Massive deep-frozen bone allografts : contamination, immunogenicity and clinical use

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Chapter 7

Summary and conclusions

This thesis addresses a number of problems and controversies regarding the use of massive deep-frozen bone allografts. The studies described in this thesis involve topics on bacterial contamination (chapter 2), immunogenicity (chapter 3) and clinical use of massive deep-frozen bone allografts (chapters 4, 5, 6).

In *Chapter 1*, a short history of the transplantation of massive frozen bone allograft is presented, and the principal advantages and disadvantages of these transplantations are discussed. Despite the long-term use and worldwide studies, questions and controversies around the use of massive deep-frozen bone allografts still exist. A number of problems and controversies are specified and presented.

Based on these specified problems and controversies, the aim of this thesis was defined and the studies that aim to solve these problems and controversies were outlined. One study addressed the bacterial contamination of allografts retrieved from postmortal donors, another one addressed the immunogenicity of transplanted allografts, and three studies addressed the indications and operative techniques of three less common types of allograft reconstructions.

In *Chapter 2* , we analysed the bacterial contamination of 1999 bone allografts retrieved from 200 cadaver donors under sterile conditions. A multivariate analysis using a multiple logistic regression model was performed to find potential risk factors for contamination. Identifying these risk factors helped to find methods to decrease the rate of contamination and increase the availability and safety of the allografts. Furthermore, the effectiveness of rinsing the allografts was assessed.

Organisms of low pathogenicity were cultured from 50% of the grafts and of high pathogenicity from 3%. The risk of contamination with low-pathogenic organisms (mainly skin commensals) increased by a factor of 1.6 for each extra member added to the procurement team. The risk of contamination with high-pathogenic organisms (mainly contaminants of the gastrointestinal tract) was 3.4 times higher in donors with a traumatic cause of death and 5.2 times higher in those with positive blood contamination. Preceding organ procurement did not significantly influence the risk of contamination. Rinsing the graft with an antibiotic solution was not an effective decontamination method.

In *conclusion*, despite careful donor selection and aseptic tissue procurement, contamination is frequent. The major source of contamination is exogenous (low-pathogenic skin commensals) and is strongly influenced by the procurement team. Contamination from endogenous sources (high-pathogenic contaminants of the gastrointestinal tract) can be controlled by donor selection. Exclusion of donors with severe trauma in highly colonised areas can diminish the endogenous contamination.

In *Chapter 3,* we analysed the cellular immune response in ten transplantations of massive frozen bone allografts. The frequency of circulating cytotoxic T lymphocytes (CTL) and T helper lymphocytes (TH) directed against mismatched donor antigens were determined by limiting dilution analysis (LDA) assays. The affinity of donor-specific CTL against the mismatched donor antigens was determined on the basis of their resistance in vitro to anti-CD8.

CTL and TH against mismatched donor antigens were found in the peripheral blood of all patients. More importantly, and reported for the first time, CTL with high affinity for donor antigens were found in five patients. These high-affinity CTL are the result of in vivo activation by donor-specific HLA antigens. They do not need CD8 molecules to stabilise the antigen and are strongly associated with rejection of heart and corneal transplant. Even after the removal of most bone-marrow cells, we found high-affinity CTL and high TH frequencies. This T-cell response could be detected over a period of years.

In *conclusion*, massive frozen bone allografts can induce high-affinity donor-specific CTL. The present assay allows qualification and quantification of the levels of CTL and TH in the blood. Since the chronic rejection of bone allografts is considered to be primarily mediated by T cells, with a key role for high-affinity CTL and TH, our approach may be helpful in establishing the effect of the immune response on the incorporation of bone grafts.

In *Chapter 4*, we evaluated the medium-term clinical results of hemicortical procedures for selected cases of low-grade malignant bone tumours. Low-grade tumours arising in or near the cortex can, theoretically, be resected while maintaining a part of the cortical circumference. These so-called hemicortical resections can ideally be reconstructed with an inlay

allograft. The oncological and allograft outcomes were analysed to deter - mine the efficacy and safety of this technique.

The 22 consecutive hemicortical procedures for low-grade parosteal osteosarcomas, peripheral (secondary) chondrosarcomas, and adamanti - nomas showed good oncological and allograft outcomes. There was no evidence of local recurrence and distant metastasis at a mean follow-up of 64 months (27 to 135). All allografts incorporated completely and there were no allograft fractures or infections. Fracture of the host's remaining cortex occurred in six patients, all before incorporation of the graft was present. They were managed successfully by cast or osteosynthesis. The functional results were excellent or good in all except one patient and compared favourable to larger intercalary procedures.

In *conclusion*, hemicortical procedures for selected cases of low-grade surface tumours give excellent oncological and allograft outcomes. Wide resection margins can be achieved, even when the medulla is involved. Precise preoperative planning using MRI is essential to define the margins of resection. Although the hemicortical procedure is technically demand - ing, gratifying clinical results make it clearly worthwhile for selected patients.

In *Chapter 5*, we compared the outcomes of 14 epi-diaphyseal interca - lary reconstructions for malignant bone tumours around the knee to 9 meta-diaphyseal and 12 diaphyseal intercalary reconstructions of femur and tibia. In these epi-diaphyseal intercalary reconstructions 1 to 2 cm of the epiphysis with adherent joint was left in place, preservation of the joint avoids the need for joint reconstruction with associated problems. Furthermore, we analysed risk factors for graft failure and complications.

All epiphyseal osteotomies had tumour-free margins and no local recurrences. Kaplan-Meier analysis showed a 10-year survival rate of the epi-diaphyseal reconstructions of 78%, which differed not significantly from the 89% of the meta-diaphyseal and the 75% of the diaphyseal recon - structions. Epi-diaphyseal complications included two infections, five fractures and three nonunion treatments. Complications for all 35 grafts included three infections, twelve fractures and nine nonunion treatments. Ultimately six of all grafts failed, infection and length of resections were significant predisposing factors. Each type of intercalary reconstruction showed good functional results with mean MSTS scores between 23 and 24.

In *conclusion*, as the epi-diaphyseal reconstruction bypasses complica - tions associated with joint reconstruction and results are comparable to other types of intercalary allografts, this type of reconstruction should be considered if at least 1 cm of tumour-free juxta-articular bone can be maintained. A pitfall of this reconstruction is the difficult fixation of the epiphyseal graft-host junction. Length of the reconstruction correlated negatively with survival of the intercalary allografts.

In *Chapter 6*, the medium- to long-term results of 20 allograft-prosthesis composite reconstructions of the proximal femur were evaluated. Contrary to others, our technique comprised press-fit fixation of a long-stem pros - thesis in both allograft and host bone. The salvaged gluteal tendons were sutured to the corresponding tendons on the graft and a bipolar cup was used when possible. The results were compared with the reported results of other composite techniques and megaprosthesis.

Good hip stability and long-term function were found after the recon- structions. No dislocations of the bipolar cup occurred. Four reconstruc - tions failed, and they were caused by stem loosening or stem fracture. In one of these a fracture of the graft also occurred. The Kaplan-Meier analysis showed a 10-year survival rate of 64% for the stem and 87% for the graft. The stem failures could be attributed to the use of an undersized graft, a small diameter stem and resultant inadequate press-fit fixation of the stem in the host bone. Once loosening of the stem in the host bone had developed, loosening in the graft followed within four years. The revi- sions were relatively simple as the non-fractured grafts could be left in place.

In *conclusion*, the used abductor reinsertion technique proved to be simple and effective. Although survival of the graft was good, revision of the stem was frequent when compared to other composite techniques. We considered the quality of the press-fit fixation of the stem in the host, more than the use of cement in the graft, the key determinant of longevity. However, a stem fixated into the graft without cement is likely to subside once loosening of the stem in the host has developed. The pitfall of our technique is the difficulty of getting adequate press-fit fixation of the stem in both graft and host bone.