From November 1994 to March 1997, we harvested 137 grafts of the femoral head from 125 patients for donation during total hip arthroplasty according to the guidelines of the American Associations of Tissue Banks (AATB) and the European Association of Musculo-Skeletal transplantation (EAMST). In addition to the standards recommended by these authorities, we performed histopathological examination of a core biopsy of the retrieved bone allograft and of the synovium.

Of the 137 allografts, 48 (35.0%) fulfilled all criteria and were free for donation; 31 (22.6%) were not regarded as suitable for transplantation because the serological retests at six months were not yet complete and 58 (42.3%) were discarded because of incomplete data. Of those discarded, five showed abnormal histopathological findings; three were highly suspicious of low-grade B-cell lymphoma, one of monoclonal plasmacytosis and the other of non-specific inflammation of bone marrow. However, according to the standards of the AATB or EAMST they all met the criteria and were eligible for transplantation.

Our findings indicate that the incidence of abnormal histopathology in these retrieved allografts was 3.6%. Since it is essential to confirm the quality of donor bones in bone banking, we advise that histopathological screening of donor bone should be performed to exclude abnormal allografts.

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The use of cancellous and/or corticocancellous allografts has become particularly common in extensive revision of failed total hip arthroplasties. Usually, frozen bone grafts from femoral heads are used. A safe bone bank is of the utmost importance. In 1984 the Musculo-skeletal Council of the American Associations of Tissue Banks (AATB) published standards for the banking of musculoskeletal tissues. Other national or supranational organisations such as the European Association of Musculo-skeletal Transplantation (EAMST) have produced similar guidelines. Since their introduction, several updates have been proposed to anticipate new clinical and epidemiological findings and improved analytical techniques.

Despite the high standards of screening tests and procurement procedures for donors, there have been several reports of the development of infectious diseases due to the transplantation of contaminated musculoskeletal allografts. Not only is there a danger of infection, but viruses such as the hepatitis B (HBV), human immunodeficiency (HIV), human T-cell leukaemia-lymphoma (HTLV1), and others may have a role in oncogenesis. Thus, the histopathological examination of retrieved allografts may be important. It is clear that allografts containing malignancies or morphologically suspect tissues should be discarded. Contaminated or pathologically abnormal tissue which may be potentially dangerous should not be used.

We have undertaken a prospective study to screen retrieved allografts histopathologically, in addition to using the standards recommended by the AATB and EAMST, in order to confirm the safety of the grafting and the quality of the donated bones. We also determined the incidence of abnormal histopathological findings and assessed the effectiveness of routine histopathological examination in bone banking.

Patients and Methods

From November 1994 to March 1997, we obtained 137 femoral heads removed at the time of primary total hip replacement from 125 patients (17 men, 108 women) according to the guidelines of the AATB and EAMST. The mean age of the donors was 69.4 years (44 to 88) with an age distribution as shown in Figure 1. All gave written and signed informed consent.
They were first screened by a questionnaire in regard to their medical, social and sexual history. The questionnaire was based on pre-existing forms, followed the guidelines of the AATB and EAMST, and was written in the national language; the patients were interviewed by a doctor. In addition, a thorough physical and routine blood examination was performed. Blood was collected to determine the blood group and Rhesus factor and screening tests were performed to exclude syphilis, HBV and hepatitis C virus (HCV), cytomegalovirus (CMV), HIV1 and 2 and HTLV type 1. An increased ESR was also used as a criterion for exclusion. All donors were retested for HIV1 and 2, syphilis, HBV, HCV and HTLV1 six months after the donation taking into account the negative window period.\textsuperscript{17,18}

After resection of the femoral heads, swabs from the corticocancellous bone and a part of the capsule were taken for aerobic and anaerobic cultures. They were incubated in Stuart’s medium and subcultured on to blood agar and culture broth for at least five to seven days. In addition, a core biopsy specimen ($1\,\text{cm}^3$) was taken from the femoral head and a part of the synovium was retrieved, fixed in neutral buffered formaldehyde and sent for histopathological investigation. The synovial material and decalcified bone samples were completely embedded in two or three paraffin blocks and, from each block, only one section was prepared and stained with haematoxylin and eosin. These stained sections were adequate in all cases. Only when immunohistochemistry was considered necessary were more blank sections cut from the blocks for further study. The grafts were packed in sterile gauze, secured in double plastic sacks and stored at $-80^\circ\text{C}$. All the data were entered on a database and also as hard copy. After this information had been collected and approved by the bone-bank co-ordinator and an authorised physician, the donor graft was rejected or approved for donation.

Results

Of the 137 grafts, 48 (35\%) were deemed eligible for donation and have been already used, mainly in revision arthroplasty. They had been examined microscopically, showed no histological abnormality and fulfilled other screening criteria. Of the remainder, 31 (23\%) have not yet been released for transplantation because not all the serological retests six months after donation are available. These allografts have been examined histologically and are suitable for use. Fifty-eight (42\%) allografts were discarded, 45 because of either a positive donor history (questionnaire), an increased ESR, or positive serological, histological or bacterial tests; in two, malignant disease was diagnosed within six months of donation (Table I). Thirteen grafts have been rejected because of incomplete information from the screening tests.

Five allografts were excluded based solely on the histopathological findings (Tables I and II). No histological abnormality was observed in the synovial tissue and this examination seems to be of little value for screening. Three (cases 1, 2 and 3) showed aggregates of small lymphocytes in the bone marrow, many of them located next to bone trabeculae. Immunohistochemistry indicated that they were composed of B-cells (CD20-positive) (Figs 2a to 2c). Light chains were not detected in these cells. According to these abnormal histopathological findings, they were suspected of being low-grade B-cell non-Hodgkin lymphoma. Another (case 4, Table II) showed scattered infiltration of bone marrow by a moderate number of monoclonal plasma cells (lambda positive, kappa negative) (Figs 3a to 3c). In the absence of manifest bone lesions on pre- and post-operative radiological examination of the skeleton and with a normal bone scan, normal serum protein electrophoresis,

\begin{table}[h]
\centering
\caption{Details of the 58 allografts which were discarded}
\begin{tabular}{|l|}
\hline
Positive screening tests & 45 \\
Questionnaire & 7 \\
ESR & 13 \\
CMV test positive & 10 \\
HBV or HCV test positive & 3 \\
Biopsy & 5 \\
Culture & 5 \\
Other & 2 \\
Incomplete screening tests & 13 \\
Total & 58 \\
\hline
\end{tabular}
\end{table}
and negative urine samples for monoclonal light chains, this lesion was diagnosed as monoclonal plasmacytosis. The fifth (case 5, Table II) showed features of a non-specific inflammatory infiltration: the bone marrow was fatty and partly fibrotic with a diffuse infiltration of polyclonal plasma cells and neutrophilic granulocytes. The haematopoietic pattern of the bone marrow was abnormal. The histopathological findings were compatible with a non-specific, acute and chronic inflammatory infiltration of the bone marrow and possibly of osteomyelitis. Cultures of swabs from this graft were negative and only the histopathological findings suggested that it should be excluded.

After establishing the diagnosis, the patients suspected of having malignant haematopoietic disease (cases 1 to 4) were referred to a haematologist-oncologist for further examination; none showed any evidence of systemic disease. The history and clinical examination were negative and abdominal ultrasound showed no abnormal pathology of the internal organs or of the lymph nodes. Repeated bone scans were negative, a biopsy of the iliac-crest bone marrow was normal and the cytology was negative. Blood smears were negative and repeated routine and additional blood tests were normal at the latest follow-up.

None of these donors had a history of malignancy or any symptoms at physical examination indicating an underlying disease. From the plain radiographs of the pelvis and hips, and the clinical findings, it was impossible to diagnose these diseases and they were treated as having osteoarthritis.
of the hip. During the procurement of the femoral head, there were no suspicious macroscopic features and the deformity of the femoral head was that typically seen in osteoarthritis of the hip. According to the guidelines of the AATB or EAMST, all five met the criteria set by these organisations and their femoral heads were eligible for donation. All five patients have no sign of systemic disease at the latest follow-up (Table II).

Discussion

Bone is probably the most frequently transplanted non-haematogenous tissue and is usually used in elective surgical procedures. The safety and quality of the graft are therefore of the utmost importance. The selection of donors, the harvesting, preparing, and storing of the retrieved allografts, and the proper selection of the recipients should be performed according to strict guidelines which are adequately documented and controllable.

Several procedures have been developed to ascertain the safety and quality of the graft. After harvesting, bone grafts are usually sterilised by gamma irradiation to avoid any potential contamination. This method, however, weakens the graft substantially and reduces its osteoinductive capacity by destroying or altering the three-dimensional structure of the bone and the inductive factors. Autoclaving or boiling damages the graft, while sterilisation by ethylene oxide is not a popular alternative because residual gases remain within the graft and cause irritation.

In addition to the standard procedure recommended by the AATB and EAMST, we performed routine histopathological screening of the graft. The incidence of abnormal histopathological findings was 3.6%, much higher than in other studies. Three of the five rejected grafts (2.2% of the screened cases) had abnormalities which possibly indicated malignancy (low-grade B-cell non-Hodgkin lymphoma). There are few comparable studies and the collected data vary. Reviewing the results of the South Australian musculoskeletal bank, Campbell and Oakeshott showed an incidence of rejection of 1.3% (31 exclusions out of 2361 bone grafts) because of histopathological screening alone. The most common reason for rejection findings was a non-specific inflammatory lesion, but others included undiagnosed Paget’s disease and undiagnosed lymphoma. The grafts, however, were collected from living and cadaver donors and there were different sites of retrieval, and therefore these findings are difficult to compare with our data. In another retrospective study six of 715 (0.84%)
consecutive cases of retrieved material during total joint arthroplasty (283 hips and 432 knees) showed pathological findings which had not been noted during preoperative screening or during the operation. In the total hip group, three out of 283 retrieved specimens (1.1%) showed pathological findings: one had lymphoid hyperplasia, one well-differentiated plasma-cell proliferation and one pannus overgrowth. In the knee group, three out of 432 specimens (0.7%) showed pathological findings: one had atypical lymphoid aggregates in the bone marrow and two others pannus formation most likely associated with rheumatoid arthritis. Although this study is comparable to ours, the mean age of the patients in the hip group differs (mean age of women 61.2 years, men 66.1 years) from that in our group (mean age 69.1 years). Since the incidence of cancer, especially lymphoproliferative diseases, increases with ageing, this may partially explain the different results.\(^\text{29}\)

Non-Hodgkin lymphoma (NHL) is particularly common in elderly patients: more than half of the cases in The Netherlands are in patients aged 65 years or over.\(^\text{30}\) The rates of incidence of these lymphoproliferative diseases increase with age except in the very young.\(^\text{31}\) Although there are no definitive explanations for this phenomenon, recent reports have shown that blood transfusion may be a risk factor for developing NHL.\(^\text{32,33}\) Allogenic bone grafting may have the same potential risk.

In our study all three lesions suspected as being low-grade NHL showed multiple aggregates which were both paratrabecular and non-paratrabecular (Figs 2b and 2c). The aggregates contained monomorphic lymphocytes. Additional immunohistochemical investigations showed that they originated from B-cells. Accordingly, these lymphoid aggregates were judged not to be benign, and the lesions were classified as possibly indicating low-grade NHL, given the clinical status. Recently, we have found another suspected case of low-grade B-cell NHL (case 6, Table II) in a patient who had a total hip arthroplasty. Although this study is comparable to ours, the mean age of the patients in the hip group differs (mean age of women 61.2 years, men 66.1 years) from that in our group (mean age 69.1 years). Since the incidence of cancer, especially lymphoproliferative diseases, increases with ageing, this may partially explain the different results.\(^\text{29}\)

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