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ORIGINAL ARTICLE

Prognostic potential of markers of bone turnover in delayed-healing tibial diaphyseal fractures

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Abstract

Introduction Clinical and radiographic examinations detect delayed or nonunion only after the event has occurred. Biochemical markers of bone turnover (BTMs) are promising laboratory tools that offer an early insight into the likelihood of delayed union. We hypothesized that BTMs display temporal variations following fractures and the behavior of BTMs differ between normal and delayed union of fractures.

Methods This was a prospective study of patients with closed fracture of tibia treated with intramedullary, interlocking nailing. BTM assays (NTX, BSAP, P1NP and osteocalcin) and clinical and radiographic assessments were obtained preoperatively and postoperatively at 8,12, 24, 36 and 72 weeks. Temporal trend of elevation of serum levels of BTMs postfracture was the primary assessment criterion and radiographic and clinical assessment of fracture union were the secondary assessment criteria.

Results The average time for fracture union was 15.24 weeks (range 15–19 weeks). The values of both bone formation and resorption markers peaked at the eighth week following the fracture. Resorption markers returned to baseline by 36 weeks. Among the formation markers, BSAP levels showed the smallest increase and returned to baseline earlier (36 weeks) than P1NP and osteocalcin (72 weeks). P1NP showed the most dramatic change, increasing to 2.5 times the mean baseline level at 8 weeks in normal union of fractures. The levels of bone formation markers (BSAP, OC, PINP) were significantly lower in patients with delayed union. There was no significant difference in the levels of the resorption marker (NTX) between normal and delayed union patients.

Conclusion Serial monitoring of biochemical markers of bone turnover can be used as an adjunct to clinical and radiological observations to predict delayed union

Level of evidence Level 2 (prospective observational study).

Keywords Bone turnover markers · Markers of bone formation · Markers of bone resorption · Fracture union · Delayed union of fractures

Introduction

Delayed union and nonunion occur in 5–10% of fractures and produce significant economic burden on patients and health care providers [1, 2]. Early detection of nonunions is essential to minimize their adverse economic and functional impact and facilitate monitoring the efficacy of recent interventions designed to accelerate fracture healing [3]. Clinical and radiographic examinations are most commonly employed for assessing union, but suffer from lack of consensus and objectivity [4, 5].

The reliability of plain radiographs and computerized tomography in determining the stage of union is poor [6, 7]. Ultrasonography has the ability to detect fracture union early, but relies heavily on the skills of the sonologist [8]. Other modalities that have been proposed for prediction of union include positron emission tomography, biomechanical testing for stiffness at the fracture site, vibrational analysis and virtual stress testing based on finite element analysis, but none have been validated thoroughly [9].

Bone turnover markers (BTMs) have the potential for early detection of nonunion [10–12]. BTMs include markers of bone formation and markers of bone resorption. Even

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though the post-fracture elevation of BTMs has been documented, there are considerable differences in the literature regarding the temporal sequence of BTM response and their effectiveness as predictors of delayed union.

We hypothesize that BTMs display temporal variations following fractures and explore the responses to the following questions—(a) whether the temporal variations in the serum levels of the currently used bone markers can predict union (in patients presenting with fresh, closed tibial fractures)? (b) Whether the behavior of bone markers was related to abnormalities in calcium and vitamin D metabolism?

Materials and methods

This is a prospective observational study of patients with closed, fresh fractures of the tibia/fibula who were managed with closed reduction and internal fixation using reamed interlocking intramedullary nails (between April 2012 and March 2014). The study was initiated after approval by the Institutional Review Committee. There were 233 patients (road traffic accidents in 221 patients and other modes of injury in 12 patients such as fall from height and sports injuries). Skeletally mature adults with closed diaphyseal fractures of the shafts of the tibia/fibula were included in the study. Exclusion criteria included tibial fractures in children, open tibial fractures, metaphyseal and intra-articular fractures, patients with abnormal renal function and patients on treatment for pre-existing metabolic bone disease and patients on long-term corticosteroid therapy [12–14]. Six patients were lost to follow-up. This left 168 skeletally mature adults with closed fractures of tibia/fibula who were eligible for inclusion [144 males and 24 females with a mean age of 31.8 years (range 20–79 years)].

Following surgical intervention, patients were advised partial weight bearing for 4 weeks followed by full weight bearing mobilization. Clinical parameters for fracture union included absence of limp and pain on bearing weight, absence of tenderness and abnormal mobility at the fracture site. Radiographic parameters included obliteration of fracture line and re-establishment of continuity in at least 3 out of 4 cortices on AP, lateral and oblique radiographs [15]. Radiographs and bone marker assays were repeated during post-operative follow-up at 8, 12, 24, 36 and 72 weeks.

The specific BTMs that were assayed included formation markers [BSAP (U/L), N-mid osteocalcin (ng/ml) and P1NP (ng/ml)] and resorption markers [NTX–β cross-laps(ng/ml)]. Blood samples were collected early in the morning after overnight fasting [16]. All patients were further assayed for serum calcium, phosphorus, vitamin D2, PTH-intact (parathormone), blood urea and serum creatinine. A total of 6 ml of blood was collected and was divided into 2 aliquots of 3 ml each. One sample was collected in a plain container (vitamin D2, calcium and phosphorous). The other sample was collected in an EDTA container (for PTH measurement). The calibrations of the measurements were done using the Siemens calibrator and Biorad control.

Delayed union was suspected when union was not evident even after 6 months and the outcomes remained uncertain [17]. Nonunion was diagnosed according to the guidelines of the FDA (Food and Drug Administration) of the US—failure of union after a minimum of 9 months from the date of fracture with no radiographic signs of progression of healing for 3 consecutive months [16]. Twenty-nine patients with delayed union underwent autologous iliac crest bone grafting after 6 months from the time of fracture and other interventions such as dynamization of the nail, fibular cuff resection, exchange reamed nailing, bone grafting. Seven out of these 21 patients failed to unite even after the initial episode of secondary interventions. Four patients required further bone grafting and exchange nailing and 3 patients underwent nail removal and application of circular Ilizarov fixation. There was no clinical evidence of infection in these patients and the erythrocyte sedimentation rate and C-reactive protein levels showed no abnormalities.

The data were analyzed using SPSS (SPSS Inc., Chicago, IL, version 19.0). Student's unpaired '*t*'' test was used to test the significance of difference between the groups and paired 't' test for within the group. A '*p*' value less than 0.05 denoted significant relationship. Sensitivity and specificity and ROC (receiver operating characteristic) curves were obtained for each of the BTMs employed in the study. AUC (area under curve) and 95% confidence intervals were calculated.

Results

The average time for union of fracture was 15.24 weeks (range 15–19 weeks). There were 29 patients with delay in union out of a total of 168 patients. Seven out of the 29 patients went on to develop nonunion even after one episode of bone grafting and required further interventions. In patients with normal union, both formation and resorption markers reached their peak values at around 8 weeks following the tibial fracture (Fig. 1a–d). The resorption markers reached baseline values by 36 weeks. Among the formation markers, BSAP levels showed the smallest increase and returned to baseline earlier (36 weeks) than P1NP and osteocalcin (72 weeks).

There was a statistically significant difference in the serum levels of bone formation markers (BSAP, P1NP and osteocalcin) at 8, 12 and 24 weeks between patients with normal fracture union and delayed union (Table 1). The levels were significantly lower in patients with delayed union. There was no statistically significant difference in the levels

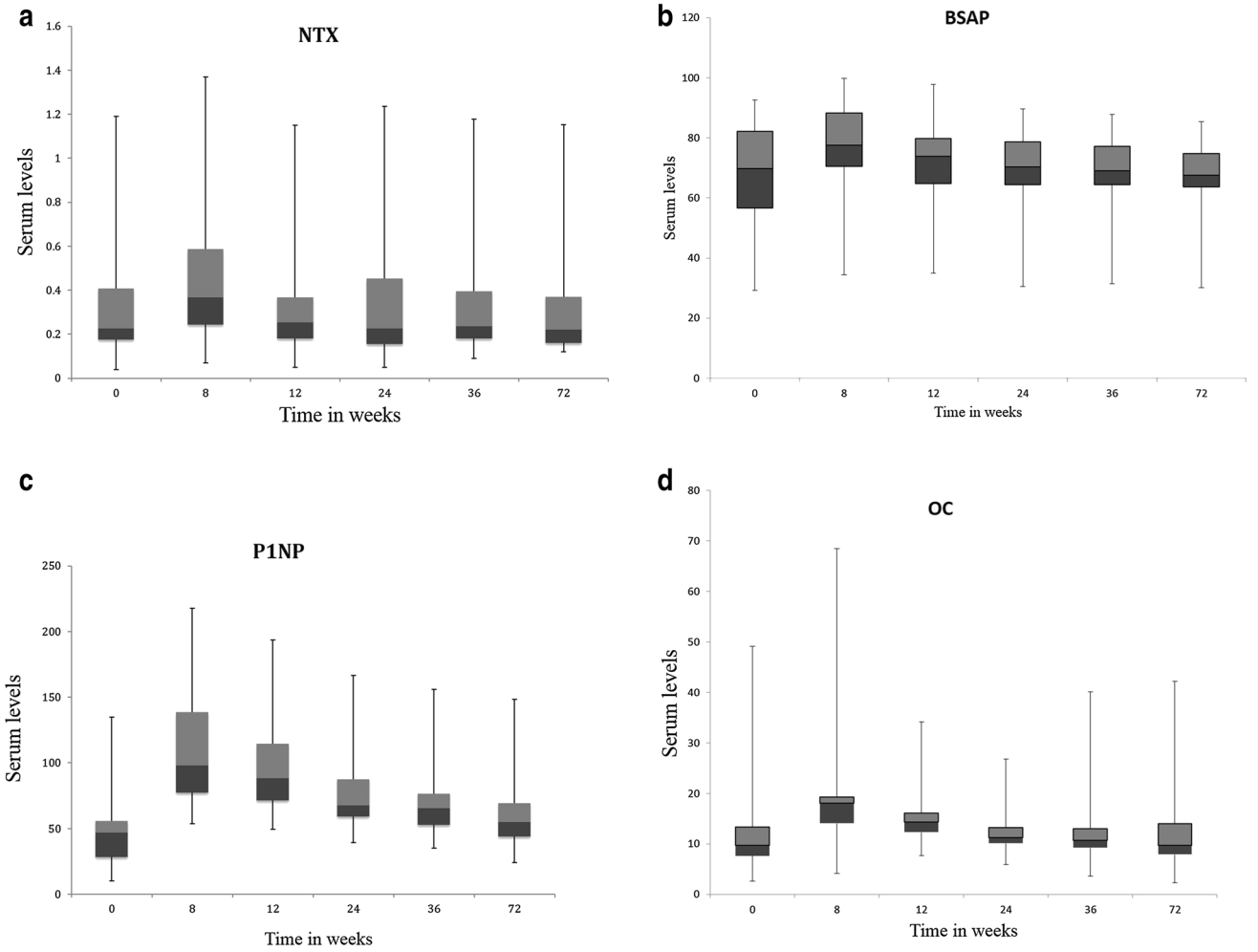

Fig. 1 **a–d** Box and whisker plots showing the serum levels of BTMs (BSAP, NTX, P1NP and OC) at specific time intervals in patients with normal union of fractures; top (grey-colored) and bottom (black-colored) of each box indicate upper and lower quartiles, horizontal line in each box indicates the median value and whiskers indicate the range between the minimum and maximum values; it can be seen that both formation markers (BSAP, P1NP, OC) and resorption marker (NTX) achieve peak levels around 8 weeks following the fracture; the levels of resorption marker (NTX) returned to baseline by 36 weeks; among the formation markers, BSAP levels returned to baseline by 24 weeks, osteocalcin levels by 36 weeks and P1NP by 72 weeks

of bone resorption markers (NTX) between patients with normal union and delayed union at baseline, 8, 12 and 24 weeks. The levels of both formation and resorption markers increased in the delayed union group after bone grafting (36th week samples).

The percentage increase in the BTM levels over time were plotted in patients with normal and delayed union (Table 2; Fig. 2a–d). Maximal differences were observed in P1NP levels between normal and delayed union groups (120% increase over baseline in normal union and by 50% in the delayed union group). Figure 3a, b illustrates the occurrence of delayed union in a patient with low peak in PINP at 8 weeks following the fracture. Union was achieved following bone grafting and fibular cuff resection.

The sensitivity and specificity were maximum at the cut-off value of 75% increase. P1NP showed the best trends with a sensitivity of 0.82 at the specificity of 0.89 at the 75% cut-off value. Other BTMs were found to be much less sensitive and specific and BSAP showed the lowest sensitivity and specificity. The receiver operating characteristic (ROC) curve of P1NP showed the most optimal curve pattern (AUC—0.9, 95% CI 0.8 and 0.95) (Fig. 4a–d).

There were no statistically significant differences in the mean serum levels of calcium, phosphorus, vitamin D and

Table 1 Difference in the post-fracture rise in the values of bone markers for patients with normal and delayed healing of fractures

BTM	Value for patients with				'*p*'
	Normal healing		Delayed healing		
	Mean	SD	Mean	SD	
Resorption marker (NTX/beta cross-laps)					
8 weeks	0.5	0.37	0.42	0.18	0.421
12 weeks	0.33	0.25	0.44	0.21	0.077
24 weeks	0.33	0.24	0.43	0.23	0.107
Formation marker (BSAP)					
8 weeks	77.9	13.5	61.9	13.5	**0.001**
12 weeks	72.3	13.0	58.9	23.4	**0.004**
24 weeks	70.4	11.8	57.5	20.0	**0.002**
Formation marker (osteocalcin)					
8 weeks	18.6	9.0	9.3	5.6	**< 0.001**
12 weeks	15.0	4.5	8.6	4.5	**< 0.001**
24 weeks	12.3	3.5	8.5	3.9	**< 0.001**
Formation marker (PINP)					
8 weeks	112.5	45.1	62.2	31.2	**< 0.001**
12 weeks	99.0	37.3	50.6	27.9	**< 0.0001**
24 weeks	74.9	21.7	46.3	20.9	**< 0.0001**

All the significant values have been marked in bold

parathormone between patients with normal union and delayed union (Table 3).

Discussion

In the present study, 29 of the 168 patients with acute, closed tibial fractures developed delayed union. The differences in the BTM levels between normal union and delayed union cohorts were statistically significant. Thus, a delayed or suboptimal peaking of BTM level at around 8-week post-fracture could be a potentially useful warning system to the possibility of eventual delay in union. This is especially applicable to P1NP which showed a 2.5 times increase in its mean value over the baseline value at 8 weeks. There were no significant differences in the metabolic profile between patients with normal union and delayed union.

Serum levels of BTMs are raised following fractures, but there are variations between the studies in the time taken by different markers to attain peak and trough values [18, 19]. Resorption markers tend to peak early and return to pre-fracture levels by around 6 months, whereas the formation markers peak later and take 1 year of more to return to normal. In our study, resorption markers peaked between 7 and 9 weeks after the fracture and returned to baseline by 6 months. Bone formation markers peaked between 7 and 9 weeks after the fracture, followed by gradual decrease towards the baseline by 1 year or more.

There is disagreement regarding the quantitative response of specific BTMs. While alkaline phosphatase was reported to be less useful than PIIINP or osteocalcin by Southwood et al. [20], Emani et al. [21] concluded that alkaline phosphatase was more responsive than osteocalcin. Mallmin et al. found no change in resorption markers and increase only in the formation markers following fractures of the distal radius [22]. Stoffel et al. [23] found a peak in PIIINP levels around 12 weeks followed by a decrease while the fracture healed.

Even though the levels of BTMs are raised following fractures, earlier studies on animal models concluded that BTMs were not useful in prediction of fracture union [24, 25]. The initial lack of support for the role of BTMs was replaced by more optimism regarding their potential, following human studies [21, 26–28]. Recent study by Granchi et al. showed that BTMs (BALP, P1CP and CTX) increased following stem cell therapy of long bone nonunions [29]. The use of multiple markers has led to considerable differences between studies and narrowing the list of candidate markers is likely to be useful for comparison between studies. The current study adds new information regarding P1NP. PINP is felt to be a more specific index of bone formation than ALP or osteocalcin; has longer half-life and less diurnal variation. It

Table 2 Percentage difference in mean values of BTM between baseline value at 0 weeks and 8, 12, 24, 36 and 72 weeks following fracture

Time interval (weeks)	BSAP—Units/liter (% increase over baseline)		NTX—ng/ml (% increase over baseline)		P1NP—ng/ml (% increase over baseline)		OC—ng/ml (% increase over baseline)	
	Normal healing	Delayed healing	Normal healing	Delayed healing	Normal healing	Delayed healing	Normal healing	Delayed healing
8	12.09	10.53	61.29	50	122.52	48.8	66.07	52.45
12	4.03	3.35	6.45	60.71	95.65	21.05	33.92	42.62
24	1.29	2.67	6.45	53.57	47.62	10.76	9.82	40.98
36	0.028	44.64*	0.96	64.28*	30.83	50.47*	9.82	163.93*
72	3.02	53.21*	5.16	75*	13.43	77.03*	0.89	172.13*

* Values are those following bone grafting in patients with delayed union

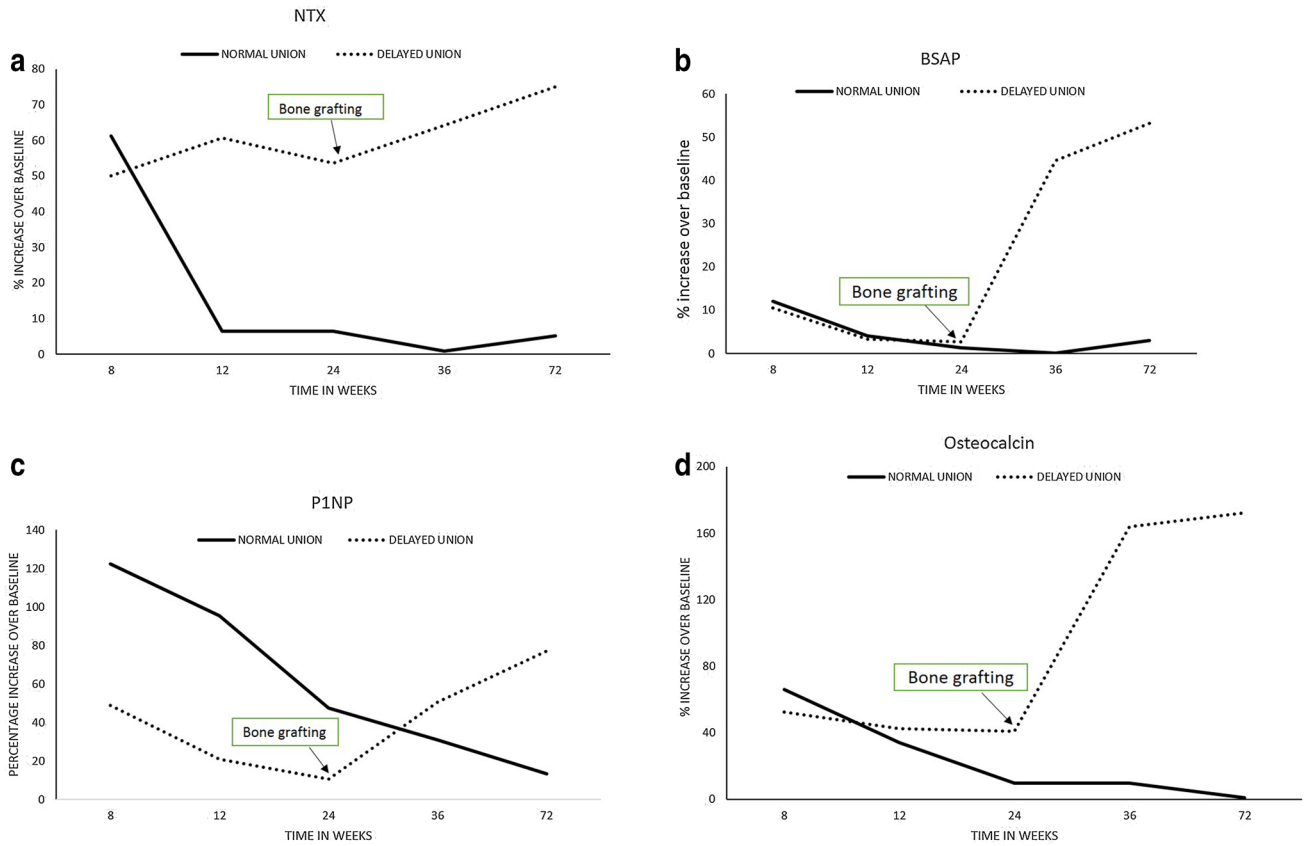

Fig. 2 **a**–**d** Line graphs comparing the percentage difference in BTM levels over the baseline value in patients with normal healing and delayed healing of fractures; bone grafting has been performed at 24 weeks in patients with delayed union

is already being used as a marker in metabolic bone diseases [12, 13].

The drawbacks of the current study are that levels of BTMs depend on the severity of the fracture and the specific bone involved and the level of the fracture in the individual bone [23, 30]. Thus, results cannot be generalized to all fractures in all long bones. In addition, BTMs are susceptible to diurnal variations and the metabolic status of the host prior to the fracture. Low specificity of the markers to bone is another important consideration (production and destruction of collagen is not limited to bone alone and BTMs may reflect these changes also). Factors other than fractures may also influence the variations in BTM levels. In the present study, we excluded children, open tibial fractures, patients with abnormal renal function, patients on treatment for pre-existing metabolic bone disease and patients on long-term corticosteroid therapy from the study. However, there may be other unknown confounders and multi-center trials involving large cohorts of patients are essential in this regard.

Conclusion

Delay in the appearance of peak levels of BTMs following the fracture appears to be associated with delayed union. However, the behavior of the BTMs depend on multiple factors including the type of bone, type and location of the fracture, age and co-morbid conditions. Caution is required in the clinical interpretation of these parameters till further studies are available. Use of multiple markers increases the difficulty in interpretation and expenditure. P1NP is a good candidate for further evaluation due to its long half-life, diurnal stability, low inter-individual variability and stability in the serum at room temperature [31]. It seems to be an interesting marker for delayed union in closed diaphyseal tibial fractures and could be a useful aid to the surgeon to decide whether a bone graft will be necessary and to inform the patient of the probability of this risk.

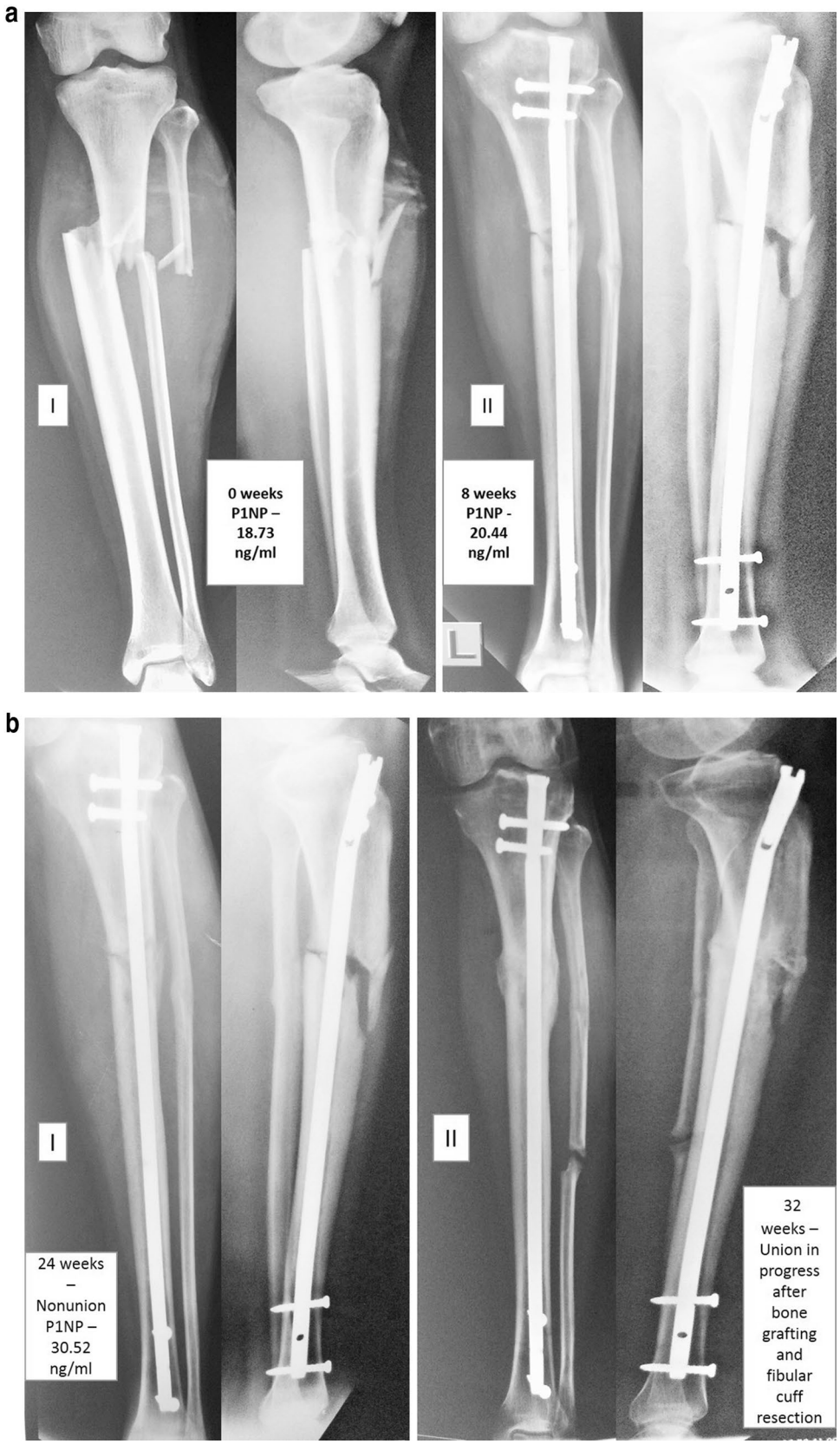

Fig. 3 **a** (1 and 2) – Plain radiographs (AP and lateral) of fracture of the tibia/fibula at 0 weeks (pre-operative) and 8 weeks (post-operative). P1NP levels have not increased to the expected peak levels at 8 weeks. **b** (1 and 2) Plain radiographs (AP and lateral) of the fracture of the tibia/fibula at 24 and 32 weeks. Nonunion is obvious at 24 weeks and P1NP levels continue to remain low. Bone grafting and fibular cuff resection were performed at 26 weeks and radiographs at 32 weeks show fracture union in progress

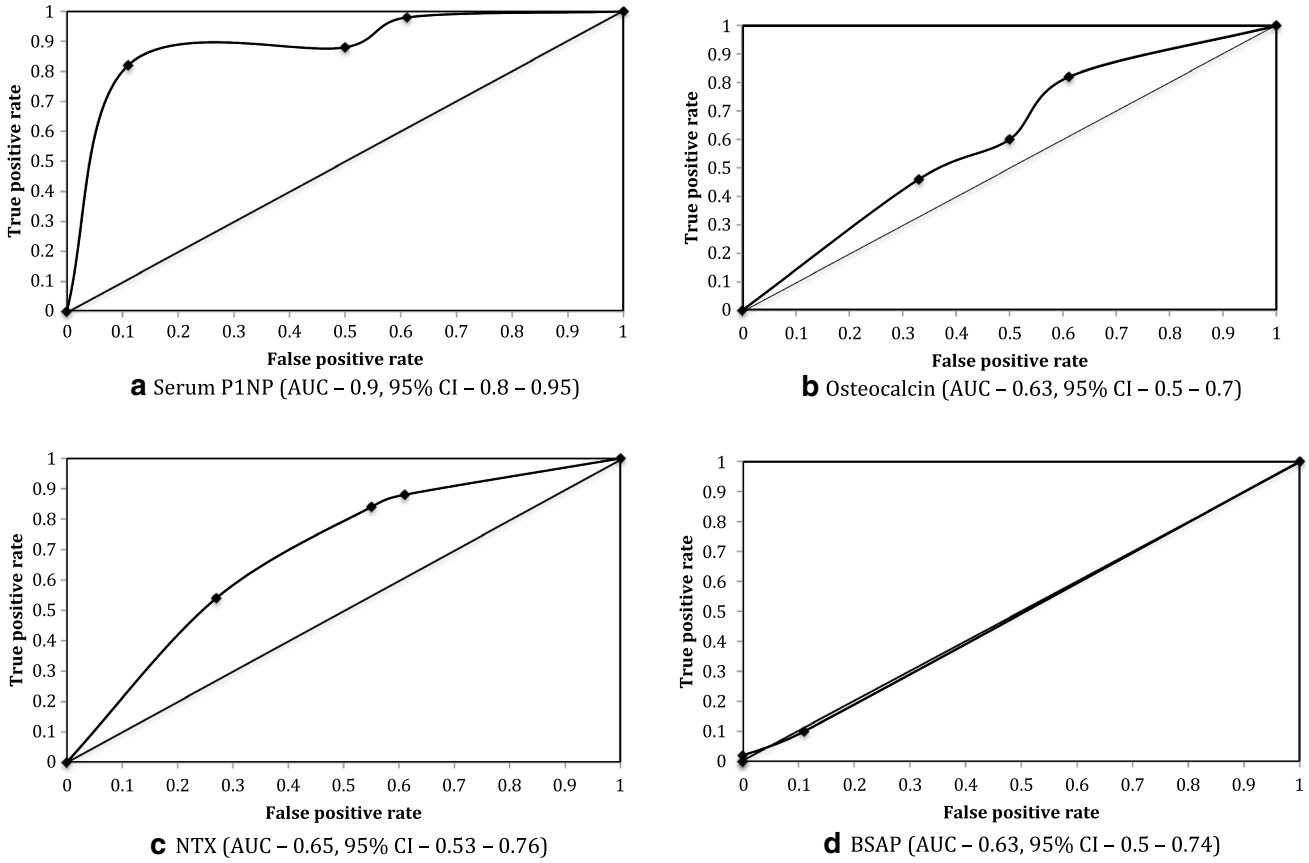

Fig. 4 **a**–**d** Receiver operating characteristics (ROC) of BTMs for percentage increase at 8 weeks over the baseline values

Table 3 Metabolic profile of patients with normal and delayed healing of fractures

Variable	Serum levels			
	Normal healing		Delayed healing	
	Mean	SD	Mean	SD
Serum calcium (mg/dl)	8.4	0.75	8.6	0.98
Serum phosphorus (mg/dl)	4.32	2.39	4.22	0.96
Vitamin D (ng/ml)	15.68	11.11	16.37	5.98
Parathormone (pg/ml)	62.3	33.8	59.3	40.1

Compliance with ethical standards

Conflict of interest Authors have no conflicts of interests.

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