Bacterial contamination of femoral head allografts from living donors

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ABSTRACT – We analyzed the bacterial contamination rate of femoral head allografts from living donors and determined the true bacterial load with cultures from the grafts in their entirety in a specially prepared medium. During 4 years we took swab cultures from 2,679 grafts of which 2,414 (90%) were negative.

In a period of 12 months, grafts rejected for reasons other than infectious disease were cultured in their entirety to determine the true bacterial load. Of the 106 grafts included, 15 were contaminated. Microorganisms were isolated from 10 of the 91 initially swab culture-negative grafts (9%) and from only 5 of the 15 swab culture-positive grafts, most of which were normal skin contaminants. Swab culture negative grafts apparently may still be contaminated.

It seems wise to subject all femoral head allografts from living donors to antibacterial processing.

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The use of femoral head allografts in revision hip arthroplasty employing the impaction grafting technique has increased the need for this type of allograft (Gie et al. 1993, Slooff et al. 1996). Many orthopedic departments still rely on femoral head bone banks run by hospitals. International standards indicate that representative samples should be cultured from the grafts obtained during total hip replacements (EAMST and EATB 1997). However, they give no guidelines for bacterial screening methods and, more importantly, on the interpretation of the results. Cultures are therefore made in different ways in accordance with each hospital's procedures. Grafts with negative cultures are commonly released for transplantation purposes without any processing.

We analyzed the contamination rate of femoral heads from living donors undergoing total hip replacement and determined whether the swab culture technique was a reliable method for assessing the bacterial load of these grafts.

Material and methods

During 4 years (1996–2000), 2,679 femoral head allografts were obtained from living donors undergoing total hip replacement. The 15 participating hospitals were given a donor protocol and procurement packages containing materials for serological and microbiological testing as well as packaging and labeling material. After retrieval of the graft, the entire surface of the femoral head was carefully swabbed with a 15 cm polyester-tipped applicator (Medical Wire and Equipment Co. Ltd., Corsham, Wits., U.K.) which was placed on transport medium (Transwab, Medical Wire and Equipment). The femoral head was then packed and labeled according to the protocol and transported with the swab cultures and the blood samples.

Following laboratory routine, the swabs were inoculated within 48 h on blood agar and chocolate plates and cultured under aerobic and anaerobic conditions. The swab sticks themselves were incubated for 5 days in a brain-heart infusion culture broth (Oxoid Unipath Ltd., Basingstoke, Hampshire, U.K.), which was subcultured on blood agar and chocolate plates for 48 h.

Table 1. Microorganisms of low and high virulence isolated from the swab cultures (broth or plate)

Microorganism	Number of cultures [a]
Low virulence	
Coagulase-negative staphylococcus	180
Propionibacterium species	17
Corynebacterium species	15
Bacillus species	12
Sporeformers, aerobic	5
Gram positive cocci [b]	5
Gram positive rods [b]	4
Micrococcus	2
Diphtheroid rods	1
Total	241
High virulence	
Streptococcus species	15
Aspergillus species	3
Molds	3
Non-fermentative gram negative rods	3
Clostridium species	2
Staphylococcus aureus	2
Gram negative rods [c]	2
Citrobacter species	1
Enterococcus faecalis	1
Yeast	1
Gram negative cocci [c]	1
Total	34

[a] more than one microorganism isolated from each swab culture.
[b] species level not determined, but were considered to be of low virulence.
[c] species level not determined, but were considered to be of high virulence.

Table 2. Comparison of swab cultures versus entire cultures

	Swab culture		
	Negative	Positive	Total
Entire culture			
Negative	81	10	91
Positive	10	5	15
Total	91	15	106

The swab cultures were interpreted by distinguishing between microorganisms of low virulence—e.g., skin contaminants such as coagulase negative staphylococci and Propionibacterium acnes—and those usually regarded as high virulence–e.g., Staphylococcus aureus, Streptococcus species and Candida species (Veen 1994). The number of organisms cultured were divided into four categories: 1. no microorganisms; 2. organisms of low virulence cultured only from the broth (low number); 3. organisms of low virulence cultured from the plate (high number) and 4. organisms of high virulence cultured from the broth or the plate.

During 12 months, the femoral heads of living donors rejected for transplantation were cultured in their entirety. The reasons for rejection were incomplete documentation and contraindications for donation, e.g., malignancies or false positive serological tests. None of the donors had an acute or chronic infectious disease. A negative or positive swab culture result was required for inclusion.

In a laminar flow unit, using aseptic techniques, the grafts were immersed in culture medium, but not cut or ground. A specially prepared multipurpose and nutritionally-enriched medium, was employed with additives ensuring growth of aerobic and anaerobic microorganisms, even fastidious organisms (3.7%g BHI, 0.5 g liquoid, 1 mL haemine, 12 g gelatine, 5 g proteose peptone/1000 cc NaCl 0.9%) (Dunne et al. 1997). The cultures were first incubated for 7 days and then the broth was inoculated on blood agar and chocolate plates and cultured for 48 h. Dispersing plates were used during the entire procedure and cultures were taken from all media as negative controls.

The results of the entire cultures were compared with the swab cultures taken during recovery of the graft. Identical organisms isolated from the swab and the entire culture were determined by means of biotyping, anti-biogram and molecular typing.

Results

Of 2,679 grafts, 2,414 (90%) swab cultures were negative. Skin contaminants were isolated only from the broth culture of 119 grafts (5%) and the plate culture of 115 grafts (4%) (Table 1). Organisms of high virulence were cultured from 31 grafts (1%) (Table 1).

Of the 106 grafts cultured in their entirety, 91 were initially swab culture negative and 15 were swab culture positive (Table 2). Microorganisms were isolated from the entire cultures of 10 swab culture-negative grafts, which resulted in a negative predictive value of 89%. Coagulase-negative

Table 3. Entire culture results from swab culture-positive grafts

Donor	Swab culture	Entire culture
1	coagulase-negative staphylococcus (broth)	No microorganisms
2	coagulase-negative staphylococcus (broth)	No microorganisms
3	coagulase-negative staphylococcus (broth)	No microorganisms
4	Bacillus species (broth)	No microorganisms
5	*Propionibacterium acnes* (plate)	No microorganisms
6	coagulase-negative staphylococcus (broth)	coagulase-negative staphylococcus
7	coagulase-negative staphylococcus (broth)	coagulase-negative staphylococcus
8	*Staphylococcus epidermidis* [a] (broth)	*Staphylococcus epidermidis* [a]
9	*Propionibacterium acnes* (plate)	Lactobacillus species
10	Bacillus species (plate)	Corynebacterium species
11	Mold (broth)	No microorganisms
12	*Aspergillus fumigatus* (broth)	No microorganisms
13	Clostridium species (broth)	No microorganisms
14	Streptococcus species (plate)	No microorganisms
15	*Staphylococcus aureus* (plate)	No microorganisms

[a] Confirmed as identical organisms by means of biotyping, antibiogram and RAPD

staphylococci were isolated from 8, diphtheroids from 1 and yeasts from 1 swab culture-negative graft.

Of the 15 swab culture-positive grafts, microorganisms were isolated from the entire culture of 5 grafts (Table 3). Identical organisms, coagulase-negative staphylococci, were cultured from 3 grafts. For technical reasons, we were unable to verify whether the microorganisms isolated from the swab and entire culture of 2 grafts were identical. The third graft yielded identical organisms from both cultures, confirmed by anti-biogram, biotyping and RAPD pattern.

No microorganisms were isolated from the control medium cultures or from the dispersal plates.

Discussion

The swab culture technique in this study was also used in postmortem donor retrieval and a contamination rate of 52% was found in these donors (Deijkers et al. 1997). In an experiment on postmortem allografts, 92% of the specimens were contaminated (entire culture), but the swab cultures were positive in only 45% (Veen et al. 1994).

In living donors, a swab contamination rate of 10% was found, which is much lower than that in postmortem donors. Other tissue banks have reported contamination rates between 1% and 19% (Hart et al. 1986, Tomford et al. 1986, Kakaiya and Jackson 1990, Chapman and Villar 1992, Ivory and Thomas 1993, Barrios et al. 1994, Campbell and Oakeshott 1995, Husted and Kramhoft 1996, Aho et al. 1998, Journeaux et al. 1999). However, the sampling techniques and laboratory methods used for bacterial screening in these reports were all different, varying from cultures of the graft, the cut surface of the femur, the capsule and the synovial fluid (Journeaux et al. 1999). These differences in screening methods make it difficult to compare the rates.

Of the 106 entire cultures made, 15 were positive and the true contamination rate was probably much lower than the rate in postmortem donors.

However, of these 15 positive entire cultures, 5 were initially swab culture-positive and 10 were swab culture-negative. Therefore, although the negative predictive value of the swab culture technique was relatively high (86%) (Veen et al. 1994), 9% of the swab culture-negative grafts proved to be contaminated. Moreover, only 3 of the 15 swab culture-positive grafts yielded identical organisms from the entire cultures. Therefore, the routine swab culture technique seems to be less suitable for assessing the bacterial load of femoral heads obtained from living donors.

The cultures were interpreted by categorizing grafts according to the virulence and the number of microorganisms isolated (Veen 1994, Deijkers et al. 1997). In postmortem donors, this system has made it possible to distinguish between con-

taminants from the skin and microorganisms of greater virulence that may have spread hematogenously from an endogenous source (Vehmeyer et al. 1999). For living donors, such distinction seems unnecessary. It is unlikely that organisms of high virulence will contaminate the femoral head hematogenously, especially since prophylactic antibiotics are given routinely to all patients undergoing total hip replacement. The femoral heads were contaminated either via air with skin flora dispersed by the operating room personnel or per continuitatem with organisms originating from the skin of the donor or the environment.

Considering the sources of contamination during or after total hip replacement, only the external surface of the graft was contaminated. The use of swabs permits culture of the entire surface. Particularly when this surface is moist, the swab culture is thought to be a reliable method for detecting superficial contamination. Tissue specimens can only be taken from a limited number of sites, but the contamination may not always be evenly spread over the surface of the graft. It is therefore unlikely that other techniques, like tissue cultures, would provide additional information.

The sources of contamination were confirmed by the types of organisms isolated from the entire cultures. Apart from Lactobacillus species, an organism frequently cultured from the genital area, and yeast, a contaminant from the environment, all organisms isolated were skin flora. Similar organisms have been cultured from contaminated surgical wounds and instruments used during orthopedic operations (Dietz et al. 1991, Davis et al. 1999). Although they are thought to be an acceptable bacterial load, and skin flora are not regarded as virulent, they can cause serious infections in the presence of foreign bodies (Hope et al. 1989, Ringberg et al. 1998).

Our findings indicate that unprocessed swab culture-negative grafts may be contaminated with organisms that can cause an infection in the recipient. It seems desirable to process grafts before they are released for transplantation. The method for processing should eradicate bacterial microorganisms. Various methods are used, each having its own effect on the biological and mechanical properties of the graft (Simonian et al. 1994, Thorén and Aspenberg 1995, Jinno et al. 2000). Antibiotic rinsing of the graft has been proposed by some, but it does not affect the risk of contamination in large studies with postmortem donors (Deijkers et al. 1997). Most femoral heads are morselized and impacted and, in these procedures, the biological properties which determine graft incorporation are more important than the mechanical strength. A low dose of gamma irradiation does not affect the biological properties of a graft and this therefore seems a suitable method for decontamination (Jinno et al. 2000). Additional chemical defatting and decontamination procedures may enhance incorporation and provide greater safety (Thorén et al. 1995, Moreau et al. 2000). However, the effect of the processing method on the mechanical properties of the graft must be taken into account since some may have harmful effects (Godette et al. 1996, Hamer et al. 1996, 1999).

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