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Cryopreserved Arterial Allografts for *In situ* Reconstruction of Infected Arterial Vessels

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Objective. *To review our experience of using cryopreserved allografts for* in situ *reconstruction in the presence of infection involving the aorta, iliac or femoral arteries.*
Design. *Retrospective clinical study.*
Methods. *From 3/2000 to 8/2003 all patients with mycotic aneurysms or secondary infection following earlier prosthetic replacement were treated with cryopreserved human allografts. Forty-two patients, 39 (93%) with a prosthetic graft infection and 3 (7%) with a mycotic aneurysm of the abdominal aorta were treated. Six (14%) had aorto-enteric fistulas, 5 (12%) had ruptured aneurysms, and 2 also had vertebral destruction. The median follow-up time was 20 months (range 1–42 months).*
Results. *Thirty-day mortality was 14%. Three patients died due to multi-organ failure, two patients died from hypovolaemic shock due to allograft rupture and one from rupture of the native aorta. The overall mortality was 24% (four additional patients). Graft patency was 100% at 30 days and 97% at follow up in the survivors. The mean actuarial survival time was 32 months (95% CI = 27–37 months).*
Conclusions. *Cryopreserved allografts for the* in situ *reconstruction of infected arteries or grafts have acceptable intermediate results.*

Key Words: Arterial graft infection; Mycotic aneurysm; Aorto-enteric fistula; Allograft.

Introduction

The traditional approach to arterial reconstruction in the presence of primary or secondary infection is complete excision and debridement, plus extra-anatomic or *in situ* prosthetic bypass grafting.[1] However, the outcome is poor regarding mortality, limb loss and eradication of infection.[2] The use of biological materials i.e. autologous vein, or human allografts, which may have better resistance to infection, has been proposed as the method of choice for *in situ* reconstruction.[1–4] The purpose of this study was to analyse the outcome of *in situ* arterial reconstructions, using cryopreserved homografts, in cases of major infection involving the aorta, iliac or femoral arteries.

Patients and Methods

Patients

From 3/2000 to 8/2003 42 patients (29 men and 13 women, median age 66, range 40–89 years) presenting with vascular infections were treated using cryopreserved human allografts for *in situ* reconstruction. The indications for surgery were prosthetic graft infection in 39 patients (93%), combined with a concomitant aorto-enteric fistula in six patients (14%), and primary mycotic aneurysms in three patients (7%). Five patients (12%) presented with a ruptured aneurysm and acute haemorrhage (Table 1). The preoperative diagnosis of graft or arterial infection was made by ultrasound and computed tomography confirming peri-graft fluid or gas formation and/or positive microbiology from open wounds. The direct aspiration of fluid was avoided preoperatively. Graft infection occurred from 7 days to 17 years (median 35 month) following the initial vascular operation. Three patients presented with mycotic aneurysms, one in aortic and two in femoral position. One patient with a femoral aneurysm was i.v. drug dependent and had no preoperative CT or ultrasound scan as he was admitted with an acute rupture. All patients presented with peripheral embolisation, fever and leukocytosis. The microbiological investigation of vessel specimens reported *Staphylococcus aureus* in all positive cases (Table 1).

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Table 1. Pathology, bacteriology and graft configuration in 42 patients who received a cryopreserved human allograft

Original graft/infection site	Microbiology	Allograft configuration	Additional pathology and procedures
(a) Graft infections ($n = 39$)			
Aortic tube graft (3)	*Escherichia coli* (1), *Proteus mirabilis* (1), no growth (1)	Descending aorta (2), aorto-biiliac (1)	Aorto-enteric fistula, small bowel segment resection (2), vertebral destruction (1)
Aorto-iliac (1)	No growth (1)	Iliac arteries (1)	Ruptured aneurysm (1)
Aorto-femoral/-profundal (5)	ORSA (1), no growth (4)	Iliac arteries (4), femoral arteries (1)	Rectus femoris muscle flap (1)
Aorto-biiliac (6)	Bacterioides spp. (1), *Candida albicans* (1), *E. coli* (1), *Enterococcus faecalis* (1), Gram negative rods (1), Gram positive cocci (2), *Salmonella* (1)	Aorto-biiliac (5), iliac artery, incomplete excision (1)	Aorto-enteric fistula, small bowel segment resection (2), vertebral destruction (1)
Aorto-bifemoral/profundal (14)	*Citrobacter koseri* (1), *Corynebacterium* (1), *E. faecalis* (4), *E. coli* (1), Gram positive cocci (6), Gram negative rods (1), ORSA (1), *Pseudomonas aeruginosa* (1), *S. aureus* (2), *Moraxella* spp. (1), no growth (5)	Femoral/iliac arteries, incomplete excision (7), aorto-bifemoral/profundal (7)	Aorto-enteric fistula, small bowel segment resection (2), ruptured aneurysm (2), rectus femoris muscle flap (1)
Femoro-distal (8), prothesio-distal (1)	*C. albicans* (2), *Corynebacterium* (1), Gram positive cocci (1), *E. coli* (1), *E. faecalis* (1), ORSA (2), *P. aeroginosa* (1), *S. aureus* (1), no growth (6)	Iliac arteries (9)	D2 amputation (1)
Femoro-femoral (1)	No growth (1)	Iliac arteries (1)	
(b) Mycotic aneurysms ($n = 3$)			
Aortic (1)	*S. aureus* (1)	Aorto-biiliac (1)	
Common femoral artery (2)	*E. coli* (1), *S. aureus* (2), ORSA (1), *P. aeruginosa* (1)	Iliac arteries (2)	Ruptured aneurysm (2), rectus abdominis muscle flap (1)

ORSA, oxacillin resistant *S. aureus*.

Surgical technique

Arterial segments were harvested from multi organ donors. Harvesting, preparation, antibiotic treatment, cryopreservation, and procurement of human allografts are largely standardised and have been described previously.[3,5] Fresh human allografts or animal xenografts were not used in this study.[6]

Thirty-four patients (81%) were treated with complete graft excision and an extensive peri-vascular debridement. Generally, the type of homograft used corresponded to the removed prosthesis in length and shape. In one patient, an infected tube graft was replaced with a bifurcated allograft (Fig. 1(a)). The following reconstructions were performed: tube graft ($n = 2$), aorto-biiliac ($n = 6$), aorto-bifemoral/profundal ($n = 7$), aorto-iliac ($n = 1$), aorto-femoral/profundal ($n = 5$), and iliaco-femoral ($n = 12$). A local debridement of the infectious focus with only 'limited' excision of the infected graft was performed in eight patients (19%), where the problem was clearly limited to only a small portion of the graft, e.g. at the site of the femoral anastomosis. In these cases the anastomosis between the intact non-infected graft and allograft was always completed in a proximal clean operating field through a separate incision and the distal end of the allograft then pulled through to the contaminated field using the distal end of the prosthetic graft. Infected prosthetic material and wound fluid was cultured for microorganisms.

Allografts were sutured end-to-end to the normal aorta, iliac or femoral artery or to a macroscopically non-infected prosthetic segment with continuous non-absorbable 3–0 to 5–0 polypropylene sutures, depending on graft diameter. Prior to implantation of the allografts the side branches were oversewn using 5/0 polypropylene and limbs were extended, using for example the internal iliac branches, to give adequate length to reach the groin, if required. Metallic clips and ligatures were avoided. All grafts were externally impregnated with neomycin and fibrin glue following implantation.

Additional surgical procedures such as muscle flaps (in case of groin wound dehiscence) or omental wrapping (in case of large areas of debridement, or vertebral destruction) were performed to protect the graft and improve healing. Wounds were drained with multiple suction drains.

Peri-operative management and follow-up

CT and duplex ultrasound scanning was performed preoperatively in every patient except for those needing emergency surgery for acute rupture and bleeding. Patients received extensive antibiotic cover according to cultures of blood, and pre- or intra-operative specimens for a minimum of two weeks. Following this period, a specific antibiotic treatment was continued for a minimum of four weeks or longer, depending on clinical and laboratory parameters. Antibiotics used included metronidazole, ciprofloxacin, amoxicillin, teicoplanin, and vancomycin. Prior to specific treatment or in those cases where no positive culture was obtained, patients received clindamycin or vancomycin.

Clinical follow-up and ultrasound graft surveillance was performed prior to hospital discharge, at 3 months, and in June 2003. Graft problems, if present, were further elucidated by means of colour duplex scans, CT scans, MRI scans, MRI angiograms, or digital subtraction angiography. In addition, all survivors except two (one aged over 90, one in prison) were seen in the outpatients department.

Statistical analysis

Continuous variables are expressed as medians and ranges. SPSS 10.0 for Windows was used for Kaplan–Meier survival analysis.

Results

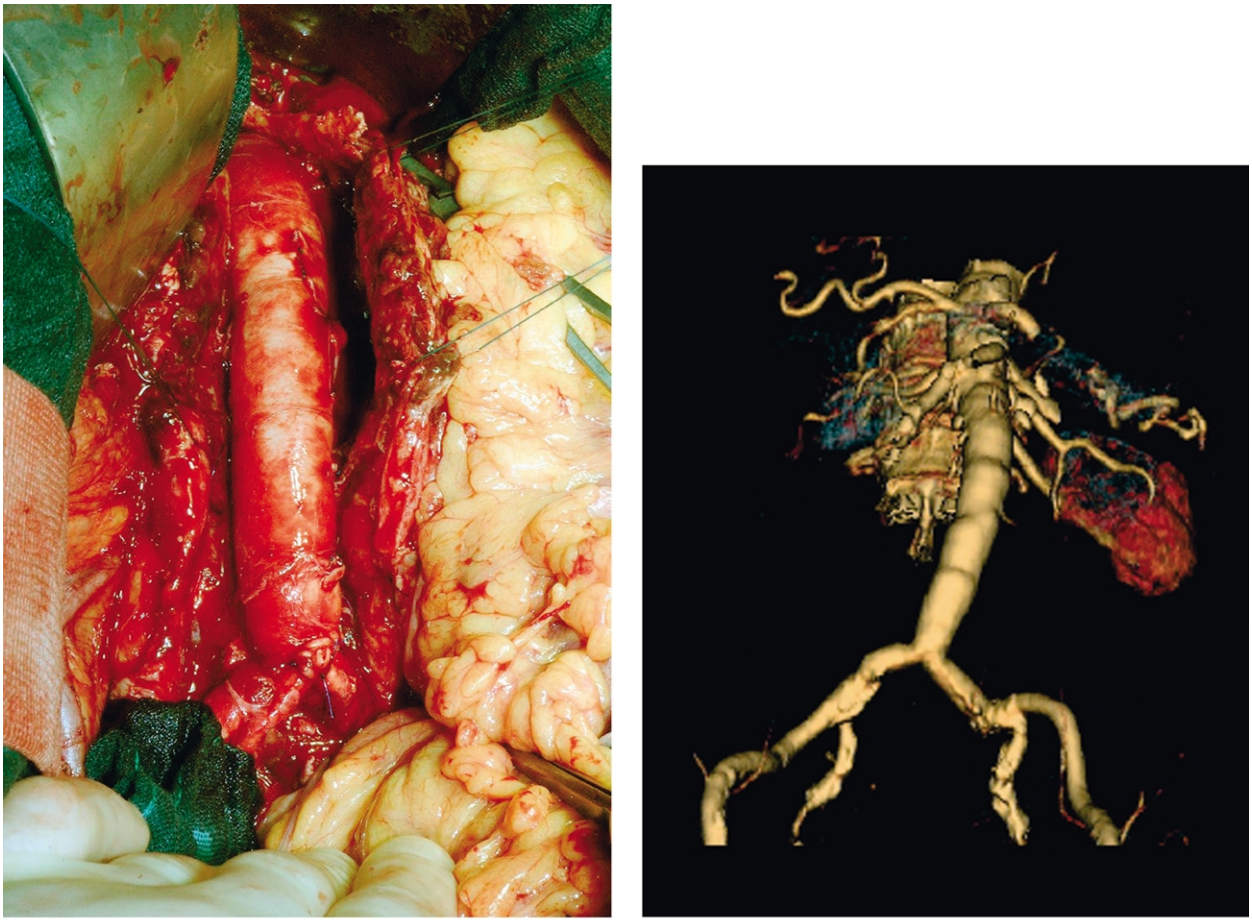
The median postoperative ICU stay was 3 days (0–41 days) and the median postoperative hospital stay was 14 days (9–132 days). The median postoperative follow-up period was 20 months (1–42 months). The mean actuarial survival time was 32 months (95% CI = 27–37 months).

Allograft disintegration at the site of the anastomosis or side branch sutures occurred in four patients (10%) due to inferior allograft quality or size mismatches. In these cases new allograft patch enhanced sutures were used following resection of the failed anastomosis. In the group of survivors, further surgical procedures were needed in 10 patients because of persistent abdominal infections requiring revisions due to aorto-enteric fistulas (three patients) or disturbed groin wound healing (six patients). One patient presented with a recurrent aorto-enteric fistula after he had already left the intensive care unit. The proximal anastomosis was revised using homograft patch enhanced polypropylene sutures. The remaining four patients with aorto-enteric fistulas did well.

In all cases, the vascular axis could be reconstructed *in situ*. Primary patency of the grafts was thus 100% initially (Fig. 1(b)). In the survivors (32 patients), there were two patients (6%) with severe vascular problems (Table 2). One presented himself too late to hospital

following thrombosis of his prothetic-femoral graft resulting in lower limb amputation. The patient with the recurrent aorto-enteric fistula was readmitted with claudication 2 month after revision of the anastomosis. Intra-arterial digital subtraction angiography revealed a haemodynamically significant stenosis of the proximal anastomosis between the aorta and the bifemoral homograft. The patient was successfully treated with percutaneous transluminal angioplasty and stent application. Two additional patients have non-significant stenoses of the femoral/profundal anastomosis and are followed up in 3 monthly intervals in our outpatients department. Secondary patency, therefore, is determined to be 97%.

The 30-day mortality was 14% (six patients). Three patients died due to multi organ failure or septic shock on postoperative day (POD) 13, 18, and 21. Postmortem examination revealed intact homografts in all three patients and an intact bowel reconstruction in the one patient who had had an aorto-enteric fistula. Despite further emergency surgery two patients died from hypovolaemic shock due to allograft rupture (POD 7 and 12) and one from rupture of the native aorta proximal to the allograft replacement (POD 27) (Table 2). Subsequent mortality was 10% (four additional patients) resulting in an overall mortality of 24%. One patient with a prolonged intensive care unit stay after aortic homograft implantation died on POD 41 due to sepsis. His relatives refused a postmortem examination. Another patient was readmitted with a groin infection that did not expose the graft. Despite debridement and vacuum drain therapy the wound and renal function deteriorated and he died from a myocardial infarction. According to their general practitioner the remaining two patients both had a fatal cardiac arrest that was not related to the vascular operation (POD 96 and 120) (Table 2).



(a) (b)

Fig. 1. Intra-operative view (a) and computed tomography scan reconstruction (b) after aorto-bi-iliac *in situ* replacement with a cryopreserved homograft made from an infrarenal aortic segment and two iliac arteries.

Table 2. Major complications after *in situ* arterial reconstruction with cryopreserved human allografts

Surgical procedure	Comorbidities	Event	Microbiology*	POD	Complication
Tube graft	CAD, PAD	Rupture of native aorta	No growth	27	Death
Tube graft	CAD, DM, AEF, VB	MOF, sepsis	*E. coli*	41	Death
Aorto-biiliac graft	CAD	MOF, sepsis	Gram positive cocci	18	Death
Aorto-biiliac graft	CAD, PAD, CRF	MOF, sepsis	*E. coli*	21	Death
Aorto-biiliac graft	CAD, PAD, DM	MOF, sepsis	Gram negative rods	13	Death
Aorto-biiliac graft	PAD	Allograft rupture (iliac limb)	*C. albicans*, *E. fecalis*	12	Death
Aorto-biprofundal graft	PAD	Allograft rupture (iliac limb)	*S. aureus*	7	Death
Aorto-femoral graft	CAD, CAD	Myocardial infarction	No growth	96	Death
Femoro-distal graft	CAD, PAD, DM	Myocardial infarction	Gram positive cocci	180	Death
Prothesio-femoral graft†	CAD, PAD, DM, CRF	Myocardial infarction	*S. aureus*	120	Death
Aorto-bifemoral graft	AEF	Proximal anastomotic stenosis	*S. aureus*, *E. faecalis*	60	PTA, stent
Prothesio-femoral graft†	CAD, CRF	Graft thrombosis	No growth	810	Leg amputation

CAD, coronary artery diease; PAD, peripheral artery disease; DM, Diabetes mellitus; AEF, aorto-enteric fistula; VB, vertebral destruction/abscess; CRF, chronic renal failure; MOF, multi organ failure; PTA, percutaneous transluminal angioplasty.
*At the time of surgery for graft infection.
†Limited excision.

Discussion

Prosthetic vascular graft infections have become a serious problem, particularly when the aorta is involved. Most graft infections are caused by inoculation of bacteria at the time of surgery, despite perioperative antibiotics. One to six percent of prosthetic grafts are affected.[7] The poor outcome is mostly due to multi organ failure, sepsis, suture line disruption and gastrointestinal bleeding when aorto-enteric fistula or erosion is associated.[8,9] Following thorough debridement, two surgical methods of arterial reconstruction have been proposed for the treatment of graft infections: extra-anatomic repair or *in situ* repair with the use of fresh or cryopreserved human arterial or venous allografts, arterial or venous autografts, prosthetic grafts, and animal xenografts.[10] However, the published results are difficult to compare due to the small number of grafts and the large number of variables involved. In addition, there are no randomised trials. In particular, the indications for *in situ* reconstruction using cryopreserved arterial homografts are not commonly agreed.[11]

Our results for early and intermediate mortality, as well as reintervention rates, are comparable to those presented by other groups.[2,11] However, Gibbons *et al*. described only one peri-operative death and no limb loss after reconstruction with autologous femoropopliteal veins in a series with a follow-up period from one month to five years. One difference compared to our patient group is the absence of aorto-enteric fistula in their patients, which is commonly associated with a high mortality.[12–14] In our series, the one patient (of six) with an aorto-enteric fistula who died, had an intact vascular graft and bowel anastomosis at postmortem examination.

The development of graft stenoses is well documented following prosthetic and biological replacement of arterial segments.[1,2,12] In our series, the one anastomotic stenosis of the infrarenal aorta was not diagnosed prior to hospital discharge. It may, therefore, be assumed that it was not related to the revision of the anastomosis but to intimal hyperplasia occurring later. Follow-up is thus required for all patients with allograft arterial reconstructions, as stenoses can often be treated by endovascular means.[1]

The alleged superior resistance of allografts to infection could be related to their viability that allows improved transfer of antibiotics and immunocompetent cells through the wall and into the peri-graft space.[2] Furthermore, allografts may have an enhanced anti-microbial activity compared to conventional grafts, since they are stored in antibiotic solution and in our practice are impregnated with neomycin fibrin glue.[15–17] However, there is no level one evidence of better resistance to infection or patency of allografts compared to prosthetic grafts.[10]

The limited availability and high cost of allografts may be overcome with improved cooperation on a European level regarding allocation, donor management, safety tests, and legal aspects. Additional research is required to detect the effect of different preparation methods, antibiotic storage solutions, cryopreservation protocols, potential modifications (e.g. decellularisation), and long-term storage on allograft quality and performance.[18]

There are several limitations of our study. The study was retrospective, the number of patients included was low and the observation period rather short, compared, for example, to the data of Szilagyi, especially with regard to long-term outcome.[19] Because of the variety of surgical procedures prior to the infection and indeed those performed for the graft infection, indications for surgery, implantation sites,

different antibiotic or anticoagulation treatments and the requirement of additional surgery, a comparative analysis of our data is difficult. However, the use of cryopreserved allografts for *in situ* reconstruction of infected arteries or grafts seems to have acceptable early and intermediate results.

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