BACTERIAL CONTAMINATION OF ALLOGRAFTS

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The risk of bacterial infection through allogeneic bone transplantation is one of the problems facing tissue banks. The purpose of this study is to report the contamination rate in 987 grafts obtained under strictly aseptic conditions, between 1989 and 1992. The grafts were stored at -80° C (cortical bone and tendons) and -40° C (cancellous bone).

The overall contamination rate was 6.6%, with Gram-positive bacteriae responsible for 80% of the positive cultures.

We discuss the sources of contamination, the most frequently isolated bacteria and the steps in the donation and transplantation procedures that help to reduce the risk of contamination.

We conclude that the methods of procurement, processing and storage of tissues are effective in making sterile allografts available.

Keywords : bone allograft ; procurement ; freezing ; microbiology.

Mots-clés : allogreffe osseuse ; prélèvement ; congélation ; microbiologie.

INTRODUCTION

The use of allografts in musculoskeletal surgery has become an accepted procedure. There are many ways for harvesting and storing them in tissue banks (1, 2). Considerable care must be taken to avoid the transmission of infectious diseases (1, 2, 3, 4). The sources of contamination can be the donor, or during procurement, storage and implantation (3). It is therefore important to make rigorous test to avoid infection and maintain strict asepsis during all these steps.

The purpose of this study is to report the incidence of bacterial contamination of allografts in our tissue bank.

MATERIAL AND METHODS

Between 1989 and 1992, 987 grafts were obtained under strictly aseptic conditions from patients. The harvested grafts were of three main types : femoral head specimens removed at the time of primary total hip replacement or hemiarthroplasty (n = 655), whole bones or segments of bones (n = 237) and tendon allografts (n = 95) taken from cadaveric donors. Each donor was studied historically, bacteriologically, and serologically and they met the selection criteria of the American Association of Tissue Banks (1, 2). For cadaveric donors, the cause of death and any co-morbid state of the donor were determined at autopsy before implantation of the grafts. All tissues from cadavers were collected in an operating room by orthopaedic surgeons and residents using routine sterile technique. Material for culture was obtained in all tissues from cadavers and patients at the time of harvesting, before the grafts were placed in the tissue bank. A swab was rubbed over the length of each graft. The swab was placed into an Amies transport medium (6) and sent with a piece of each graft for microbiological testing. These samples were inoculated in thioglycollate broth and incubated at 37 degrees Celsius. After 48 hours, a subculture was done using blood agar incubated at 37 degrees Celsius, during 48 hours, in aerobic and anaerobic conditions and afterwards the culture was reported. Cultures of blood from cadavers at the time of procurement were also performed. All grafts were packed in three sterile plastic bags and wrapped in labelled water-proof paper and then stored by freezing in electrical freezers at -40 degrees Celsius (femoral

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heads) and -80 degrees Celsius (whole bones or segments of bones and tendons). The freezers were equipped with an alarm to ensure that the tissues were kept frozen until they were used clinically. An allograft was used only if all the cultures were negative and after the freezing period was completed (minimum 3 weeks). All the contaminated allografts were discarded on receipt of these results. In all the noncontaminated allografts further cultures were done when the grafts were transplantated.

RESULTS

Of the 987 allografts, there were 65 positive cultures (6.6%) : 45 in cancellous bone grafts (6.8% of all the cancellous bone grafts), 18 in cortical grafts (7.6% of all the cortical grafts) and 2 in tendons (2.1% of all the tendons). The most frequently isolated bacteria was *Staphylococcus epidermidis* which represented 47% (30 grafts) of all the cases of contamination, followed by *Streptococcus viridans* in 26% of the cases (17 grafts). If we add the contamination by *Staphylococcus aureus* (7%), Gram-positive bacteria represent 80% of the contamination cases. Other isolated organisms were : *Neisseria sicca* (3.3%), Corynebacterium species (3.3%), *Enterococcus* (3.3%), Bacillus species (3.3%), *Salmonella enteritidis* (3.3%) and *Proteus mirabilis* (3.3%). Only 2 cultures done at the time of implantation from 763 grafts were positive for *Staphylococcus epidermidis*. There was no clinical evidence of infection after 3 years, in the 2 patients who received these grafts. Three blood cultures from 81 cadaveric donors were positive : 2 for *Staphylococcus epidermidis* and 1 for Bacillus species. Of all the bones procured, the femur (12%) and the hemipelvis (20%) were the most frequently contaminated ones.

DISCUSSION

Considering the low number of positive cultures at the time of implantation, bacterial control of the harvested grafts, using the methods that have been advocated by the American Association of Tissue Banks (1, 2) appears to be effective in making sterile grafts available at the time of implantation. When these standards are strictly followed, the risk of transmitting bacterial diseases to the recipient is significantly diminished.

As reported by other authors (5, 8, 9), as much as 10% of the harvested bone is discarded, due to demonstrable bacterial contamination that originated from the donor or was incurred during the procurement, preservation, or storage of the graft. Delloye *et al.* (4) reported an overall contamination rate of 12.2% of sterile-procured bone. Our lower percentage of overall contamination could be related to the low number of positive blood cultures in the donors, or to the sensitivity of the bacterial contamination screening methods.

The rate of contamination is higher in whole bones, especially femur and hemipelvis, because the handling of these grafts is greater and the time of procurement is longer. It is useful to avoid delay between sampling and culture and it is also important to pack and place each graft in the bank immediately after deperiostization and swabbing, rather than at the end of the operation.

The most frequently isolated bacteria are Gram-positive organisms as reported by others (3, 4, 9). Since the major source of contamination is the skin, efforts should be made to sterilize it. Skin preparation with a 2% iodine solution and drapping in addition to the use of two pairs of gloves and periodic glove changes appears to be a valuable aid in diminishing bone contamination.

Although some authors (3) gamma-irradiate allografts with positive cultures we prefer to discard them because it is known that it exposes the bone to mechanical weakening (7).

Clinically, it seems that in most cases of infection after the use of massive allografts there is no evidence that the source was the graft (9). Tomford *et al.* (9), however, reported 3 cases of infections in patients in whom bones from the same cadaver were used and one possible explanation for the infections was the contamination at the time of procurement and failure of swab cultures to detect the contaminating organisms.

To avoid this problem some authors (10) have recently proposed the rinsing of bones in a sterile solution followed by aerobic and anaerobic incubation of the solution. They have proved in an experimental model that this is a better screening

method for bacterial contamination than the conventional swab.

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SAMENVATTING

R. H. BARRIOS, M. LEYES, S. AMILLO, C. OTEIZA. Bacteriële contaminatie van allogene botenten.

Het risico van bacteriële contaminatie via allogene bottransplantatie is een zeer belangrijk probleem voor de botbanken. Het doel van deze studie is de analyse van het aantal contaminaties bij 987 enten, die onder strenge aseptische techniek gepreleveerd werden tussen 1989 en 1992. Al de enten werden steriel bewaard op -80° C (cortex en pezen) en op -40° C (spongieus bot).
Het algemeen contaminatie percentage beliep 6,6%, met in 80% van de positieve culturen vaststelling van Gram-positieve bacteria.
De contaminatiebronnen, de meest frekwente geïsoleerde bacteria en de technieken bij het preleveren en het transplanteren worden beschreven, met het oog op een vermindering van het risico voor contaminatie.
De auteurs konkluderen dat de technieken voor preleveren, manipulereen en stockeren van de verschillende weefsels doelmatig zijn om steriele allogene enten te bekomen.

RÉSUMÉ

R. H. BARRIOS, M. LEYES, S. AMILLO, C. OTEIZA. Contamination bactérienne des allogreffes osseuses.

Le risque de contamination bactérienne par transplant d'os allogène est un des plus importants problèmes auxquels sont confrontées les banques d'os. Le but de cette étude est l'analyse du taux de contamination pour 987 greffes, prélevées dans des conditions strictement aseptiques entre 1989 et 1992. Les greffes furent conservées à -80° C (os cortical et tendons) et à 40° C (os spongieux).
Le taux de contamination général était de 6,6% ; des bactéries Gram-positives étaient présentes dans 80% de cultures positives.
Les auteurs discutent des différentes sources possibles de contamination, des bactéries isolées le plus fréquemment et des différentes étapes dans le prélèvement et la transplantation en s'attachant aux procédés que permettent de réduire le risque de contamination.
Les auteurs concluent à l'efficacité des méthodes de prélèvemet, de manipulation et de stockage pour la préparation des allogreffes.