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Quantitative evaluation of mineralization during multi-level bone transport for large segmental lower limb defects using pixel value ratio: a retrospective cohort study

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Abstract

Background

To quantitatively evaluate bone mineralization following bifocal (BF), trifocal (TF), tetrafocal (TBT), and pentafocal (PBT) bone transport using the pixel value ratio (PVR), and to compare the clinical efficacy of different bone transport strategies in treating large segmental defects (≥ 6 cm) of the lower limb.

Methods

A retrospective study was conducted on 122 patients with large lower-limb bone defects treated with bone transport at the First Affiliated Hospital of Xinjiang Medical University between 2017 and 2023. Patients were divided into four groups: BF (n = 50), TF (n = 37), TBT (n = 20), and PBT (n = 15). Standardized digital radiographs were obtained at 2, 4, 6, 8, 10, and 12 months postoperatively to measure PVR. Demographic and clinical data were collected, including age, sex, lengthening distance, healing time, healing index (HI), external fixation time, and external fixation index (EFI). Limb function recovery was assessed using the ASAMI scoring system.

Results

No significant differences were observed among the four groups regarding age, sex, BMI, lengthening distance, or high-risk factors (smoking, alcohol consumption, obesity, hypertension, diabetes) ($P > 0.05$). The PVR values in the TF, TBT, and PBT groups were significantly higher than those in the BF group at all time points ($P < 0.001$). Significant differences in HI and EFI were found among groups ($P < 0.001$), and both were negatively correlated with PVR. No statistically significant differences were found in the distribution of ASAMI bone or functional outcomes among the four groups ($P > 0.05$).

Conclusions

Multi-level bone transport enhances early mineralization efficiency and shortens external fixation duration without compromising final clinical outcomes. PVR serves as a reliable quantitative indicator for monitoring regenerate maturation and may assist in clinical decision-making regarding dynamization and frame removal.

Introduction

Since the 1950s, distraction osteogenesis has been widely applied in the management of bone defects caused by trauma, infection, tumors, and limb deformity correction.[1][2][3][4], However, patients with long segmental defects often require prolonged external fixation, which may lead to psychological disorders such as anxiety and sleep disturbance, as well as complications including poor regenerate formation, soft tissue interposition, intolerable pain due to soft tissue tension, and docking site nonunion.[5][6][7]To reduce treatment duration and complications, surgical techniques have evolved from single-segment to multi-segment bone transport. However, there is still a lack of quantitative radiographic evidence directly reflecting mineralization outcomes among different bone transport strategies.

Currently, evaluation of callus formation largely depends on subjective interpretation of radiographs and clinical symptoms, which may result in inconsistent clinical decision-making.[1][2]Premature weight-bearing or fixator removal may increase the risk of refracture.[8]

Although methods such as dual-energy X-ray absorptiometry and quantitative CT have been proposed, they are costly or involve additional radiation exposure.[3][9]Recently, the pixel value ratio (PVR), calculated as the ratio between regenerated bone and adjacent normal cortical bone on digital radiographs, has emerged as a practical and reproducible quantitative indicator.[3]Previous studies have confirmed its reliability in assessing callus maturation and guiding fixator removal.[10][11]Therefore, this study aimed to quantitatively compare mineralization outcomes among bifocal (BF), trifocal (TF), tetrafocal (TBT), and pentafocal (PBT) bone transport for defects ≥ 6 cm, and to establish evidence-based guidance for selecting optimal transport strategies based on defect length.

Patients and methods

Patient enrollment

From January 2017 to January 2023, the patients who underwent bone lengthening for reconstruction of large segmental bone defects of the lower extremity in the First Affiliated Hospital of Xinjiang Medical University were collected. The patient's age, gender, lengthening distance, osteotomy times, PVR value, healing index, external fixation time, external fixation index, and ASAMI scoring system were used to evaluate the postoperative limb function recovery.

Inclusion criteria: (1) Adult patients (≥ 18 years old) who underwent lower limb bone lengthening surgery due to bone defect. (2) Single-level, two-level, three-level, and four-level techniques were used for bone lengthening. (3) The length of bone defect was ≥6cm.

(4) Complete medical history and imaging data from bone lengthening surgery to removal of external fixator. (5) The follow-up time was up to 24 months after operation.

Exclusion criteria: (1) Patients under 18 years old. (2) Incomplete imaging data or missing follow-up. (3) patients with systemic diseases that significantly affect bone metabolism (e.g., uncontrolled diabetes, severe osteoporosis).

A total of 122 patients were enrolled in the study. The patients were divided into four groups according to the number of osteotomies: 1. Group BF, n = 50: bone defect ≥6cm treated with single-level bone lengthening. TF group, n = 37: bone defect ≥6cm treated with two-segment bone lengthening. TBT group, n = 20: bone defect ≥6cm treated with three-segment bone lengthening. PBT group, n = 15: bone defect ≥6cm treated with four-segment bone lengthening.

Surgical Technique and Postoperative Management

Patients underwent corticotomy and bone transport under either epidural or general anesthesia. Based on our previous experience in managing large tibial defects with distraction osteogenesis, the length of each individual transported bone segment was designed to be no less than 3–4 cm to ensure biological viability.

A circular external fixator (Ilizarov frame) or a unilateral rail external fixator was assembled and applied according to an individualized preoperative plan. Corticotomy was performed using a Gigli saw at the predetermined osteotomy site in order to preserve periosteal blood supply.

After a latency period of 7 days, distraction was initiated at a rate of 1 mm per day, divided into four increments. Early weight-bearing and passive joint exercises were encouraged beginning on the first postoperative day, as tolerated.

Patients underwent regular clinical examinations by specially trained surgeons, with weekly radiographic assessments during the distraction phase. The distraction rate and rhythm were adjusted when necessary based on clinical and radiographic findings.

Frame dynamization was performed once clear bridging callus was observed between distraction zones on radiographs and limb length equalization had been achieved, in order to enhance mechanical stability. After removal of the external fixator, limb protection with a cast or brace was maintained for at least 4–6 weeks, and partial weight-bearing was allowed following frame removal.

At the final clinical follow-up, bone and functional outcomes were evaluated using the ASAMI scoring system. Patients were encouraged to continue early joint mobilization and

partial weight-bearing within tolerance throughout the rehabilitation period.

PVR measurement

Standardized anteroposterior and lateral digital radiographs were taken at 2, 4, 6, 8, 10, and 12 months after surgery. Measurements were taken from anteroposterior and lateral views, including callus, proximal bone area, and distal bone area. For normal cortical bone, we selected an area extending approximately 2 cm from the edge of the bone defect. The average pixel value obtained for each region was recorded. The PVR was calculated using the following formula:

$$PVR=\frac{\text{Regenerated medial/lateral/posterior/anterior callus pixel value}}{(\text{Distal normal bone pixel value þ Proximal normal bone pixel value}) \div 2}$$

The anterior, posterior, medial, and lateral PVRS were calculated and the average of these four values was used as the final PVR (Fig. 1) (Fig. 2). A higher PVR value indicates better bone regeneration, and a value of 1 indicates mineralization equivalent to adjacent normal bone. In this study, the mean PVR of multiple regenerated bone sites with multi-segmental bone lengthening was calculated, resulting in a comprehensive and representative data that can be directly compared with the single-segment group. The use of mean values helps to reflect the overall picture and reduce the potential bias introduced by individual measurement points.

Three independent observers were assigned to perform the measurements separately, and the mean values were used for analysis to compare the PVR differences among the groups.

Figure 2 Measurement of pixel value ratio (PVR) in digital radiography. **a** Bifocal bone transport.**b** Trifocal bone transport.**c** Tetrafocal bone transport.**d** Pentafocal bone transport.

Statistical Methods

Statistical analysis was performed using IBM SPSS Statistics 27.0.1 (IBM Corp., Armonk, NY, USA). Shapiro-wilk test was used to assess normality for continuous variables. The normal distribution data were expressed as mean ± standard deviation ($\bar{x}\pm s$), and the non-normal distribution data were expressed as median (interquartile range). The categorical variables were expressed as the number of cases (percentage). one-way ANOVA was used to analyze the continuous variables among the four groups if normality and homogeneity of variance were satisfied, otherwise Kruskal-Wallis H test was used. The Chi-square test or Fisher's exact test was used for categorical variables. To compare the pixel values at

different time points, the linear mixed-effects model (LMM) was used to analyze the time effect, group effect and interaction of PVR: PVR was used as the dependent variable, group (BF/TF/TBT/PBT), time point (2/4/6/8/10/12months) and the interaction term "group × time" were used as fixed effects, and individual patients were used as random effects to deal with the intra-individual correlation. If the interaction term was significant, further post hoc comparisons between groups were performed at each time point, and Bonferroni correction was used to control type I error for multiple comparisons. All tests were two-sided, and P values<0.05 were considered to indicate statistical significance.

Results

Baseline characteristics

A total of 122 patients were enrolled in this study, including 50 patients in BF group, 37 patients in TF group, 20 patients in TBT group and 15 patients in PBT group. Male patients accounted for 82% (41/50) in BF group, 89.2% (33/37) in TF group, 80% (16/20) in TBT group and 66.7% (10/15) in PBT group. The mean age of the four groups was 34.16 ± 7.89 years in BF group, 36.00 ± 9.14 years in TF group, 39.45 ± 8.19 years in TBT group and 37.13 ± 8.42 years in PBT group. (Table 1.)

Tibia was the main bone lengthening site in the four groups, accounting for 72% (36/50) in BF group, 81.08% (30/37) in TF group, 80.0% (16/20) in TBT group, and 86.78% (13/15) in PBT group. This distribution reflects the high incidence of tibial defects requiring bone lengthening in clinical practice. The average lengthening distances of BF, TF, TBT and PBT groups were 8.48 ± 1.67 cm, 8.49 ± 1.96 cm and 7.67 ± 1.54 cm, respectively. There was no significant difference among the four groups.

Table 1

Demographic and clinical characteristics of patients undergoing bone transport.

Longitudinal PVR analysis, EFI and HI among the four groups

At all postoperative time points, multi-level groups (TF, TBT, PBT) exhibited significantly higher PVR values compared with the BF group ($P < 0.001$).(Table 2. Figure 3) A linear mixed-effects model was used to longitudinally analyze the PVR at 2, 4, 6, 8, 10, and 12 months after surgery. The results of the model showed that the time effect was significant ($P < 0.001$), suggesting that the degree of mineralization of regenerated bone continued to increase with the extension of time after surgery. The group effect was significant ($P < 0.001$), suggesting that the overall mineralization level of different segmental bone

transport techniques was different. The interaction effect of group × time was significant ($P < 0.001$), suggesting that there were differences in the trajectories of PVR over time in different groups. (Table 3) At 2 months after surgery, the overall PVR values of each group were low, indicating that the bone regeneration in the distraction area was still in the early stage. The PVR increased significantly in the first 4 months after surgery, with the highest increase, suggesting that this stage is a critical time window to evaluate the bone regeneration efficiency of different segmental bone lengthening techniques. From 6 to 12 months after surgery, the PVR value further increased and tended to be stable. Although there were still statistical differences among the groups, the differences gradually decreased over time, reflecting the gradual maturation of the regenerated bone and the final state of bone healing.

The external fixation index and healing index of each group are shown in Table 2, which reflect the bone healing efficiency of the bone lengthening technique. The HI and EFI were significantly lower in the other three groups than in the BF group, indicating that the bone defect treated with the multi-segment technique healed more quickly and required less external fixation for each centimeter extension. There was no significant difference in bone defect length among the four groups, but both HI and EFI were significantly lower in the PBT group than in the other three groups. This finding suggests that four-segment bone lengthening may reduce treatment cycles more than other options when treating large bone defects.

Table 2 :PVR at various time points ,external fixation index and healing index in the four groups

Table 3 Longitudinal PVR analysis

Figure 3 Trend of PVR in each group, single-segment bone lengthening group (BF), two-segment bone lengthening group (TF), three-segment bone lengthening group (TBT), four-segment bone lengthening group (PBT).

ASAMI outcomes

No statistically significant differences were observed among groups in final ASAMI bone or functional outcomes.Although the PBT group demonstrated numerically higher excellent/good rates, differences did not reach statistical significance.This suggests that while multi-level techniques improve process efficiency, final clinical outcomes are comparable.

Table 4 ASAMI scores of patients during follow-up

Criteria:Bone results Excellent: Union, no infection, deformity < 7°, limb length discrepancy (LLD) < 2.5 cm. Good: Union plus any two of the following: absence of infection, deformity < 7°, LLD < 2.5 cm.Fair: Union plus any one of the following: absence of infection, deformity < 7°, LLD < 2.5 cm.Poor: Nonunion/refracture/union plus infection plus deformity > 7° plus LLD > 2.5 cm.Functional results Excellent: Active, no limp, minimum stiffness (loss of < 15°knee extension/<15°ankle dorsiflexion) no reflex sympathetic dystrophy (RSD), insignificant pain.Good: Active, with one or two of the following: limb, stiffness, RSD, significant pain.Fair: Active, with three or all of the following: limb, stiffness, RSD, significant pain.Poor: Inactive (unemployment or inability to return to daily activities because of injury).Failure: Amputation.

Discussion

This study included 122 patients with large segmental lower limb defects (≥ 6 cm) treated between 2017 and 2023. For the first time within a single cohort, pixel value ratio (PVR) was used as the core quantitative indicator to dynamically compare the mineralization process of single-, double-, triple-, and quadruple-level bone transport (BF, TF, TBT, and PBT) from 2 to 12 months postoperatively.

The main findings were as follows. First, at all follow-up time points, PVR values in the multi-level groups (TF/TBT/PBT) were significantly higher than those in the single-level BF group, demonstrating a graded relationship in which a greater number of segments corresponded to higher PVR values. Second, PVR increased most rapidly during the first four postoperative months, suggesting that this interval represents a critical observation window for distinguishing mineralization efficiency among different segmental strategies. Third, both the healing index (HI) and external fixation index (EFI) were significantly lower in the multi-level groups and negatively correlated with PVR, indicating that improved mineralization quality may occur concurrently with shortened external fixation duration. Fourth, although significant differences were observed in radiographic quantitative parameters, no significant differences were found in the final ASAMI bone and functional outcome distributions, suggesting that different strategies can ultimately achieve comparable clinical results, while prolonged external fixation duration represents the direct trade-off.

Previous evaluations of distraction osteogenesis have largely relied on empirical radiographic interpretation, which is inherently subjective and may increase the risk of premature weight-bearing or frame removal, particularly in complex defect reconstruction [12][13][14].In contrast, PVR reflects regenerate mineralization by calculating the grayscale pixel ratio on digital radiographs. It can be obtained during routine outpatient follow-up and offers advantages of cost-effectiveness, reproducibility, and ease of clinical implementation[10][11].In the present study, PVR increased progressively over time in all

four groups. However, PBT, TBT, and TF consistently demonstrated significantly higher PVR values than BF at 2, 4, 6, 8, 10, and 12 months, with PBT showing the highest values and BF the lowest. These findings suggest that multi-level bone transport not only accelerates lengthening but, more importantly, enhances mineralization efficiency per unit time, enabling regenerated bone to approach normal cortical density more rapidly (PVR→1).

From a mechanistic perspective, the multi-level strategy distributes the total distraction length across multiple osteotomy sites, thereby reducing stress concentration and microenvironmental burden at each individual distraction zone. This may optimize vascular supply, mechanical stability, and tissue differentiation within the biological window of distraction osteogenesis, leading to more favorable early callus formation and mineralization trajectories. This interpretation is consistent with the principles emphasized by Ilizarov[14].Moreover, infection-related bone defects are inherently associated with delayed healing and increased complication risk. Therefore, optimizing transport strategies to shorten external fixation duration has practical implications for infection control and reconstruction stability throughout the treatment course[1][8].

Notably, the greatest increase in PVR occurred between 2 and 4 months postoperatively. Thereafter (6–12 months), PVR continued to rise but gradually plateaued, and intergroup differences narrowed over time. This dynamic pattern is clinically meaningful, as it indicates that differences among segmental techniques primarily influence the initiation and acceleration phases of mineralization rather than the final maturation stage. This observation is consistent with previous studies suggesting that early PVR can reliably predict regenerate quality[3], More importantly, it highlights a clinically actionable concept: the first four postoperative months should be regarded as a key management window. Within this period, PVR may be used to: 1. dynamically adjust distraction rate and rhythm; 2. assist in determining the timing of dynamization—previous studies have demonstrated its correlation with regenerate strength and maturity and its utility in guiding weight-bearing and frame removal decisions[11];3.identify patients with delayed mineralization and guide early intervention[16][17][18].

In this study, HI and EFI were significantly lower in the TF, TBT, and PBT groups compared with the BF group, and both indices were negatively correlated with PVR within each group. These findings are consistent with those reported by Liu et al.Liu[18].When treating similar defect lengths, multi-level bone transport shortens the distraction distance per segment, allowing earlier formation of high-quality regenerate bone. Consequently, stable weight-bearing and dynamization conditions may be achieved sooner, thereby reducing the duration of external fixation. In other words, multi-level transport does not sacrifice quality for speed; rather, it enhances lengthening efficiency through structured allocation of distraction length [9][20].This conclusion aligns with previous comparative

studies showing that double-level transport often outperforms single-level transport in terms of treatment duration and efficiency for tibial defects following infection[8][9].For large segmental defect reconstruction, a shared objective of optimization strategies is to reduce external fixation time and minimize the risk of complication[1][23].Additionally, prolonged external fixation has been associated with increased psychological burden, including anxiety and sleep disturbances[5].Therefore, shortening frame duration represents a meaningful clinical benefit, not only in terms of bone healing but also regarding overall rehabilitation experience and patient compliance.

Although significant differences were observed in PVR, HI, and EFI, no significant differences were detected in ASAMI bone or functional outcome grades. This finding is not contradictory. Rather, it suggests that by the mature stage (12-month follow-up), most patients achieve a comparable level of healing and function. As a grading system, ASAMI has limited sensitivity for detecting differences in process efficiency[13][22][23].The advantage of multi-level strategies is therefore more evident in reduced time cost and shorter risk exposure required to achieve equivalent final outcomes.

For lower limb defects ≥ 6 cm, multi-level bone transport may be considered one of the preferred strategies. Furthermore, using PVR as a core quantitative monitoring parameter allows the establishment of a reproducible follow-up and decision-making framework: during the 2–4 month window, close monitoring of the PVR slope can help identify insufficient mineralization; for dynamization timing, PVR may supplement or partially replace subjective assessment of cortical continuity; and for frame removal, combining PVR with conventional radiographic criteria may improve safety and interpretability.

Nevertheless, selection of the reconstruction strategy should continue to consider soft tissue conditions, infection control, transport distance, feasibility of osteotomy sites, and surgeon experience. This study provides a quantitative comparative framework that addresses the longstanding lack of direct mineralization-based evidence supporting multi-level transport strategies.

This study has several limitations. As a retrospective analysis, selection bias and unmeasured confounding cannot be completely excluded, despite comparable baseline characteristics among groups. In addition, although PVR offers accessibility and quantitative advantages, it may be influenced by radiographic parameters, ROI selection, soft tissue coverage, and metallic artifacts. Standardized imaging protocols and blinded dual-observer measurements were used to minimize bias. Future prospective or multicenter studies are warranted to validate these findings. Integrating PVR with complication profile and patient-reported outcomes may better reflect the comprehensive benefits in terms of efficiency, safety, and patient experience. Furthermore, predictive

models based on early PVR trajectories may facilitate individualized adjustment of distraction protocols and weight-bearing schedules.

Conclusion

Multi-level bone transport significantly enhances early mineralization efficiency and reduces external fixation duration without compromising final clinical outcomes.Pixel value ratio serves as a practical, reliable, and reproducible quantitative tool for monitoring regenerate maturation and guiding clinical decision-making.For large lower limb defects ≥ 6 cm, multi-level bone transport combined with PVR-guided follow-up may represent an optimized reconstruction strategy.

Abbreviations

BF
Bifocal bone transport
TF
Trifocal bone transport
TBT
Tetrafocal bone transport
PBT
Pentafocal bone transport
PVR
Pixel value ratio
HI
Healing index
EFI
External fixation index
ASAMI
Association for the Study and Application of the Method of Ilizarov
LMM
Linear mixed-effects model

Declarations

Ethics approval and consent to participate

This study was a retrospective, single-center comparison that was conducted in January 2017 and January 2023. The Institutional Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University approved the study protocol and waived the need for participant informed consent. Written informed consent was obtained from the

individual(s) for the publication of any potentially identifiable images or data included in this article.

Consent for publication

Not applicable.

Availability of data and materials

The data sets generated and analyzed during the current study are not publicly available due to restrictions on ethical approvals involving patient data and anonymity but can be obtained from the corresponding author as reasonably required.

Competing interests

The authors declare that they have no conflict of interest.

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Authors’ contribution

DX , KT and YW contributed equally to study design and manuscript drafting.

KT and AS collected clinical data.

AY supervised the study and revised the manuscript.

All authors read and approved the final manuscript.

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Not applicable.

Clinical trial number

Not applicable.

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Tables

Tables are available in the Supplementary Files section.

Figures

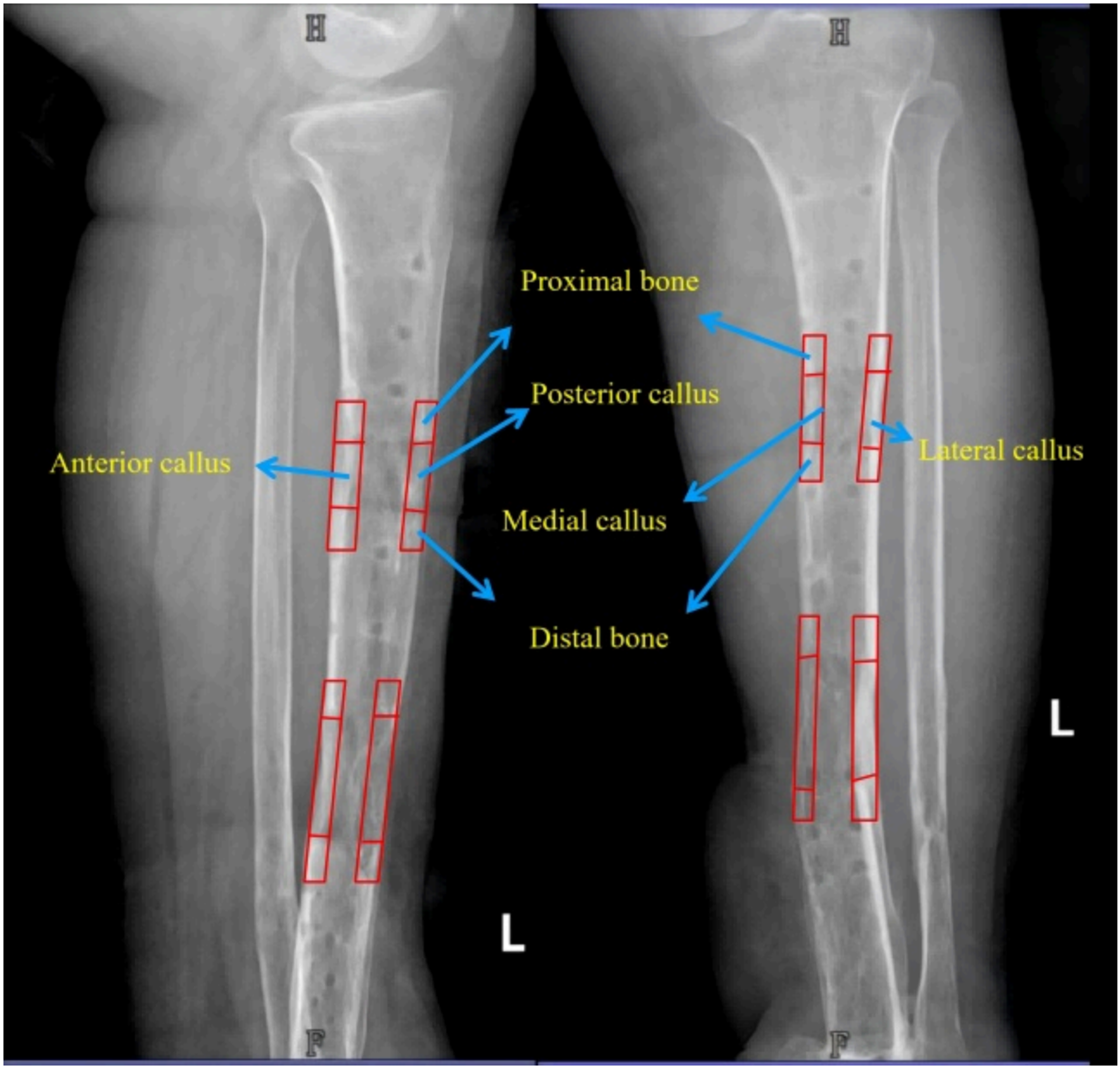

Figure 1

The assesment nof lateral, medial, anterior and posterior callus pixel value ratio.

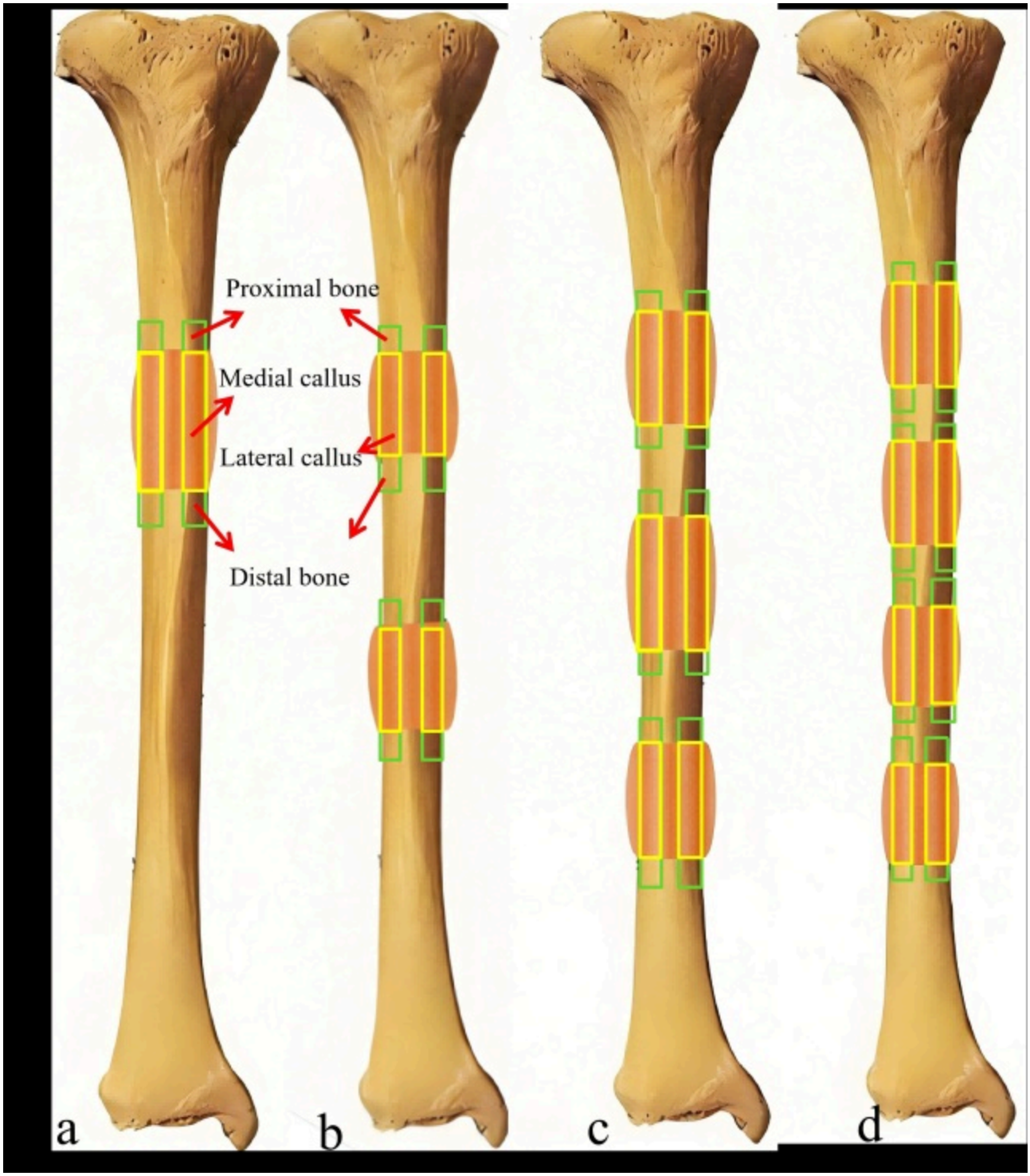

Figure 2

Measurement of pixel value ratio (PVR) in digital radiography. **a** Bifocal bone transport.**b** Trifocal bone transport.**c** Tetrafocal bone transport.**d** Pentafocal bone transport.

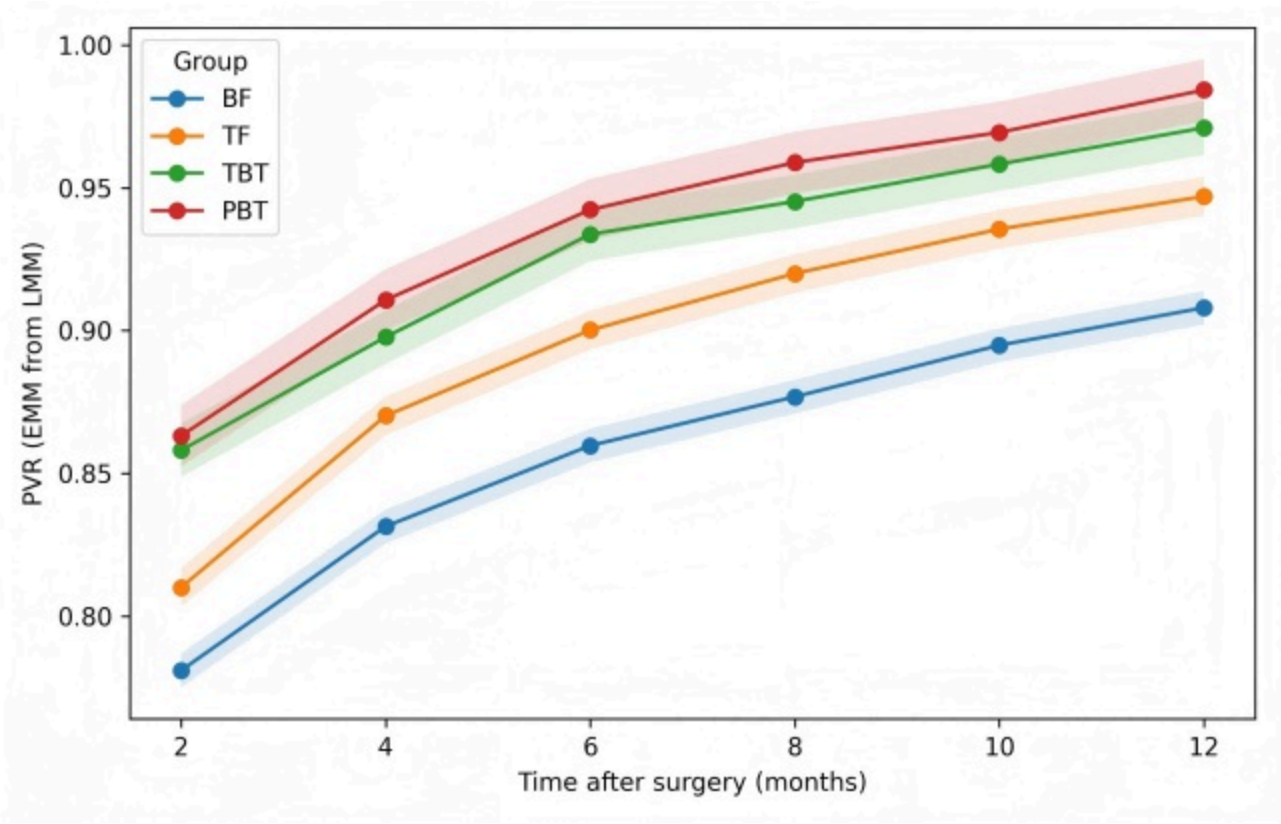

Figure 3

Trend of PVR in each group, single-segment bone lengthening group (BF), two-segment bone lengthening group (TF), three-segment bone lengthening group (TBT), four-segment bone lengthening group (PBT).

Supplementary Files

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