The real contamination of femoral head allografts washed with pulse lavage

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ABSTRACT – At the Tampere Bone Bank, all the discarded femoral heads from September 1997 to May 2000 were recultured. The grafts had been washed with pulse lavage at harvesting. 48 grafts had been discarded because of a positive culture and 85 with negative cultures because of positive or insufficient serological information. The femoral heads were split into halves, which were recultured as a whole in thioglycolate broth for 14 days. The contamination of previously culture positive and negative femoral heads did not differ. In only 2 cases did we find the same type of bacteria in the primary as in the new culture. Most of the primary contamination proved to be false positive. The real contamination seems to be very low, at least after pulse lavage washing of the femoral head.

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Allografts entail a risk of bacterial infection, which is by far the largest problem in bone banking (Aspenberg 1998). This risk can be minimized by using various sterilizing methods. However, these methods are complex, expensive and damage the osteogenic properties of the allograft (Ijiri et al. 1994, Fideler et al. 1995, Thoren and Aspenberg 1995, Currey et al. 1997, Aspenberg and Lindqvist 1998, Boyce et al. 1999). In our previous study, we found pulse lavage washing very effective in reducing bacterial contamination of the allograft bone (Hirn et al. 2001). We used a pressure of 6 psi which has no noticeable biological effects on the bone (Bhandari et al. 1999). Since September 1997, all harvested allografts have been washed with pulse lavage in our clinic. Surprisingly, it has not reduced the contamination of the allografts as much as we might have expected from the experimental studies. Therefore, we wanted to determine the real contamination of the washed fresh frozen femoral heads by reculturing the contaminated grafts as a whole. The clean grafts, which were rejected for other reasons, served as controls.

Material and methods

At Tampere University Hospital, femoral heads are harvested from primary hip arthroplasty operations under strict aseptic conditions. Donors are screened for transmissible diseases and urinary or systemic infections. The soft tissue remnants of the graft are removed. The graft is washed with sterile saline. Micro-Aire Pulse Lavage 4740 with an output pressure up to 7 bars (6psi) is used (Hirn et al. 2001). After that, a bacterial culture is taken and the graft packed in a 3-fold plastic bag. During the procedure, the graft is touched only by forceps. Contact of the graft with the glove and the patient's skin are carefully avoided. The graft is stored at –70° to –80 °C.

Bacterial screening is done by taking 3–4 small pieces of bone from the surface of the graft for one culture in thioglycolate broth for 14 days. A sample of the broth is stained with acridine orange. Positive tubes are subcultured on blood and chocolate agar plates under aerobic and anaerobic conditions. Bacteria are then identified using conventional biochemical and immunological methods.

Between September 1997 and May 2000, 923 femoral heads were harvested. The discarding rate was 14%. All the discarded femoral heads were

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Table 1. The bacteria isolated at harvesting from the 48 contaminated femoral heads

Coagulase-negative staphylococci	18
Bacillus sp.	12
Streptococcus viridans-group	5
Micrococcus sp.	4
Propionibacterium sp.	2
Corynebacterium sp.	1
Acinetobacter sp.	1
Staphylococcus aureus	1
Pseudomonas sp.	1
Serratia sp.	1
Rhodococcus sp.	1
Mixture	1

Table 2. The bacteria isolated from the contaminated (96) and the clean (170) femoral head halves in the new cultures. The difference between the groups is not statistically significant (p = 0.7, Fisher's exact test)

Contaminated halves (n 96)	
Coagulase-negative staphylococci	2
Rhodococcus sp.	2
Propionibacterium sp.	1
Clean halves (n 170)	
Coagulase-negative staphylococci	5
Propionibacterium sp.	1
Micrococcus sp.	1

included in the study. Of these, 48 femoral heads (5.2%) had a positive bacterial culture (Table 1), and 85 (9.2%) bacteriologically clean femoral heads were discarded because of positive or insufficient serological information.

In this study, we split the discarded femoral heads into halves. 96 halves were contaminated according to the primary culture at harvesting, and 170 halves verified as clean. Each half was recultured as a whole in thioglycolate broth for 14 days and bacterial screening was done according to the same principles described above.

Contamination with the same bacteria as in the primary and new cultures was considered as real contamination.

Fisher's exact test was used for the statistical analysis.

Results

New positive cultures from both halves of the femoral head were found only in one case with growth of Rhodococcus. In all the others, one other half was clean (Tables 1–2).

A new culture was positive in 4 of the 48 primarily contaminated femoral heads. The figure among the primarily clean femoral heads was 7 of 85.

5 of 96 primarily contaminated halves of femoral heads and 7 (4%) of 170 halves of the clean femoral heads were contaminated on the second culture (p = 0.7, Table 2).

In 2 femoral heads, the same type of organism as in the primary culture was detected in the new culture. The first case was coagulase-negative staphylococcus, which grew only from one half in the new culture. The second case was the previously-mentioned one with Rhodococcus on both halves.

Discussion

Femoral heads harvested from healthy living donors should be sterile inside the body. Slight contamination always occurs during harvesting. Therefore, stored fresh frozen femoral heads can never be considered as sterile products. With proper handling, the bacterial contamination is kept to a level which does not cause clinical infection. It is important that the graft is packed immediately after bacterial screening and touched as little as possible by gloves. Contamination may arise from the operation team, environment or donor (Veen 1994, Deijkers et al. 1997, Sommerville et al. 2000). However, the specimen may also become contaminated in the laboratory during preparation for culture (Farrington et al. 1998).

The contamination percentage has varied between 3–40% depending on the tissue taken and the screening method used (Veen 1994, Deijkers et al. 1997, Sommerville et al. 2000). In one report, the contamination of femoral heads was 22% (Sommerville et al. 2000). The grafts were screened by taking 4 different samples, in the belief that the increase in the number of cultures more accurately assesses the contaminating bioburden of the graft and thereby increases the safety of the bone bank. It is true that the likelihood of detecting contamination increases with the number of cultures (Hirn et

al. 2001). However, at the same time you also get more false positive results. In our series, more than 90% of the positive primary cultures proved to be false positive.

The most important question is the true number of negative grafts and whether they can cause a clinical infection. We have previously shown that even with high levels of contamination, bacterial screening is unreliable and about 25% are false negative (Hirn et al. 2001). Therefore, the most reliable method may be to sterilize all tissues and not only those with a positive primary culture.

According to the literature, the infection rate in revision arthroplasties has been the same whether or not allografts were used (Tomford et al. 1990, Wang and Chen 1997, Hanssen and Rand 1998, Head and Emerson 1999). Sterilizing all tissues seems to be an overreaction. In our series, we found only one real contaminant—i.e, Rhodococcus—which grew on both halves of the femoral head. The case with coagulase-negative staphylococci in the primary culture and on one half in the new culture may also be true contamination. However, we cannot be certain because the genotype of the bacteria was not determined. All of the bacteria encountered were of low virulence and their appearance was similar in both new cultures. The high virulent bacteria seen in the primary cultures could not be detected in the new ones. Moreover, the contamination percentages in the primary and both new cultures were about the same. In our laboratory and with our methods, the contamination seems to remain at about 5% if cultures are taken from "clean" human tissues.

The clinical importance of the low virulent bacteria depends on the amount of the bacteria. Pulse lavage reduces the presence of these contaminants on the surface of the graft and is therefore recommended to improve safety (Hirn et al. 2001). The level of the washing pressure seems to be important. Adherent bacteria may be removed more effectively by higher pressure (70 psi) (Bhandari et al. 1999). However, the higher pressure seems to cause macroscopic damage of the bone (Bhandari et al. 1998, Dirschl et al. 1998). It may also carry surface contaminants deeper intramedullarly (Bhandari et al. 1998). We used a pressure of 6 psi, which damages bone less and causes a real contamination of less than 1%.

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