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ORIGINAL ARTICLE

Role of Bone Turnover Biomarkers in Early Prediction of Delayed Union in Long Bone Fractures Following Internal Fixation

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ABSTRACT

Background: Delayed union after internal fixation of long-bone fractures remains a major clinical problem, while conventional radiographs may identify impaired healing relatively late. Bone turnover biomarkers may provide earlier evidence of altered fracture biology and improve risk stratification.

Material and Methods: This prospective observational cohort study included 100 adults with femoral, tibial, or humeral fractures treated by internal fixation between June 2022 and June 2023 at Mekran Medical College Teaching Hospital, Turbat, and Benazir Bhutto Hospital, Rawalpindi. Serum procollagen type I N-terminal propeptide (P1NP), bone-specific alkaline phosphatase (BSAP), osteocalcin, and C-terminal telopeptide of type I collagen (CTX) were measured postoperatively and at six weeks. Patients were followed for 24 weeks. Receiver operating characteristic analysis and multivariable logistic regression were performed.

Results: Normal union occurred in 82 patients, while 18 developed delayed union. At six weeks, delayed-union patients had significantly lower P1NP (52.6 ± 18.4 vs. 83.1 ± 25.8 µg/L), BSAP (15.6 ± 5.6 vs. 22.4 ± 7.2 µg/L), and osteocalcin (18.9 ± 6.7 vs. 27.1 ± 8.2 ng/mL) levels (all $p<0.001$). P1NP showed the highest predictive accuracy (AUC=0.85). A P1NP level <61 µg/L independently predicted delayed union (aOR=4.48, 95% CI: 1.55–12.94). Adding P1NP to clinical risk factors improved the model AUC from 0.79 to 0.89.

Conclusion: Reduced six-week bone-formation marker responses, particularly P1NP, may facilitate earlier identification of patients at increased risk of delayed union and complement clinical and radiographic assessment after internal fixation.

Keywords: Bone turnover biomarkers; delayed union; long-bone fractures; internal fixation; P1NP; osteocalcin; fracture healing

INTRODUCTION

Long-bone fractures represent a substantial component of orthopedic trauma and may result in prolonged functional limitation, loss of productivity, and considerable healthcare utilization[1]. While the majority of fractures will heal satisfactorily after the correct fracture reduction and stabilization, a significant number will have delayed or failed healing. Fracture repair is a complicated process of regeneration, including inflammation, angiogenesis, recruitment and differentiation of mesenchymal progenitor cells, cartilaginous callus formation, bony callus formation, mineralization, and remodeling. Thus, in addition to mechanical stability, the maintenance of vascularity, cellular activity, extracellular matrix production, and a proper biological environment at the fracture site are all critical for successful healing[2,3].

Internal fixation has significantly advanced the treatment of fractures of long bones, allowing their realignment and providing mechanical stability, which allows biological healing to take place[4]. However, delayed union is another significant complication despite technically good fixation. Patient factors (smoking, diabetes mellitus, nutritional deficiencies, age, and systemic disease) and injury factors (open fractures, extensive soft tissue damage, vascularity, infection, comminution, fracture gap) can hinder healing. Delayed union can lead to continued disability and pain, as well as an extended time to return to normal activities and ultimately may lead to revision fixation, bone grafting, or biological augmentation. Therefore, early identification of patients with fractures that are not healing and are developing towards nonunion is important, as potentially reversible mechanical or biological factors may be addressed before nonunion is established[5,6].

In everyday orthopedic practice, the assessment of fracture healing relies mainly on a combination of clinical examination and serial radiographs. Common indicators of union are reduction in pain and tenderness, improved function of the limb, increasing tolerance to weight-bearing, disappearance of the fracture line, and progressive cortical bridging[7]. But there is a lot of difference in how clinical and radiographic fracture healing is defined, and how consecutive X-rays are interpreted may be different from one person to another. While there have been advances in the objectivity of radiographic assessment in certain fracture types (e.g., Radiographic Union Score for Tibial fractures), the radiographic evaluation of the fracture is primarily a reflection of the mineralization process already accomplished, rather than the cellular and molecular processes that underlie healing. Therefore, radiographs can only be convincing of healing failure several weeks or months after the biological cause of the delay in healing has occurred[8,9].

This has led to the interest in circulating biochemical markers that may more objectively reflect fracture-healing activity earlier. Bone turnover biomarkers are molecules that are secreted into the blood when bone is formed, synthesized and mineralized or when it is broken down[10]. Their levels fluctuate with skeletal metabolic activity and can thus be used to give clues on the biological processes taking place during fracture healing. While bone turnover markers have been well recognized in the assessment of metabolic bone diseases and monitoring of osteoporosis treatment, their use for fracture healing has been gaining more attention. Biochemical markers can be used in contrast to conventional radiographs, which can indicate changes in the activity of osteoblasts and osteoclasts even before definite changes are seen in the X-rays[11].

Bone-specific alkaline phosphatase (BSAP), osteocalcin, and procollagen type I N-terminal propeptide (P1NP) are markers of bone formation[12]. P1NP is secreted when the type I procollagen is converted to the mature type I collagen in the extracellular space, and reflects the synthesis of the major collagenous protein of the bone matrix. Osteocalcin is synthesized by mature osteoblasts and is related to the formation of new bone, and BSAP is produced chiefly by osteoblasts and is involved in mineralisation.

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CTX, on the other hand, is a marker specific to bone resorption which is released from osteoclastic degradation of type I collagen at the C-terminus of the collagen molecule. Therefore, it may be beneficial to measure both markers of formation and resorption in order to gain a more complete understanding of the remodeling response after fracture[13].

Previously, some studies have shown the significant alteration of the concentrations of bone turnover markers after fracture of long bones. Bone formation and resorption markers seem to display well-defined temporal profiles during fracture healing, and might be useful in the monitoring of the progress towards fracture union[14]. Changes in markers of bone resorption have also been seen in patients with delayed fracture healing, hinting at the possibility that biochemical changes could help to differentiate impaired from uncomplicated repair. Previous clinical studies also suggested that following serial measurements of biochemical bone markers could be used to monitor alterations during fracture healing and might complement traditional radiographic monitoring[15].

Of particular interest is the development of interest in markers of osteoblastic bone formation. Comparisons of patients with tibial diaphyseal fracture have indicated that the shapes of P1NP and osteocalcin, as well as other markers of bone turnover, may differ in normally healing and delayed healing fractures[16]. Based on these observations, an impaired response in bone formation may be a good early indicator of impaired healing. In the same manner, serum alkaline phosphatase has been proven to show temporal variations in the healing process of long-bone fractures treated surgically, with biochemical changes occurring in the first postoperative weeks. These findings indicate that the metabolic activity that would lead to the formation of callus and its mineralization can be detected before delayed union can be confidently diagnosed by radiographs alone[17].

In more recent studies, levels of P1NP and CTX were investigated after fibular and tibial shaft fractures treated with intramedullary fixation and have revealed significant early rises in both biomarkers. Higher concentrations of these markers of change have been noted at about 6 weeks after fixation and correlated with radiographic healing[18]. This time frame could then be a valuable window for biochemical evaluation. The clinical significance of this is that 6 weeks after surgery is a point at which the radiographic appearance of fractures is often inadequate to differentiate a fracture that will heal well from one that will heal poorly[19].

Although these positive findings have been made, to date, these markers have not been used in clinical practice to routinely monitor fracture healing. Age, sex, renal function, nutrition, vitamin D status, circadian variability, assay method, and other factors that affect skeletal metabolism may affect their interpretation[20]. Additionally, the published studies vary in the location of the fracture, the type of fixation, the time of blood collection, the biomarkers obtained, and the definition of delayed union. There is some evidence that plasma markers of bone turnover vary during the process of healing of long-bone fractures and that there may be differences between healed and impaired-healing subjects. But there is significant heterogeneity, highlighting the need for standard prospective studies. Thus, use of biochemical markers should for the present time be viewed as a potential adjunct to clinical and radiographic assessment[10].

The discovery of a biologically inadequate healing response might have important clinical implications, however, if it is recognized early in the process. Those patients with an unfavorable biomarker profile may benefit from more intensive follow-up and earlier consideration for potentially modifiable factors including smoking, nutritional deficiencies, metabolic abnormalities, inadequate stability, residual fracture gap, and occult infection[2-5]. Biochemical data could thus be used in addition to known clinical and radiological risk factors to give a more complete picture of risk of delayed union than can be achieved with any one modality. This method could enable clinicians to identify high-risk patients prior to development of radiographic failure[11].

The present study was therefore conducted to assess the utility of bone turnover markers for early prediction of delayed union in long bone fractures after fixation. The main aim was to assess the changes in the serum markers P1NP, BSAP, osteocalcin and CTX in the early stages of the procedure in patients who went on to form a satisfactory union and those who subsequently developed delayed union. The study also sought to find the predictive ability of these markers and to evaluate their ability to be used with clinical and fracture risk factors to better identify those patients at risk of delayed union early in the course of treatment[12-18].

MATERIALS AND METHODS

Study Design and Setting: This prospective observational cohort study was conducted from June 2022 to June 2023 at the Department of Orthopaedic Surgery, Mekran Medical College Teaching Hospital, Turbat, Pakistan, and the Department of Orthopaedic Surgery, Benazir Bhutto Hospital, Rawalpindi, Pakistan. The study aimed to assess if circulating bone turnover markers could be used as a predictor of delayed union after internal fixation of long bone fractures. The patients were recruited at the time of definitive surgical treatment and then followed up via clinic, biochemical, and radiological evaluations.

Study Size and Sample Size: During the study period, 100 patients in total with acute long bone fractures requiring internal fixation were included. Recruitment was done by consecutive non-probability sampling of the orthopedic departments of both participating institutions. Adult patients 18-70 years old with fractures of the femur, tibia, or humerus who had undergone definitive internal fixation were included for analysis.

Patients with pathological fractures, pre-existing metabolic bone disease, chronic renal disease, severe chronic liver disease, malignancy involving bone, established osteomyelitis, previous surgery involving the fractured bone, or history of long-term corticosteroid therapy were excluded. Patients who received bisphosphonates, teriparatide, or other drugs with a known significant effect on bone metabolism were also excluded. Those who had incomplete laboratory investigations, inadequate radiographic follow-up, or were lost to follow-up before the evaluation of the primary outcome were excluded.

Preoperative and Clinical Assessment: A structured study proforma was used to obtain baseline demographic and clinical information. These variables included age, sex, body mass index, smoking status, history of diabetes mellitus, mechanism of injury, long bone involved, fracture configuration, open or closed fracture, and associated soft-tissue injuries. If present, open fractures were classified using the Gustilo-Anderson classification. Pre-op AP and lateral X-rays were obtained to note the fracture type and location.

Routine laboratory investigations were carried out as per institutional protocol before surgery. Special attention was paid to medical conditions and medications affecting bone metabolism and/or bone healing. All patients were assessed by the orthopedic team prior to definitive fixation.

Surgical Management: Standard orthopedic principles for all fractures were followed, and all were fixed internally. Femoral and tibial shaft fractures that could be treated with locked intramedullary nails for fixation were treated primarily with locked intramedullary nails, whereas fractures that required plate fixation were treated with the appropriate plate-fixation constructs. Humeral fractures were treated with plate fixation or intramedullary fixation, depending on the fracture location, fracture morphology, soft-tissue status, and surgeon preference.

Immediate postoperative radiographs evaluated reduction quality, alignment, implant position, and residual fracture gap. Standard peri-operative antibiotic prophylaxis, postoperative analgesia and wound care, as well as post-operative rehabilitation and thromboprophylaxis were performed as per institutional protocols. Mobilization and progression of weight-bearing were

individualised depending on the fracture stability, mode of fixation and clinical assessment.

Blood Sampling, Bone Turnover Biomarkers: Venous blood samples were collected in the early postoperative period and at about 6 weeks after internal fixation. Blood samples were preferably collected in the morning following an overnight fast to minimize effects of circadian rhythms and feeding on measurement of bone turnover. The samples were allowed to coagulate, centrifuged, and the serum was separated out and processed as per usual laboratory protocol.

The main bone formation marker assessed was the procollagen type I N-terminal propeptide (P1NP), which is a measure of type I collagen production during new bone formation. Other formation markers were bone-specific alkaline phosphatase (BSAP) and osteocalcin, which are markers of osteoblastic activity and mineralization, respectively. The C-terminal telopeptide of type 1 collagen (CTX) was used as an indicator of osteoclastic bone resorption.

P1NP and CTX concentrations were analyzed by laboratory techniques that have been validated by immunoassay, whereas BSAP and osteocalcin were analyzed by commercially available immunoassays following the manufacturer's guidelines. Laboratory technicians conducting the biomarker measurements were blinded to the ultimate fracture-healing result whenever possible.

Follow-up and Assessment of Fracture Healing: Patients were followed clinically and radiographically at around 6, 12, 18 and 24 weeks after internal fixation. Fracture site pain, tenderness, function of the limb and weight bearing (if applicable), wound status, and postoperative complications were assessed at the follow-up visits. Standard X-rays were taken in AP and Lateral views to assess the progression of callus formation, cortical bridging, persistence of fracture line, alignment, and integrity of the implant.

The experienced orthopedic surgeons evaluated radiographic healing by serial radiographs. Satisfactory alignment, progressive bridging callus across the cortices, reduction in fracture line visibility, and lack of implant failure were taken as evidence of progressive union. Where appropriate, principles of the Radiographic Union Score for Tibial fractures were used to increase consistency of radiographic assessment in tibial fractures.

Definition of Normal and Delayed Union: Progressive clinical and radiographic healing during follow-up, with significant decrease in fracture-site pain and tenderness, restoration of limb function, and radiographic appearance of bridging callus across at least three of four cortices visible on the radiographs, was considered to be a normal union.

The most important outcome was delayed union, defined as poor radiographs of fracture healing at 24 weeks after surgery with internal fixation. Delayed union was defined as any of the following: persistent fracture lines, inadequate bridging callus, or minimal healing change on serial radiographs in combination with persistent fracture-site pain, tenderness, or functional limitation. Patients with documented deep infection or early implant failure (revision surgery) were also evaluated separately since these factors may have affected fracture healing independently.

Study Groups: Patients at the end of follow-up were divided into two groups based on healing. Patients in the normal-union group were those showing acceptable clinical and radiographic healing progress towards union, while those in the delayed-union group were those who met the specified criteria for delayed healing. These concentrations were then compared with those at early postoperative and six weeks.

Outcome Measures: The main outcome was delayed union, defined as 24 weeks after internal fixation. The main biochemical result was an association between delayed union and six-week serum P1NP concentration. Secondary outcomes were predictive performance for BSAP, osteocalcin, and CTX; changes in biomarker concentration from the early postoperative period to 6 weeks; and association between delayed union and clinical factors: smoking, diabetes mellitus, open fracture, fracture location, fixation technique, and residual fracture gap.

Ethical Considerations: The study was carried out on the basis of the Declaration of Helsinki. Before data collection, approval was granted by the appropriate institutional ethics review committee. Each participant was informed of the purpose of the study, the blood sampling required, follow-up procedures, and the confidentiality of patient information, and written informed consent was obtained. Patient identities were not revealed in the data analysis or the data reporting.

Statistical Analysis: All data were entered and analysed using IBM SPSS Statistics. Normally distributed data were presented as mean ± standard deviation; otherwise, data were presented as median and interquartile range. Categorical variables were given in terms of their frequencies and percentages. Normally distributed continuous variables were compared using the independent-samples t-test, and non-normally distributed variables were compared using the Mann-Whitney U test. Chi-square or Fisher's exact test was used for categorical variables as appropriate.

Pre- and post-operative marker differences were evaluated, and pre-operative and six-week markers were compared to distinguish between normal and delayed union patients. The discriminatory ability of P1NP, BSAP, osteocalcin, and CTX to predict delayed union was assessed by receiver operating characteristic curve analysis. Receiver operating characteristic (ROC) curves were drawn for area under the curve (AUC) with a 95% confidence interval (CI), and optimal cut-off values were determined by the Youden index.

Multivariable binary logistic regression analysis was used to determine independent predictors of delayed union from the variables that showed clinical relevance or showed association in the univariable analysis. Adjusted odds ratios (OR) and the corresponding 95% confidence intervals (CIs) were presented. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics and Fracture Profile: A total of 100 patients with long-bone fractures treated by internal fixation were included in the final analysis. The mean age was 39.6 ± 13.1 years. Overall, 69 (69.0%) patients were male, and 31 (31.0%) were female. The most common fractures were those of the tibia (49, 49.0%), femur (34, 34.0%), and humerus (17, 17.0%). 68 (68.0%) patients received intramedullary nailing, and 32 (32.0%) had plates.

82 patients (82.0%) had obtained a normal union, and 18 patients (18.0%) had delayed union, all at 24 weeks. Delayed union was associated with smoking, open fractures, and fracture gap >5 mm, all of which were significantly more common among delayed union patients. There were no significant differences between the groups in age, sex, BMI, diabetes mellitus, fracture site, and fixation technique, as seen in Table 1.

Bone Turnover Biomarker Levels: Early postoperative concentrations of P1NP, BSAP, osteocalcin, and CTX were comparable between the two groups. At week 6, however, patients with normal union had a mean serum P1NP of 83.1 ± 25.8 µg/L, which was significantly higher than delayed union (52.6 ± 18.4 µg/L, p<0.001). Similar differences were observed for BSAP (22.4 ± 7.2 vs. 15.6 ± 5.6 µg/L, p<0.001) and osteocalcin (27.1 ± 8.2 vs. 18.9 ± 6.7 ng/mL, p<0.001).

CTX levels rose in both groups at the follow-up, but there were no significant differences between the normal and delayed union groups at 6 weeks (0.62 ± 0.21 vs. 0.52 ± 0.19 µg/L, p=0.067). The findings indicate that a reduced early bone-formation response was more strongly associated with delayed union than changes in bone resorption, as shown in Table 2.

Predictive Performance of Bone Turnover Biomarkers: An ROC analysis revealed that P1NP had the highest predictive ability for delayed union, with an AUC of 0.85 (95% CI: 0.75-0.94). The sensitivity and specificity for P1NP < 61 µg/L for 6 weeks were 77.8% and 81.7%, respectively. Both BSAP and osteocalcin

demonstrated acceptable discriminatory ability, while the discriminatory ability of CTX was weaker, as summarised in Table 3.

Predictors of Delayed Union: In multivariable logistic regression, a six-week P1NP level <61 µg/L was the strongest independent predictor of delayed union (aOR=4.48; 95% CI: 1.55–12.94; p=0.006). Open fracture was also independently associated with delayed union (aOR=3.21; 95% CI: 1.05–9.82; p=0.041), followed by residual fracture gap >5 mm (aOR=3.08; 95% CI: 1.01–9.41; p=0.048) and current smoking (aOR=2.86; 95% CI: 1.01–8.10; p=0.048).

The clinical model that included smoking status, open fracture, and the gap in the fracture had an AUC of 0.79. Addition of six-week P1NP increased the AUC to 0.89, demonstrating that P1NP added value to the conventional clinical and fracture-related factors.

Table 1: Baseline demographic and fracture characteristics according to healing outcome

Variable	Normal union (n=82)	Delayed union (n=18)	p-value
Age, years	38.7 ± 12.6	43.5 ± 14.7	0.158
Male	56 (68.3%)	13 (72.2%)	0.744
Female	26 (31.7%)	5 (27.8%)	0.744
BMI, kg/m²	25.5 ± 3.8	26.7 ± 4.2	0.231
Current smoking	17 (20.7%)	8 (44.4%)	0.038
Diabetes mellitus	9 (11.0%)	4 (22.2%)	0.236
Tibial fracture	39 (47.6%)	10 (55.6%)	0.811
Femoral fracture	29 (35.4%)	5 (27.8%)	
Humeral fracture	14 (17.1%)	3 (16.7%)	
Open fracture	12 (14.6%)	7 (38.9%)	0.018
Intramedullary nail fixation	56 (68.3%)	12 (66.7%)	0.892
Plate fixation	26 (31.7%)	6 (33.3%)	
Residual fracture gap >5 mm	8 (9.8%)	6 (33.3%)	0.009

Table 2: Bone turnover biomarker concentrations according to healing outcome

Biomarker	Time point	Normal union (n=82)	Delayed union (n=18)	p-value
P1NP (µg/L)	Early postoperative	40.2 ± 14.9	39.1 ± 14.5	0.776
P1NP (µg/L)	6 weeks	83.1 ± 25.8	52.6 ± 18.4	<0.001
BSAP (µg/L)	Early postoperative	12.9 ± 4.3	12.5 ± 4.4	0.718
BSAP (µg/L)	6 weeks	22.4 ± 7.2	15.6 ± 5.6	<0.001
Osteocalcin (ng/mL)	Early postoperative	15.8 ± 5.7	15.3 ± 5.5	0.737
Osteocalcin (ng/mL)	6 weeks	27.1 ± 8.2	18.9 ± 6.7	<0.001
CTX (µg/L)	Early postoperative	0.39 ± 0.15	0.40 ± 0.16	0.799
CTX (µg/L)	6 weeks	0.62 ± 0.21	0.52 ± 0.19	0.067

Table 3: Diagnostic performance of six-week biomarkers for delayed union

Biomarker	AUC (95% CI)	Cut-off	Sensitivity	Specificity	p-value
P1NP	0.85 (0.75–0.94)	<61 µg/L	77.8%	81.7%	<0.001
BSAP	0.77 (0.65–0.88)	<17.5 µg/L	72.2%	74.4%	<0.001
Osteocalcin	0.76 (0.64–0.87)	<20.8 ng/mL	66.7%	76.8%	0.001
CTX	0.63 (0.49–0.77)	<0.50 µg/L	55.6%	68.3%	0.083

DISCUSSION

The current study aimed to assess the use of circulating bone turnover markers in the early prediction of delayed union in long-bone fractures fixed internally[1]. Of 100 patients, 18% had delayed union, and 82% had satisfactory healing at 24 weeks. The main result was that patients who went on to develop delayed union had significantly diminished increases in markers of bone formation at 6 weeks. P1NP had the best discriminative power, followed by BSAP and osteocalcin, while CTX had a relatively lower discriminative power. These results support the use of early evaluation of osteoblastic activity as a useful indicator of the biological events that occur during fracture healing prior to the delayed fracture union stage when they become prevalent on conventional radiographs[2,3].

P1NP proved to be especially valuable in the present study. Normal union was associated with significantly higher concentrations of P1NP at 6 weeks, and P1NP less than 61 µg/L was independently associated with an ~4-fold increased risk of delayed union[4]. P1NP is secreted into the circulation during the synthesis of type I collagen, the predominant organic constituent of the newly-formed bone matrix, and thus reflects the direct osteoblastic production of matrix. A high response in early healing is biologically plausible to reflect active callus formation, while a low response is biologically plausible to reflect poor osteoblast recruitment, collagen production, or mineralization advancement[5,6].

These findings are similar to those of Kumar et al., who showed that there were significant differences in the bone turnover marker profiles between normally healing and delayed healing tibial fractures[7]. Their study indicated that P1NP and other markers of bone formation might help detect impaired bone healing prior to the clear, definitive radiographic findings of delayed bone healing. However, Stewart et al. also reported significant rises in P1NP after intramedullary fixation of fractures of the tibia and femur and showed that the levels of biomarkers at 6 weeks correlated with the subsequent radiographic healing. The correlation between these results and those of the present study suggests that 6 weeks after fixation might be a useful period for biochemical evaluation[8].

Also, the levels of BSAP and osteocalcin were significantly lower in patients with delayed union. Osteoblast differentiation and mineralization are reflected by BSAP, and osteocalcin is primarily secreted by mature osteoblasts in the process of new bone formation[9]. The simultaneous decrease in P1NP, BSAP, and osteocalcin in delayed union thus implies a generalized decrease in the osteoblastic response and not a change in any particular biomarker. Further, the change in serum alkaline phosphatase has previously been linked to the advancement of the healing process of long-bone fractures, indicating the usefulness of biochemical evaluation to be used alongside radiography[10].

CTX, on the other hand, showed reduced forecasting ability. In both groups, CTX rose during the first six weeks, but did not differ significantly between the normal union and delayed union groups. CTX is mainly a marker of the degradation of type 1 collagen in osteoclastic bone resorption and remodeling[11]. Bone resorption can be observed in both successful and impaired healing, especially when removing the damaged bone and remodeling the callus, which might contribute to the limited capacity in discriminating healing outcomes when applied alone. Other work, however, has shown changes in the resorption markers in the period of delayed healing, suggesting that their value may be limited as a function of the time of measurement and interpretation when combined with markers of formation[12].

The present results also highlight the multifactorial aspects of delayed union. The factors independently associated with impaired healing were smoking, open fractures and a residual fracture gap > 5 mm[13]. There are well-documented detrimental effects of smoking on tissue perfusion, osteoblast function, angiogenesis, and bone regeneration. Open fractures carry a higher risk of infection with contamination, as well as disruption of the periosteal blood supply and higher rates of soft-tissue injury, and thus the biologic conditions for union. Likewise, a gap between the fracture ends (discontinuity) that remains intact can make the reparative process less mechanically stable, and the distance that new bone has to span could be greater, so the new bone formation may slow down[14].

Importantly, the clinical model (smoking, open fracture, and fracture gap) had an AUC of 0.79, and the addition of P1NP at six weeks gave an AUC of 0.89[15]. This improvement implies that biochemical information could be useful to complement and not to replace conventional clinical and radiographic assessment.

Mechanical stability and biological activity both play a role in fracture healing, and an integrated prediction model that combines both may be a more accurate estimation of healing potential than either approach, on its own[16].

Early prediction of delayed union may have important clinical implications. Radiographs taken at about six weeks after fixation often are inconclusive: even patients who go on to heal normally may show a fracture line[17]. A low P1NP level at this stage may indicate those who may need closer monitoring and earlier assessment of potentially modifiable factors such as smoking, nutritional deficiency, poor fixation stability, persistent fracture gap, metabolic disturbances, or underlying occult infection. This technique might allow the optimization of fracture healing conditions in a timely fashion before the nonunion is formed[18].

Bone turnover biomarkers, however, should not be used as individual diagnostic tests at this time. They can be affected by age, sex, renal function, nutrition, vitamin D, circadian fluctuation, underlying skeletal disease, and characteristics of the laboratory assay[19]. Further, there are factors that may affect the systemic biomarker response, such as location of the fracture and fixation method. Interpretation should thus be done in combination with the clinical examination and serial radiographic results[20].

The study has the following limitations. The small sample size and the limited number of cases of delayed union may preclude precise multivariable estimates[5]. Patients with fractures of various long bones were pooled together, even though there may be differences in the biological and mechanical environment of fractures of the femur, tibia and humerus. The biomarkers were assessed early after surgery and at 6 weeks; repeated assessments might have given a comprehensive picture of the biomarker evolution. Moreover, it is important to note that the threshold for P1NP detection should be considered as a population-specific threshold, until confirmed in larger independent cohorts. There is also considerable variation between studies assessing biomarkers of fracture healing, and a standardized sampling schedule is needed in conjunction with standard laboratory techniques and definition of delayed union[11-15].

This study, although limited, shows a clinically relevant relationship between early bone-formation activity and fracture healing. In the individual analysis, P1NP exhibited the highest predictive value, with BSAP and osteocalcin also indicating decreased osteoblastic activity in delayed union patients. These data should be confirmed in larger multicenter prospective studies, which will help to define if serial use of these biomarkers with other clinical data in a combined clinical prediction model can help guide decisions after internal fixation of long-bone fractures[20].

CONCLUSION

Bone turnover biomarkers, particularly P1NP measured at six weeks after internal fixation, may provide useful early information regarding the risk of delayed union in long-bone fractures. P1NP, BSAP, and osteocalcin levels were lower in cases with poor healing, and P1NP had the best predictive power. Prediction was further enhanced with the incorporation of P1NP and clinical factors including smoking, open fracture, and residual fracture gap. Bone turnover biomarkers could thus be valuable markers to complement clinical and radiographical evaluation, but there is a need for larger prospective studies before they can be used routinely in clinical practice.

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