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

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Chapter 1

Introduction and aim of the thesis

INTRODUCTION

Increased number of limb-salvage operations for malignant bone tumours

In the last decades, the number of limb-saving resections of bone tumours has increased due to advances in chemotherapy and diagnostic imaging. Reconstruction of large bone and osteoarticular defects remains a challenge. The reconstruction can either be performed using a mega - prosthesis, a massive bone allograft, or an allograft-prosthesis composite. The use of allografts represents a well-accepted and useful alternative and may even be preferred in specific cases.

History of massive bone allograft transplantation

The first well-documented transplantation of allogenic massive bone was reported by Macewen[1] in 1881. He successfully reconstructed a destructed humerus of a child with the use of a tibia from a just deceased boy. However, it was not until the reports of Lexer [2] in the nineteen twenties that orthopaedic surgeons became intrigued by the use of bone allograft in the reconstruction of large bone defects. Due to the high percentage of failures, however, the use of bone allografts remained limited. In the nineteen sixties, after the discovery that deep-freezing of the allografts prior to implantation could diminish the adverse immunogenic reaction, varying degrees of success of allograft implantations were reported by Parrish[3] in the United States, Volkov [4] in the USSR and Ottolenghi [5] in Argentina. The development of better techniques for osteosynthesis also contributed to these improved clinical results. The successful long-term reports by Mankin [6,7] dating from the nineteen eighties until today have been a stimulus for many orthopaedic surgeons to use bone allografts. The development of modern bone banks and their ability to supply safe and effective massive deep-frozen bone allografts has facilitated the increased use of these allografts. [8]

In 1988, the Orthopaedic Department of the Leiden University Medical Center started to use massive bone allografts to reconstruct bone defects after the resection of bone tumours. To increase the availability of safe and effective bone allografts, a cadaveric bone bank was founded in 1989 by Eurotransplant and Bio Implant Services (BIS) Foundation in close cooperation with the Orthopaedic Department of the Leiden University Medical Center.

Advantages and disadvantages of the use of massive deep-frozen bone allografts

The principal advantages of the use of massive bone allografts over metallic implants are the versatility in sizing, the possibility of ingrowth at the graft-host junctions, and the physiological attachment of tendons. The versatility in sizing, which allows intra-operative cutting of the allograft to a large variety of forms, limits the unnecessary resection of healthy bone. The ingrowth may result in a better longevity [6,7,9] and the effective physiological attachment of tendons may result in a better clinical outcome for reconstruction of osteoarticular bone defects.

The use of massive bone allografts, however, also has a number of disadvantages and pitfalls. Clear disadvantages are the possibility of disease transmission [8] and the limited availability of the grafts. Furthermore, despite the good overall long-term results, the high complication rates and the unpredictable outcomes in individual cases remain troublesome. The main complications are infections, fractures, and nonunions. The complication rate depends on location, size, and complexity of the reconstructions, but remains partly unpredictable. The varying and poorly understood immune response is believed to play an important role in the occurrence and unpredictability of complications. [6,7,9]

Problems and controversies around massive deep-frozen bone allografts

The effect of bacterial contamination on the availability of massive bone allografts

Limited availability of bone allografts can hinder the necessary size matching. One reason for this is that a large part of the allografts retrieved from postmortal donors cannot be used as massive bone allografts due to bacterial contamination. If a graft is contaminated it must be discarded or additionally sterilised to avoid transmission of the micro-organisms to the recipient. As this sterilisation process can alter biomechanical and biological properties,[10,11] the massive bone grafts are preferably used without additional sterilisation. To prevent this wastage of retrieved grafts, the risk of contaminations should be minimised. Although general guidelines to prevent contamination are given,[9] no thorough multivariate analysis of risk factors for bacterial contaminations has been performed.

The poorly understood and varying immunogenicity of massive deep-frozen bone allografts

One important question that is still not solved conclusively is the effect and precise mechanism of the immunological reaction on the behaviour and ingrowth of the implanted allograft. Deep-freezing kills most bone cells and through this reduces the immunogenicity significantly so that the graft can be transplanted successfully without HLA matching or using immunosuppressive drugs. [3-7] Nevertheless, an immune response can still be evoked. Donor-specific antibodies, cell-mediated immunity, and histological signs of immunological rejection have been found after transplantation of massive frozen bone allografts. [9,12] Most clinical studies into the effect of the immune response have focussed on antibodies. However, the chronic rejection of allografts is considered to be mediated by T cells rather than by antibodies. Therefore, the most direct way to asses the immune response is to analyse the T-cell characteristics. New sophisticated immunological assays are capable of quantifying and qualifying this T-cell response, but they have not yet been applied in clinical studies concerning bone transplantation.

Controversies around the clinical use of massive deep-frozen bone allografts

Despite worldwide studies into the transplantation of massive frozen bone allografts, controversies around the indications and operative techniques still exist. We believe that the indications for certain types of allograft reconstructions could be extended as the principal advantages of massive bone allografts have not yet been used to their full potentials.

Recent advances in chemotherapy and diagnostic imaging allow more precise and localised resection of malignant bone tumours. Due to this, the large versatility in sizing of the massive bone allograft has created new challenges for the clinical use of these grafts. Firstly, after hemicylindrical

(hemicortical) *en-bloc* resection of a bone tumour while maintaining part of the cortical circumference the bone defect can ideally be reconstructed with a tailor made hemicortical allograft. These hemicortical procedures may be used more often for low-grade tumours arising in or near the cortex. Secondly, indications for certain types of intercalary allograft reconstruction may be extended. Intercalary allografts are traditionally used to reconstruct a diaphyseal or meta-diaphyseal segment. As oncological safe resections of bone tumours extending into the epiphysis while maintaining the juxta-articular bone have become possible, reconstruction with an epi-diaphyseal intercalary graft seems a very attractive option. In doing so, a larger osteoarticular reconstruction can be avoided.

When a standard prosthesis is encased in an allograft (allograft-prosthesis composite) for the reconstruction of an osteoarticular defect, the merits of both types of reconstruction can be combined. Effective reattachment of the host tendons to the preserved allograft tendons and the ingrowth of the graft may result in better function and longevity of this composite reconstruction compared to a reconstruction with megaprosthesis only. Nevertheless, controversies on the optimal operative techniques of the composite exist.

AIM OF THE THESIS

The aim of this thesis was to address a number of problems and controversies around the use of massive deep-frozen bone allografts. Three different aspects of the transplantation of massive deep-frozen bone allograft were studied. Firstly, the contamination of the allografts retrieved from postmortal donors was studied in order to increase safety and availability of the allografts. Secondly, the immune response to the transplanted allografts was studied, to asses whether qualification and quantification of the cellular immunity was possible. Thirdly, the clinical results of three less common types of allograft reconstructions were evaluated to specify the indications and operative techniques.

OUTLINE OF THESIS

In *Chapter 2*, a multivariate analysis of the incidence and potential causes of bacterial contamination of bone allografts at time of retrieval from postmortal donors is performed. The effectiveness of rinsing the allografts

is assessed. Methods to decrease the contamination and increase the availability and safety of the graft are given.
In *Chapter 3*, the presence of activated (primed) cytotoxic T cells and T helper cells in the peripheral blood of recipients of massive frozen bone allografts was analysed with the use of a relatively new immunological assay. Qualifications and quantifications of the cellular response were performed. The clinical relevance of measuring the chronic T-cell response is discussed.
In *Chapter 4* , the medium-term results of the hemicortical procedure for selected cases of low-grade malignant bone tumours arising in or near the cortex are evaluated. The oncological and allograft outcomes were analysed to determine the efficacy and safety of this technique. The question whether the indications for such hemicortical procedures can be expanded, is assessed.
In *Chapter 5*, the medium to long-term results of epi-diaphyseal reconstructions were compared to the more traditionally used meta-diaphyseal and diaphyseal intercalary reconstructions for malignant bone tumours. Pitfalls of the epi-diaphyseal intercalary reconstructions are noted. An analysis of possible risk factors for low allograft survival and high complication rates for the intercalary reconstructions is performed.
In *Chapter 6*, the medium to long-term results of allograft-prosthesis composite reconstructions of the proximal femur were evaluated. The operative technique with press-fit fixation of the stem in both allograft and host bone is described. The results are compared with the reported results of other composite techniques and megaprosthesis.
In *Chapter 7*, a summary of the results and conclusions of the preceding chapters is given.

REFERENCES

1. *Macewen W.* Observations concerning transplantation of bone. *Proc R Soc Lond* 1881;32:232-47.
2. *Lexer E* . 20 jahre transplantationsforschung in der chirurgie. *Arch Klin Chir* 1925;138:251-302.
3. *Parrish FF.* Treatment of bone tumors by total excision and replacement with massive autologous and homologous grafts. *J Bone Joint Surg [Am]* 1966;48-A:968-90.

4. Volkov M. Allotransplantations of joints.*J Bone Joint Surg [Br]*1970;52-B:49-53.

5. Ottolenghi CE. Massive osteo and osteo-articular bone grafts. Technic and results of 62 cases. *Clin Orthop* 1972;87:156-64.

6. Mankin HJ, Doppelt S, Tomford WW . Clinical experience with allograft implantation. The first ten years. *Clin Orthop* 1983;174:69-86.

7. Mankin HJ, Gebhardt MC, Jennings LC, Springfield DS, Tomford WW. Long-term results of allograft replacement in the management of bone tumors. *Clin Orthop* 1996:324:86-97.

8. Tomford WW, Doppelt SH, Mankin HJ, Friedlaender GE . 1983 Bone bank procedures. *Clin Orthop* 1983;174:15-21.

9. Enneking WF, Mindell ER. Observations on massive retrieved human allografts. *J Bone Joint Surg [Am]* 1991;73-A:1123-42.

10. Munting E, Wilmart JF, Wijne A, Hennebert P, Delloye C. Effect of sterilization on osteoinduction: comparison of five methods in demineralized rat bone. *Acta Orthop Scand* 1988;59(1):34-8.

11. Pelker RR, Friedlaender GE. Biomechanical considerations in osteochondral grafts. In: Friedlaender GE, Goldberg VM, eds. *Bone and cartilage allografts: biology and clinical applications* . Park Ridge: AAOS, 1989:155-62.

12. Stevenson S, Li XQ, Martin B. The fate of cancellous and cortical bone after transplantation of fresh and frozen tissue-antigen-matched and mismatched osteochondral allografts in dogs. *J Bone Joint Surg [Am]* 1991;73-A:1143-56.