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INFECTION

Radiological and clinical outcomes using induced membrane technique combined with bone marrow concentrate in the treatment of chronic osteomyelitis of immature patients

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Aims

Treatment of chronic osteomyelitis (COM) for young patients remains a challenge. Large bone deficiencies secondary to COM can be treated using induced membrane technique (IMT). However, it is unclear which type of bone graft is optimal. The goal of the study was to determine the clinical effectiveness of bone marrow concentrator modified allograft (BMCA) versus bone marrow aspirate mixed allograft (BMAA) for children with COM of long bones.

Methods

Between January 2013 and December 2017, 26 young patients with COM were enrolled. Different bone grafts were applied to repair bone defects secondary to IMT procedure for infection eradication. Group BMCA was administered BMCA while Group BMAA was given BMAA. The results of this case-control study were retrospectively analyzed.

Results

Patient infection in both groups was eradicated after IMT surgery. As for reconstruction surgery, no substantial changes in the operative period ($p = 0.852$), intraoperative blood loss ($p = 0.573$), or length of hospital stay ($p = 0.362$) were found between the two groups. All patients were monitored for 12 to 60 months. The median time to bone healing was 4.0 months (interquartile range (IQR) 3.0 to 5.0; range 3 to 7) and 5.0 months (IQR 4.0 to 7.0; range 3 to 10) in Groups BMCA and BMAA, respectively. The time to heal in Group BMCA versus Group BMAA was substantially lower ($p = 0.024$).

Conclusion

IMT with BMCA or BMAA may attain healing in large bone defects secondary to COM in children. The bone healing time was significantly shorter for BMCA, indicating that this could be considered as a new strategy for bone defect after COM treatment.

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Keywords: Bone marrow concentrate, Induced membrane technique, Allograft, Bone defect, Osteomyelitis

Article focus

- Bone marrow concentrator modified allograft (BMCA) and bone marrow aspirate mixed allograft (BMAA) are both effective and safe bone arthroplasty procedures for immature patients with infectious bone defects caused by osteomyelitis treatment with induced membrane technique (IMT).

Key messages

- BMCA demonstrates a more rapid healing speed than BMAA and was found to be effective in achieving healing of bone defects.

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Strengths and limitations

- BMCA may be considered as a new strategy for immature patients with infectious bone defects.
- The adherence rate and enrichment were not measured in Group BMCA. The length of follow-up was comparatively short.

Introduction

The management of chronic osteomyelitis (COM) for young patients is a major challenge for orthopaedic surgeons. There are still many controversies regarding infection control and the optimal reconstruction method. Conventionally, sequestrectomy, bone grafting or bone transport, and systemic antibiotic treatment are standard therapies. Since the 1960s, when the distraction osteogenesis was introduced by Ilizarov and Ledyaev,[1] management of infected bone defects has been revolutionized.[2] Kucukkaya et al[3] suggested that the bifocal method of the Ilizarov treatment is the best alternative for the bone defect caused by chronic hematogenous osteomyelitis in children. In a retrospective study, children with COM obtained good results with both tibiofibular synostosis and Ilizarov distraction osteogenesis.[4] Major disadvantages of the Ilizarov device are the difficult application, poor patient tolerance, and frequent pin tract infection.[5] However, through increasing familiarity with the technique the number of major complications decreased, while the minor complications rate remained stable.[6] Induced membrane technique (IMT) is a verified operative technique used to treat larger bone deficiencies ancillary to bone tumour, pseudarthrosis, trauma, and/or COM.[7-9] This method was initially explained in 1986.[10] However, information regarding its use in young people is limited. Additionally, the majority of published paediatric series are diverse, and comprise children with different diseases.[7-9,11-14] Furthermore, it is unclear which kind of bone arthroplasty is appropriate to rebuild large bone deficiencies post-IMT.[15]

Bone marrow aspirates have been utilized to encourage osteogenesis, whether as an add-on to bone substitutes with osteoconductivity[16] or through direct inoculation.[17] An animal study demonstrated that coagulated bone marrow aspirates can be considered as an alternative autogenous therapy for long bone healing.[18] Nevertheless, the straight-forward use of bone marrow, through soaking or injection, is inadequate due to a reduced proportion of osteogenic progenitors therein. The selective cell retention (SCR) technique was developed, as osteogenesis-associated progenitors of bone marrow can be specifically attained in polyporous bioscaffolds to formulate bone grafts during an operation. Lee and Goodman[19] and Yousef et al[20] reported that the clinical results are very encouraging. Fitzgibbons et al[21] suggested that the concentrated bone marrow may be combined with allograft preparations, producing a product that promotes osteoconduction, osteoinduction, and osteogenesis with limited morbidity. However, reports of this technique combined with IMT in treating segmental bone defects are scarce. Therefore, this retrospective case-control study set out to assess the outcome and effectiveness of IMT combined with bone marrow concentrator modified allograft (BMCA) or bone marrow aspirate mixed allograft (BMAA) in children with COM of long bones.

Methods

With approval from the ethics committee of Southwest Hospital Chongqing, China (No. KY201878), we carried out a case-control analysis. Patients under 18 years old with COM of long bones who registered between January 2013 and December 2017 were included in the analysis. The following patients were excluded: four patients underwent reconstruction using autologous bone grafts or artificial bone grafts, and three patients who failed to complete follow-up. Finally, 26 patients (19 males and seven females) were included in the present retrospective case-control study. Different bone grafts were applied to repair bone defects secondary to IMT procedure for infection eradication. Group BMAA was given BMAA while Group BMCA was administered BMCA. There was no essential difference in the selection of surgical indications between the two groups.

Surgical techniques. All participants underwent two-stage surgery using general anaesthesia. Initially, patients underwent surgical debridement and antibiotic polymethyl methacrylate (PMMA) bone cement was implanted to fill bone defect. We routinely added 5 g vancomycin in 40 g PMMA bone cement (containing gentamicin; Heraeus, Hanau, Germany). Antibiotic therapy was quickly initiated with a wide-spectrum intravenous antibiotic, which was altered once isolation and classification of bacteria was accomplished. The PMMA spacer was used until infectious markers (white blood cells (WBCs) and CRP) were normalized. It was resected in an additional operation. For subsequent surgery, the bone defect was completed with BMCA for Group BMCA or BMAA for Group BMAA in the induced membrane, with concurrent osteosynthesis conducted as necessary. The protocols for preparation of BMCA were adapted from a similar study and the Bone Marrow Concentrator (patent number: ZL 2009 2 0128747. X) was used for Group BMCA. For group BMAA, first, the same volume of bone marrow aspiration was extracted from anterior iliac crest, which was directly mixed with the allograft intraoperatively. After construction using BMCA or BMAA, all the grafts were implanted in the induced membrane to fill the bone defects. Drainage was applied before the closure. The second stage intraoperative process of Group BMCA and Group BMAA is shown in Figure 1.

Postoperative treatment and evaluation. All patients were treated with antibiotics intravenously for two weeks after the first- and second stage-surgery, and then switched to oral antibiotics for four weeks. The surgical time, intraoperative blood loss, hospital stay, and associated

Fig. 1

Diagram for Group BMCA and Group BMAA intraoperative process. BMAA, bone marrow aspirate mixed allograft; BMCA, bone marrow concentrator modified allograft.

complications were recorded in detail during the second-stage operation. Radiograph examination was conducted on the first day after surgery. Functional exercise of the affected limb was started on the third day, and the patient was allowed to partially bear weight with crutches two months after surgery.

Follow-up assessment. Follow-up, comprising of clinical and radiological evaluation, was conducted every month after surgery until the bone healed. Thereafter, patients were followed up every six months. Bone healing was thought to have occurred radiologically depending on whether the bridging callus was obvious on three of four cortices as seen on two scans.[22] The follow-up radiographs were independently assessed by two authors (JS, DS) in an unblinded fashion. Disagreements between the two authors were judged by another author (ZX). Post-surgical functional recovery was assessed utilizing the activities of daily living scale (ADLs),[23] and the occurrence of bone resorption and the incidence of other complications in both cohorts were recorded.

Statistical analysis. Results were assessed utilizing SPSS v22.0 (IBM, Armonk, New York, USA). Comparing categorical variables was conducted utilizing chi-squared tests and Fisher's exact tests if adequate. Continuous variables were analyzed utilizing an independent-samples *t*-test. Kaplan-Meier survival curve and log-rank test helped compare healing time. Differences among groups were significant if $p < 0.05$.

Results

A comparison of patients in both groups is displayed in Table I. Overall, 26 patients (19 males and 7 females) comprised this study. There were no statistical differences with regards to sex, age, volume of bone defect, location, and positive rate of bacterial culture between the two groups. No medical complications occurred in the perioperative period. The mean operating time (start of incision to closing the wound) was 101.5 minutes (SD 33.2; 50 to 160) in Group BMCA and 105.4 minutes (SD 65.5; 40 to 260) in Group BMAA. The mean intraoperative blood loss was 350.8 ml (SD 635.6; 20 to 2,400) in Group BMCA and 235.4 ml (SD 353.1; 10 to 1,000) in Group BMAA. The mean hospital stay was 17.8 days (SD 5.8; 9 to 28) and 15.9 days (SD 4.2; 11 to 21) in Groups BMCA and BMAA, respectively. No substantial differences were seen in the surgical time ($p = 0.852$, independent-samples *t*-test), intraoperative blood loss ($p = 0.573$, independent-samples *t*-test), and hospital stay ($p = 0.362$, independent-samples *t*-test) between the two groups. Patients were managed for a mean 24.9 months (SD 16.3; 12 to 60) in Group BMCA and 21.6 months (SD 10.8; 12 to 48) in Group BMAA. No disagreement on bone healing time by assessing follow-up radiographs between three authors (JS, DS, ZX). Median time to bone healing was 4.0 months (interquartile range (IQR) 3.0 to 5.0; range 3 to 7) and 5.0 months (IQR 4.0 to 7.0; range 3 to 10) in Groups BMCA and BMAA, respectively. No significant differences were observed in mean follow-up time ($p = 0.548$, independent-samples *t*-test) among both groups. However, there was significant variation in bone healing time among the two groups. By analyzing the Kaplan-Meier survival curve (Figure 2), the median healing time in Group BMCA was statistically lower than that in Group BMAA ($p = 0.024$, log-rank test). The absence of impairment in ADLs was similar for Group BMCA and Group BMAA (85% (n = 11/13) vs 77% (n = 10/13) at the last follow-up; $p = 1.0$, independent-samples *t*-test). The bone healing process of Group BMCA and Group BMAA is shown in Figures 3 and 4, respectively. There was no recurrent infection in either group. However, one case (Figure 5a) from Group BMAA developed significant bone resorption in the grating site and implant failure

Table I. The comparative analysis of patients in two groups.

Variable	Group BMCA	Group BMAA	p-value
Number of patients	13	13	N/A
Mean age, yrs (SD)	11.8 (4.17)	11.8 (4.18)	1.000*
Sex (M/F), n	3/10	4/9	1.000†
Mean volume of bone defect, ml (SD)	51.4 (29.4)	35.4 (17.3)	0.103*
Location, n			0.879‡
Radius and/or ulna	2	2	
Femur	3	2	
Tibia	8	9	
Bacterial strain, n			0.695†
Negative	6	8	
Positive	7	5	
Staphylococcus aureus (MRSA)	6 (4)	4 (4)	
Enterobacter cloacae	1	N/A	
Micrococcus luteus	N/A	1	
Mean operation time, mins (SD)	101.5 (33.2)	105.4 (65.5)	0.852*
Mean intraoperative blood loss, ml (SD)	350.8 (635.6)	235.4 (353.1)	0.573*
Mean hospital stay, days (SD)	17.8 (5.8)	15.9 (4.2)	0.362*
Median healing time, mths (IQR)	4.0 (3.0 to 5.0)	5.0 (4.0 to 7.0)	0.024§
Success rate, %	100	100	1.000†
Mean follow-up time, mths (SD)	24.9 (16.3)	21.6 (10.8)	0.548*
ADLs, n			1.000†
None	11	10	
Slight	2	3	
Moderate	0	0	
Severe	0	0	

*Independent-samples *t*-test.
†Fisher's exact test.
‡Chi-squared test.
§Log-rank test.
ADLs, activities of daily living scale; IQR, interquartile range; MRSA, methicillin-resistant *Staphylococcus aureus*; N/A, not applicable.

seven months post-reconstruction (Figure 5b). The female patient underwent a second bone graft surgery and achieved bone union three months afterwards (Figure 5c).

Discussion

The IMT is a validated, consistent choice to treat large bone defects with different causes, such as tumour, pseudarthrosis, and osteomyelitis (OM). In a new systematic review and meta-analysis, Morelli et al[11] assessed the effectiveness of IMT. They discovered that the technique led to 89% bone union rate and 91% of infection removal among patients.[11] Gouron et al[14] described how the method can be safely utilized in children with large bone defects with various aetiologies, such as those with congenital pathologies, pseudarthrosis, and malignant tumours.[14,24]

IMT induces a white pseudosynovial membrane by the bone cement implanted in the bone defect. In the second stage, autologous cancellous bone grafting is carried out within the pseudosynovial membrane six to eight weeks after the first stage. Induced membrane has certain similarities to periosteum in terms of biological properties,[25] and may be described as an "induced-periosteum".[26] The membrane is largely composed of type I collagen, fibroblasts, and blood vessels (inner surface), and it releases bone morphogenetic protein 2, vascular endothelial growth factor, core-binding factor α1, interleukin 6, collagenase-1, and additional growth factors to encourage bone defect rebuilding.[27] Therefore, it has incomparable advantages in the rebuilding of long segmental bone defects, and the presence of the induced membrane enables rapid osteogenesis of the grafts in the defect area.[28] One of our previous studies has shown that IMT promotes bone union of graft in an induced membrane in segmental bone defects.[15]

In spite of the fact that IMT was described in 1986,[10] its utilization in children has seldom been described. Additionally, paediatric series are extremely diverse, as varied populations comprise the data (malignant tumours, congenital pathologies, chronic infections, trauma).[7,12,13] Recently, Rousset et al[29] reported the results of treatment for immature osteomyelitis. They confirmed the efficacy of IMT in treating large bone defects in young patients.[29]

However, IMT requires a large number of autografts for reconstruction, which poses a major issue for patients without sufficient source of autologous bone, including young patients.[10] In addition, donor site complications are frequently reported with autologous bone grafting.[30] The use of allograft avoids donor site complications, but osteoconduction and osteoinduction have both been reported to be reduced when the allograft was processed in a standard fashion.[31] Furthermore, allograft has been reported to have a risk of bone resorption.[32]

To solve this above problem, several methods have been developed to increase osteogenesis of bone substitutes.[33] Bone marrow is full of pro-osteogenic constituents, including mesenchymal stem cells (MSCs), which may differentiate in osteoblasts. Bone marrow aspirates have been utilized to encourage osteogenesis, whether as an add-on to bone substitutes with osteoconductivity or through direct inoculation. Nevertheless, the straightforward use of bone marrow, through soaking or injection, is inadequate due to a reduced proportion of osteogenic progenitors therein. To progress the effectiveness of bone marrow in spinal fusion, it is extremely important to identify methods that can heighten the amount of osteogenic mechanisms. Utilizing a cell separator (COBE 2991 Cell Processor; Caridian BCT, Lakewood, Colorado, USA), Gan et al[34] gathered post-enriched bone marrow with alkaline phosphatase level, which heightened it by 4.3-fold. Improved spinal fusion rate was 95.1%, at a mean follow-up of 34.5 months. In spite of the favourable outcome, this method is

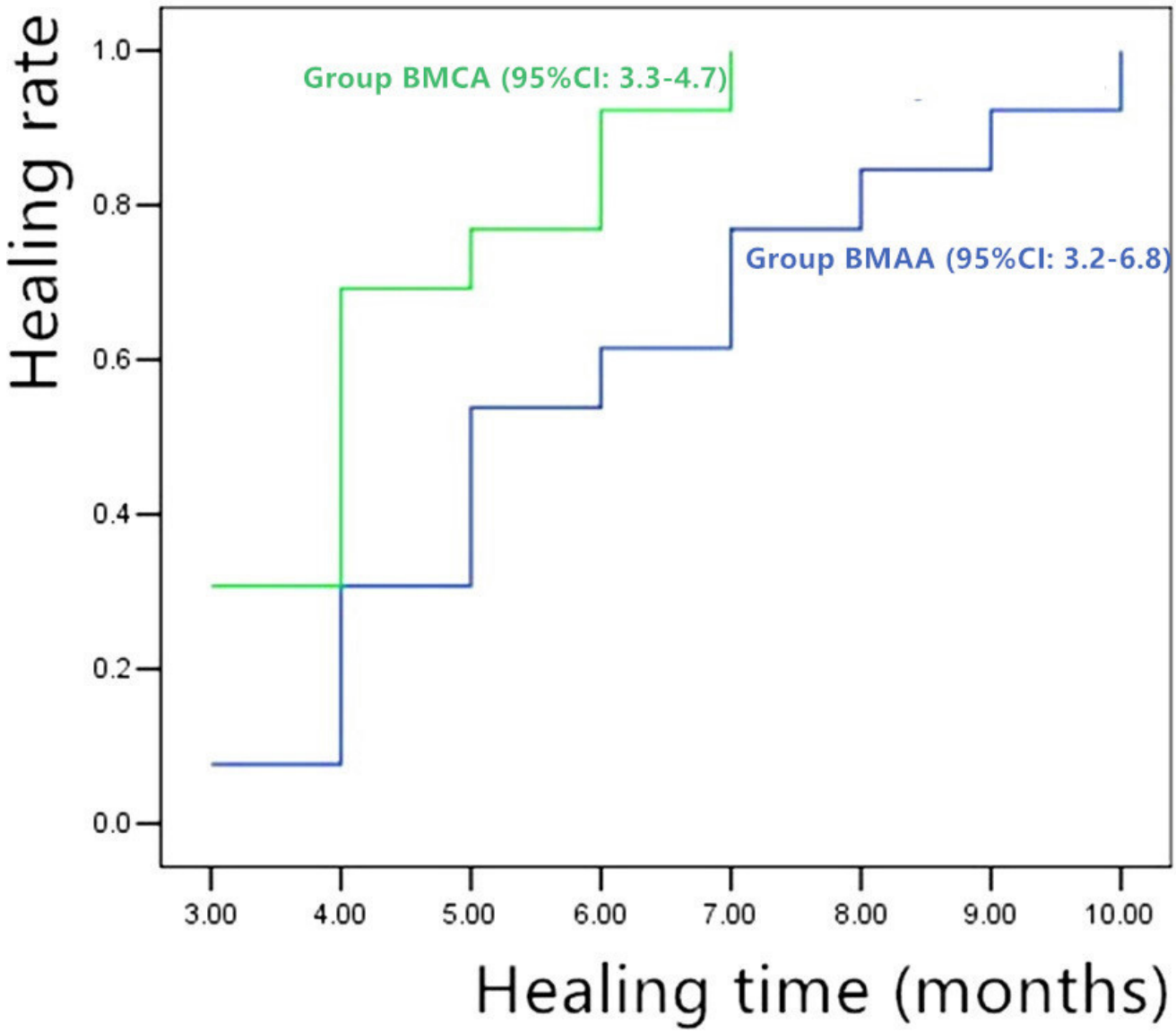

Fig. 2

Kaplan-Meier survival curve for comparison of healing time in treating bone defects between Group BMCA and Group BMAA. The median healing time in Group BMCA was significantly shorter than that in Group BMAA (p = 0.024, log-rank test). BMAA, bone marrow aspirate mixed allograft; BMCA, bone marrow concentrator modified allograft; CI, confidence interval.

associated with relatively increased amount of bone marrow, use of elaborate instruments, and an additional preparation room. The SCR technique was developed, as osteogenesis-associated progenitors can be specifically attained in polyporous bioscaffolds to formulate bone grafts during an operation. Along with the aid of Cellect DBM System (Depuy Spine, Raynham, Massachusetts, USA), Lee and Goodman[19] effectively fabricated bone grafts utilizing SCR technology and established that the amount of osteoprogenitors was heightened by three- to four-fold. Three individuals with secondary osteonecrosis of the femoral condyle underwent grafting and attained exceptional outcomes with no obstacles through a two-year follow-up. Fitzgibbons et al[21] discovered that the osteoprogenitor population in the bone marrow can be concentrated through the use of a selective retention system. The concentrated bone marrow, combined using allografts, was associated with osteoconductivity, osteoinductivity, and osteogenesis with inadequate morbidity for the majority of foot and ankle arthrodesis. However, reports of this technique combined with IMT in treating segmental bone defects are scarce.

In our study, we compared the outcomes of BMCA with those of BMAA. Our clinical observations indicated that the median bone healing time of the BMCA group was significantly shorter than that of the BMAA group (p = 0.024, log-rank test). Furthermore, one patient from the BMAA group had bone nonunion and required additional

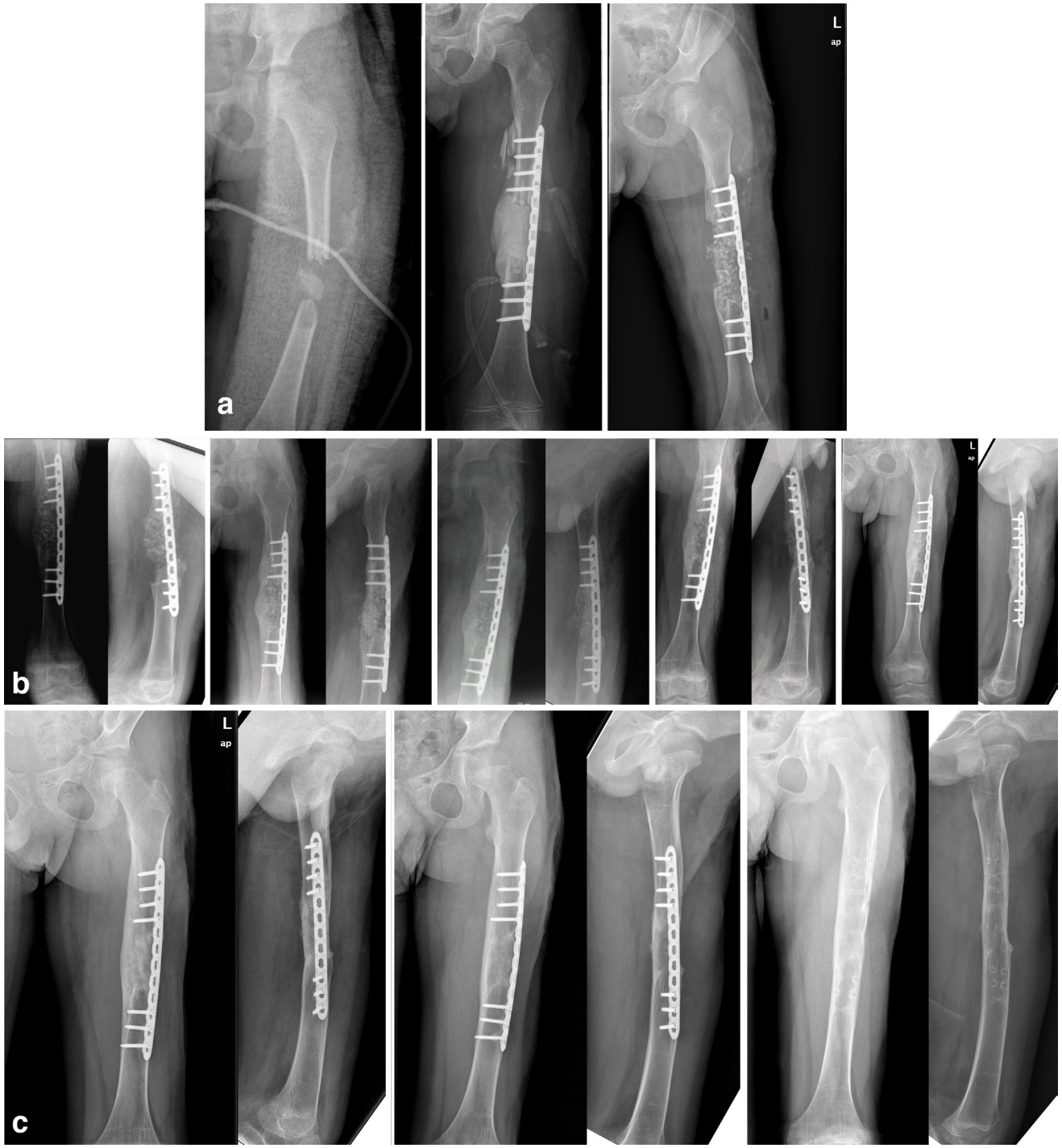

Fig. 3

Radiographs demonstrating the healing process of an eight-year-old male patient with a bone volume defect of 45 ml in Group BMCA. a) Anteroposterior (AP) radiographs before first operation, after first operation, and after second operation. b) AP and lateral radiographs from 1, 2, 3, 4, and 6 months postoperatively, demonstrating bony consolidation in the fourth month. c) AP and lateral radiographs from 1, 2, and 3 years postoperatively. BMCA, bone marrow concentrator modified allograft.

procedures to achieve bone union. This suggests that BMCA is superior to BMAA in osteogenesis capabilities, despite the fact that there was no substantial difference in the success rate. Meanwhile, there was no statistical variation in intraoperative blood loss, surgical time, or hospital stay between the two groups.

In our study, a success rate of 100% with only one patient suffering from a complication (implant failure)

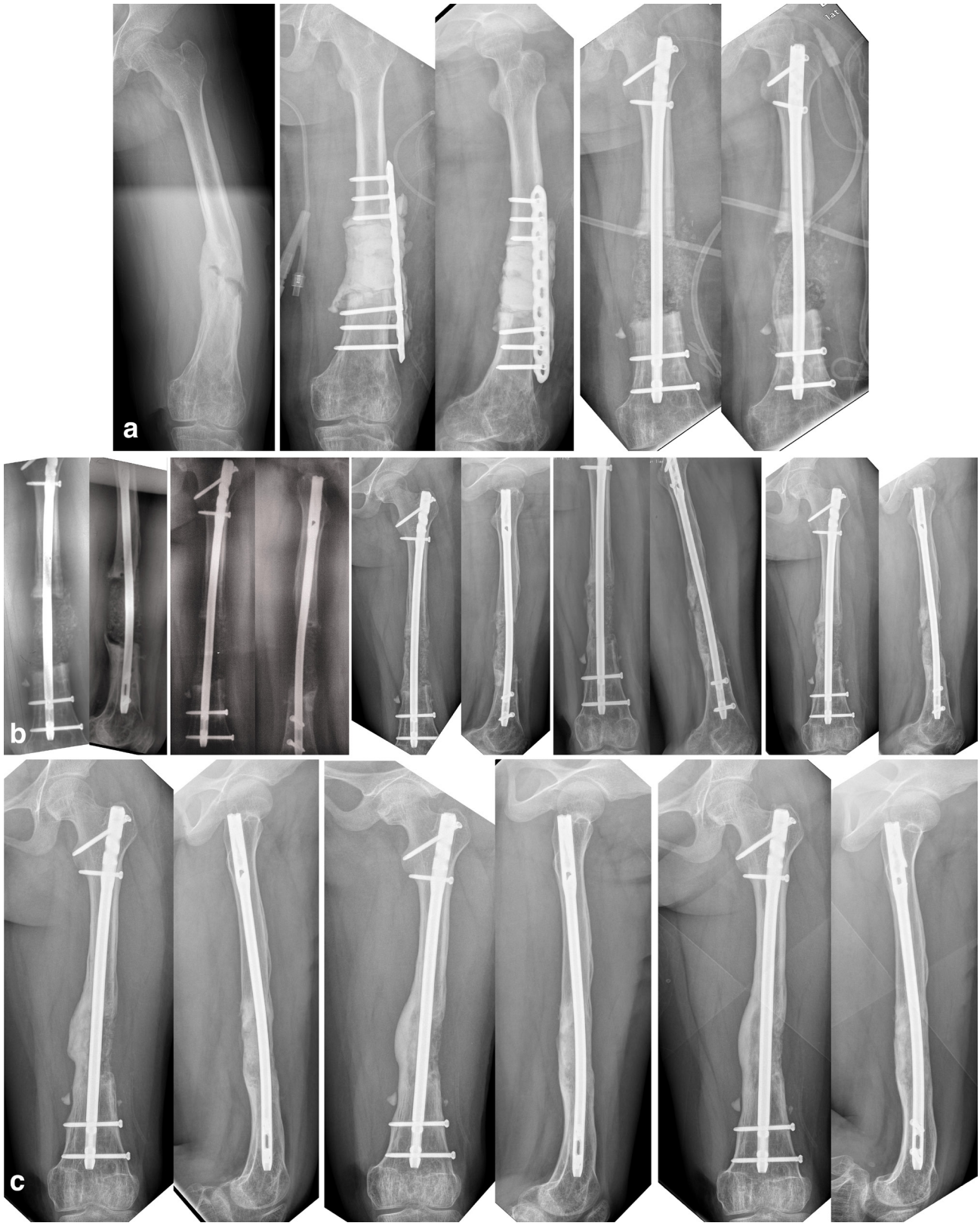

Fig. 4

Radiographs demonstrating the healing process of a 14-year-old female patient with bone defect of 70 ml in Group BMAA. a) Anteroposterior (AP) radiographs before first operation, AP and lateral radiographs after first and second operation. b) AP and lateral radiographs from 1, 3, 5, 7, and 9 months postoperatively demonstrating bony consolidation in the seventh month. c) AP and lateral radiographs from 1, 1.5, and 2 years after the second operation. BMAA, bone marrow aspirate mixed allograft.

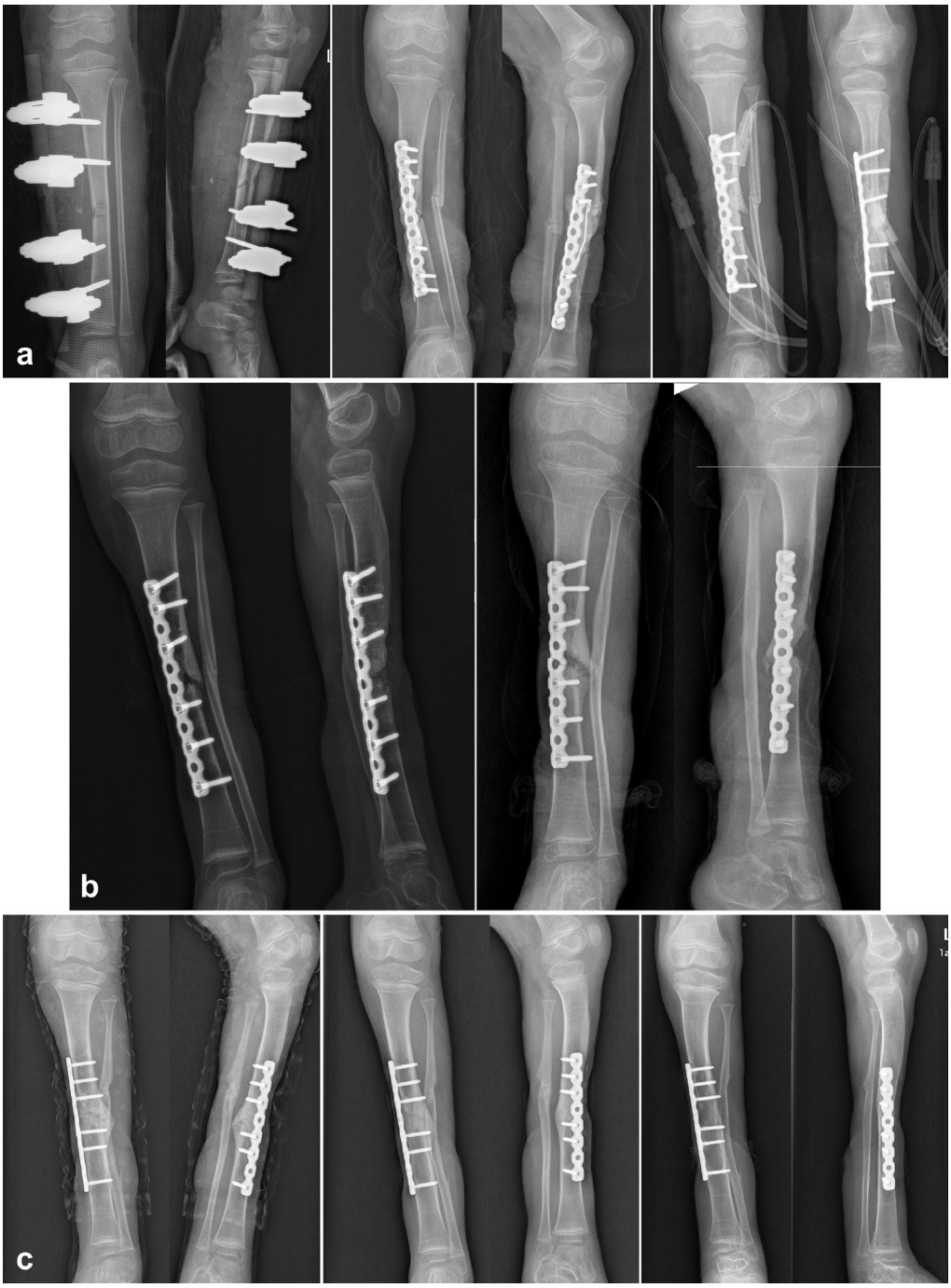

Fig. 5

Radiographs demonstrating the healing process of a seven-year-old female patient with bone defect of 20 ml in Group BMAA. a) Anteroposterior (AP) and lateral radiographs before first operation, after first operation, and after second operation. b) AP and lateral radiographs from 2 and 7 months postoperatively of second stage, demonstrating bone resorption in the grafting site and implant break. c) Additional procedures of bone grafting with bone marrow aspirate were done. AP and lateral radiographs from 3, 5, and 18 months postoperatively of the third revision surgery, demonstrating bony consolidation in the third month. BMAA, bone marrow aspirate mixed allograft.

is very impressive compared to the review by Morelli et al,[11] reporting a complication rate of 54% (n = 29/54) and a need of additional surgery in order to achieve bone union in 39% (n = 21/54) of the cases. The major aetiologies of the initial bone pathology were tumours (69%) and congenital pseudoarthroses (24%),[11] which is a very different occurrence from our patients with COM. Heterogeneity in terms of diagnosis among patients may lead to differences in treatment outcomes. Mansour and Ghanem[35] identified chemotherapy as one of the risk factors for failure. The spacer was left in situ for a long time in oncological cases to coincide with chemotherapy

and/or radiotherapy, which may have an impact on the success rate of treatment. Recently, Rousset et al[29] confirmed the efficacy of IMT in treating large bone defects secondary to chronic osteomyelitis (COM) and infected nonunion in children and adolescents, which is similar to our results.

This study has a few limitations. A relatively low number of patients was assessed, although there was diversity between patients with regards to bone defects. Additionally, the adherence rate and enrichment were not measured in Group BMCA. The impact of different fixations (plate or nail) on bone healing cannot be reliably judged due to the limited samples of nail, and it is difficult to make a significant statistical conclusion between bone healing time and fixation method. The length of follow-up was comparatively short. Studies with larger populations and longer time periods are needed to confirm the outcomes of SCR combined with IMT.

In conclusion, IMT with BMCA and BMAA can attain bone healing in large bone defects secondary to COM among children and adolescents. The selection of bone substitute is vital. Our initial data indicate that graft integration and bone healing may be anticipated earlier if BMCA is utilized as a bone void filler, and BMCA may be considered a new strategy for young patients with bone defects with limited autograft sources for repair.

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