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Explanted cryopreserved allografts: a morphological and and allograft heart valves from infants and adults[1]

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Abstract

Objective: Life expectancy of cryopreserved allografts implanted in infants is different from those implanted in adults. A morphological study of explanted allograft heart valves was performed to determine the mechanism of deterioration and to compare cryopreserved arterial and heart valve allografts from adult patients with those explanted from infants. **Method**: Between 1987 and 1996, 209 cryopreserved allografts were implanted: 125 valved conduits or monocusps to reconstruct the right ventricular outflow tract in congenital heart disease, 50 allograft heart valves to treat native aortic and prosthetic aortic valve endocarditis and 34 cryopreserved arterial allografts to replace mycotic aortic aneurysms or infected aortic prosthetic grafts. Two months to 8 years after implantation, 23 heart valve allografts, 11 right-sided and 12 left-sided, and four arterial allografts had to be explanted for reasons such as degeneration, recurrent infection, aneurysm formation or rupture. Besides conventional staining, immunohistochemical detection of cell populations was performed as follows: CD45RO, CD3 and CD43 for T lymphocytes, CD20 for B lymphocytes, CD68 for macrophages, protein S100 for Langerhans-cells, vimentin for fibroblasts, α-actin for smooth muscle cells and factor VIII for endothelial cells. **Results**: Explanted cryopreserved allografts were all fibrotic, acellular, non-vital and without endothelial cells. The fibrous tissue was preserved. T lymphocytes, indicating rejection, were found in all right-sided allografts from the paediatric population, but only in 9% of left-sided valves explanted from adults and in one of the four of arterial allografts. Macrophages and Langerhans-cells were found only in right-sided allografts from paediatric patients. **Conclusion**: Right-sided cryopreserved allografts from a paediatric population showed ongoing cellular rejection. By contrast, there was only a weak T-cell mediated rejection to adult heart valve and arterial allografts. Therefore, similar long-term results can be expected in adult arterial and heart valve allografts, whereas longevity of right-sided heart valve allograft in the paediatric age group seems endangered by cellular rejection.

Keywords: Explant studies; Cryopreserved allografts; Infants; Adults

1. Introduction

Despite the changing methods of procurement and various sterilization protocols such as gamma irradiation, pretreatment with glutaraldehyde or dry-freezing, a durable cardiovascular allograft has not yet been found.

The cryopreservation of heart valves has been reported to result in a viable allograft capable of maintaining its collagenous matrix by constant self-repair resulting in a durable homologous heart valve substitute [1]. The presence of a variable degree of cellularity, the ability to metabolize glucose and the successful culture of fibroblasts from explanted cryopreserved heart valve allografts was thought to indicate preserved viability late after implantation [2]. In fact, valve leaflet tissue from an aortic allograft, explanted up to 10 years after implantation was considered as viable due to the presence of intact donor fibroblasts, identified by

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DNA fingerprint studies [3]. Thus, viability seems to confer durability on allograft valves.

However, the degree of antigen expression is determined by the viability of the allograft and especially of the allograft endothelial cells which are able to express class I and class II major histocompatibility antigens [4]. Allografts that are used to reconstruct the right ventricular outflow tract in congenital cardiac surgery are much more prone to degeneration compared with allograft heart valves implanted in the adult left ventricular outflow tract [5]. Thus, durability of the allograft seems to be determined by its antigenicity and by the degree of the immunological response of the host; the latter seems to be stronger in infants than in adults.

In this report, results from morphological and immunohistochemical examinations performed in cryopreserved allografts explanted from the right ventricular outflow tract, from the left ventricular outflow tract and from explanted cryopreserved arterial allografts were compared between adult patients and patients from a paediatric age group. The purpose of the present study was, first, to determine the viability of explanted allografts; second, to elucidate the question, if early allograft degeneration is paralleled by an active immunological rejection; third, to identify differences in the rejection reaction on implanted allografts between infants and adults; and fourth, to evaluate the immunological reaction on arterial allografts to predict the long-term behaviour of implanted arterial allografts comparing the immune response of arterial and heart valve allografts.

2. Materials and methods

2.1. Patients

Morphological and immunohistochemical examinations were performed on 27 explanted cardiovascular allografts, 11 heart valve allografts from the right ventricular outflow tract in infants, aged 2–16 years, 12 heart valve allografts from the left ventricular outflow tract in adults and four arterial allografts from the infrarenal aorta and pelvic vessels.

The 11 right ventricular to pulmonary artery allografts, valved conduits and monocusps, were explanted 2 weeks–7 years after implantation [6]. Reasons for explantation were: monocusp patch regurgitation 2 weeks after implantation in one, marked aneurysm formation of a valved allograft in two, heart transplantation 5 years after total correction for double-outlet right ventricle in another child and calcifying allograft stenosis at the distal anastomosis to the native pulmonary artery in five and along the conduit in the remaining two children. Thus, 9% of right-sided allografts had to be replaced during this 9-year observation period. Besides, the reoperative mortality rate was 0%.

Twelve left-sided heart valve allografts, used to treat acute infectious endocarditis [7], had to be replaced 4 weeks–8 years after implantation. Reasons for reoperation were: allograft endocarditis in three, allograft degeneration in five, pseudoaneurysm formation causing allograft regurgitation in three patients and recurrent mitral valve regurgitation in one. During the observation period, the reoperation rate for allografts, used to treat acute destructive aortic root endocarditis was 24% with a reoperative mortality rate of 8% due to a fatal perioperative neurological event in one patient.

Since 1990, cryopreserved arterial allografts were increasingly used to treat mycotic aortic aneurysm and prosthetic aortic graft infection [8]. Four out of 34 implanted arterial allografts (12%), removed at the time of autopsy, were available for morphological and immunohistochemical examination: one patient died from a sudden cardiac death early postoperatively, one from multi-organ failure due to multiple intraabdominal abscesses after perforation of the sigmoid colon with faecal peritonitis and two died late from allograft-enteric fistulae 10 and 18 month after implantation.

2.2. Methods

2.2.1. Histology

Intraoperatively, the allografts were macroscopically evaluated and carefully removed. After immersion fixation (paraformaldehyde/glutaraldehyde/picric acid), Paraffin sections were cut and stained with Haematoxylin-Eosin and the elastin van Gieson method. In case of suspected recurrent endocarditis or persistent vascular infection, perigraft exudates and tissue specimens were sent to microbiological examinations. Rapid Gram's staining and examination for aerobes and anaerobes and fungi were performed. In addition, polymerase chain reaction (PCR) was used to identify the responsible infectious agents.

2.2.2. Immunohistochemistry

Immunohistochemical studies using an immunoperoxidase method were performed on all allografts. For the identification of inflammatory cells the following antibodies were used: CD20 for B lymphocytes, CD45RO, CD3 and CD43 for T lymphocytes and CD68 for macrophages. Monoclonal antibodies against alpha actin were used to identify muscle cells, anti-S100 protein was used to detect infiltration of antigen-presenting cells. Anti-vimentin detected fibroblasts and anti-factor VIII identified endothelial cells on the luminal surface of allografts.

3. Results

3.1. Macroscopy

Allografts explanted from infants showed extensive, circumferential calcification of the allograft wall. If allograft

stenosis was the reason for reoperation, then it was found at the distal anastomosis from the allograft to the native pulmonary artery bifurcation. Proximal, infundibular or valvular allograft stenosis was not observed. Surprisingly, the valve leaflets, either from valved allografts or from monocusps, were delicate, mobile and seemed to be spared from the calcifying process. In one child, a true aneurysm of the entire allograft was found intraoperatively causing severe pulmonary valve regurgitation. Due to the small number of patients, we did neither expect nor find any difference between pulmonary and aortic allografts with regard to long-term function or degree of degeneration.

Similar intraoperative findings were observed in the left ventricular outflow tract from adult patients although the degree of calcification was less with spotty calcifications observed in the aortic root and allograft wall. The leaflets were delicate and not involved in the degenerative allograft process. In case of recurrent endocarditis, leaflets were destroyed similar to the acute infection of the native aortic valve. Annular abscesses or allograft-ventricular disconnection was not found. In three patients, originally operated with the scalloped-freehand implantation technique, pseudoaneurysm formation distorting the geometry of the aortic root, led to compression of the allograft and subsequent regurgitation.

Results from explanted cryopreserved arterial allografts are scarce and were all found at autopsy. In three patients, an allograft-enteric fistula was found, originating from a small allograft side-branch artery. The distal and proximal anastomoses were found to be normal except in one patient with a leakage of the proximal infrarenal anastomosis of a bifurcation allograft due to persistence of *Candida albicans* infection (Fig. 1). Dilatation or calcification of arterial allografts was not found.

3.2. Histology

Explanted allografts, heart valves and arteries, were almost acellular in the media with only few donor fibrocytes. The lamina elastica showed partial fragmentation and total desquamation of the endothelial layer. A dense fibrous band, a neointima, was observed on the luminal surface of the allograft wall.

The interface between the allograft and the native tissue was fused containing few fibrocytes, smooth muscle cells, collagen and elastic fibers. Host fibroblasts and monocytes seemed to invade the allograft at the interface from the adventitial layer giving the impression of a localized inflammatory reaction. In case of endocarditis, significant inflammatory cell infiltration was found and Gram staining revealed gram-positive bacteria in one patient.

The leaflets showed plasma and fibrin insudation but were acellular and, in infants as well as in adults, free from any inflammatory cell infiltration. In addition, calcification was not found in allograft valve leaflets. By contrast, the wall of the heart valve allograft was heavily calcified. In adults, spotty calcifications were noted, however in children, circumferential calcifications as soon as 18 months after implantation and even bone formation was observed. Light microscopy demonstrated a preserved structure of the matrix constituents, especially the collagenous framework and the glycosaminoglycane content, in heart valve as well as in arterial allografts. Besides, arterial allografts, although with a shorter follow-up, were free from any calcifications.

3.3. Immunohistochemistry

Multiple foci of inflammatory infiltration were found in the wall of allografts explanted from the paediatric population. They were made up predominantly from T lymphocytes (Fig. 2), indicating active, ongoing allograft rejection. B lymphocytes were sparse. In older allografts, positive staining reactions with CD68 and S100 revealed the presence of macrophages and some antigen-presenting cells. In addition multiple foci of plasma cell infiltration were found as well (Fig. 3).

By contrast, allografts from the left ventricular outflow tract in adults were free from T lymphocytes except in one

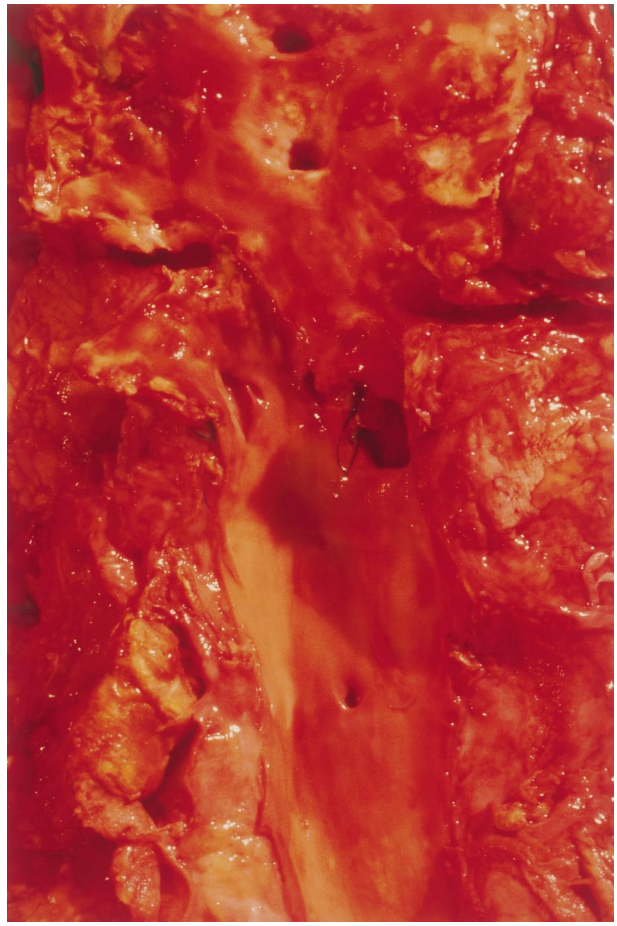

Fig. 1. Infrarenal anastomotic leakage of a cryopreserved aorto-bifemoral bifurcation allograft with persistence of *Candida albicans* infection.

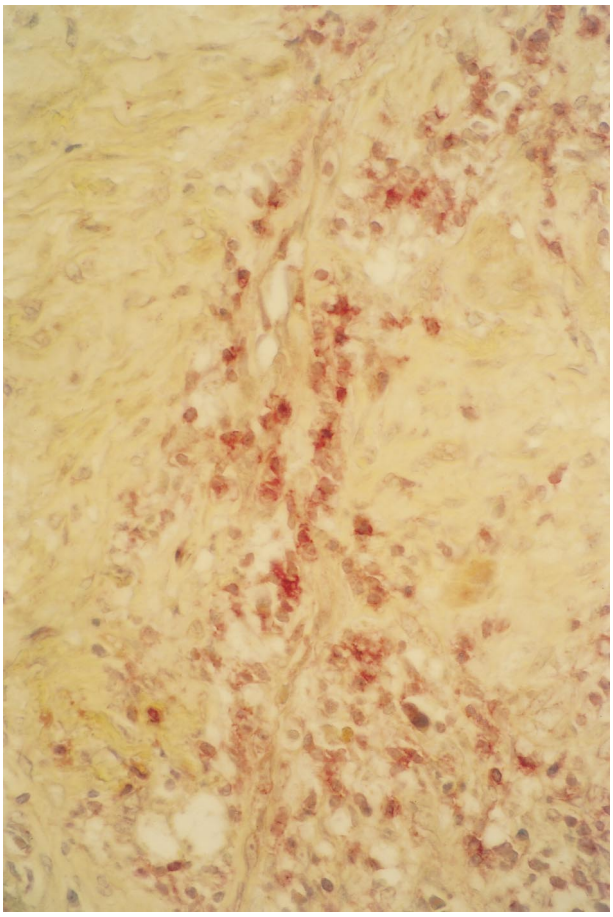

Fig. 2. Immunohistochemical stain for CD3 demonstrating T lymphocyte infiltration in a 2-year-old aortic allograft from right ventricular outflow tract (original magnification ×300).

patient who died after several aortic root reoperations caused by an unidentified destructive process, which affected every type of valve replacement device. Macrophages and antigen-presenting cells could not be found in allografts explanted from adults and B lymphocytes were sparse and only in the wall of the valved allograft. Neutrophils, macrophages and lymphocytes were not found in the allograft valve leaflets.

Results for arterial allografts were similar to explanted left-sided allografts. In general, inflammatory cell infiltration was limited and T lymphocytes were found only in one short iliac segment of an aorto-biiliac bifurcation graft, implanted for a huge right retroperitoneal abscess after prosthetic graft infection with *Aspergillus fumigatus*, *Candida albicans* and *Staphylococcus aureus*.

4. Discussion

The results of the present study demonstrate an almost complete loss of cellular elements and a total desquamation of endothelial cells in cryopreserved cardiovascular allografts explanted form infants and adults. Furthermore, an active T lymphocyte mediated rejection, as well as macrophages and antigen-presenting cells have been found in the wall of the allografts used to reconstruct the right ventricular outflow tract in congenital heart disease. By contrast, explanted allografts from the left ventricular outflow tract, used to treat acute infective endocarditis in adult patients, showed a limited humoral rejection with only few B lymphocytes in the wall of the allograft. Macrophages and antigen-presenting cells were not observed. Inflammatory cell infiltration was not found in the valve leaflets neither in heart valve allografts from adults nor from infants.

The ideal cardiovascular conduit has not yet been found for the use in children. This is especially true for the ventricular to pulmonary artery connection, the last durable part of an otherwise successful biventricular repair. The use of cryopreserved allografts facilitated reconstruction of the right ventricular outflow tract and a lower overall mortality has been observed with its use [9]. However, the freedom from allograft-related reoperation has not improved and allograft replacement is inevitable [10]. Inflammatory infil-

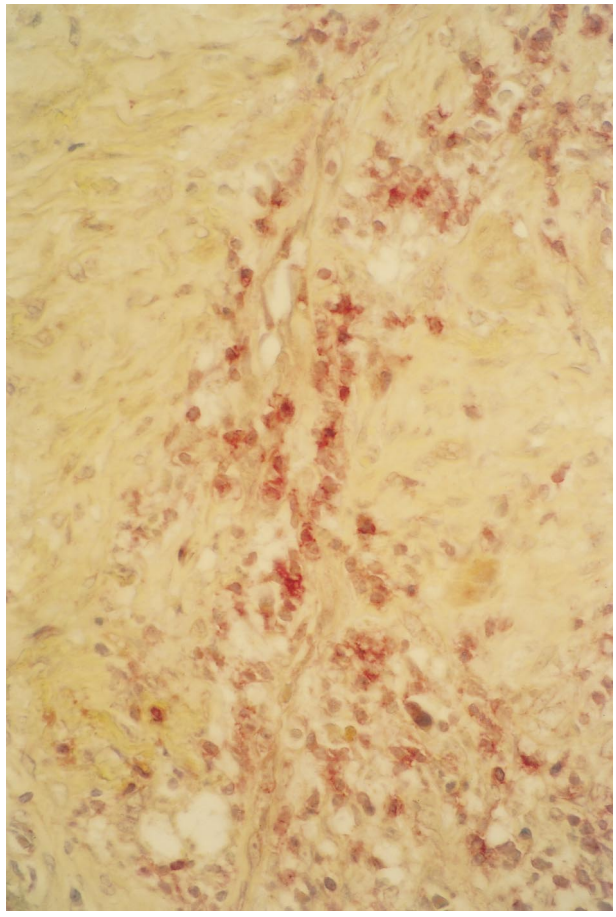

Fig. 3. Plasma cell infiltration in a 4-year-old pulmonary allograft from the right ventricular outflow tract (Haematoxylin-Eosin; original magnification ×300).

tration of T lymphocytes altering tissue structure and function, is the major finding in our explanted allografts from the paediatric age group and is accepted as active rejection. The presence of B lymphocytes, macrophages, antigen-presenting cells and plasma cell infiltration completes the impression of a strong and continuous immunological response to the allografts implanted in children, decreasing its life expectancy considerably. In addition, the right-sided allograft stenosis observed at the distal anastomoses from the allograft to the native pulmonary bifurcation was caused by extensive and circumferential calcification, preventing adequate growth even of the adjacent native tissue.

By contrast, allografts from the left ventricular outflow tract from adult patients showed only a weak immunological reaction. B lymphocyte infiltration was weak and macrophages, antigen-presenting cells and T lymphocytes were absent except in one patient. This is an interesting finding, as these allografts, used to treat destructive infectious endocarditis [7], were placed into an immunologically upregulated environment. However, the adult host's immunological response to these left-sided allografts was weak, partially contributing to the long-term allograft durability in adults.

Besides the rejection reaction of the host, the preserved viability of allografts has been thought to confer durability [11]. However, our explanted cryopreserved allografts were almost acellular and without endothelial cells and we considered all as non-viable. This loss of viability has been confirmed by others [12] and human valves prepared for use as allografts have found to be non-viable even before implantation [13]. Cryopreservation, on the other hand, represents a cell- and tissue-protective preparation method [14], preserving the collagenous skeleton and the ground substance, rendering the allograft relatively immunologically inert and mechanically superior to freshly implanted allografts [15]. We believe, that the preservation of the collagenous network and not the presumed viability improves the durability of cryopreserved allografts.

The degree of the rejection reaction observed in infants, however, is more important in determining the durability of allografts than the preservation of the ground substance and this seems to be confirmed by the rather limited life expectancy of allografts implanted in the immunologically active paediatric age group [16]. This interpretation is supported by another study, observing the same predominant T lymphocyte infiltration in allograft cardiac valves in infants [17] and further by the fact, that donor-specific T lymphocytes can be cultivated from explanted allografts [18]. By contrast, compared with the relatively weak rejection reaction of the adult host, the preservation of the collagen skeleton seems to be more important in determining the long-term durability of left-sided allografts in adult patients [19,20].

The results with allografts in the treatment of infective endocarditis stimulated the use of arterial allografts for the treatment of major vascular infection. Although early results are satisfactory [8], the long-term behaviour of these grafts is unknown. Freshly implanted arterial allografts were found to be unsuitable as vascular substitutes [21] and their early failure has been associated with a strong immunological reaction [22]. However, our morphological and immunohistochemical findings on explanted cryopreserved arterial allografts, although limited by a small number of patients and a rather short follow-up, demonstrate a rejection reaction similar to what is observed in left-sided cryopreserved heart valve allografts explanted from adult patients. Despite the occasional presence of T lymphocytes, this moderate inflammatory cell infiltration in arterial allografts, observed in this study, has been confirmed by Goffin et al. [23]. Thus, the long-term outcome of arterial and heart valve allografts implanted in adults may be quite similar, rendering cryopreserved arterial allografts a more durable vascular substitute than as freshly implanted arteries. This is further supported by the preservation of the collagen skeleton which does not differ between our adult heart valve and vascular allografts.

There are several limitations to this study: first, it is a descriptive study, which does not quantify the degree of inflammatory cell infiltration nor the amount of allograft wall calcification. Thus, we were not able to determine a relationship between cell rejection and propensity for allograft calcification; second, due to the small number of explanted allografts, comparison between pulmonary and aortic allografts has not been possible; third, this morphological study was designed to detect differences in immunological rejection of various allografts. The results observed do not represent the actuarial survival of right-sided allografts used in congenital heart surgery nor from those used to treat acute infectious aortic endocarditis in adults.

In conclusion, allografts from infants showed active T lymphocyte mediated cellular rejection limiting the durability of these right-sided allografts in the paediatric age group. By contrast, heart valve and arterial allografts in adults are subjected to a relatively weak humoral rejection. The additional preservation of the ground substance, which is comparable for cryopreserved heart valve and vascular allografts expects similar long-term results for left-sided heart valve and arterial allografts.

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Appendix A. Conference discussion

Dr U. Althaus (*Bern, Switzerland*): I think that is a very interesting study with quite an important message. Your observation that right-sided homografts explanted from a paediatric population showed clear signs of an active cellular rejection might imply that some form of immunosuppressive therapy could be beneficial with regard to the long-term prognosis of this particular subgroup of patients. In clinical practice, do you actually use some kind of an immunosuppressive modality after implantation of a cryopreserved homograft in a child?

Dr Vogt: No, we do not use immunosuppressive treatment. In these patients, immunosuppression has been discussed by various groups, but, to the best of my knowledge, has not yet been tried. I think it is difficult to estimate complications and side effects of such a prolonged therapy. In addition, the mortality rate for the exchange of degenerated right-sided allografts has been 0%.

Dr M. O'Brien (*Brisbane, Australia*): Your excellent study does confirm the work of many who are demonstrating the immune response. I wonder if you are really seeing the effect of age and not the difference between right and left-sided implants. Some workers have shown that the left-sided implants in the infant or young child also demonstrate a similar immune response producing a much earlier failure. In fact in our hands, if we look at the left-sided implants across the age bracket, the highest failure is in that young child as opposed to the good durability in the older, middle-aged adult. So I just wondered if you were just looking at the effect of age. What is your opinion?

The second question I have is to do with viability. Would it be better to have totally dead, non-viable valves for infants?

Dr Vogt: We do not have a fair number of left-sided allografts explanted from the paediatric age group. Thus, we cannot compare right-sided with left-sided allografts from infants only. Up to the age of 16 years, the morphological as well as immunohistochemical studies did not reveal a difference with regard to rejection. We've found T-lymphocytes in all our allografts explanted from children.

There is some controversy about allograft viability. On the one hand, viability may prolong durability, on the other hand, viability may provoke a stronger rejection reaction, again limiting the durability of these allografts. This problem is not solved.

I know, Dr O'Brien, that you use early cryopreserved allografts suggesting improved durability. However, we cannot determine the ischemia time in our allografts since we use allografts from an European pool.

Dr O'Brien: I would just like to make one last comment on immunosuppression. We have got rats immunosuppressed with abdominal allograft aorta valve implants. These are unlike rats and are compared with a rat group that previously were not immunosuppressed. I think our immunology department will get the answer to this and to use the model to decide on the duration of immunosuppression that may be needed. I think these very important answers will come out over the next year.

Dr Z. Religa (*Zabrze, Poland*): Our clinical and experimental results are totally different, maybe due to the fact that the ischemic time that we accept is after 6 h. So what is the ischemic time of your homografts?

Dr Vogt: I cannot give you an exact answer. We explant allografts from multiorgan donors with a very short warm ischemia time. They are immediately stored in cold saline solution with a cold ischemia time of less than 24 h. As far as we get allografts from a European pool, I cannot answer your question. However, a shorter period of cold ischemia time is not proven to improve the long-term results of implanted allografts. We believe that rejection phenomenon's, as described, are more important determining their outcome.