Intercalary frozen autografts for reconstruction of bone defects following meta-/diaphyseal tumor resection at the extremities

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Research Article

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Abstract

Background: To evaluate the clinical outcome and complications of limb malignant tumors treated with intercalary resection and frozen autograft reconstruction.

Methods: We reviewed 40 patients whose malignant bone tumors were treated by wide resection and recycling liquid nitrogen-treated autograft reconstruction between 2006 and 2017. Limb function, bone union at the osteotomy site and complications were evaluated. Functional outcome was assessed using the Musculoskeletal Tumor Society (MSTS) scoring system.

Results: The most common tumor was osteosarcoma (11 cases), followed by Ewing's sarcoma (7 cases). Tumors were located in the humerus (5), ulna (1), femur (13) and tibia (21). The mean follow-up period was 48.4 months (range 11-127 months). Of the 40 patients, 19 remained disease-free, and 3 were alive with disease. The mean size of the defect after tumor resection was 12.1 cm (range 5-24 cm). Bone union was achieved in 37 patients, with a mean union time of 8.7 months (range 4-18 months). Fifteen complications were encountered in 14 patients, including 3 graft nonunions, 3 infections (1 superficial, 2 deep infections), 4 limb length discrepancies, 2 graft fractures and 3 local recurrences. The mean MSTS score was 82.5% (range 50-100%). Excellent functional results were achieved in 24 patients (60%) and good results in 14 (35%).

Conclusions: Liquid nitrogen-treated tumor-bearing autografts are a simple and effective option for biologic reconstructions after meta-/diaphyseal tumor resection of long bones. This method has excellent clinical outcomes and is especially recommended for patients with nonosteolytic primary or secondary bone tumors.

Introduction

With the application of neoadjuvant chemotherapy and surgical staging, there has been great progress in the treatment of patients with limb malignant tumors[1]. In recent years, limb salvage surgery has become increasingly popular after tumor resection because of its better overall survival rate and functional outcome than amputation [2, 3].

When the tumor is located in the diaphyseal or metaphyseal regions of long bones, intercalary excision is usually performed to remove the tumor and preserve the adjacent joints. The bone defect after tumor resection can be reconstructed with nonbiologic (endo-prosthesis) or biologic (such as allograft, recycling tumor-bearing autograft, vascularized or nonvascularized fibular graft, distraction osteogenesis) reconstruction.

In recent years, reconstruction with recycling tumor-bearing autografts has become popular because it is adaptable and easily performed and has no risk of disease transmission. Several methods of recycling have been developed, such as autoclaving[4], irradiation[5] and pasteurization[6]. However, these recycling methods require special equipment or strict thermal control and usually cause bone weakness, resulting in bone absorption and pathological fracture.

In 1999, based on in vitro and in vivo studies of the hypothermic effects of liquid nitrogen on osteosarcoma cells[7], Tsuchiya et al. introduced and clinically applied a reconstruction technique with tumor-bearing autografts treated with liquid nitrogen, and the long-term outcome was satisfactory[8, 9]. This technique has many advantages: simplicity, a perfect fit, a shorter union period, sufficient biomechanical strength, osteoinduction, osteoconduction, ease of soft-tissue attachment, low cost and no risk of disease transmission.

In this study, we reviewed and analyzed the clinical outcome and complications of patients treated with intercalary frozen autografts after resection of limb malignant tumors.

Methods

From 2006 to 2017, 40 patients with malignant bone tumors of the limbs underwent intercalary tumor resection and reconstruction with recycled frozen autografts (Table 1). All lesions were located in the diaphyseal or metaphyseal region of a long bone. At least a 2-cm resection margin was obtained, and adjacent joints were preserved. If the tumor was close to the epiphysis (< 2 cm), the osteotomy line was transphyseal to achieve the surgical margin.

Table 1
, Details of patients who underwent intercalary frozen autograft reconstruction.

No.	Gender /age(yrs)	Site	Histology	Length (cm)	Fixation	Bony union (months)	MSTS score (%)	Complications	Follow-up (months)	Outcome
1	F/54	tibia	lymphoma	7.5	nail	7	100	superficial infection	52	AWD
2	F/11	tibia	Ewing's sarcoma	13	plate	-	66	deep infection, nonunion	11	DOD
3	M/59	humerus	Metastasis (lung)	6	plate	10	93		45	AWD
4	F/21	femur	osteosarcoma	12	plate	8	83		118	NED
5	M/59	femur	malignant peripheral schwannoma	13	nail	6	90	recurrence	32	DOD
6	M/13	femur	Ewing's sarcoma	15	plate	10	86		26	DOD
7	F/16	tibia	osteosarcoma	10	nail	12	80		28	DOD
8	F/17	femur	osteosarcoma	15	plate	9	83		28	DOD
9	F/12	tibia	Ewing's sarcoma	8	plate	9	70	limb shortening (2cm)	48	NED
10	M/16	tibia	Ewing's sarcoma	5	plate	4	93		54	NED
11	F/19	humerus	malignant peripheral schwannoma	12.5	nail	6	90	recurrence	44	NED
12	M/26	humerus	chondrosarcoma	10	plate	8	86		92	NED
13	M/19	tibia	Ewing's sarcoma	11	nail	10	90		36	NED
14	F/51	humerus	chondrosarcoma	11.5	plate	10	93		88	NED
15	F/68	tibia	Metastasis (breast)	12	nail	12	86		11	DOD
16	M/14	tibia	Ewing's sarcoma	8	plate	8	76	limb shortening (4cm)	42	NED
17	F/15	tibia	malignant peripheral schwannoma	10	plate	8	83		60	NED
18	M/71	ulna	Metastasis (kidney)	9	plate	10	96		20	DOD
19	M/22	femur	osteosarcoma	25	plate	15	80	fracture	75	NED
20	M/70	femur	Metastasis (lung)	24	plate	-	86	nonunion	17	DOD
21	F/49	femur	Metastasis (multiple Myeloma)	14.5	nail	8	86		127	AWD
22	F/14	tibia	osteosarcoma	12	plate	5	96		56	NED
23	F/32	tibia	synovial sarcoma	6	plate	6	93		53	DOD

Yrs years, M male, F female, MSTS musculoskeletal tumour society, AWD alive with disease, DOD died of disease, NED no evidence of disease

No.	Gender /age(yrs)	Site	Histology	Length (cm)	Fixation	Bony union (months)	MSTS score (%)	Complications	Follow-up (months)	Outcome
24	F/10	tibia	osteosarcoma	13.5	plate	5	70	limb shortening (4cm)	76	DOD
25	M/55	femur	chondrosarcoma	15	nail	7	80	recurrence	40	NED
26	F/45	tibia	malignant fibrous histiocytoma	8	plate	6	93		36	DOD
27	M/16	femur	Ewing's sarcoma	16	nail	8	90		32	DOD
28	M/16	femur	osteosarcoma	18	nail	10	93		50	DOD
29	M/17	tibia	chondrosarcoma	7.5	plate	11	86		63	NED
30	F/22	tibia	synovial sarcoma	13	plate	18	96		126	NED
31	M/12	tibia	osteosarcoma	17	plate	12	83	limb shortening (3cm)	36	NED
32	M/56	humerus	malignant fibrous histiocytoma	11	nail	9	90		30	DOD
33	M/76	tibia	lymphoma	11	nail	8	70	deep infection	33	DOD
34	F/17	tibia	malignant fibrous histiocytoma	9.5	plate	10	90		48	NED
35	F/15	tibia	osteosarcoma	10	plate	10	70	fracture	32	NED
36	M/31	tibia	fibrosarcoma	11	nail	6	86		14	NED
37	M/10	femur	osteosarcoma	16	plate	8	83		50	DOD
38	F/34	femur	synovial sarcoma	10	nail	5	86		42	DOD
39	F/55	tibia	Metastasis (lung)	7	nail	11	83		22	DOD
40	F/13	femur	osteosarcoma	23	plate	-	50	nonunion	45	NED
Yrs years, M male, F female, MSTS musculoskeletal tumour society, AWD alive with disease, DOD died of disease, NED no evidence of disease										

The cohort comprised 19 males and 21 females, with a mean age of 31.2 years (10–76 years). The diagnoses were 11 osteosarcomas, 7 Ewing's sarcomas, 6 metastases, 4 chondrosarcomas, 3 synovial sarcomas, 3 malignant fibrous histiocytomas, 3 malignant peripheral schwannomas, 2 lymphomas, and 1 fibrosarcoma. Tumors were located in the humerus (5), ulna (1), femur (13) and tibia (21). This study was approved by the Institutional Ethics Committee of our hospital.

All diagnoses were confirmed by preoperative biopsy. Patients with osteosarcomas and Ewing's sarcomas received neoadjuvant chemotherapy. All patients received postoperative chemotherapy, except for those with chondrosarcomas. The length of resection was determined based on preoperative Magnetic Resonance Imaging (MRI).

The tumor-bearing autografts were treated with free freezing techniques using liquid nitrogen. Briefly, the intercalary diaphysis with the tumor was excised en bloc to achieve a wide margin or intentional marginal margin. Intraoperative frozen sections of marrow/bone samples from osteotomy sites were taken to confirm the clean margin. Any attached soft tissues and gross tumors were removed from the resected bone, and the canals were curetted. Then, the bone was soaked in liquid nitrogen for 20 minutes, thawed at room temperature (~26°C) for 15 minutes, and rinsed in distilled water for 15 minutes. The recycled bone was implanted into the bone defect and fixed by a locking plate (Fig. 1) or intramedullary nail (Fig. 2). Allografts or cement were used to fill bone defects as well as to increase strength after curettage in the recycled bone.

Bone union was assessed with X-ray every month until the grafted bone united. Follow-up radiographs were obtained every 3 months for the first year and then every 6 months to check for recurrences and metastases.

Patients were encouraged to begin range of motion exercises immediately after the surgery. Partial weight bearing was allowed at one month after the operation. Full weight bearing was permitted until solid bone union was achieved. Radiological evidence of bone union in osteotomy sites included obscured osteotomy lines or sufficient bridging callus at the host-graft junction.

We evaluated the reconstruction methods, function of the affected extremities, bone union at the osteotomy site and complications. The functional outcome at the final follow-up was evaluated using the Musculoskeletal Tumor Society (MSTS) scoring system[10].

Results

Details on the treatment results are presented in Table 1. The mean follow-up was 48.4 months (range 11–127 months). At the last follow-up, 19 patients remained disease-free, 3 patients were alive with disease, and 18 patients had died of the disease or metastasis. The mean size of the defect after tumor resection was 12.1 cm (range 5–24 cm). During follow-up, the frozen autografts were removed in four cases (10%): two cases due to local recurrence, one due to severe infection and one due to fracture.

Bone union was achieved in 37 patients, with a mean union time of 8.7 months (range 4–18 months). Primary union (< 12 months) occurred in 32 of the 40 patients (80%), delayed union ($\geq$ 12 months) in 5 (12.5%), and nonunion in 3 (7.5%). Fixation with intramedullary nailing was performed in 15 patients (37.5%); fixation with plating was carried out in the remaining 25 patients (62.5%).

Fifteen complications occurred in 14 patients, including 3 graft nonunions, 3 infections (1 superficial, 2 deep infections), 4 limb shortenings, 2 graft fractures and 3 local recurrences.

Nonunion occurred in 3 patients. One patient with tibial Ewing's sarcoma developed nonunion because of delayed infection during postoperative chemotherapy and died of the disease at 11 months. Another patient with femoral metastasis of lung cancer did not heal at 17 months after the operation and died of the disease uneventfully. Both of them only had slight limb pain and could walk on canes during the survival period. In the third patient with femoral osteosarcoma, nonunion of the frozen graft and aseptic loosening of the plate were observed at 36 months postoperatively. Revision surgery was performed to remove the original fixation, graft with autogenous iliac bone and fix with an intramedullary nail. The patient is still followed up closely.

Infections developed in 3 patients. One patient with superficial infection was treated successfully with debridement. Another patient with deep infection was treated conservatively; the patient died of the disease at 11 months after surgery with nonunion of autograft. The third patient with deep infection underwent amputation.

Of three cases of local recurrence, two originated from surrounding soft tissues. One patient underwent amputation because of vascular involvement, and the other underwent local resection. Another patient with local recurrence after autografting underwent wide resection and prosthesis reconstruction.

One patient with fracture of a tibial graft was successfully managed with internal fixation and allograft. The other patient with femoral graft fracture underwent prosthesis reconstruction.

Four patients had a shortened lower extremity (2–4 cm), which was due to involvement of the epiphysis. All cases were managed conservatively using shoe lifts. No additional limb lengthening was performed.

Functional outcomes were assessed with the MSTS scoring system, with a mean score of 82.5% (range 50–100%). All but 2 patients had a score higher than 70%. The function of the limb was rated as excellent in 24 patients (60%), good in 14 (35%), and fair in 2 (5%).

Discussion

Liquid nitrogen has been used to treat bone tumors for some time. In 1969, Marcove et al. utilized liquid nitrogen for palliative treatment in patients with primary and metastatic bone tumors [11]. Many authors have reported the clinical use of liquid nitrogen as an adjuvant to curettage for benign, aggressive or malignant tumors [12–15]. In 2003, Yamamoto et al. verified the efficacy of exposing tumor cells to liquid nitrogen and found that the treated bone could maintain intact mechanical strength [7]. Tsuchiya et al. reported in 2005 clinical results of reconstruction with tumor-bearing bone treated with liquid nitrogen [8]. Since then, several authors have described the use of frozen autografts for reconstruction after excision of the tumor (Table 2) [8, 9, 16–21]. The freezing technique comprises free-freezing and pedicle-freezing methods depending on the tumor location. Compared to the free freezing procedure, the pedicle freezing method only cuts

one side of the tumor site and maintains the continuity of the other side. Fewer osteotomy sites lead to a shorter bone union time and lower rate of postoperative complications because of early blood flow recovery. In this study, all autografts were treated with a free freezing method to preserve adjacent joints.

Table 2
Demographic data and outcomes of patients treated with frozen autograft in previous studies.

Author	Tsuchiya	Tsuchiya	Igarashi	Subhadrabandhu	Paholpak	Zekry	Garg
Published year	2005	2010	2014	2015	2015	2017	2020
Cases	28	33	36	22	12	34	10
Follow-up (months) Mean (range)	28.1 (10–54)	30 (7–69)	101 (16–163)	65 (24–138)	Median 32 (12–86)	62 (24–214)	39.6 (6–97)
Rate of bony union	92.9%	93.9%	83.3%	90.9%	100%	97.1%	93.8%
Union period (months) Mean (range)	6.7	7.3	6.2 (2–11)	9.5 (6–15)	8.2 (3–12)	10.2 (3–36)	5.2 (4–7)
Function (MSTS score) Mean (range)	71.4% Excellent 10.7% Good 10.7% Fair 3	75.7% Excellent 15.1% Good 9% Fair	72.2% Excellent 19.4% Good 8.3% Fair	89.3%	79% (66.7–93.3%)	86.8% (30–100%)	80.2% (75–85%)
Complications							
Infection (NO./percentage)	3/10.7%	4/12.1%	4/11.1%	2/9.1%	3/25%	2/5.9%	0
Recurrence (NO./percentage)	2/7.1%	3/9.1%	4/11.1%	3/13.6%	1/8.3%	4/11.8%	0
Fracture(NO./percentage)	2/7.1%	3/9.1%	7/19.4%	1/4.5%	1/8.3%	6/17.6%	0
Nonunion(NO./percentage)	2/7.1%	2/6.1%	6/16.7%	2/9.1%	0	5/14.7%	1/10%

There have been many studies demonstrating the strong ability of liquid nitrogen treatment to kill tumor cells and the low possibility of local recurrence within recycled tumor-bearing autografts. The main mechanism of cryosterilization is crystal formation and dehydration of tumor cells, which are more sensitive to low temperatures than normal cells [22, 23]. The process of rapid freezing and slow thawing may cause fatal damage to tumor cells. It was also found that the number of cycles of liquid nitrogen treatment, either one or two, resulted in no significant difference with regard to effects of cell killing[7]. We used one-cycle steps of freezing in liquid nitrogen for 20 minutes, thawing at room temperature for 15 minutes and then rinsing in distilled water for 15 minutes. Only 3 local recurrences (7.5%) occurred in this cohort. The reported recurrence rate was approximately 7.1–13.6% (Table 2) [8, 9, 16–21]. Tsuchiya et al. reported 28 cases of frozen autograft use for reconstruction, and only 2 patients experienced recurrence (7.1%) [8]. In 2005, Subhadrabandhu et al. reported 3 cases of local recurrence among 22 patients (13.6%) [19]. Most recurrences were located in the surrounding soft tissue rather than the recycled bone. This was probably due to insufficient surgical resection. In addition, liquid nitrogen-treated bone retains certain antigens that might initiate an immune response against the tumor. In vivo experiments have shown that cryoablation can cause release of cytokines and inhibit the growth of tumors [24–26]. It may further reduce the rate of local recurrence.

The osteoinductive and osteoconductive properties of frozen autografts be retained [27]. Histological sections showed newly formed bone, osteocytes and microvessels in all portions of the grafted bone removed from patients, and good bone fusion was seen at the host-graft junction [28]. The reported bony union rate was between 83.3% and 100%, with typically 5.2 to 10.2 months to reunion (Table 2) [8, 9, 16–21]. Tsuchiya et al. reported bony union in 26 of 28 patients (92.9%), with a mean time of 6.7 months [8]. In another report, bony union of the frozen autografts was achieved in 33/34 patients (97.1%), with an average union time of 10.2 months [21]. In our study, bone union was seen in 37 of 40 patients (92.5%), with a mean union period of 8.7 months. Nonunion was seen in only three patients, two of whom had died of the disease before bony union; revision surgery was performed for the third patient. Union times and union rates in this study were comparable to those in other reports.

As tumor patients survive longer, there are more concerns about autograft fracture. Reported fracture rates range from 4.5–19.4% (Table 2) [8, 9, 16–21]. Subhadrabandhu et al. reported only one fracture in 22 patients [19]; Igarashi et al. documented seven fractures out of 36 patients [9]. Overall, the rate of fracture was low because of early bony union and intact initial strength of the recycled bone. Biomechanical tests have revealed that the initial strength of liquid nitrogen-treated bones is comparable to that of allografts and pasteurized bones but higher than that of autoclaved bones [7]. In addition, bone remodeling and revitalization in frozen bones occurs earlier than that in autoclaved or pasteurized bone [28]. Fracture thus remains a remote possibility, and only two occurred in our group as of the latest follow-up. Nevertheless, in most patients, bone cortices are usually destroyed by malignant tumors, and the biomechanical strength of the frozen autograft might decrease depending on the extent of destruction. Therefore, careful assessment of bone quality is needed before the resected bone is recycled. It is best for osteoblastic tumors but not osteolytic lesions.

In addition to bone nonunion and fracture, other complications, such as infection and limb shortening, have been documented. In the current cohort, infection was seen in three patients. The reported infection rate was between 5.9% and 25% (Table 2) [8, 9, 16–21]. Tsuchiya et al. reported three patients with deep infections among 28 patients (10.7%) [8]. Zekry et al. reported 2 cases of infection in a group of 34 patients (5.9%) [21]. Additional surgeries are usually needed to control infection, including debridement and irrigation or removal of the autograft. Garg et al. suggest that vancomycin-mixed normal saline be used to prevent infection during the recycling process [16].

Limb length discrepancy is inevitable in pediatric patients, especially when the epiphysis is invaded by the tumor or sacrificed during resection. Excessive limb shortening requires additional salvage surgery, such as distraction osteogenesis. Takeuchi et al. reviewed 12 children with osteosarcoma around the knee joint and investigated the growth of residual epiphysis following epiphyseal-preservation surgery [29], finding limb shortening (> 3 cm) in 6 patients, with two experiencing limb lengthening [29]. Zekry et al. suggested that bone lengthening should be considered if the leg-length discrepancy is more than 2cm [21]. In our cohort, all four patients with shortened lower extremities (2–4 cm) were managed conservatively using shoe lifts. No additional limb lengthening was performed.

The MSTS functional score has been reported to be 79–89.3%, and “excellent” results were seen in most patients (over 70%) (Table 2) [8, 9, 16–21]. Tsuchiya et al. reported that limb functional results were excellent in 20 (71.4%) out of 28 patients [8]. Subhadrabandhu et al. reviewed 22 patients and reported that 16 patients had excellent results with a mean functional MSTS score of 89.3% [19]. In our group, the mean MSTS score was 82.5%, and “excellent/good” results were seen in 95% of patients (38/40), with a good range of joint motion. The functional results are comparable with those reported in previous studies. With this reconstruction method, adjacent joints could be preserved, and recycling autografts could be easily reattached by soft tissues and muscles, which led to better muscle control and superior functional scoring.

This study has some limitations. The number of patients was small, and the research methodology was a clinical review. This study included a heterogeneous study sample with different diagnoses, tumor locations, and fixation methods. All of these factors made it difficult to analyze differences in outcome and complications. Further prospective studies in a larger series of patients with long-term follow-up should be conducted.

Conclusion

Our results suggest that liquid nitrogen-treated tumor-bearing autografts are a simple and effective option for biologic reconstructions after meta-/diaphyseal tumor resection at the extremities. Once incorporated, this approach offers a permanent form of reconstruction with excellent clinical outcomes, including high functional scores and a low complication rate. The application of this method is especially recommended for patients with nonosteolytic primary or secondary bone tumors.

Abbreviations

MSTS, Musculoskeletal Tumor Society.

MRI, Magnetic Resonance Imaging.

Declarations

Ethics approval and consent to participate: This study was approved by the Institutional Ethics Committee of Shangdong Provincial Hospital Affiliated to Shandong First Medical University. Informed consents were obtained from all subjects and/or their legal guardian(s). All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication: Written informed consents were obtained from all subjects and/or their legal guardian(s) for the use of their clinical data.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Author contributions: Jingyan Yang and Dong Li designed the study and wrote the manuscript. Wenze Li and Rongjie Feng collected and analyzed the data. Wenze Li prepared Figures 1-2. All authors reviewed the manuscript.

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Figures

Figure 1

A 14-year-old female with osteosarcoma involving the shaft of the left tibia (case 22).

a Preoperative MRI showed a tumor located in the diaphysis of the left tibia. **b** Intercalary resection using a wire saw. **c** Resected bone. **d** Resected bone treated with liquid nitrogen. **e** The frozen autograft was reimplanted and fixed with a locking plate and screws. **f** Postoperative X-rays. **g** X-rays at 5 months after reimplantation showing union at both osteotomy sites.

Figure 2

A 16-year-old male with osteosarcoma involving the shaft of the right femur (case 28).

a, b and c X-ray, CT and MRI showed the tumor involving the diaphysis of the right femur. **d** Postoperative X-rays after intercalary resection and recycling autograft reconstruction with intramedullary nail fixation. **e** X-ray at 10 months after reimplantation showing union at both osteotomy sites.