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What Are the Challenges and Complications of Sterilizing Autografts with Liquid Nitrogen for Malignant Bone Tumors? A Preliminary Report

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Abstract

Background Reconstruction of defects after resection of malignant bone tumors with liquid nitrogen-sterilized recycled autografts is an alternative to bone allografts and endoprostheses in resource-constrained environments. Most studies reporting favorable outcomes with liquid nitrogen-sterilized autografts for bone reconstruction are geographically restricted to a few countries, and the technical challenges of routinely using liquid nitrogen intraoperatively, especially when using the pedicle freezing technique, has not been documented.

Questions/purposes (1) What are the technical challenges of liquid nitrogen sterilization of bone tumors for inexperienced surgeons? (2) What are the complications associated with the procedure?

Methods Between May 2017 and October 2019, 88 patients underwent limb salvage procedures for malignant bone tumors of the extremities at our institution. An endoprosthesis was used for reconstruction of the defect following resection in 45% (40 of 88) of these patients, mostly in adults (median age 21 years; range 9 to 68). In the remaining 55% (48 of 88) of patients undergoing biological reconstruction, liquid nitrogen-sterilized autograft was used in 90% (43 of 48), extracorporeal irradiation-sterilized autograft was used in 4% (2 of 48) and allograft was used in 6% (3 of 48). Of the 43 patients receiving liquid nitrogen-sterilized autograft, 5% (2 of 43) were excluded due to loss to follow-up and the remaining 95% (41 of 43) were included for the analysis. Liquid nitrogen-sterilized autograft was the preferred method of reconstruction at our institution during the study period, unless the patient had an indication for prosthesis reconstruction; extracorporeal irradiation-sterilized autograft was used due to resource constraints with liquid nitrogen and allograft was used when patients insisted.

All surgical procedures were performed by the same team of trained orthopaedic oncology surgeons. The medical records of the included 41 patients were retrieved using an institutional database search in this retrospective study, and all were used to ascertain technical challenges associated with the operations as well as early (within 3 weeks of the index procedure) and late complications (those occurring 3 weeks or more after surgery). The technical challenges were defined as follows: the quantity of liquid nitrogen to be used; arranging, storing and handling of liquid nitrogen in the operating room, type and size of the container to be used for sterilization, the positioning of the container during pedicle freezing, level of fibular osteotomy for pedicle freezing of tibia, soft tissue protection, limb rotation

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during pedicle freezing, managing tourniquet time, and any other intraoperative factors with the use of liquid nitrogen for sterilizing the autograft. As our experience with the technique gradually grew, the answers to the above-mentioned factors were determined. Considering the removal of autograft as the endpoint of interest, survival of the autograft was determined by Kaplan-Meier analysis.

The median (range) patient age was 14 years (2 to 49), and 54% (22 of 41) were males. Osteosarcoma was the most common diagnosis (68%, [28 of 41]) followed by Ewing's sarcoma (20%, [8 of 41]). On presentation, 27% of patients (11 of 41) had radiological evidence of pulmonary metastasis. Tumors were seen frequently around the knee (39% [16 of 41] proximal tibia and 22% [9 of 41] distal femur). Before resection 85% (35 of 41) underwent neoadjuvant chemotherapy. Sixty-six percent (27 of 41) underwent pedicle-freezing and the remaining 34% (14 of 41) underwent free-freezing of the tumor segment of the bone. The median (range) duration of surgery was 280 minutes (210 to 510). The patients were followed up for a median (range) duration of 21 months (5 to 30); two patients were lost to follow-up.

Results With gradual experience using liquid nitrogen-sterilization over time at our institution, we determined that the following factors helped us in performing liquid nitrogen-sterilization more efficiently. For every operation 15 L to 20 L of unsterilized liquid nitrogen was arranged, 1 or 2 days before the procedure, and stored in industrial-grade cryocylinders in the operating complex. During the procedure, the operating surgeons wore additional plastic aprons under the surgical gowns, surgical goggles, and rubber boots. The staff managing the liquid nitrogen in the operating room wore thermal protective gloves. For most of the pedicle freezing procedures, we used a cylindrical stainless-steel container that was 30 cm in height and 15 cm in diameter, with a narrow opening. The container was kept on a separate moveable cart that was placed next to the operating table at a slightly lower level, and it was wrapped in multiple cotton rolls, plastic sheets, surgical sheets, and a crepe bandage. For pedicle freezing of the tibia, we performed the fibular osteotomy at least 5 cm away from the planned surgical margin, roughly around the axis of rotation of the limb. The soft tissue at the base of the delivered bone segment was dissected for at least 5 cm beyond the planned surgical margin of bone, and was protected with multiple layers of cotton rolls, plastic drapes, a single roll of Esmarch and crepe bandage. The tumor segment was externally rotated during pedicle freezing for all anatomic sites (proximal tibia, distal tibia, proximal humerus, and proximal femur). The tourniquet was inflated just before pedicle freezing to prevent tumor dissemination and not before the initial incision in all pedicle freezing procedures.

Thirty-nine percent of patients (16 of 41) experienced complications associated with the procedures, and 15% (6 of 41) underwent revision surgery. Early complications (occurring within 3 weeks of the index procedure) were skin necrosis in four of 16 patients, intraoperative fracture in one of 16, superficial infection in one of 16, and neurapraxia in one of 16 patients. Late complications (occurring 3 weeks or more after surgery) were resorption of the recycled bone in four of 16 patients, nonunion of the osteotomy site in two of 12, delayed union of the osteotomy site in one of 16, collapse of the recycled bone in one of 16, and local recurrence in 1 of 16 patients. Kaplan-Meier survivorship free from removal of autograft at 2 years after surgery was 92% (95% confidence interval 89 to 96).

Conclusion Liquid nitrogen-sterilization is an alternative technique that requires some training and experience for the surgeon to become proficient in treating primary malignant bone tumors. Because it is widely available, it may be an option worth exploring in resource-constrained environments, where allografts and endoprostheses cannot be procured. The methods we developed to address the technical challenges will require more study and experience, but we believe these observations will aid others who may wish to use and evaluate liquid nitrogen sterilization of extremity bone sarcomas.

Level of Evidence Level IV, therapeutic study.

Introduction

Although biological reconstructions after resection of bone tumors can have excellent durability and function [2], allografts may not be available in all locations around the world, particularly in resource-constrained environments [1, 17, 21, 32]. For that reason, recycled autografts are sometimes used; sterilization of a bone segment in a patient with a malignant tumor can be achieved with irradiation, autoclaving, or pasteurization [4, 5, 16, 17]. However, these techniques require special equipment and strict thermal control. Additionally, the hyperthermic process may cause weakness of the recycled bone and loss of the bone's osteoinductive potential [9, 28]. Cryosterilization of malignant bone tumors with liquid nitrogen at -196 °C devitalizes tumor cells by inducing formation of ice crystals and cell dehydration. Liquid nitrogen-sterilized bones have superior osteoinductive properties to bone sterilized with hyperthermic techniques because of better preservation of bone morphogenic proteins [9, 29].

In 2005, Tsuchiya et al. [32] described reconstruction using tumor-bearing autografts treated with liquid nitrogen; since then, studies with long-term follow-up have reported good functional and oncologic outcomes in patients with liquid nitrogen-sterilized autografts for malignant bone tumors [12, 23, 35]. However, most of these studies are mainly from the Southeast and East Asian countries, as use of allografts in some of these geographical regions is restricted due to socioreligious reasons [12, 23, 35]. Moreover, to our knowledge, no studies have reported the technical details and challenges of using liquid nitrogen intraoperatively.

In this preliminary report, we asked: (1) What are the technical challenges of liquid nitrogen sterilization of bone tumors for inexperienced surgeons? (2) What are the complications associated with the procedure?

Patients and Methods

Study Design, Setting, and Participants

Between May 2017 and October 2019, 88 patients underwent limb salvage procedures for malignant bone tumors of the extremities at our institution. An endoprosthesis was used for reconstruction of the defect following resection in 45% (40 of 88) of these patients, mostly in adults (median [range] age 21 years [9 to 68]), for the following indications: (1) when patients desired early weightbearing and unassisted ambulation, (2) osteolytic lesions with more than one-third cortical destruction, (3) more than three pulmonary metastatic nodules at the time of surgery, (4) progression of local or metastatic disease with neoadjuvant chemotherapy, and (5) extrapulmonary metastasis on presentation. In the remaining 55% (48 of 88) of patients undergoing biological reconstruction, liquid nitrogen-sterilized autograft was used in 90% (43 of 48), extracorporeal irradiation-sterilized autograft was used in 4% (2 of 48) and allograft was used in 6% (3 of 48). Of the 43 patients receiving liquid nitrogen-sterilized autograft, 5% (2 of 43) were excluded due to loss to follow-up and the remaining 95% (41 of 43) were included for the analysis. Liquid nitrogen-sterilized autograft was the preferred method of reconstruction in our institution during the study period, unless the patient's tumor was more conducive to reconstruction with a prosthesis; extracorporeal irradiation-sterilized autograft was used due to resource constraints; liquid nitrogen and allograft were used when patients insisted. Institutional ethical board approval was obtained for the study.

Description of the Treatment

All 41 patients had histologic confirmation of a malignant bone tumor before treatment. Preoperative CT images, MR images, and radiographs of the affected bone were used to determine the extent of the tumor, length of resection, and the type of reconstruction needed.

All surgical procedures were performed by the same team of trained orthopaedic oncology surgeons with no previous experience with liquid nitrogen-sterilization in the treatment of malignant bone tumors at the start of this study. Fourteen patients underwent "free freezing" sterilization (Fig. 1) and 27 underwent "pedicle freezing" sterilization (Fig. 2). The goal was to perform pedicle freezing and avoid osteotomy in all patients, but when it was difficult to rotate the limb to submerge the tumor-bearing segment in the liquid nitrogen container or when the bone tumor was long, free freezing was performed (in nine distal femurs, two femur shafts, two humerus shafts and one shaft of ulna). In the free-freezing technique, the tumor was resected with wide margins following the standard oncologic principles of resection. Soft tissue was removed from the resected bone segment, bone marrow and the tumor in the medullary canal were curetted, and samples were sent for a histologic examination. Soft tissue removal was performed on a separate table; the table, instruments used, and the surgical gown and gloves of the surgeon performing it were discarded to prevent tumor tissue contamination. If the lesion was sclerotic and dense, one or two cortical holes were drilled (with a 2.5-mm drill bit) into the medullary canal, preferably in the same direction in which the cortical screws of the plate to fix the graft would be passed, to prevent fracture because of water volume expansion during freezing. The resected bone was frozen in liquid nitrogen for 20 minutes, thawed at room temperature for 15 minutes, and thawed in distilled water for 10 minutes. All 14 patients who underwent free freezing had two osteotomies to deliver the bone segment for sterilization. In patients who underwent the pedicle freezing technique, the tumor segment, along with its soft tissue and skeletal margins, was skeletonized and separated from the surrounding tissue. If the tumor was away from the articular surface (Fig. 3A), a single osteotomy was performed approximately 2 to 3 centimeters from the tumor. The tumor segment was delivered from the long axis of the limb, maintaining bony continuity on the side opposite the osteotomy (Fig. 3B). If the tumor was close to the joint and an osteotomy was not possible, the adjacent joint was dislocated and the tumor segment was delivered, maintaining bony continuity away from the dislocated joint. The soft tissue at the base of the delivered bone segment was dissected for at least 5 cm beyond the planned surgical margin of bone and was protected with multiple layers of cotton rolls, plastic drapes, and a single roll of Esmarch and crepe bandage (Fig. 3C). The medullary canal was gentle curetted, marrow tissue was obtained for a histologic examination ,and the limb was cautiously rotated to position the skeletonized tumor-bearing segment in a container with liquid nitrogen for freezing (Fig. 3D). Patients with joint-sparing pedicle freezing underwent a single osteotomy, and the patients with pedicle freezing whose adjacent joint was dislocated did not need an osteotomy. The results of a frozen section histologic examination from the osteotomy sites were negative for malignant cells in all patients. There was no macroscopic tumor contamination of the tumor bed during the procedure.

For pedicle freezing of the tibia, an osteotomy of the fibula was performed opposite from the osteotomy of the tibia to rotate the limb. The bone segment was rotated into the container, with the tourniquet inflated to prevent tumor dissemination. The duration of the sterilization process was the same as that of the free freezing technique: 20 minutes in liquid nitrogen, 15 minutes in room-temperature air, and 10 minutes in

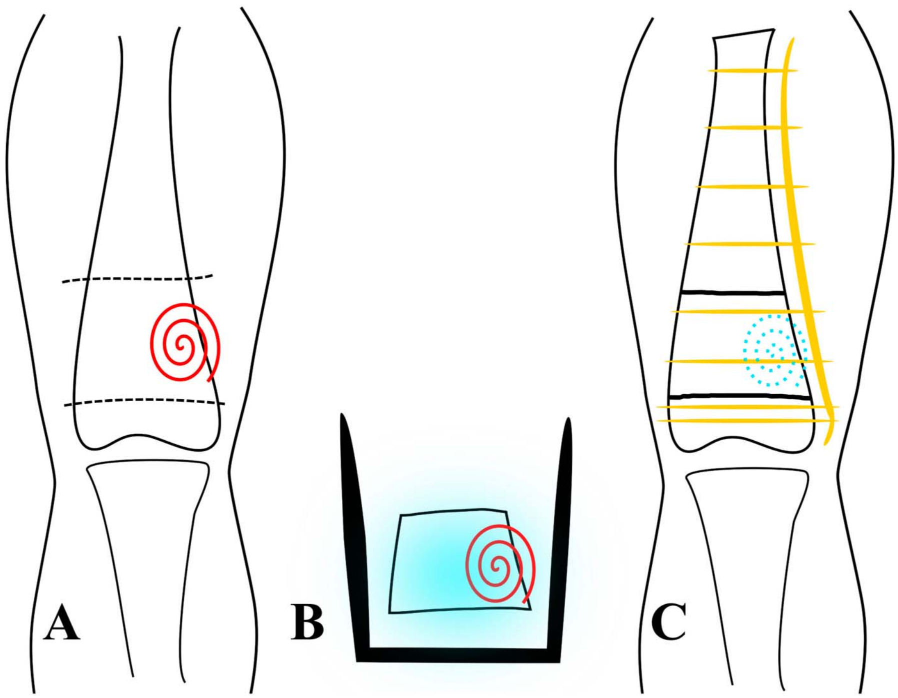

Fig. 1 A-C (**A**) This diagram represents the "free freezing technique" with a tumor in the distal femur, which was (**B**) resected en bloc and sterilized in liquid nitrogen. (**C**) This image shows the recycled bone used for skeletal reconstruction.

distilled water. After sterilization, bone cement was finger packed into the recycled bone without a cement gun in all patients before reimplantation. Reconstruction was performed with a plate and screws in patients with an osteotomy, and in patients with a recycled articular surface, prosthetic replacement of the joint was performed (Fig. 4A-B). For the replaced knee, a cemented rotating hinged knee prosthesis was used, and for the proximal femur, a cemented bipolar prosthesis was used. For the distal tibia, joint replacement was not performed, and the ankle was fused; for the proximal humerus, recycled cartilage was used for reconstruction without replacing the shoulder. In all patients with pedicle freezing, the recycled bone was stabilized to the native bone that was not frozen with a long plate or with a long intramedullary stem that spanned at least 10 cm into the unfrozen bone. Soft tissue was reconstructed with adequate coverage of the recycled autograft, and the limb was immobilized in a slab. Primary wound closure was achieved in 76% (31 of 41), 17% (7 of 41) underwent skin grafting, and the remaining 7% (3 of 41) had a free vascularized flap to cover the wound after the primary procedure.

Aftercare and Follow-up

Postoperative adjuvant chemotherapy began approximately 2 weeks after the procedure, when indicated. If soft-tissue healing was needed after a tendon or articular capsule reconstruction, the adjacent joint was immobilized for 3 to 6 weeks after surgery. Weightbearing was restricted in all patients for 3 months, after which progressive weightbearing as tolerated was started with crutch support. Full weightbearing ambulation without support was encouraged by 6 months, and strenuous activities such as running, jumping, or lifting heavy weights were restricted for 1 year postoperatively. Serial radiographs were used to assess the time to union of the osteotomy site and to note changes in the recycled autografts. Delayed union was defined as radiological union at the osteotomy 6 months or more after the procedure without any additional surgery; and nonunion was defined as no radiological evidence of callus formation at the osteotomy site 9 months after the procedure necessitating additional surgery.

Demographics and Description of the Study Population

The median (range) age of the patients was 14 years (2 to 49), and 54% (22 of 41) of patients were males. Osteosarcoma was the most common diagnosis (68% [28 of 41]) followed by Ewing's sarcoma (20% [8 of 41]). Tumors were seen frequently around the knee (39% [16 of 41] proximal tibia and 22% [9 of 41] distal femur). On presentation 27% (11 of 41) of patients had pulmonary metastasis, and none of the patients had a skip metastasis or bone metastasis. Before surgery 85% (35 of 41) underwent

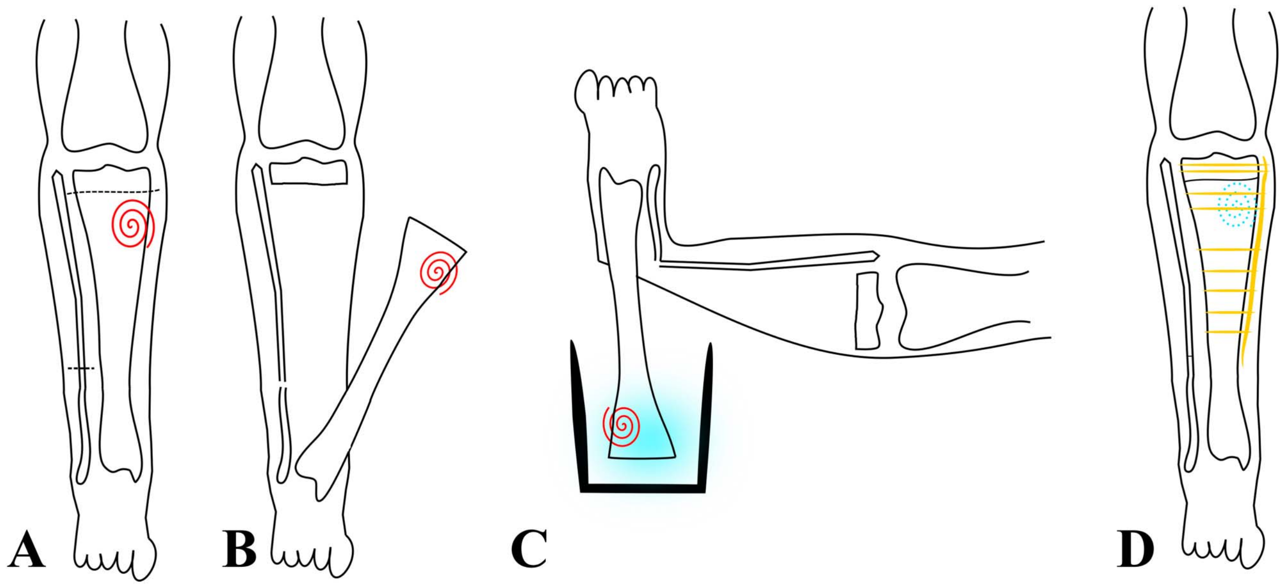

Fig. 2 A-C (**A**) This figure shows the "pedicle freezing technique" with a tumor in the proximal tibia. (**B**) A single osteotomy was performed in the proximal tibia and the tumor segment was delivered after another osteotomy in the distal fibula. (**C**) The limb was rotated into a container with liquid nitrogen to eradicate the tumor, maintaining continuity with the limb. (**D**) The recycled proximal tibia was reconstructed using a plate and screws, and the joint was spared.

neoadjuvant chemotherapy. Patients with Ewing's sarcoma (n = 8) received four to eight cycles of vincristine (2 mg/m^2 of body-surface area), doxorubicin (75 mg/m^2) and cyclophosphamide (1.2 g/m^2) alternating with ifosfamide (9 g/m^2) and etoposide (100 mg/m^2), with cycles beginning every 14 days [34]. Patients with high-grade osteosarcomas (n = 26) received two 5-week cycles of doxorubicin (75 mg/m^2), cisplatin (120 mg/m^2) and methotrexate (12 g/m^2) [7]. Sixty-six percent (27 of 41) of patients underwent pedicle-freezing and the remaining 34% (14 of 41) underwent free-freezing of the tumor segment of the bone. The median (range) duration of surgery was 280 minutes (210 to 510). The patients were followed for a median (range) duration of 21 months (5 to 30); two patients were lost to follow-up (Table 1).

Primary and Secondary Study Outcomes

Though the technique of liquid nitrogen-sterilization has been described by Tsuchiya et al. [31, 32], technical challenges associated with the sterilization technique have not been described or studied before. To address our primary study outcome, technical challenges, we aimed at determining: the quantity of liquid nitrogen to be used; arranging, storing and handling of liquid nitrogen in the operating room; type and size of the container to be used for sterilization; the positioning of the container during pedicle freezing; the level of osteotomy for pedicle freezing, soft tissue protection, limb rotation during pedicle freezing; and managing tourniquet time. Based on our gradually accumulated experience using liquid nitrogen-sterilization, we wanted to report the methods or techniques we used to answer the above-mentioned challenges, which helped us perform liquid nitrogen-sterilization more efficiently. The other objective of the study was to determine the complications associated with liquid nitrogen sterilization. To answer this question, we report the early complications attributable to the procedure which occurred during or within 3 weeks of the procedure and late (those occurring 3 weeks or more after surgery) complications.

Statistical Analysis

Categorical variables were presented as frequencies and percentages, continuous variables were presented as median and range. The survivorship free from graft removal of the patients at 2 years after surgery was estimated using the Kaplan-Meier method.

Results

What Are the Technical Challenges of Liquid Nitrogen Sterilization for Inexperienced Surgeons? Lessons Learned

Liquid nitrogen was not readily available in our hospital and had to be arranged and stored in cryocylinders 1 or 2 days before surgery. In two instances, the liquid nitrogen

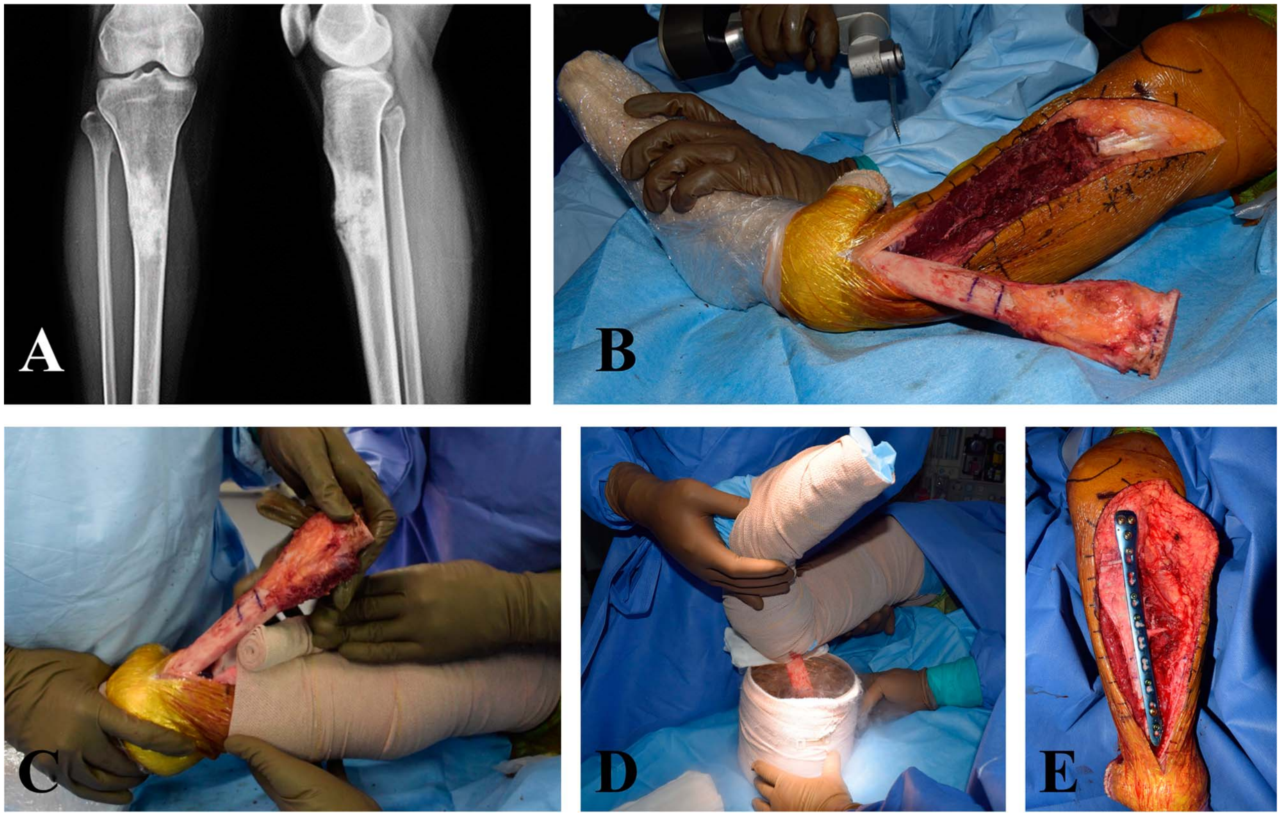

Fig. 3 A-E (**A**) This is a plain radiograph from a patient with low-grade osteosarcoma of the proximal tibia and (**B**) a tumor segment that was skeletonized from the surrounding tissue after joint-sparing osteotomy of the proximal tibia. (**C**) The surrounding soft tissue was protected to prevent thermal injury, and (**D**) the tumor segment was treated in liquid nitrogen by rotating the limb and maintaining continuity with the limb. (**E**) The reconstructed bone was stabilized with a locking plate and screws. A color image accompanies the online version of this article.

arrived 5 days before surgery, and a large amount had evaporated, even though it was stored in an industrial-grade 30-L capacity cryocylinder. Approximately 15 L to 20 L were ordered for each operation, and no special sterilization was performed of the liquid nitrogen to be used. Twice, during the initial days of employing this technique, the quantity of liquid nitrogen arranged was not enough to perform pedicle freezing, and the resected tumor bone had to be sterilized by extracorporeal irradiation instead.

The operating surgeons wore additional plastic aprons under the surgical gowns, surgical goggles, and rubber boots for all operations. The staff managing the liquid nitrogen in the operating room wore thermal protective gloves. Because the liquid nitrogen is denser than air, we found that if the surgeon

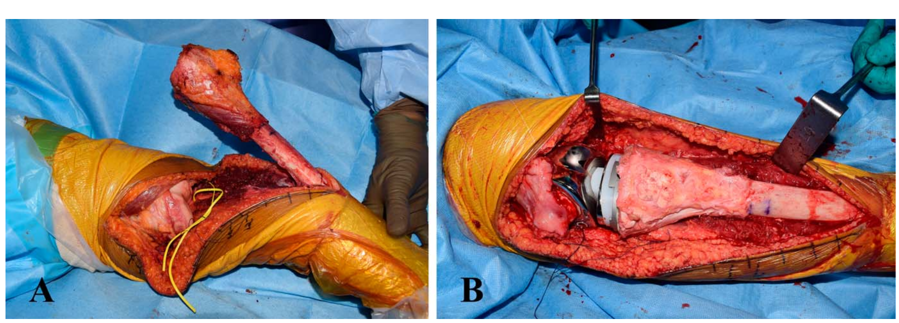

Fig. 4 A-B (**A**) This image shows an osteosarcoma of the proximal tibia involving the metaphysis and the epiphysis, which we dissected from the surrounding soft tissue and skeletonized, maintaining bony continuity with the limb after the knee was dislocated. (**B**) The knee was replaced with a long-stem tibial plate after sterilization in liquid nitrogen. A color image accompanies the online version of this article.

Table 1. Characteristics of patients who underwent liquid nitrogen sterilization and recycled autograft reconstruction for malignant bone tumors of the extremities

Number	Age (years)	Sex	Diagnosis	Tumor site	Pulmonary metastasis	NAC	Type of freezing	Number of osteotomy	Adjacent joint	Stabilization of recycled autograft	Duration of surgery (minutes)	Operative site closure after surgery	AC	Follow-up (months)	Clinical status
1	13	Male	Osteosarcoma	DF	No	Yes	Free	2	Retained	Plate	230	Primary	Yes	30	ADF
2	14	Female	Ewing's sarcoma	PT	No	Yes	Pedicle	0	Replaced	Long stem	280	Primary	Yes	28	ADF
3	11	Male	Osteosarcoma	PT	No	Yes	Pedicle	0	Replaced	Long stem	310	Skin graft	Yes	30	ADF
4	28	Male	Ewing's sarcoma	DT	No	Yes	Pedicle	1	Retained	Plate	400	Flap	Yes	28	DDD
5	15	Female	Osteosarcoma	PT	Yes	Yes	Pedicle	0	Replaced	Long stem	380	Skin graft	Yes	29	DDD
6	11	Female	Ewing's sarcoma	PF	Yes	Yes	Pedicle	0	Replaced	Long stem + plate	410	Primary	Yes	27	AWD
7	2	Female	Ewing's sarcoma	PT	No	Yes	Pedicle	1	Retained	Plate	510	Primary	Yes	23	DOR
8	15	Male	Osteosarcoma	PH	No	Yes	Pedicle	0	Retained	Plate	290	Primary	Yes	25	ADF
9	45	Male	Malignant giant cell tumor	PF	Yes	No	Pedicle	0	Replaced	Long stem	300	Primary	Yes	25	DDD
10	12	Male	Osteosarcoma	PT	Yes	Yes	Pedicle	0	Retained	Plate	270	Primary	Yes	27	AWD
11	14	Female	Osteosarcoma	PT	No	Yes	Pedicle	0	Replaced	Plate	260	Skin graft	Yes	28	ADF
12	16	Male	Osteosarcoma	PT	No	Yes	Pedicle	0	Replaced	Long stem and plate	280	Primary	Yes	29	ADF
13	15	Female	Osteosarcoma	FS	Yes	Yes	Free	2	Retained	Plate and nail	310	Primary	Yes	26	AWD
14	21	Female	Osteosarcoma	PT	No	No	Pedicle	1	Retained	Plate	210	Flap	No	26	ADF
15	16	Female	Osteosarcoma	PT	No	Yes	Pedicle	0	Replaced	Long stem	290	Primary	Yes	26	ADF
16	30	Male	Malignant giant cell tumor	PH	No	No	Pedicle	0	Retained	Plate	220	Primary	No	25	ADF
17	43	Female	Osteosarcoma	DT	No	No	Pedicle	0	Fused	Plate	270	Primary	No	23	ADF
18	27	Male	Chondrosarcoma	PT	No	No	Pedicle	0	Replaced	Long stem + plate	230	Skin graft	No	22	ADF
19	11	Male	Osteosarcoma	PT	No	Yes	Pedicle	1	Retained	Plate	260	Primary	Yes	22	ADF
20	9	Male	Osteosarcoma	DF	Yes	Yes	Free	2	Retained	Plate	210	Primary	Yes	24	ADF
21	11	Male	Osteosarcoma	DF	No	Yes	Free	2	Retained	Plate	220	Primary	Yes	21	ADF
22	9	Female	Osteosarcoma	PT	No	Yes	Pedicle	1	Retained	Plate	270	Primary	Yes	20	ADF
23	28	Male	Adamantinoma	DT	No	No	Pedicle	0	Fused	Plate	260	Primary	No	19	ADF
24	21	Male	Osteosarcoma	HS	Yes	Yes	Free	2	Retained	Plate	290	Primary	Yes	21	ADF
25	31	Male	Osteosarcoma	DF	No	Yes	Free	2	Retained	Plate	260	Primary	Yes	20	ADF
26	4	Female	Ewing's sarcoma	US	No	Yes	Free	2	Retained	Plate	230	Primary	Yes	19	ADF
27	14	Female	Osteosarcoma	DF	No	Yes	Free	2	Retained	Plate	250	Primary	Yes	19	ADF
28	12	Male	Osteosarcoma	DF	No	Yes	Free	2	Retained	Plate	230	Primary	Yes	18	ADF

Table 1. continued

Number	Age (years)	Sex	Diagnosis	Tumor site	Pulmonary metastasis	NAC	Type of freezing	Number of osteotomy	Adjacent joint	Stabilization of recycled autograft	Duration of surgery (minutes)	Operative site closure after surgery	AC	Follow-up (months)	Clinical status
29	49	Male	Chondrosarcoma	DF	No	No	Free	2	Retained	Plate + vascularized fibula	320	Primary	No	14	ADF
30	11	Female	Osteosarcoma	DF	No	Yes	Free	2	Retained	Plate + vascularized fibula	310	Primary	Yes	17	ADF
31	9	Female	Osteosarcoma	DF	Yes	Yes	Free	2	Retained	Plate + vascularized fibula	320	Primary	Yes	13	ADF
32	16	Female	Ewing's sarcoma	FS	No	Yes	Free	2	Retained	Nail	290	Primary	Yes	16	ADF
33	10	Female	Osteosarcoma	PT	No	Yes	Pedicle	1	Retained	Plate	270	Flap	Yes	13	ADF
34	17	Male	Ewing sarcoma	PF	No	Yes	Pedicle	0	Replaced	Long stem	260	Primary	Yes	12	AWD
35	14	Female	Osteosarcoma	PT	Yes	Yes	Pedicle	0	Replaced	Long stem	240	Skin graft	Yes	11	AWD
36	13	Male	Osteosarcoma	PH	No	Yes	Pedicle	0	Retained	Plate	260	Primary	Yes	14	ADF
37	12	Male	Osteosarcoma	PH	Yes	Yes	Pedicle	0	Retained	Plate	290	Skin graft	Yes	13	AWD
38	29	Female	Osteosarcoma	PT	No	No	Pedicle	1	Retained	Plate	300	Primary	Yes	9	ADF
39	31	Male	Osteosarcoma	PT	No	Yes	Pedicle	0	Replaced	Long stem	310	Skin graft	Yes	7	ADF
40	12	Male	Ewing's sarcoma	HS	Yes	Yes	Free	2	Retained	Plate	310	Primary	Yes	6	AWD
41	12	Female	Osteosarcoma	PF	No	Yes	Pedicle	0	Replaced	Long stem	320	Primary	Yes	5	ADF

NAC = neoadjuvant chemotherapy; AC = adjuvant chemotherapy; DF = distal femur; PT = proximal tibia; PF = proximal femur; DT = distal tibia; PH = proximal humerus; FS = femoral shaft; HS = humeral shaft; US = ulnar shaft; ADF = alive, disease-free; DDD = dead due to disease; AWD = alive with disease; DOR = dead due to other reason.

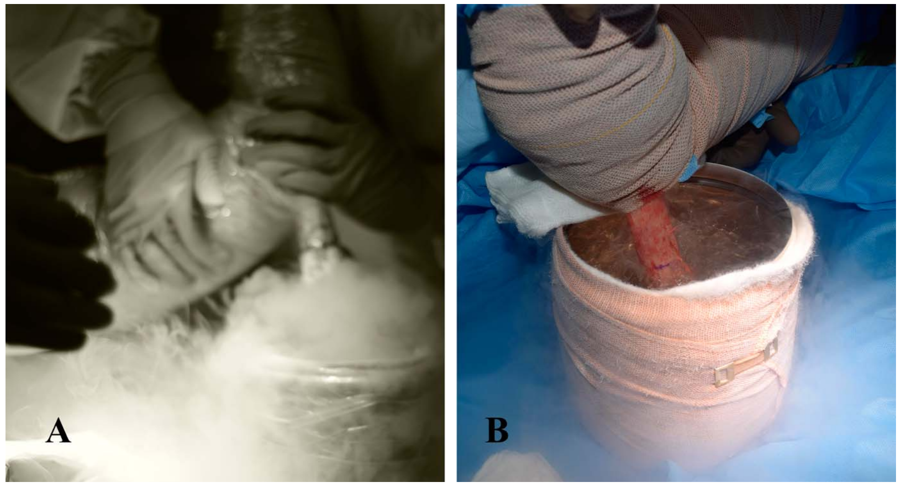

Fig. 5 A-B (**A**) Dense vapors of liquid nitrogen formed when the limb was rotated into the container, which prevented us from visualizing the bone level, which must be entirely submerged in liquid nitrogen for complete sterilization. (**B**) The vapors settled down in 2 to 3 minutes. A color image accompanies the online version of this article.

or the assistant did not wear boots, the spill over from the cart caused discomfort during pedicle freezing, which could disturb the process of maintaining stable limb position in the container for a prolonged period during sterilization. Also, because water is a good heat conductor, we realized that the gloves of the person handling the container must be completely dry to prevent possible thermal injury.

For most of the pedicle freezing procedures, we used a cylindrical stainless-steel container that was 30 cm in height and 15 centimeters in diameter and had a narrow opening. Wider containers were used initially, but we learned that they required more liquid nitrogen because they were associated with increased spilling and faster evaporation. The container was kept on a separate moveable cart that was placed next to the operating table at a slightly lower level. The container was wrapped in multiple cotton rolls, plastic sheets, surgical sheets, and a crepe bandage to reduce injury to the patient and facilitate management by the surgeon and assistants. Before filling the container with liquid nitrogen, we performed trials using an empty container to check whether the limb could be rotated smoothly. The height and tilt of the operating table required to place the tumor segment into the container were determined before freezing because the limb's position had to be maintained in liquid nitrogen without much movement for 20 minutes continuously. The tumor segment was externally rotated in the coronal plane during pedicle freezing for all anatomic sites (proximal tibia, distal tibia, proximal humerus, and proximal femur). Marking the bone level (with a marking pen or electrocautery) to be sterilized helped to confirm that the segment of the tumor, including the planned surgical margin (2 cm to 3 cm), was submerged in liquid nitrogen during freezing.

Liquid nitrogen was slowly poured directly from the cryocylinder into the stainless-steel container. During pedicle freezing, the marking on the bone was challenging to identify for the first 2 to 3 minutes because of liquid nitrogen vapors (Fig. 5A). Hence, for the first 3 minutes, the liquid nitrogen level in the container was maintained almost to the brim; the liquid nitrogen later evaporated, and the marking became evident as the vapors settled (Fig. 5B). When the tumor bone was in the container, care was taken to prevent its contact with the opening and walls of the container. Cotton pads dipped in warm saline were placed at the opening of the container and were continuously irrigated with warm saline to prevent thermal injury to the surrounding soft tissue. Since the bone adjacent to the frozen segment was the sole source of blood supply to the recycled bone, it was protected during pedicle freezing by regular application of warm saline with a syringe. The vascular bundle in the protected soft tissue supplied the frozen bone and the distal extremity, which was well wrapped and thermally insulated as mentioned earlier. A small quantity of liquid nitrogen was stored in a sterile thermal flask and kept in the cart for refilling the container whenever the level of liquid nitrogen fell below the marked bone level, as refilling from the cryocylinder was very difficult when the limb was positioned in the container. Frequent refills were needed because liquid nitrogen evaporated rapidly.

For pedicle freezing of the tibia, determining the level of the osteotomy of the fibula was important. During the initial procedures, an osteotomy was made around the planned surgical margin of the tibia, but rotating the tibia into the container was difficult and often required more soft-tissue dissection. With experience, we performed the osteotomy at least 5 cm away from the planned surgical margin, roughly around the axis of rotation of the limb for smoother rotation of the limb (Fig. 6).

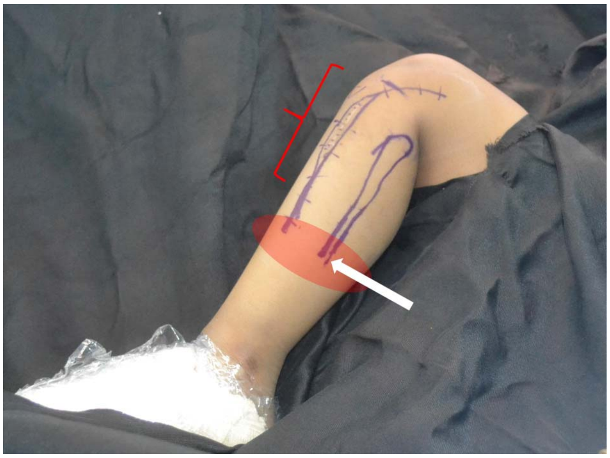

Fig. 6 This preoperative photograph shows a patient with Ewing's sarcoma of the proximal tibia; the bracket indicates the extent of the tumor, the shaded area indicates the axis of rotation for pedicle freezing, and the arrow indicates the site of fibula osteotomy to rotate the limb. A color image accompanies the online version of this article.

The tourniquet should ideally be inflated before pedicle freezing to prevent tumor dissemination. We found that when the tourniquet was inflated before the initial incision for pedicle freezing procedures, the tourniquet time often exceeded the recommended limit and could not be used for the duration of liquid nitrogen sterilization (20 minutes). Deflating the tourniquet for reperfusion added to the overall surgical time and a messier surgical field. Thus, for recently procedures, the tourniquet was inflated before pedicle freezing of the tumor segment instead of before the initial incision. We used a tourniquet in all but four patients: four of the proximal humerus and four of the proximal femur, in whom the axillary and femoral vessels, respectively, were clamped during freezing.

What Are the Complications Associated with the Procedure?

The proportion of patients who experienced complications associated with this procedure was 39% (16 of 41), and 15% (6 of 41) underwent revision surgery. Early complications (occurring within 3 weeks of the index procedure) were skin necrosis in four of 16 patients, intraoperative fracture in one of 16, superficial infection in one of 16, and neurapraxia in one of 16 patients (Table 2). Skin necrosis was observed in the limb near the opening of the liquid nitrogen container and was observed only in pedicle freezing procedures (Fig. 7). One patient with skin necrosis underwent débridement and split-skin grafting; wounds healed spontaneously in the other three patients by secondary epithelialization. An infection of the recycled autograft (distal tibia) developed in one patient 2 months after the procedure, which subsided with multiple débridements and the recycled autograft was retained. One resected bone (free-frozen distal femur) had a fracture that was noticed during thawing of the recycled bone in distilled water. We decided to use the same bone for reconstruction because it had just a small crack in the posterior cortex, with good bone stock and strength in the other three cortices. We observed no adverse consequences in follow-up of this patient using our standard rehabilitation protocol. Late complications (occurring 3 weeks or more after surgery) were resorption of the recycled bone in four of 16 patients, nonunion of the osteotomy site in two of 12, delayed union of the osteotomy site in one of 16, collapse of the recycled bone in one of 16, and local recurrence in one of 16 patients. The collapsed recycled autograft of one patient with proximal tibia pedicle freezing and knee arthroplasty had implant loosening 9 months postoperatively. During revision surgery in this patient with collapse, the recycled autograft was weak with no signs of vascularization and was hence replaced with a proximal tibia endoprosthesis. Resorption of the recycled autograft was evident on serial radiographs in four patients after a median (range) duration of 6 months (4 to 10) from surgery; three of these four patients were asymptomatic, and one patient had a painless limp (Fig. 8). Infection was ruled out in all four of these patients. No surgical intervention was performed, and protected weight-bearing with a brace was recommended to prevent a pathologic fracture, because all four resorptions were in the tibia (three in the proximal tibia; one in the distal tibia). At the final follow-up examination of these four patients, two had evidence of new bone formation at the site of resorption.

One patient with a low-grade osteosarcoma of the proximal tibia who underwent joint-sparing pedicle freezing and plate reconstruction had local recurrence 19 months postoperatively. Local recurrence was located proximal to the osteotomy site in the bone that was not recycled. This patient with local recurrence underwent resection of the proximal tibia and prosthesis reconstruction. However, none of the recycled bone showed evidence of active disease or local recurrence.

Discussion

Allografts are a viable option for reconstruction of skeletal defects after resection of malignant bone tumors at all sites and in all age groups, but associated complications (such as infection, fracture, and nonunion), and lack of availability of matched allografts impedes its routine use in many centers and countries globally. A recycled autograft is an alternative to endoprostheses and effective methods such as irradiation, autoclaving, and pasteurization have been described to sterilize tumor bone before reimplantation, with good long-term outcomes [4, 5, 17]. Tsuchiya et al. [32] first reported the method of sterilizing tumor bone with liquid nitrogen after resection. To

Table 2. Complications of liquid nitrogen sterilization and recycled autograft reconstruction for malignant bone tumors

Number	Complication	Time*	Age	Type of freezing	Site	Revision surgery	Autograft retained	Final outcome
1	Skin necrosis	Early	16	Pedicle	PT	No	Yes	Healed without intervention
2	Skin necrosis	Early	30	Pedicle	DT	No	Yes	Healed without intervention
3	Skin necrosis	Early	11	Pedicle	PT	No	Yes	Healed without intervention
4	Skin necrosis	Early	27	Pedicle	PT	Yes	Yes	Débridement and skin grafting
5	Infection	Early	43	Pedicle	DT	Yes	Yes	Subsided, autograft retained
6	Neuropraxia	Early	14	Pedicle	PT	No	Yes	Recovered
7	Fracture	Early	14	Free	DF	No	Yes	Fractured bone used for reconstruction
8	Delayed union	Late	15	Pedicle	FS	No	Yes	United
9	Collapse	Late	16	Pedicle	PT	Yes	No	Autograft replaced with prosthesis
10	Resorption	Late	28	Pedicle	DT	No	Yes	New bone seen; patient died
11	Resorption	Late	12	Pedicle	PT	No	Yes	No fracture risk, ambulant with support
12	Resorption	Late	15	Pedicle	PT	No	Yes	Patient died
13	Resorption	Late	14	Pedicle	PT	No	Yes	New bone seen, no complaints/ fracture risk, ambulant without support
14	Nonunion	Late	21	Pedicle	PT	Yes	Yes	United after autologous bone grafting
15	Local recurrence	Late	21	Pedicle	PT	Yes	No	Autograft replaced with prosthesis
16	Nonunion	Late	49	Free	DF	Yes	Yes	United after autologous bone grafting

Early = less than or equal to 3 weeks after surgery; late = more than 3 weeks after surgery; PT = proximal tibia; DT = distal tibia; DF = distal femur; FS = femoral shaft.

prevent nonunion at the osteotomy site during resection, they described a new technique of pedicle freezing [31]. In contrast to the hyperthermic methods, liquid nitrogen-sterilization of primary malignant bone tumors retains the osteoinductive potential of the recycled bone. Liquid nitrogen sterilization is a potent option, with multiple studies reporting effective destruction of tumor cells and good long-term functional outcomes [12, 18, 23, 35]. However, the practicality of using liquid nitrogen in the operating room is not well understood. The first or subsequently published studies did not address the technical challenges of the procedure [10, 11, 30, 31]. The current study was conducted to report our early experience with liquid nitrogen sterilization in the treatment of malignant bone tumors of the extremities. With gradually increasing experience with using liquid nitrogen sterilization, we were able to perform the technique more efficiently.

Limitations

This study had several limitations. First, the limited number of patients with a short-term follow-up is the major limitation. Since we aimed at reporting our early experience addressing the technical challenges of the procedure, and also considering that all the procedures were performed by the same team of surgeons over a relatively short period, we feel 41 patients is good number to comment on the technicalities of the procedure and also short-term complications. The retrospective study design is the second limitation and the factors reported in the study were based on personal experience of the surgical team. As published detailed description of procedure of liquid nitrogen sterilization is limited, we feel these factors provide a platform to be analyzed as variables in future studies. Third, technical challenges vary from one center to another depending on the resources available. But this study reports more about using the commonly available resources considering liquid nitrogen is widely available in most countries. Last, selection bias is another limitation because patients with extensive osteolytic lesions, those who desired early weightbearing, and those with more than three pulmonary metastatic nodules were not included because they underwent endoprosthetic reconstruction. In in this study, those patients who were considered for biological reconstruction were all planned for liquid nitrogen sterilization and not any other method of recycling, except for in 10% when the patients either

Fig. 7 This clinical photograph shows thermal skin necrosis around the axis of rotation in a patient who underwent the pedicle freezing technique for an osteosarcoma of the proximal tibia. A color image accompanies the online version of this article.

insisted on bone allografts or when liquid nitrogen was a constrained resource.

What Are the Technical Challenges of Liquid Nitrogen Sterilization for Inexperienced Surgeons?

The standard operating procedure for using and transporting liquid nitrogen must be followed strictly because there have been accidental harm and deaths from mishandled liquid nitrogen in medical practice [13, 14, 22]. As liquid nitrogen evaporates, it reduces the oxygen concentration in the air and may cause asphyxia, especially in confined spaces [20]. Because liquid nitrogen may not be readily available for surgical use in many centers, a prior arrangement may be necessary; we felt that approximately 15 L to 20 L was sufficient for most pedicle freezing procedures, and a smaller quantity could be used for free freezing. Ultraviolet irradiated decontamination of liquid nitrogen is recommended before in vivo procedures and are performed because of the possibility of microbial contamination [24]. However, no sterilization techniques were used in our study; our infection incidence of 6% was comparable to that of other studies [10, 31, 32]. A sterilized flask has been used as the container for liquid nitrogen during freezing in some centers [10, 11]. We used a lightweight, sterile, cylindrical, stainless-steel container wrapped in multiple sterile cotton and plastic rolls and a crepe bandage for easy, protective handling. During pedicle freezing, the planned tumor margin must be submerged in liquid nitrogen to prevent inadequate sterilization and local recurrence. Spray devices have been used to scatter additional liquid nitrogen to the wide margin of bone (2 cm to 3 cm) near the level of liquid nitrogen in the container [31]; we used bone marking, constant observation, and frequent refilling of the container to serve this purpose.

The use of a tourniquet for cryosurgery is controversial [8]. Studies have argued for using a tourniquet to decrease circulation and increase bone necrosis during freezing [19], while opponents believe that tourniquets decrease skin and nerve vascularization, which can lead to skin necrosis or neurapraxia [25]. Tsuchiya et al. [31] recommended using a tourniquet to prevent possible tumor dissemination during freezing, which can occur when the water volume expands and pressure is subsequently increased in the bone marrow. This can also cause fracture if the bone is not adequately decompressed. We used a tourniquet during all pedicle freezing procedures; alternatively, if a tourniquet could not be applied, the major vessels supplying the limb were clamped.

A shorter operating time is an advantage of the pedicle freezing technique [31]. However, in our study, the operating time for pedicle freezing was more than that of free freezing procedures. Although the overall surgical time may reduce with experience, we feel that the duration of the pedicle freezing technique will be longer than that of free freezing because of increased dissection of the soft tissue and the additional procedure of protecting the soft tissue and rotating the limb.

What Are the Complications Associated with the Procedure?

A total of 39% (16 of 41) of our patients experienced complications, of whom 15% (6 of 41) of patients were treated with further surgery. Thermal skin necrosis (25%)

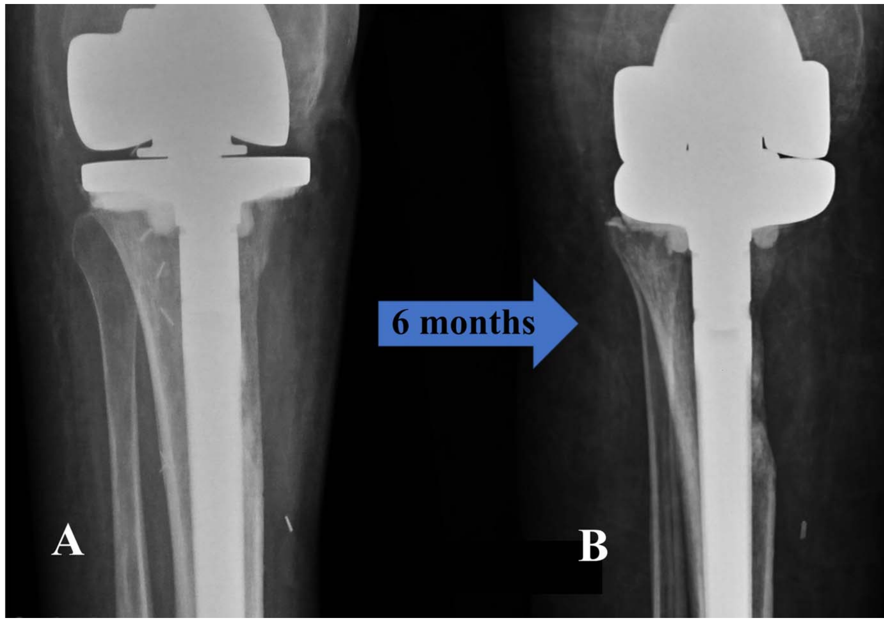

Fig. 8 A-B (**A**) This radiograph is from a patient who underwent pedicle freezing and joint arthroplasty for an osteosarcoma of the proximal tibia. (**B**) Six months later, this patient experienced resorption of the metaphysis of the recycled tibia.

and resorption of the recycled autograft (25%) were the most common complications in our study. The increased incidence of skin necrosis may be attributed to the learning curve of the procedure because all patients with skin necrosis underwent pedicle freezing during the first year of the study. In contrast to initial reports of liquid nitrogen sterilization, more recent studies with longer follow-up have reported higher complication proportions of up to 50% in frozen autograft reconstruction [27, 31, 32]. The incidence of commonly reported complications such as infection, nonunion, fracture, and neurapraxia were comparable to the incidence in our series [8, 10, 11, 31]. Aponte-Tinao et al. [3] reported that 54% of the allografts used for reconstruction in the knee after surgery for osteosarcoma underwent additional operations; which may be due to a high risk of infection and to the degradation of tissue's mineral properties and bone density of an allograft with time [3, 33]. Although resorption of a frozen autograft is not commonly reported, recycled autografts are known to undergo time-dependent resorption [15]. We believe that following reconstruction with cryosterilized autograft, protected weight bearing is essential to prevent fracture; as vascularization and new bone formation in the recycled bone which may occur as a response to loading, is a gradual process. Nonunion of the osteotomy site is a frequent complication of biologic reconstructions. Active bone morphogenic proteins in frozen bones may contribute to better osteoinduction and hence lower nonunion, compared with commonly used hyperthermic sterilization technique of extracorporeal irradiation [9]. Nonunion may be also be prevented by pedicle freezing because early blood flow to the recycled bone aids in faster recovery at the osteotomy site [16, 26, 31]. But our rate of complications must be considered with caution due to the very short follow-up period; the complications of biological reconstruction can occur after 2 or 3 years, and the complication rates of our study will only worsen with time.

Similar to one patient with local recurrence in our series, Tsuchiya et al. [31] reported local recurrence in two of their patients, with the foci of the tumor not in the recycled bone but in the surrounding soft tissue. Following the oncologic principles of tumor resection may prevent such recurrences. The primary focus of surgery should be tumor eradication, not facilitating the process of liquid nitrogen sterilization.

In our small series of patients with a short follow-up duration, the survivorship of liquid nitrogen-sterilized recycled autografts was 92%. Although the short-term results of survival of frozen autografts have been promising, Shirai et al. [27] reported graft survival of 57.4% ± 10.2% at 10 years after surgery in 62 patients with frozen autograft reconstruction. Recently Wu et al. [35] demonstrated that the survival of recycled bone treated with liquid nitrogen is similar to treatment with extracorporeal irradiation. Immediately after cryosurgery, the cortex of the frozen bone showed minimal changes, and the extent of an injury became apparent at approximately 1 week [6]. Repair of the frozen bone is first evident at 2 months after freezing, and it may take up to 6 months for new bone to form. The changes in a recycled autograft afterwards are multifactorial and mainly time-dependent. Multiple studies with longer follow-up durations are needed to assert the longevity of frozen autografts.

Conclusions

Liquid nitrogen sterilization is an alternative technique that requires some training and experience for the surgeon to become proficient in treating primary malignant tumors of the bone. Because it is widely available, it may be an option worth exploring in resource-constrained environments, where allografts and endoprostheses are difficult to procure. We identified several technical factors that have not been fully evaluated, but may be helpful to consider for others contemplating the use in future studies. Considering the short-term follow-up of this study, we anticipate that the proportion of patients with complications of these frozen autografts will increase with longer follow-up.

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