Evaluation of the Efficacy of Pasteurized Autograft and Intramedullary Vascularized Fibular Transfer for Osteosarcoma of the Femoral Diaphysis

Tang Liu, PhD, MD¹,², Lin Ling, PhD, MD², Qing Zhang, PhD, MD², Yong Liu, PhD¹, Xiaoning Guo, PhD, MD²

¹State Key Laboratory of Powder Metallurgy and ²Department of Orthopaedics, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

Objective: To assess the treatment of osteosarcomas of the femoral diaphysis through wide en bloc excision and reconstruction of the defect by pasteurized autograft combined with vascularized fibular transfer.

Methods: A single-center, retrospective study was performed of 15 selected patients (six females, nine males) whose lesion in the middle diaphysis of the femur were treated by en bloc excision and reconstruction of the defect with recycled pasteurized autograft combined with vascularized fibular graft between January 2000 and December 2011. The primary diagnoses of the patients were osteosarcoma (15 patients), and one of these patients had a pathological fracture. The mean patient age at the time of surgery was 22.3 years (range, 10–40 years). All these cases of bone tumors were staged according to Enneking’s criteria with three stage IIA cases, and 12 stage IIB cases. The patients were examined clinically and radiologically every month during the first 6 months after surgery to exclude the evidence of infection and local recurrence, then at 3-monthly intervals for 2 years and then at 6-monthly intervals for life. Function was assessed using the Musculoskeletal Tumor Society Scoring system (MSTS).

Results: At a mean follow-up of 65.1 months (range, 31–131 months), all the patients had achieved bony union at the final follow-up. The mean time to bone union of the proximal junctions of vascularized fibula was 8.7 months (range, 6.0–13.0 months) and that of the distal junctions was 9.2 months (range, 6.0–12.0 months). Mean union time of the proximal junctions of the pasteurized autogenous bone was 14.3 months (range, 10.0–25.0 months) and that of the distal junctions was 15.6 months (range, 10.0–27.0 months). There were two fractures of the pasteurized bone during the operation. One patient, in whom the plate had been removed after union at 3 years postoperatively, sustained a fracture in a fall. This was treated with external fixation and united uneventfully. One patient, in whom two of the proximal screws had been broken, developed coxa vara. There was no infection. There were three pulmonary metastases and no local recurrence. The mean function score was 81.8%. Five patients died of their underlying disease, and the disease-specific survival of patients was 69.1%.

Conclusions: Pasteurized autogenous bone graft combined with a vascularized fibula graft is a useful reconstruction method for large bone defects after resection of osteosarcoma in the femur.

Key words: Autografts; Femur; diaphyses; Limb Salvage; Osteosarcoma

Introduction

Osteosarcoma is one of the most common primary bone tumors, which mainly affects children and adolescents. Current treatment for osteosarcoma involves surgical resection and multi-agent neoadjuvant and adjuvant chemotherapy. This combined treatment has significantly improved the 5-year survival rate. The introduction of chemotherapy combined with the development of imaging and surgical techniques have made it possible to perform bone tumor resections and limb-sparing surgery in more than...
The inclusion criteria were as follows: (i) the primary diagnoses of the patients were osteosarcoma and the site of lesion in the middle diaphysis of the femur; (ii) patients were treated by en bloc excision and reconstruction of the bone defect with recycled pasteurized autograft combined with vascularized fibular graft; (iii) after an overview of our references, we compared our results with endoprosthetic reconstruction after wide resection cases in studies by Abudu et al. We picked out 13 cases from the study by Abudu et al. All these selected cases were patients with primary sarcomas of the femoral diaphysis; and (iv) the outcome measures are local recurrence, metastasis, bone union, revision, function measure (mean Musculoskeletal Tumor Society [MSTS] scoring system), and 5-year survival rate.

Study exclusion criteria included: (i) the major neurovascular bundle cannot be free of tumor; (ii) the resection of the affected bone cannot leave a wide margin or a normal muscle cuff in all directions; and (iii) the patients who have undergone endoprosthetic reconstruction of the bone defect.

General Data
A total of 15 selected patients (six females and nine males) were enrolled in this study, with one patient having a pathological fracture. The mean patient age at the time of surgery was 22.3 years (range, 10–40 years). All these cases of bone tumors were staged according to Enneking’s criteria with three stage IIA cases, and 12 stage IIB cases (Table 1).

Management Before Surgery
Before operation, systematic examinations were performed to determine the degree of severity of the local disease and to distinguish the presence of metastasis including clinical assessment, X-ray radiograph, SPECT scan, chest radiographs, and CT scan of lungs. Magnetic resonance imaging (MRI) was also performed to define the degree of the tumor, involvement of the soft tissues (particularly neurovascular bundle), and the level of bone resection. At diagnosis, there was no patient presenting lung metastases or skip metastasis. All patients with osteosarcoma received preoperative chemotherapies with a high dose of methotrexate, doxorubicin, ifosfamide, and cisplatin.

Operative Techniques
All patients were anesthetized and in a supine position. Except in rare instances in which biopsy sites or soft tissue extension of tumor was prohibitive, a lateral femur approach was used and biopsy tracks, when present, were elliptically excised en bloc with the specimen. Wide resection was performed in all cases and all the bone defects occurred only in the diaphysis. To avoid intraosseous tumor extension, bone was resected 3.0 cm beyond abnormal uptake, as determined by preoperative MRI studies.

An ipsilateral vascularized fibula segment was taken with its periosteum and peroneal vessels. The length of the vascularized fibula exceeded the length of the resected bone defect by at least 4.0 cm. Microscopic anastomosis was
achieved using the distal end of the femoral circumflex descending artery.

The bone was pasteurized in the following manner. After resection of the bone, soft tissue, gross tumor, and the intramedullary macroscopic portion of the tumor were cleared thoroughly from the specimen. It was then treated in saline, pre-heated at 65°C for 45 min.

The pasteurized bone was then reassembled at the original anatomical site. All patients had an intercalary autogenous bone graft. Fixation was accomplished as follows: the vascularized fibula was inserted into the pasteurized autogenous bone and host femur, and then fixed with a plate and screws (Fig. 1A-E). Schematic diagrams of the surgical incisions and techniques are showed in Figs 2A-F.

Management After Surgery
The degree of tumor necrosis in response to the preoperative chemotherapy was rated according to the Huvos grading system. Ten patients had grade IV response (complete tumor necrosis), two patients had grade III response (more than 90% necrosis), and three patients had grade II response (less than 90% necrosis). All the patients received postoperative chemotherapies except the three patients who had grade II response and were not compliant with the post-operative chemotherapy regimen.

After operation, the reconstructed limbs were cast for 3 months, and a brace was adopted thereafter until radiographs showed union. Partial weight-bearing was started when union of the vascularized fibula was noted, and weight-bearing was done when union of the pasteurized autogenous bone was demonstrated on radiographs.

Outcome Measurement

Local Recurrence
Local recurrence means the tumor has relapsed in the same place as the original tumor. The patients were examined clinically and radiologically every month during the first 6 months after surgery to exclude the evidence of infection and local recurrence, then at 3-monthly intervals for 2 years, and then at 6-monthly intervals for life.

Metastasis
Osteosarcoma that has spread from the initially affected bone to one or more sites in the body, distant from the site of origin, is called metastatic. The most common site to which osteosarcoma spreads is the lungs. A CT scan of the chest was performed every 3 months in the first year, and every 6 months thereafter. A bone scan was performed every 6 months in the first year and annually thereafter for life.

Bone Union
Bridging across three of four cortices in biplanar radiographs was considered evidence of consolidation.
Revision
Revision means surgery must be re-done to replace a worn-out joint replacement or amputation. The patients were examined clinically and radiologically to judge the evidence of infection, prosthesis loosening, tumor relapse, and so on.

Function Measure
Function was assessed using the Musculoskeletal Tumor Society Scoring system (MSTS)\textsuperscript{2}.

Clinical Effect Evaluation
After an overview of other studies, we compared our results with endoprosthetic reconstruction after wide resection cases from reports by Abudu et al. We selected 13 cases from the study by Abudu et al. (Table 2)\textsuperscript{5}. All of these selected cases were patients with primary sarcomas of the femoral diaphysis. For evaluation of endoprosthetic and biological reconstruction methods, the two groups of patients were compared regarding their oncological, surgical, and functional outcome.

Ethics and Consent to Participate
This study was approved by the Second Xiangya Hospital committee for clinical research (NO. 2012-S231) and informed consent was obtained from the patients and the parents or guardians of the patients participating in the study.

Statistical Analysis
SPSS v13.0 (SPSS\textsuperscript{TM} Inc., Chicago, Illinois) was adopted to analyze statistics. Therapeutic variables (revision and function), pathological variables (Enneking stage, local recurrence, and metastatic disease) and demographic variables (gender, age, and duration of follow-up) were examined. The endpoints of the study were local recurrence, progression of disease, and revision for any cause, such as infection, loosening, and so on. Descriptive summary statistics included...
means and ranges. Age and time intervals were regarded as continuous variables. All other covariates were modeled as categorical variables. Differences between means and proportions were tested with the Fisher’s exact test or the Rank-Sum test, and the Kaplan–Meier estimate was used to measure survival proportions. All tests were two-sided and a P-value < 0.05 was considered significant.

**Results**

**General Results**

In the present study, the mean length of the femur defect after excision of the tumor segment was 19.8 cm (range, 15.5–25.0 cm). The mean length of surgical time was 309.2 min (range, 243–351 min). The mean blood loss during operation was 937.3 mL (range, 810–1251 mL). The mean wound drainage was 321.7 mL (range, 210–450 mL).

**Assessment of Bone Union**

At a mean follow-up of 65.1 months (range, 31–131 months), all the patients had achieved bony union at the final follow-up. The mean time to bone union of the proximal junctions of vascularized fibula was 8.7 months (range, 6.0–13.0 months) and that of the distal junctions was 9.2 months (range, 6.0–12.0 months). The mean union time of the proximal junctions of the pasteurized autogenous bone was 14.3 months (range, 10.0–25.0 months) and that of the distal junctions was 15.6 months (range, 10.0–27.0 months) (Figs 3A-D and 4A-D).

**Assessment of Complications**

There were two fractures of the pasteurized bone during the operation. One patient, in whom the plate had been removed after union at 3 years post-operatively, sustained a fracture in a fall. This was treated with external fixation and united uneventfully. One patient, in whom two of the proximal screws had been broken, developed coxa vara. There was no infection.

Pulmonary metastasis occurred in three patients. Those patients’ pulmonary metastasis occurred at the time of 39 months, 57 months, and 45 months after surgery, respectively. One patient received revision at the time of 47 months postoperatively. Five patients died of their underlying disease. There was no local recurrence.

**Clinical Effect Evaluation Outcomes**

We compared our results with endoprosthetic reconstruction after wide resection cases from reports by Abudu et al. There were no significant differences between the patients in our study and the study by Abudu et al. in age at surgery, gender, or tumor location.

**Metastasis**

Pulmonary metastasis occurred in three patients (20.0%) in our study while no pulmonary metastasis occurred in the patients in the study by Abudu et al. (Table 3 and Fig. 5A).
Local Recurrence
There was no local recurrence in our study, while local recurrence occurred in one patient (7.69%) in the study by Abudu et al. (Table 3 and Fig. 5A). In our study, there was one revision (6.7%), while revisions were indicated in four patients (30.8%) in the study by Abudu et al. (Table 3 and Fig. 5A).

Function Measure
All patients were functionally assessed at the latest follow-up. The mean MSTS2 score was 81.8% in our study and 83.5% in the study by Abudu et al. (Table 3 and Fig. 5B).

Five-Year Survival Rate
In our study, five patients died of their underlying disease while all other patients were alive without evidence of disease at latest follow-up. Consequently, the disease-specific survival of patients in the present study was 69.1% at 5 years, where it was 74.1% in the study by Abudu et al. (Fig. 5C). We were under-powered to detect statistically significant differences between groups, and thus lack of a significant finding should not be intercepted as no difference. Some of the differences that failed to reach statistical significance, such as the revision rate (7% in our study vs. 30.8% in the Abudu et al. study), was large.

Discussion
In this study, we used a pasteurized intercalary autogenous bone graft combined with a vascularized fibula graft for bony defects after tumor resection in the patients with osteosarcoma and acquired good oncological and functional outcomes. Furthermore, we were able to prove that prosthetic reconstruction was similar to reconstruction with recycled pasteurized autograft in terms of local recurrence and metastasis, while a higher rate of revision was noted in patients with prosthetic reconstruction.

Limitation of the Study
We note several limitations in this study. The study was retrospective, and the number of patients was relatively small. Maybe it related to the fact that there wasn’t any statistically significant difference between our study and that of Abudu et al. Although only three patients developed pulmonary metastases and none developed local recurrence, longer follow-up is needed as these were malignant lesions with a possibility of late recurrence.

Fig. 3 (A) The radiographs of a 10-year-old boy (case 6) with osteosarcoma of the right femur are shown. The femur was pathological fracture. (B) Radiographs taken 1 week postoperatively are shown. The length of the bone defect was 21 cm. (C) Radiographs taken 6 months postoperatively are shown. (D) Radiographs taken 12 months postoperatively are shown. Bone union and incorporation were achieved.
Complication Rates
High complication rates were reported in the use of recycled pasteurized autograft without a vascularized fibular graft. Manabe et al. described a complication rate of 52% in 25 patients who had reconstruction surgery with pasteurized autogenous bone. In their study, fracture (12%) and infection (20%) were the main complications. Ahmed et al. reported 22 sarcoma patients whose bone tumor excision followed by pasteurization and reimplantation. In their study, fracture (13.6%), graft collapse (9.0%), and infection (9.0%) were the major complications encountered. Sugiura et al. described a series of 19 patients who received only pasteurized autogenous bone. Infection was noted in one patient, fracture was present in two patients, and pseudoarthrosis was found in six patients. In their study, the complication rate was nearly 50%. In a previous study, we treated malignant bone tumors of the distal tibiae with en bloc intra-articular excision and ankle arthrodesis using recycled pasteurized autograft; infection occurred in two patients (20.0%) while the nonunion rate was 54.5%. Manfrini et al. reported on 24 patients with bone tumors that had intercalary segments of tibia or femur reconstructed with the fibula inside the massive allograft. In their study, the rate of fracture or nonunion of allograft was 33.3%. Li et al. reported 11 patients that had intercalary resection of lower extremity malignancy underwent reconstruction with an allograft and vascularized fibular construct. There were no allograft fractures or infections. Nonunion was noted in one patient (9.1%). In the current study, bone defects were reconstructed by the combination of vascularized fibula and pasteurized autogenous bone grafts. All the patients had achieved bony union without infection. There were two fractures of the pasteurized bone during the operation and one patient sustained a fracture in a fall 3 years post-operatively.

| TABLE 3 Main patient and outcome characteristics | Abudu et al. study | Our study | P-value |
|-----------------------------------------------|------------------|------------|---------|
| Group names | Total 13 | 15 | - |
| Mean age (years) | 25.9 ± 15.6 | 22.3 ± 8.8 | 0.7860† |
| Sex | 11M / 2F | 9M / 6F | 0.2210† |
| Specimen Length (cm) | 21.3 ± 3.3 | 19.8 ± 2.7 | 0.4960† |
| Mean follow-up (months) | 70.1 ± 55.2 | 65.1 ± 28.0 | 0.7510† |
| Local recurrence | 1 | 0 | 0.4640† |
| Metastasis | 0 | 3 | 0.2260† |
| Mean MSTS | 83.5 ± 10.6 | 81.8 ± 7.1 | 0.5560† |
| Revision | 4 | 1 | 0.1530† |
| Five-year survival rate | 74.1% | 69.1% | 0.8940† |

* Rank-Sum test; † Fisher’s exact test.
Chemotherapy has been reported to increase the infection and decrease bony modeling in a dose-dependent manner. A series of 112 patients treated with massive bone allografts for osteosarcoma was reported. The study showed the incidence of delayed union was 49% and identified that the incidence of delayed union was significantly increased by the use of chemotherapy. Another study also indicated that the incidence of the nonunion was significantly increased in the setting of chemotherapy. Li et al. reported that the time of osseous union in the patients who had chemotherapy was longer than that in patients who did not receive chemotherapy. In our series, all the patients received chemotherapy. Mean union time of the proximal junctions of the pasteurized autogenous bone was 14.6 months and that of the distal junctions was 13.3 months, which was comparable with that in previous studies, with ranges between 6.1 months and 25.0 months. We had no cases of bony infection, which is a significant improvement in comparison to previous studies. The lower infection rate may be attributed to better vascular circumstance provided by the grafted vascularized fibula.

**Functional Evaluation**

The MSTS score is commonly used for functional evaluation after grafting. Several studies have been done using this evaluation system. Manabe et al. reported an MSTS score of 86% in the lower limbs in 25 patients who had autogenous pasteurized bone grafting. Suk et al. reported an MSTS score of 77% in the lower limbs in 12 patients who had autogenous pasteurized bone grafts. Sugiura et al. reviewed pasteurized intercalary autogenous bone graft combined with a vascularized fibula graft in 15 patients with malignant bone tumors. The mean total postoperative functional evaluation score was 81%, and the mean MSTS score for 12 patients with bone defects at sites from the diaphysis to the metaphysis was 78%. In the present study, the mean post-operative functional score was 80.8%, which was comparable to others.

**Fibula Graft Outcome**

As described by Sugiura et al., plate fixation is technically difficult in bone defects of 15 cm or larger. For such cases, they fixed the fibula outside the pasteurized autogenous bone and used an intramedullary nail inside the marrow, while in our study all the patients adopted plate fixation and achieved good outcomes.

The study by Manfrini et al. suggests that free fibular flaps will incorporate into the allograft. Li et al. observed that abundant callus originated from the outerlayer of the fibula and united the fibula with the host bone and allograft. They also found that even if contact between the allograft and host bone is less than optimal, vascularized bone flap facilitates the host–allograft union at the level of the osteotomy. Pasteurized autogenous bones are similar to allografts, in that both possess bone induction ability and bone conductive ability. Sugiura et al. reviewed pasteurized intercalary autogenous bone graft combined with a vascularized fibula graft and stated that the addition of a vascularized fibula graft seems to promote the theoretically anticipated remodeling process and more complete bone was contained than would be expected without the graft. Our results indicate that pasteurized intercalary autogenous bone graft combined with a vascularized fibula graft is a useful reconstruction method for large bone defects after resection of osteosarcoma in the femurs.

Therefore, we make a conclusion that pasteurized autogenous bone graft combined with a vascularized fibula graft is a useful reconstruction method for large bone defects after resection of osteosarcoma in the femurs.

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**Author contributions**

Q Z., T.L., L. L., and X. G. conceived the study and designed the study. Y. L., T.L., L. L., and X. G. provided the study materials. L. L., T.L. and X.G. performed the data analysis. All authors contributed to the interpretation and discussion of the results and wrote the manuscript. All authors have approved the manuscript for submission.
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