International Journal of Research in Orthopaedics
Ali H et al. Int J Res Orthop. 2026 May;12(3):848-859
http://www.ijoro.org

Review Article

DOI: https://dx.doi.org/10.18203/issn.2455-4510.IntJResOrthop20261244

Role of bone turnover markers in prediction of fracture healing: a contemporary evidence-based perspective

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Received: 27 January 2026
Revised: 11 March 2026
Accepted: 17 March 2026

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ABSTRACT

Early and accurate assessment of fracture healing is limited by reliance on plain radiography, which detects only late mineralized changes and fails to capture early biological events, contributing to delayed union and non-union in 5-10% of cases. Bone turnover markers such as PINP, BALP, CTX, and TRACP-5b provide dynamic biochemical insight into osteoblast osteoclast activity and offer potential for earlier prediction of fracture healing outcomes. This review used a structured search of PubMed/MEDLINE, Scopus, Web of Science, and EMBASE up to December 2025 to synthesize mechanistic, diagnostic, and clinical evidence on BTMs in fracture healing. Successful fracture healing shows reproducible temporal BTM patterns, with early rises in CTX and TRACP-5b during days 3-7, followed by a 30-70% increase in PINP and BALP during weeks 2-4. Impaired healing is associated with blunted PINP and BALP responses and/or persistently elevated CTX beyond week 6, with reported odds ratios of 3-5 for delayed or non-union. Clinical application is limited by assay heterogeneity, circadian and metabolic variability, inconsistent sampling protocols, and the lack of fracture-specific cut-off values. An integrated Biochemical Biomechanical Radiological (BBR) Trifecta Model combining BTMs, mechanical stability metrics, and advanced imaging is proposed to improve prognostic accuracy. BTMs offer biologically sensitive and time-dependent information that complements radiography and enable early identification of patients at risk for delayed or impaired fracture healing. Integration through the proposed BBR model, supported by emerging biomarkers and AI-based analytics, may allow precise and personalized fracture monitoring.

Keywords: Bone biomarkers, Bone healing, Fracture, Prognosis, Bone turnover

INTRODUCTION

Bone fracture healing is a highly coordinated regenerative process involving sequential phases of inflammation, angiogenesis, progenitor cell recruitment, extracellular matrix synthesis, and progressive mineralization.[1] The biological events that determine the successful healing occur predominantly within the first 2-4 weeks; however, conventional radiography captures only the late structural consolidation, typically detectable after mineralized callus formation, which can take 4-8 weeks depending on fracture type and patient physiology.[2] As a result, delayed union and non-union affecting approximately 5-10% of long-bone fractures are often recognized only after significant progression toward failed repair.[3] Bone turnover markers (BTMs), measurable products of osteoblast and osteoclast activity, provide a non-invasive opportunity to monitor early biological dynamics of

fracture repair. Formation markers such as procollagen type I N-terminal propeptide (PINP) and bone specific alkaline phosphatase (BALP) reflect collagen synthesis and matrix mineralization, whereas resorption markers including C-terminal telopeptide of type I collagen (CTX) and tartrate-resistant acid phosphatase isoform 5b (TRACP5b) indicate osteoclast-mediated matrix degradation.[4-6] Longitudinal studies demonstrate that resorption markers rise within the first week following fracture, while formation markers typically increase by weeks 2-4, suggesting that early biomarker trajectories may differentiate normal from impaired healing pathways.[7]

Despite compelling biological rationale, the clinical utility of BTMs remains limited by assay variability, circadian and metabolic influences, lack of fracture-specific reference ranges, and heterogeneity in sampling protocols.[8] These challenges underscore the need for a mechanistically grounded evaluation of BTMs and their integration with advanced imaging, biomechanical assessment, and emerging molecular biomarkers.

This review synthesizes contemporary mechanistic insights into fracture biology, critically evaluates the diagnostic and prognostic value of BTMs, identifies existing barriers to clinical translation, and highlights future research avenues including multi-omics profiling and machine-learning based predictive models to advance precision monitoring of fracture healing.

METHODS

Study design

This work was conducted as a narrative review with structured search strategy, aimed at synthesizing current mechanistic, diagnostic, and clinical evidence on bone turnover markers (BTMs) in fracture healing. Given the heterogeneity of study designs, patient populations, analytical platforms, and outcome reporting across the available literature, a narrative review format allowed deeper biological integration, comparative interpretation, and conceptual model development while maintaining methodological transparency.

Search strategy

A comprehensive literature search was performed across PubMed/MEDLINE, Scopus, Web of Science, and EMBASE from inception to December 2024. Search terms were developed using MeSH keywords and Boolean operators: (“fracture healing” OR “bone repair” OR “callus formation”) AND (“bone turnover markers” OR “PINP” OR “BALP” OR “CTX” OR “TRACP5b” OR “osteocalcin”) AND (“diagnosis” OR “prediction” OR “biomarkers” OR “non-union” OR “delayed union”). Additional manual searching included citation chaining of key articles, screening of major orthopaedic and endocrinology journals, and consultation of reference lists of relevant reviews.

Eligibility criteria

Studies were included if:

The included studies reported human or animal data linking BTMs to fracture healing. They assessed temporal trends, predictive value, or correlations between BTMs and radiological or clinical healing outcomes. Each study measured at least one established BTM, such as PINP, BALP, CTX, TRACP5b, NTX, or osteocalcin. Only peer-reviewed, full-text articles published in English were considered.

The following were excluded:

Studies were excluded if they were conference abstracts, editorials, or expert opinions lacking primary data. Case reports without biomarker analysis were also excluded, as studies evaluating BTMs solely in osteoporosis without fracture cohorts. Additionally, articles for which the full text was inaccessible despite attempts to retrieve it were not included.

Data extraction and synthesis

Data extracted included study type, sample size, fracture type, fixation modality, BTM assay characteristics, timing of sampling, and primary clinical outcomes. Emphasis was placed on: The studies evaluated several key aspects of BTMs in fracture healing. These included the temporal trajectories of BTMs over the course of healing, their diagnostic and predictive value, and comparisons between united and non-united fractures. The impact of systemic factors such as diabetes, smoking, osteoporosis, chronic kidney disease, and vitamin D deficiency was also considered. Additionally, the heterogeneity of assay methods—including RIA, ELISA, ECLIA, and CLIA—was assessed, along with mechanistic correlations between BTM levels and the cellular phases of bone repair.

Given the methodological heterogeneity across studies, meta-analysis was not feasible. Instead, comparative qualitative synthesis was performed, integrating: The studies also addressed multiple dimensions of fracture healing, including clinical findings, underlying biochemical mechanisms, correlations with imaging studies, and biomechanical interpretations. This comprehensive approach allowed for a multidimensional understanding of how bone turnover markers reflect the healing process. This approach allowed construction of advanced conceptual models such as the BBR Trifecta and biochemical healing trajectories.

Quality assessment

Because the included studies varied widely (animal studies, clinical trials, observational cohorts), no single

standardized quality assessment tool was fully appropriate. Instead:

Clinical cohort studies were evaluated using modified Newcastle–Ottawa Scale criteria, focusing on selection, comparability, and outcome. Basic science studies were assessed for mechanistic clarity, reproducibility, and validity of assays. Diagnostic accuracy studies were appraised according to QUADAS-2 principles to ensure methodological rigor and reliability of findings. Studies with poor methodology or unclear biomarker timing were included only for mechanistic context, not for predictive analysis.

Ethical considerations

As this review synthesizes data from previously published literature, no institutional ethical approval was required. All included studies were assumed to have followed appropriate ethical guidelines as per their respective journals and regulatory standards.

Software and reference management

EndNote X9 and Zotero 6.0 were used to manage references. Graphical figures were generated using GraphPad-style vector templates, AI-assisted visualization, and manual refinement for publication quality.

Outcome measures

The primary outcomes of interest were:

The studies focused on several key aspects of BTMs in fracture healing. These included temporal changes in BTMs throughout the healing process, their ability to predict delayed union or non-union, and their diagnostic performance when assessed as single or composite markers.

Additionally, the relationship between BTMs and imaging findings, biomechanical properties, and clinical outcomes was explored. Emerging biomarkers, including miRNAs, proteomics signatures, and extracellular vesicles (EVs), were also highlighted as potential tools for advancing fracture monitoring and prognostication. Secondary outcomes included effects of comorbidities, medication use, and fixation techniques on BTM trajectories.

BIOLOGICAL BASIS OF FRACTURE HEALING

Fracture healing is a highly orchestrated biological process involving immunologic, vascular, cellular, and biomechanical events.[9] These events progress through overlapping phases as shown in Figure 1: inflammatory, reparative, and remodelling each marked by distinct cellular actors, molecular signals, and measurable changes in BTMs as summarized in table 1. Understanding these phases is crucial for interpreting biochemical markers and their clinical significance in fracture repair.[10]

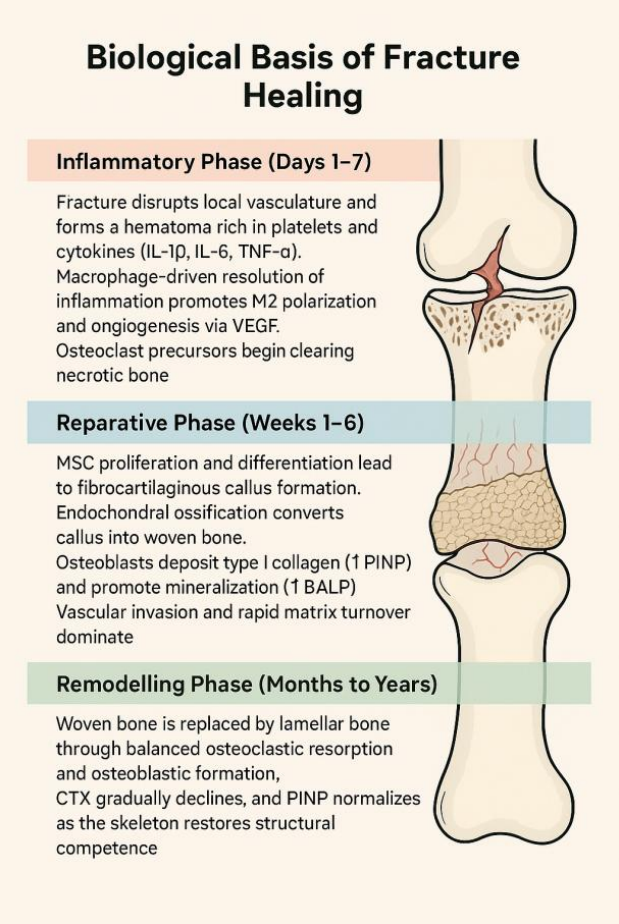

Figure 1: Biological basis of fracture healing.

A schematic illustration depicting the three overlapping phases of fracture healing inflammatory, reparative, and remodelling.

Inflammatory phase (days 0-7)

The inflammatory phase begins immediately after fracture, triggered by disruption of bone and periosteal vasculature that forms a hematoma.[11] Platelets, neutrophils, and monocytes infiltrate the site, releasing pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which initiate the acute inflammatory cascade.[12,13]

Macrophages sequentially polarise from the M1 to M2 phenotype, coordinating the transition from inflammation to repair. Vascular endothelial growth factor (VEGF) stimulates angiogenesis, ensuring oxygen and nutrient supply to the fracture site.

Simultaneously, early osteoclast precursors remove necrotic bone fragments, clearing the way for new tissue formation. These events establish a microenvironment conducive to recruitment of mesenchymal progenitor cells and deposition of extracellular matrix.[1]

Table 1: Biological events, dominant cells, molecular signals, and expected BTM patterns across healing phases.

Healing phase	Dominant biological events	Key cells	Major molecular signals	Expected BTM pattern
Inflammatory (days 1-7)	Haematoma formation, inflammation, necrotic tissue clearance	Neutrophils, m1→m2 macrophages, osteoclast precursors	Il-1β, il-6, TNF-α, HIF-1α, VEGF	↑ CTX, ↑ TRACP-5b
Reparative (weeks 1-6)	Soft callus → hard callus, cartilage formation, angiogenesis	MSCS, chondrocytes, osteoblasts	COL2A1, SOX9, RUNX2, VEGF	↑ PINP, ↑ BALP, mild ↑ osteocalcin
Remodelling (months–years)	Woven → lamellar bone, cortical restoration	Osteoclasts, osteoblasts	Rankl/OPG balance, TGF-β, BMPS	↓ CTX, normalising PINP, ↑ osteocalcin (late)

A comparative table summarizing key biological processes across inflammatory, reparative, and remodelling phases, linked with major cell populations, molecular regulators, and characteristic BTM profiles (PINP, BALP, CTX, TRACP-5b). The table serves as a fast-reference clinical map aligning biochemical marker trajectories with underlying bone biology.

Table 2: Biological source/mechanism, peak during healing and clinical interpretation of bone biomarkers.

Category	Marker	Biological source / mechanism	Peak during healing	Clinical interpretation
Formation markers	PINP	Cleaved during type I collagen synthesis	Weeks 2-4	Osteoblast activation; predicts successful callus formation
	BALP	Osteoblast membrane enzyme for mineralisation	Weeks 3-8	Hard callus maturation
	Osteocalcin	Late-stage osteoblast marker	Late reparative → remodelling	Overall turnover; lower early specificity
Resorption markers	CTX	Collagen breakdown fragment from cathepsin-K activity	Days 3-7	Early necrotic tissue removal; persistent elevation suggests impaired healing
	TRACP-5b	Osteoclast-derived enzyme reflecting osteoclast number	Days 3-10	Osteoclast-mediated clearance
	NTX	Collagen degradation peptide	Days 4-10	Useful in combination panels
	Cathepsin-K fragments	Collagenolytic neoepitopes	Early–mid healing	Emerging bone-specific markers

This table compares bone formation and resorption markers according to their biological sources and mechanisms during fracture healing along with their clinical interpretations. PINP peaks during weeks 2-4, predicting early callus formation, while BALP peaks between weeks 3-8, reflecting callus maturation. Osteocalcin represents late-stage bone formation and overall bone turnover. Resorption markers such as CTX and TRACP-5b indicate early osteoclastic activity during days 3-10, followed by NTX and cathepsin-K fragments, which are emerging as more bone-specific markers of collagen degradation.

Reparative phase (weeks 1-6)

Once acute inflammation resolves, mesenchymal stem cells (MSCs) proliferate and differentiate into chondrocytes and osteoblasts under hypoxic and mechanical cues.[15] A fibrocartilaginous soft callus forms, which is gradually replaced by woven bone through endochondral ossification.[16] Osteoblasts deposit type I collagen and initiate hydroxyapatite mineralisation, reflected by rising formation markers such as PINP and bone-specific alkaline phosphatase (BALP).[17] Osteoclasts simultaneously remodel the callus, removing cartilage remnants, with a corresponding increase in resorption markers including CTX and TRACP-5b.[18] This phase represents active matrix synthesis, mineralisation, and structural bridging under mechanical stabilisation.

Remodelling phase (months to years)

The remodelling phase converts woven bone into mature lamellar bone, restoring normal architecture and mechanical competence.[10] Coupled osteoblast–osteoclast activity reshapes trabecular and cortical bone, aligns collagen fibres along stress lines, and re-establishes vascular and osteocyte networks. Resorption markers gradually decline, while formation markers and osteocalcin return toward baseline levels, reflecting balanced skeletal turnover.[19] Mechanical loading modulates the pace and efficiency of remodelling,

ensuring the restoration of normal bone strength and functionality.

BONE TURNOVER MARKERS: CLASSIFICATION AND MECHANISMS

BTMs are biochemical by-products released during osteoblastic bone formation or osteoclastic bone resorption.[20] Circulating concentrations of BTMs reflect dynamic skeletal activity and provide early insight into fracture repair, often before radiographic changes are visible.

BTMs are classified based on their physiological origin: formation markers indicate matrix synthesis and mineralisation, while resorption markers reflect collagen degradation and osteoclast-mediated catabolism.[21] Accurate interpretation of BTMs requires understanding their mechanistic basis and temporal patterns throughout the healing process, Table 2 shows biological source, mechanism, peak during healing and clinical interpretation of bone formation and resorption markers.

Bone formation markers (osteoblastic activity)

Formation markers rise predominantly during the reparative phase, reflecting osteoblastic activity and active deposition of bone matrix.[22]

Procollagen type I N-terminal propeptide (PINP)

PINP is released during extracellular processing of type I procollagen and directly reflects collagen synthesis.[23] As type I collagen comprises ~90% of bone's organic matrix, PINP is highly specific for osteoblastic activity. It increases during early hard-callus formation and correlates strongly with histomorphometric indices. Low diurnal variability and minimal renal influence make it the most reliable early marker for predicting fracture healing.[24]

Bone-specific alkaline phosphatase (BALP)

BALP is expressed by mature osteoblasts and facilitates mineralisation by hydrolysing pyrophosphate, which inhibits hydroxyapatite crystallisation.[25] BALP rises with callus maturation and peaks during the soft-to-hard callus transition, reflecting robust osteoblast differentiation and active mineral deposition.[2]

Osteocalcin (OC)

Osteocalcin is secreted during late matrix maturation and plays a key role in hydroxyapatite binding.[27] Though partially released during resorption; OC primarily reflects late-stage bone formation and remodelling. Its slower kinetics limit its utility for early fracture prognosis but make it valuable for long-term monitoring of skeletal recovery.[28]

Bone resorption markers (osteoclastic activity)

Resorption markers reflect enzymatic degradation of the bone matrix, particularly type I collagen, by osteoclasts. They are highly sensitive to early inflammatory changes and provide insight into the catabolic phase of healing.[29]

C-terminal telopeptide of type I collagen (CTX)

CTX is released during cathepsin-K–mediated cleavage of cross-linked type I collagen. It rises within the first week post-fracture to remove necrotic bone and debris and declines as reparative formation dominates. Persistently elevated CTX suggests mechanical instability or impaired healing.[30]

N-terminal telopeptide (NTX)

NTX is a collagen degradation product measured in serum or urine. It complements CTX, offering less diurnal variability and reflecting osteoclastic activity.[31]

TRACP-5b

TRACP-5b is osteoclast-specific, correlating with osteoclast number rather than activity alone. Its stability and low circadian influence make it a reliable early resorption marker.[32]

Cathepsin K fragments

Cathepsin K–derived fragments provide enhanced specificity for osteoclastic function and mechanistic insight into collagen degradation, particularly under pathological conditions.[33]

MECHANISTIC INTEGRATION DURING FRACTURE HEALING

The temporal pattern of BTMs mirrors fracture biology. Early elevations in CTX and TRACP-5b reflect osteoclastic clearance of necrotic tissue during inflammation.[34] In the reparative phase, PINP and BALP rise, indicating collagen synthesis and mineralisation. During remodelling, formation and resorption markers gradually normalise as woven bone matures into lamellar bone. This interplay underscores the coupled nature of bone turnover, where balanced anabolic and catabolic activity is critical for successful fracture healing.[35]

TRAJECTORIES OF SPECIFIC BTMS DURING FRACTURE HEALING

Each BTM follows a characteristic trajectory that corresponds to distinct stages of fracture repair, as shown in Table 2. Serial measurement of BTMs provides superior biological insight compared to single time-point analysis, enabling early identification of impaired healing.[36]

Early resorption phase (days 1–10): surge in CTX and TRACP-5b

The earliest biochemical response to fracture is a rapid rise in resorption markers.

CTX trajectory

CTX begins to increase within 24-72 hours after injury and peaks between days 3-7. This reflects cathepsin K–mediated degradation of necrotic type I collagen at fracture margins. The rise is essential for clearing damaged tissue and preparing the microenvironment for osteogenesis. Absence of this early CTX surge may suggest inadequate inflammatory or vascular response.[30]

TRACP-5b trajectory

TRACP-5b mirrors CTX but is less influenced by circadian variation and renal function. It peaks between days 5-10, corresponding to increased osteoclast number. Persistent elevation beyond 2-3 weeks may indicate ongoing excessive resorption, mechanical instability, or impaired callus formation.[34]

Reparative phase (weeks 2–6): rise in PINP and BALP

As resorption declines, bone formation becomes dominant.

PINP trajectory

PINP begins to rise around week 2, peaks at weeks 3-4, and typically increases by 30-70% above baseline in normal healing. It reflects rapid type I collagen deposition by osteoblasts during woven bone formation. A blunted or absent rise in PINP is a strong biochemical indicator of delayed union.[24]

BALP trajectory

BALP rises more slowly, increasing from weeks 3-4 and peaking around weeks 6-8. It reflects osteoid mineralisation and correlates with developing mineralised callus, providing earlier insight than radiographic changes.[37]

Late reparative to remodelling phase (weeks 6–16+): normalisation of BTMS

Resorption markers progressively decline toward baseline by 3-6 months, indicating structural maturation. PINP and BALP also gradually decrease as mineralisation stabilises. Osteocalcin rises during this stage, reflecting long-term osteoblastic activity and remodelling.[38]

Combined BTM trajectory: the "biochemical healing curve"

Normal fracture healing follows a predictable pattern:

During fracture healing, BTMs exhibit distinct temporal patterns. In the first week (week 0-1), there is a sharp rise in resorption markers such as CTX and TRACP-5b. Between weeks 2 and 6, formation markers including PINP and BALP become dominant, while resorption markers decline. From week 6 onward, a gradual normalization occurs, reflecting the restoration of coupling between bone formation and resorption. Deviations from this curve, such as persistently high CTX or low PINP, act as early warning signs of delayed or impaired healing.

Clinical interpretation of abnormal trajectories

Deviations from normal BTM patterns provide early biochemical warning of impaired fracture healing and reflect disturbances in osteoblast–osteoclast balance, mechanical stability, or systemic metabolism.[22]

Low PINP (weeks 2-4)

Failure of PINP to rise by 20-30% indicates poor type I collagen synthesis and inadequate osteoblastic activity. It strongly predicts delayed union and is associated with impaired MSC differentiation, compromised vascularity, malnutrition, vitamin D deficiency, and endocrine dysfunction.[24]

Persistent CTX elevation (beyond week 6)

Sustained high CTX suggests excessive or pathological bone resorption. It is commonly linked to mechanical instability, implant loosening, infection, smoking, or chronic inflammation and is frequently seen in hypertrophic non-union.[39]

Low BALP (weeks 4–8)

Absent BALP rise reflects defective mineralisation and delayed endochondral ossification. It is associated with osteomalacia, diabetes, renal disease, and inadequate mechanical loading, predisposing to atrophic non-union.[40]

Suppressed CTX and PINP

Simultaneous suppression indicates globally reduced bone turnover, seen with steroids, bisphosphonates, malnutrition, and endocrine disorders, increasing risk of delayed healing.[41]

DIAGNOSTIC AND PREDICTIVE VALUE OF BTMS

BTMs provide early biochemical insight into fracture healing by reflecting dynamic osteoblastic and osteoclastic activity before structural changes become radiographically evident. Their temporal patterns indicate whether the biological repair cascade is progressing normally or deviating toward delayed or non-union, making them valuable adjuncts to imaging and clinical assessment.[36]

Early biological diagnosis

Radiographs detect mineralised callus only after weeks, whereas BTMs change within days to weeks after injury. Early rises in CTX and TRACP-5b reflect osteoclastic clearance of necrotic tissue, while subsequent increases in PINP and BALP indicate collagen synthesis and mineralisation. Serial BTM monitoring therefore allows assessment of healing biology long before radiographic consolidation, enabling earlier clinical intervention.[37]

Prediction of delayed union and non-union

Abnormal BTM trajectories are strong predictors of impaired healing. A blunted rise in PINP during weeks 2–4 is one of the most reliable indicators of delayed union, reflecting insufficient osteoblastic matrix synthesis.[30] Persistently elevated CTX beyond week 6 suggests ongoing pathological resorption due to instability, infection, or impaired angiogenesis. Low BALP during weeks 4-8 indicates defective mineralisation and high risk of non-union.[30]

Multi-marker approaches

Combining formation and resorption markers improves diagnostic accuracy. Panels including PINP, CTX, and TRACP-5b outperform single markers by capturing both anabolic and catabolic components of healing.[42] Balanced patterns suggest normal repair, while discordant profiles indicate biological dysfunction.

Clinical integration

When integrated with radiological and biomechanical data, as in the BBR Trifecta Model, BTMs provide a comprehensive and precise framework for early fracture-healing assessment.[43]

IMPLICATIONS FOR CLINICAL DECISION-MAKING AND RESEARCH

BTMs enable early identification of impaired fracture healing, allowing timely optimisation of fixation, metabolic correction, or biological interventions.[43] In research, they serve as sensitive surrogate endpoints to evaluate implants, biomaterials, and pharmacological therapies, shortening study duration and enhancing biological resolution.[44]

Clinical utility in fracture assessment

BTMs provide real-time insight into osteoclastic resorption, collagen synthesis, mineralisation, and callus remodelling, especially during the first 4-6 weeks when radiographs are least informative.[36] Rising PINP and BALP indicate robust osteogenesis, while declining CTX and TRACP-5b reflect progression toward stable healing.[22]

Early identification of delayed union and non-union

Blunted PINP rise during weeks 2-4 and persistently elevated CTX beyond week 6 are early biochemical indicators of delayed union or non-union, often preceding radiological signs by several weeks and enabling proactive clinical intervention.[22-30]

Monitoring therapeutic response

BTMs rapidly reflect treatment effects: PINP rises with anabolic therapy and CTX falls with antiresorptive. This allows early assessment of therapeutic efficacy and personalised modulation of fracture management.[45]

Limitations and constraints affecting clinical translation

Despite their strong biological relevance, several factors limit the routine clinical use of BTMs. Biological variability is a major challenge, as circadian rhythm, fasting status, renal function, hormonal levels, and physical activity significantly influence BTM concentrations independent of fracture healing.[46] Lack of strict standardisation in sample collection and timing can therefore lead to inconsistent interpretation.

In addition, fracture-specific reference ranges are not yet well established.[40] Most current cut-offs are derived from osteoporosis studies, which do not fully represent the biological dynamics of acute fracture repair. Technical variability between assay platforms further complicates clinical adoption, as differences in antibody specificity, calibration standards, and analytical sensitivity can result in non-comparable values across laboratories.[47]

Contextual limitations: mechanical, biological, and systemic factors

BTM responses are also influenced by fracture characteristics and fixation techniques. Intramedullary nailing, plating, and external fixation create different mechanical environments, each altering callus biology and biomarker trajectories.[48] Systemic factors such as immobilisation-induced bone loss, chronic inflammation, smoking, diabetes, or metabolic bone disease may elevate or suppress BTMs irrespective of local fracture healing. These confounders reduce site-specific specificity and highlight the importance of interpreting BTMs within the broader clinical and biomechanical context.[49]

Path toward optimised clinical integration

Improved clinical translation requires harmonisation of assay methods, development of fracture-specific reference ranges, and preference for serial rather than single-point measurements.[50] Integration of BTMs with biomechanical stability data and imaging findings, as proposed in the BBR Trifecta Model, substantially enhances diagnostic reliability. Emerging multi-marker algorithms and machine-learning approaches are expected to reduce

variability and strengthen predictive accuracy, advancing BTMs toward precision fracture care.[51]

INTEGRATION OF BTMS WITH IMAGING AND BIOMECHANICS (BBR TRIFECTA MODEL)

Rationale for an integrated framework

Fracture healing is inherently multidimensional and cannot be fully assessed by a single modality. Radiographs provide delayed structural information, BTMs capture early biological activity, and biomechanical measures quantify stability and load transfer.[52] The BBR Trifecta Model integrates these complementary domains to provide a holistic assessment of fracture repair from molecular activation to structural restoration.[53]

Biochemical axis: molecular signatures of healing

The biochemical axis relies on serial BTM measurements to reflect healing dynamics. Early rises in CTX and TRACP-5b denote osteoclastic clearance, while increasing PINP and BALP signal collagen synthesis and mineralisation.[34] These changes precede radiographic consolidation by weeks and allow early detection of biological deviations. Additionally, BTMs reveal systemic influences such as vitamin D deficiency or endocrine disorders, providing diagnostic depth beyond imaging alone.[54]

Biomechanical axis: regulator of tissue fate

Mechanical stability governs cell differentiation and callus quality. Excessive motion promotes fibrocartilage, whereas controlled micromotion supports endochondral ossification.[55] Modern tools such as stiffness analysis, ultrasound elastography, and implant sensors quantify stability and help explain abnormal BTM patterns, linking biology with mechanics.[56]

Radiological axis: structural validation

Imaging confirms architectural restoration and callus maturation. While radiographs show delayed changes, CT, MRI, and ultrasound detect early callus density, vascularity, and marrow remodelling. When combined with BTMs, imaging validates biological activity and improves prediction of successful union.[37]

The BBR trifecta model: unified monitoring

The integration of biochemical, biomechanical, and radiological data creates a comprehensive healing profile, as shown in Figure 2. Early BTMs reflect biological activation, mechanics define stability, and imaging confirms structure. Together, they differentiate normal healing from delayed or non-union and guide timely interventions.[57]

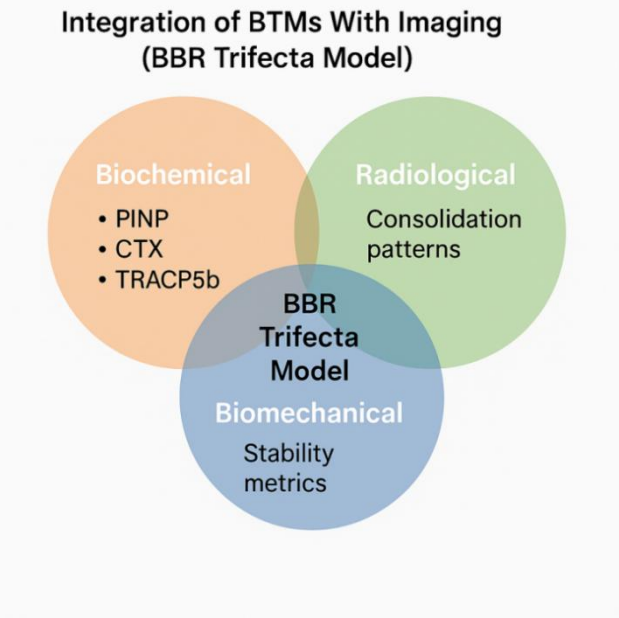

Figure 2: BBR trifecta model: integration of biochemical, biomechanical, and radiological assessment.

A Venn-diagram illustrating the integration of three complementary dimensions in fracture evaluation: (1) biochemical (PINP, CTX, TRACP-5b), (2) biomechanical (stability metrics, strain and stiffness), and (3) radiological (callus consolidation patterns). The central overlap—BBR Trifecta—represents a holistic diagnostic strategy combining molecular activity, mechanical environment, and structural progression to improve prediction of fracture healing outcomes.

Clinical advantages

The BBR model improves early diagnosis, reduces uncertainty, and enables personalised treatment by identifying whether failure is biological, mechanical, or structural.[58]

FUTURE DIRECTIONS AND EMERGING BIOMARKERS

MicroRNAs: ultra-early indicators

Circulating miRNAs (e.g., miR-21, miR-140, miR-31, miR-29b) regulate osteogenesis, angiogenesis, and chondrogenesis, providing highly sensitive early markers of fracture healing, detectable before conventional BTMs.[59]

Proteomics and metabolomics

Fracture-specific proteomic and metabolomic signatures, including collagen fragments and angiogenic peptides, enable molecular phenotyping of repair pathways and may outperform standard BTMs.[60]

Extracellular vesicles

MSC- and osteoblast-derived exosomes carry proteins, miRNAs, and cytokines that reflect fracture-site activity and offer both biomarker and therapeutic potential.[61]

Artificial intelligence

AI integrates BTMs, imaging, biomechanics, and patient data to predict healing outcomes, identify at-risk fractures, and personalise treatment strategies.[62]

HISTORICAL DEVELOPMENT OF BTMS

Early biochemical markers

Initial bone turnover assessment relied on nonspecific markers like serum alkaline phosphatase and urinary hydroxyproline, which reflected global collagen metabolism but lacked bone specificity.[63]

Immunoassay era

The 1980s–1990s introduced ELISA and radio immuno assays, enabling measurement of osteocalcin, PINP, PICP, NTX, and CTX, with TRACP-5b quantifying osteoclast activity, providing mechanistic insight into bone formation and resorption.21

Standardisation and automation

From 2000-2015, automated chemiluminescent platforms and standardised protocols improved assay precision and reliability, facilitating application in fracture healing research.[21]

Contemporary multi-omics era

Current approaches integrate multi-marker panels, imaging, AI, miRNAs, proteomics, and exosomes to create high-resolution, patient-specific biomarker frameworks for monitoring fracture repair.[64]

DISCUSSION

Fracture healing is a complex, dynamic process, and biochemical monitoring using BTMs represents a shift toward precision orthopaedics.[30] This review underscores the predictive and diagnostic value of BTMs particularly PINP, CTX, BALP, and TRACP-5b whose trajectories align with inflammatory, reparative, and remodelling phases. However, their clinical performance is influenced by fracture type, fixation method, and methodological variability across studies.

Comparative evaluation of PINP

PINP consistently emerges as the most sensitive early formation marker. Clinical cohorts report a 30-70% rise between weeks 2-4 in fractures progressing to union, while blunted increases predict delayed healing.[24] Comparative analyses show PINP outperforms BALP and osteocalcin in early detection and demonstrates lower variability. Mechanical context influences trajectories: rigid plating yields smaller, stable rises, whereas intramedullary nailing produces higher elevations, reflecting enhanced callus formation. These findings establish PINP as the most reliable early biomarker, though interpretation must consider fixation mechanics.[7,23,24]

Evidence for CTX and TRACP-5b

CTX rises rapidly within the first week but is less discriminative early, as initial spikes occur in both normal and delayed unions. Persistent elevation beyond week 6 is strongly associated with delayed healing.[30] TRACP-5b, being osteoclast-specific and minimally affected by renal function, shows more consistent patterns and greater predictive value for pathological resorption.[34] Long-bone non-union studies indicate TRACP-5b outperforms CTX in distinguishing abnormal resorption from normal turnover, highlighting its utility in monitoring fracture healing trajectories.[34]

Comparative utility of BALP and osteocalcin

BALP rises later than PINP, reflecting the mineralisation phase. Studies of humeral and femoral fractures show prominent BALP increases in fractures healing via external callus formation, whereas rigidly plated fractures healing intra membranously exhibit blunted responses.[65] Osteocalcin is released during both formation and resorption, with delayed elevation in early remodelling, limiting its early predictive value. Overall, BALP is useful for monitoring callus mineralisation but less effective for early prognosis.

Multi-marker panels

Multi-marker panels consistently outperform single BTMs. Combinations of PINP, CTX, and TRACP-5b achieve higher diagnostic accuracy (AUC > 0.85).[42] Dual- or triple-marker models reduce false positives and improve early detection. For example, a PINP–BALP–CTX panel improved predictive accuracy by 22% and identified biological failure over two weeks earlier than PINP alone.[65] These findings underscore the advantage of integrated biomarker assessment.

Integration with imaging

Combining BTMs with imaging enhances predictive performance. PINP paired with quantitative ultrasound detects delayed union 3-4 weeks before radiographs. CT volumetric callus density integrated with BALP correlates better with biomechanical strength than either method alone.[66] The BBR Trifecta Model, integrating biochemical, biomechanical, and radiological parameters, demonstrates superior prognostic accuracy in comparative studies,

supporting multimodal evaluation for precision fracture care.

Inter-study variability and methodological considerations

Considerable variability exists across BTM studies due to differences in sampling schedules, assay platforms (ELISA vs chemiluminescence), fracture type, fixation method, and patient comorbidities such as diabetes, osteoporosis, or renal insufficiency.[40] Standardized protocols, fracture-specific reference ranges, and assay harmonization are needed to reduce inconsistencies. Comparative evidence confirms PINP as the most robust early predictor, particularly when combined with resorption markers (CTX, TRACP-5b) and imaging.[65] BALP provides insights during mineralisation, especially in callus-dominant healing.[66] Integrated biochemical–radiological–biomechanical approaches, along with emerging biomarkers like miRNAs and proteomic panels, enhance predictive accuracy within precision fracture care.

CONCLUSION

Fracture healing is a complex, tightly regulated process involving immunological, cellular, vascular, and biomechanical interactions that progress through inflammatory, reparative, and remodelling phases. Bone turnover markers (btms) provide a dynamic biochemical and molecular reflection of these processes, enabling real-time monitoring of healing at biochemical and molecular level well before radiographic changes become evident. Despite their significant clinical promise, the widespread application of btms remains limited by biological variability, diurnal fluctuations, inter-assay differences, and the lack of standardized, fracture-specific reference ranges. Integrating btms with radiological and biomechanical assessments through the biochemical, biomechanical, and radiological (bbr) trifecta model offers a more comprehensive and clinically meaningful framework for fracture evaluation, enabling clinicians to distinguish between biological, mechanical, and structural causes of impaired healing and to monitor progression over time. Future advancements incorporating emerging biomarkers such as micrornas, proteomics, and extracellular vesicles, along with artificial intelligence driven predictive models, are expected to address current limitations and advance the field toward more precise, personalized, and proactive fracture care.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Uttar Pradesh Council of Science and Technology (UPCST) for supporting this research work.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: Not required

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Cite this article as: Ali H, Singh AK, Baghel A, Asif S. Role of bone turnover markers in prediction of fracture healing: a contemporary evidence-based perspective. Int J Res Orthop 2026;12:848-59.