

# Non-Bacterial Thrombotic Endocarditis in an Adult 14 Months after Cryopreserved Aortic Allograft Valve Implantation

Mervyn B. Forman<sup>1</sup>, James B. Atkinson<sup>2</sup>, J. Alan Wolfe<sup>3</sup>, Richard A. Hopkins<sup>4</sup>

<sup>1</sup>Emory University and North Atlanta Cardiovascular Associates PC, Atlanta, GA, <sup>2</sup>Vanderbilt University School of Medicine, Nashville, TN, <sup>3</sup>Peachtree Cardiovascular and Thoracic Surgeons, Atlanta, GA, <sup>4</sup>Children's Mercy Hospital, Kansas City, MO, USA

Cryopreserved aortic allograft tissue is used to correct aortic valve disease in adults and to reconstruct the right ventricular outflow tract in children with congenital heart disease. In adults, allograft durability is regarded as comparable to or better than that of manufactured bioprostheses, with failure usually due to slow fibrocalcific degeneration. Normally, allograft semilunar valves have excellent hemodynamics and low rates of infectious endocarditis and thromboembolism. The role of immune-mediated inflammation in post-implant allograft valve performance is slow in onset, and variable. Herein is

The availability of cryopreserved aortic allograft tissue has resulted in its use to correct aortic valve disease in adults and for reconstruction of the right ventricular outflow tract in children with congenital heart disease (1,2). In contrast to infants and young children, in whom accelerated degeneration occurs secondary to calcification of the leaflets, durability in adults is typically regarded as comparable to or better than that of manufactured bioprostheses. When failure does occur, it is usually due to slow fibrocalcific degeneration. Allograft (note, from hereon, allograft and homograft are used interchangeably to acknowledge historical literature) semilunar valves are usually associated with excellent hemodynamics and very low rates of classical infectious endocarditis and thromboembolism (1-4). Whilst antigenic with demonstrable donor HLA antigens and provokable recipient antibody titers, the role of immune-mediated inflammation in post-implant allograft valve performance has been unclear, typically slow in onset, and certainly variable. In the present adult patient, rapid deteriora-

presented the case of a male adult with rapid deterioration of the aortic valve homograft wall, without loss of the valve leaflets, resulting in severe aortic regurgitation. The pathological findings were consistent with classical marantic (sterile) endocarditis with acute and chronic inflammatory changes associated with advanced atherosclerotic lesions in the allograft aortic wall tissue resulting in thrombosis and subsequent cerebral embolization.

The Journal of Heart Valve Disease 2007;16:410-416

tion of the wall of the aortic valve homograft occurred without loss of the valve leaflets, resulting in severe aortic regurgitation. The pathological findings were consistent with classical marantic (sterile) endocarditis (now termed non-bacterial thrombotic endocarditis, NBTE) with acute and chronic inflammatory changes associated with advanced atherosclerotic lesions in the allograft aortic wall tissue resulting in thrombosis and subsequent cerebral embolization.

## Case report

A 35-year-old Caucasian male with a bicuspid aortic valve diagnosed during infancy presented with reduced exercise tolerance associated with mild dizziness. The patient denied exertional chest pain or syncope. There was no family history of congenital heart disease, hyperlipidemia or autoimmune disorders, and the patient was a non-smoker. An electrocardiogram revealed left atrial enlargement and marked left ventricular hypertrophy with a strain pattern. A two-dimensional echocardiogram was recorded which demonstrated severe concentric left ventricular hypertrophy (1.7 mm) with hyperdynamic function. The aortic valve was heavily calcified, and deformed with reduced opening and a peak valvular gradient of 175 mmHg. Transesophageal echocardiography (TEE) con-

---

Address for correspondence:  
Dr. Richard A. Hopkins MD, Cardiac Surgical Research Institute,  
Children's Mercy Hospital, 4 West Tower, 2401 Gillham Road,  
Kansas City, MO 64108, USA  
e-mail: rahopkins@cmh.edu

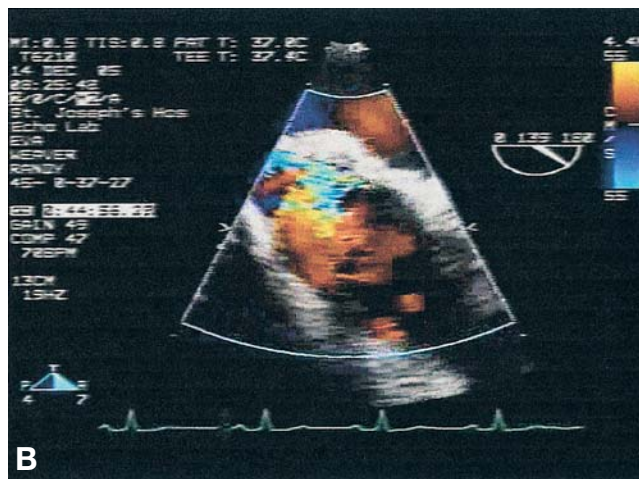
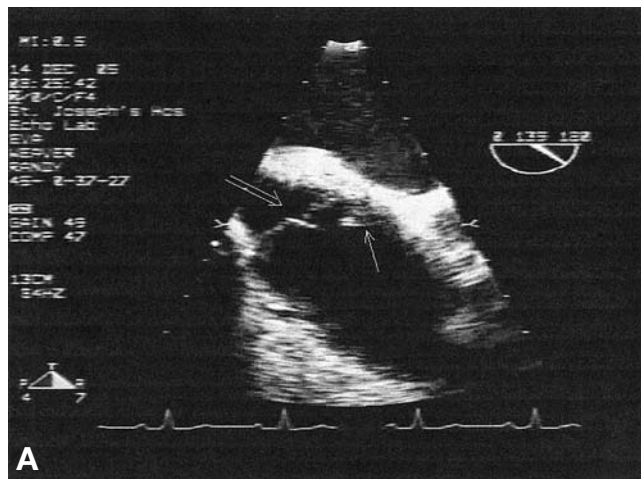


Figure 1: a) Long-axis view of aortic homograft on transesophageal echocardiogram showing prolapse and failure of coaptation of two leaflets (double arrow), with the third leaflet appearing immobile and retracted (single arrow). b) Color Doppler view showing eccentric jet compatible with severe aortic regurgitation.

firmed the presence of a severely calcified bicuspid valve with doming of the anterior leaflet and immobility of the posterior leaflet.

The ascending aorta was dilated (measuring 5.5 cm), and there was moderate aortic regurgitation. Left heart catheterization showed angiographically normal coronary arteries with a dominant circumflex system. All routine laboratory studies and the lipid profile were normal. The patient's blood group was A-positive.

The patient did not wish to receive long-term anticoagulation, and elected to have a homograft placed. The operative findings included the presence of a severe bicuspid aortic valve with fixed opening, 30-mm annulus and a dilated ascending aorta. A reduction annuloplasty was performed, followed by replacement of the ascending aorta to the level of the innominate artery with a #25 aortic homograft with reimplantation of the left and right coronary arteries. The homograft was obtained from a 23-year-old male farm worker who died from traumatic abdominal injuries in a hunting accident. The donor was blood type A positive, had a negative medical history, and chewed tobacco but did not smoke. Procedural records demonstrated that the freezing profile, storage and shipping of the homograft were within acceptable parameters; a warm ischemic time of 11.3 h and a cold ischemic time of 20.8 h. The tissue was cryopreserved with a standard 1°-per-minute freezing protocol in 10% dimethyl sulfoxide, 15% fetal calf serum and RPMI medium. Postoperatively, the patient developed hypertension which required beta-blocker therapy. A two-dimensional echocardiogram performed at five weeks after surgery showed a normally functioning aortic valve with trivial aortic regurgitation.

The patient remained asymptomatic for 14 months, at which time he experienced sudden onset of severe

headache with bitemporal radiation, associated with intermittent nausea and vertigo. He also complained of exertional dyspnea and denied any fever or recent dental surgery, but did report being treated with a course of antibiotics for a flu-like illness four weeks prior. A new murmur was documented; this was a long, early diastolic murmur compatible with severe aortic regurgitation. The patient was afebrile and there were no peripheral stigmata of endocarditis. Laboratory tests showed a normal leukocyte count, C-reactive protein level, and erythrocyte sedimentation rate. Although numerous blood cultures were negative, a presumptive diagnosis was made of endocarditis and the patient was treated empirically with

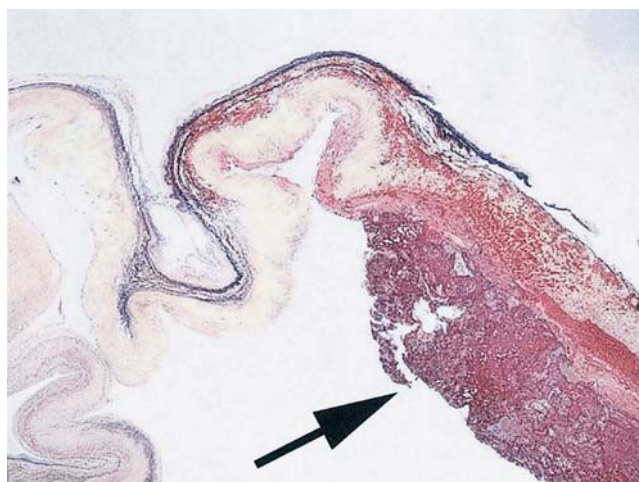


Figure 2: Photomicrograph of a homograft valve leaflet, showing a fibrin thrombus (arrow) overlying an intact valve leaflet. The thrombus does not contain inflammation and the valve leaflet appears intact. (Movat pentachrome stain; original magnification,  $\times 100$ .)

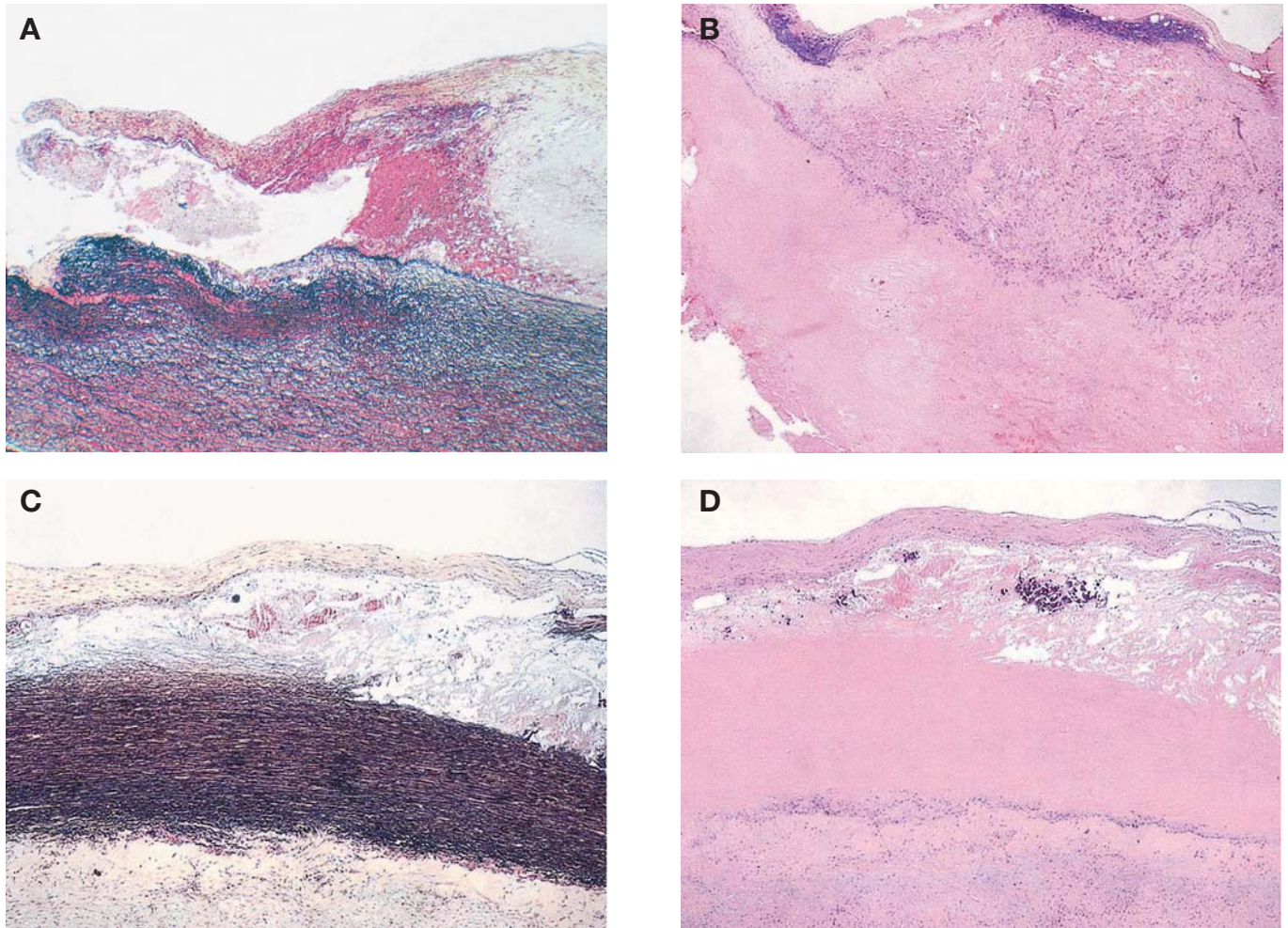


Figure 3: A) Sinus wall lesion. The wall of the homograft contains a complicated atherosclerotic plaque which consists of a large lipid-rich and necrotic core that contains acute hemorrhage with rupture of the overlying fibrous cap. The media has a normal pattern and arrangement of the elastic lamellae. (Movat pentachrome stain; original magnification,  $\times 100$ .) B) Hematoxylin and eosin staining of the adjacent section (original magnification,  $\times 100$ ). C) Fibrous sheathing on the lumen side of homograft sinus wall, the acellular transplanted wall based on elastin (black) and collagen (yellow). The adventitial side (bottom) is involved with granulation tissue, typical of surgical healing. (Movat pentachrome stain; original magnification,  $\times 40$ .) D) Hematoxylin and eosin staining of the adjacent section (original magnification,  $\times 40$ ).

ceftriaxone and vancomycin, administered intravenously. A magnetic resonance imaging scan performed five days after admission revealed a large intraparenchymal hematoma of the left frontal lobe, with no significant mass effect. A gadolinium magnetic resonance angiogram was reported as being normal. Selective cerebral angiography demonstrated irregularities in arterial segments suggestive of vasculitis, but no evidence of mycotic or Berry aneurysms or of any arterio-venous malformation. Subsequent TEE demonstrated marked thickening of the homograft aortic wall which was most prominent at the level of the leaflets. The leaflets were well visualized and did not contain any vegetations. However, two of the leaflets had prolapsed, while a third leaflet appeared fixed and immobile, resulting in poor coaptation and

an eccentric regurgitant jet which filled the majority of the left ventricular outflow tract, compatible with severe aortic regurgitation (Fig. 1a and b). No thrombi were seen in the left atrium or left atrial appendage, and a bubble study demonstrated no evidence of intra-atrial shunt.

In view of the large intracerebral hemorrhage, surgery was delayed for six weeks. During that time the patient received (as an outpatient) intravenous antibiotic therapy, in addition to treatment with steroids, propranolol and enalapril. Prior to cardiac surgery, computed tomography of the head showed complete resolution of the previously noted frontal hemorrhage. Due to the operative findings, the entire homograft was explanted and the aortic root and ascending aorta replaced with a #23 ATS<sup>®</sup> valve conduit and #26

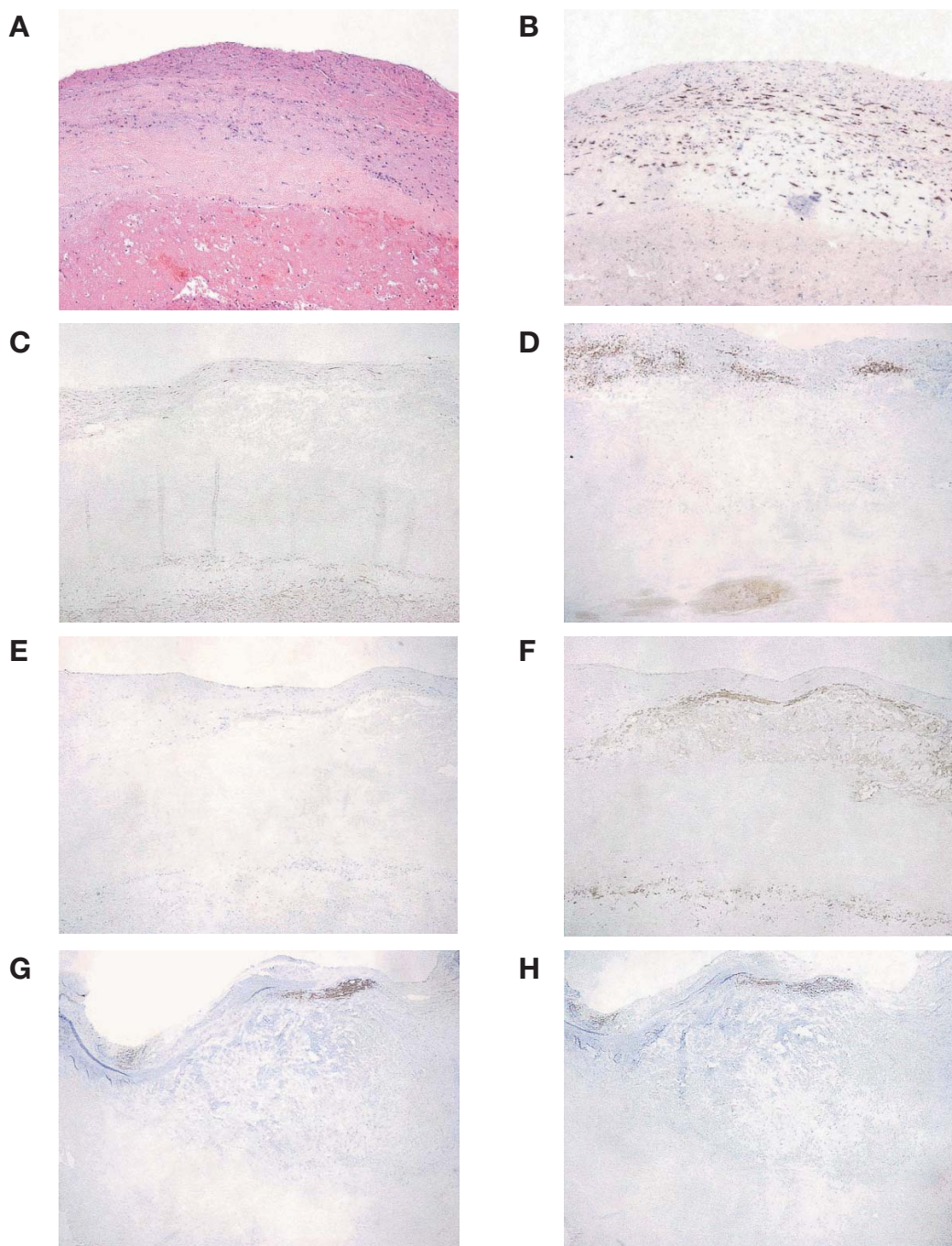


Figure 4: A) Homograft wall (hematoxylin and eosin staining; original magnification,  $\times 100$ ). Note the presence of some 'recellularization' yet hypocellular graft wall, the sheathing or pseudointima formation on lumen side (top) and granulation tissue healing from outside (bottom). Panels (A) to (F) are from the same tissue block location. B) Immunohistochemistry (IHC) for HSP-47, colligin, a molecular collagen chaperone protein (stains brown), indicating that the cells that are actively synthesizing collagen. Note predominance in the migrating wave of cells in the aortic wall. C) IHC for alpha-smooth muscle actin. Note the presence in the lumen sheathing and adventitia, but minimally when cells have in-migrated into the non-viable homograft wall, suggesting a more fibroblast phenotype (i.e., scar). D) IHC for CD5 (T and B lymphocytes), indicating presence throughout the homograft, but with the greatest density on the lumen side at the sheath-homograft wall junction. E) IHC for Factor VIII (endothelium). Note the single cell layer overlying the pseudointima and also scattered throughout the granulation tissue; this is indicative of classic neovascularization as part of the healing/inflammatory response. F) IHC for CD68 (macrophages). The gray-black staining indicates greatest macrophage densities along interfaces of homograft with host cells. G) IHC for CD20 (B lymphocytes) accumulating in the 'endocarditic' lesions in the lumen. H) IHC for CD3 (T lymphocytes) accumulating along with the B lymphocytes demonstrated in (G).

Dacron® graft. The patient had an uneventful postoperative course, with transthoracic echocardiography performed at one week after surgery showing a normally functioning prosthetic valve.

### Operative findings

Gross inspection at surgery showed the aortic homograft external appearance to be unremarkable, with no evidence of calcification or thickening. Following transection of the homograft at the previous suture lines, the most striking macroscopic findings were observed on the intimal surface of the homograft wall tissue, with only minor changes in the valve leaflets. The entire conduit (wall) section of the graft was involved, extending up to the anastomosis but not crossing the native aortic tissue, including the intimal surface surrounding each coronary button. Widespread atherosclerotic-type changes interspersed with hemorrhagic changes were present, resulting in marked thickening of the aortic wall, which measured 4-6 mm. A wide spectrum of atherosclerotic lesions including fatty streaks, stable non-calcified lesions and complex ulcerated plaques with fibrinous exudation were observed. Incision of these plaques produced soft liquid atheroma. In contrast, the homograft valve leaflets appeared relatively spared, with translucent free edges becoming more opaque towards the annulus. Two of the valve leaflets showed fibrin and thrombus predominantly within the sinuses, resulting in asymmetric leaflet retraction and central aortic insufficiency.

### Microscopic changes

The vegetations consisted microscopically of platelet-fibrin thrombi that were partially hyalinized, indicative of early organization (Fig. 2). These thrombi did not appear infective in that they contained few leukocytes, and tissue stains for bacteria and fungal organisms were negative. Cultures of the vegetations were also negative. The architecture of the valve remained intact and there was no evidence of focal calcification on the valve leaflets, even with special stains (Alizarin red).

Microscopic examination of the homograft aorta showed a wide variation of changes, ranging from normal intima to various degrees of atherosclerosis; these included fatty streaks and raised fibrinous plaque. Within some of the lipid-rich atheromatous lesions there was evidence of recent intra-plaque hemorrhage with focal rupture of the fibrous caps and associated recent mural thrombus (Fig. 3). Examination of the media showed a largely acellular wall with smooth muscle appearing non-viable on hematoxylin and eosin (H&E)-stained sections. However, elastic tissue was present in normal amounts and distribution within the media, with the exception of one focal area of

medial disruption and hemorrhage that was associated with an overlying intimal plaque that exhibited rupture and hemorrhage. The adventitia was densely fibrotic and contained focal collections of lymphocytes.

Immunohistochemical studies (Fig. 4) were performed utilizing B lymphocytes (CD20), T lymphocytes (CD3 and CD5), macrophages (CD68), smooth muscle cells (alpha-smooth muscle actin), and endothelial cells (Factor VIII). Neither the valve leaflets nor the associated leaflet thrombus contained B or T cells. In samples of the aorta from the homograft, scattered intimal T cells and macrophages were present within fatty streaks, as well as the raised atheromata typical of that seen in atherosclerosis. Focal collections of lymphocytes within the adventitia consisted of both B and T cells, and macrophages were also scattered within the adventitia. In addition, linear collections of macrophages were found along the border (interfaced between the media and adventitia). Only rare smooth muscle cells in the media stained with alpha-smooth muscle actin. Factor VIII-positive endothelial cells covered the intimal surface of the valve leaflets but were focally absent from the intimal surface of the aortic homograft.

### Discussion

The findings of the present case provided a number of unique and important clinical and pathological observations. As far as the present authors are aware, this is the first report of 'pseudo' marantic endocarditis in an adult cryopreserved allograft aortic valve recipient. The sterile aortitis caused cerebral emboli, a classic presentation for NBTE endocarditis. The pathological process was extremely rapid and confined predominantly to the wall of the homograft. Unlike adults with porcine xenografts or infants in whom fibrocalcific degeneration of the allograft valve leaflets is the most prominent finding, the degenerative process in the present patient resulted in extensive changes of atherosclerosis in the wall of the aortic homograft. The presence of numerous macrophages and both T and B lymphocytes in the aortic wall supports the concept that the process was inflammatory and likely immune driven.

Aortic valve replacement with cryopreserved aortic allograft tissue has resulted in favorable long-term results in adults, with greater than 90% freedom from reoperation at 10 years (1-6). In adults aged less than 60 years, longevity of the valve is superior to that of porcine xenografts and to freshly stored allografts (5). These exhibit excellent hemodynamic profiles with a low incidence of endocarditis or thromboembolic events (1). Whilst degenerative changes are relatively rare in adults with cryopreserved conduits, they occur

more frequently in patients aged under 60 years, as exemplified by the present patient in whom the allograft was inserted at the age of 35 years (5). However, in contrast to the study of Doty et al. (1), where the mean time to explantation was 6.9 years, severe valvular incompetence occurred rapidly in the present patient, necessitating explantation of the allograft at 14 months after surgery. This time course was more similar to the experiences in neonates and young infants, which are often attributed to the robust immune system and elevated calcium metabolism in these young subjects.

Several large studies have demonstrated that aortic valve allografts are associated with a very low incidence of infective endocarditis and thromboembolism (1-7). The present patient had a large frontal hemorrhage secondary to embolization of friable atherosclerotic and thrombotic material present on complex plaques on the intimal surface of the wall of the graft. Numerous blood cultures and pathological stains revealed no evidence of bacterial endocarditis on the valve leaflets. Cerebral angiography failed to demonstrate a mycotic or congenital aneurysm or arteriovenous malformation. As far as the present authors are aware, this is the first report of NBTE embolization secondary to degeneration of the wall of an aortic allograft.

The pathological features of explanted dysfunctional cryopreserved allografts in infants, children and young adults have been described and differ markedly from those of the present patient (4-8). The most striking findings in infants and young children are extensive calcification of both the conduit wall and leaflets whereby the leaflets are thickened, calcified and retracted and, in many cases, absent (4). In young adults, calcification appears more prominent in the arterial wall than the leaflets, yet leaflet changes are often present and include thickening, retraction, and focal calcification with loss of trilaminar structure, reduced cellularity, and absent endothelial cells (8).

In contrast, the pathological changes in the present patient were confined to the wall of the allograft, with only minor changes seen in the leaflets. Macroscopically, the leaflets were well preserved with no evidence of calcification microscopically. Small non-infective thrombi were seen on two of the leaflets at the sinotubular junction, and these appeared to represent an extension of the pathologic process in the aortic wall. The entire wall of the graft manifested severe atherosclerotic changes, with various stages of plaque development including complicated ulcerated plaques with associated thrombi formation. The loss of structural support to the leaflets by the severe pathological changes in the wall resulted in leaflet prolapse, retraction and incomplete closure producing severe aortic

regurgitation. There is no vaso vasorum in semilunar valve leaflets, and thus exposure to host inflammatory attack occurs via the bloodstream flowing over the leaflets. In contrast, the surgical healing of homograft wall tends to occur from the outside (adventitial) in towards the lumen and appears as granulation tissue, including neovascularization which exposes all elements of the wall to macrophages and lymphocytes and thus, potentially, the entire inflammatory/immune cascade.

Numerous hypotheses have been proposed to explain the phenomenon of accelerated calcification in children and young adults with cryopreserved allografts, including both non-immune and immune factors. Blood group incompatibility is associated with premature homograft failure (although both donor and recipient were type A in this case). A robust serum antibody response directed to HLA class I and II antigens typically appears within one month and persists for at least three years (3,10-12). Pathologic studies conducted in infants and children have shown increased numbers of both T cells and macrophages in the leaflets and walls of explanted allografts (8). T cells appear the likely culprits of allograft injury as they secrete numerous cytokines and interferon which are both pro-inflammatory and cytotoxic, and have been shown to mediate valve destruction in the experimental model (13-16). Increased calcific destruction of allografts in children may be secondary to a more virulent T-cell response.

The pathologic findings in the present patient support the hypothesis that allograft failure was mediated by an immune process differing from usual fibrocalcific degeneration, neointima formation and vascularized neoadventitia found in mature fibrous tissue (13,17). The findings support the hypothesis that the aortitis in this patient was immune-driven and had resulted in verucous atheromatous disease, reminiscent of the classical features of NBTE (marantic) endocarditis and consistent with the presumed etiologic autoimmune process (18). Autoimmune, inflammatory and sterile immunogenic heart valve diseases are well recognized: for example, rheumatic, Kawasaki's disease, carcinoid, seronegative spondyloarthropathies associated valvular disease with +HLA-B27 antigen and negative rheumatoid factor, juvenile rheumatoid arthritis, NBTE associated with malignant wasting disease (the usual patients for whom the term marantic is currently applied), and classic Libman-Sacks *marantic* endocarditis associated with systemic lupus erythematosus, marked clinically by thromboembolism (18,19).

The precipitating factor which initiated this peculiar form of allograft degeneration is unknown. Ross (20) has postulated that atherosclerosis is an immune disorder in which endothelial dysfunction is the trigger-

ing event. Similarly, circulatory cytokines such as tumor necrosis factor and interleukin-1 have been proposed as 'pathogens' for NBTEs. Rapid and severe atypical degeneration of the wall of an aortic allograft in the present adult resulted in secondary leaflet dysfunction causing severe aortic regurgitation and the classical presentation of sterile endocarditis with cerebral embolization. The absence of any previous report of the clinical presentation of symptomatic NBTE developing on a homograft aortic valve is somewhat surprising, since immune and inflammatory host responses to homograft valves have been repeatedly documented (9,11,13-16,21). Cryopreserved allograft valve transplants can now be listed as one etiology for a marantic or NBTE type of endocarditis with sterile vegetations associated with platelet rich thrombotic depositions and accelerated atherosclerotic-type lesions.

### References

1. Doty JR, Salazar JD, Liddicoat JR, Flores JH, Doty DB. Aortic valve replacement with cryopreserved aortic allograft: Ten-year experience. *J Thorac Cardiovasc Surg* 1998;115:371-380
2. Bando K, Danielson GK, Schaff HV, Mair DD, Julrud PR, Puga FJ. Outcome of pulmonary and aortic homografts for right ventricular outflow tract obstruction. *J Thorac Cardiovasc Surg* 1995;109:509-518
3. Christenson JT, Vala D, Sierra J, Beghetti M, Kalangos A. Blood group incompatibility and accelerated homograft fibrocalcification. *J Thorac Cardiovasc Surg* 2004;127:242-250
4. Clarke DR, Campbell DN, Hayward AR, Bishop DA. Degeneration of aortic valve allografts in young recipients. *J Thorac Cardiovasc Surg* 1993;105:934-942
5. Yap CH, Skillington PD, Matalanis G, et al. Anti-HLA antibodies after cryopreserved allograft valve implantation does not predict valve dysfunction at three-year follow up. *J Heart Valve Dis* 2006;15:540-544
6. McGiffin DC, Galbraith AJ, O'Brian MF, et al. An analysis of valve re-replacement after aortic valve replacement with biologic devices. *J Thorac Cardiovasc Surg* 1997;113:311-318
7. Kirklin JK, Smith D, Novick W, et al. Long-term function of cryopreserved aortic homografts: A ten year study. *J Thorac Cardiovasc Surg* 1993;106:154-166
8. Koolerger DR, Hazelkamp MG, de Heer E, et al. The pathology of fresh and cryopreserved homograft heart valves: An analysis of forty explanted homograft valves. *J Thorac Cardiovasc Surg* 2002;124:689-697
9. Shaddy RE, Hawkins JA. Immunology and failure of valved allografts in children. *Ann Thorac Surg* 2002;74:1271-1275
10. Baskett RJF, Nanton MA, Warren AE, Ross DB. Human leukocyte antigen-DR and ABO mismatch are associated with accelerated homograft valve failure in children; implications for therapeutic interventions. *J Thorac Cardiovasc Surg* 2003;126:232-239
11. Hawkins JA, Breinholt JP, Lambert LM, et al. Class I and Class II anti-HLA antibodies after implantation of cryopreserved allograft material in pediatric patients. *J Thorac Cardiovasc Surg* 2000;119:324-330
12. Hogan P, Duplock L, Green M, et al. Human aortic valve allografts elicit a donor specific immune response. *J Thorac Cardiovasc Surg* 1996;112:1260-1267
13. Hopkins RA. *Cardiac Reconstructions with Allograft Tissues*. Springer-Verlag, New York, 2005
14. Hopkins RA. Tissue engineering of heart valves. Decellularized valve scaffolds. *Circulation* 2005;111:2712-2714
15. Hawkins JA, Hillman ND, Lambert LM, et al. Immunogenicity of decellularized cryopreserved allografts in pediatric cardiac surgery: Comparison with standard cryopreserved allografts. *J Thorac Cardiovasc Surg* 2003;126:247-253
16. Rieder E, Seebacher G, Kasimir M-T, et al. Tissue engineering of heart valves. Decellularized porcine and human valve scaffolds differ importantly in residual potential to attract monocytes. *Circulation* 2005;111:2792-2797
17. Yacoub MH, Klieverik LM, Melina G, et al. An evaluation of the Ross operation in adults. *J Heart Valve Dis* 2006;15:531-539
18. Libman E, Sacks B. A hitherto undescribed form of valvular and mural endocarditis. *Arch Intern Med* 1924;33:701-737
19. Lopez JA, Ross RS, Fishbein MC, Seigel RJ. Nonbacterial thrombotic endocarditis: A review. *Am Heart J* 1987;773-776
20. Ross R. Atherosclerosis - an inflammatory disease. *N Engl J Med* 1999;340:115-126
21. Ketchedjian A, Krueger P, Lukoff H, et al. Ovine panel reactive antibody assay of HLA response to allograft bioengineered vascular scaffolds. *J Thorac Cardiovasc Surg* 2005;129:159-166