Faster integration of human allograft bone than of the bovine substitute Lubboc

Non-randomized evaluation of 20 cases with benign tumors or tumor-like conditions

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ABSTRACT **In 20 patients, we studied healing and time for complete integration of human cancellous bone allografts and the bovine substitute Lubboc on plain radiographs. They were all operated on because of benign tumors or tumor-like conditions, and followed until integration of the graft or for at least 1 year after operation. The median follow-up was 10 (2–40) months. Allografts showed a better integration than the bovine substitute. We conclude that allografts are to be preferred for these indications.**

Postoperative pain after graft harvesting is a disadvantage of autologous bone in the treatment of osseous defects (Kurtz et al. 1989, Arrington et al. 1996). Moreover, limited availability of autologous material makes human bone allografts and bovine substitutes good alternatives. We studied human cancellous bone allografts and the bovine substitute Lubboc as regards ingrowth ability and time until integration. 20 benign tumors or tumor-like conditions were treated by curettage. Human bone allografts or Lubboc were implanted into the defects. Healing of the bony defects was investigated by evaluating serial plain radiographs.

Patients and methods

In this retrospective cohort study, we evaluated 21 consecutive patients who underwent surgical treatment of benign tumors or tumor-like conditions during a 2-year period. 1 patient was excluded because of explantation of the bone replacement material, which was due to recurrence of an enchondroma. The median age of the 20 patients was 24 (5–67) years and 11 were male.

The diagnoses included aneurysmal bone cyst, enchondroma, nonossifying fibroma and juvenile bone cyst (Table). No pathological fractures occurred. The patients were followed until complete integration of the implant, or for at least 1 year after operation (mean 7 (2–40) months). 3 persons who failed to show complete integration were lost to follow-up after 3, 4 and 5 months. The 7 patients who underwent surgery in the first year of the study received Lubboc. The following 13 operations were performed, using human bone allografts.

Human bone allografts were produced in the institute's bone bank. After harvesting under sterile conditions and cryopreservation, we thawed the bones for the following mechanical preparation. Between steps in preparation, the grafts were stored entirely covered in an antibiotic solution (Ringer Lösung (Mayrhofer, Linz, Austria) with gentamicin (Refobacin, Merck, Darmstadt, Germany) in a concentration of 10 mg/L and vancomycin (Vancomycin "Lilly", Lilly, Gießen, Germany) in a concentration of 330 mg/L, to avoid surface contamination. The products used in this study were half femoral heads and cancellous chips. After mechanical preparation, the bone was shaken (3

Data on patients

Case	A	B	C	D	E	F	G	H
1	13	M	JC	L	Humerus	A	I	12
2	12	M	AC	R	Humerus	A	I	6
3	9	W	NF	R	Distal femur	A	I	9
4	14	M	AC	R	Humerus	A	I	5
5	36	W	E	L	Proximal humerus	A	I	2
6	6	M	AC	R	Proximal femur	A	I	7
7	5	M	JC	R	Proximal tibia	A	I	4
8	14	M	NF	L	Distal femur	A	I	3
9	55	M	FD	R	Proximal femur	A	P	5
10	12	M	AC	L	Proximal femur	A	I	4
11	17	F	FD	R	Proximal femur	A	P	3
12	13	F	JC	R	Calcaneum	A	I	3
13	39	F	C	R	Distal tibia	A	P	4
14	35	F	C	R	Fifth metacarpal	L	I	40
15	58	F	E	L	Femur	L	P	13
16	37	F	IG	L	Tibia	L	I	28
17	7	M	AC	R	Humerus	L	I	21
18	13	M	NF	R	Tibia	L	I	10
19	67	F	E	L	Second toe	L	I	7
20	17	M	AC	R	Femur	L	I	6

A Age at operation (years)
B Gender
C Diagnosis
  AC Aneurysmal cyst
  C Chondroma
  E Enchondroma
  FD Fibrous dysplasia
  IG Intraosseous ganglion
  NF Nonossifying fibroma
  JC Juvenile cyst
D Side
E Location
F Material
  A Allograft
  L Lubboc
G Healing
  I Integration
  P Partial Integration
H Time-to-healing (mo)

times for 10 minutes each) to remove bone marrow residues. Then, excessive fluid was allowed to drip onto sterile drapes. The grafts were double-packed in sterile containers. To ensure microbial safety, swabs were taken on explantation, before and after processing and prior to implantation.

Lubboc (Transphyto (now: Ost Developpement), Clermont-Ferrand, France) consists of bovine cancellous bone, manufactured in four stages including high-pressure washing to remove the bone marrow, delipidation by extraction using organic solvents, two treatments with 8 M urea to extract proteins and sterilization by accelerated electron beams at 25 kGy.

The radiographs were evaluated independently by 3 investigators who examined the plain radiographs taken before surgery, and at 0, 1.5, 3, 6 and 12 months after surgery. The data were then checked for inter-observer agreement. In case of differences, the patient's radiograph was reevaluated by all 3 observers together. The radiographic integration was classified as: I complete, II partial, III resorption, IV inert graft (Nigro and Grace 1996).

Integration was judged to be complete only when host bone and graft appeared radiographically homogeneous and trabecular remodeling was seen. Partial integration was associated with heterogenous radiopacity of the graft and the formation of a partial gap between the graft and host bone containing a fibrous layer. Resorption was defined as lack of osseous contact between the graft and host bone, caused by fibrous encapsulation of the entire graft. The radiopacity of the graft gradually decreased until complete resorption. In contrast, inert grafts showed no change or even an increase in radiopacity, no signs of osseous contact, or of remodeling during the entire follow-up.

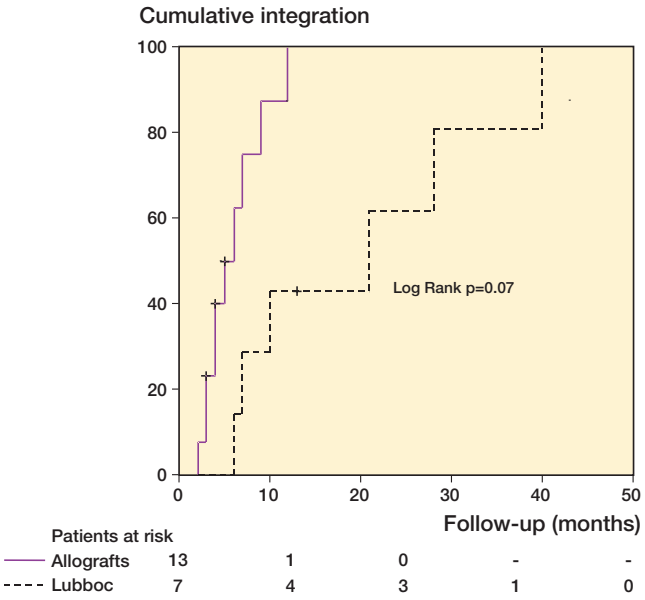
We measured the size of the defect on plain radiographs and calculated its volume with the ellipsoid approximation formula ($a \times b \times c \times 0.6$).

Statistics

Continuous data are presented as the median and total range. Discrete data are given as counts and percentages. The Mann-Whitney U-test was used to compare continuous variables. We analyzed the integration of implants during the follow-up with the Kaplan Meier method, and compared the integration of allografts and Lubboc implants with the log rank test. A two-sided p-value < 0.05 was considered statistically significant. Calculations were performed using SPSS for Windows Version 10.0 (Chicago, IL, USA).

Results

The integration of allografts was faster than that of Lubboc (log rank $p = 0.007$). At the 6-month follow-up, complete radiographic integration had occurred in 7 of the allograft patients, but in only 1 of those who received Lubboc.



Kaplan-Meier curve showing cumulative integration of the implant after follow-up.

After 12 months, all allografts showed complete radiographic integration. 3 patients, who did not complete the first year, had had partial integration at the last follow-up. Of the 7 patients who received the bovine substitute Lubboc, 3 showed complete integration after 1 year, 1 had partial integration after 13 months, and 3 complete integration at 21, 28 and 40 months.

After the follow-up, complete integration of allografts and bovine substitutes was seen in 10 and 6 patients, respectively, partial integration in 3 patients with allografts and 1 who had received the bovine substitute. The percentages of patients with integration were similar in the two groups. However, allografts integrated significantly faster (Figure).

We found no significant differences in the size of the defect between patients with an allograft (median 27 (4.8–58) cm^3) and those who had received the bovine material (median 36 (0.6–58) cm^3, p = 0.3). We therefore evaluated the relation between the defect's size and integration by combining the two groups, but found no association at follow-up. Thus patients with complete integration had a defect size of median 27 (0.6–58) cm^3 vs. median 30 (0.6–58) cm^3 in patients with graft integration (p = 0.2).

Discussion

No data are avilable on the application of the bovine substitute Lubboc after curettage of benign tumors, although good results have been described for allografts (Glancy et al. 1991). We found that allografts integrated faster than Lubboc. This may be due to the way Lubboc is manufactured. After an effective delipidation process using organic solvents, the bovine bone is treated twice with 8M urea to extract proteins (Chappard et al. 1993). Treatment with urea extracts bone morphogenetic proteins (BMP) (Urist et al. 1982). BMPs can induce bone formation in primates (Ripamonti et al. 2001) and a lack of BMPs in substitute materials could reduce stimulation of bone formation. However, the allografts used in this study were deep-frozen directly after harvesting and prepared while adding an antibiotic solution. Although we have no reliable evidence, it seems unlikely that BMPs are inactivated after a single deep-freezing cycle. Therefore, intact BMPs in allografts may be beneficial as regards integration of the transplant. We used deep-frozen allografts because lyophilization and irradiation reduce the mechanical properties of homologous bone (Hamer et al. 1996). Irradiated material may even release compounds that are toxic for osteoblast-like cells (Moreau et

al. 2000). Finally, cancellous bone prepared by adding of an antibiotic solution is also an antibiotic carrier (Witsø et al. 2000).

No fractures of implanted materials occurred in our patients, which may be due to the fact that the compressive strength of the bovine substitute Lubboc is similar to that of human cancellous bone (Purmarat and Squire 1993).

The present situation concerning Bovine Spongiform Encephalopathy makes it necessary to restrict the use of materials of bovine origin as much as possible. Upon request, the distributor provided certificates for Lubboc from the French Medication Agency, which assured us that no relation with BSE had been reported. However, it has now become possible to test for PrP^{Sc}, the only known component of the infectious prion particles (Prusiner 1998). 5 tests have been evaluated by the European Commission (http://europa.eu.int/comm/food/fs/bse/bse42_en.pdf). Rigorous use of these tests would enable manufacturers to show that their materials are free from infectious prion particles.

Our study suggests that human allograft is better than Lubboc. Another and more important problem is whether bone substitutes from animals may transmit bovine spongiform encephalopathy. These types of materials should not be used unless the manufacturer can guarantee that they are free of transmissible diseases.

No competing interests declared.

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