Value of Screening Procedures in Femoral Head Procurement for Allograft Transplantation : The National University of Singapore Bone Bank Experience

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ABSTRACT : Eighty patients undergoing hemi-arthroplasty for fracture of the neck of the femur during September 1989 to March 1991 were studied regarding their suitability as donors of femoral heads for the National University of Singapore Bone Bank. Donor history alone revealed medical contraindications in sixteen patients; four had old pulmonary tuberculosis, two bronchopneumonia, one urinary tract infection, two bedsore, one chronic otitis media, one infected fracture, three malignancy, one rheumatoid arthritis and one systemic lupus erythematosus. Laboratory investigations performed in the remaining sixty-four patients showed that further nine femoral heads were unsuitable and discarded (three were positive for syphilis, two for hepatitis and four had positive bacteriologic cultures). Of the four femoral heads positive on bacteriologic culture, three grew skin organisms. Thus out of eighty patients, only fifty-five; the femoral heads were suitable for use (69%). This study showed that strict screening procedures were vital to prevent the transmission of diseases to recipients. Bacteriologic tests performed on twenty-six femoral heads used for allograft transplantation in twenty recipients at the time of implantation showed negative cultures, and no infection has occurred in any recipient so far.

INTRODUCTION

"Many hospitals have household refrigerators or freezers containing small quantities of allografts removed in the course of routine surgical procedures that are used for subsequent transplantation needs limited to that institution. This practice is not only inadequate to ensure reliable preservation but it is potentially hazardous because unexpected pathogenic factors of diseases may be transferred with the graft."[1].

Such practices are performed in some institutions in Singapore. The bone material most readily available for the allograft is the femoral head removed in the course of a Moore's or Thompson's arthroplasty performed for patients with fractured neck of femur. The femoral heads are stored in ordinary freezers at temperatures of -10°C or warmer. No proper screening procedures are performed. To promote the use of allograft transplantation in Singapore, the National University of Singapore (NUS) Bone Bank was started by the author in October 1988[2] using proper banking facilities and strict donor screening procedures to minimise the risk of transmitting dangerous diseases.

The NUS Bone Bank Protocol follows the American Multi-Centre Protocol with Massachussets General Hospital Bone Bank as the Central Registry[3]. It strictly follows the guidelines for banking of musculo-skeletal tissues set up by the American Association of Tissue Banks.[4]

MATERIALS AND METHODS

Study Population

Eighty patients above the age of 60 undergoing hemi-arthroplasty for fracture of the neck of femur of Garden's Stage III or IV[6] during September 1989 to March 1991 were studied regarding their suitability as donors for femoral heads.

Screening Procedures

Detailed Medical History : A detailed medical history is taken to exclude presence of active systemic infection, active infection involving the bone to be procured, active or past history of slow- virus disease, history of malignancy, history or presence of active hepatitis or unexplained jaundice, history of diffuse connective tissue disease, metabolic bone disease, chronic drug abuse, or heavy radiation to the bone to be procured, or of toxic substance or steroid use for more than five to seven days.[4,6,7,8] This history is taken by the research assistant of the NUS Bone Bank verified by that taken by our house-officers or residents. In cases of doubt, decision as to its suitability is made by the author. A positive history of any of these diseases or conditions is a contraindication to bone donation.

Clinical Examination : A thorough physical examination is also conducted by the house-officers or residents to

exclude any malignancy or infection present in any part of the body.

Blood Investigations : If history and clinical examination do not reveal any contraindication, blood investigations are performed. These include:-

* HIV-tested by Enzyme Immunoassay (EIA)
* Hepatitis Screen-testing for HBs Ag, HBe Ag, Anti-HBs Ag and Anti-HBe Ag. The markers are also tested by Enzyme Immunoassay (EIA)
* Rapid Plasma Reagin (RPR) to screen for Syphilis using a rapid agglutination test. RPR reactive sera is tested for TPHA (Treponema Pallidum Haemagglutination)[4,7,8]
* Blood Grouping of Patient-performed in preparation for the operation
* Tissue Typing-originally performed for HLA (A,B and DR). Subsequently, tissue typing is not done.

Culture and Sensitivity Test : At the time of the hemiarthroplasty, a piece of bone taken from the femoral neck was sent for culture and sensitivity[4,7,8] for both aerobic and anaerobic organisms. A piece of the bone tissue itself was sampled in preference to a swab from the bone tissue.

A similar sample is taken and sent for the culture and the sensitivity from all femoral heads used in recipients at the time of implantation.

Chart Review

This is also performed to exclude unexplained fevers, increasing white cell counts and increasing ESR.

Procedure for Procurement of Femoral Head : The method employed was the standard sterile, double, jar technique.[7,9] The femoral head removed from the donor was passed to the procurer, a surgeon who is a part of the NUS Bone Bank team. All soft tissues were removed from the femoral head including the ligamentum teres. The bone was then flushed with 1 to 2 liters of normal saline until no more fat globules were seen in the returning fluid and until the fluid became clear. At this point, a bone rongeur was used to take a sample of bone from the neck of the femur for bacteriologic tests. Once the culture sample had been taken, the femoral head was soaked in a solution of the normal saline containing Ampicillin and Cloxacillin for about 20 to 30 minutes.

The femoral head was then placed in an inner, sterile, red cap plastic bottle which was in turn put inside a bigger, outer sterile glass jar which was sealed and then carefully labelled for proper identification.

The specimens in their jars were then stored in their Revco- Freezer in the Operating Room Complex at a cabinet temperature of-80°C.

In the majority of cases, the femoral head was stored with its intact articular cartilage (Fig.1). In some cases (six femoral heads), the articular cartilage was removed and the head osteotomised into small, convenient pieces ready for use.

Documentation: This is extremely vital. A proforma was used for all patients with Grade III and IV fracture neck of femur. All data are computerised.

Twenty-six femoral heads were subsequently used as allografts in twenty patients; in 12 for spinal fusion, in 3 for subtalar fusion, in 2 following curettage of giant cell tumours, in 2 for revision hip surgery and in 1 for augmenting a hypoplastic mandible.

RESULTS

Of the eighty patients with the femoral neck fracture, screening by the detailed medical history and thorough the clinical examination revealed that sixteen patients were not suitable as donors (Table 1). Blood tests were not performed on these patients and their femoral heads were not collected.

Table 1
Medical Contraindications

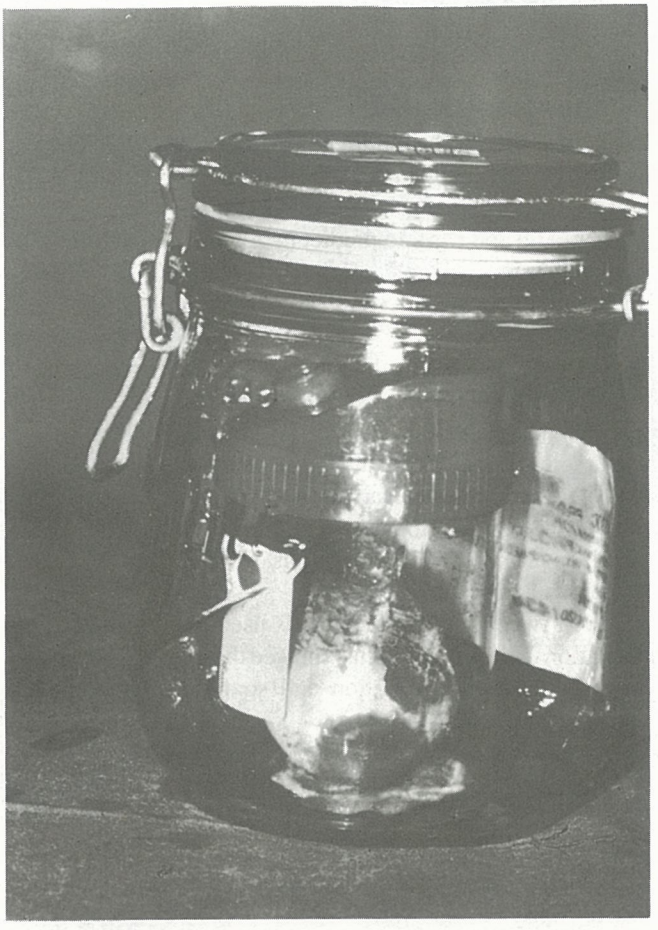
Medical Conditions	No. Femoral Heads
Old Pulmonary Tuberculosis	4
Bronchopneumonia	1
Bronchopneumonia, Herpes Zoster	1
Urinary Tract Infection	1
Bedsore	2
Chronic Otitis Media	1
Thyroid Malignancy	1
Carcinoma Rectum	1
Pathological Fracture, Breast Cancer	1
Infected Fracture	1
Rheumatoid Arthritis	1
Systemic Lupus Erythematosus	1

Blood and bactaeriologic tests were performed in the remaining sixty-four patients and their femoral heads were collected. The results of laboratory investigations revealed that further nine femoral heads were not suitable for use and had to be subsequently discarded (Table 2).

Table 2
Contraindications due to positive laboratory test

Positive Laboratory Test	No. Femoral Heads
Syphilis	3
Hepatitis B	2
Positive Culture	4

In four instances, the femoral heads procured were contaminated. In the femoral heads contaminated, three were



due to skin organisms. In two cases, the organism was being Staphylococcus epidermidis (on enrichment) and in one case, Staphylococcus aureus (on enrichment). The remaining contamination was Bacteroides fragilis.

Thus, out of eighty patients, only fifty-five femoral heads were found to be safe for use (69%).

The culture and the sensitivity of bone samples from the twenty- six femoral heads used for allograft transplantation in twenty recipients revealed no growth on culture media.

DISCUSSION

The donor selection represents the single most important step in providing safe effective use of human tissue for transplantation[7].

Another key element in the safety and effectiveness of a bone bank is supervision by an experienced doctor. A medical director must be available to ensure quality control and to resolve questions regarding the appropriateness of any specific graft[9]. In the NUS Bone Bank, this role is played by the first author.

One very important reason for setting up a bone bank may be the fact that the donor history alone is inadequate to ensure a safe graft[9]. Further screening tests are indicated. In Hart et al series, [9] 79 out of 180 grafts procured were discarded (44%) because they were unable to meet the established criteria.[4,6,7,8] However,in our series, the discard rate is lower, nine out of sixty-four femoral heads (14%) could not meet established criteria.[4,6,7,8]

As for our screening tests, tissue typing for HLA antigens has been dropped from our protocol because the preponderance of evidence suggests that freezing of bones at temperatures of-70°C or colder decreases allograft antigenicity.[9] Whilst fresh bone allografts are strongly antigenic and whilst freezing coupled with drying greatly diminishes the antigenicity of bone, deep-freezing (without drying) causes a decrease in antigenicity intermediate between that of fresh allografts and those that have been freeze-dried. Recently, most workers have considered deep-frozen bone to be almost non-immunogenic.

The bacterial contamination is a major problem besides the transfer of viral or malignant disease. Many pathogens can survive the freezing or even freezing and drying and retain the capacity to produce clinical infection in the recipient. Whilst this problem is of limited significance to the kidney recipient where the transplanted tissue is viable and suffused with blood and antibiotics, it is a very serious problem to the individual receiving a "dead" allograft containing viable bacteria.[7]

In our series, four femoral heads were contaminated. In three cases, the organism was cultured only on enrichment medium, implying that the numbers of organism present were too few to be detected by primary cultures. This is a picture usually obtained with contaminants. Indeed, the organisms in these three cases were skin organisms, two being Staphylococcus epidermidis and one Staphylococcus aureus.

The experience by others have also shown that most of the organisms cultured from bones at time of procurement are skin contaminants. Of 91 bones procured in 1979 in Massachussetts General Hospital,[6] 31 (32%) were contaminated with either Staphylococcus epidermidis alone or a combination of Staphylococcus and other skin contaminants like Proprionibacterium, Peptostreptococcus, Lactobacillus and Micrococcus. Of these, 21 (62%) grew Staphylococcus epidermidis only in thioglycollate broth[8], This organism is found on the skin of healthy subjects and is considered as a part of normal flora. But recently, this organism has attracted increasing attention among orthopaedic surgeons because of its association with infected joint replacements.[12] The organism has also been shown to produce infection with neurosurgical shunts[13] and cardiovascular valves.[14]

However, in a study on the freeze-dried allograft bone performed at the US Navy Tissue Bank,[15] out of 11 infections proven in a series of 303 cases, only one case was due to Staphylococcus epidermidis. The remainder were Staphylococcus aureus or a mixture of gram negative organisms. This suggests that skin organisms cultured on procured bone may not be important.

The NUS Protocol like others laid great emphasis on

maintaining bacteriologic sterility of the allografts during procurement, storage in the bank and preparation for implantation. In our protocol, any organism cultured including skin organism, even on enrichment, is considered to be significant and the bone discarded from use.

Antibiotics are not routinely used during bone procurement because their usage may make the interpretation of the graft cultures to be difficult and preclude the use of grafts in patients sensitive to these drugs.[7] The NUS Bone Bank Protocol uses the antibiotics routinely but makes sure that the specimen for culture is taken before soaking the bone in Ampicillin/Cloxacillin solution.

It is also important to check quality control on bone sterility. There are two alternatives. One is to culture a percentage of the stored bones at regular intervals. The other is to culture the bones at the time of implantation. The disadvantage of the first alternative is the possibility of contaminating them. The disadvantage of culturing at implantations that the contamination would not be revealed until the bone had been implanted. The NUS Protocol adopts the second alternative because of the low contamination rate at the time of the procurement.

Boston experience has shown that our emphasis on bacteriologic sterility is indeed justified. They reported a series of seven patients who developed infections in the allografts and required either resection of the graft (four patients, two receiving a second allograft) or amputation (three patients).[16]

Fortunately, the incidence of infection in allograft transplantation is low. The US Navy Tissue Bank experience [15] revealed an incidence of only 11 proven infections in 303 cases of the freeze-dried allograft bone ie. 3.6%. This compares favourably with a report by Cruse[17] in which there was 3.9% incidence of the infection in 986 fresh autografting procedures.

Over the last two years, twenty operations have been performed using twenty-six femoral heads procured by the NUS Bone Bank. The cultures from all these heads taken at the time of the implantation were all negative. Furthermore, so far,no infection has occurred in any of these twenty recipients, although it is too early to predict the long term outcome.

CONCLUSION

The donor history alone was found to be inadequate to exclude unsuitable donors. The laboratory investigations revealed that further nine out of sixty-four patients were also unsuitable for bone procurement and the femoral heads collected had to be discarded in these nine patients. This showed that the additional screening procedures were vital to avoid the transmission of dangerous diseases from the donors to the recipients.

All twenty-six femoral heads used for the bone transplantation in twenty recipients showed the negative cultures at the time of the implantation and so far no infection has occurred in any recipient.

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