Autologous Bone Marrow Grafting Combined with Demineralized Bone Matrix Improves Consolidation of Docking Site After Distraction Osteogenesis

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Background: Distraction osteogenesis is used for the reconstruction of extensive osseous defects. Delay in docking site consolidation results in significant prolongation of this surgical procedure. The primary aim of the present study was to retrospectively compare three different treatment options, all aimed at improving and accelerating docking site consolidation. We further sought to clarify whether the application of autologous bone marrow cells combined with demineralized bone matrix would substantially improve docking site consolidation.

Methods: Between 1995 and 2008, forty-three patients (mean age, 38.28 years) were managed with bone transport for the treatment of a tibial bone defect (mean length, 9.49 cm). The patients were divided into three groups according to the ''docking site procedure'' used: closed compression (Group A), surgical debridement of the docking site and application of autologous iliac bone graft (Group B), or surgical debridement and local application of bone marrow concentrate and demineralized bone matrix (Group C). Docking site consolidation was assessed both radiographically and clinically, and the results were statistically analyzed.

Results: The median ''healing time'' required for docking site consolidation was significantly longer in the compression group as compared with the demineralized bone matrix plus bone marrow group ($p = 0.021$), whereas there was no difference between the other groups. There was no significant difference among the groups in terms of complication rates ($p = 0.702$). Docking site consolidation was completed prior to regenerate consolidation in nine of the ten patients in Group C and in 13.6% of the patients in Group B, whereas in all of the remaining patients, completion of regenerate healing always preceded docking site consolidation.

Conclusions: The application of demineralized bone matrix and autologous bone marrow is at least equivalent to autologous cancellous bone graft in terms of substantially reducing docking site healing time compared with closed compression alone. The application of demineralized bone matrix and autologous bone marrow is an effective treatment option, with minimal donor site morbidity, for reducing consolidation time of the docking site in tibial defects treated with distraction osteogenesis.

Level of Evidence: Therapeutic Level III. See Instructions to Authors for a complete description of levels of evidence.

The treatment of skeletal defects constitutes a major challenge in modern orthopaedic reconstructive surgery. The rate of segmental skeletal defects of traumatic origin in the United States is approximately 150,000 per year[1]. Adding the cases of skeletal defects resulting from the treatment of osteomyelitis or tumor resection further emphasizes the importance of the problem.

Distraction osteogenesis is one treatment method that is widely used for dealing with skeletal defects; this method produces new, vascularized bone that is structurally and qualitatively comparable with "normal" bone. The advantages of this method include the avoidance of extensive procedures to obtain large autogenous or free vascularized bone grafts, thus substantially lowering morbidity, providing the ability for early weight-bearing due to the support of the powerful external fixator, and leading to an increase of local blood flow through the stimulation of small-vessel angiogenesis[2,3].

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The technique consists of performing an osteotomy proximal or distal to the defect, then gradually transporting a healthy segment of bone while the remaining gap at the osteotomy site is gradually filled with regenerated bone, known as "regenerate." The site where the transported segment meets the target segment on completion of the bone transport is called the "docking site."

Although the docking site has been recognized as a frequent source of problems in terms of its ability to consolidate[1,4-7], resulting in substantial prolongation of the entire procedure and subsequent delay of fixator removal, most research has been focused on improving the maturation of the regenerate, not on achieving more effective docking site consolidation.

The primary aim of the present study was to compare three different treatment options to improve and accelerate docking site consolidation. We retrospectively reviewed three groups of patients with tibial segmental defects who were managed with distraction osteogenesis. The three treatment methods were (1) closed compression, (2) surgical debridement of the docking site and application of autologous iliac bone graft, and (3) surgical debridement of the docking site followed by local application of a mixture of autologous bone marrow concentrate and demineralized bone matrix (DBM).

Hence, in the present study, we aimed to compare a historical standard of compression with a gold standard of autogenous bone graft as well as with a newer method for healing at the "docking site" during bone transport. We hypothesized that both the time to consolidation and the rate of healing would be improved in association with the use of either autograft or demineralized bone matrix supplemented with bone marrow aspiration.

Materials and Methods

This retrospective case series study was approved by our hospital's ethical committee for clinical studies and was performed in accordance with the Greek guidelines for clinical studies. Between 1995 and 2008, forty-three patients (thirty-eight male and five female) with a mean age of 38.28 years (range,

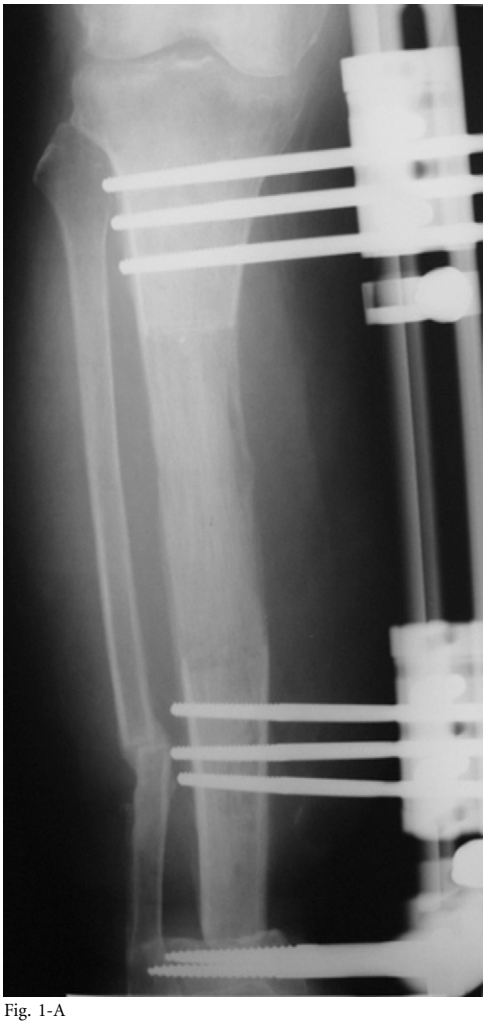

Fig. 1-A

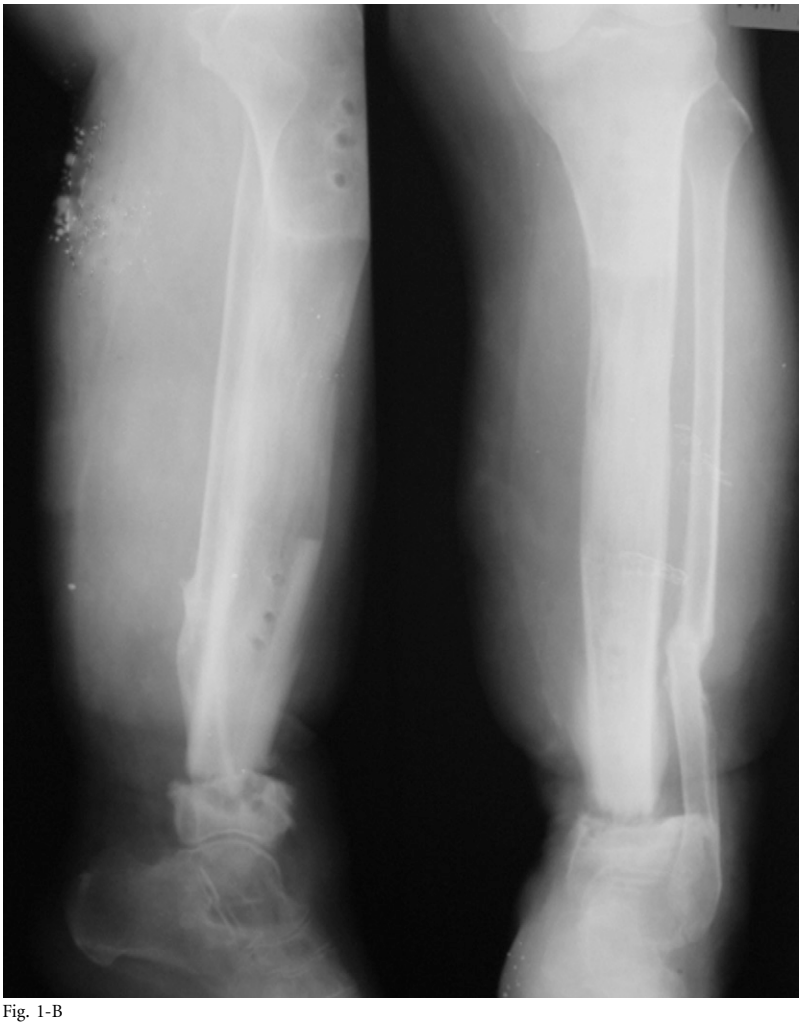

Fig. 1-B

Figs. 1-A and 1-B Radiographs for one of the two patients in Group A who had a nonunion that necessitated further surgical intervention. **Fig. 1-A** Radiograph showing the docking site immediately after the completion of compression. **Fig. 1-B** Although the consolidation of the regenerate was successfully completed, a nonunion of the docking site developed.

sixteen to seventy-four years) were managed with bone transport with an external fixator, according to the principles of distraction osteogenesis, for the treatment of a tibial bone defect. Thirty-four patients were managed with a monolateral frame, four were managed with a circular frame, and five were managed with a hybrid fixation system. Hydroxyapatite-coated pins were used in all cases in which a monolateral fixator was applied. Fixation was not extended across the ankle in any patient. The mean length of the bone defect was 9.49 cm (range, 3 to 22 cm). The defect was the result of septic pseudarthrosis in twenty-eight patients (65.1%), osteomyelitis in nine (20.9%), trauma in five (11.6%), and tumor resection in one (2.3%) (see Appendix). The location of the defect was the proximal metaphysis in five cases (11.6%), the diaphysis in twelve (27.9%), and the distal metaphysis in twenty-six (60.5%).

Through the distraction period, standard anteroposterior and lateral radiographs were made every three to four weeks to assess the distraction process. The patients were divided into groups according to the natural progression of the techniques that were used to achieve docking site consolidation as practiced during three different time periods. Completion of the distraction phase was dictated by the transported segment having reached the docking site, with no gap being >4 mm as verified on standard anteroposterior and lateral radiographs. Each technique was applied in a group of consecutive patients, and the order of the groups represents the chronological order of the different techniques as performed at our institution. More specifically, the closed compression method was used between 1995 and 1999 (Group A), the application of autologous cancellous iliac bone graft was used between 1999 and 2005 (Group B), and the application of a mixture of demineralized bone matrix and centrifuged autologous bone marrow was used between 2005 and 2008 (Group C) (see Appendix).

Group A

In Group A, the closed compression method, as proposed by Ilizarov, was applied in eleven patients (nine male, two female) with a mean age of 43.45 years (range, sixteen to seventy-four years). In this group, the mean length of the bone defect was 9.45 cm (range, 3 to 16 cm). The defect was the result of septic pseudarthrosis in eight cases, osteomyelitis in two, and tumor resection in one (see Appendix). After the transferred segment had reached the docking site, an overcompression of 3 mm was applied at the apposed segments through the external fixator at a rate of 1 mm per day in 0.25-mm increments every six hours. The whole procedure was performed closed, and the docking site was not surgically exposed. Static compression was initially used in all cases. The "accordion technique," as described both by Ilizarov and by others, was subsequently utilized in patients in this group with delayed union or nonunion.

Group B

Group B included twenty-two patients (twenty male and two female) with a mean age of thirty-six years (range, eighteen to sixty-seven years). In this group, surgical debridement of the interposed tissues and application of autologous cancellous iliac bone graft was performed just prior to docking completion.

With the patient in the supine position and under general anesthesia and with the application of a tourniquet, the docking site was surgically exposed and all interposing soft tissues at the apposed bone surfaces were meticulously removed, followed by freshening of the apposed bone surfaces (by means of the removal of 1 to 2 mm of bone) with use of rongeurs. Corticocancellous autogenous bone graft was harvested from the iliac crest to fill the docking-site gap. The bone surfaces were then apposed but not compressed. The mean length of the bone defect was 9.82 cm (range, 4 to 22 cm).The defect was the result of septic pseudarthrosis in thirteen cases, osteomyelitis in five, and trauma in four (see Appendix).

Group C

In Group C, surgical debridement of the docking site was followed by the application of a mixture of demineralized bone matrix and centrifuged autologous bone marrow aspirated from the ilium. This group consisted of ten patients (nine male, one female) with a mean age of 37.4 years (range, twenty-one to fifty-two years). The mean length of the bone defect in Group C was 8.8 cm (range, 4.5 to 14 cm). The defect was the result of septic pseudarthrosis in seven cases, osteomyelitis in two, and trauma in one (see Appendix).

Patients in this group underwent the same docking site preparation as did those in Group B. However, instead of autologous iliac bone graft application at the docking site, 60 mL of bone marrow was aspirated from the patient's iliac wing with an 11-gauge biopsy needle. The aspiration site was changed every 30 mL, and the obtained aspirate was centrifuged under aseptic conditions with a standardized procedure (BMAC; Harvest, Plymouth, Massachusetts). The resulting 8 to 10 mL of concentrate was mixed with 5 to 10 mL of Grafton Putty DBM (OST Developpement SA, Clermont-Ferrand, France), and the mixture was applied on the docking site interface.

Docking site consolidation was assessed on standard anteroposterior and lateral radiographs that were made every six weeks. When there was radiographic and clinical evidence of consolidation of the docking site and the regenerate with manual stress techniques, the external fixator was removed. The docking site consolidation process was considered to be successful when there was clear radiographic evidence of union within six months after "docking." In cases in which docking site consolidation occurred after the six-month period, the patient was considered to have a delayed union. In cases in which signs of union were not evident nine months after the completion of the distraction phase, the patient was considered to have a nonunion. Nonunion and fracture of the docking site were considered to be complications of the treatment method.

Statistical Analysis

Quantitative variables were compared among the groups with use of the Kruskal-Wallis test, and qualitative variables were compared among the groups with use of the Fisher exact test. The healing times were compared with use of the log-rank test, and the data are presented with Kaplan-Meier curves. The Bonferroni correction was used for the pairwise differences between groups, and the

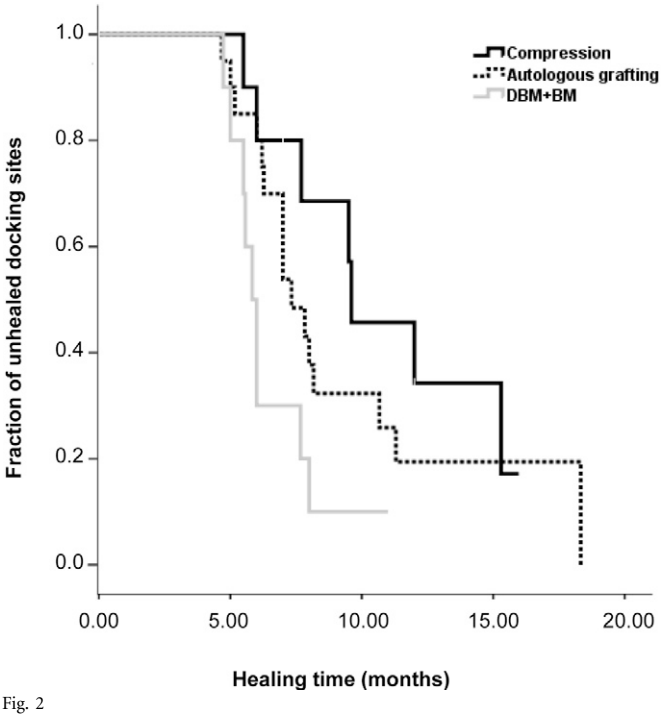

Fig. 2

Kaplan-Meier curves showing healing times in the compression group (Group A) (black solid line), the autologous bone graft group (Group B) (black dotted line), and the bone marrow-demineralized bone matrix (DBM+BM) group (Group C) (gray solid line).

corrected p values were reported. The p values were two-tailed, with the level of significance set at 0.05. All analyses were conducted with SPSS 16.0 (SPSS, Chicago, Illinois).

Source of Funding
No funding of any kind was received for this study.

Results

Statistical analysis confirmed the homogeneity of all patient groups as there were no significant differences in terms of patient age, sex, or reason for surgery (see Appendix). There were no significant differences among the groups in terms of the type of external fixator used, the location of the tibial defect, the length of the transported segment, the length of the bone segments adjacent to the docking sites, or the length of treated bone defect (see Appendix).

In the compression group (Group A), successful consolidation of the docking site within six months was achieved in two of the eleven patients. Six patients had delayed union, and two patients had a nonunion (Figs. 1-A and 1-B) that necessitated further bone-grafting for the docking site to consolidate. Fracture of the docking site occurred in one patient.

The median time needed for docking site consolidation was twelve months (range, 5.5 to 15.3 months) (Table I).

In the autologous bone-grafting group (Group B), successful consolidation of the docking site (within six months) was evident in four (18.2%) of twenty-two patients. Thirteen patients (59.1%) had delayed union, and four patients (18.2%) had nonunion of the docking site. Fracture of the docking site occurred in one patient (4.5%). The median time required for docking site consolidation was 7.33 months (range, 4.7 to 18.3 months) (Table I).

In the bone marrow-demineralized bone matrix group (Group C), successful consolidation of the docking site occurred in seven of ten patients, delayed union was diagnosed in

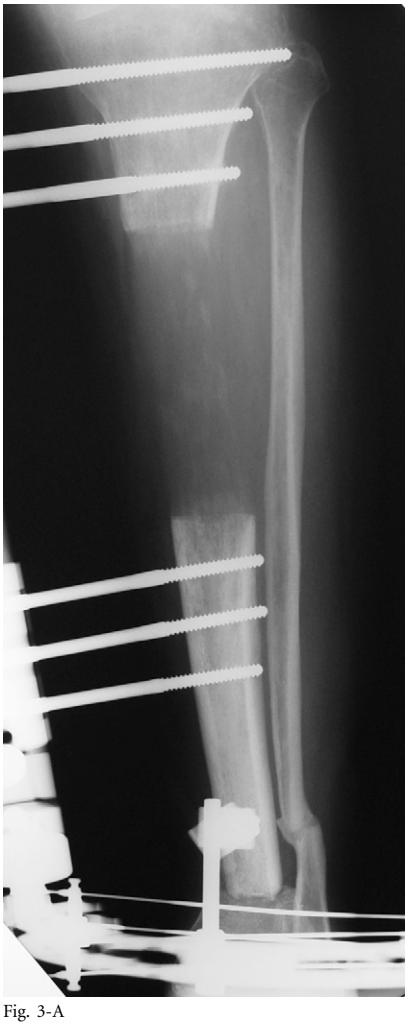

Fig. 3-A

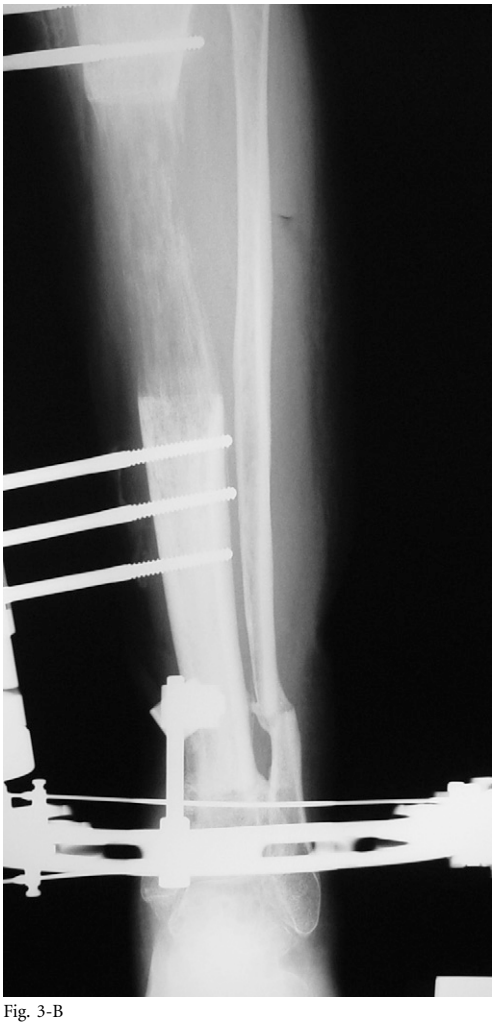

Fig. 3-B

Figs. 3-A and 3-B Radiographs for one of the nine patients in Group C in whom docking site consolidation was completed prior to regenerate consolidation. **Fig. 3-A** Radiograph showing the docking site immediately after bone marrow-demineralized bone matrix application. **Fig. 3-B** Radiograph showing consolidation of the docking site prior to regenerate consolidation.

TABLE I Results of Treatment

	Overall	Group A	Group B	Group C
No. of patients	43	11	22	10
Result *(no. of patients)*				
Consolidation within 6 months	13	2	4	7
Delayed union	21	6	13	2
Nonunion	7	2	4	1
Docking site fracture	2	1	1	—
Median time for consolidation *(mo)*		12*	7.33	5.83*

*Significant difference between the two groups ($p < 0.05$).

two patients, and nonunion developed in only one patient. The median time required for docking site consolidation was 5.83 months (range, 4.7 to 8 months) (Table I).

Overall, there were three cases of pin loosening in three patients (with one loose pin per patient). As four pins had been inserted initially in each clamp, there was no need for pin

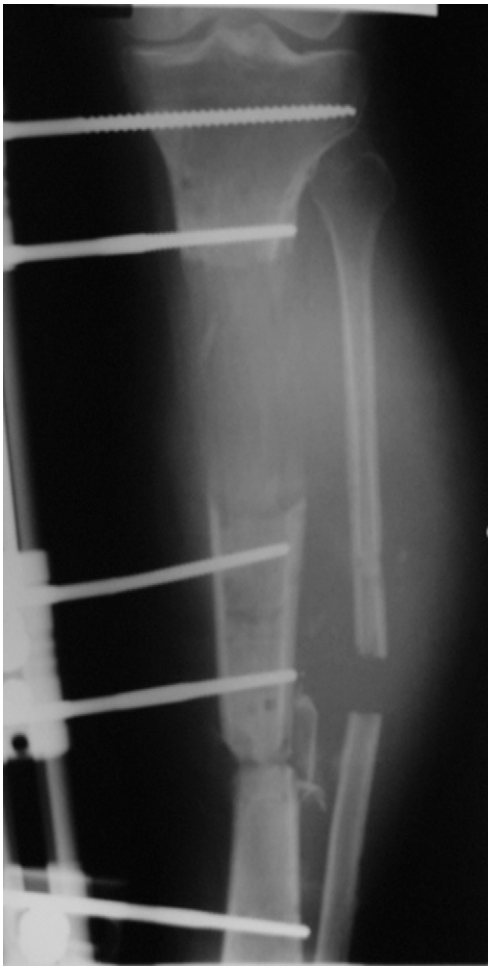

Fig. 4-A

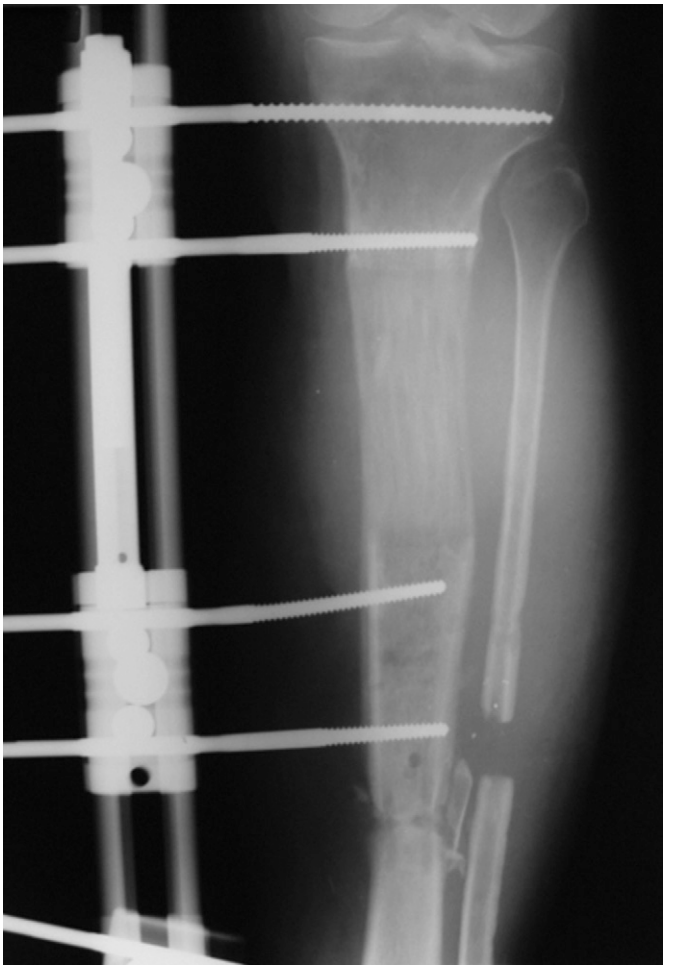

Fig. 4-B

Figs. 4-A and 4-B Radiographs for one of the patients in Group B in whom completion of regenerate healing preceded docking site consolidation. **Fig. 4-A** Radiograph showing the docking site immediately after autologous bone graft application. **Fig. 4-B** Radiograph showing consolidation of the regenerate prior to docking site consolidation.

exchange because at least three pins per clamp were left in situ after removal of the loose ones.

Statistical analysis showed that the median "healing time" required for docking site consolidation was significantly longer in the compression group as compared with the bone marrow-demineralized bone matrix group (Bonferroni adjusted p value = 0.021), whereas there was no difference between the other groups (Table I).There was no significant difference among the groups in terms of complication rates (p = 0.702). Healing times in the three groups are presented with Kaplan-Meier curves (Fig. 2).

The docking site consolidation was completed prior to regenerate consolidation in nine of ten patients in Group C (Figs. 3-A and 3-B) and in only three (13.6%) of twenty-two patients in Group B. This finding was not observed in any of the patients in Group A. In the remaining patients (including nineteen of twenty-two patients in Group B and in all patients in Group A), completion of regenerate healing always preceded docking site consolidation (Figs. 4-A and 4-B).

Discussion

The docking site has been recognized as a frequent source of problems, especially in terms of its ability to heal[1,4-7], resulting in substantial prolongation of the entire procedure and subsequent delay of fixator removal. In a recent study investigating the results of various methods that are used for improving docking site consolidation, it was suggested that when coaptation of apposed bone surfaces is delayed, spontaneous union at the docking site remains unpredictable and it is likely that a further surgical intervention will be needed[8].

Initial efforts to enhance and expedite docking site consolidation with the closed compression method did not yield as satisfactory results as initially anticipated. Subsequently, this technique was gradually replaced with autologous bone graft application, which is the current "gold standard" with which all other grafts are compared. It is the only graft material that has osteogenic and osteoinductive as well as osteoconductive properties[9-11]. The osteoblasts, preosteoblasts, and mesenchymal stem cells that are present in the autologous bone graft are capable of responding to local stimuli and release growth factors of their own, which accelerate angiogenesis and promote new bone formation[11,12]. Autologous bone-grafting has been widely used and has been found to be effective for the treatment of nonunion, with union rates reaching 87% to 100% in cases of tibial nonunion[10,13-15]. It has also been successfully applied for improving consolidation of the regenerate following distraction osteogenesis and to complete the healing at the docking site of nonunions treated with the same technique[8,16]. The most important disadvantage of autologous bone-grafting is the need for a separate surgical procedure, which often is associated with some donor-site morbidity[17,18].

Autologous bone marrow aspirate has been found to be effective in various experimental animal models of fracture nonunion[19-23] and also has been used for the treatment of tibial nonunion[24-28] as well as for expediting the maturation process of the regenerate following distraction osteogenesis[29,30]. Autologous bone marrow aspirate contains osteoprogenitor cells and has osteogenic and osteoinductive but not osteoconductive properties[11].

Because of its lack of osteoconductivity, autologous bone marrow aspirate is often combined with demineralized bone matrix (DBM), whose bone collagen matrix has osteoconductive[31] as well as osteoinductive properties[32,33]. Numerous animal studies have confirmed the synergistic effect of these two materials[11,23,34].

Multiple bone marrow aspirations combined with cell concentration techniques have been proposed to increase the density of progenitor cell populations because patient-related variation in the number and osteogenic potential of the bone marrow cells is known to exist[11]. Hernigou et al. reported that, in order for autologous bone marrow to be effective for the treatment of tibial nonunion, it must contain at least 1500 progenitor cells per mL[27]. In a study of sixty tibial nonunions, they reported that the concentration of progenitors cells in the seven cases in which the graft failed to unite was significantly lower than that in the fifty-three cases in which the treatment was successful (634 ± 187 compared with 2835 ± 1160 per mL; p = 0.001). These findings were in accordance with those of a previous study, which demonstrated in an animal model that the osteogenic capacity of bone marrow was related to cell density[35].

According to the manufacturer of the collection and processing kit used in our series, the mean concentration of autologous progenitor cells was found to be 3500/mL (range, 1764 to 4760/mL), well over the threshold defined by the aforementioned studies.

There is a growing body of literature dealing with the improvement of regenerate quality with use of various techniques, and bone marrow aspirate has been shown to enhance and expedite regenerate maturation[29,30]. However, a recent experimental study suggested that one of the major biological differences between docking site healing and regenerate healing is that the docking site heals primarily by means of endochondral bone formation and creeping substitution, which usually takes longer than regenerate healing, which occurs primarily by means of intramembranous ossification[36]. Hence, the assessment of the effect of any regenerate treatment should be exerted with great caution on the docking site, since the regenerate and the docking site are two different biological entities.

In the present study, we aimed to clarify whether the application of autologous bone marrow cells combined with demineralized bone matrix would significantly improve consolidation of the docking site by accelerating bone healing and reducing the rate of pseudarthrosis. Our results suggest that the application of demineralized bone matrix and bone marrow significantly reduces docking site consolidation time compared with the older compression method and provides a comparable if not better clinical outcome in comparison with autologous bone grafts. Although the difference between autologous bone-grafting and bone marrow application combined with demineralized bone matrix failed to reach significance, a clear trend could be noted in favor of the bone marrow-demineralized bone matrix group (Group C).

It could be argued that the higher healing rate observed in Group C was surprising, but we assume that the increased healing potential of osteoprogenitor cells combined with the better filling

of the defect by the demineralized bone matrix-aspirate paste (which, unlike bone grafts, leaves no voids) could be the reason for the better results in terms of docking site consolidation.

There appears to be a discrepancy between the excellent healing rates of 87% to 100% that have been reported when tibial nonunions have been treated with autograft[10,13,15] and the healing rate observed in our autograft group. We believe that our cases could not be simply considered as nonunions because a substantial number of them were defects resulting from septic nonunions with poor surrounding soft-tissue quality. Moreover, in most of our cases, the defect was located in the distal third of the tibia, an area of known poor vascularity.

Our most important clinical observation was the reversal of consolidation completion between the regenerate and the docking site that was observed in Group C. Specifically, while the completion of consolidation of the regenerate preceded that of the docking site in all patients in Group A and in 86.4% of the patients of Group B, we found that healing of the docking site was the first to occur in nine of the ten patients in Group C. Because in these "reverse consolidation" cases regenerate healing serves as an "internal control" neutralizing all other parameters that might have influenced docking site healing, it is believed that this acceleration of docking site consolidation can be attributed to the effect of bone marrow and demineralized bone matrix application.

A previous retrospective study highlighted the importance of surgeon experience when performing distraction osteogenesis procedures[37]. A possible positive influence of the accumulated surgical experience on the improved results of the third group cannot be entirely ruled out.

Another issue possibly affecting the healing time is the location of the docking site. Experimental evidence has demonstrated that, compared with diaphyseal bone, metaphyseal bone possesses a significantly greater osteogenic potential, rendering the metaphysis the optimal location for distraction osteogenesis[38].

The limitations of the present study include its retrospective nature and the fact that the three different treatment options were used sequentially and not randomly. The closed compression method was the first treatment option that was used in an attempt to achieve consolidation of the docking site; this method was later abandoned and replaced initially with autologous bone graft and later with autologous bone marrow application. Moreover, the relatively large number of monolateral frames may explain, in part, the particularly long delays in docking site healing in all three groups. Statistical analysis did not demonstrate any significant difference among the three groups with regard to the frame types. In conclusion, it appears that the application of demineralized bone matrix and autologous bone marrow is at least equivalent to autologous cancellous bone graft, both of which appear to substantially reduce docking site healing time compared with closed compression alone. The application of demineralized bone matrix and autologous bone marrow is an effective treatment option, with minimal donor-site morbidity, for reducing consolidation time of the docking site in tibial skeletal defects treated with distraction osteogenesis.

Appendix

eA Tables showing demographic data, reasons for surgery, treatment method, defect position, and the lengths of the transported segment, docking segment, and defect are available with the electronic version of this article on our web site at jbjs.org (go to the article citation and click on "Supporting Data"). ■

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