Positive culture in allograft ACL-reconstruction: what to do?

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ABSTRACT

The transmission of disease or infection from the donor to the recipient is always a risk with the use of allografts. We carried out a research study on the behavioural pattern of implanted allografts, which were initially stored in perfect conditions (all cultures being negative) but later presented positive cultures at the implantation stage. Because there is no information available on how to deal with this type of situation, our aim was to set guidelines on the course of action which would be required in such a case. We conducted a retrospective study of 181 patients who underwent an ACL reconstruction using BPTB allografts. All previous bone and blood cultures and tests for hepatitis B and C, syphilis and HIV were negative. An allograft sample was taken for culture in the operating theatre just before its implantation. The results of the cultures were obtained 3–5 days after the operation. We had 24 allografts with positive culture (13.25%) after the implantation with no clinical infection in any of these patients. Positive cultures could be caused by undetected contamination while 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 – other than an antibiotic protocol – would be required when facing a case of positive culture of a graft piece after its implantation.

KEYWORDS

Allografts contamination; Bone–patellar tendon–bone allograft; ACL reconstruction; Positive culture; Infection.

INTRODUCTION

Although the performance of bone–patellar tendon-bone (BPTB) autografts sets the gold standard in the reconstruction of the anterior cruciate ligament (ACL), more allografts are being used since the paper by Shino and colleagues [24]. Most published articles show that similar results can be achieved with BPTB allografts [8, 13, 15, 18, 20, 23]. There is always a potential risk of transmitting disease or infection from the donor to the recipient when using allografts [27, 28]. Since 1988, only eight cases of bone transplantation-associated HIV infection have been reported, although bacterial allograft infection is more common [14]. However, to the best of the author's knowledge there are no papers published on what to do with a contaminated implanted allograft. Although we suppose that septic arthritis after ACL reconstruction is caused by allograft contamination, there are no clinical studies which prove this [3]. This lack of information does not provide us with any tools whatsoever as to the course of action that should be taken. This paper focuses on the actual incidence of implanted BPTB allografts which are initially non-contaminated, and on their possible clinical repercussion.

MATERIALS AND METHODS

A retrospective review was conducted of the medical records of all 181 patients with anterior cruciate ligament reconstruction using BPTB allograft between December 1987 and December 2001. Part of this series was previously published showing our clinical results with BPTB allografts [29, 30, 31]. All patients were operated on at our knee surgery division.

International bone bank screening procedures were followed for all donors. All of our tissue bank allografts were extracted in an operating room from donors of cadavers by means of sterile routine techniques (many of them were also organ donors), and immediately put in a sterile plastic recipient that was enclosed in two closed sterile plastic bags and frozen at –80°. Prior to storage a sample for culture was taken from all tissues, [1, 17, 22]. Blood cultures and tests for hepatitis B and C, syphilis and HIV (via PCR techniques) were also carried out. All allografts whose culture resulted positive, or whose donor was affected with any of the mentioned infections, were discharged. Grafts free of bacterial contamination or donor infections were kept and stored frozen at –80°C in another electric freezer equipped with an alarm to ensure that tissues were stored in perfect conditions until they were to be used. Allografts were stored for a maximum of 2 years. No secondary sterilization methods were used, because none of them ensures the complete sterilization of the graft without damaging the structure of the graft [11, 25].

A graft sample was taken in each case for culture in the operating theatre just before its implantation in the patient. In a previous study we found that this is the most reliable method for cultures [17]. The graft had previously been washed in three litres of sterile physiologic fluid. The same culture methods were used for all samples. The sample was collected in a sterile container. In total, 4ml of trypticase soy broth (TSB) was added and was shacked with a vortex. This was inoculated in blood agar, chocolate blood agar and thioglycollate broth. The blood agar and chocolate blood agar 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 underwent observation for 5 days. The same method of culturing allografts was used during the recovery and processing phases and when opened in the operating room for clinical implantation.

If after the 5-day period no growth whatsoever was found on the plates, in the thioglycollate broth, or in TSB the sample was considered negative. If there was growth on the plates, it was considered important, whereas if growth was detected in the thioglycollate broth or in TSB, but not on the plate it was considered to be poor.
We reviewed 181 patients who underwent an anterior cruciate ligament reconstruction. The average age was 25 years (16–48). BPTB allograft from our bone bank was used in all cases. Oncologic patients or those with immunodeficiency were excluded from this revision.

We indicated in all patients the prophylactic antibiotic protocol recommended by the Clinical Infections Committee of our hospital. This protocol consisted of administering endovenous Cefazolin 1 g/day 30 min before surgery and another gram every 8 h for 2 days. Clindamicine 600 mg/8h and Gentamicine 1.7 mg/kg per day were used if Cefazolin was contraindicated. In the case of positive culture, oral antibiotic treatment was prescribed for 2 weeks following the antibiogram.

Routine wound and temperature controls were performed until the patient was discharged from the hospital, as well as in posterior clinical examinations. We considered laboratory tests to be unnecessary because of the lack of clinical signs.

RESULTS

Positive cultures appeared in 24 allografts after their implantation (13.25%). Cultures tests proved positive for Staphylococci coagulase negative (ECN) in ten grafts (41.6%), Corynebacterium jeikeium in six grafts (25%), Micrococcus and Propionibacterium granulosus in two grafts each (8.3%), and each of the following appeared once (4.1%): E. Colli, Bacillus brevis, Agribacterium radiobacter/SCN, Propionibacterium acnes. We found abundant bacterial growth in 3 cases (12.5%; ECN, Corynebacterium jeikeium, Agribacterium radiobacter/ and SCN), and in 21 cases (87.5%) growth was classified as poor (Table 1). Using our prophylactic antibiotic protocol, and with an average follow-up of 49.5 months (range 12–150), no clinical infection was detected in any of these patients.

DISCUSSION

The first bone banks appeared in the 1940s but thanks to a long series published by Malinin (1976) and Mankin (1983) the use of human allografts became universal practice [26]. Today this practice in Spain is controlled by the National Transplantation Organization (NTO), who sets the guidelines for proper donor selection, graft extraction storage distribution and implantation. In the year 2000 a total of 60 bone banks were registered at the NTO.

Although autografts are the gold standard in ACL reconstruction, the use of BPTB allografts has been shown to be increasing progressively [15, 23]. The use of autografts

does have its drawbacks due to harvesting and donor site morbidity. Furthermore, autograft harvesting increases operating time, and gives rise to the risk of patella fracture, patella tendonitis and residual tender scars [12]. On the other hand, advantages such as the unlimited supply of grafts, diminished surgical time and the absence of donor site morbidity [23] make allografts a very interesting option. Moreover, several studies have shown that the risk of transmitting diseases is acceptable and results are almost similar to the ones obtained with autografts [2, 17, 19, 27].

The transmission of disease or infection by the donor to the recipient is always a risk when resorting to the use of allografts, but the prevalence is low [16, 28], being lower than the risk of transmission by the transplantation of organs. Since 1988, only eight cases of bone transplantation-associated HIV infection have been reported [14]. Gamma irradiation of allografts is not effective in HIV inactivation at the levels currently used. Therefore, good screening procedures are the most effective means for providing the safest possible allografts [9, 10, 21, 25]. No viral transmission has been registered in our bone bank since it was set up in 1986. Tomford et al. found an incidence of infection related to the use of allografts of 5% in patients who had treatment for bone tumours, and of 4% in those who had revision of a hip arthroplasty [27] 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. By March 2002, the CDC had received 26 reports of bacterial infections associated with musculoskeletal tissue allografts, 13 infected with Clostridium spp., including one death [3].

The best way to avoid infection or diminish its incidence is careful donor selection and the application of routine sterile techniques when dealing with bacterial cultures at the extraction, storage and implantation stages of the allografts.

Nevertheless, what course of action can be taken when a culture from an implanted BPTB allograft (with all extraction and storage cultures proving negative) turns out to be positive? Although we always perform routine cultures just before implantation, the results are not available until 5 days later. Consequently, we are faced with the task of determining what to do when any of those cultures prove to be positive. Should the implanted graft be removed?

We have reviewed more than 50 papers on general bone banking, and to the best of the author’s knowledge there is no information concerning the issue covered in our research study in any of these papers or in the greatly recommended books by Friedlander et al. [7], the EAMST (European Association of Musculo-Skeletal Transplantation) [6] and Czitrom [4]. Therefore, the experience that we are reporting now could prove to be useful in broadening our knowledge of potential problems that occur during bone and soft tissue transplantation and their treatment.

The satisfactory behaviour of our contaminated grafts, the poor growth and the type of bacteria found in most of our positive cultures all indicates the possibility of a laboratory contaminant. However the positive culture rate of our grafts was higher than our laboratory contamination rate, which is under 5%. We also found extensive growth in three of the implanted grafts. In short, there is substantial evidence to state that positive cultures could be caused by non-detected contamination during the stages of

harvesting, storage or their manipulation before implantation. Davis and colleagues [5] studied the contamination rates in samples from the surgical suckers, bladders and needles; the organisms and contamination rates found were similar to those in our study (skin commensals) and the rate of infection was 1% (with the infecting organism different from that found in the surgical instruments). Our results show that contamination of the allografts in the operating theatre is as frequent as the contamination of the surgical equipment.

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 needed when facing the case of a positive culture of a graft piece after implantation. Therefore, in our opinion no other special antibiotic preventive therapy or surgical treatment is required.
At any rate, we must take great care as to the micro-organism and the antibiogram in order to remove the allograft and use specific antibiotics in cases where very pathogenous bacteria, such as Clostridium, are found. Therefore it is important to obtain cultures before and after processing the allograft in order to identify any contaminant.
In cases in which skin commensals are the contaminant, the possibility of obtaining the same results with a simpler preventive antibiotic therapy – such as the one we currently use when not implanting allografts, or when no positive cultures are negative after implantation – is now under consideration by the Clinical Infections Committee of our Hospital. A prospective study will follow.

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Table 1. Patients and results					
Patient	**Age**	**Gender**	**Bacteria**	**Growth**	**Follow-up**
1	17	Male	Staphylococci coagulase negative	Poor	150
2	22	Female	Staphylococci coagulase negative	Poor	138
3	27	Male	Staphylococci coagulase negative	Important	90
4	20	Male	Propinebacterium granulosus	Poor	90
5	34	Male	Propinebacterium acnes	Poor	90
6	35	Male	Corynebacterium jeikeium	Important	78
7	29	Female	Staphylococci coagulase negative	Poor	66
8	23	Male	Corynebacterium jeikeium	Poor	66
9	21	Female	Corynebacterium jeikeium	Poor	66
10	33	Male	Agribacterium radiobacter/SCN	Important	54
11	33	Male	Staphylococci coagulase negative	Poor	54
12	29	Male	Corynebacterium jeikeium	Poor	54
13	25	Male	Bacillus brevis	Poor	54
14	32	Male	Micrococcus	Poor	30
15	23	Male	E. colli	Poor	30
16	30	Male	Staphylococci coagulase negative	Poor	30
17	46	Female	Staphylococci coagulase negative	Poor	30
18	27	Male	Micrococcus	Poor	18
19	35	Male	Corynebacterium jeikeium	Poor	18
20	41	Male	Propionibacterium granulosus	Poor	18
21	17	Male	Staphylococci coagulase negative	Poor	12
22	16	Female	Corynebacterium jeikeium	Poor	12
23	32	Male	Staphylococci coagulase negative	Poor	12
24	23	Male	Staphylococci coagulase negative	Poor	12