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Frozen cancellous bone allografts: positive cultures of implanted grafts in posterior fusions of the spine

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Abstract We have carried out a study on the behaviour pattern of implanted allografts initially stored in perfect conditions (aseptically processed, culture-negative and stored at –80 °C) but which presented positive cultures at the implantation stage. There is no information available on how to deal with this type of situation, so our aim was to set guidelines on the course of action which would be required in such a case. This was a retrospective study of 112 patients who underwent a spinal arthrodesis and in whom a total of 189 allograft pieces were used. All previous bone and blood cultures and tests for hepatitis B and C, syphilis and HIV (via PCR techniques) were negative. The allografts were stored by freezing them at –80 °C. A sample of the allograft was taken for culture in the operating theatre just before its implantation in all cases. The results of the cultures were obtained 3–5 days after the operation. There were 22 allografts with positive culture results (12%) after implantation. These allografts were implanted in 16 patients (14%). Cultures were positive for staphylococci coagulase negative (ECN) in 10 grafts (46%), *Pseudomonas stutzeri* in two grafts (9%), *Corynebacterium jeikeium* in two grafts (9%), staphylococci coagulase positive in two grafts (9%) and for each of the following organisms in one case each (4%): *Corynebacterium* spp., *Actinomyces odontolyticus*, *Streptococcus mitis*, *Peptostreptococcus* spp., *Rhodococcus equi* and *Bacillus* spp. No clinical infection was seen in any of these patients. Positive cultures could be caused by non-detected contamination at harvesting, storing or during manipulation before implantation. The lack of clinical signs of infection during the follow-up of our patients may indicate that no specific treatment different from our antibiotic protocol is required in the case of positive culture results of a graft piece after implantation.

Keywords Allograft contamination · Bone bank

Introduction

Although autografts, which are usually taken from the iliac crest, are still considered to be the gold standard, bone allografts are being used increasingly often for long spinal fusions. There is always a potential risk of disease or infection transmission from the donor to the recipient with the use of allografts [8, 10, 19, 20], though since 1988 only eight cases of bone-transplantation-associated HIV infection have been reported and bacterial allograft infection is almost negligible (4–5%) [2, 12, 19, 21]. However, we could find no previous cases reports on positive cultures detected after graft implantation even though all the bank cultures were initially negative. There is therefore a lack of information as to the course of action to be taken in these circumstances.

The aim of this paper is to report the rate of contamination of allografts which were in supposed perfect condi-

tion before their implantation, and to analyse its possible clinical repercussions.

Materials and methods

Between January 1995 and December 2000, 112 patients operated on at our Spinal Surgery Division received frozen cancellous bone allografts associated with surgical bed autograft as bone augmentation in long spinal arthrodesis. Surgical time was between 3 and 5 h in all cases. Grafts were taken from the freezer half an hour before implantation and were always processed in sterile conditions.

We followed the EAMST international bone bank screening procedures for all donors [7]. Most allografts in our tissue bank were obtained from donor cadavers (that were also organ donors) in an operating room by using routine sterile techniques. Some femoral heads were collected from patients undergoing a hip replacement. A sample of bone for culture was obtained from all tissues of cadavers and patients before storage [1, 14, 16]. Cultures of blood and tests for hepatitis B and C, syphilis and HIV (via PCR techniques) were also carried out. The allografts were stored by freezing to –80 °C in an electrical freezer equipped with an alarm to ensure that tissues were kept frozen until needed. The grafts were kept in three closed sterile plastic bags. No secondary sterilization methods were used [11, 17]. We store the grafts for a maximum of 5 years although they are usually used in the first year after extraction. For long spinal arthrodesis we used morsellized cancellous bone allografts.

A graft sample was obtained in each case for culture in the operation room just before implantation into the patient. Previous studies carried out by us showed that this is the most reliable method for cultures [14]. Previous to the implantation and taking the sample for the culture, the graft was washed with 3 l of sterile physiological fluid. We used the same culture methods for all samples. The sample was collected in a sterile container. Four millilitres of trypticase soy broth (TSB) were added and shaken with a vortex. This was used to inoculate a blood agar plate, a chocolate blood agar plate and a thioglycollate broth. The blood agar and chocolate blood agar plates were incubated at 35 °C in the presence of 10% CO_2. The thioglycollate broth and the TSB with specimen were incubated at 35 °C. The cultures were observed every day for5 days. The same method of culturing allografts was used during the recovery and processing phases and when the allografts were opened in the operating room for clinical implantation.

If there was no growth on the plates, in the thioglycollate broth or in TSB the sample was considered negative at 5 days. When some bacterial growth was observed on the plates then the growth was considered as important, and if there was no growth on the plates but some in the thioglycollate broth or in TSB, the growth was considered as poor. Any bacterial growth in any medium was cause for the tissue to be refused for banking. Differentiation between poor and important growth concerns the bacterial behaviour not the amount of contamination.

We reviewed 112 patients who underwent a spinal arthrodesis in which a total of 189 allograft pieces were used. The grafts were 72 femoral heads, 62 tibial plateaux, 54 femoral condyles and one calcaneus.

Long posterior fusions were performed for deformities such as scoliosis, degenerative disorders or fractures. We excluded patients with oncological disease or vertebral infection. Our own universal fixation system was used including rods, pedicle screws, sublaminar wires and hooks, depending on the operation [3].

All patients received the prophylactic antibiotic protocol recommended by the Clinical Infections Committee of our hospital. This protocol consisted of intravenous cefazolin 1 g/day 30 min before surgery, every 3 h during surgery and 8 h after finishing the operation. Clindamicin 600 mg/8 h and gentamicin 1.7 mg/kg per day were used if cefazolin was contraindicated.

In those cases in which the postoperative culture was positive, patients received the regimen we currently use for massive (structured) bone allografts: oral administration of cefadroxile 500 mg/12 h for 3 weeks. None of our patients was allergic to penicillin. This is an empiric antibiotic protocol and, despite the fact that we

Table 1 Patients with positive cultures

Patient	Pathology	Age (years)	No. of allografts	Bacteria	Growth	Follow-up (months)
1	Scoliosis	16	2	*Corynebacterium* spp.	Important	66
				Rhodococus equi	Important	
2	Scoliosis	17	2	Staphylococci coagulase positive	Poor	66
				Staphylococci coagulase positive	Poor	
3	Scoliosis	14	2	Staphylococci coagulase negative (ECN)	Poor	60
4	Scoliosis	12	2	*Peptostreptococcus* spp.	Important	60
5	Scoliosis	10	2	ECN	Poor	42
				ECN	Poor	
6	Scoliosis	13	1	ECN	Poor	42
7	Scoliosis	16	2	*Bacillus* spp.	Poor	42
				Corynebacterum jeikeium	Poor	
8	Scoliosis	12	3	ECN	Poor	42
				ECN	Poor	
9	Arthritis	70	1	ECN	Poor	30
10	Scoliosis	14	1	ECN	Poor	30
11	Scoliosis	17	3	*Corynebacterium jeikeium*	Poor	30
12	Arthritis	54	1	*Pseudomonas stutzeri*	Poor	30
13	Spinal fracture	45	2	*Pseudomonas stutzeri*	Poor	30
14	Scoliosis	14	2	ECN	Poor	18
15	Arthritis	50	2	ECN	Poor	12
16	Arthritis	45	2	*Streptococcus mitis*	Poor	
				Actinomyces odontolyticus	Poor	18

have no evidence of its efficacy, is the recommended protocol of our hospital's Clinical Infections Committee.

Routine wound and temperature controls were done until the patient was discharged and in subsequent clinical revisions. The presence or absence of signs or symptoms of infection was assessed by clinical and laboratory controls.

Results

From the 189 allografts used (126 from cadaver donors and 63 from live donors) we had 22 allografts with positive culture (12%) after implantation (14 from cadaver donors and 8 from live donors). These allografts were implanted in 16 patients with an average age of 26 years (range 10–70 years). Cultures were positives for staphylococci coagulase negative (ECN) in 10 grafts (46%), *Pseudomonas stutzeri* in two grafts (9%), *Corynebacterium jeikeium* in other two grafts (9%), staphylococci coagulase positive in two grafts (9%) and for *Corynebacterium* spp., *Actinomyces odontolyticus*, *Streptococcus mitis*, *Peptostreptococcus* spp., *Rhodococcus equi and Bacillus* spp. in graft each (4%). We found important bacterial growth in three cases (13%) and in 18 cases (85%) growth was classified as poor (Table 1). Using our prophylactic antibiotic protocol with an average follow-up of 38.6 months (range 6–60 months), no clinical infection occurred in any of these patients.

Discussion

The first bone banks appeared in the 1940s but it is thanks to the long series published by Malinin (1976) and Mankin (1983) that the use of human allografts became a universal practice [18].

More than 4,000 bone extractions are performed in Spain every year, from both live and cadaver donors. In the year 2000, a total of 60 bone banks were registered. The National Transplantation Organization (ONT) takes care of the co-ordination of the bone banks and gives them all directives for the correct extraction, storage, distribution and implantation policy. The ONT states that grafts should be obtained from live or dead donors in which AIDS, viral hepatitis C, viral hepatitis B and tumoral disease have been excluded. Grafts should be taken in sterile conditions and immediately stored at –80 °C. Cultures of all extracted pieces should be taken before storage, and in every case in which the culture or the blood test is positive, the piece should be discarded. When all cultures are proved to be negative, the piece is stored in a new freezer ready for its use. Pieces can be stored for a maximum of 5 years; in this series none of the grafts had been stored for longer than a year. All manipulations should be done in sterile conditions.

Although autografts are still the gold standard, bone allografts are being used progressively and are increasingly necessary for spinal arthrodesis [8, 10, 19, 21]. The use of autograft bone is not without complications or problems because of harvesting and donor site morbidity. Autograft harvesting also adds operating time and additional blood loss [10]. The advantages of allografts are their unlimited quantity, the time saving during surgery and the avoidance of donor site morbidity [8]. Several studies have shown that the risks are acceptable and results similar to those obtained with autografts [8, 10, 14, 15, 19, 21]. Bioactive ceramics may also be considered a good alternative when doing a posterior fusion, with no risk of transmitting disease. We have no experience with their use.

The transmission of disease or infection from the donor to the recipient is always a risk with the use of allografts, but the prevalence is low [13, 20] – lower than the risk of transmission through transplantation of organs. Since 1988, eight cases of bone-transplantation-associated HIV infection have been reported [12]. Gamma irradiation of allografts is not effective in HIV inactivation at the levels currently used. Thus, good screening procedures are the most effective means for providing the safest possible allografts [17]. No viral transmission has been registered in our bone bank since its creation in 1986. The incidence of bacterial infection is higher with the use of massive (structured) bone allografts than with cancellous bone allografts [2]. Tomford et al. [19] found an incidence of infection related to the use of allografts of 5% in patients having treatment for bone tumours and 4% in those who had revision of a hip arthroplasty. These rates of infection were not substantially different from those that have been reported in similar series in which large allografts or sterilized prosthetic devices were used. The causes of infection were difficult to determine, but contamination of the allograft was probably not a factor in most patients [19]. In March 2002, the Centers for Disease Control had received 26 reports of bacterial infections associated with musculoskeletal tissue allografts, 13 infected with *Clostridium* spp., including one death [4].

We can try to avoid infection or diminish its incidence by careful donor selection and the application of routine sterile techniques with bacterial cultures at extraction, storage and implantation of the allografts. But what should be done when a culture from an implanted allograft (with all extraction and storage cultures being negative) is positive? Although we always make routine cultures just before implantation, the results are not available for 5 days. If the culture is positive should the graft be removed?

In the course of the present study we have reviewed more than 50 papers on general bone banking or allograft policy and management, and about the use of bone allografts in spinal surgery. We have not found any data concerning the topic of our study, either in these papers or in the authoritative books by Friedlander et al. [9], the EAMST (European Association of Musculo-Skeletal Transplantation) [7] and Czitrom and Winkler [5]. These books study all basic sciences and their clinical application related to bone allografts. Furthermore, behind the one by the EAMST

are the works and statements of a whole scientific society specifically dedicated to the study of musculoskeletal tissue transplantation. Therefore, the experience that we are reporting now would be useful to improve knowledge about possible problems occurring in bone transplantation and their treatment.

As an explanation of the satisfactory behaviour of our contaminated grafts, the poor amount of growth and the type of bacteria found in most of our positive cultures could be interpreted as laboratory contamination. However, the positive culture rate of our grafts is higher than the positive culture rate considered as contamination in our laboratory (less than 5%). Thus, positive cultures could be caused by non-detected contamination at harvesting, storing or during manipulation before implantation. Davis et al. [6] found similar contamination rates to us in samples taken from the sucker tips, light handles, blades and needles used in 100 elective primary hip and knee arthroplasties. Organisms found were similar to those in our study (skin commensals) and the rate of infection was 1% (with the infecting organism different from the single identified contaminant) [6].

The lack of clinical signs of infection during the follow-up of our patients may indicate that no specific treatment – other than our antibiotic protocol – is necessary when facing a case of positive culture of a graft piece after implantation. Prophylactic antibiotics and the patient's immune system are enough to avoid infection. Therefore, in our opinion, no other special antibiotic preventive therapy or surgical treatment is required in most patients.

We must look carefully to the microorganism involved and the antibiogram in order that where very pathogenic bacteria (such as *Clostridium*) are found the allograft is removed and appropriate antibiotics used. It is therefore important to obtain cultures before and after processing the allograft in order to identify any contaminant.

We think that the contamination of these allografts happened while processing them for implantation in the operating theatre, as has already been reported with surgical needles and suckers [6]. In any case, as we have shown in our series, this contamination has no clinical relevance if antibiotic prophylaxis is used. To the best of our knowledge this is the first paper about this matter, and this high rate of allograft contamination is not known by most orthopaedic surgeons. The differences among the supposed or known rates of contamination at implantation may arise from the fact that cultures are not currently taken following protocol. That is the reason why we use prophylactic antibiotics but in most cases, where skin commensals are the contaminant, the possibility of achieving the same results with simpler preventive antibiotic therapy (such as the one we currently use in cases in which we do not implant allografts or when no positive culture is found after implantation) is now under consideration by the Clinical Infections Committee of our hospital. A prospective study will follow.

Authors' note. The authors realize that many of the references listed are not included in a major database and not readily available. However, we consider that they are important for this article. We will gladly provide them for interested readers.

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