Reimplantation of the resected tumour-bearing segment after recycling using liquid nitrogen for osteosarcoma

Mohamed Abdel Rahman · Ayman Bassiony ·
Hisham Shalaby

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Abstract Reconstruction after en bloc resection of malignant tumours is still the subject of debate. We questioned the effectiveness of reconstruction by reimplanting the tumour-bearing segment after recycling in liquid nitrogen. Ten patients with osteosarcoma around the knee were included, with a mean age of 21 years. The operative technique included wide en bloc excision, debridement, and management of the resected segment with liquid nitrogen followed by reimplantation and internal fixation. At a mean follow-up of 4.5 years there was no local or systemic recurrence and the mean functional score was 82.4%. The frozen graft united proximally and distally in all but one patient in a period ranging from six to ten months. The effectiveness of this reconstruction technique in properly selected patients with osteosarcoma is comparable to other techniques of biological reconstruction with the added benefit of being simple, cheap and durable.

Introduction

Extremity amputation with wide or radical surgical margins was the primary treatment for bone and soft tissue

malignancies involving the extremities until the 1970s [2]. The advances in radiological imaging and the increased effective use of adjuvant treatment, both chemotherapy and irradiation, share the credit for increasing successful limb salvage surgery [26]. Currently, limb salvage is the standard procedure for treatment of malignant bone tumours in many centres [8, 9, 20, 23, 24].

Limb salvage surgery involves wide resection of the bone tumour, followed by reconstruction of the defect [6, 22]. Various techniques for reconstruction of the bony and soft tissue defect following resection of malignant bone tumours have been described including the use of endoprosthesis [8, 19–21], autograft [23], allograft [18], distraction osteogenesis [28] or recycling of the resected segment by a variety of methods including autoclaving [5], freezing [14], Pasteurisation [1, 12] or extracorporeal irradiation [4].

Freezing or cryosurgery was first used in the management of bone tumours in 1964, as a palliative procedure [14]. The use of liquid nitrogen for the management of primary osteosarcoma was first described in 1984. Repetitive freezing and thawing destroyed any tumour cells present at the margin of the curettage and immediate histological studies, and those carried out at a second-look procedure showed no evidence of residual tumour [13].

The advantages of reconstruction using tumour-bearing massive frozen autograft treated by liquid nitrogen are simplicity, osteoconduction, a short treatment time, a perfect fit, easy attachment of tendons and ligaments and desirable bone stock [27].

In this prospective case series of patients with osteosarcoma of the femur and tibia we questioned the effectiveness of reconstruction of the skeletal defect using tumour-bearing massive frozen autograft treated by liquid nitrogen, with regard to the radiological and functional outcome at a mean follow-up of 4.5 years.

M. Abdel Rahman · A. Bassiony · H. Shalaby
Orthopaedic Surgery Department, Demerdash Hospital,
Ain Shams University,
Abbaseia Square,
Cairo, Egypt

M. Abdel Rahman
e-mail: melniny2000@yahoo.com

A. Bassiony
e-mail: aymanbassiony@yahoo.com

H. Shalaby (✉)
3B Foad Basha St., Heliopolis,
Cairo, Egypt
e-mail: hishamshalaby@yahoo.com

Patients and methods

We report here a prospective clinical study initiated in 2002 in a tertiary referral centre for bone tumours. Any patient with a clinical and radiological picture suggestive of a malignant or an aggressive benign tumor was considered a potential candidate. The initial assessment of these patients was carried out according to the current protocol of our institution which includes plain X-ray, MRI, CT scan, bone scan, angiography, CT scan of the lungs and standard laboratory investigations. Following the imaging survey, all patients underwent a carefully planned open biopsy and histopathological examination. Following the biopsy the tumours were staged according to the modified system of the Musculoskeletal Tumour Society [7].

Patients with metastatic lesions or with primary malignant tumours other than osteosarcoma were excluded from the study. Other exclusion criteria included the presence of distant metastases, extensive cortical bone destruction preventing the reuse of bone, as evaluated by X-rays and CT scan, presence of distant skip lesions that could not be included in the en bloc resection, occurrence of a pathological fracture and neurovascular involvement. Patients younger than the age of 15 years were also excluded.

Participation in this clinical study was entirely voluntary. All patients who met the inclusion criteria (29 patients) were provided with a detailed explanation of the nature of the proposed procedure and the possible complications with an opportunity to ask questions. They were assured non-participation would not jeopardize their care. Other treatment options were also explained to them. Nineteen

patients declined to enroll in the study or preferred other treatment options, leaving ten patients who met the inclusion criteria and who accepted enrolment in the study. All the included patients had a high grade extra-compartmental osteosarcoma of the femur or tibia (stage II B). Six patients had conventional high grade osteosarcoma and four patients had high grade surface osteosarcoma with intramedullary extension as confirmed by the MRI scan.

The study was approved from the scientific and ethical point of view and all patients signed an informed consent form.

The study included six females and four males with a mean age at the time of surgery of 21 years (range 16–27 years). All patients were managed with the same preoperative chemotherapy protocol of three cycles at three-week intervals. In each cycle the patient was given Adriamycin 75 mg/m² and Cisplatin 150 mg/m² for three days. After completion of the three cycles, restaging of the tumour was done using the same preoperative imaging studies (Fig. 1). High dose methotrexate is not part of the chemotherapy protocol used at our institute because it is not available for technical reasons related to unavailable resources to monitor these patients appropriately.

All patients were managed with the same perioperative regimen, including administration of antibiotics, prophylaxis against venous thrombosis, postoperative pain control and physical therapy.

The operative technique included three main steps. The first step was a wide en bloc excision including all involved compartments as planned through the MRI scan. Intercalary resection was done in six cases (five femoral and one

Fig. 1 Preoperative radiological investigations of a case of proximal tibial osteosarcoma. **a** Plain X-rays (anteroposterior [AP] and lateral) **b** MRI scan. **c** Plain X-rays (AP and lateral) after finishing the three cycles of chemotherapy

tibial). Osteoarticular resection was done in the remaining four cases (three femoral and one tibial).

The second step involved a thorough debridement of all the tumour and soft tissues from the resected bony segment. This included gross dissection of all the soft tissue components of the tumour, reaming of the medullary canal, and removal of all attached soft tissues. The debrided tissue was sent for histopathological examination.

In the third step the excised segment was frozen in liquid nitrogen for 20 minutes, thawed at room temperature for 15 minutes, thawed in distilled water for ten minutes and then reimplanted and fixed in place by an appropriate fixation device (plates and screws for intercalary resection and intramedullary nailing for osteoarticular resection). Cancellous bone graft harvested from the iliac crest was applied at the osteotomy site. Knee arthrodesis was carried out, in the four cases where osteoarticular resection was performed, by preparation of the opposite joint surface (e.g. proximal tibia in case of distal femoral osteosarcoma) and fixation with intramedullary nailing.

The mean follow-up period was 54 months (range 34–69 months). While the patients were receiving the postoperative chemotherapy, they were examined monthly for early detection of infection and early recurrence. After finishing the postoperative chemotherapy, patients were examined every three months for detection of local recurrence, pulmonary metastasis and to assess graft union.

Fig. 2 Plain X-rays (anteroposterior [AP] and lateral) four years after biological reconstruction with tumour bearing frozen autograft

Table 1 Details of the study group

Case no.	Gender	Age (y)	Diagnosis	Bone Involved	Location	Resected segment length (cm)	Reconstruction type	Time for graft union (mo)	Complications	Follow-up (mo)	Functional score ^a
1	F	16	Osteosarcoma	Tibia	Proximal	14	Intercalary	6	Skin sloughing	53	80%
2	F	27	HGSOS	Femur	Shaft	20	Intercalary	10	None	44	80%
3	F	17	Osteosarcoma	Tibia	Proximal	18	Knee arthrodesis	7	None	34	80%
4	F	23	HGSOS	Femur	Shaft	22	Intercalary	8	None	43	90%
5	M	22	Osteosarcoma	Femur	Distal	20	Knee arthrodesis	10	None	60	77%
6	M	25	Osteosarcoma	Femur	Distal	23	Intercalary	15	Delayed healing of proximal osteotomy	52	77%
7	F	21	Osteosarcoma	Femur	Distal	20	Knee arthrodesis	10	None	69	80%
8	M	23	HGSOS	Femur	Shaft	19	Intercalary	6	None	64	90%
9	M	18	Osteosarcoma	Femur	Distal	19	Knee arthrodesis	9	None	66	80%
10	F	19	HGSOS	Femur	Shaft	17	Intercalary	7	None	55	90%

HGSOS high grade surface osteosarcoma, F female, M male

^a Functional score according to a modified system of the Musculoskeletal Tumor Society

Results

At the most recent follow-up all patients were alive and free from any signs of local or systemic recurrence. The mean disease-free survival was therefore calculated as 54 months (range 34–69 months).

The functional evaluation was performed using a modified system of the Musculoskeletal Tumour Society [7], which is based on six parameters including pain, functional activities, emotional acceptance, the use of external support, walking ability and gait. The mean functional score for the study group was 82.4% (range 77–90%).

In the six patients who had intercalary reconstruction, the knee range of movement at the most recent follow-up ranged between 50 and 90 degrees with a mean of 80 degrees.

Radiological union of the reimplanted segment proximally and distally was assessed according to Hsu et al. with graft union defined as uninterrupted external bony borders between the graft and the recipient bone in addition to obscured or absent osteotomy lines at both junctions [11]. According to this system, the frozen graft united proximally and distally in all but one patient in a period ranging from six to ten months with a mean of eight months (Fig. 2). In the remaining patient additional grafting at the proximal osteotomy site with cancellous bone graft from the iliac crest was required 11 months postoperatively, and four months later full union was achieved.

Skin sloughing occurred in one patient at the site of the previous biopsy site and was managed by debridement and coverage by split thickness skin graft. Details of the study group are shown in Table 1.

Discussion

Limb salvage can result in survival rates and disease-free periods that equal those achieved with amputation. It also offers a better psychological acceptance and an intact body image [25]. Although it is generally accepted that limb salvage

surgery leads to an increased risk of local recurrence, there is no convincing evidence that it leads to decreased survival, and any higher rate of local recurrence suggests either inadequate margin of resection or inadequate adjuvant treatment [2].

The ideal reconstruction of the defect created after en block resection of the tumour is still debated. Endoprosthetic replacement incurs a high financial cost and involves many complications including a 12% deep infection rate, loosening, breakage, a 70% probability of further surgical procedures being required within ten years and an amputation risk of 25%, as reported in some studies [8, 19–21]. Massive allografts are widely used in many centres. However, bone banking requires substantial time, energy, money and for a variety of reasons, is not available in many countries [18]. Infection remains the most severe complication of this technique [17]. Distraction osteogenesis with bone transport techniques for such large bony defects involves a lengthy period of time in a cumbersome frame and a high incidence of complications making it far from an ideal solution for these challenging cases [28]. The use of vascularised and non-vascularised fibular grafts with the circular frame appears to be a better option to reduce the frame time and has been described for osteosarcoma of the distal tibia [23].

Recycling of the resected segment is a good alternative method which is more economic as well as durable because of its biological nature and can be achieved by heating, irradiation or freezing.

Liquid nitrogen, stored at -197°C , is an effective cryogenic agent that can be used for either tissue preservation or destruction. A slow freeze and quick thaw allow tissue preservation, while a quick freeze and slow thaw lead to its destruction [3]. Several studies reported the use of liquid nitrogen to treat the wall of bone cavities after extended curettage [14–16, 30].

To the best of our knowledge, reimplantation of the resected tumour-bearing segment after recycling using freezing by liquid nitrogen has been described only in one study involving 28 patients with different malignant bone

Table 2 A comparison between our study and other techniques of biological reconstruction using recycling of the resected segment

Parameter	Technique of recycling			
	Pasteurisation [1]	Extracorporeal irradiation [3]	Liquid nitrogen [28]	Our study
Number of patients	23	8	28	10
Mean functional score	86% ^a	92% ^a	71.4% excellent ^b 10.7% good10.7% fair7.1% poor	82.4% ^a
Percentage of bony union	91%	100%	93%	95%
Re-fracture / fixation failure	13%	50%	7.5%	0%
Local or systemic recurrence	0	37%	7.5%	0%
Deep infection	22%	0	10.5%	0%

^a Functional score according to a modified system of the Musculoskeletal Tumour Society

^b No mean functional score defined by the authors

tumours in different locations [29]. In this study bony union was seen at a mean of 6.7 months after the operation in 26 patients. Complications were encountered in seven patients (25%), including three deep infections (10.5%), two fractures (7.5%), and two local recurrences (7.5 %). Local recurrence was encountered in two cases for which only marginal excision was done rather than wide en block.

In our prospective clinical study ten patients with non-metastatic osteosarcoma of the femur or tibia were treated using reimplantation of the tumour-bearing frozen autograft. At a mean follow-up of 4.5 years, all the patients were alive and free from any signs of local or systemic recurrence, with the graft united proximally and distally. Our results are comparable with the published results of other techniques of limb salvage and biological reconstruction using recycling of the resected segment [4, 12]. We also encountered fewer complications compared to the earlier reports of the same technique [29] (Table 2), including no local recurrence, which we believe is due to the wide en block resection together with the effectiveness of this technique in destruction of the tumour cells. Due to the short follow-up period it was not possible to judge the incidence of late complications as fracture, necrosis and collapse of the bone yet none of these complications were encountered till the time of the last followup.

The technique of recycling of the resected segment using freezing by liquid nitrogen has the disadvantages of preventing histological analysis of the whole specimen, which is useful to tailor further chemotherapy regimes appropriately. However, a good response documented by at least two of four radiological methods, including plain radiography (good delineation of the tumour and appearance of sclerotic changes), MRI (marked shrinkage of any extension of the tumour into the soft tissues), angiography (disappearance of “tumour” vessels), and thallium-201 scintigram (disappearance of the abnormal accumulation), has been shown to indicate more than 90% tumour necrosis, confirming a good response to preoperative chemotherapy [10].

Recycling is also unsuitable for patients with destructive malignant bony lesions. However, this technique is simple, cheap and durable and can be particularly useful in geographic regions where expensive endoprostheses are difficult to obtain. We can therefore recommend this technique as an appropriate method of reconstruction in properly selected patients with osteosarcoma. The use of liquid nitrogen should not substitute en block resection. Long-term follow-up studies will provide further useful information.

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Ethical approval The board of the Orthopaedic Department at Ain Shams University has approved this study from the scientific and ethical point of view. This study meets the ethical standards and complies with the national as well as the local standards set within the department.

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