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Cadaveric allograft microbiology

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Abstract This study aims to determine the contamination rate of cadaveric bone allograft and blood cultures retrieved from 119 donors within Leicester between 1990 and 2003. A contamination rate of 27% was present, with 120 of 437 bone allografts culturing positive at the time of retrieval. Similarly, a contamination rate of 37% was present, with 40 of 107 blood samples culturing positive. The time interval between death and procurement did not influence blood contamination. Coagulase-negative *Staphylococcus* was the commonest organism isolated in both blood and bone cultures. One donor had *Clostridium* grown in their blood culture. The available evidence confirms similar contamination rates with other studies. The majority of organisms isolated were skin commensals with a low rate of contamination of highly pathogenic organisms such as *Clostridium*.

Résumé Cette étude a pour but de déterminer le taux de contamination des allogreffes osseuses cadavériques et des hémocultures chez 119 donneurs, entre 1990 et 2003, à Leicester. Un taux de contamination de 27% était présent avec 120 des 437 allogreffes avec une culture positive au moment du prélèvement. De la même façon, un taux de contamination de 37% était présent avec 40 des 107 prélèvements de sang avec une culture le positive. L'intervalle de temps entre la mort et l'acquisition du prélèvement n'a pas influencé la contamination du sang. Le staphylocoque coagulase négative était l'organisme plus fréquent isolé dans le sang et dans l'os. Un donneur avait un Clostridium dans l'hémoculture. Les résultats confirment des taux de contamination voisins de ceux des autres études. La majorité des organismes isolés sont des commensaux de la peau avec un bas taux de contamination d'organismes hautement pathogènes tels que Clostridium.

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Introduction

The utilisation of cadaveric bone allograft for revision arthroplasty and tumour surgery takes place in many orthopaedic units [10]. With increasing demand for allografts, bone banks have been established to provide safe and effective allografts to patients. The Leicester Bone Bank was established in 1989 and is one of a few hospital-based tissue banks across the United Kingdom (UK) that collects and stores massive cadaveric musculoskeletal allografts. Stringent standards and quality control measures in bone banks have been implemented to minimise contamination and protect prospective recipients from the risk of transmitted infection [2, 6].

Previous papers have identified skin organisms such as coagulase-negative *Staphylococcus* as the organism most commonly isolated from donated allografts [10, 13]. However, organisms such as *Clostridium* have become particularly important following a recent study by Malinin et al. [12] who showed a significant number of clostridial contamination in musculoskeletal allografts. This paper aims to determine the contamination rate of blood and bone allograft at the Leicester Bone Bank over 14 years and whether the time interval between death and procurement of allograft influences blood culture results.

Patients and methods

This study was based on information collected prospectively by the Leicester Bone Bank between January 1990 and September 2003. We reviewed the culture results of all blood samples and bone allografts donated to the bone bank and number and type of organisms isolated were determined. Cadavers with a positive blood culture result

that had bone allografts retrieved in less than 24 h were compared to cadavers with allografts retrieved after 24 h following death.

Donors

During the study period, there were 119 cadaveric donors who were selected and screened according to the guidelines of the British Association of Tissue Banking (BATB) and European Association of Musculoskeletal Transplantation (EAMST). The mean age was 46 (17–69) years. There were 93 males and 26 females. Most donors died from cardiovascular disease (63). Other causes of death included cerebrovascular disease (23) and trauma (20). Of the donors, 63 (53%) were referred to the transplant team from the emergency department in comparison to 56 (47%) from wards and intensive care units.

Retrieval and storage

Allografts were harvested from cadaveric donors soon after death. Allografts procured from a single donor were either whole or segments of bones. Procurement is performed under aseptic techniques in an operating theatre. The cadavers are draped and the skin is decontaminated with 10% iodine solution. The allografts are taken from the cadavers and passed to a second person working at a separate table. They are washed in normal saline and then stripped of their soft tissue. The allografts are swabbed for culture and wrapped in sterile towels and plastic bags. All cadaveric allografts are stored at −80°C.

Microbiology

Blood from either a central or peripheral vessel is taken for culture before procurement. The blood samples are injected into blood culture bottles and cultured for aerobic and anaerobic organisms for 5 days. Positive blood cultures are gram stained and subcultured onto agar. The allografts are cultured by having a swab rubbed over the entire surface of each graft. The swab is cultured aerobically and anaerobically on agar and then placed into broth. Primary plates are incubated for 48 h before reporting. Broth samples are subcultured after 48 h on agar before reporting.

Out of 455, 262 (58%) bone allografts were gamma irradiated (secondary sterilisation) because of positive culture results from blood or bone, or the time interval between death and retrieval was greater than 24 h. Six of 455 (1%) bone allografts, all from one donor, were discarded because of *Clostridium* organisms being isolated from the blood culture. The same patient was positive for syphilis serology.

Statistical analysis

Analysis involved using the chi-squared test with significance at $p<0.05$ and the calculation of the difference in proportion confidence interval.

Results

Blood contamination

Blood cultures were taken from 107 donors, of which 67 (63%) were negative and 40 (37%) cultured positive with organisms. Coagulase-negative *Staphylococcus* (n=27) and *Streptococcus* (n=12) species were the commonest isolates. Other organisms isolated are shown in Table 1.

The time interval between death and retrieval was not recorded for 38 of the earlier donors. Culture-positive blood samples retrieved in less than 24 h following death were compared to culture-positive samples retrieved after 24 h of death. The details of the two donor groups are listed in Table 2. Out of 54 blood cultures, 22 (41%) were contaminated in the less than 24 h group compared to six of 20 (30%) in the greater than 24 h group. The difference in proportion of culture-positive samples was 11% (p=0.4, 95% CI: −14%–31%) and not significant.

Graft contamination

From 119 donors, 445 allografts were obtained. Results of seven cultures were not available. From those allografts harvested, organisms were cultured from 120 (27%). The commonest isolates were coagulase-negative *Staphylococcus* (n=93) and *Bacillus* species (n=12). Other species cultured are shown in Table 1. A single organism was cultured from 117 allografts. The distribution of different types of allografts harvested and their contamination rates are shown in Table 3.

Table 1 Organisms isolated from blood cultures and bone allografts

Organisms	Number of times cultured	
	Blood culture[a]	Bone allograft[b]
Coagulase-negative *Staphylococcus*	27	93
Streptococcus species	12	4
Bacillus species	3	12
Pseudomonas species	0	5
Staphylococcus aureus	2	1
Propionibacterium acnes	1	0
Diphtheroids	1	4
Escherichia coli	1	2
Haemophilus influenzae	1	0
Clostridium species	1	0
Enterococcus species	0	2

[a]Multiple organisms were cultured from 11 blood samples.
[b]Multiple organisms were cultured from three bone allografts.

Table 2 Details of the two donor groups

	Group 1, less than 24 h	Group 2, greater than 24 h
Age[a]	46	49
Time interval of retrieval[a]	15 (3–23)	27 (24–33)
Total number of donors	57	24
Male	43	19
Female	14	5
Positive BC	22	6
Negative BC	32	14

BC blood culture.
[a]Mean (range).

Table 3 Types of allografts and their contamination rate

Allograft	Number	Contamination rate[a]
Proximal femur	101	27 (27%)
Distal femur	101	30 (30%)
Whole femur	72	18 (25%)
Proximal tibia	133	35 (27%)
Distal tibia	8	2 (29%)
Humerus	26	7 (27%)
Acetabulum	4	1 (33%)

[a]Culture results were not available for seven allografts.

Discussion

Strict precautions are taken according to regional and national guidelines to minimise cross-infection of potential recipients. Amongst these precautions is the microbial screening of allografts for contamination. Despite adherence to these rigourous standards, the rate of contamination of retrieved bone allografts range from 5% to 44% [7, 8, 11, 15]. This rate varies due to differences in quality control and microbiological screening [1].

There was a 27% contamination rate of bone allografts in our study. This compares favourably with those of other reported studies. The majority of positive samples cultured coagulase-negative *Staphylococcus*. This may represent contamination from handling, as the method used to culture the specimens can exaggerate the presence of one organism to many colonies. However, 12 of our bone allografts grew *Streptococcus*. Deijkers et al. [4] divided contaminating organisms into low and high pathogenicity. They considered organisms of low pathogenicity to be skin commensals. Organisms of high pathogenicity were thought to originate from endogenous sources in the donor and more likely to cause infection in the recipient allograft.

Any contaminated bone allograft is normally discarded or irradiated to minimise the risks of cross-infection, depending on the pathogenicity of the organisms isolated. Our policy is to irradiate bone that is retrieved after 24 h of death, bone from donors with contaminated blood samples or positive cultures. Discarding contaminated bone would result in a high discard rate and wastage of resources in our bone bank where there is a shortage of donors. However, it is possible that positive cultures could play a role in influencing antibiotic therapy should the recipient develop subsequent infection. Infection following the implantation of bone allograft is a serious complication. Tomford et al. [15] showed a 5% and 4% incidence of infection related to the use of allografts in patients who had surgery for bone tumour and revision hip arthroplasty, respectively. Other studies have demonstrated infection rates as high as 12.2% when banked allograft is used for reconstructive surgery [14]. Any clue to identify the causative organism in treating infected allografts would be an invaluable tool.

Our blood sample contamination rate was 38%, and this compares favourably with other studies [13]. The majority of allograft retrieval occurred within 24 h of death. EAMST recommends that harvesting of bone allograft should take place between 12 h and 24 h after death [6], whereas BATB suggests retrieval of tissue within 48 h of death [2]. However, numerous logistic difficulties may hinder the retrieval of allograft within 24 h. Kumta et al. [9] showed that it may be possible to extend the period within which cadaveric bone allograft may be harvested for transplantation to up to 96 h following death provided sterile procurement and storage techniques are utilised. Other investigators such as Dolan et al. [5] reported that the incidence of positive cultures with cadaveric tissue does not increase with post-mortem time up to 48 h after death. We were unable to demonstrate any evidence of increased blood contamination in donors who had allografts harvested after 24 h of death compared to the recommended period of 12–24 h by EAMST.

Other organisms such as *Clostridium* have recently become a concern following the death of a young patient due to infection with *Clostridium sordelli* [3]. Malinin et al. [12], in a recent study, showed a significant number of clostridial contamination in musculoskeletal allografts. In our study, *Clostridium* was isolated in one blood sample (1%) compared to a contamination rate of 8.1% reported by Malinin et al. [12].

There are limited guidelines for microbiological surveillance of bone banking across the UK besides the standards of EAMST that suggest “all tissues retrieved shall be tested for aerobic and anaerobic bacteriological contamination using appropriate testing”. We suggest that methods of allograft retrieval and the number and type of specimens sent for microbial analysis should be standardised. This would allow tissue banks to use this information for quality control and identify any breakdown if their rates of contamination are higher than expected.

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