental signaling pathway regulating osteoclast differentiation, activation, and survival. RANKL, expressed by osteoblasts, promotes osteoclastogenesis, while OPG, a soluble decoy receptor produced by osteoblasts, inhibits it. The ratio of RANKL to OPG is a critical determinant of bone resorption. While their measurement in serum is challenging due to instability and the existence of different forms, they remain powerful biomarkers for understanding the pathophysiology of bone diseases and the mechanism of action of drugs like denosumab (a RANKL inhibitor).<sup>[56]</sup>

**2.4.2. Sclerostin.** Produced by osteocytes, sclerostin is a potent inhibitor of the Wnt signaling pathway, which is essential for osteoblast proliferation, differentiation, and activity. By inhibiting this pathway, sclerostin suppresses bone formation. It has emerged as a key target for OP therapy, as demonstrated by romosozumab, an antisclerostin antibody that increases bone formation and decreases bone resorption. Circulating sclerostin levels are being investigated as a potential marker of bone formation activity and a predictor of response to anabolic therapies.<sup>[57]</sup>

**2.4.3. Periostin.** This matricellular protein is primarily expressed in periosteum and periodontal ligament, where it plays a crucial role in regulating collagen fibrillogenesis and mediating bone's response to mechanical loading. It is involved in both bone formation and repair. Serum periostin levels have been associated with bone mineral density (BMD) and fracture risk in some studies, although its precise role as a clinical marker is still under investigation.<sup>[58]</sup>

**2.4.4. Cathepsin K.** This is the primary protease secreted by osteoclasts to degrade type I collagen. While its measurement as a circulating protein is complex, its enzymatic activity is central to bone resorption. It is also a key therapeutic target, as evidenced by the development

of cathepsin K inhibitors like odanacatib. The inclusion of these emerging markers in research studies is enhancing our understanding of bone biology and holds promise for future clinical applications, particularly in guiding treatment selection and monitoring response to newer targeted therapies. For a quick reference, the key features, sample types, clinical applications, and important preanalytical notes for the major BTMs discussed above are summarized in Table 1.

### 3. Clinical applications of BTMs: potential and limitations

With the accelerating aging of the global population, OP has become a major public health concern. Although dual-energy X-ray absorptiometry is the gold standard for diagnosis, many fractures occur in individuals with osteopenia ( $T$  score  $\geq -2.5$ ) due to their larger population size.<sup>[59,60]</sup> Therefore, additional tools are needed to refine fracture risk prediction and identify patients who will benefit most from pharmacotherapy. Advanced imaging techniques like trabecular bone score can provide supplementary data,<sup>[61,62]</sup> and measuring BTMs has been extensively explored for this purpose.

#### 3.1. BTMs in predicting bone loss

Around menopause, increased bone turnover due to estrogen deficiency is a key driver of bone loss.<sup>[63]</sup> This is characterized by a rapid increase in bone resorption, which, due to the coupling of resorption and formation, is followed by a secondary increase in formation markers.<sup>[64]</sup> While studies have shown a clear relationship between elevated BTM levels at menopause and the rate of subsequent bone loss, this association is less clear in older women, limiting the utility of

single BTM measurements for predicting bone loss in elderly individuals.<sup>[65,66]</sup> Consequently, current evidence does not support the use of BTMs as a public health screening tool for identifying individuals at high risk of bone loss.<sup>[67]</sup> An innovative approach to potentially enhance the predictive utility of BTMs is the “Bone Balance Index”. This method uses regression analysis to determine the relative contributions of a bone formation marker (e.g., OC) and a bone resorption marker (e.g., NTX) in a population with stable bone mass. An individual patient's deviation from this established relationship might provide a more accurate reflection of net bone loss.<sup>[68]</sup> While preliminary studies suggest this index could be a robust predictor, it is important to emphasize that this is an emerging research tool. Its general applicability and clinical utility require validation in larger, prospective studies before it can be considered for routine clinical use.

#### 3.2. BTMs in predicting fracture risk

The ability of BTMs to predict fracture risk is a distinct question from their ability to predict bone loss. For a given BMD, fracture risk can vary significantly due to other factors like bone microarchitecture and turnover rate. While some prospective studies have shown that elevated bone resorption markers are associated with an increased risk of fragility fractures, particularly in the short term (e.g., within 5 years), the evidence is not entirely consistent.<sup>[69–71]</sup> A meta-analysis by Johansson et al. did find a significant association between reference BTMs (PINP and CTX) and fracture risk in untreated older individuals.<sup>[69]</sup> However, a major limitation is the lack of data on how BTMs interact with other risk factors included in fracture risk algorithms like FRAX. Therefore, they are not currently integrated into these widely used clinical tools.<sup>[72]</sup> While BTMs are a

**Table 1**  
Key bone turnover markers in clinical use.

| Marker   | Type       | Sample type         | Key clinical applications                                                                    | Important preanalytical notes                                                                  |
|----------|------------|---------------------|----------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| PINP     | Formation  | Serum               | IOF/IFCC reference marker for bone formation; monitoring anabolic and antiresorptive therapy | Minimal diurnal variation; stable sample; preferred marker for clinical trials                 |
| PICP     | Formation  | Serum               | Assessing bone formation                                                                     | Cleared by liver; levels influenced by hormones (e.g., thyroid)                                |
| BALP     | Formation  | Serum               | Assessing bone formation, especially in CKD patients (not renally cleared)                   | No fasting required; minimal diurnal variation; cross-reactivity with liver ALP (about 7%–18%) |
| OC       | Formation  | Serum/plasma        | Assessing bone formation and overall turnover                                                | Significant diurnal variation; sensitive to freeze–thaw cycles; cleared renally                |
| CTX      | Resorption | Serum/plasma (EDTA) | IOF/IFCC reference marker for bone resorption; monitoring antiresorptive therapy             | Fasting morning sample essential; significant diurnal variation; unstable at room temperature  |
| NTX      | Resorption | Urine               | Monitoring bone resorption; expressed as ratio to creatinine                                 | Corrected for dilution by urinary creatinine; less preferred due to variability                |
| TRACP-5b | Resorption | Serum/plasma        | Specific marker of osteoclast number; useful in CKD patients (cleared by liver)              | No fasting required; no diurnal variation; stable; reflects cell number, not activity          |
| DPD      | Resorption | Urine               | Historical marker of bone resorption                                                         | More bone-specific than PYD; assay complex (HPLC), largely replaced                            |

ALP = alkaline phosphatase, BALP = bone-specific alkaline phosphatase, CKD = chronic kidney disease, CTX = C-terminal telopeptide of type I collagen, DPD = deoxypyridinoline, IFCC = International Federation of Clinical Chemistry and Laboratory Medicine, IOF = International Osteoporosis Foundation, HPLC = high-performance liquid chromatography, NTX = N-terminal telopeptide of type I collagen, OC = osteocalcin, PICP = procollagen type I carboxy-terminal propeptide, PINP = procollagen type I N-terminal propeptide, PYD = pyridinoline, TRACP-5b = tartrate-resistant acid phosphatase 5b.

powerful tool for epidemiological studies of fracture risk, current evidence is insufficient to support their routine use for identifying individuals who require OP treatment.

### 3.3. BTMs in monitoring treatment for OP

The primary goal of OP treatment is fracture risk reduction. Given the slow and relatively small magnitude of BMD changes, repeat BMD measurements are typically not recommended within the first 1 to 2 years of therapy. In contrast, BTMs exhibit rapid and significant changes soon after treatment initiation, making them invaluable for monitoring early treatment response.<sup>[73]</sup>

The pattern of BTM change depends on the class of medication:

- Antiresorptive drugs (e.g., bisphosphonates, denosumab) rapidly inhibit osteoclast activity, leading to a swift decline in bone resorption markers (CTX) within weeks, followed by a slower decrease in formation markers (PINP) due to physiological coupling.<sup>[74]</sup>
- Anabolic drugs (e.g., teriparatide) directly stimulate osteoblasts, causing a rapid and robust increase in bone formation markers (PINP), which peaks within months and is followed by a later increase in resorption markers.<sup>[75,76]</sup>
- Dual-acting agents (e.g., romosozumab, an antiscleerostin antibody) have a unique effect, initially increasing formation markers while transiently decreasing the resorption marker CTX.<sup>[77]</sup>
- Combination therapy with agents like denosumab and teriparatide can result in complex BTM profiles, where the potent antiresorptive effect of denosumab can blunt the teriparatide-induced increase in bone formation.<sup>[78,79]</sup>

This rapid and mechanism-specific response makes BTMs essential tools in clinical trials for demonstrating drug pharmacodynamics and efficacy. In clinical practice, a decrease in resorption markers (e.g.,  $\geq 30\%$ – $50\%$  reduction in CTX) after initiating antiresorptive therapy is a strong indicator of adherence and adequate response.

### 3.4. BTMs in guiding pharmacotherapy selection

In principle, baseline BTM levels could guide treatment choice: patients with low bone turnover might benefit more from anabolic agents, while those with high turnover might respond well to antiresorptives. Although some studies show a correlation between baseline BTMs and the subsequent BMD increase, the antifracture efficacy of OP treatments has been shown to be largely independent of baseline BTM levels. Consequently, current guidelines do not recommend using BTMs to select the initial pharmacological agent for OP.<sup>[80,81]</sup>

### 3.5. BTMs in optimizing treatment adherence

OP is often asymptomatic until a fracture occurs, which can lead to poor long-term adherence to oral medications.<sup>[82]</sup> While the evidence from randomized controlled

trials on whether monitoring BTMs improves adherence is inconclusive,<sup>[83–85]</sup> many experts and guidelines suggest a practical role for BTM measurement in this context.<sup>[73,86–88]</sup> A common recommendation is to measure a baseline BTM (PINP, CTX) before starting therapy and again 3 to 6 months later. If the marker has not changed appropriately (e.g., a significant decrease in CTX on an antiresorptive), it prompts a discussion with the patient to verify adherence before assuming treatment failure and considering an alternative therapy.<sup>[89,90]</sup> In this way, BTMs serve as a valuable objective feedback tool to engage patients and support persistence with therapy, acting as a useful adjunct to, or in some cases an alternative to, early repeat BMD testing.<sup>[91,92]</sup>

## 4. Conclusions

BTMs are increasingly applied in OP management, offering unique insights that complement BMD measurement. When their preanalytical and biological variabilities are properly controlled, they can accelerate clinical decision-making, provide early assessment of treatment response, help identify poor adherence, and contribute to fracture risk prediction. PINP and  $\beta$ -CTX are the IOF/IFCC recommended reference markers for bone formation and resorption, respectively. BALP and TRACP-5b are particularly valuable in patients with CKD, as their levels are not confounded by impaired renal function. Despite historical concerns about variability, the advent of automated assays and a better understanding of influencing factors have solidified the role of BTMs in both clinical trials and practice. In clinical settings, they are particularly useful for identifying poor response or non-adherence, serving as a practical tool to enhance patient management and optimize long-term outcomes.

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