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# Pasteurized Tumoral Autograft and Adjuvant Chemotherapy for the Treatment of Canine Distal Radial Osteosarcoma: 13 Cases

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**Objective—**To report outcome in 13 dogs with distal radial osteosarcoma, without evidence of metastasis, treated by a combination of adjuvant chemotherapy and a pasteurized autograft limb-sparing procedure.
**Study Design—**Prospective clinical study.
**Animals—**Thirteen dogs with distal radial osteosarcoma.
**Methods—**Limb-sparing procedure was performed using an autograft from the excised tumoral segment, pasteurized at 65°C for 40 minutes. Adjuvant chemotherapy (cisplatin or cisplatin and doxorubicin) was administered in all dogs.
**Results—**Mean and median survival times were 531 and 324 days, respectively (range, 180 to 1,868 days). Overall survival was 100% at 6 months, 50% at 12 months, 44% at 18 months, and 22% at 24 months. Lung metastasis occurred in 5 (38%) dogs. Observed complications were local recurrence (2 dogs, 15%), allograft infection (4 dogs, 31%), and implant failure (3 dogs, 23%). Limb function was good in 12 dogs (92%) and fair in 1 dog.
**Conclusions—**Pasteurized bone autograft derived from the tumoral bone segment was an effective alternative to cortical bone allograft for limb sparing in canine distal radial osteosarcoma, in terms of feasibility, pattern of healing, complications, and survival.
**Clinical Relevance—**Use of a pasteurized bone autograft eliminates the need for a canine bone allograft bank and has the added advantage of good fit to the recipient site.


APPENDICULAR osteosarcoma (OSA), a locally aggressive and highly metastatic tumor, is the most common primary bone cancer of dogs and has a poor prognosis, with a low 2-year survival rate.[1] Local disease control can be achieved by amputation[2,3] or limb sparing.[4-7] These techniques are not curative if used alone[8-10] because nearly 90% of affected dogs have undetected metastatic disease at admission.

Limb amputation is the local treatment of choice for canine appendicular OSA because it is almost complication free; however, it is not always feasible because of concurrent orthopedic or neurologic problems, or owner refusal. Consequently, several limb-sparing techniques have been described, mostly for distal radial OSA. These techniques include use of fresh-frozen cortical bone allografts from a bone bank[5-7] or application of a modified Ilizarov apparatus to facilitate bone regeneration.[11,12]

A novel procedure, using an autograft derived from the excised tumoral bone and pasteurized to kill tumoral cells, has been reported.[13] Our purpose was to report outcome in 13 dogs (including a previously reported case[13]) with distal radial OSA treated by limb sparing using a pasteurized autograft and adjuvant chemotherapy.

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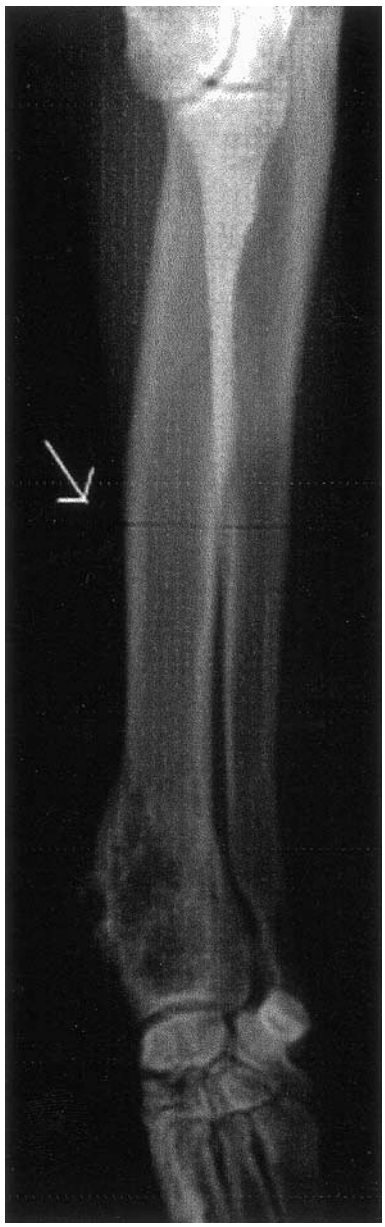

**Fig 1. Preoperative lateral radiograph of a distal radial osteosarcoma (dog 12). Cortical lysis and periosteal reaction can be noted. The arrow shows the line drawn on the radiograph to mark the ostectomy site.**

## MATERIALS AND METHODS

### *Criteria for Inclusion*

Between January 1996 and August 2002, dogs with distal radial OSA that involved no more than half the diaphyseal length (Fig 1) and had no clinical or radiologic evidence of metastasis were included. OSA was confirmed by histologic examination of a Jamshidi needle biopsy specimen.

### *Limb-Sparing Technique*

We used a previously reported technique for OSA of the distal radius.[6,14] Briefly, proximal ostectomy was performed 4 cm proximal to the radiographic margin of the tumor then the bone segment was excised after disarticulation of the carpal joint. The diaphyseal host stump bone marrow was cytologically evaluated for neoplastic cells. Using other surgical instruments, the tumoral bone segment was aseptically cleaned of most surrounding soft tissue and bone marrow. Tumoral cells were killed by pasteurization (65°C for 40 minutes) in a thermostat-controlled, sterile saline (0.9% NaCl) solution.[13] The autograft was then modeled, trimming any exuberant, necrotic tumor.

After preparation of the carpus for arthrodesis, the autograft was repositioned and stabilized with a plate and screws using AO/ASIF technique. Autogenous cancellous bone (harvested from the proximal humerus or the wing of the ilium) was inserted at the host graft interfaces in most dogs. In the last 2 dogs, corticocancellous strips (collected from the wing of the ilium with a Slocum modified gouge [Slocum B, Slocum TD: DARthroplasty: the surgical technique; Slocum Enterprises Brochure, 1998]) were also inserted near the host graft interfaces. The ulna was spared in 9 dogs and resected and pasteurized with the radius in 4 dogs (Fig 2). A modified Robert Jones bandage was applied for 10 days. Postoperatively, cefazolin (20 mg/kg intramuscularly, every 12 hours) was administered for 7 to 10 days.

Dogs were reassessed for metastasis, limb function, autograft healing, and complications on a monthly basis for the first 3 months and then every 3 months thereafter. Limb function was evaluated as the following: excellent (slight or no lameness), good (mild lameness), fair (evident lameness), and poor (severe lameness or no use of the limb).[6]

### *Chemotherapy*

Cisplatin (Platamine; Pharmacia Upjohn, Milan, Italy) was administered intravenously [IV] at 70 mg/m$^2$ in 5 dogs. It was administered in the middle of a 6-hour hydration period (17 mL/kg/hr of saline solution) every 3 weeks for 3 or 4 treatments, starting the day after surgery. A combination of cisplatin (70 mg/m$^2$ IV) and doxorubicin (30 mg/m$^2$ IV; Adriblastina; Pharmacia Upjohn) was administered to 6 dogs on an alternate basis every 3 weeks for a total of 2 cisplatin and 2 doxorubicin administrations, respectively. Two dogs were administered cisplatin (50 mg/m$^2$ IV concurrent with saline-induced diuresis), and doxorubicin was administered 24 hours later (15 mg/m$^2$ IV). This protocol was administered on a 21-day cycle for 4 cycles.[15]

Because of potential side effects of these drugs, peripheral blood cell count and renal function were monitored during treatment. Cardiac function was evaluated in all dogs. In particular, in dogs administered doxorubicin, car-

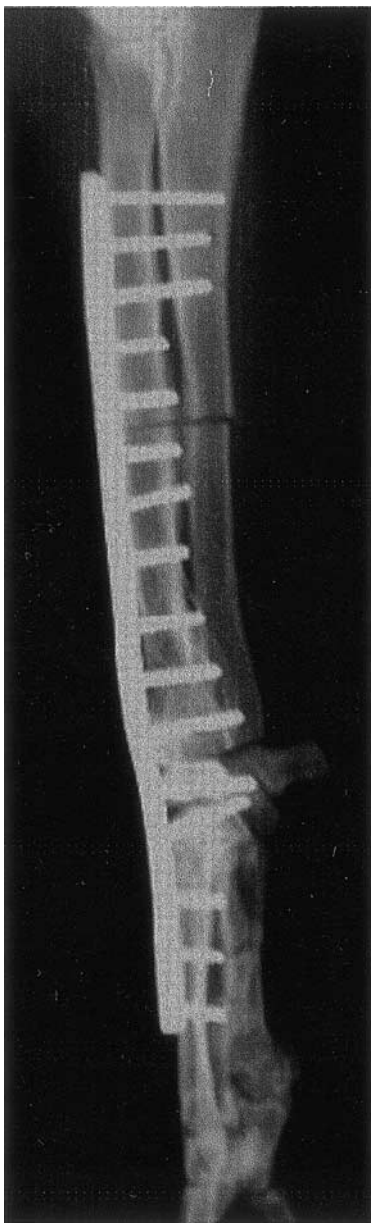

**Fig 2. Immediate postoperative lateral radiograph (dog 12) after autograft replacement and plate fixation.**

diac function was evaluated clinically and by echocardiography preoperatively, during treatment, and after the last doxorubicin administration. Metoclopramide (Plasil; Lepetit, Milan, Italy; 0.2 to 0.4 mg/kg subcutaneously) was administered when nausea and vomiting occurred after cisplatin administration.

**Outcome.** The time between surgery and death because of lung metastasis or tumor-related causes was considered "overall survival." The time between surgery and radiographic evidence of lung metastasis and/or local recurrence was considered "disease free period." Deaths because of chemotoxicity were included in the group of tumor-related deaths.

## RESULTS

Twelve male and 1 female dogs were included. All were >30 kg (range, 31 to 78 kg) and had a median age of 7 years (range, 3.5 to 11 years). OSA (staged T2N0M0)[16] was localized to the right (7 dogs) or left (6 dogs) distal radius (Table 1). Mean and median survival times were 531 and 324 days, respectively (range, 180 to 1,868 days). Mean and median disease-free intervals were 487 and 255 days, respectively. Overall survival was 100% at 6 months after surgery; 50% at 12 months (5 of 10 dogs; 3 dogs were excluded, 1 dog because of a non-tumor-related death [polycystic kidney], and 2 because of a follow-up <12 months); 44% at 18 months (4 of 9; 1 dog was excluded because of gastric dilatation-volvulus); and 22% (2 of 9 dogs) at 24 months. Lung metastasis was radiographically detected in 5 dogs (38%), 2 of which also had metastasis to regional lymph nodes and bone, respectively. Two dogs (15%) likely died of chemotoxicity (renal failure and dilated cardiomyopathy).

Observed complications were local recurrence (2 dogs), allograft infection (4 dogs), and implant loosening (3 dogs; Table 1). The 2 dogs with local recurrence, detected 143 and 169 days after surgery, respectively, had their leg amputated. Recurrence was confirmed by histology. Local infection manifested as recurrent wound drainage, was mild in all dogs, and was controlled by administration of antibiotics (cefazolin, enrofloxacin, amoxicillin-clavulanic acid) that were selected based on microbial culture and susceptibility tests.

Implant failure occurred in 3 dogs. In dog 3, on radiographs taken 350 days after surgery, there was evidence of multiple screw loosening, and 1 screw was removed. In dog 4, the distal screw became loose and was removed 76 days after surgery. At 187 days, 3 more screws were removed and replaced because they were loose and broken. The implants were removed 708 days after surgery.[13] In dog 5, on radiographs taken 316 days after surgery, there were 2 loose screws that were not removed because the dog also had renal failure.

Limb function, evaluated in all treated dogs, was good in 12 dogs (92%) and fair in 1 dog. Four dogs were still alive after 180, 255, 1,357, and 1,868 days, respectively.

Table 1. Treatment and Outcome Data for 13 Dogs With Distal Radial Osteosarcoma Treated by Limb Sparing With a Pasteurized Autograft and Adjuvant Chemotherapy

| Dog No. | Breed | Age (yr) | Sex | Weight (kg) | Limb | Chemotherapy (No. of injections) | Metastasis | Local Recurrence | Complications* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Great Dane | 3.5 | M | 78 | R | C (4) | Y | N | None |
| 2 | Irish setter | 11 | M | 31 | R | C (3) | N | N | Infection |
| 3 | Mixed | 7 | M | 65 | L | C (3) | Y | N | Implant failure |
| 4 | Abruzzean shepherd | 9 | M | 50 | R | C (3) | N | N | Infection<br>Implant failure |
| 5 | Bobtail | 10 | M | 35 | L | C (3) | N | N | Implant failure |
| 6 | Rottweiler | 8 | M | 55 | R | C (2)<br>D (2) | N | Y (169 days)<br>Amputated | None |
| 7 | Great Dane | 10 | M | 67 | L | C (2)<br>D (2) | Y | N | None |
| 8 | Great Dane | 5 | M | 72 | R | C (2)<br>D (2) | Y | Y (143 days)<br>Amputated | None |
| 9 | Newfoundland | 7 | M | 70 | L | C (2)<br>D (2) | Y | N | Infection |
| 10 | Saint Bernard | 7 | M | 73 | L | C (2)<br>D (2) | N | N | None |
| 11 | Mixed | 7 | M | 68 | R | C (2)<br>D (2) | N | N | Infection |
| 12 | Rottweiler | 7.5 | F | 52 | R | C/D (4) | N | N | None |
| 13 | Great Dane | 5 | M | 60 | L | C/D (4) | N | N | None |

Abbreviations: M, male; F, female; R, right; L, left; C, cisplatin; D, doxorubicin; Y, yes; N, no.
*Some died from tumor-unrelated causes.

All dogs were administered adjuvant chemotherapy. Few signs of toxicity were observed. Nausea and vomiting were observed for 2 or 3 days, after cisplatin administration. Metoclopramide was used to alleviate these signs. Neutrophil counts did not drop below $3 \times 10^3$/mL (range, 3 to $11.5 \times 10^3$/mL), so chemotherapy was not delayed. Dog 5 died of renal failure about 11 months after surgery. Dog 10 died of dilated cardiomyopathy. Left ventricular dilatation and decreased fractional shortening were noted by echocardiography after the second doxorubicin administration (85 days after surgery). Congestive heart failure treatment was initiated (enalapril, digoxin, and furosemide); the dog died 203 days after surgery.

## DISCUSSION

Difficulties finding donor dogs and national legal limitations on establishing a cortical bone graft bank encouraged us to find an alternative to the cortical allograft limb-sparing procedure. We reasoned that a pasteurized autograft derived from the primary bone neoplasm might be an alternative bone source.[17,18] The pasteurized bone segment fits the recipient site, and it mitigates the need for a bone bank.

Our results suggest that complications related to limb sparing with a pasteurized autograft are similar to those encountered with fresh frozen cortical allograft, ie, local recurrence, allograft infection, and implant failure. To put our results in perspective, we compared them with other reports[4,5] and a previous report of our own experience with cortical allograft limb sparing in dogs[6] (Table 2).

Local recurrence is the major tumor-related complication. Recurrence reported with limb sparing using cortical bone allografts is approximately 25%. A reduction in recurrence (15%) has been obtained through a slow release of cisplatin within the surgical wound.[4] Our recurrence rate (15%) was similar and

Table 2. Comparison of Outcome Between Dogs With Distal Radial Osteosarcoma Treated by Limb Sparing With Cortical Allografts[6] or by Pasteurized Autograft

| | Allografts[6] (18 dogs) | Pasteurized Autografts (13 dogs) |
|---|---|---|
| Mean survival | 478 days | 531 days |
| Median survival | 266 days | 324 days |
| Survival rate | 35% 1 year, 19% 2 years | 50% 1 year, 22% 2 years |
| Local recurrence | 28% | 15% |
| Metastasis | 55% | 38% |
| Implant failure | 11% | 23% |
| Infection | 39% | 31% |

together with a long, recurrence-free, follow-up suggests that pasteurization, together with tumor devascularization through surgical excision, effectively killed tumor cells in the neoplastic segment. Local recurrence did not occur at the ostectomy site, confirming a free margin "en bloc" excision. Therefore, evaluation of recent radiographs represents a reliable method for assessing a neoplastic-free proximal osteotomy.[19] Other authors suggest that additional imaging studies, including MRI (the modality of choice in human medicine), should be beneficial to evaluate the extent of neoplasia before limb-sparing ostectomy.[20] We used intraoperative cytology on the proximal ostectomy bone marrow to increase our confidence in the probability of a free margin "en bloc" resection.

Because the authors are of the opinion that microscopic involvement of the ulna can represent a source of recurrence, the ulna was also removed in the last 4 dogs. This action, together with an increased ability of surgeons to achieve a free margin resection, may explain the different recurrence rates between the groups compared in Table 2. Ulna and/or surrounding soft-tissue microscopic tumoral involvement may have been the cause of the 2 local recurrences that we observed.

Our technique did not prevent infection of the implant system, which is the primary complication, associated with any bone graft limb-sparing procedure. Our infection rate was comparable with that reported for cortical allograft limb sparing.[4-6] Variable degrees of infection occurred in our dogs, with recurrent wound drainage being most frequent. This was usually controlled by administration of systemic antibiotics and wound management. It is of interest that development of infection has been associated with longer survival, possibly because of increased immune system stimulation.[4]

An important concern of our technique may be a limitation on the number of screws that can sometimes be placed in the autograft because of tumor erosion. In fact, as evident in Table 2, there is an increased implant failure rate when the groups are compared. Although the implant complications were higher in the autograft group, this did not always result in loss of stability of the entire implant system. Furthermore, we observed no appreciable loss of implant stability when the ulna was removed. If potential instability is of concern, use of bone cement could be considered in the graft to increase stability, even if it can delay allograft healing.[21]

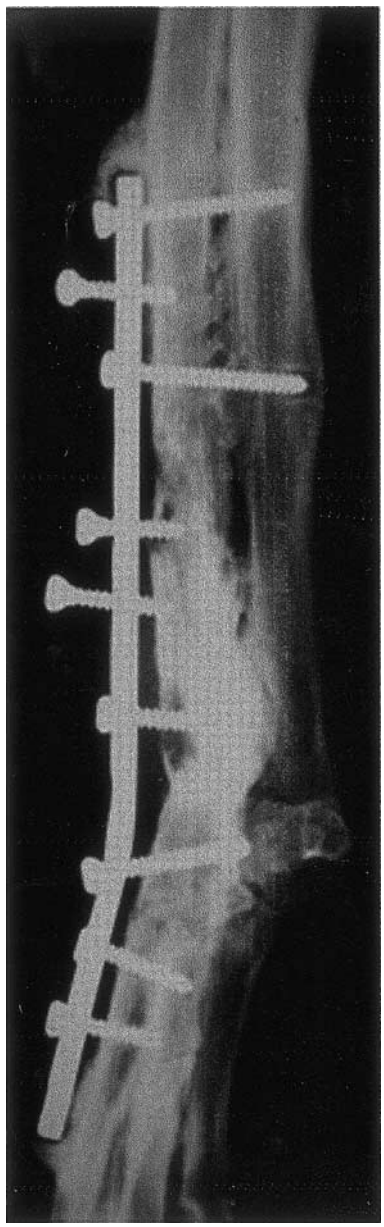

**Fig 3. Dog 3, 11 months after surgery. The autograft shows signs of an intense remodelling, and it appears proximally and distally fused to the host bone stumps. Most screws are loose; the plate is no longer adherent to the bone, but the autograft appears to be incorporated.**

Radiographic evaluation of healing with pasteurized autografts was similar in all phases (Fig 3) of healing when cortical allografts are used.[5,22] Healing is also directly related to implant stability. This observation suggests that pasteurization did not interfere with bone healing. Histopathologic examination would have

been a more accurate method to assess healing; however, it was not performed. We implanted corticocancellous strips, in addition to autogenous cancellous bone, in 2 dogs in an attempt to stimulate osteoinduction. It would be premature to comment on the clinical value of these grafts, based on our current experience.

Two dogs probably died from chemotoxicity. One dog died of renal failure. Because serum biochemical values before, during, and after cisplatin chemotherapy were normal, no further renal function tests were performed. We speculate that cisplatin may have contributed to progressive worsening of renal function. In the second dog, cardiotoxicity was evident after the second doxorubicin administration. The owner declined necropsy examination, so doxorubicin-induced cardiac abnormalities could not be confirmed histologically. These cases were classified as tumor-related deaths.

The different metastatic rate observed between groups in Table 2 may be related to differences in chemotherapy protocols. Metastatic disease still remains the main reason of failure of the OSA treatment, irrespective of surgical technique and the chemotherapy protocol used.

Our experience with these 13 dogs leads us to conclude that a pasteurized bone autograft technique is an effective alternative to cortical bone allograft for limb sparing for canine distal radial OSA. A major advantage of this approach is that it eliminates the need for a canine bone allograft bank; however, it does not eliminate the main complication, infection, that is still associated with use of a cortical graft.

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