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Factors influencing cryopreserved allograft heart valve degeneration

A thesis by

Cheng-Hon Yap (MBBS)

This thesis is being submitted in total fulfilment of the requirements for the degree of Master of Surgery (by research).

Submitted: September 2006

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ABSTRACT

Heart valve replacement is becoming more commonplace in developed nations. Despite this the ideal valve prosthesis has not been found. The allograft valve has been used for over 40 years and remains an important prosthesis with many advantages. However, like other biological valve prosthesis, they have a finite durability. The causes of allograft valve degeneration are still unknown. The study aims to identify factors associated with cryopreserved allograft valve degeneration. Knowledge of such factors will improve our understanding of the potential causes and mechanisms of allograft heart valve degeneration.

A historical cohort study was conducted on 115 adult recipients of cryopreserved aortic and pulmonary allograft valves who had valves implanted between 1/06/1998 and 31/03/2003 at 9 hospitals in the state of Victoria, Australia. Recipients were human leukocyte antigen (HLA) typed and tested for anti-HLA antibodies by enzyme-linked immunosorbent assay (ELISA) and lymphocytotoxicity (CDC) methods. Specificities of recipient anti-HLA class I antibodies were defined by CDC testing against a panel of T-lymphocytes from 80 blood donors. Donor valve HLA typing was performed on stored donor deoxyribonucleic acid samples using molecular methods (sequencing and sequence-specific oligonucleotide typing). Valve function at most recent echocardiographic follow-up was examined for an association with the following variables: HLA A, B and DR donor-recipient mismatch, recipient anti-HLA class I and II antibody status, donor-specific anti-HLA class I antibody status, valve

ischaemic time, donor age, recipient age, ABO blood group donor-recipient match and allograft size.

Mean recipient age was 45 years (18-75 years), 75% were male. 74 pulmonary (62 Ross and 12 non-Ross procedure) and 41 aortic allografts were implanted. Echocardiographic follow-up was 96% complete (110/115), at a mean of 41 (± 18) months, range 6-85. Median allograft ischemic time was 31 hours, range 20-48. Longer ischaemic time and younger recipient age were associated with valve dysfunction. HLA A, B or DR mismatch, HLA class 1 mismatch, total HLA mismatch, donor age, ABO mismatch and allograft size were not associated with valve dysfunction. Serum samples were taken at a mean of 36 months (range 3 - 65 months) after allograft implantation. By ELISA testing 96% (87/92) of patients had anti-HLA class I antibodies, and 82% (75/92) had anti-HLA class II antibodies. By CDC testing 68% (61/90) of patients were positive for anti-HLA class I antibody. Where donor HLA type was defined, 72% (49/68) were positive for anti-HLA class I antibodies by CDC and 54% (37/68) of recipients had antibodies specific to at least 1 donor HLA class-I antigens. There was no correlation between antibody status and valve dysfunction.

In this study of adult recipients of allograft valves longer ischaemic times and younger patient age predicted valve dysfunction at a mean of 3 years follow-up. Ideally, allograft ischaemic times should be kept below 48 hours. Despite a detectable anti-HLA antibody response against allograft valve antigens, the immunological factors studied (HLA mismatch and anti-HLA antibody status) were not significantly associated with allograft valve dysfunction. The

significance of a host immune response in causing allograft valve degeneration remains unclear.

DECLARATION

This is to certify that

i. the thesis comprises only my original work towards the Master of Surgery except where indicated in the Preface,

ii. due acknowledgement has been made in the text to all other material used,

iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies and appendices.

Signature: …….

PREFACE

The following work was performed in collaboration with the Victorian Transplantation and Immunogenetics Service (VTIS), the Donor Tissue Bank of Victoria (DTBV) and participating surgeons (Peter Skillington, George Matalanis, Bruce Davis, Ian Nixon, Michael Yii, Brain Buxton, and Jai Raman).

The VTIS performed donor and recipient HLA-typing, and the detection and characterisation of anti-HLA antibodies in the recipient serum samples. The DTBV collected donor blood samples during allograft valve harvest for later HLA-typing. Participating surgeons assisted with patient recruitment by providing written invitations to their respective patients. They also assisted with data collection by allowing access to medical records held at the surgeons' consulting rooms.

Parts of Chapters 2 and 3 have been published as review articles in peer-reviewed journals:

- Yap C-H, Yii M. Allograft aortic valve replacement in the adult. Heart, Lung and Circulation 2004; 13(1): 41-51.
- Yap C-H, Yii M. Factors influencing late allograft valve failure. Scandinavian Cardiovascular Journal 2004; 38(6):325-333.

Results arising from this thesis have been published in a peer-reviewed journal:

- Yap CH, Skillington PD, Matalanis G, Davis BB, Tait BD, Hudson F, Ireland L, Nixon I, Yii M. Anti-HLA antibodies after cryopreserved allograft valve implantation does not predict valve dysfunction at 3-year follow-up. Journal of Heart Valve Disease 2006; 15:540-544.

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I am grateful for the support and guidance of my research supervisors, Mr Michael Yii and Professor Wayne Morrison. Michael's energy and enthusiasm for research and the clinical aspects of cardiac surgery has been an inspiration.

To the patients, without which, none of this would have been possible.

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LIST OF ABBREVIATIONS

AVR	Aortic valve replacement
DNA	Deoxyribonucleic acid
DTBV	Donor Tissue Bank of Victoria
HLA	Human leukocyte antigen
IgG	Immunoglobulin gamma
OD	Optical density
PCR	Polymerase chain reaction
PRA	Panel reactive antibody
RVOT	Right ventricular outflow tract
SSO	Sequence-specific oligonucleotide
TOF	Tetralogy of Fallot
VTIS	Victorian Transplantation and Immunogenetics Service

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CHAPTER 1: Introduction

1.1 Study background

Heart valve replacement is becoming more commonplace in developed nations.[1] However, the search for the ideal valve replacement prosthesis continues. Allograft heart valves have been used since 1964 for the surgical treatment of patients with valvular and congenital heart disease. Whilst allograft valves offer the benefits of near-perfect haemodynamics, freedom from thromboembolism and an intrinsic resistance to infection, these valves have a limited lifespan. Despite their use for over 40 years, little is known regarding the causes of allograft valve degeneration.

Numerous factors are thought to contribute to allograft valve degeneration. Many surgeons now believe that host immune-mediated destruction of valves is an important cause of allograft valve failure. However, this remains unproven as there is little evidence to suggest that such a host immune response leads to clinically significant valve dysfunction.

Although the allograft valve is currently in widespread use, the causes of allograft valve degeneration and the role of the immune response in this is still unclear. This represents a serious gap in our knowledge which is required to better treat patients with valvular and congenital heart disease.

1.2 Aim and scope of study

The aim of the study is to identify the factors associated with cryopreserved allograft valve degeneration in the context of current adult cardiac surgical practice. Knowledge of such factors will improve our understanding of the potential causes and mechanisms of allograft heart valve degeneration.

The study is limited to examining valve allografts in two settings:

- aortic valve allografts implanted in the aortic position to replace a diseased aortic valve or root, and
- pulmonary valve allografts, implanted in the pulmonary position to replace a diseased pulmonary valve or reconstruct the right ventricular outflow tract.

Mitral valve allografts and non-valvular allograft material are not examined in this study.

The allografts studied were all cryopreserved prior to use in an attempt to preserve cellular and connective tissue integrity. Fresh allografts, or those that are antibiotic-treated and stored at $4^{o}C$, are less frequently used worldwide and not used in the state of Victoria. They were thus not examined in this study. No attempt at modification of the allografts (e.g. decellularisation or cellular seeding) was performed prior to implantation in this study.

Paediatric patients are important recipients of allograft valves. This study only examines the adult recipients of allograft valves. It is recognised that allografts implanted in paediatric patients may be subjected to different degenerative factors such as growth and enhanced immune reaction, as evidenced by their accelerated degeneration.

1.3 Overview of the study

To achieve the above aim, the next two chapters provide the background to the study. Chapter 2 gives an overview of prosthetic heart valves, and then examines the history and current status of allograft valve use, with a focus on the advantages and limitations of allograft valves. Finally, the chapter describes the patterns of allograft valve dysfunction. Chapter 3 reviews the factors that are implicated in valvular degeneration. In particular, the current view on the role of the immune system in mediating allograft valve failure is discussed.

Based on the knowledge of the current use of allografts and the various factors implicated in valvular degeneration outlined in Chapters 2 and 3, Chapter 4 describes a study method for investigating the causes of allograft valve degeneration. The details of a historical cohort study design are given. Chapter 5 examines the results of this study in the aortic and pulmonary allograft valve setting. Then, in light of the study design and results outlined in the preceding two chapters, Chapter 6 evaluates the results of the study. Finally, the study conclusions are drawn in Chapter 7.

CHAPTER 2: The Allograft Valve

2.1 Introduction

The allograft valve and the causes of its degeneration are the subjects of this study. This chapter aims to describe the current place of allograft valves as a valve replacement conduit in the field of cardiac surgery.

Section 2.2 provides an overview of prosthetic heart valves. It describes the options available to the surgeon when replacing a diseased valve at surgery. Section 2.3 reviews the history of allograft valve replacement. Section 2.4 reviews the current use of allograft valves. In particular, it describes the advantages and rationale for its use, and reviews the current published results obtained with the use of allograft valves. Finally, section 2.5 describes the patterns of allograft valve degeneration. It highlights the different modes of failure seen in aortic and pulmonary allograft valves.

2.2 Overview of prosthetic heart valves

The number of valve replacements continues to increase in developed nations, including Australia.[1] This is largely due to the aging population and overall improvements in medical care. However, the search for the ideal valve replacement prosthesis continues. The characteristics of an ideal valve replacement conduit were defined by Harken in the 1950s during the infancy of valve replacement surgery and have remained essentially unchanged.[2] A valve prosthesis should be non-thrombogenic, non-traumatic to the blood elements,

offer no resistance to physiological flows, be durable and permanent, resistant to infection, quiet and technically practical to insert.

Prosthetic heart valves used to replace diseased heart valves at surgery can be divided into two groups: mechanical and biological (bioprosthetic).[3] Mechanical prostheses are manufactured entirely from man-made materials, mainly of metals or carbon alloys. Bioprostheses are composed primarily of material that originated as living tissue. They include porcine aortic valves, valves manufactured from bovine pericardium, valves transplanted from other human beings (allografts), and autografts from the patient. Bioprostheses may be stented (stented heterografts) or unstented (unstented heterografts, allograft and autograft). Commonly used prosthetic heart valves are summarised in Table 2.1 below. More recently, tissue-engineered valves have emerged.[4,5] Although still undergoing preliminary clinical trials, they aim to combine the proven advantages of unstented heterografts and allografts with improved durability, through the process of autologous seeding of a valve tissue matrix.

The major advantage of a mechanical valve is an extremely low rate of structural deterioration. Most series have not observed any such complication.[6,7] The disadvantages are primarily related to the need for anticoagulation therapy; there is an increased incidence of bleeding and requisite lifestyle limitations.[8] The incidence of major bleeding or death related to anticoagulation therapy is in the order of 1-2 percent per patient year.[9,10] In children or the active younger adult the freedom to participate in sport and to lead a full active lifestyle free from the worries and complications of anticoagulation is invaluable. Hence, the impact of restricting younger patients on anticoagulation from leading full active lives

cannot be underestimated. Management of anticoagulation during pregnancy poses a difficult problem, as the patient and foetus are both at risk of serious complications. There is a real risk of valve thrombosis in the patient,[11,12] and warfarin is known to have teratogenic effects on the foetus.[13] Additionally, some mechanical valves show poor haemodynamic performance which can result in late ventricular dysfunction.[14,15]

The advantage of a bioprosthesis is freedom from anticoagulation therapy. Additionally, the rate of structural valve deterioration in patients over 65 years of age is lower than in patients under 65, especially in the aortic position. In patients over 65 years undergoing aortic valve replacement with a porcine bioprosthesis, the rate of structural deterioration is less than 10 percent at 10 years. In the older patient, the dual effect of freedom from anticoagulation and improved durability is particularly advantageous as the risk of major bleeding from anticoagulation therapy, with resultant serious morbidity or death, is markedly increased in the older age-group.[16,17] The disadvantage of the bioprosthetic valve is a higher rate of structural valve deterioration and consequent need for reoperation, particularly in patients under 65 years or age.[8,7,18]

The allograft may be more durable than heterograft prosthesis. In the aortic position approximately 30 to 35 percent of heterograft prostheses[3,6,7] and 10 to 30 percent of allograft prostheses fail within 10 to 15 years of implantation.[19-23] With both types of prostheses failure is inevitable if the patient lives long enough.

If the patient has an indication for anticoagulation therapy for any reason, for instance atrial fibrillation or presence of a mechanical valve in another position, the major advantage of a biological valve is reduced substantially.

Biological valves have a much higher rate of structural deterioration when implanted in patients with renal failure, those on haemodialysis, and those with hypercalcaemia. Paediatric and adolescent patients who are still growing have a high risk of accelerated biological valve calcification.[8]

Other factors such as valve availability, cost, local surgical skill and experience, valve related noise and patient preference must also be considered. Since there is no single ideal valve choice, consideration of these issues in the context of an individual patient will allow selection of a suitable valve.

Table 2.1. Types of prosthetic heart valves. [24]

Type	Manufacturer	Model	Year of first clinical use
Mechanical			
Caged ball	Baxter-Edwards	Starr-Edwards	1965
Tilting disk	Medtronic	Medtronic-Hall	1977
	Medical Inc	Omniscience	1978
Bileaflet	St. Jude	St. Jude	1977
	Baxter-Edwards	Duromedics	1982
	Carbomedics	Carbomedics	1986
Biological			
Porcine	Baxter-Edwards	Carpentier-Edwards (CE) Standard, CE SupraAnnular	1971, 1982
	Medtronic	Hancock Standard, Hancock Modified Orifice	1970, 1978
	St. Jude	Toronto Stentless	1991
	Medtronic	Freestyle	1992
Bovine pericardium	Baxter-Edwards	CE	1982
Allograft	Non-commercial, Cryolife		1962 1984
Autograft	Non-commercial	Pulmonary autograft	1967

2.3 History of allograft valve use

Ross of Guy's Hospital in London[25] and Barratt-Boyes of Green Lane Hospital in Auckland[26] independently performed the first orthotopic allograft aortic valve replacements in 1962. This was based on earlier reports by Murray[27] and Kerwin[28] who inserted allograft aortic valves in the descending thoracic aorta of patients with severe aortic incompetence. Such heterotopic placement of aortic valve allografts showed reasonable function up to six years. These early valves were fresh untreated allografts implanted shortly after collection. However, unpredictable supply necessitated the use of sterilization and preservation techniques to allow valve storage. These methods of sterilization and preservation included freeze-drying,[29,30] irradiation,[29] chemical preservation with beta-propiolactone,[29] buffered formaldehyde[31] and ethylene oxide.[26] Such early methods led to a high level of early failure due to cusp rupture.[32] Many centres subsequently abandoned this procedure due to early failures, technical difficulties and an inconsistent supply of suitable valves.

The late 1960s saw early methods of preservation replaced by antibiotic sterilization and storage in culture medium at 4^{o}C.[33-35] It was hoped that the physiological use of antibiotics and nutrient media would maintain some viability of donor valves with a resultant improvement in valve durability. Angell[36] and O'Brien[37] pioneered the use of cryopreservation and storage in liquid nitrogen in the early 1970s. This allowed indefinite storage of donor valves thereby reducing wastage of this limited resource. It also allowed valve banking which permitted a full range of valve sizes to be available for use.

There has been a renewed interest in aortic valve replacement (AVR) using the allograft since the late 1980s as good mid to long-term results of cryopreserved[38] and antibiotic sterilised valves were published.[39-41] Furthermore, since 1984 the commercial availability of cryopreserved allografts from CryoLife Inc. has reduced the problem of availability.

Autologous valves were used for valve replacement surgery on the basis that allografts may provoke a deleterious immune response. Animal studies showing that the autologous pulmonary valve could be successfully transposed to the mitral position were performed by Lower and Shumway of Stanford in 1961.[42] Pillsbury and Shumway of Stanford subsequently showed in 1966 that the autologous pulmonary valve could be used to replace the aortic valve in the dog.[43] Ross applied this work to humans reporting in 1967 the replacement of diseased aortic valves using the pulmonary valve autograft in 14 patients.[44] An aortic or pulmonary valve allograft (or occasionally, a xenograft or autologous pericardial valve) was implanted in the pulmonary position to replace the pulmonary autograft. This procedure has since been termed the Ross procedure or pulmonary autograft procedure. It was hoped that because of its close structural similarity to the normal aortic valve, its perfect viability, and its autologous nature, the pulmonary valve would be an ideal aortic valve replacement. Many surgeons have avoided this procedure due to its technical demands, the long bypass time required and due to the fact that it is essentially a “double valve replacement” for disease of a single valve, with the potential for creating pulmonary valve disease. However, there has been renewed interest in the Ross Procedure since the late 1980s when reports confirming its excellent long-term results began to emerge.[41,45]

2.4 Current practice and results of allograft valve replacements

The choices of valve replacement prosthesis currently available are mechanical or tissue. Tissue valves consist of stented xenograft, unstented xenograft and the allograft. Despite the known finite durability of tissue valves, the long-term results have been satisfactory, particularly in older patients, patients with a limited life expectancy, and those undergoing valve replacements in the aortic position. Amongst tissue valves, allograft valves continue to hold a place in valve replacement surgery with proven durability out to 15-20 years.[19,20] Additionally, they offer benefits in valve surgery for endocarditis.[46-48] Most of the evidence favouring the use of allograft valves in the literature is based on the extensive experience with aortic valve allografts in the aortic position. With several exceptions, these results and principles can be applied to pulmonary allografts. The following two sub-sections outline the current practice and use of aortic and pulmonary allograft valves in the treatment of heart valve disease.

2.4.1 Allograft aortic valve replacement

Primary aortic valve replacement using the allograft as the valve replacement conduit of choice has been employed by several hospitals continuously since the 1960s.[19,20,41,49] Limited supply and lack of experience with the use of allografts has prevented the wider use of allografts in many centres. However, in the past two decades the availability of commercially available cryopreserved allografts (CryoLife Inc. Marietta, Georgia, USA) and encouraging results from long-term series have led to greater use of valvular allografts in the aortic position.

From a haemodynamic perspective, aortic allografts have consistently shown minimal transvalvular gradients in vivo. This is identical to or only slightly higher than native valves and significantly lower than those seen in stented bioprosthesis and mechanical devices.[15,50-53] The lower transvalvular gradients may be clinically significant in patients with a small annulus or in larger patients with an undersized replacement valve. Use of the allograft valve preserves the aortic root complex, which plays an important role in maintaining the intricate relationship between valve leaflet and aortic wall distensibility. This acts uniquely to maintain optimal flow in coronary arteries during both systole and diastole. The superior haemodynamic performance of the allograft translates to more complete regression of ventricular hypertrophy and greater improvement in left ventricular function after allograft aortic valve replacement (AAVR), compared to after mechanical valve or stented bioprosthetic valve replacement.[14,54,55] It may also equate to better long-term survival and could also explain the lower incidence of sudden death after allograft replacement,[41,49] compared to mechanical valve replacement. Recent increase in use of unstented and low-profile prosthetic valves

represents attempts to extend these haemodynamic benefits to bioprosthetic and mechanical prosthesis.[56,57]

The allograft valve has long been known to have an intrinsic resistance to infection. The risk of endocarditis after AAVR is low in contrast to after prosthetic valve replacement. Recently reported series have shown 92-98% freedom from endocarditis at 10 years and 83-95% at 20 years (Table 2.2). The post-operative incidence of endocarditis shows an even distribution over time[38,41] unlike that for prosthetic valve endocarditis, which peaks in the first six months post-operatively[58] and has a high mortality of 20-50%.[59] It is also the valve replacement device of choice in the setting of active aortic valve endocarditis as the risk of recurrent endocarditis is lower and overall survival higher when an allograft aortic valve (AAV), as compared to a prosthetic valve, is used to replace an infected native or prosthetic valve.[46,47,60,61] Furthermore, the root replacement method of AAVR allows aortic root repair in those cases with extensive root destruction or aortoventricular discontinuity.[48,59]

The allograft is considered non-thrombogenic, regardless of the method of preservation. Anticoagulation is not routinely employed in patients post-AAVR. This affords an active lifestyle free from haemorrhagic or valve-related thromboembolic complications. Published figures for freedom from thromboembolism at 10 and 20 years are 89-100% and 80-99% respectively (Table 2.2). The true valve-related rate of thromboembolism is likely overestimated in these figures for three reasons.[20] Firstly, accepted guidelines for reporting complications after valve replacement requires any vascular event to be recorded “unless proved to have resulted from another cause”.[62] This means that

unless a neurovascular or peripheral embolic event is shown to be due to haemorrhage or large vessel thrombus, it must be attributed to the valve. Secondly, thromboembolism is partially a patient dependent event with a low baseline incidence in the normal population. Barratt-Boyes suggested that strokes in allograft valve patients should not be attributed to the allograft valve itself as clots have never been seen on valves at any reoperation, and these events may simply represent the background incidence in the patient cohort.[49] It is conceivable that the true allograft valve-related rate of thromboembolism is zero. Finally, many AAVR patients within these reported series undergo concomitant cardiac procedures which may independently increase their thromboembolic risks. For instance, O'Brien noted a 92% 15-year actuarial freedom from thromboembolism in AAVR patients without mitral valve surgery, compared to 75% in those undergoing concomitant mitral valve surgery.[19]

Hazard analysis shows that thromboembolic risk is greatest in the immediate post-operative period, presumably associated with calcium, air and thrombus embolisation from the surgical field.[38] Lund and colleagues examined the risk factors for thromboembolism in his series of 618 patients. In addition to the well-recognized risk factors of age and hypertension, he identified long harvest time (duration between donor death to harvest of valve), as an independent risk factor for embolisation.[20] This could be explained by the loss of endothelial cells, rendering the valve potentially thrombogenic.

Outcomes are assessed by rate of early mortality, overall survival, structural valve failure, and need for redo AVR. Reported early mortality rates (30-day mortality) of 1.1 to 8.0% are low and comparable to that of xenograft or

mechanical valve replacements (Table 2.3). Early deaths are usually unrelated to valve failure or dysfunction, with the leading cause of early mortality being non-valvular cardiac failure.[19,20]

Subgroup analyses of long-running series show that cardiac surgery has become safer over the years, with early mortality rates dropping accordingly. O'Brien reported an early mortality rate of 3% overall, 8.9% between 1969 and 1975, and 1.13% between 1985 and 1998.[19] Subgroup analysis reveals an expected lower early mortality in low risk subgroups, such as patients who undergo primary allograft AVR with no other concomitant procedures. In Kirklin's series of 178 patients, a relatively high overall early mortality rate of 8.0% was noted. However, in this series patients undergoing primary isolated AAVR (n = 92) had an early mortality of only 1%.[63] Hence, for the majority of AAVR patients today who undergo a primary isolated procedure, a low early mortality rate of less than 3% can be expected.

Overall survival is consistently reported in AAVR series (Table 2.4). Survival is usually determined by non-valve patient-related factors and is not indicative of valve function.[64] Hence, its usefulness is limited and it cannot be used to make meaningful comparisons between different valves. Direct comparisons of allograft valve failure between series are difficult as they differ in patient selection, methods of sterilization and preservation, surgical techniques and duration of follow-up. A further confounding factor is the different ways in which valve failure is reported: valve-related deaths,[41,64] reoperation for structural deterioration,[19] reoperation for any cause,[21 19] reoperation for valve-related causes, [64] redo AVR,[20] primary tissue failure,[20,41] degenerative valve failure,[21] structural

valve degeneration[19] and significant valve incompetence.[49] Careful examination of their exact definitions is required to determine if comparisons can be made between series. For instance, "primary tissue failure" is most commonly defined as valve dysfunction requiring reoperation or causing death. However, Langley[23] uses "valve failure" instead, while Lund[20] uses "primary tissue failure" to include survivors with clinical, echocardiographic and angiographic evidence of moderate to severe valve failure. Internationally agreed guidelines for reporting morbidity and mortality after cardiac valvular surgery now exist.[62] With adherence to these guidelines we can look forward to more meaningful comparisons to be made between tissue valve series in the future.

The main reason for valve failure requiring repeat AVR is aortic regurgitation secondary to structural degeneration. Infective endocarditis and early failure due to technical errors are minor causes of valve failure. Freedom from repeat aortic valve replacement for any cause at 5, 10, 15, 20 and 25 years are 93-98%, 79-92%, 55-76%, 35-50% and 24-31% respectively (Table 2.4). This demonstrates that the aortic allograft valve has a high risk of failure requiring reoperation beyond 15 years. Hence, series that fail to reach significant numbers of patients past 15 years should be viewed with caution as they may not show the true failure rate of the allograft valve.

There is evidence to suggest that the viable allograft (homovital and cryopreserved) is superior to the non-viable antibiotic-sterilised allograft stored at four degrees Centigrade. The Harefield Hospital series of Lund and Yacoub reported freedom from primary tissue failure at 10 and 15 years of 71% for the homovital allograft compared to 61% and 32% for the antibiotic sterilised

allograft ($p < 0.05$).[20] They also showed a survival benefit with 77% survival in patients receiving the homovital allograft and 46% survival in the non-viable allograft group ($p < 0.05$).[20] O'Brien's Brisbane series had been reporting an impressive superiority in favour of the viable cryopreserved valve over the non-viable antibiotic-sterilised allograft stored at four degrees Centigrade.[65,66] However a more recent report with follow-up to 29 years showed that the superiority was evident only within the second decade, there being no difference by 20 years.[19]

Table 2.2. Endocarditis and thromboembolism after aortic allograft replacement.

Series	Era	Patients	Max/mean follow-up (years)	Freedom from endocarditis (%) *	Freedom from thromboembolism (%) *
Harefield[20]	1969-93	618	27.1/ 10.1	- / 93/ 91/ 89/ 89	- / 89/ 85/ 80/ 77
Brisbane[19]	1969-98	1022	29/ 7.3	97-99/ 92-96/ 88-93/ 86-89/ -	98/ 94/ 92/ 83/ 79 † 88/ 82/ 75/ - / - ‡
Salt Lake City[64]	1985-96	117	11 / -	- / 98/ - / - / -	- /100/- / - / -
Southampton[23]	1973-94	249	21 / 12.4	- / 98/ 96/ 95/ -	
London[41]	1964-86	555	21/ -	- / - / - / 83/ -	-/99 /99 /99 / -
Birmingham[63]	1981-91	178	9.2/ 4.0	3 events	1 event

* Actuarial rates at 5/10/15/20/25 years. † AVR ± CABG. ‡ AVR + Mitral valve surgery

Table 2.3. Early mortality and overall survival with allograft aortic valve replacement. Series subgroups shown in italics.

Series	Era	Patients	Preservation *	Operative method†	30-day mortality (%)	Overall Survival (%)‡
Harefield[19]	1969-1993	618	AB(479), CP(12), HV(127)	SC(551), RR(67)	5.0	84/ 67/ 48/ 35/ 26
Brisbane[18]	1969-1998	1022	AB (124), CP(898)	SC(635), RR(352), CI(35)	3.0	90/ 77/ 60/ 42/ 19
	1969-1975	*124*	AB	*SC*	8.9	
	1975-1995	*546*	CP	*SC(511), CI(35)*	2.9	
	1985-1998	*352*	CP	*RR*	1.1	
Salt Lake City [64]	1985-1996	117	CP	SC(106), RR(12)	3.4	76% at 10 yrs
Southampton [23]	1973-1994	249	AB	SC	2.4	- / 78/ 66/ 54/ -
Southampton [22]	1973-1983	200	AB	SC	1.5	- / 81/ 68/ 58/ -
Birmingham [63]	1981-1991	178	CP	SC(155), RR(23)	8.0	87/ 85 at 8 yrs
London[41]	1964-1986	555	AB(337), CP(218)	SC		52% at 20 yrs
Auckland[49]	1968-1974	252	AB	SC	6.0	77/ 57/ 38 at 14 yrs
Rotterdam[67]	1987-2001	267	CP	SC(95) RR(180)	5.5	87/ 73 at 9yrs

* AB = Antibiotic sterilised and stored in culture medium at 4° C. CP = Cryopreserved. HV = Homovital.

† RR = root replacement, SC = subcoronary, CI = cylinder implantation.

‡ Overall survival at 5/10/15/20/25 years.

Table 2.4. Long-term valve-related outcome after allograft aortic valve replacement. Series subgroups shown in italics.

Series	Era	No. of Patients	Mean/median age (yr)	Preservation *	Operative method †	Max / mean follow-up (years)	Freedom from repeat AVR, any cause (%) ‡	Freedom from valve-related mortality (%) ‡	Freedom from structural valve failure (%) ‡ ‖	Freedom from primary tissue failure (%) ‡ ¶
Harefield[20]	1969-1993	618	51 ± 16		RR(67), SC(551)	27.1 / 10.1	- / 81/ 55/ 35/ 31	-	-	- /62/34/18/14 §
	1980-1993	*127*	-	*HV*	-	*17.1 / -*	-	-	-	*- /71/71/ - / -*
	1969-1993	*491*	-	*AB(479), CP*	-	-	-	-	-	*- /61/32/ - / -*
Harefield[21]	1980-1993	275	45.8 ± 19	HV	RR(128), SC(147)	14 / 4.8	98/ 91/ - / - / -	-	94/89/ - / - / -	-
Brisbane[19]	1969-1998	1022	-			29 / 7.3		-	-	-
	1969-1975	*124*	*47.2*	*AB*	*SC*	-	*98/ 84/ 58/ 50/ 24*	-	-	-
	1975-1995	*546*	*48.7*	*CP*	*SC(511),CI(35)*	-	*96/ 87/ 76/ 48/ -*	-	-	-
	1985-1998	*352*	*43.1*	*CP*	*RR*	-	*96/ 83/ - / - / -*	-	-	-
Southampton[22]	1973-1983	200	50.0 ± 14.0	AB	SC	24 / 15.6	- / 86/ 70/ 39/ -	- / 98/ 95/ 95/ -	- / 81/ 62/ 31/ -	-/ 81/ 60/ 32/ -
Salt Lake City[64]	1985-1996	117	45.6	CP	SC(106), RR(12)	11 / 4.6	- / 92/ -/ -/ -	93% at 10 yrs	-	-
London[41]	1964-1986	555	40.5 ± 14.0	FD, AB, CP	SC	21/ -	-	67% at 20 yrs	-	- / 57 / - / 12/ -
Rotterdam[67]	1987-2001	267	46.1 ± 16	CP(268), HV	SC(95), RR(180)	13.8 / 4.8	90 / 77(9 yrs)	-	-	-
Auckland[49]	1968-1974	252	51.1	AB	SC	16.5 / 10.8	93/ 79 / - / - / -	-	95/ 78/ - / -/ -	-

* AB = Antibiotic sterilised and stored in culture medium at 4° C. CP = Cryopreserved. HV = Homovital. FD = Freeze-dried.

† RR = root replacement, SC = subcoronary, CI = cylinder implantation.

‡ Freedom from event at 5/10/15/20/25 years.

‖ Defined as moderate or severe valve malfunction discovered at clinical examination, echocardiography, reoperation or post mortem examination. Patients with endocarditis and technical failures excluded.

¶ Defined as valve dysfunction, either requiring reoperation or causing death (unless otherwise stated).

§ Defined as: significant regurgitation (at least grade 3/4) or stenosis (≥ 35 mm Hg) at angiography or Doppler echocardiography, discovered at redo AVR or post-mortem examination, or when following clinical criteria fulfilled: diastolic murmur, wide pulse pressure (>50 mm Hg), increased cardiothoracic index (>0.5), and increasing left ventricular hypertrophy and strain pattern electrocardiogram, all in the absence of infectious endocarditis.

2.4.2 Allograft reconstruction of the right ventricular outflow tract

Allograft valved conduits are used for reconstruction of the right ventricular outflow tract (RVOT) in paediatric patients with congenital heart disease, such as pulmonary atresia,[68] truncus arteriosus,[69] transposition of the great vessels,[70] Tetralogy of Fallot (TOF)[71] and many other complex congenital lesions.[72-74] In the adult patient with congenital heart disease, definitive correction of the congenital defect has usually been performed in childhood. The allograft valved conduit is then used for RVOT conduit replacement, as the conduit used in childhood at the time of definitive repair fails and requires re-replacement. The advantages of the allograft over prosthetic valved conduits in patients with congenital heart disease include technical ease of implantation as they are soft and flexible,[75] moulding easily to the patient's cardiac tissue and improving haemostasis[76]; better haemodynamics thus improving right ventricular function post-operatively[76,77]; and the potential to utilise branches of the allograft to reconstruct distal pulmonary artery stenosis.[77,78]

Pulmonary and aortic allografts are also used in both the adult and the paediatric population for the replacement of the harvested pulmonary valve and artery in the Ross Procedure.[44] In this procedure the reconstruction of the right ventricular outflow tract (RVOT) can be achieved with any prosthetic valve depending on the technique employed. In Ross's early series, RVOT reconstruction was performed with xenograft valved Dacron conduits, autologous pericardial valved and non-valved conduits, autologous fascia lata conduits and aortic or pulmonary allografts. The majority of patients in Ross's early series received the aortic allograft.[79] More recently the pulmonary allograft has become the conduit of choice to reconstruct the RVOT in the Ross procedure. It is used exclusively in all reported contemporary series.

The Ross procedure is based on the premise that the pulmonary valve is anatomically identical to the aortic valve. It has the dual advantage of being autologous and therefore non-immunogenic, and completely viable allowing for potential growth in the young recipient. The pulmonary autograft in the aortic position is very durable compared to other biological prosthesis. Need for replacement of the autograft valve in reported series is very low to non-existent.[79-82] Histological analysis of explanted autografts have shown a full complement of living cells with no evidence of calcification, thus suggesting preserved growth potential.[45,83] Echocardiographic studies of children who have had the Ross procedure showing increase in autograft diameter provide further evidence for growth.[84-87]

The development of allograft dysfunction in the RVOT and the consequent need for re-replacement of the allograft is a major concern with the Ross procedure. Allografts in the RVOT show a slower rate of degeneration than in the higher pressure aortic position. The most reliable data on the long-term performance of allografts in the pulmonary position comes from Ross's pioneer series. This landmark series reported on 131 Ross procedure hospital survivors with a follow-up of 9-26 years, mean 20 years.[80] Of this patient cohort, 113 received a valve allograft to reconstruct the RVOT, 107 aortic and 6 pulmonary allografts. Ten and twenty-year freedom from reoperation on the allograft was 89% and 80%, respectively. Indication for allograft reoperation was 2 early allograft re-operations for infection, and 20 late re-operations, for stenosis in 15, regurgitation in 4, and operation damage in 1. Many valves in this series were sterilised with irradiation or ethylene oxide which are now known to have deleterious effects on valve performance.[80] Hence, contemporary use of fresh or cryopreserved allografts may show lower rates of reoperation.

Table 2.5 summarises the need for re-replacement of the allograft in the RVOT in contemporary series. These freedom from reoperation results need to be interpreted with caution as they are influenced by the average age of the recipients in each series as well as the preoperative diagnosis of the patients. Series with larger numbers of paediatric patients with complex congenital heart disease will show greater rates of re-replacement of the RVOT allograft since allograft failure is accelerated in the young recipient[88] and in those with extra-anatomic (non-Ross procedure) implantation.[89-91]

In contrast to allografts in the aortic position, allografts in the RVOT tend to fail by stenosis.[89,90,92-95] Most series show this trend with the predominant indication for valve reoperation being allograft valvular or conduit stenosis. As an example, Gerestein and co-workers reported a series of 316 allografts implanted in the RVOT in which 21 reoperations for structural valve failure were performed over mean follow-up duration of 4 years; 19 patients failed by stenosis and 2 by regurgitation.[89]

While rates of reoperation for the RVOT allograft may be relatively low, the degree of allograft dysfunction may be underestimated if one looks at reoperation rates alone. Echocardiographic studies reveal that pulmonary allografts exhibit early levels of dysfunction in up to 30 percent of Ross procedure patients.[52,96-98] Such pulmonary allograft valve dysfunction is likely to be well tolerated for many years as long as pulmonary artery pressure remains low.[99] Hence, reoperation is not required in most cases of pulmonary allograft dysfunction as patients remain asymptomatic. Additionally, in patients in whom severe allograft failure develops necessitating reoperation on the RVOT allograft, the operation appears to be a less complicated procedure, especially in the setting of a previous Ross procedure where a morphologically normal right ventricular outflow tract and pulmonary artery exists.[100]

In summary, whilst a large variation in RVOT allograft durability exists (Table 2.5), depending on the ages of the recipient and the nature and complexity of the underlying cardiac disease (complex congenital heart disease vs. Ross procedure), a significant number of patients undergo reoperation on their failing RVOT allograft. The predominant indication for reoperation is valvular or conduit stenosis causing ventricular dysfunction or symptoms.

Table 2.5. Reoperation for allograft valve failure in the right ventricular outflow tract.

Series	Era	No. of Patients	Age (yr) Mean /median	Type of allograft (P= pulmonary) (A= aortic)	Max / mean or median follow-up (years)	Freedom from reoperation of the allograft (any cause) +/- valve- related deaths
Ross Registry[101]	1987-1996	2523	29	Unspecified	25 (max)	96% at 5 yrs 91% at 10 yrs 88% at 15 yrs 84% at 20 yrs
Netherlands[102]	1988-2000	341	26	P (329) A (12)	12.5 / 4.0	96% at 7 yrs
Rotterdam[89]	1986-99	297	18	P (246) A (70)	12 / 4	91% at 5 yrs 87% at 7 yrs
Oklahoma[103]	1986-99	328	20	P	11.5 / 3	90% at 8 years
Oklahoma[90]	1985-97	331	14	P (304) A (27)	11.2 / 3.8	90% at 5 yrs 82% at 8 years
Duke[88]	1985-99	185	53 <1 yr 46 1-10 yr 86 > 10 yr	A (18) P (163)	14 (max)	At 5 years 25% for pts <1 yr 61% for pts 1-10 yr 81% for pts > 10 yr
London[80]	1967-84	113	32	A (107) P (6)	26 / 20	89% at 10 yrs 80% at 20 yrs
Toronto [92]	1985-90	219	5.0	P (132) A (87)	2.4 (mean)	85% at 3 years 55% at 5 years
Leuven[91]	1987-96	187	25	P (153) A (34)	10 / 2.8	90% at 5 years
Cincinnati[94]	1985-90	89	4.3	P (54) A (41)	5.3 / 2.6	81% at 4.4 years

Denver[77]	1985-91	115	3.5	P	6.7 / 3.3	92% at 6 yrs
Texas[104]	1990-2000	191	39	P	10 (max)	98%
Los Angeles[87]	1992-99	111	16	P	7.6 / 3.6	97%
New York [99]	1987-97	145	43	P	10 / 2.5	100 %
Brussels[105]	1991-97	80	31	P	6.3 / 2.7	100%
Mayo[95]	1985-93	326	12.4	P (118) A (230)	8.8 / 2.9	94%(pulmonary), 70%(aortic) at 5 years
Wisconsin[106]	1985-99	205	4.4	P (150) A (70)	13 / 3.6	74% at 5 years 54% at 10 years
London[107]	1971-99	405	6.8	P (94) A (293)	23 / 5.4	84% at 5 years 58% at 10 years 31% at 15 years

2.5 Patterns of allograft dysfunction

Allograft valves fail by stenosis, regurgitation or both. Early valvular dysfunction is usually caused by technical errors.[45,79] It manifests as immediate valvular regurgitation and may be due to factors such as allograft malposition or suture line dehiscence. This problem has been reduced to some degree with greater use of the root replacement and inclusion cylinder methods of implantation[108-110] and the recognition that a learning curve applies to allograft valve implantation, especially with the subcoronary method of implantation.[111-113] Furthermore, any misalignment of valvular leaflet and sinus geometry due to technical errors at implantation may cause increased stress on the valve-root complex and thus predispose the allograft to accelerated late degeneration.

Infective endocarditis is another important cause for valve failure. It carries a high mortality.[58] As mentioned previously, allograft valves are intrinsically resistant to infection and are seen by many surgeons as the valve replacement conduit of choice when an infected native or prosthetic valve requires replacement. Nevertheless, implanted allografts show a very low and constant rate of infection. Linearised rates of 0.2 - 1.0 percent per year have been reported.[114] In the setting of endocarditis allograft valve failure occurs by acute valvular regurgitation. The need for replacement can be acute in the setting of active endocarditis with severe valvular regurgitation causing cardiac failure, cardiogenic shock or overwhelming sepsis. Alternatively, the need for replacement may be delayed with progressive valvular dysfunction following an episode of treated endocarditis.

Most cases of allograft dysfunction are not related to technical errors or infective endocarditis. They manifest as late progressive dysfunction, over months to years, and are known variously in the literature as “primary tissue failure”[20,22] or “structural

valvular deterioration".[115] The cause for this late degeneration is likely to be multifactorial. The remainder of this chapter and the focus of this thesis explore this category of allograft valve dysfunction.

2.5.1 Allografts in the aortic position

Late degenerative aortic allograft failure occurs predominantly by progressive regurgitation; stenosis is uncommon.[116,117] As indicated by the freedom from reoperation rates previously mentioned and echocardiographic studies, it occurs in a time related manner.[113,116]

An important cross-sectional echocardiographic follow-up study by O'Brien and colleagues described the late echocardiographic profile of 570 aortic allograft patients. [116] This study is unique in the high numbers of patients and the completeness of follow-up. At an average time to an echocardiogram of 6.8 years after aortic allograft replacement (standard deviation 4.1 years; range 1.0 to 22.9 years) only 28% of patients were free from any allograft dysfunction. 54% had grade 1 dysfunction (44% regurgitation, 10% stenosis), 13% had grade 2 dysfunction (11% regurgitation, 2% stenosis), 4.6% had severe dysfunction (3.9% had grade 3 or 4 regurgitation, <1% grade 3 stenosis). Hence, whilst 59% of patients had allograft regurgitation, only 13% had any allograft stenosis. Furthermore, the mean time after surgery to the presence of allograft regurgitation increased with higher grades of allograft regurgitation. This confirms that late allograft failure occurs by regurgitation, is a progressive process and increases in severity proportional to the duration from surgery.

2.5.2 Allografts in the RVOT

The pulmonary allograft is similarly susceptible to stenosis, regurgitation, degeneration and calcification.[118] In adult patients undergoing the Ross procedure, pulmonary allograft dysfunction is usually manifest as an early post-operative gradient that is sustained and non-progressive in most patients. Savoye and co-workers reported an echocardiographic follow-up of 100 Ross procedure patients with a mean follow-up of 33 months. Average peak transvalvular gradients were 7.8 and 15.8 mmHg at discharge and most recent follow-up, respectively. Twenty-one patients had gradients between 20 and 40 mmHg and 2 patients had gradients above 40 mmHg.[97] Briand and co-workers reported on 132 patients at a mean follow-up of 2.5 years and showed that the pulmonary valve effective orifice area (EOA) was on average 31% less than immediately after surgery, with the fall in EOA occurring predominantly in the first 6 months after the Ross procedure. Twenty percent of patients developed significant pulmonary stenosis, with reoperation required in 4 due to symptoms or right ventricular dysfunction.[52,119] Linden and Cohn's series of 37 Ross procedure patients showed a pulmonary stenosis rate of 33%, with 11% having high peak gradients of 40-80mmHg. [120]

Raanani and co-workers followed 109 patients after the Ross procedure showing allograft stenosis with a peak transvalvular gradient of more than 20mmHg in 28.5% of patients (30/105) and more than 40mmHg in 3.8% (4/105) of patients. Moderate or severe allograft regurgitation was seen in only 9.5% (10/105) of patients. [98] Carr-White and co-workers reported on 144 consecutive patients after the Ross procedure with up to 7 years follow-up. 17% developed peak transvalvular gradients of 30mmHg or greater across the pulmonary allograft; of these 8 had gradients greater than 50mmHg.

Calculated instantaneous hazard function for allograft stenosis was high in the first year after surgery and rapidly dropped to zero after the fourth year. Four patients required reoperation for allograft stenosis; in all 4 there was macroscopic and microscopic evidence of a chronic adventitial reaction with perivascular infiltration, causing extrinsic compression.[96]

In conclusion, these echocardiographic data and others[121-123] indicate that pulmonary allograft dysfunction occurs predominantly by stenosis in the adult Ross procedure population. It occurs in up to 30% of patients following the Ross procedure and this occurs early on during the post-operative period. The cause for pulmonary allograft stenosis is not known although many authors believe it is due to an immunological phenomenon.[85,98,104,121,124] Its significance remains unclear, as it is largely well-tolerated. However, some patients do develop symptoms or right ventricular dysfunction, requiring replacement of the pulmonary valve. Longer echocardiographic follow-up will reveal if such pulmonary allograft dysfunction increases with time, as it does with aortic allograft dysfunction.

In contrast to the reasonable outcomes in adult pulmonary allograft Ross procedure recipients as outlined above, early failure of the allograft valve in the RVOT is a significant problem in the paediatric population undergoing reconstruction of the RVOT for congenital heart disease or the Ross procedure.[124-128] Allograft dysfunction in the paediatric RVOT occurs by both regurgitation and stenosis. As an example, in Baskett and co-workers echocardiographic follow-up study of 90 allograft valves implanted in the RVOT of 77 children with a mean age at operation of 5.1 years and a mean follow-up period of 4.6 years, a high 67% of valves failed by their echocardiographic criteria.[125] 53% of allografts developed regurgitation of at least grade

2 severity, and 29% of valves developed stenotic gradients of 50mmHg or greater. The failures occurred early, with a mean time to echocardiographic failure of 11.2 months. 18 valves required reoperation, 3 for regurgitation, 9 for stenosis and 6 for both. Freedom from echocardiographic valve failure at 5 years was only 27%. In an older paediatric group of patients with a mean age of 10.4 years, 93 patients were studied following the Ross procedure with a mean follow-up of 3.2 years by Elkins and co-workers.[124] They found that 25% of patients developed peak allograft gradients over 25mmHg and 20% developed allograft regurgitation. Most of those with allograft regurgitation were of mild severity.

It remains unclear why the pulmonary allograft in the RVOT but not the allograft in the aortic position is prone to developing an early stenotic gradient. A proposed hypothesis is that allografts in either position are subjected to the same immunological or inflammatory shrinkage process. However the high pressure systemic circulation prevents or lessens the shrinkage process from being haemodynamically apparent, unlike in the lower pressure pulmonary circulation. A second hypothesis is that the lower pressure RVOT could allow greater leukocyte adhesion to the endothelium of the RVOT because of lower intraluminal shear stress, thus allowing a more severe immune/inflammatory process to occur.[52]

2.6 Conclusion

We have seen that allograft valves have been used since the early 1960s as replacements for diseased heart valves with acceptable outcomes. They continue to hold a place in the current treatment of valvular heart disease because of their near perfect haemodynamics, low thrombogenicity, resistance to infection and flexibility in complex reconstructions.

However, they continue to exhibit limited durability, despite improvements in valve processing methods designed to optimise cellular and connective tissue integrity. This is particularly a problem in paediatric patients who exhibit accelerated degeneration of their allografts. In general, allograft deterioration is a gradual process with greater degrees of valvular dysfunction seen during increasing post-operative intervals.

In order to extend the use of allograft valves we need to understand the causes of allograft degeneration. Apart from the rare cases of infective endocarditis and technical errors of implantation the precise cause of allograft degeneration remains poorly understood.

CHAPTER 3: Factors influencing late valve failure

3.1 Introduction

As discussed in Chapter 2, allograft valves continue to hold a place in the treatment of valve disease. However, the main limitation of allograft valve use at present is the gradual long-term degeneration that leads eventually to the need for re-replacement. Therefore, the key to improving allograft usability is unravelling the causes of allograft valve dysfunction.

This chapter aims to examine the potential causes for allograft valve failure. Section 3.2 provides an overview and framework for discussing the potential contributing factors to allograft failure. Section 3.3 discusses the donor, recipient, technical and valve processing factors that have been implicated in valve failure. Section 3.4 examines the mounting body of evidence which suggest an immunological basis for valve failure.

3.2 Overview

Identifiable causes of allograft failure, such as endocarditis and technical error at time of implantation, are responsible for only a minority of allograft valve failures. The majority of allograft failure occurs in the absence of the above causes. The causes and mechanisms of such valve failure are not clearly understood. It is likely to be complex and multifactorial, with an interaction between:

- Donor factors
- Recipient factors

- Technical factors
- Valve procurement, processing and storage factors
- Host immune response towards the allograft

The interaction and magnitude of factors such as mechanical stress, ischaemia, chemical insults and/or immunological destruction is likely to be highly variable. Nevertheless, it is vital that the role of these factors be identified and understood. This would allow modification of important variables, such as better patient selection, host response, technical/operative and valve processing variables, in order to improve long-term results of allograft valve implantation.

3.3 Donor, recipient, technical and valve processing factors

3.3.1 Donor Factors

Quality of the donor valve

Quality of the donor valve, although never formally assessed in the literature, is an important factor. Most tissue valve banks have strict criteria in assessing allografts for quality. The Donor Tissue Bank of Victoria, for instance, adopts a three category system assessing for tears, lacerations, fenestrations, contusions, atheroma or calcific deposits in the allograft valve or conduit. Allografts with major structural anomalies or pathological lesions are not harvested as they could lead to valve failure. Even minor lesions may cause undue mechanical stress within the valve-root complex of the implanted allograft, leading to accelerated failure over time.

Donor allograft age

Increasing donor allograft age has been reported as a significant factor by several authors.[20,21,49,67,116] Yacoub and co-workers in their series of homovital aortic allografts identified donor age over 55 years as significant.[21] Barratt-Boyes showed freedom from significant valve incompetence of 94% at 10 years when donor age was less than 20 years and 64% when donor age was over 50 years.[49] Lund and co-workers identified donor age greater than 65 as an independent determinant for tissue failure with an odds ratio of 1.75.[20] In view of this some groups do not accept valves from donors greater than 50-60 years of age. Langley's[23] and Kirklin's[63] groups limited allografts to donors up to 50 years and 55-60 years respectively. Consequently, neither series could identify donor age as a significant predictive factor for valve failure. The increasing failure rates with rising donor age are presumably due to age-related structural changes in the valve connective tissue or cellular components. In contrast, Gerestein and co-workers in their analysis of 316 allografts used for RVOT reconstruction identified donor age less than 30 years as a strong risk factor (hazard ratio 3.5) for valve-related reoperation by univariate, but not multivariate, analysis.[89]

Furthermore, it is possible that the donor to recipient age relationship may more accurately predict valve survival than either the donor or recipient age taken in isolation. Lund and co-workers showed in their large aortic allograft valve series that rising donor age minus recipient age may be the more important factor, rather than either donor or recipient age alone, in accurately predicting valve dysfunction.[20]

In conclusion, most authors agree on a donor age cut-off of 50 to 55 years as being significant and would prefer to implant a younger allograft. However, on this

point, supply problems are a great limiting factor in many centres forcing surgeons to accept older donor valves on occasion.

3.3.2 Host Factors

Younger age at operation.

A consistent finding in almost all reported series of allografts in the aortic or pulmonary position is that long-term allograft durability falls with younger age at operation. This relationship of age at operation and allograft durability is linear.

In long-term series of aortic allograft replacement, a younger recipient age has consistently been associated with allograft failure and need for allograft valve reoperation due to accelerated valve degeneration.[19,20,49,67,113] The mode of failure in the young is usually leaflet and annular calcification leading to aortic stenosis. O'Brien's group noted a 10-year freedom from reoperation for structural deterioration of only 47% in the under-20 age group compared to 94% in the over-60 group.[19] Yacoub's group identified a recipient age of less than 30 as a risk factor for late structural degeneration on multivariate analysis.[21]

A similar age trend, identifying younger recipient age as predictive of allograft failure, is seen in allografts implanted in the RVOT.[77,89,91,95,106,125,129,130] For instance, Forbess and co-workers showed that five-year survival of the allograft in the RVOT was 25% for recipients less than 1 year old, 61% for those between 1 and 10 years, and 81% for those older than 10 years.[88]

A similar phenomenon, to a much greater extent, has long been observed in the structural failure of xenograft valves.[131,132] The reason for this age-related degeneration

is unclear. Because of this phenomenon, the optimal valve replacement conduit in children and young adult with aortic valve or root disease may be the pulmonary autograft,[133] with its potential for life-long autograft growth.

Small allograft valve size

Smaller allograft size has frequently been found to be associated with valve failure in studies of RVOT allografts.[88,89,95,106,125] Only one large series of RVOT allografts did not find conduit size as predictive of failure. [107]Two speculative explanations for this finding are possible. Firstly, all implanted allografts undergo a similar destructive process but in smaller allografts the resultant haemodynamic impact is greater, in the form of a higher transvalvular gradient or greater valve incompetence. Secondly, in younger patients smaller valves are more rapidly outgrown with increasing body size. The size of the heart and pulmonary artery increases out of proportion to the implanted allograft, resulting in either progressive valvular incompetence due to annular dilatation and failure of cusp coaptation, or conduit stenosis. This is particularly evident in infants, who generally triple their size within 18 months. As a consequence, most surgeons prefer to implant an oversized allograft conduit in the RVOT.

Extra-anatomic implantation

Allografts implanted in the RVOT in an anatomical position occur in patients undergoing the Ross procedure. In contrast, extra-anatomic implantation occurs in patients requiring RVOT reconstruction for complex congenital heart disease. Allograft survival is greater in the former compared to the latter. [88-91] This is probably because

greater haemodynamic stress is placed on the allograft in patients with congenital heart disease, due to high pulmonary vascular resistance and abnormal pulmonary vascular anatomy. [77,134,135] It could also be related to other technical factors inherent to the non-anatomical placement of the allograft such as utilisation of non-allograft material for conduit extension and sternal compression of the allograft. [130]

3.2.3 Technique of implantation

There are four main surgical techniques for implantation: subcoronary 120-degree rotation, subcoronary intact non-coronary sinus, aortic root replacement or aortic root inclusion (miniroot or cylinder). There has been long-standing controversy amongst surgeons as to which are the best technique.

Those advocating careful subcoronary implantation[136,137] argue that reimplantation of the coronary arteries is unnecessary and the patient's aortic tissue is retained, providing more flexible support for the graft and reducing leaflet stress. Removal of the allograft aorta should reduce long-term root calcification. However, this technique may be technically more demanding, as it requires precise positioning of valve commissures to prevent valve leaflet distortion. A learning curve has been identified with the subcoronary implantation technique. [111-113]

Those preferring root replacement argue that this technique allows preservation of the aortic root complex, maintaining sinotubular and commissural geometry, thereby reducing the possibility of valve distortion at implantation and consequent early regurgitation. [108-110] This technique also treats associated annulus or root pathology, such as dilatation, which may have an adverse long-term effect on an implanted subcoronary allograft valve. This method may be technically easier than subcoronary

implantation, accounting for a reduction in early reoperations for technical failure seen in some studies. [19,67,113] It is also more forgiving with size mismatches. The ability to size up and down by up to 3 mm is an advantage where limited sized allografts are available. [53] However, the allograft aortic root can undergo hardening and shrinkage due to calcification over time, placing stress on the valve leaflets. Inclusion cylinder techniques retain the patient's native aorta, but distortion of the allograft at aortic closure may occur if the space between graft and the natural aortic root is either too great or too small. [64] Furthermore, tamponade of the allograft or coronary ostial dehiscence may occur if suture lines are not secure.

Whilst it is likely that no single technique is applicable to all types of aortic valve and root disease, [138] current evidence is in favour of the root replacement technique. Root replacements, compared to subcoronary implantation, have suggested a reduced incidence of redo AVR, valve failure and reoperation for technical reasons, [19,67] as well as improved survival. [20] Echocardiographic follow-up studies have also shown a greater incidence of aortic allograft regurgitation and higher transvalvular gradients in the subcoronary compared to the root replacement groups at various institutions. [108,110,113,116] Lower incidence of autograft valve reoperation and dysfunction has also been reported with the use of the root replacement technique. [103] Furthermore, the learning curve with subcoronary techniques suggest that early in the surgeon's allograft experience, root replacement may allow more reproducible results. [111]

3.2.4 Valve procurement, processing and storage factors

Current valve procurement, processing and storage methods are designed to maximise cellular and connective tissue viability of allografts. Hence, ischaemic times (duration

between death and cryopreservation, or in the case of homovital and antibiotic-preserved valves, duration between death and implantation) and the use of potentially damaging chemical compounds are minimised.

In relation to ischaemic times, conflicting results are found in the literature. Some authors have found that short duration (less than 4 hours) between allograft procurement and cryopreservation, and shorter antibiotic preservation times prior to cryopreservation, were predictive of valve failure.[139] [125] Others have found that longer duration (in excess of 5.5 hours) of donor death to organ procurement,[106] warm ischaemic time,[140] and duration between valve harvest and implantation[20] are predictive of valve failure. Given that longer ischaemic times reduce cellular viability[90,141] and perhaps connective tissue integrity, long-term allograft durability may be thus expected to be reduced.

In conclusion, considering the above studies there may be an optimal duration of allograft ischaemia which would maximise the long-term durability of the implanted allograft. The duration should be long enough to reduce the detrimental expression of elements responsible for allograft antigenicity, for instance HLA expression by endothelial cells or dendritic antigen-presenting cells, whilst preserving the elements conducive to long-term durability such as the connective-tissue matrix and fibroblast cell viability.

Previous sterilisation techniques such freeze-drying, irradiation, chemical preservation with beta-propiolactone, buffered formaldehyde and ethylene oxide are no longer in use because they were implicated in early valve failure due to cusp rupture. More recently, the use of amphotericin B has also been identified as deleterious to allograft viability.[141] Most centres have thus avoided the use of amphotericin B in their

sterilisation procedure. Currently, most allograft valves implanted are of the cryopreserved variety. Cryopreservation maintains greater than 50% of cellular viability, provided the duration between death and cryopreservation is kept relatively short (<24 to 48 hours).[90] Although difficult to prove, preserved viability with cryopreservation and the use of viable "homovital" valves is believed to be the basis for the improved durability of currently used allograft valves.[19,21]

3.4 Influence of immune system on valve failure

Despite an abundance of evidence that allograft valves are immunogenic[142-146] and that a host immune response to implanted valves exists,[147-151] the clinical relevance of this remains unclear. Hence, several clinically important questions remain unresolved:

- Will immunosuppression of allograft recipients post-operatively improve allograft valve survival?
- Is there an advantage to prospective pre-operative HLA-matching of donors and recipients?
- What modifications can we make to the sterilisation and preservation process to reduce the immunogenicity of allograft valves?

In examining the myriad of evidence for a role of the host immune system in allograft valve failure, perhaps the strongest evidence is a simple observation made by Donald Ross, the pioneer of allograft and autograft valve surgery. Ross suggests that the simple fact that the pulmonary autograft shows improved durability over the aortic allograft in the aortic position (80%[80] vs 50% [19,20] valve survival at 20 yrs) stands alone as evidence for a role of the immune system in mediating allograft valve failure.[80] The evidence for

the role of host immune system in the development of allograft valve dysfunction is examined in this section.

3.4.1 Valve antigenicity & viability

Currently used allografts fall into one of three groups:

- Homovital or fresh valves are harvested using sterile technique from heart transplant recipients or brain-dead multi-organ donors and kept in nutrient medium (e.g. Medium 199) containing extremely low doses of antibiotics. They are stored at 4^{o}C and used at the earliest opportunity. They are considered viable if used within 4-8 days. Valve viability is defined as viability of fibroblasts at time of implantation.
- Cryopreserved valves are obtained from either heart transplant recipients, brain-dead multi organ donors or, as is most often the case, routine autopsies. They are sterilised in an antibiotic and nutrient medium solution for a variable amount of time, usually between 6 and 48 hours, then cryopreserved and stored in liquid nitrogen. They are seen as viable if cryopreserved within two to three days of donor death.[37]
- Antibiotic-sterilised valves stored at 4^{o}C are usually harvested from routine autopsies and kept in an antibiotic and nutrient medium until used at a later date. They are considered non-viable.

The aortic valve consists of endothelial cells and fibroblasts that produce and remodel the valve matrix of collagen, elastin and mucopolysaccharides. These cells maintain the haemodynamically perfect structure of the valve and ensure its non-thrombogenicity. Current sterilisation and preservation techniques aim to ensure the

cellular and molecular matrix remain intact and functional at implantation, thereby allowing for greatest valve durability.[152]

Although the subject of controversy, valve cellular viability is believed by most surgeons to ensure greatest valve durability and provides the rationale for the current use of homovital[21] and cryopreserved[38] valves. It remains unclear as to what constitutes valve viability. Most studies have been centred on culturing fibroblasts as preservation of these cells is felt to be a marker of the integrity of the extracellular matrix. However, as Albertucci points out, "cell viability at implantation does not necessarily mean the valve will remain viable", and "lack of viability does not necessarily mean the valve will not remain functional".[53]

To validate their cryopreservation technique, O'Brien[37] and Angell[36] performed studies showing their cryopreserved valves were viable at implantation. O'Brien cultured fibroblasts from cryopreserved valves on thawing, indicating fibroblast viability at time of implantation, and on valve explantation two months to ten years after implantation. These explanted valves have shown variable but largely preserved leaflet cellularity.[153] Furthermore, chromosomal studies on an explanted valve from a female patient at nine years post-AAVR suggested that persisting cells were from the male donor.[38] O'Brien concluded that the superior durability seen in cryopreserved over antibiotic sterilised valves in his series is due to ongoing leaflet donor cell viability many years post-implantation.[38]

Others have not been able to consistently replicate these findings. Several studies on explanted allograft valves have shown reduced to absent leaflet cellularity, absent endothelial cells and altered gross architecture of valves.[154-156] Such studies suggest that cryopreserved valves become largely acellular post-implantation due to loss

of donor cellularity, but retain their connective-tissue framework that alone maintains the structural basis of function. In-situ hybridization[157] and chromosomal[158] studies have shown leaflet cellularity to be of host origin. This has led some authors to believe that host fibroblast ingrowth after implantation, not persisting donor cells, is responsible for the leaflet cellularity in explanted valves and is responsible for preserving the structure and function of implanted valves. The lack of leaflet cellularity and evidence of host cell invasion both indicate that cellular viability at time of implantation may not be important for the long-term durability of the allograft valve. Hence, the issue of valve viability and durability remains unresolved.

Retaining cellular viability of the allograft has the disadvantage of preserving the antigenic endothelium and dendritic cells of the valve, thereby potentially inducing a destructive recipient immunologic response. This may be akin to the major role played by HLA mismatch and HLA-specific antibodies in rejection of cardiac transplants.[149,159-161] Cellular elements of the allograft valve express HLA antigens. Endothelial cells on allograft valves have been shown to express HLA class I and II antigens,[142,143] and immune stimulatory and adhesion molecules that are known to play a role in inflammatory and rejection responses.[162] Dendritic cells, the antigen presenting cells of the human heart valve within the subendothelial valve matrix, also express HLA class II antigens.[142]

3.4.2 Host-immune response

Given that valvular elements are antigenic, it is not surprising that allograft valves are capable of triggering both cell-mediated and humoral immune responses that are donor-

specific. In vitro studies by Hoekstra showed that fresh and cryopreserved allograft valve leaflets stimulated human peripheral blood lymphocytes in a lymphocyte proliferation assay.[144,145] Hoekstra then studied T lymphocytes in explanted allografts valves and showed cytotoxicity was directed against donor-specific class I and II antigens.[163] Yacoub's group also showed that the endothelial cells and fibroblasts of cardiac valves were immunogenic, in that each stimulated peripheral blood mononuclear cells when co-cultured in vitro.[146] In vivo studies in allograft valve and conduit recipients have consistently shown humoral immune responses directed against donor-specific HLA class I and II antigens.[147-151] Furthermore, this response remained positive for up to 15 years after allograft implantation, suggesting that the presence of allograft material leads to a persistent and prolonged antibody response.[164] In conclusion, recipients of currently used viable allograft valves show donor-specific humoral and cell-mediated immune responses, which may play a role in the subsequent dysfunction of allograft valves.

As previously mentioned, some studies have linked short times between death and cryopreservation (ie. ischaemic time) with long-term allograft dysfunction.[98,139] This would be consistent with the theory of immune-mediated valve dysfunction as it is known that allograft viability[90] and immunogenicity[142] decreases with increasing ischaemic times. This suggests that a clinically significant role for immune-mediated allograft dysfunction exists and that this role is modifiable by defining and applying the optimal duration of allograft ischaemia.

In Stark and co-workers' series of 405 allografts implanted in the RVOT for congenital heart disease it was observed that patients receiving second or subsequent allografts had higher allograft failure rates than those receiving their first allografts.[107] It

is likely that this is caused by an enhanced immune response to the allograft in a patient sensitised by previous allografts. Hence, it may be that patients, particularly in the paediatric population, receiving a second or subsequent allograft should be the target of careful HLA-matching or immunosuppression.

In contrast to human studies, animal studies have shown good direct evidence for immune mediated allograft valve destruction. Moustapha and associates showed that both fresh and cryopreserved cardiac allograft valves in the rat model undergo changes that are characteristic of cell-mediated rejection and lead to structural valve failure. This is in contrast to the syngeneic valves that were used as controls in their study which showed no cellular infiltration and remained structurally preserved. [165] Oei and colleagues showed, in a similar rat allograft model, valve infiltration with recipient helper and cytotoxic T-cells and antigen-presenting dendritic cells occurred within seven days of implantation. Subsequent influx of macrophages was associated with complete stromal cell loss and structural leaflet failure by day 21.[166,167]

Legare and colleagues showed that in a rat model of allograft valve transplantation the immune destruction is T cell-mediated. In their study T-cell deficient rats who received allograft valves showed none of the cellular infiltration or valvular structural deterioration seen in the immune competent rats.[168] They also showed that immune-modulatory measures, such as cyclosporin therapy and anti-integrin monoclonal antibodies are effective in preventing allograft valve structural failure in the rat model.[169]

Despite the abundance of evidence as described above, until recently the magnitude and clinical significance of a destructive immune response in allograft valve degeneration was unclear as no direct association with functional outcomes in humans

had been established. Such direct evidence can only be found by showing that the performance of allograft valves is associated with donor-recipient matching of the major transplantation antigen systems, namely the ABO and HLA histocompatibility systems in humans. This is akin to the well-established role of ABO and HLA matching in solid organ and bone marrow transplantation.[170-173]

3.4.3 ABO blood group compatibility

The A and B histo-blood group antigens are coded on the ABO locus on Chromosome 9. This locus codes for two glycotransferase enzymes which transfer a terminal carbohydrate unit to a core H chain, giving either A or B antigenic properties to the cell membrane. The H chain is a transmembrane saccharide chain that is part of a glycolipid or glycoprotein complex present on the surface of most nucleated cells.[174] The addition of N-acetylgalactosamine to the H chain as the terminal carbohydrate confers blood group A specificity, and the addition of D-galactose confers group B specificity. In humans, A and B antigens are widely distributed on the surface of almost all cells, principally on vascular endothelium, epithelium, primary sensory neurons, bone, cornea, skin, red blood cells and platelets. They are also found within the cells, such as within Golgi apparatus, secretory granules and cell nuclei. Furthermore, these antigens are secreted in plasma and secretions, such as saliva and urine.[175] The importance of the ABO system in transplantation is that humans have antibodies against those ABO antigens absent in their own tissue. These antibodies can then induce an immune rejection process against transplanted tissue expressing foreign A or B antigens. Hence, ABO matching is usually performed in solid organ transplants (ie. kidney, heart and liver) and in bone marrow transplantation.[175]

The issue of ABO incompatibility in allograft valves is unsettled. Some authors have advocated ABO matching of allograft valves.[95,143] However, most series have historically implanted allograft valves without matching donor and recipient for ABO antigens. The many series which have retrospectively examined ABO mismatch have not found it to be a significant predictor for allograft valve degeneration. [21,89,92,95,107,116,124,127,130,139,176] Thus, the need to prospectively ABO match donor and recipients of allograft valves is unresolved and requires further study.

3.4.4 Human leukocyte antigen compatibility in allograft valve surgery

The human leukocyte antigen (HLA) molecules are encoded on chromosome 6 in a region known as the Major Histocompatibility Complex (MHC), which spans over 4 megabases. The MHC contain the 6 major HLA loci which encode structurally similar molecules, known as HLA class I and II molecules based on their distribution, structure, source of peptide antigen and the class of responding T-cells. These molecules function as receptors for peptide fragments (ie. antigens) that are displayed on the cell surface where they are recognised by appropriate T-cells. By facilitating antigen presentation to helper and cytotoxic T-cells they regulate specific immunity and play a vital role in determining rejection of tissue between genetically distinct individuals.[177,178]

There are approximately 20 class I HLA genes, 3 of which, HLA-A, B and C are the main players in regulating antigen presentation. The 3 main class II HLA genes are HLA-DR, DQ and DP. Class I molecules are expressed on most nucleated cells and interact with antigen-specific T cells of the CD8 phenotype, also known as cytotoxic T-cells. In contrast, class II molecules are only expressed by specialised antigen-

presenting cells such as B-cells, dendritic cells and macrophages. The class II molecules specifically interact with the antigen-specific T cells of the CD4 phenotype. These are the helper T-cells which play a central role in the immune system by augmenting macrophage function and promoting the proliferation and differentiation of B cells and cytotoxic T cells.[177]

The importance of HLA-matching in bone marrow and certain solid-organ transplants, such as kidney and heart transplantation, is well established. In the international multi-centre Collaborative Transplant Study a highly significant advantage of HLA matching on patient and graft survival rates was found in the analysis of kidney transplants. Ten years post-transplantation graft survival rates were 17% lower in first cadaver kidney transplants with a complete mismatch (6 HLA-A + B + DR mismatches) compared to those with no mismatch.[170] The effect was similarly strong in first heart transplants where at 3 years graft survival was 84% in those with 0 or 1 mismatch and 71% in those with 6 mismatches.[159] The accepted mechanism behind HLA-mismatch related rejection of allogeneic tissue is a host T-cell response triggered by recognition of non-self class II HLA antigens on antigen-presenting and endothelial cells present in transplanted tissue. This is followed by an amplification phase against class I and II non-self antigens present on most transplanted cells, with a resultant destructive donor-specific cell-mediated and humoral response against graft cells possessing these non-self HLA antigens.[177] There is good evidence for this mechanism applying in allograft valve transplantation in a rat model. As previously mentioned, Legare and associates found that T-cell nude mice did not show valve immune cell infiltration and destruction after allograft valve implantation.[168]

At present allograft valve recipients and cadaveric allograft donors are not routinely tissue-typed, as unlike solid organ transplantation, implantation of allograft valves occurs with no preoperative HLA matching. Fresh allograft valves are antigenic in that valvular endothelial and dendritic cells, and possibly fibroblasts, within allograft valves express HLA class I and II antigens.[142,143] Accounting for the fact that the preservation and storage process may reduce this antigenicity (as described above) by down-regulating HLA antigen expression or by reducing cellular viability, what then is the role of HLA compatibility in the contemporary use of allograft valves? Several single-institution studies have attempted to answer this question with inconsistent results.[179,180] [125,139]

In summary, there is emerging clinical evidence supporting a role for HLA-DR, but not HLA class 1, antigen matching in determining long-term allograft valve performance. This is supported by in-vitro tissue culture studies by Hoekstra and co-workers, which show that HLA-DR matching between responder T-lymphocytes and stimulatory allograft valve pieces and allograft valve endothelial cells reduces the intensity of T-lymphocyte stimulation.[144,145] However, in the absence of studies utilising prospective HLA-matching of allograft valve donor and recipients, the precise role of the immune response in mediating the long-term deterioration in valve function remains unresolved.

3.4.5 Use of immunosuppression

Perhaps the strongest evidence for immune-mediated allograft valve dysfunction comes from the observation that valves of transplanted hearts show no signs of deterioration, with preservation of near-normal architecture and cellularity.[155] This is a result of long-

term immunosuppressive therapy. Convincing evidence in the animal model for the role of immunosuppression exists. Studies in the rat model have shown that cyclosporine therapy may decrease immune cellular infiltration and preserve structural integrity of implanted allograft valves.[169,181] However, the optimal duration of cyclosporin therapy required to arrest allograft valve destruction is unclear. These studies suggest that a 14 or 28 day course is effective whereas a 7 day course is insufficient. Long-courses of immunosuppression are undesirable in the clinical setting due to the risks of infection and malignancy. These risks would have to be balanced against those of accelerated valve failure.

Clinical efforts have been made to use immunosuppressive therapy for the prevention of allograft rejection in paediatric patients.[127,182,183] However, there is no convincing evidence that it is efficacious, and many have abandoned such therapy for fear of deleterious side-effects. Nevertheless, some groups currently use routine immunosuppression in younger[85] and other selected patients deemed at high-risk for allograft valve rejection.[125,181]

3.5 Conclusion

We have seen in this chapter that many host, recipient, surgical and valve processing variables have been implicated in allograft valve failure. Furthermore, there is an accumulating body of evidence which suggests a significant host immune response which may have a role in valve destruction post-allograft valve implantation. The absence of any prospective controlled studies in human subjects to examine these variables precludes the drawing of any definitive conclusions on the causes of allograft valve failure. Given the current “acceptable” results of allograft performance, it is

highly unlikely that any such future studies will be performed due to ethical issues of patient safety and the lack of clinical equipoise.

CHAPTER 4: Study design

4.1 Introduction

As outlined in Chapters 2 and 3, a review of the literature was performed to assess the current level of understanding of the causes of allograft valve failure. This revealed that a probable cause for allograft failure is a recipient-mediated immunological response against the donor allograft valve material. Furthermore, there may be an interaction between immunological and other non-immunological factors. These other factors include allograft size, ABO blood group mismatch, valve ischaemic time, donor age, recipient age and technical factors.

The aim of this historical cohort study was to investigate the causes for cryopreserved allograft heart valve failure. The study involved an historical cohort of patients operated on between 1998 and the commencement of the study in 2003. Data was collected and analysed to evaluate a relationship between variables thought to potentially influence allograft valve function and valve function as assessed by routine post-operative echocardiography. This remainder of this chapter explains in detail the study design.

4.2 Study conception

Allograft valve recipient and cadaveric donors are not routinely HLA-typed, as unlike solid organ transplantation, implantation of allograft valves occurs with no preoperative HLA matching. The possibility of investigating donor-recipient HLA-mismatch to explore the causes of allograft degeneration had been conceived in 1997 by staff of the

Donor Tissue Bank of Victoria (DTBV, Southbank, Melbourne, Australia). To this end, the DTBV in collaboration with the Victorian Transplantation and Immunogenetics Service (VTIS) and several allograft valve surgeons initiated the routine collection of cadaveric donor blood since 1997. The samples were forwarded to the Victorian Transplant and Immunogenetics Service (Parkville, Melbourne, Victoria, Australia) where they were stored for possible future HLA typing. This study was designed to utilise the availability of the stored cadaveric DNA specimens for donor HLA-typing.

The DTBV began collecting cadaveric donor blood samples ine 1997. Allograft valves taken from these cadavers were implanted from early 1998 onwards. Hence, donor HLA-typing data was only potentially available for recipient patients operated on since early 1998.

Clinically significant valvular dysfunction is usually a time-dependent phenomena; it occurs with greater frequency with increasing post-operative duration. Severe valve dysfunction and the consequent need for reoperation is expected to occur with significant frequency beyond 10 years post-operatively. Thus severe valve dysfunction or reoperation for valve failure was not expected to be present with any significant frequency during the post-operative follow-up duration covered by this study. Hence the assessment of valve degeneration was obtained by routine post-operative echocardiographic assessment.

4.3 Participant selection

4.3.1 Inclusion criteria

All adult patients (persons over 18 years of age at time of surgery) who received an allograft heart valve supplied by the DTBV between 1st June 1998 and 31st March 2003, and operated on by participating surgeons, were considered for this study.

4.3.2 Exclusion criteria

Patients whose informed consent for participation was not obtained were not included in the study. Reasons for not obtaining informed consent included: declination to participate, mental incompetence (as judged by the principal investigator), deceased, or inability to contact for consent.

4.3.3 Ethical considerations

No serious ethical issues were identified in the performance of this study. The study was carried out in accordance with National Health & Medical Research Council of Australia guidelines as detailed in *"National Statement on Ethical Conduct in Research Involving Humans", National Health & Medical Research Council of Australia, June 1999.* The study was conducted following approval from the St. Vincent's Hospital Melbourne (SVHM) Human Research Ethics Committee (HREC Protocol 157/02) and the Victorian Institute of Forensic Medicine Ethics Committee.

4.3.4 Patient recruitment

In accordance with SVHM Human Research Ethics Committee recommendations, initial patient contact was made by the participating surgeons in writing inviting the

potential participant to take part in the study. Following this a letter of invitation accompanied by the "Patient Information and Consent Form" (PICF) (see Appendix A) was mailed to each participant. A follow-up telephone call to each potential participant was then made by the principal investigator to discuss the study details and answer any questions. Verbal informed consent to participate in the study was obtained at this time. To participate in the study patients were required to

1. return to the investigators a signed copy of the PICF; and
2. present to a regional commercial pathology laboratory and have 40ml of blood taken for HLA type and antibody testing. This blood specimen was collected in three 10ml acid-citrate-dextrose and one 10ml plain tube. It was then forwarded to the VTIS.

Upon recruitment, the medical records held at surgeon's rooms and the hospital were searched for basic demographic, medical and operative details.

4.3.5 Participating surgeons

Participating surgeons were:

1. Peter Skillington (Royal Melbourne, Epworth and Melbourne Private Hospitals). 110 patients.
2. George Matalanis (Austin, Epworth and Warringal Private Hospitals). 15 patients.
3. Bruce Davis (Alfred and Cabrini Hospitals). 11 patients.
4. Ian Nixon (St. Vincent's Melbourne and St. Vincent's Private Hospitals). 10 patients.

5. Michael Yii (St. Vincent's Melbourne and St. Vincent's Private Hospitals). 8 patients.
6. Brian Buxton (Austin, Epworth and Warringal Private Hospitals). 4 patients.
7. Jai Raman (Austin and Warringal Private Hospitals). 4 patients.

During the study period 18 other patients received DTBV allografts and were operated on by 8 non-participating surgeons. These 18 patients were excluded from the study.

4.4 Study method

4.4.1 Variables studied

The following variables were analysed with regard to post-operative valve function:

i. HLA A, B and DR donor-recipient mismatch
ii. Recipient anti-HLA class I and II antibody status
iii. Donor-specific anti-HLA class I antibody status
iv. ABO blood group donor-recipient match
v. Allograft ischaemic time (ie. time between death and valve harvest)
vi. Donor age
vii. Recipient age
viii. Allograft size

The allograft processing and preservation variables were not studied as these were standardised by the DTBV. However, processing and preservation methods should be considered when reviewing the possible effect of the variables studied on allograft

performance. Hence, a summary of the harvest, processing and cryopreservation protocol used by the DTBV is given in Appendix B.

HLA A, B and DR donor-recipient mismatch

Following recruitment and obtaining written informed consent from the allograft recipient patients, blood samples (30ml acid citrate dextrose, 10 ml plain tubes) were collected from recipient patients for HLA typing and antibody studies. HLA typing of allograft recipients and their corresponding donors was performed by the VTIS using serological and molecular methods. Recipient HLA typing was performed on recipient blood samples collected between May 2003 and April 2004. Stored cadaveric donor blood samples were typed in June of 2004.

Recipient HLA class I typing was performed by standard complement-dependent microlymphocytotoxicity assay[184] using a panel of anti-sera from 140 blood donors defining all the known Class I specificities. T-lymphocytes were isolated with Dynabeads coated with antibody specific for CD8 receptor (Dynabeads® HLA Class I, Dynal Biotech, ASA, Oslo, Norway) and processed according to manufacturer's instructions. Recipient lymphocytes were incubated with antisera at 20-25^{o}C for 40 minutes. Rabbit complement was added followed by a further incubation period of 60 minutes. Lymphocyte viability was assessed with the addition of Ethidium Bromide / Acridine Orange solution. A positive reaction was defined as greater than 10% dead cells per well.

Recipient HLA class II typing was performed by polymerase chain reaction amplification (PCR) sequence-specific oligonucleotide (SSO) typing methods. PCR

amplification of exon 2 was performed, followed by detection of hypervariable regions by a set of oligo-probes specific for sequence differences.

DNA was extracted from cadaveric blood by conventional salting out methods.[185] Donor HLA class I typing was performed by PCR amplification and sequencing methods,[186] using in-house PCR primers and Big Dye terminator chemistry and ABI 3730 48 capillary sequencer (Applied Biosystems Inc., Foster City, CA, USA). Donor HLA- class II typing was performed by PCR SSO methods as previously described.

Recipient and donor HLA types were recorded by converting SSO and sequencing designations back to the corresponding serological equivalents. The degree of HLA mismatch between donor and corresponding recipient was graded as a whole number value between 0 and 2 for each HLA locus. When a donor was homozygous at an A, B or DR locus for a HLA antigen for which the recipient was heterozygous, the degree of mismatch at this locus was designated as 0, and not 1, because there is no functional immune recognition of the donor antigens at this locus by the recipient.

Recipient anti-HLA class I and II antibody status

Recipient anti-HLA class I and II antibody status testing was performed by the VTIS. Ten ml of recipient blood was collected in plain blood tubes for anti-HLA antibody testing. Testing was performed using both qualitative solid phase enzyme-linked immunosorbent assay (ELISA) and by standard lymphocytotoxicity methods for anti-HLA class I antibodies in recipient serum. Testing for anti-HLA class II antibodies was performed by the ELISA method only due to funding restrictions.

Two methods were employed for the detection of recipient HLA class I antibodies because there are known differences in the ability of each test to detect the presence of clinically significant anti-HLA class I antibodies. Previous studies have failed to detect a correlation between allograft valve function using standard microlymphocytotoxicity methods,[179,187] whilst a more recent study using an ELISA method had yielded a positive correlation.[188]

Patient serum was tested for Immunoglobulin gamma (IgG) antibodies against HLA class I antigens using a commercially available qualitative solid phase ELISA (QUIKSCREEN®, GTI Diagnostics, Waukesha WI, USA).[189] Patient serum is added to microwells coated with affinity-purified HLA class I (HLA-A, B and C) glycoproteins, obtained from platelets of White, Black and Hispanic blood donors, allowing antibody, if present, to bind. Unbound antibodies are then washed away. An alkaline phosphatase labeled anti-human globulin reagent (Anti-IgG) is added to the wells and incubated. The unbound Anti-IgG is washed away and the substrate p-nitrophenyl phosphate is added. After a 30-minute incubation period, the reaction is stopped by a sodium hydroxide solution. The optical density (OD) of the colour that develops is measured in spectrophotometer. Test results showing OD values equal to or greater than two times the value obtained for the mean of the negative controls are regarded as positive results.

Patient serum was tested for Immunoglobulin G (IgG) antibodies against HLA class II antigens using a commercially available qualitative solid phase ELISA (B-SCREEN®, GTI Diagnostics, Waukesha WI, USA).[190] The testing procedure is identical to that described for QUIKSCREEN® above, with the exception that the HLA class II glycoproteins are obtained from EBV transformed B lymphocyte cell lines, which were carefully chosen to provide a wide range of HLA class II antigens.

Patient serum was also tested for antibodies against HLA class I antigens using a standard complement-dependent microlymphocytotoxicity technique, as described below. The level of panel reactive antibody (PRA) is the number of wells against which the patient's serum reacted, as a percentage of the total number of wells tested. It is thus an estimate of the frequency of the population against which the patient had an anti-HLA class I antibody. For the qualitative testing for the presence of anti-HLA class I antibody in the recipient serum, a PRA level greater than 5% was taken as a positive result.

Donor-specific anti-HLA class I antibody status

Anti-HLA class I antibody present in the recipient serum was typed using standard complement-dependent microlymphocytotoxicity method. Recipient sera were tested against a panel of T-lymphocytes from 80 blood donors of known HLA-type. The panel covered all known HLA class-I specificities. Recipient serum was incubated with lymphocytes at 20-25^{o}C for 40 minutes. Rabbit complement was added followed by a further incubation period of 60 minutes. Lymphocyte viability was assessed with the addition of Ethidium Bromide / Acridine Orange solution. A positive reaction was defined as greater than 10% dead cells per well. The panel reactive antibody (PRA) level is the number of wells against which the patient's serum reacted, as a percentage of the total number of wells tested. HLA-Class I specificity was assigned if PRA level was above 5%. Specificities were assigned by an experienced laboratory scientist, blinded to the donor HLA type.

Recipient anti-HLA class I antibody specificities were than checked against the corresponding serologically-equivalent donor HLA-class I (A and B) types to ascertain if donor-specific anti-HLA class I antibodies were present.

ABO blood group donor-recipient match

A possible relationship between ABO blood group mismatch and post-operative allograft valve degeneration was investigated. The hospital records of each recipient were examined for ABO blood group type. Records of the DTVB were examined for donor blood group type. A positive match was assigned based on donor and recipient types as follows; all other combinations constituted ABO mismatch:

Donor – O, Recipient – any

Donor – A, Recipient – A or AB

Donor – B, Recipient – B or AB

Donor – AB, Recipient – AB

Allograft ischaemic time

Records at the DTVB were examined for time of death and time of cryopreservation. Where the precise time of death is uncertain, the time the patient was last seen or known to be alive was taken as the time of death. Allograft ischaemic time is defined as the interval between time of death and time of cryopreservation.

Donor age

Donor age was determined from the records at the DTVB. Age was recorded as years at last birthday prior to death.

Recipient age

Recipient age was determined from the medical records held at the surgeon's rooms. Recipient age was taken as the age at time of operation. Age was recorded as years at last birthday prior to surgery.

Allograft size

Allograft size was obtained from the records of the DTBV. Sizing occurs during the harvest process. The diameter of the aortic or pulmonary valve is measured using a graduated teflon cone. The cone is gently inserted into the valve until there is a snug fit and the graduation measurement corresponding to the level of the valve leaflets is read.

4.4.2 Outcomes

The outcome measured was the valve function at most recent post-operative echocardiographic study with regards to:

i. Trans allograft valve mean gradient in mmHg

ii. Degree of allograft valve regurgitation (trivial, mild, moderate, or severe)

The degree of allograft valve dysfunction on echocardiography was categorically classified as follows:

i. Nil dysfunction – Nil or trivial valvular regurgitation. Mean gradient less or equal to 10mmHg.
ii. Mild dysfunction - Mild valvular regurgitation, or mean gradient 11 to 30mmHg.
iii. Moderate dysfunction - Moderate valvular regurgitation, or mean gradient 30 to 50 mmHg.
iv. Severe dysfunction – Severe valvular regurgitation, or mean gradient greater than 50 mmHg.

Echocardiography was performed in the participating patients as part of their routine follow-up as ordered by their treating cardiologist or cardiothoracic surgeons. In the absence of symptoms, routine echocardiography is performed every 1-2 years depending on the individual surgeon or physician preference. No echocardiograms were performed specifically for the purpose of this study. Copies of the most recent post-operative echocardiograms were obtained from the surgeons' rooms, cardiologist's rooms or directly from the echocardiography testing laboratories.

Velocities across the allograft valve were calculated by a continuous-wave Doppler imaging transducer. For determination of the transvalvular pressure gradient, a modified Bernoulli equation was used:

$$PG = 4V^2 \ mmHg$$

where *PG* is the transvalvular pressure gradient and *V* is the transvalvular jet velocity. Hence, the peak pressure gradient, was:

$$PG_{peak} = 4\ V_{max}^{2}\ mmHg$$

where PG_{peak} is the peak transvalvular pressure gradient and V_{max} is the maximal transvalvular jet velocity. The mean transvalvular pressure gradient was determined by averaging the instantaneous gradients over the systolic ejection period. Where mean gradients was not stated in the echocardiogram report, it was estimated from the maximal transvalvular jet velocity using the formula[191]:

$$PG_{mean} = 2.4(V_{max})^{2} + 0.75\ mmHg$$

where PG_{mean} is the mean transvalvular pressure gradient and V_{max} is the maximal transvalvular jet velocity. Allograft valve regurgitation was evaluated with conventional pulsed wave, continuous wave and colour flow Doppler. Semiquantitative assessment of regurgitation (trivial, mild, moderate, or severe) was based on the length and width of the regurgitant jet and the distance it reaches into the left or right ventricular outflow tract.

The outcomes of all allograft recipients with available post-operative echocardiogram reports were analysed. A subgroup analysis was performed on pulmonary allograft recipients. Pulmonary allograft recipients were analysed separately because, as described in section 2.4.2, the incidence and pattern of early allograft dysfunction may be different in this group of patients compared to those who receive aortic allografts.

4.5 Data handling

Data was entered into an inter-relational database created using Microsoft Access version 2000. Data analysis was performed using SPSS version 11.5 (SPSS, Chicago, IL, USA).

Univariate and multivariate analysis was performed to assess for a relationship between each variable and the outcomes. Data was analysed for normality by assessing the frequency histogram and the Kolmgorov-Smirnov statistic. Parametric continuous data was compared using the unpaired t-test. Non-parametric continuous data was compared using the Mann-Whitney log-rank test. Nominal and categorical data was compared with the chi-square test or Fisher's exact test (for data with low expected frequencies). Data was assessed for a correlation between two numerical variables by calculating the Pearson correlation coefficient (r).

Multivariate analysis was performed using linear multiple regression analysis for continuous outcomes (e.g. mean valve gradient) and logistic regression analysis for categorical outcomes (e.g. valve dysfunction, valve regurgitation). Standard, rather than stepwise or sequential, methods were used in that variables were entered simultaneously. In all analyses p values less than 0.05 were considered significant.

CHAPTER 5: Results

5.1 Introduction

Using the study design outlined in Chapter 4, this chapter presents the findings of the study. In the first section the characteristics of the patient cohort under study are described. Patient demographics, valvular pathology and operative details are summarised. In the next section, the results of the most recent echocardiogram are shown with reference to the transvalvular mean gradient, degree of regurgitation and degree of allograft dysfunction. Finally, the variables under study are analysed in relation to the echocardiographic outcomes. Conclusions are then drawn from these results.

5.2 Patient cohort

One hundred and sixty-two patients met the inclusion criteria of the study. Of these, 115 (71%) participated in the study. The remaining 47 were excluded because consent was not obtained for the following reasons:

i. Not contactable, 30.
ii. Declined to participate, 11.
iii. Deceased, 3.
iv. Mental incompetence, 2.
v. Language difficulties, 1.

None of the 115 participants had had their allograft valve explanted or replaced at time of recruitment.

5.2.1 Demographics

There were 83 (72.2%) males and 32 (27.8%) females. Patients were aged 18-75 at time of surgery. Mean age at time of surgery was 44.9 years.

Valve disease was predominantly stenosis in 48 patients (42%), regurgitation in 48 patients (42%) and mixed in 19 patients (16%). The aetiology of valve disease was predominantly congenital unicuspid or bicuspid aortic valve disease. This was the sole aetiology in 86 patients (75%). The remaining 25 % of patients had various other valve etiologies:

i. Active endocarditis in 10 patients.
ii. Congenital heart disease in 7 patients.
iii. Previous endocarditis in 4 patients.
iv. Degenerative RVOT conduit or valve prosthesis in 4 patients.
v. Degenerative aortic valve disease in 3 patients.
vi. Degenerative aortic valve prosthesis in 2 patients.
vii. Rheumatic aortic valve disease in 1 patient.
viii. Traumatic pulmonary valve injury in 1 patient.

Previous cardiac surgery had been performed in 20 patients (17.4%). The procedures are shown in Table 5.1. Allografts had been implanted in 5 patients previously. In the 3 patients who had received an aortic allograft in the aortic position, 2 patients each had had aortic allograft replacements on 2 occasions, for recurrent endocarditis.

With regard to the number of previous cardiac operations in each patient, 16 patients had had a single operation previously. Two patients had had 2 operations previously. The remaining 2 patients had had 3 operations previously.

Table 5.1. Previous cardiac surgery.

Type of operation	***Number of patients***
TOF correction	3
Pulmonary valvotomy	3
Aortic valvotomy	1
Pulmonary valvotomy and patent foramen ovale closure	1
Pulmonary valvotomy and ventricular septal defect closure	1
RVOT reconstruction using aortic allograft and ventricular septal defect closure	1
Ross procedure with pulmonary allograft reconstruction of RVOT	1
AVR using aortic allograft	3
RVOT reconstruction using Dacron conduit and porcine valve prosthesis	1
CABG x2 and AVR using bovine pericardial prosthesis	1
AVR using bovine pericardial prosthesis	1
AVR using mechanical prosthesis	1
Patent ductus arteriosus ligation	1
Coarctation of aorta repair	1

5.2.2 Operative details

Operations of the 115 patients were performed between 3rd June 1998 and the 26th of March 2003. They were performed at the following Victorian hospitals:

i. Epworth Hospital, 29 patients.
ii. Melbourne Private Hospital, 26 patients.
iii. Royal Melbourne Hospital, 24 patients.
iv. St. Vincent's Hospital Melbourne, 11 patients.
v. Cabrini Hospital, 7 patients.
vi. Austin and Repatriation Hospital, 7 patients.
vii. Warringal Private Hospital, 5 patients.
viii. The Alfred Hospital, 3 patients.
ix. St. Vincent's Private Hospital, 3 patients.

The patients were operated on by 7 surgeons. Mr Peter Skillington was the surgeon in 77 cases (67%), Mr George Matalanis in 12, Mr Bruce Davis in 10, Mr Ian Nixon in 8, Mr Michael Yii in 6, Prof. Brian Buxton in 1, and Assoc Prof Jai Raman in 1 case.

Forty one aortic allografts were implanted in the aortic position and 74 pulmonary allografts were inserted in the pulmonary position. Type of operation preformed were

i. Aortic allograft implantation in the aortic position in 41 patients.
ii. Ross procedure in 62 patients.
iii. Pulmonary allograft reconstruction of the RVOT in 12 patients.

Seventy-three patients (63%) had at least one concomitant procedure. The mean number of additional procedures in these patients was 1.4. No concomitant procedures were performed in 42 patients. The various concomitant procedures as outlined in Table 5.2.

Table 5.2. Concomitant procedures.

Procedure	***Number of patients***
Reduction of aortic annulus	48
Tailoring of aneurysmal ascending aorta	14
Replacement of aneurysmal ascending aorta	12
Coronary artery bypass grafting	7
Tailoring of main pulmonary artery (aneurysmal or stenotic)	6
Right ventricular outflow tract enlargement	3
Closure of patent foramen ovale	3
Pulmonary annulus reduction	2
Mitral valve repair	2
Aortic valve replacement	2
Patent ductal arteriosus closure	1
Tricuspid valve repair	1
Pulmonary annulus enlargement	1
Left ventricular outflow tract myomectomy	1

5.3 Echocardiographic follow-up

Echocardiographic follow-up was 95.7% complete, in that 110 of the 115 patients had a recent post-operative echocardiogram available for analysis. The echocardiograms were performed over a 40 month period between 18th March 2002 and 18th July 2005. Mean interval between the date of operation and the date of most recent available

echocardiogram was 40.80 months (standard deviation 18.39, range 6.18 - 84.73 months).

With regard to the 110 patients, 36 patients had aortic valve allografts and 74 had pulmonary valve allografts. Overall mean allograft transvalvular gradients showed a median of 7.00 mmHg, and a range of 1-31 mmHg. Table 5.3 details the echocardiography results. No patients had severe regurgitation. No patients were classified as having severe allograft valve dysfunction (ie. severe regurgitation or mean gradient >50mmHg).

Table 5.3. Echocardiography data at most recent follow-up.

	Entire cohort	Pulmonary allografts	Aortic allografts	
Patient number	110	74	36	
Postoperative duration (months). Mean (standard deviation)	40.8 (±18.4)	42.9 (±17.8)	36.4 (±19.0)	
Mean transvalvular gradient (mmHg) Median (range)	7.0 (1-31)	7.0 (3-31)	6.0 (1-31)	
Regurgitation				
nil or trivial	83 (75.5%)	61 (82.4%)	22 (61.1%)	
mild	23 (20.9%)	10 (13.5%)	13 (36.1%)	
moderate	4 (3.6%)	3 (4.1%)	1 (2.8%)	
severe	0	0	0	
Degree of allograft dysfunction*				
nil	66 (60.0%)	45 (60.8%)	21 (58.3%)	
mild	38 (34.5%)	25 (33.8%)	13 (36.1%)	
moderate	6 (5.5%)	4 (5.4%)	2 (5.6%)	
severe	0	0	0	

* See section 4.3.2 for definitions

5.4 Analysis of variables affecting allograft function

5.4.1 HLA match

All allograft recipients were successfully tissue-typed. However, donor HLA-typing was incomplete in that DNA extraction from the donor samples failed in approximately a third of donor samples. This was thought to be due to delayed sample delivery from the DTVB to the VTIS and prolonged warm ischaemic time of some cadaveric donors resulting in time-related degradation of donor DNA. Hence, HLA matching data was available for 84 donor-recipient pairs at the HLA-A locus, for 81 donor-recipient pairs at the HLA-B locus and for 80 donor-recipient pairs at the HLA-DR locus.

Table 5.4 shows the frequency of HLA mismatch at each of these loci. As expected in a random population the degree of HLA match is low. 52-64% of donor-recipient pairs are completely mismatched, whilst only 7-8% of donor-recipient pairs are matched at each HLA locus.

The presence of allograft dysfunction showed no relationship to the degree of HLA-A, B or DR mismatch (Table 5.5-5.7). However, there was a non-significant trend ($p = 0.25$) for the HLA-DR locus, as 40% (19/47) of patients with complete HLA-DR mismatch showed allograft valve dysfunction compared to 28% (8/29) of patients with 0-1 HLA DR mismatch. There was no relationship between allograft dysfunction and HLA class I mismatch (0-2 vs 3-4 HLA-A and B mismatch) or total HLA mismatch (0-4 vs 5-6 HLA-A, B and DR mismatch). See Table 5.8 and 5.9.

Table 5.4. Degree of HLA-A, B and DR mismatch in allograft valve donor-recipient pairs.

	HLA-A	**HLA-B**	**HLA-DR**
Number for analysis	84	81	80
Degree of mismatch			
0	7 (8.3%)	6 (7.4%)	6 (7.5%)
1	33 (39.3%)	24 (29.6%)	23 (28.8%)
2	44 (52.4%)	51 (63.0%)	51 (63.8%)

Table 5.5. Valve dysfunction and degree of HLA-A mismatch (n=80).

	Degree of HLA-A mismatch	
	0-1 mismatch	*2 mismatches*
No valve dysfunction	24	24
Mild or moderate valve dysfunction	14	18

Chi-square statistic 0.30, p=0.58

Table 5.6. Valve dysfunction and degree of HLA-B mismatch (n=77).

	Degree of HLA-B mismatch	
	0-1 mismatch	*2 mismatches*
No valve dysfunction	17	30
Mild or moderate valve dysfunction	11	19

Chi-square statistic 0.00, p=0.96

Table 5.7. Valve dysfunction and degree of HLA-DR mismatch (n=76).

	Degree of HLA-DR mismatch	
	0-1 mismatch	*2 mismatches*
No valve dysfunction	21	28
Mild or moderate valve dysfunction	8	19

Chi-square statistic 1.29, p=0.25

Table 5.8. Valve dysfunction and degree of HLA-class I (A and B) mismatch (n=75).

	Degree of HLA-class I (A and B) mismatch	
	0-2 mismatch	*3-4 mismatches*
No valve dysfunction	11	34
Mild or moderate valve dysfunction	10	20

Chi-square statistic 0.70, p=0.40

Table 5.9. Valve dysfunction and degree of HLA-class I and II (A, B and DR) mismatch (n=68).

	Degree of HLA-class I and II (A,B and DR) mismatch	
	0-4 mismatch	*5-6 mismatches*
No valve dysfunction	20	22
Mild or moderate valve dysfunction	9	17

Chi-square statistic 1.11, p=0.29

Mean valve gradients did not show a relationship with the degree of HLA mismatch at the A, B or DR locus. Table 5.10 shows the median values of "mean valve gradient" for degrees of mismatch at each of the 3 HLA loci. No difference in the mean valve gradients was observed between those with 0-1 mismatches versus those with complete mismatch at each locus, by the log-rank test (Table 5.10). Furthermore, no difference in mean valve gradients, by log-rank test, was found either according to degree of matching at the HLA-class I (A and B) locus (0-2 vs 3-4 mismatches, p=0.77), or for total HLA (A, B and DR) match (0-4 vs 5-6 mismatch, p=0.94).

Table 5.10. Mean valve gradient and degree of HLA mismatch.

	HLA-A	**HLA-B**	**HLA-DR**
Number for analysis	80	77	76
Mean valve gradient mmHg (median, range)			
0 mismatch	5 (5-31)	10 (5-16)	6.5 (5-9)
1 mismatch	7 (3-31)	7 (2-24)	7 (2-24)
2 mismatch	7 (2-24)	6 (3-31)	7 (3-31)
0-1 vs 2 mismatch by log-rank test, p-value	0.68	0.86	0.53

There was no relationship between the degree of valvular regurgitation and the degree of HLA-mismatch at the A, B or DR locus, by Chi-square testing (Table 5.11-5.13). There was also no relationship between degree of valvular regurgitation and

degree of HLA-class I mismatch (0-2 vs 3-4 HLA-A and B mismatch) or total HLA mismatch (0-4 vs 5-6 HLA-A, B and DR mismatch). See Table 5.14 and 5.15.

Table 5.11. Valvular regurgitation and degree of HLA-A mismatch (n=80).

Degree of valvular regurgitation	Degree of HLA-A mismatch	
	0-1 mismatch	*2 mismatches*
Nil or trivial	29	32
Mild or moderate	9	10

Chi-square statistic 0.00, p=0.99

Table 5.12. Valvular regurgitation and degree of HLA-B mismatch (n=77).

Degree of valvular regurgitation	Degree of HLA-B mismatch	
	0-1 mismatch	*2 mismatches*
Nil or trivial	22	38
Mild or moderate	6	11

Chi-square statistic 0.11, p=0.92

Table 5.13. Valvular regurgitation and degree of HLA-DR mismatch (n=76).

Degree of valvular regurgitation	Degree of HLA-DR mismatch	
	0-1 mismatch	*2 mismatches*
Nil or trivial	24	37
Mild or moderate	5	10

Chi-square statistic 0.18, p=0.67

Table 5.14. Valvular regurgitation and degree of HLA-class I (A and B) mismatch (n=75).

Degree of valvular regurgitation	Degree of HLA-class I (A and B) mismatch	
	0-2 mismatch	*3-4 mismatch*
Nil or trivial	15	43
Mild or moderate	6	11

Chi-square statistic 0.58, p=0.45

Table 5.15. Valvular regurgitation and degree of HLA-class I and II (A, B and DR) mismatch (n=68).

Degree of valvular regurgitation	Degree of HLA-class I and II (A,B and DR) mismatch	
	0-4 mismatch	*5-6 mismatch*
Nil or trivial	24	30
Mild or moderate	5	9

Chi-square statistic 0.35, p=0.56

Subgroup analysis – Pulmonary allografts

In the subgroup of 74 patients who received a pulmonary allograft, there was no significant relationship between HLA mismatch and allograft valve dysfunction (tables C1- C3, in Appendix C). However, there was a non-significant trend (p =0.16) for the HLA-DR locus, in that 46% (16/35) of patients with complete HLA-DR mismatch showed allograft valve dysfunction compared to 25% (4/16) of patients with 0-1 HLA DR mismatch. There was no relationship between mean valve gradient (table C11) or degree of valve regurgitation (table C12-C14) and degree of HLA mismatch.

5.4.2 Anti-HLA antibody status

Anti-HLA antibody status was investigated in 92 of the 115 patients. There was insufficient suitable blood for testing in the remaining patients due to collection error at the pathology laboratory. Blood samples were taken between 3.4 and 64.8 months after allograft implantation. The mean duration between allograft implantation and antibody testing was 35.87 months (SD 16.34, 95% CI 32.48 - 39.25).

By ELISA testing 96% (87/92) of patients were positive for anti-HLA class I antibody, and 82% (75/92) of patients were positive for anti-HLA class II antibody. There was no correlation between ELISA class I or II antibody status and post-operative interval. Testing for anti-HLA class I status by the microlymphocytotoxicity method was performed in 90 patients. After the ELISA testing, there was insufficient serum sample to perform the microlymphocytotoxicity test in the remaining 2 patients. Median PRA was 24.50%, range 0-99%. There was a significant inverse correlation between the PRA level and post-operative interval (Pearson correlation -0.306, p=0.003). Anti-HLA class I positive status was defined as PRA levels greater than 5%. By this method, 68% (61/90) of patients were positive for anti-HLA class I antibody.

There was no difference in the frequency of valve dysfunction according to anti-HLA class I antibody status by microlymphocytotoxicity (positive 22/58 vs negative 15/27, p=0.13) or ELISA (positive 34/82 vs negative 4/5, p=0.16) testing. There was also no difference in the frequency of valve dysfunction according to anti-HLA class II status by ELISA testing (positive 28/70 vs negative 10/17, p=0.16)

Using the log-rank test there was no difference in mean valve gradient according to anti-HLA class I status by microlymphocytotoxicity (p=0.57) or ELISA (p=0.17) testing. There was also no significant difference in mean valve gradient according to

anti-HLA class II antibody status by ELISA (p=0.053) testing. Furthermore, there was no difference in the frequency of mild-moderate valve regurgitation according to anti-HLA class I status by microlymphocytotoxicity (positive 15/58 vs negative 7/27, p=0.99) or ELISA (positive 20/82 vs negative 3/5, p=0.11) testing, or according to anti-HLA class II status (positive 18/70 vs negative 5/17, p=0.76).

Subgroup analysis – Pulmonary allografts

Anti-HLA antibody status was investigated in 61 of the 74 patients who received a pulmonary valve allograft. Blood samples were taken between 5.09 and 64.85 months after allograft implantation. The mean duration between allograft implantation and antibody testing was 36.73 months (SD 15.93, 95% CI 32.65 – 40.82).

By ELISA testing 93% (57/61) of patients were positive for anti-HLA class I antibody, and 75% (46/61) of patients were positive for anti-HLA class II antibody. Testing for anti-HLA class I status by the microlymphocytotoxicity method was performed in 60 patients. After the ELISA testing, there was insufficient serum sample to perform the microlymphocytotoxicity test in 1 patient. Median PRA was 22.00%, range 0-99%. Anti-HLA class I positive status was defined as PRA levels greater than 5%. By this method, 65% (39/60) of patients were positive for anti-HLA class I antibody.

There was no difference in the frequency of valve dysfunction according to anti-HLA class I antibody status by microlymphocytotoxicity (14/39 vs 11/21, p=0.22) or ELISA (23/57 vs 3/4, p=0.30) testing. There was also no difference in the frequency of valve dysfunction according to anti-HLA class II antobody status by ELISA testing (positive 18/46 vs negative 8/15, p=0.33).

Using the log-rank test there was no difference in mean valve gradient according to anti-HLA class I status by microlymphocytotoxicity (p=0.34) or ELISA (p=0.65) testing. There was also no difference in mean valve gradient according to anti-HLA class II status by ELISA (p=0.11) testing. Furthermore, there was no difference in the frequency of mild-moderate valve regurgitation according to anti-HLA class I status by microlymphocytotoxicity (positive 8/39 vs negative 3/21, p=0.55) or ELISA (positive 10/57 vs negative 2/4, p=0.11), or according to anti-HLA class II status (positive 9/46 vs negative 3/15, p=0.97).

5.4.3 Donor-specific anti-HLA class I antibody status

Specificities of anti-HLA class I antibody were determined by the microlymphocytotoxicity method in 90 patients. Corresponding donor HLA specificities were available in 68 of the 90 cases (76%). The remaining 22 donor HLA types were unknown because of failure to extract suitable DNA for typing as mentioned previously in section 5.4.1. The 22 corresponding allograft recipients were thus excluded from the analysis. Appendix D shows anti-HLA class I antibody specificity for the 68 patients tested with known corresponding donor HLA typing.

Figure 5.1 summarises the results. Of the 68 patients, 19 (28%) were anti-HLA class I antibody negative (ie. PRA less or equal to 5%). Of the remaining 49 patients who were anti-HLA class I antibody positive, 37 (54%) had antibodies specific to 1 or more donor HLA class-I antigens. The other 12 patients (18%), who were antibody positive, had non-donor specific anti-HLA class I antibodies. Anti-HLA class I antibodies in 6 of these 12 patients were considered multi-specific, precluding the

determination of specificity. These 6 patients had high PRA levels of between 91 and 99%.

The frequency of valve dysfunction between those patients with donor-specific anti HLA-1 antibodies and those without was similar (15/36 vs 12/28, p=0.92). Mean valve gradient was slightly lower for those with donor-specific antibodies (median 6.5 mmHg) compared to those without donor-specific antibodies (median 8.5 mmHg). The difference was not significant by log-rank testing, p= 0.07. The frequency of mild-moderate valve regurgitation in patients with donor-specific anti-HLA class 1 antibodies was higher at 31% (11/36) compared to those without, 18% (5/28). However, this difference did not reach significance by Chi-square testing, p=0.24.

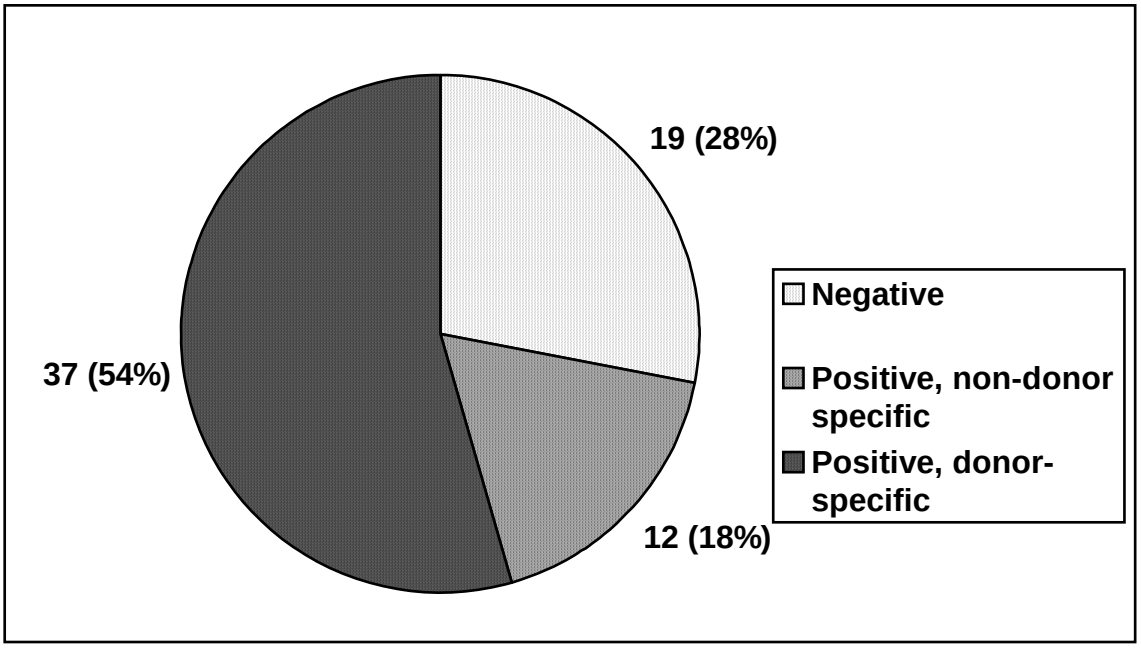


Figure 5.1. Anti-HLA class I antibody status by microlymphocytotoxicity testing (n=68).

Subgroup analysis – Pulmonary allografts

Specificities of anti-HLA class I antibody were determined by the microlymphocytotoxicity method in 60 patients. Corresponding donor HLA specificities were available in 48 of the 60 cases (80%). The remaining 12 donor HLA types were unknown because of failure to extract suitable DNA for typing as mentioned previously. The 12 corresponding allograft recipients were thus excluded from the analysis, leaving 48 available for analysis.

Of the 48 patients, 15 (25%) were anti-HLA class I antibody negative (ie. PRA less or equal to 5%). Of the remaining 33 patients who were anti-HLA class I antibody positive, 25 (52%) had antibodies specific to 1 or more donor HLA class-I antigens. The other 8 patients (17%), who were antibody positive, had non-donor specific anti-HLA class I antibodies. Anti-HLA class I antibodies in 5 of these 8 patients were considered multi-specific, precluding the determination of specificity.

The frequency of valve dysfunction between those patients with donor-specific anti HLA-1 antibodies and those without was similar (10/25 vs 10/23, p=0.81). Mean valve gradient was slightly lower for those with donor-specific antibodies (median 7.0 mmHg) compared to those without donor-specific antibodies (median 8.0 mmHg). The difference was not significant by log-rank testing, p= 0.11. The frequency of mild-moderate valve regurgitation in patients with donor-specific anti-HLA class 1 antibodies was higher at 24% (6/25) compared to those without, 13% (3/23). However, this difference did not reach significance by Chi-square testing, p=0.47.

5.4.4 ABO blood group donor-recipient mismatch

Of the 115 patients in the study, echocardiographic follow-up data was missing in 5 patients and donor ABO type data was unknown in 6 donors. Hence, 104 patients had ABO match and echocardiographic follow-up data available for analysis. 73 (70%) patients were ABO matched with their allograft valve and 31 (30%) were ABO mismatched.

There was no difference in the frequency of allograft valve dysfunction between the ABO matched and mismatched groups (28/73 vs 14/31, p=0.52). Mean valve gradient in the ABO matched (median 7.0 mmHg, range 2-31 mmHg) and mismatched groups (median 6.0 mmHg, range 1-24 mmHg) were similar. There was no significant difference in the mean allograft valve gradient between the ABO matched and mismatched groups, by log-rank test (p = 0.64).

There was no difference in the frequency of mild-moderate valve regurgitation in the ABO matched versus mismatched groups (17/73 vs. 9/31, p=0.54). There was no difference in frequency of ABO mismatch in those with and without mild-moderate valve regurgitation (9/26 vs 22/78, p=0.54).

In the subgroup of pulmonary allografts, there was a non-significant trend towards a greater frequency of allograft valve dysfunction in the ABO mismatched group (11/20 vs16/50, p=0.074). There was no difference in the mean valve gradient between ABO matched and mismatched groups (median 7 vs 7 mmHg, log rank test p=0.80). There was a non-significant trend towards a greater frequency of allograft valve regurgitation in the ABO mismatched group (6/20 vs 6/50, p=0.071).

5.4.5 Allograft ischaemic time

Median ischaemic time was 31.1 hours, with a range of 20.0 - 47.6 hours (n=109). Allograft ischaemic time was higher in those with valve dysfunction. Median ischaemic time was 32.1 hours in those with valve dysfunction compared to 30.0 hours in those without. Log-rank testing showed that the difference in ischaemic times between the groups was significant (p= 0.03). Longer ischaemic time correlated with the presence of valve regurgitation. The group with mild-moderate regurgitation had a higher median ischaemic time of 32.3 hours compared to the group with nil-trivial regurgitation, which had a median ischaemic time of 30.3 hours. The difference was significant by log-rank test (p=0.04). Ischaemic time did not correlate with mean valve gradient (r = 0.04, p=0.67).

In the subgroup of pulmonary allograft (n=74), there was no association between ischaemic times and the presence of allograft dysfunction, mild-moderate valvular regurgitation or mean valve gradients. Median ischaemic time was 31.8 hours in those with pulmonary valve dysfunction and 29.3 hours in those without pulmonary valve dysfunction. This difference did not reach significance by log-rank testing (p=0.16). There was no difference between the ischaemic times in the patients with (n=13, median 31.8 hours) and without (n=61, median 30.5 hours) mild-moderate valvular regurgitation by log-rank testing (p=0.40). Ischaemic time did not correlate with mean valve gradient (r = 0.12, p=0.33).

5.4.6 Donor age

Donor age was available for 99.1% of participants (114/115). Mean donor age was 37.1 years (SD 12.4 years). Figure 5.2 shows the distribution of donor age. Donor age was

not associated with valve dysfunction. Mean donor age was 34 years for those with mild-moderate valve dysfunction compared to 38 years for those without valve dysfunction (p=0.11 by t-test). Donor age did not correlate with mean valve gradient (r= -0.09, p=0.36), nor was it associated with valve regurgitation. Mean donor age for those with and without mild-moderate valve regurgitation was similar (36 vs 37 years, p=0.70 by t-test).

In the analysis of the subgroup of pulmonary allografts (n=74), younger donor age was associated with valve dysfunction. Mean donor age was lower at 32 years for those with mild-moderate valve dysfunction compared to 41 years for those with no valve dysfunction (p = 0.003 by t-test). Age of pulmonary valve donors inversely correlated with mean gradients, r= -0.29 (p=0.011). Donor age was not significantly associated with valve regurgitation. Mean donor age was not significantly different in those with and without valve regurgitation (32 vs 39 years, p=0.20 by t-test).

In conclusion, donor age was not significantly associated with parameters of valve failure by univariate analysis of the whole cohort. However, in the analysis of pulmonary valve recipients, younger age of donor was associated with valve dysfunction and higher degrees of valve stenosis.

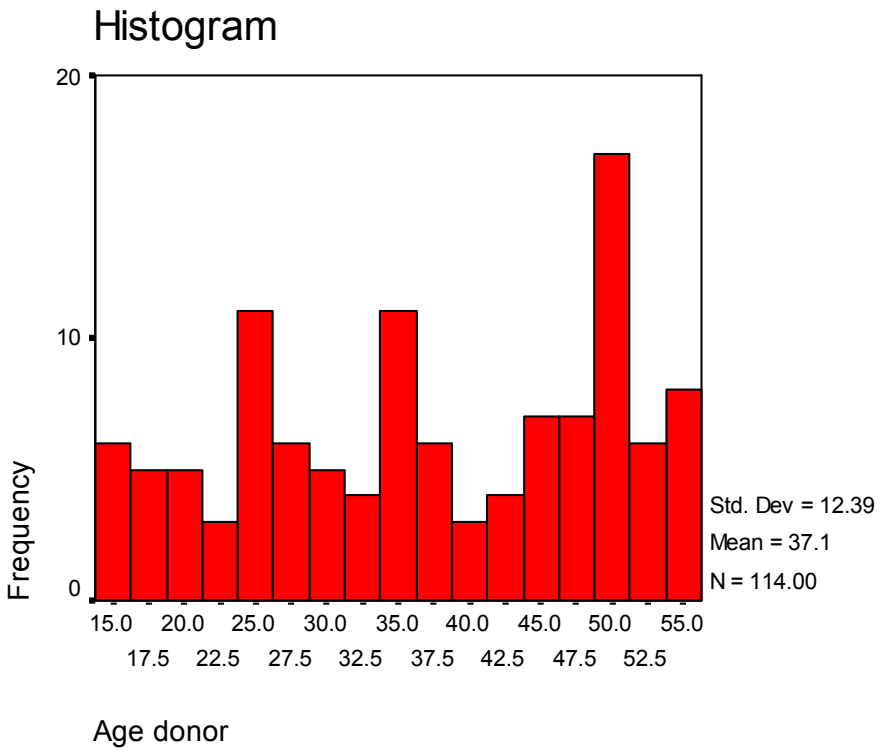


Figure 5.2. Allograft valve donor age.

5.4.7 Recipient age

Patient (recipient) age at operation was available for all participants. Mean recipient age was 44.9 years (SD 14.0 years). Figure 5.3 shows the distribution of recipient age. Recipient age was associated with mild–moderate valve dysfunction. Mean recipient age of those with valve dysfunction was lower (n=44, 40 years) than for those with no valve dysfunction (n=66, 47 years). This difference was significant by t-test, p=0.02. Donor age inversely correlated with mean valve gradient (r 0= -0.41, p=0.001). It was not however, associated with valve regurgitation. The mean recipient age was 41 in those with mild/moderate regurgitation, compared to 45 in those with nil-trivial

regurgitation, p=0.22 by t-test. Hence, younger recipient age is associated with greater allograft dysfunction primarily by greater levels of allograft valve stenosis.

In the analysis of pulmonary allograft recipients (n=74), lower recipient age was associated with valve dysfunction. Mean recipient age of those with valve dysfunction was lower (n=29, 37 years) than for those with no valve dysfunction (n=45, 44 years), p=0.012 by t-test. Recipient age inversely correlated with mean valve gradient (r 0= -0.36, p = 0.002). Lower recipient age was associated with valve regurgitation. Mean age was lower at 34 years for those with mild-moderate regurgitation, compared to 43 years for those with nil-trivial regurgitation, p=0.024 by t-test.

In conclusion, lower recipient age was significantly associated with parameters of valve failure by univariate analysis of the whole cohort and for the subgroup of pulmonary allografts. The mode of failure was by stenosis only for the whole cohort, and by both stenosis and regurgitation for the pulmonary allograft cohort.

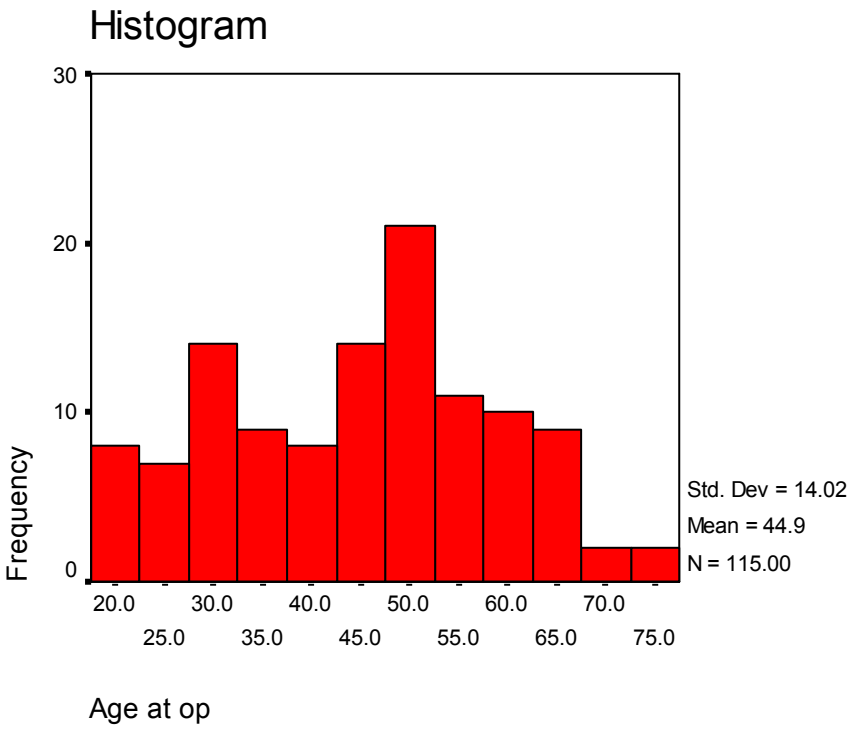


Figure 5.3. Recipient age at operation.

5.4.8 Allograft size

Mean allograft size (n=115) was 24mm, with a standard deviation of 2.38mm (figure 5.4). Allograft size was not associated with valve dysfunction. Mean valve size was identical in those with and without valve dysfunction (24.0 vs 24.0mm, p=0.95). There was no correlation between valve size and mean valve gradient, r= -0.14, p=0.15), or with valve regurgitation. The mean valve size was similar in those with and without mild-moderate valve regurgitation (24.3 vs 23.9, p= 0.50).

In the sub analysis of pulmonary allografts (n=74), no significant association was found between valve size and valve dysfunction. Mean valve size was slightly lower in those with compared to those without valve dysfunction (23.9 vs 24.5mm,

p=0.35). There was a non-significant inverse correlation between valve size and mean gradient, r= -0.20 (p=0.084). The mean valve size was similar in those with and without mild-moderate valve regurgitation (24.4 vs 24.2, p= 0.85).

In conclusion, there was no correlation between allograft size and valve dysfunction, mean gradient or valve regurgitation in the analysis of the whole cohort nor in the subgroup of pulmonary allografts. However, there was a trend towards a correlation between smaller valve sizes and higher mean valve gradients in the pulmonary allograft group.

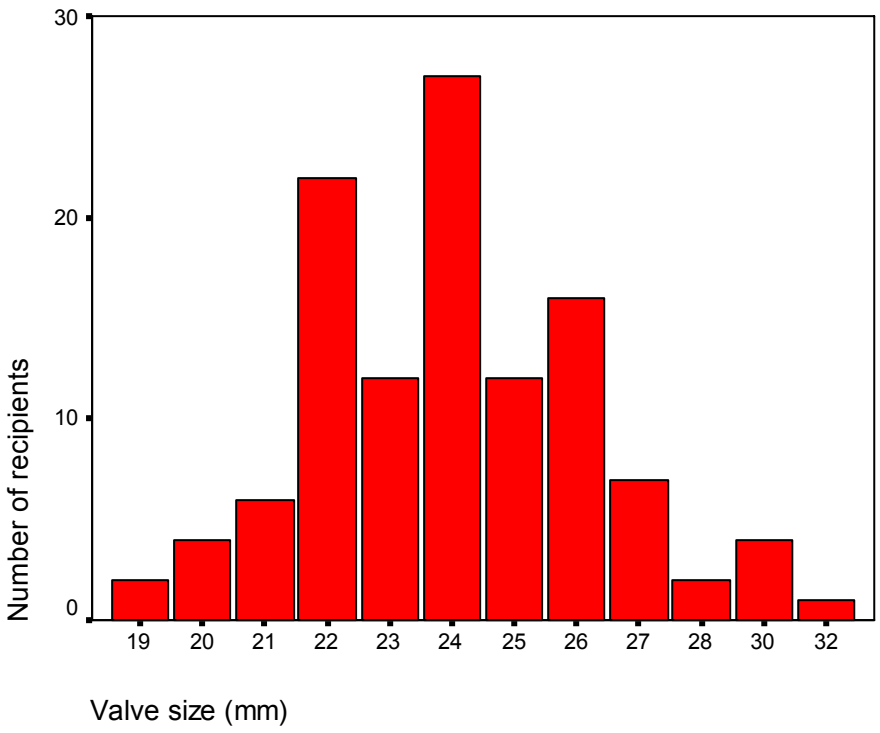


Figure 5.4. Distribution of allograft valve sizes.

5.4.9 Multivariate analyses

Multivariate analysis was performed with the following variables for the outcomes of valve dysfunction, mean valve gradient and valve regurgitation:

i. Number of HLA-A mismatch
ii. Number of HLA-B mismatch
iii. Number of HLA-DR mismatch
iv. Anti-HLA class I antibody status by ELISA
v. Anti-HLA class II antibody status by ELISA
vi. Anti-HLA class I antibody status by microlymphocytotoxicity
vii. Donor-specific anti-HLA class I antibody status
viii. ABO blood group match
ix. Allograft ischaemic time
x. Age of donor
xi. Age of recipient at operation
xii. Allograft valve size

Only younger age of recipient (p=0.009) and higher allograft ischaemic time (p=0.044) were found to be significant in predicting valve dysfunction (nil vs mild-moderate) by logistic regression analysis. Only younger age of recipient (p=0.001) was found to predict higher mean valve gradient. In the analysis of valve regurgitation (nil/trivial vs mild/moderate) only younger age of recipient was found to be a significant predictor (p=0.008).

In summary, younger age of recipient was a strong predictor of allograft valve dysfunction, stenosis and regurgitation. Higher allograft ischaemic time predicted valve dysfunction, but not higher gradients or valve regurgitation separately. Multivariate analysis was not performed on the subgroup of pulmonary allografts because of the relatively small numbers of participants in this group.

5.5 Conclusion

In this study of 110 adult allograft valve recipients, with a mean echocardiographic follow-up duration of 41 (±18) months, 2 factors were significantly associated with echocardiographic parameters of valve dysfunction. In the overall cohort, univariate analysis revealed that longer ischaemic times were associated with valve dysfunction and regurgitation, and younger recipient age was associated with both valve dysfunction and higher mean valve gradients. There was a trend (not statistically significant) observed for a higher frequency of valve dysfunction in those with complete mismatch compared to those with 0-1 HLA-DR mismatch at the HLA-DR locus. In the multivariate analysis longer ischaemic times predicted valve dysfunction, whilst younger recipient age predicted valve dysfunction, regurgitation and higher valve gradients.

In the subgroup analysis of 74 pulmonary allograft recipients with a mean echocardiographic follow-up duration of 43 (±18) months, univariate analysis revealed lower donor age was associated with valve dysfunction and stenosis, whilst lower recipient age was associated with valve dysfunction, stenosis and regurgitation. In addition, there was a non-significant trend for HLA-DR mismatch and valve

dysfunction, ABO mismatch and valve dysfunction and regurgitation, and smaller pulmonary allograft size and valve stenosis.

There was no significant association for the immunological variables of HLA-A, B or DR, HLA-class I, HLA class I and II, anti-HLA class I or II antibody, or donor-specific HLA class I antibodies, and the echocardiographic parameters of valve dysfunction.

CHAPTER 6: Discussion

6.1 Introduction

This chapter will discuss the results outlined in chapter 5. The significance of the results will be placed in the context of the current understanding of allograft degeneration and immunology. Furthermore, limitations of the study and areas of future research will be explored.

6.2 Factors influencing allograft degeneration

As outlined in chapter 3, many factors have been implicated in allograft degeneration. However, the exact causes remain unclear. Current evidence demonstrates a measurable host immune response directed against the allograft valve. Whether such a response causes clinically significant valve degeneration is unknown. Furthermore, it is likely that any degenerative host immune response will interact with other non-immunological factors in causing valve degeneration. In light of this, the current study aimed to add to the current understanding of allograft valve degeneration by examining the association between post-operative valve function and immunological factors (HLA and ABO mismatch and the antibody response against the allograft), and other factors such as donor and recipient age, valve size and ischaemic time.

Young patient age

The current study identified young recipient age as significant in allograft dysfunction by both univariate and multivariate analysis. This was true also for the

pulmonary allograft subgroup. Young recipient age has long been known to be related to aortic[19,20,49,67,113] and pulmonary[77,88,89,91,95,106,125,129,130] allograft valve degeneration, as well as bioprosthesis[131,132] degeneration. However, mechanical valve prosthesis does not show a similar recipient-age association. Whilst this problem is seen throughout the paediatric and adult age range, it is in the paediatric patients where this problem is most significant.[127,192] Allograft valves and non-valvular allograft material are used extensively in cardiac surgery to reconstruct a variety of congenital cardiac defects and abnormalities. The mode of failure in the young is thought to be by leaflet and annular calcification leading to valvular stenosis or cuspal rupture leading to regurgitation. In this study, patients were found to have allograft dysfunction by both mild-moderate stenosis and regurgitation. No valves in this study were explanted and thus, direct examination of the allograft to determine the mechanism of dysfunction was not performed.

The cause of this age-related phenomena is unclear, although many authors believe it to be immunogenic[127,130,193] or relating to differences in calcium metabolism. However, there is little evidence to suggest that there is increased immunogenicity directed against the implanted allograft in younger patients. Alternatively, early allograft valve failure in the young may be due to outgrowth of the implanted allograft, with progressive growth of the native heart and great vessels. A third factor is the increased haemodynamic demands placed on the allograft by younger patients who are both more active and have greater metabolic needs.

ABO blood group

No difference was found in allograft dysfunction according to ABO blood group matching by univariate and multivariate analysis. Despite the solid-organ transplant experience, it has not been routine practice to prospectively ABO match donors and recipients of allograft heart valves, as the vast majority of other series of adult and paediatric allograft replacement in the pulmonary and aortic positions have not identified either ABO or Rheuses incompatibility as a significant factor in predicting allograft dysfunction. [21,89,92,95,107,116,124,127,130,139,176]

Nevertheless, some authors have advocated ABO matching of allografts[95,143] based on endothelial cell ABO antigen expression[194] and allograft heart transplantation experience[195]. In studies on paediatric recipients of allograft valved conduit for reconstruction of the RVOT, ABO blood group mismatch was found to be predictive for allograft failure in two series. [125,128] Notably, both were series of infant and paediatric valve allograft recipients.

There may be several reasons why ABO matching is unnecessary. Firstly, it may be that cardiac valvular endothelium, unlike other vascular endothelium, does not express blood group antigens. Kadner and co-workers, using antibodies to ABO antigens, showed that fresh and cryopreserved allograft valvular endothelium does not express ABO antigens in contrast to microvascular cardiac endothelium. [196]Secondly, most allografts may be non-ABO immunogenic, due to pre-implantation endothelial cell loss caused by prolonged warm ischemic times, and the sterilisation and preservation process. Finally, it may be that as predicted, allograft valvular endothelium is rejected and destroyed post-implantation in ABO-incompatible cases. However, this may not translate to a difference in valvular function, as unlike solid-organ transplants, intact

endothelium in the transplanted allograft valve may not be vital to its long-term function. In support of this final possibility, Fischlein and associates showed that all cryopreserved allografts valves provoked an immunologic reaction post-operatively, which was more intense in ABO-incompatible, compared to ABO-compatible allografts. However, the significance of the detected immunologic reaction was unclear as it was reversible without immunosuppression after an average of 3 days. [197]

Ischaemic times

The current study found that longer ischaemic time was associated with allograft valve dysfunction and valvular regurgitation. No association was found with valve stenosis. This finding occurred in the context of a fairly narrow range of time between death and cryopreservation of between 20 and 48 hours. Median ischaemic time was 31 hours. This encompassed the time required for the cadaver to be transported to the coronial offices, permission from the family to harvest tissue to be obtained, harvest and 6-8 hour antibiotic sterilisation process to be performed, and finally the cryopreservation process initiated. It is somewhat surprising that in this narrow window of time of 28 hours, an associated difference in valve outcome is found.

Authors have found that both long[20,106,140] and very short [125,139] ischaemic times may be deleterious to allograft valve function. However, interpretation of these studies is difficult as the total ischaemic time (ie. time between death and cryopreservation) is not stated. Rather a subset of intervals such as time between harvest and cryopreservation[139], duration of antibiotic preservation[125], duration between death and harvest[106], duration between harvest and implantation[20], were reported as significant.

Dignan and colleagues reported short time (<4 hours) between allograft procurement and cryopreservation as significant in predicting structural deterioration in their series of 168 aortic allograft valve replacements[139]. Baskett and co-workers similarly found that shorter antibiotic preservation time was predictive of valve failure in their paediatric series of 90 allograft valves placed in the RVOT. They concluded that shorter ischaemic times may be undesirable as antigenicity and immunogenicity is retained. This is consistent with previous findings that HLA antigen expression is lost with increasing ischaemic times.[142] Tweddell and co-workers, in their study of 220 cryopreserved aortic and pulmonary allografts used in the reconstruction of the RVOT in 183 late surviving patients, found warm ischaemic time (time from donor death to organ procurement) in excess of 5.5 hours to be associated with allograft failure.[106] Yacoub's group has found warm ischaemic time[140] and duration between valve harvest and implantation[20] is predictive of valve failure. Given that longer ischaemic times reduce cellular viability[90,141] and perhaps connective tissue integrity, long-term allograft failure may be a consequence of reduced structural integrity.

Based our findings and the above studies, we conclude that there exist an optimal duration of allograft ischaemia. This duration should allow down-regulation or degradation of the antigenic elements within the allograft whilst allowing the structural and cellular elements required for long-term valve durability to be preserved. The exact duration of the "desirable" allograft ischaemia time is difficult to define and may lie under the 20-48 hour range studied here. Prospective clinical studies varying valve ischaemia time is unlikely to ever be performed. However, careful analysis of existing valve bank data in existing allograft patient series may reveal the answer.

Allograft valve size

Allograft valve size was not found to be associated with valve dysfunction on either univariate or multivariate analysis. In the sub analysis of pulmonary valve allografts, valve size remained non-significant. Many reported series of allografts implanted in the RVOT have found smaller valve size to be associated with valve failure. [88,89,95,106,125] One large series of RVOT 405 allografts did find conduit size as predictive of failure on univariate analysis, but not on multivariate analysis. [107] These published series contained a large number of infant and paediatric patients where the RVOT reconstruction using an allograft was performed for congenital defects.

The age to annulus diameter relationship is proportional and linear in the early years of life. Upon reaching adulthood, maximum body and annulus size is reached. Thus, there ceases to be a relationship between age and annulus diameter within the adult age range. In this study, there was no correlation between valve size and patient age at surgery ($r = 0.024$, $p = 0.84$). Furthermore, we know that young age, especially in the infant and paediatric ages, is a very strong predictor for valve failure as previously discussed. Given that the above published series contained a large number of infant and paediatric patients we can conclude that small allograft sizes were a marker for young patient age in these studies and therefore were found to be related to valve failure. Conversely, in the current study of adult allograft recipients valve size was not found to be related to valve dysfunction because the current study did not include any paediatric patients and valve size was not a marker for patient age in the adult age range.

In conclusion, small allograft size is unlikely to be a true predictor of valve failure. Rather, it is a marker for very young patient age, which is a strong predictor of valve failure. As the current study did not include infant and paediatric patients, and as

allograft valve size does not correlate with recipient age in our patient age range, we did not find allograft valve size to be associated with valve dysfunction.

Donor age

This study found younger donor age to be associated with valve dysfunction and higher degree of valve stenosis in the pulmonary valve cohort. However, donor age was not significant in the univariate or multivariate analysis of the whole cohort. Historically, older donor age has been found to be significant in predicting allograft valve failure. [20,21,49,67,116] For instance, Yacoub and co-workers in their series of 275 homovital aortic allografts identified donor age over 55 years as predicting valve failure. [21] Hence, most allograft valve banks apply a donor age limit of 55-60 years of age. The DTBV applies a donor valve age limit of 55 years, whilst the European Homograft Bank limits aortic valve donors to 60 years. [198] The increasing failure rates with older donor age are presumably due to age-related changes in the connective tissue and cellular components of the allograft leaflets. The absence of an association between higher donor age and valve dysfunction in the present study is consistent with the donor age limit of 55 years applied by the DTBV.

There is a single study of allograft valves which has found younger donor age as predictive of valve failure. Gerestein and co-workers in their analysis of 316 allografts used for RVOT reconstruction identified donor age less than 30 years as a strong risk factor (hazard ratio 3.5) for valve-related reoperation by univariate, but not multivariate analysis. [89] However, their study included a large number of paediatric patients and infants. The median age of patients was 16 years and 25% of patients were less than 4 years of age. They further stated that patient age at operation was highly correlated

(Pearson's R >0.50; p<0.01) with donor age and allograft diameter. Whilst their finding would be consistent with that of this study, it is probable that in their series with a large number of paediatric patients, donor age was a marker of young patient age at operation, which is the strong predictor of valve failure.

It is possible that the finding of an association between younger donor age and valve dysfunction by stenosis in the pulmonary allograft cohort represents a type 1 error in this study. Alternatively, other valid reasons may exist for this finding. However, based on the study, the author is unclear as to what such reasons may be.

Antibody status

In this cross-sectional study of allograft recipients out to 5 years post-operatively, we found that 96% and 82% of recipients had anti-HLA class I and II antibodies respectively, by ELISA testing. In a similar study by Dignan and coworkers anti-HLA class I antibodies were found in 83% and class II antibodies were found in 61% of recipients at a mean of 6.5 years post-operatively, using a different commercially available ELISA test. Other studies have used microlymphocytotoxicity methods and have found anti-HLA class I antibodies in 54%-100% of patients. [164,179,180,187,188,199,200] Anti-HLA class II antibodies have found in 19% - 77% of recipients using various testing methods. [150,188] The difference in frequency of antibodies found in these studies are likely due to varying valve preservation methods[164], ischaemic time, post-operative interval[201] and method of testing[188]. Our high prevalence rates of anti HLA class I and II antibodies may be due to the relatively short post-operative interval, short valve ischemic time and the high sensitivity of the ELISA method used. To our knowledge, the QUIKSCREEN® and B-SCREEN® qualitative ELISA kits employed in this study

have not been previously used in allograft valve studies. They have however, been widely used in the setting of solid organ transplants, and have proven to have high sensitivity when compared to other methods. [202,203]

In this study 76% of recipients positive for anti-HLA class I antibodies by MLC had detectable donor-specific antibodies. This is consistent with previous studies which found 81-100% of HLA-antibodies were donor-specific. [150,164,179,180,187,199] Six other recipients in this study had broad panel reactivity precluding HLA specificity designation. It is probable that these patients also had donor-specific antibodies. It is therefore most likely, that the allograft valve is the cause of the formation of anti-HLA antibodies in recipients as the allograft material provides a long-term source of foreign HLA antigens. It is worth considering that patients may form antibodies to HLA antigens in 2 other settings: blood transfusion and fetal-maternal transfusion. We did not look at the rate of blood product usage or multi-parity in this study. However, previous studies of allograft valve recipients have found blood transfusions not to be significant in the formation of anti-HLA antibodies. [147,164] In addition, blood transfusion in those not pre-sensitised is known to produce very low rates of antibody formation; 2.7% in one study. [204] Further evidence that allografts is the source of the anti-HLA antibodies comes from studies of patients receiving decellularised allograft material. Two such studies have shown a reduced antibody response in those receiving decellularised allografts. [205,206]

This study did not reveal a correlation between anti-HLA antibodies and the echocardiographic evidence of valve dysfunction. To date only a single study by Dignan and associates have shown that the presence of anti-HLA antibodies are associated with greater valve dysfunction. [188] In that study an association was found between aortic

valve allograft structural deterioration and a high titre of anti-HLA class II antibodies detected by ELISA. Significantly, they did not find a link between valve dysfunction and antibodies when either MLC or flow cytometry methods were used. It remains unclear at present whether valve-specific antibodies are a causative factor in allograft valve degeneration. Larger studies with longer follow-up and younger patients are required to answer this issue. Alternatively, improved durability of decellularised allograft valves over conventional allografts may settle this issue.

The presence of anti-HLA class I and II antibodies may have significant deleterious consequences for those who could require future heart or other organ transplantation. [148,201] It may also have implications for those requiring a subsequent allograft valve replacement or conduit as is often the case in paediatric patients with congenital heart defects reaching adulthood. Such patients may benefit from avoiding the use of allograft material on their second or subsequent operation, or benefit from preoperative cross-matching. [200]

HLA match

This study did not find a significant association between HLA match at each of the A, B and DR locus, or HLA match at the class I locus (A and B) or total HLA match (A,B and DR) and allograft dysfunction. The probability of good HLA matching was low because no preoperative HLA typing or matching was performed. At each of HLA locus studied, 7-8% of patients were completely HLA matched, whilst 52-64% was completely mismatched. The low rates of HLA matches found in the study were in keeping with the known high diversity of HLA types in a random population. This represents an inherent limitation in the study design, which can be overcome only by

conducting large international registry studies or prospective randomised matching studies, both of which have not been performed to date in the field of allograft valve implantation.

Two studies by Smith and colleagues[179] and Bechtel and associates[180] have shown no association between HLA mismatch and valve performance. In contrast, 2 studies by Baskett and co-workers[125] and Dignan and associates[139] have linked HLA-mismatch with poorer valve performance.

Baskett and colleagues studied 47 paediatric allograft valve recipients who had reconstructions of the RVOT, with a mean age of 5 years. They found that HLA-DR mismatch was associated with echocardiographic valve failure by multivariate analysis, after a mean follow-up period of 4.6 years (maximum 11 years). It was unclear in their paper as to whether HLA class 1 antigen matching also was analysed. [125] Dignan and associates studied 162, mainly adult, recipients of cryopreserved aortic allografts with a maximum follow-up of 12 years. They found that HLA A, B and DR was not associated with echocardiographic allograft failure in the overall analysis of the entire cohort. However, when the subset of 92 patients with at least 5 years follow-up was analysed separately HLA-DR (but not HLA-A or B) mismatch was associated with allograft valve failure by bivariate analysis. It is possible that the 2 studies that failed to reveal an association between HLA match and valve performance did so because of the bias of small numbers[180], short follow-up[180], older recipient cohort and long durations between donor death and cryopreservation or implantation (in the case of homovital valves[179]).

The current study of HLA match in 80 patients, although limited by a short echocardiographic follow-up duration, did not find HLA matching to be significant. It remains to be seen if the absence of an association persists on longer follow-up. Given

the study limitations of low rates of HLA matching, relatively small numbers and short follow-up, it is possible that the current results may represent a type II error.

6.3 Technical Factors

The current study employed 2 techniques for detecting the presence of anti-HLA class 1 antibodies in recipient sera. This was performed because previous studies used the lymphocytotoxicity methods and were unable to find any correlation between anti-HLA antibodies and allograft degeneration. [147,148,150,151,164,179] Both methods have significant limitations. Lymphocytotoxicity testing has been the historical method for detecting HLA-specific antibodies in patients awaiting organ transplantation. Limitations of this method include reliance on good cell viability of lymphocytes used in the panels, variability in the effectiveness of rabbit serum (used as a source of complement and heterophile antibody), cross-reaction with non-HLA specific cytotoxic antibodies, and inability to detect non-complement fixing antibodies. [202,203] These factors may in turn affect the sensitivity and specificity of lymphocytotoxicity methods. The ELISA methods also suffer from limitations. For instance, it may detect anti-HLA antibodies that are non-cytotoxic. Furthermore, it does not detect IgM or IgA anti-HLA antibodies, and there is a possibility that immune complexes or other immunoglobulin aggregates in the patient serum may cause an increased non-specific binding resulting in false-positive assay results. [189] Considering these limitations, it has been unclear as to which technique has greater sensitivity and specificity in detecting antibodies against allograft valves.

Studies have shown that PRA levels fall with increasing post-operative duration. [147] [179,201] This study found that PRA levels were lower with increasing post-operative

duration, whereas ELISA status did not correlate with post-operative duration. Furthermore, of the 29 patients with PRA levels of 5% or less (ie. antibody negative by lymphocytotoxicity) 24 (83%) were positive for anti-HLA class 1 antibodies by ELISA testing. In light of this, it is possible that ELISA methods are more specific and sensitive for detecting low levels of anti-HLA antibodies as compared to traditional lymphocytotoxicity methods in long-term follow-up of allograft recipients. [202,203] ELISA methods may thus, be the preferred method of detecting anti-HLA antibodies in future studies of allograft recipients. This is supported by a recent study by Dignan and colleagues which showed a link between anti-HLA antibodies in recipient sera and aortic allograft degeneration. Dignan's study employed a commercially-available ELISA kit (LATM, One Lambda) different to the kit used in the current study. Of importance, they did not demonstrate a similar link when antibody status was tested using lymphocytotoxicity or flow PRA methods.

A point of contention is the different PRA levels used in various studies as a cut-off for determining qualitative anti-HLA antibody status by the lymphocytotoxicity method. The current study used >5% as indicating anti-HLA antibody positive. This cut-off level is used in studies by Smith[164,179] and Bechtel.[180,187] Hoekstra however, used PRA level >9% [199] whilst Dignan used PRA >20%.[188] The 5% level is used by the VTIS as the cut-off below which HLA specificities cannot be confidently assigned by lymphocytotoxicity testing. Hence, it makes intuitive sense that this level is used for qualitative testing in the current study. Furthermore, in this study none of the 5 patients who were ELISA negative for anti-HLA class I antibodies had PRA levels above 5%.

6.3 Study limitations

The current study has several limitations. The numbers of patients are relatively small. Larger numbers of recipients are required to detect a measurable influence of the variables studied as with less than 10 years of follow-up, the rates of valve dysfunction in adult recipients are low. The study was limited in the number of patients available for recruitment as it was dependent on the collection of donor DNA by the DTBV, which has been performed only since 1997. The participation rate of 71% was good considering 19% of patients that were lost to follow-up. In the area of HLA mismatch analysis, further attrition of numbers occurred due to loss of donor HLA type information from degraded DNA samples. It is thought that delayed sample delivery from the DTVB to the VTIS and prolonged warm ischaemic time of some cadaveric donors is responsible for this. We have addressed the issue by minimising the interval between the taking of donor samples and processing (DNA extraction) at the VTIS. The problem of prolonged warm ischaemic time could not be avoided as valves are taken from routine coronial cadavers, which have a variable cause and circumstances of death and storage prior to arrival at the Coroner's Office.

The small number of subjects may also have implication for the ability to generalise the results of the multivariate analysis in the study. In view of current biostatistical theory, and considering the number of variables studied relative to the number of subjects included in the study, the results of the multivariate analysis may not be generalisable.

The post-operative duration represented by the echocardiographic follow-up in the study is a further limitation. Greater duration of follow-up would allow higher rates of valve degeneration to accrue, increasing the power of the study to detect an

association between the variables studied and valve dysfunction. Again, the limitation imposed by the collection of donor DNA only since 1997 represents the limiting factor in the duration of post-operative follow-up represented by this study. Considering the limitations above, it is concluded that there is a possibility of type 2 errors in the analysis of the variables studied.

The clinical significance of mild to moderate allograft valve dysfunction in the RVOT is uncertain. The right ventricle can tolerate mild to moderate degrees of pulmonary valve regurgitation well, provided pulmonary arterial pressures are not excessively elevated. Hence, such degrees of pulmonary valve dysfunction may have little, if any, clinical significance. Ideally, the outcome measure which should be addressed by future studies is the incidence of severe valve dysfunction and reoperation for allograft valve degeneration. Such studies would require larger numbers and follow-up in significant numbers of subjects beyond the 10-15 year period. Because of the short follow-up duration of the study, no patients had severe valve dysfunction or required reoperation for allograft valve dysfunction.

The scope of the study excluded allograft mitral valves, non-cryopreserved allograft valves, non-valvular allograft material (e.g. patches or non-valvular conduits) and paediatric subjects. Hence, the extent to which the findings can be translated to these areas is uncertain.

6.4 Areas of future study

Future studies are required to unravel the mechanisms behind the accelerated degeneration of tissue valves seen in young recipients. This would allow modification of tissue valves, beyond the current focus on anti-calcification treatments, which may

enhance the durability of tissue valves. This is urgently required as allografts remain the most important replacement material in paediatric patients.

A different approach to study design should be considered in this area of research. Historically, single-centre cohorts of allograft recipients are retrospectively analysed for factors that affect allograft durability. Apart from a few centres such as Brisbane and Harefield, large numbers of patients are seldom accrued. National or international registries should be created, through which allograft recipients can be followed in larger numbers with uniform data collection.

The possibility of prospective HLA-type matching in paediatric patients should also be considered. Studying the significance of HLA-matching in unmatched cohorts such as this one is difficult because of the low incidence of HLA match occurring by chance. Prospective HLA matching studies will unequivocally settle the issue of the significance of host immune reaction in the degeneration of allograft valves. If applied to the paediatric age group, smaller numbers and shorter follow-up will be required, as compared to similar studies in adults. Problems here may be the need for large valve banks containing a variety of valve sizes and HLA types. Here, the role of a large national or regional bank, or a network of valve-sharing banks, is required. Non-commercial organisations such as the European Homograft Bank, or commercial enterprises such as CryoLife Inc, are ideally placed to take on the prospect of large-scale HLA matching.

Finally, ongoing modifications of the allograft such as the current focus on decellularisation may settle the issue of immunogenicity. If decellularised valves (with pre or post implantation recellularisation) show improved durability over conventional allografts, then a destructive immunological host response is likely to be a factor in

current allograft degeneration. The use of decellularised valves will also allow us to determine the significance of non-immunological factors such as recipient age and technical implantation factors, without the confounding effect of immunological factors.

CHAPTER 7: Conclusion

This historical cohort study of adult recipients of aortic and pulmonary allograft heart valves analysed the association between immunological and non-immunological variables, and echocardiographic parameters of allograft valve dysfunction, at a mean of 3.4 years post-operatively. Young recipient age and longer ischaemic times were associated with allograft valve dysfunction in the whole cohort, whilst in the pulmonary allograft recipients, lower donor age and lower recipient age were associated with pulmonary allograft dysfunction. HLA-DR complete mismatch showed a non-significant trend towards valve dysfunction. The immunological variables of HLA-A mismatch, HLA-B mismatch, anti-HLA antibody status, and ABO blood group mismatch were not associated with valve dysfunction.

This study confirms that young patient age at time of surgery represents a strong predictor of allograft valve dysfunction. The study also adds to the current state of knowledge by defining an optimal warm ischaemic time conducive to valve durability. The ideal ischaemic time would be less than 48 hours. The lower limit of the ideal ischaemic time remains unclear. On a practical note, ischaemic times of less than 20 hours (ie. this study's lower limit) would be impractical as the mandatory harvest process of transport, family consent to bank tissue, sterilisation and cryopreservation, would require a minimum time of approximately 15 hours in most cases.

A cross-sectional study of recipient sera revealed 96% and 82% of recipients had detectable anti-HLA class I and II antibodies by ELISA testing. Also 72% of recipients had anti-HLA class I antibodies by lymphocytotoxicity testing; of these the majority (76%) had anti-HLA class I antibodies against at least 1 donor-specific HLA antigen.

Despite a detectable humoral response against the allograft material implanted, no correlation with valve dysfunction was found. The study of HLA donor-recipient mismatch at the A, B and DR loci also failed to find a correlation with valve dysfunction. Hence, the clinical significance of any host immune response against the allograft remains unclear, and requires further study.

The lack of significance of ABO blood group mismatch in this study is consistent with the majority of historical cohort studies published thus far. The findings thus support the current practice of not ABO matching donor and recipients of allograft valves. Small allograft size was not associated with allograft dysfunction as, unlike in paediatric series, small allograft size was not a surrogate marker for younger patient age in this study. Finally, in this study with a donor age limit of 55 years, it is unclear why young donor age was found to be associated with pulmonary allograft dysfunction.

This study has several limitations. Most importantly, the number of subjects is relatively small and the duration of follow-up relatively short given the outcome being studied. The effect of each variable studied is likely to be confounded by many other known and unknown variables which make type 2 errors a possibility. It is important to note that limitations to the study design were imposed by the protocol-driven collection of DNA by the DTBV since 1997. Finally, it must be recognised that given the follow-up duration encompassed by the study, the study uses echocardiographic parameters of mild to moderate valve dysfunction as surrogate markers for the clinically relevant outcomes of severe valve dysfunction and ultimately, reoperation for valve dysfunction.

Considering the limitations shared by this and many other studies, a different approach to the design of future cohort studies is warranted. Registries at the national or international level are required to collect uniform prospective data regarding the factors

implicated in valve dysfunction, as well as data on post-operative valve performance and relevant clinical outcomes. In this way, the confounding effect of multiple factors can be addressed. Furthermore, prospective HLA matching studies or the performance of decellularised valves may resolve the issue of the host immune reaction.

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APPENDIX A: Participant information and consent form

ST. VINCENT'S HOSPITAL

PARTICIPANT INFORMATION AND CONSENT FORM

Version 3 Dated 13th Feb. 2003

PROTOCOL NO. (SVH): 157/02

NAME OF PARTICIPANT:

U.R. NO:

FULL PROJECT TITLE:

Donor and recipient human leukocyte antigen mismatch: implications for allograft heart valve function.

NAMES OF INVESTIGATORS: Dr. Cheng-Hon Yap and Mr. Michael Yii

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This Participant Information and Consent Form is **6** pages long. Please make sure you have all the pages.

1. Your Consent

You are invited to take part in this research project.

This Participant Information contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project before you decide whether or not to take part in it.

Please read this Participant Information carefully. Feel free to ask questions about any information in the document. You may also wish to discuss the project with a relative or friend or your local health worker. Feel free to do this.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form,

you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Participant Information and Consent Form to keep as a record.

2. Purpose and Background

Human tissue heart valves used in valve replacement operations are known as allograft valves. You are invited to participate in this research project because this type of valve was used in your valve replacement operation.

Up to 400 people will participate in this project.

Previous experience has shown that allograft heart valves may be better than mechanical or animal tissue valves for some patients. They avoid the need for thinning the blood with medications and may last longer than animal valves. However, because it is a tissue valve and not a mechanical valve it may eventually wear out and need to be replaced.

We think that one reason it wears out is that our body's immune system recognises the valve as foreign (i.e. not belonging to us) and attacks it.

Each person has a unique tissue type, much like a fingerprint, which allows the immune system to recognise if a tissue is foreign. We think that if the tissue type of the valve is similar to the tissue type of the person who receives it, the immune system may not recognise the valve as being foreign and therefore not attack the valve. This will cause less wear and allow the valve to last longer. This research project will try to test this idea.

To do this we first need to find out how different your tissue type is compared to your valve. We will then try to work out if the difference is linked to how well your valve functions. We can determine your tissue type by a blood test.

The results of this research may be used to help researcher Dr. Cheng-Hon Yap to obtain a Masters of Surgery Degree.

3. Procedures

Participation in this project will involve

- Agreeing to have the researchers access information from your medical records.
- A blood test (a tablespoon of blood) to work out your tissue-type. This blood test will be similar to those you have had in the past.

- A 10-minute phone interview in 2003, and another in 2008-2010. To assess your general health, we will ask questions about symptoms such as shortness of breath and chest pain. We will also ask questions to find out about any problems you may have had related to your heart valve, such as reoperation, stroke or infection.

4. Possible Benefits

This study may benefit people with heart valve disease, as it will help doctors work out the best type of heart valve replacement operation to perform. We cannot guarantee that you will receive any direct benefits from this project.

5. Possible Risks

Possible risks, side effects and discomforts include the small amount of bleeding and bruising related to having a blood test.

6. Alternatives to Participation

You may of course choose to not participate in this project. This will not affect in any way your current or future medical treatment.

7. Privacy, Confidentiality and Disclosure of Information

Information we collect will be kept on a computer at the Department of Cardiothoracics, St. Vincent's Hospital, Melbourne. Access to information will be restricted to researchers involved in this project. This information will be kept for 15 years and then destroyed.

Any information in this project that can identify you will be kept confidential. It will only be disclosed with your permission, except as required by law. The results of the study will be presented at scientific conferences and in medical journals. In any presentation or publication, information will be provided in such a way that you cannot be identified.

Upon request to the Principal Investigator, you (or your power of attorney) are entitled to be informed of your tissue type and the tissue type of the donor.

8. Results of Project

A copy of any published results can be given to you at your request.

9. Further Information or Any Problems

If you require further information or if you have any problems concerning this project you can contact the researchers. The researchers responsible for this project are

- Dr. Cheng-Hon Yap

Post-graduate student/Principal Researcher; Department of Cardiothoracics, St. Vincent's Hospital Melbourne. Telephone: 03 9288 2211 or 0402 015001.

- Mr. Michael Yii

Cardiothoracic Surgeon/ Associate Researcher; Department of Cardiothoracics, St. Vincent's Hospital Melbourne. Telephone: 03 9288 2211 or 03 9419 2477

10. Complaints and Research Participant Rights

If you have any complaints about any aspect of the study or the way in which it is being conducted you may contact the Patient Representative at St Vincent's Hospital on Telephone: 03 9288 2211. You will need to tell the Patient Representative the name of the person who is noted above as principal investigator.

11. Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your routine treatment, your relationship with those treating you or your relationship with St. Vincent's Hospital Melbourne.

Before you make your decision, a member of the research team will be available to answer any questions you have about the research project. You can ask for any information you want. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team before you withdraw.

12. Ethical Guidelines

This project will be carried out according to the National Statement on Ethical Conduct in Research Involving Humans (June 1999) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

The ethical aspects of this research project have been approved by the Human Research Ethics Committee of St. Vincent's Hospital Melbourne.

13. Reimbursement for your costs

You will not be paid for your participation in this project.

ST. VINCENT'S HOSPITAL

CONSENT FORM

Version 3 Dated 13th Feb. 2003

FULL PROJECT TITLE:

Donor and recipient human leukocyte antigen mismatch: implications for allograft heart valve function.

I have read, or have had read to me in my first language, and I understand the Participant Information version 3 dated 13th Feb. 2003.

I freely agree to participate in this project according to the conditions in the Participant Information.

I will be given a copy of the Participant Information and Consent Form to keep.

The researcher has agreed not to reveal my identity and personal details if information about this project is published or presented in any public form.

Participant's Name (printed) ……

Signature Date

Name of Witness to Participant's Signature (printed)
……

Signature Date

Researcher's Name (printed) ……

Signature Date

Note: All parties signing the Consent Form must date their own signature.

ST. VINCENT'S HOSPITAL

REVOCATION OF CONSENT FORM

FULL PROJECT TITLE:

Donor and recipient human leukocyte antigen mismatch: implications for allograft heart valve function.

I hereby wish to WITHDRAW my consent to participate in the research proposal described above and understand that such withdrawal WILL NOT jeopardise any treatment or my relationship with St. Vincent's Hospital.

Participant's Name (printed) …….

Signature Date

APPENDIX B: Donor valve harvest, processing and thawing

This section details the methods by which the donor valves are procured, processed, and when needed for use, thawed just prior to implantation. As the processing conditions may modify the viability, antigenicity and ultimately, the durability of the allograft valves, the procurement and processing variables should be carefully considered in the analysis of the study results.

All allografts of the Donor Tissue Bank of Victoria, Melbourne, Australia (DTVB), are obtained from routine coronial cadavers. Donor age limit is 55 years of age. The donor heart is obtained within 24 hours of the presumed time of death. Where time of death is not accurately known it is taken as the time at which the deceased person was last seen or known to be alive.

The heart is procured under clean conditions. A swab is from the pericardial cavity and cadaveric blood is obtained for routine microbiological culture and serological testing, and for blood group typing. A tube of acid-citrate-dextrose (ACD) blood is forwarded to the Tissue Typing Laboratory at the Victorian Transplant and Immunogenetics Service to be stored for future HLA typing. The heart is removed from the cadaveric chest cavity en-bloc and removal of the valve block from the heart is performed immediately. The heart valve is trimmed as soon as possible, at a maximum of 2 hours after collection. If a delay beyond 2 hours is anticipated to trimming (e.g. transport of tissue from a country area), the heart block is placed in Medium 199 (Medium 199 with Hank's salts Single Strength, JRH Biosciences) with antibiotics

(Penicillin 30µg/ml and Streptomycin 50µg/ml), with trimming then performed at the earliest opportunity.

At trimming the valve is trimmed, sized and valve quality graded. Poor quality valves are not used. Valves are rinsed with Medium 199 solution and samples taken for microbiological culture. The aortic and pulmonary valves are each placed in 100ml of Medium 199 and antibiotics (as above). The valves are incubated at 37 degrees Celsius for between 6 and 8 hours. In exceptional circumstances, due to staffing levels, the valves are incubated for up to 12 hours.

After the incubation period the valve is prepared freezing under sterile conditions. The valve is placed in the freezing solution, comprising a mixture of 20ml DMSO and 180ml of cold Medium 199 without antibiotics, and sealed in a nylon bag. Incubated tissue trimmings, a sample of incubation solution and freezing solution are taken for microbiological culture. The valve is then frozen to minus 40 degrees Centigrade in a controlled rate liquid nitrogen freezer, at a rate of 1 degree Centigrade per minute. The freezing process is completed over 60 minutes. Heart valves are then stored in the vapour phase of a liquid nitrogen freezer until removed for clinical use.

A blood sample is sent to Blood Bank for ABO group and Rhesus factor typing. All solutions and tissue trimmings are cultured for aerobes and anaerobes. The pericardial swab is cultured for aerobes, anaerobes and fungi. Cadaveric blood is tested for antibodies to human immunodeficiency virus (HIV) 1 and 2, hepatitis B surface antigen, antibodies to hepatitis C virus, anti-human T-cell lymphotropic 1 and 2 and syphilis. All microbiology testing is performed by the microbiology section of the DTBV. All microbiological tests must return a negative result for the valve to be cleared for clinical use.

The valve is delivered to the operating theatre in a portable dewar at liquid nitrogen temperatures. When the valve is required a circulating nurse removes the valve package from the portable dewar. The valve package is left to equilibrate at room temperature for three minutes, and then gradually rewarmed in a warm normal saline bath (35-37°C). When all visible ice has thawed the package is opened. The valve and accompanying tricuspid leaflet are then gently removed from its package and placed into a large bowl containing Hartmanns solution. It is left to completely thaw in the Hartmanns solution for a minimum of 5 minutes. It is then placed in another bowl containing Hartmanns solution for a further 5 minute passive rinse, to ensure the removal of all DMSO. Next the valve is transferred to a small bowl containing Medium 199 and is ready for trimming and implantation by the surgeon. Valve trimmings, the accompanying tricuspid valve leaflet and the freezing and rinsing solutions are sent for microbiology testing.

APPENDIX C: Subgroup of pulmonary allografts - results

Table C.1. Valve dysfunction and degree of HLA-A mismatch in subgroup with pulmonary allografts (n=55).

	Degree of HLA-A mismatch	
	0-1 mismatch	*2 mismatches*
No valve dysfunction	16	17
Mild or moderate valve dysfunction	9	13

Chi-square statistic 0.31, p=0.58

Table C.2. Valve dysfunction and degree of HLA-B mismatch in subgroup with pulmonary allografts (n=53).

	Degree of HLA-B mismatch	
	0-1 mismatch	*2 mismatches*
No valve dysfunction	11	20
Mild or moderate valve dysfunction	8	14

Chi-square statistic 0.00, p=0.95

Table C.3. Valve dysfunction and degree of HLA-DR mismatch in subgroup with pulmonary allografts (n=51).

	Degree of HLA-DR mismatch	
	0-1 mismatch	*2 mismatches*
No valve dysfunction	12	19
Mild or moderate valve dysfunction	4	16

Chi-square statistic 1.98, p=0.16

Table C.4. Mean valve gradient and degree of HLA mismatch in subgroup with pulmonary allografts.

	HLA-A	**HLA-B**	**HLA-DR**
Number for analysis	55	53	51
Mean valve gradient mmHg (median, range)			
0-1 mismatch	7 (3-31)	7 (3-24)	7.5 (4-24)
2 mismatch	8 (4-24)	7.5 (3-31)	7 (3-31)
0-1 vs 2 mismatch by log-rank test, p-value	0.55	0.70	0.90

Table C.5. Valvular regurgitation and degree of HLA-A mismatch in subgroup with pulmonary allografts (n=55).

Degree of valvular regurgitation	Degree of HLA-A mismatch	
	0-1 mismatch	*2 mismatches*
Nil or trivial	21	25
Mild or moderate	4	5

Fisher's exact test p=1.00

Table C6. Valvular regurgitation and degree of HLA-B mismatch subgroup with pulmonary allografts (n=53).

Degree of valvular regurgitation	Degree of HLA-B mismatch	
	0-1 mismatch	*2 mismatches*
Nil or trivial	16	28
Mild or moderate	3	6

Fisher's exact test p=1.00

Table C.7. Valvular regurgitation and degree of HLA-DR mismatch in subgroup with pulmonary allografts (n=51).

Degree of valvular regurgitation	Degree of HLA-DR mismatch	
	0-1 mismatch	*2 mismatches*
Nil or trivial	15	28
Mild or moderate	1	7

Fisher's exact test p=0.41

APPENDIX D: Anti-HLA class I antibody specificities

Patient #	Anti HLA antibody *
1	NEG
2	*BW6*
3	NEG
4	*A1*,A11,A24,*B8*
5	*A1*
6	*A1*
7	*A2*
8	*BW6*, A2
9	NEG
10	NEG
11	MULTI
12	B13
13	*A1,*A25,A26,*B7,B8*
14	NEG
15	NEG
16	*A3*, B35, B15, BW6
17	NEG
18	*A1*,A3,A11
19	*A1,B8,B44*,B45
20	*A1*,23,24,B51,52,57,58,38,49,63
21	A25,A26
22	*A2, B13*
23	B51,B53
24	C7, *B7*,B8
25	*A3,B57*,B58
26	NEG
27	NEG
28	*A2,A32*,A68
29	NEG
30	*A2,A3*,A68
31	MULTI
32	*B57*, 58, 63
33	MULTI
34	WEAK
35	*BW4, A1*,A23,A24,A25,A32
36	NEG
37	MULTI
38	NEG
39	*BW6*
40	A19,A24,A25
41	A23,A24,*BW4*
42	MULTI
43	*BW4*
44	*BW4*
45	*A1,A2,* B57,B58,B63,A68,A23,A24
46	NEG

47	MULTI
48	*A3,B7*
49	B62
50	*BW6*
51	*A1*,A11,A24
52	A23,*A24*,*B7*,B8,B48,B60,B61
53	*A11*,*B44*,B45
54	*A2*, BW6
55	NEG
56	NEG
57	*BW4, A1*,A23,A24,A25
58	*A2, BW6*
59	*A1*,A23,A24,*B44*,B45
60	NEG
61	*A2*,A68,B51,B52,B53,*B35*,B18,B70, B57,B58
62	NEG
63	NEG
64	*B44*
65	*A1,B8*
66	*A1*,A3,B44,*B45*,B18,B35,B60,B61
67	*A2*,A68,*B7*
68	NEG

* Italics indicate antibodies that correspond to donor antigens.

APPENDIX E: Publications and Presentations

Publications

- Yap C-H, Yii M. Allograft aortic valve replacement in the adult. Heart, Lung and Circulation 2004; 13(1): 41-51.
- Yap C-H, Yii M. Factors influencing late allograft valve failure. Scandinavian Cardiovascular Journal 2004; 38(6):325-333.
- Yap CH, Skillington PD, Matalanis G, Davis BB, Tait BD, Hudson F, Ireland L, Nixon I, Yii M. Anti-HLA antibodies after cryopreserved allograft valve implantation does not predict valve dysfunction at 3-year follow-up. Journal of Heart Valve Disease 2006; 15:540-544.
- Yap CH, Skillington PD, Matalanis G, Davis BB, Tait BD, Hudson F, Ireland L, Nixon I, Yii M. Factors influencing cryopreserved allograft valve degeneration. *Manuscript in preparation.*

Presentations

- Yap CH. Nixon I, Skillington PD, Matalanis G, Davis B, Tait BD, Hudson F, Ireland L, Yii M. Anti-HLA antibodies after cryopreserved allograft valve implantation does not predict valve dysfunction at 3-year follow-up. Australasian Society of Cardiac and Thoracic Society Annual Scientific Meeting, Noosa Heads, Queensland, Australia. 29th October 2005. Heart, Lung and Circulation 2006; 15:80.

- Yap CH, Skillington PD, Matalanis G, Davis BB, Tait BD, Hudson F, Ireland L, Yii M. HLA-mismatch and other factors affecting cryopreserved allograft valve degeneration. European Association for Cardiothoracic Surgery and the European Society of Thoracic Surgeons 5th Joint Meeting, Stockholm, Sweden, 13 Sept 2006. Interactive Cardiovascular and Thoracic Surgery 2006; 5(Suppl 2):S245.

Current Review

Allograft Aortic Valve Replacement in the Adult: A Review

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Aortic valve replacement using an allograft has been used continuously for over 40 years. Its advantages are excellent haemodynamic function, low thrombogenicity, resistance to infection and avoidance of the complications of anticoagulation. The main concern is its long-term durability, with the high hazard phase for failure between 10 and 20 years. We have only recently been able to judge the true long-term behaviour of the contemporary allograft with two recently published series of patients having reached follow-up beyond 20 years in significant numbers. This review of allograft aortic valve replacement in the adult covers the areas of history, benefits, techniques of sterilisation and preservation, operative methods and outcomes.

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Introduction

Aortic valve replacement (AVR) using an allograft has been used continuously for over 40 years. Its advantages are excellent haemodynamic function, low thrombogenicity, resistance to infection and avoidance of the complications of anticoagulation. The main concern is its long-term durability, with the high hazard phase for failure between 10 and 20 years. We have only recently been able to judge the true long-term behaviour of the contemporary allograft with two recently published series of patients having reached follow-up beyond 20 years in significant numbers.[1,2]

This review of allograft aortic valve replacement (AAVR) in the adult covers the areas of history, benefits, techniques of sterilisation and preservation, operative methods and outcomes.

History

Ross[3] and Barratt-Boyes[4] independently performed the first allograft aortic valve replacements in 1962. This was based on earlier reports by Murray[5] and Kerwin et al.[6] who inserted allograft aortic valves in the descending thoracic aorta of patients with severe aortic incompetence, showing reasonable function up to 6 years. These early donor valves were fresh untreated allografts. However, unpredictable supply necessitated the use of sterilisation and preservation techniques, which included freeze-drying,[7,8] irradiation,[7] chemical preservation with beta-propiolactone,[7] buffered formaldehyde[9] and ethylene oxide.[4] These methods led to a high level of early failure due to cusp rupture.[10] Many centres then abandoned this procedure due to early failures, technical difficulties and an inconsistent supply of suitable valves.

The late 1960s saw early methods of preservation replaced by antibiotic sterilisation and storage in culture medium at 4 °C.[11–13] It was hoped that the physiological use of antibiotics and nutrient media would maintain some viability of donor valves with a resultant improvement in valve durability. Angell et al.[14] and O'Brien et al.[15] pioneered the use of cryopreservation and storage in liquid nitrogen in the early 1970s. This allowed indefinite storage of donor valves, reducing wastage, and valve banking which permitted a full range of valve sizes to be available for use.

There has been a renewed interest in allograft AVR since the late 1980s as good mid to long-term results of cryopreserved[16] and antibiotic sterilised valves were published.[17–19]

Benefits

From a haemodynamic perspective, aortic allografts have consistently shown minimal transvalvular gradients in vivo. This is only slightly higher than native valves and significantly lower than those seen in stented bioprosthesis and mechanical devices.[20,21] The lower transvalvular gradients may be clinically significant in patients with a small annulus or in larger patients with an undersized

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Table 1. *Endocarditis and Thromboembolism after Allograft Aortic Valve Replacement*

Series	Era	Patients	Maximum/Mean Follow-up (Years)	Freedom from Endocarditis (%)[a]	Freedom from Thromboembolism (%)[a]
Harefield[1]	1969–1993	618	27.1/10.1	–/93/91/89/89	–/89/85/80/77
Brisbane[2]	1969–1998	1022	29/7.3	97–99/92–96/88–93/86–89/–	98/94/92/83/79[b], 88/82/75/–/–[c]
Salt Lake City[69]	1985–1996	117	11/–	–/98/–/–/–	–/100/–/–/–
Southampton[75]	1973–1994	249	21/12.4	–/98/96/95/–	
London[19]	1964–1986	555	21/–	–/–/–/83/–	–/99/99/99/–
Birmingham[85]	1981–1991	178	9.2/4.0	Three events	One event

[a]Actuarial rates at 5/10/15/20/25 years.
[b]AVR ± CABG.
[c]AVR + mitral valve surgery.

replacement valve. Use of the allograft valve preserves the aortic root complex, which has an intricate relationship between valve leaflet and aortic wall distensibility. This acts uniquely to maintain optimal flow in coronary arteries during both systole and diastole. The superior haemodynamic performance of the allograft translates to more complete regression of ventricular hypertrophy and greater improvement in left ventricular function after AAVR, compared to after mechanical valve or stented bioprosthetic valve replacement.[22] It may also equate to better long-term survival and could also explain the lower incidence of sudden death[19,23] after allograft replacement, compared to mechanical valve replacement.

The allograft valve has long been known to have an intrinsic resistance to infection. The risk of endocarditis after AAVR is low in contrast to after prosthetic valve replacement. Recently reported series have shown freedom from endocarditis of 92 to 98% at 10 years and 83 to 95% at 20 years (Table 1). The post-operative incidence of endocarditis shows an even distribution over time[16,19] unlike that for prosthetic valve endocarditis, which peaks in the first 6 months post-operatively[24] and has a high mortality of 20–50%.[25] It is also the valve replacement device of choice in the setting of active aortic valve endocarditis as the risk of recurrent endocarditis is lower and overall survival higher when an allograft aortic valve (AAV), as compared to a prosthetic valve, is used to replace an infected native or prosthetic valve.[26–29] Furthermore, the root replacement method of AAV implantation allows aortic root repair in those cases with extensive root destruction or aortoventricular discontinuity.[25,30]

The allograft is considered non-thrombogenic, regardless of the method of preservation. Anticoagulation is not routinely employed in patients post-AAVR. This affords an active lifestyle free from haemorrhagic or valve-related thromboembolic complications. Published figures for freedom from thromboembolism at 10 and 20 years are 89 to 100 and 80 to 99%, respectively (Table 1). The true valve-related rate of thromboembolism is likely overestimated in these figures for three reasons.[1] Firstly, accepted guidelines for reporting complications after valve replacement requires any vascular event to be recorded "unless proved to have resulted from another cause".[31] This means that unless a neurovascular or peripheral embolic event is shown to be due to haemorrhage or large vessel thrombus, it must be attributed to the valve. Secondly, thromboembolism is partially a patient dependent event with a low baseline incidence in the normal population. Barratt-Boyes et al.[23] suggested that strokes in allograft valve patients should not be attributed to the allograft valve itself as clots have never been seen on valves at any reoperation, and these events may simply represent the background incidence in the patient cohort. It is conceivable that the true allograft valve-related rate of thromboembolism is zero. Finally, many AAVR patients within these reported series undergo concomitant cardiac procedures which may independently increase their thromboembolic risks. For instance, O'Brien et al.[2] noted a 92% 15-year actuarial freedom from thromboembolism in AAVR patients without mitral valve surgery, compared to 75% in those undergoing concomitant mitral valve surgery.

Hazard analysis show that thromboembolic risk is greatest in the immediate post-operative period, presumably associated with calcium, air and thrombi embolization from the surgical field.[16] Lund et al.[1] looked at risk factors for thromboembolism in his series of 618 patients. In addition to the well-recognised risk factors of age and hypertension, he identified long harvest time (death of donor to harvest of valve), as an independent risk factor for embolization. This could be explained by the loss of endothelial cells, rendering the valve potentially thrombogenic.

Sterilisation, Preservation and Viability

Current use of allograft falls into one of three general groups. Homovital or fresh valves are harvested using sterile technique from heart transplant recipients or brain-dead multiorgan donors and kept in nutrient medium (e.g. Medium 199) containing extremely low doses of antibiotics. They are stored at 4 °C and used at the earliest opportunity. They are considered viable if used within a few days. Valve viability is defined by viability of fibroblasts at time of implantation. Cryopreserved valves are obtained from either heart transplant recipients, brain-dead multiorgan donors or, as is most often the case, routine autopsies. They are sterilised in an antibiotic and nutrient medium solution for a variable amount of time, usually between 6 and 48 h, then cryopreserved and stored in liquid nitrogen. They are seen as viable if cryopreserved within 2–3 days of donor death.[15] Antibiotic-sterilised valves stored at 4 °C are usually harvested from routine autopsies and kept in an antibiotic and nutrient medium until used at a later date. They are considered non-viable.

The aortic valve consists of endothelial cells and fibroblasts that produce and remodel the valve matrix of collagen, elastin and mucopolysaccharides. These cells maintain the haemodynamically perfect structure of the valve and ensure its non-thrombogenicity. Sterilisation and preservation techniques aim to ensure the cellular and molecular matrix remain intact and functional at implantation, thereby allowing for greatest valve durability.

Although the subject of controversy, valve cellular viability is believed by most surgeons to ensure greatest valve durability and provides the rationale for the current use of homovital[32] and cryopreserved[16] valves. It remains unclear as to what constitutes valve viability. Most studies have been centred on culturing fibroblasts as preservation of these cells is felt to be a marker of the integrity of the extracellular matrix. However, as Albertucci[21] points out, "cell viability at implantation does not necessarily mean the valve will remain viable", and "lack of viability does not necessarily mean the valve will not remain functional".

To validate their cryopreservation technique, O'Brien et al.[15] and Angell et al.[14] performed studies showing their cryopreserved valves were viable at implantation. O'Brien cultured fibroblasts from cryopreserved valves on thawing, indicating fibroblast viability at time of implantation, and on valve explantation 2 months to 10 years after implantation. These explanted valves have shown variable but largely preserved leaflet cellularity.[33] Furthermore, chromosomal studies on an explanted valve from a female patient at 9 years post-AAVR suggested that persisting cells were from the male donor.[16] O'Brien et al.[16] concluded that the superior durability seen in cryopreserved over antibiotic sterilised valves in his series is due to ongoing leaflet donor cell viability many years post-implantation.

Others have not been able to consistently replicate these findings. Several studies on explanted allograft valves have shown reduced to absent leaflet cellularity, absent endothelial cells and altered gross architecture of valves.[34–36] Such studies suggest that cryopreserved valves become largely acellular post-implantation due to loss of donor cellularity, but retain their connective-tissue framework that alone maintains the structural basis of function. In-situ hybridisation[37] and chromosomal[38] studies have shown leaflet cellularity to be of host origin. This has led some authors to believe that host fibroblast ingrowth after implantation, not persisting donor cells, is responsible for the leaflet cellularity in explanted valves and is responsible for preserving the structure and function of implanted valves. The lack of leaflet cellularity and evidence of host cell invasion both indicate that cellular viability at time of implantation may not be important for the long-term durability of the allograft valve. The issue of valve viability and durability remains unresolved.

Immunology of the Allograft

Retaining cellular viability of the allograft has the disadvantage of preserving the antigenic endothelium and dendritic cells of the valve, thereby potentially inducing a destructive immunologic response. This may be akin to the major role played by human leukocyte antigen (HLA) mismatching and HLA-specific antibody in rejection and long-term function of cardiac transplants.[39–42]

Cellular elements of the AAV express HLA antigens. Endothelial cells on allograft valves have been shown to express HLA class 1 and class 2 antigens,[43,44] and immune stimulatory and adhesion molecules that are known to play a role in inflammatory and rejection responses.[45] Dendritic cells, the antigen presenting cells of the human heart valve, within the subendothelial valve matrix also express HLA class 2 antigens.[43]

Allograft valves are capable of triggering both cell-mediated and humoral immune responses that are donor-specific. In vitro studies by Hoekstra showed that fresh and cryopreserved AAV leaflets stimulated human peripheral blood lymphocytes in a lymphocyte proliferation assay and that HLA-DR matching reduces the degree of stimulation.[46,47] Hoekstra et al.[48] then studied T lymphocytes in explanted allografts valves and showed cytotoxicity directed against donor-specific class I and class II antigens. Johnson et al.[49] showed that the endothelial and fibroblasts of cardiac valves were immunogenic, in that each stimulated peripheral blood mononuclear cells when co-cultured in vitro. Three in vivo studies in AAV recipients have shown humoral immune responses directed against donor-specific class I and class II antigens.[50–52]

AAVR patients are not routinely HLA-matched or administered immunosuppressive drugs. Immune responses may thus play a role in the long-term degeneration seen with allograft valves, particularly in the young recipient. Previous experimental use of immunosuppression with cyclosporine, azathioprine and steroids, and use of non-steroidal anti-inflammatory drugs, have been abandoned due to lack of any measurable benefit and fear of their adverse effects.[53,54] Two small retrospective studies have looked at HLA mismatch and markers of long-term valve function. A study with 127 patients by Smith was unable to demonstrate any correlation.[55] However, in a retrospective study of 162 patients Dignan et al.[56] were able to demonstrate that class II HLA mismatch had a significant association with AAV structural deterioration in a small subset of patients with long-term follow-up greater than 5 years.

The issue of ABO incompatibility is also unsettled. While some authors[44] have advocated ABO matching of aortic allografts based on endothelial cell ABO antigen expression[57] and allograft heart transplantation experience, long-term series have not shown a benefit in valve function related to ABO matching. This may be because cardiac valvular endothelium, unlike micro vascular endothelium, does not express blood group antigens.[58] It may also be due to endothelial cell loss from prolonged warm ischemic times, and the sterilisation and preservation process. However, Fischlein et al.[59] showed that all cryopreserved allografts valves provoked an immunologic reaction post-operatively, which was more intense in ABO-incompatible, compared to ABO-compatible allografts. This reaction was reversible without immunosuppression.

In summary, the role of the immune system in the degeneration of allograft valves remains uncertain. We await the results of large HLA-matching outcome studies in AAVR patients to resolve this issue.

Operative Technique

There are four main surgical techniques for implantation: subcoronary 120° rotation, subcoronary intact non-coronary sinus, aortic root replacement or aortic root inclusion (miniroot or cylinder). There is long-standing controversy amongst surgeons as to which is the best technique.

Those advocating careful subcoronary implantation[60,61] indicate reimplantation of the coronary arteries is unnecessary and patient's aortic tissue is retained, providing more flexible support for the graft and reducing leaflet stress. Removal of the allograft aorta should reduce long-term root calcification. However, this technique may be technically more demanding, as it requires precise positioning of valve commissures to prevent valve leaflet distortion. A learning curve has been identified with the subcoronary implantation technique.[62–64]

Those in favour of root replacement argue that this technique allows preservation of the aortic root complex, maintaining sinotubular and commissural geometry, thereby reducing the possibility of valve distortion at implantation and consequent early regurgitation.[65–67] This technique also treats associated annulus or root pathology, such as dilatation, which may have an adverse long-term effect on an implanted subcoronary allograft valve. This method may be technically easier than subcoronary implantation, accounting for a reduction in early reoperations for technical failure seen in some studies.[2,62,68] It is also more forgiving with size mismatches. The ability to size up and down by up to 3 mm is an advantage where limited sized allografts are available.[21] However, the allograft aortic root can undergo hardening and shrinkage due to calcification over time, placing stress on the valve leaflets. Inclusion cylinder techniques retain the patient's native aorta, but distortion of the allograft at aortic closure may occur if the space between graft and the natural aortic root is either too great or too small.[69] Furthermore, tamponade of the allograft or coronary ostial dehiscence may occur if suture lines are not secure.

Whilst it is likely that no single technique is applicable to all types of aortic valve and root disease,[70] current evidence is in favour of the root replacement technique. Root replacements, compared to subcoronary implantation, have suggested a reduced incidence of redo AVR, valve failure and reoperation for technical reasons,[2,68] as well as improved survival.[1] Echocardiographic follow-up studies have also shown a greater incidence of aortic regurgitation and higher transvalvular gradients in the subcoronary compared to the root replacement groups at various institutions.[62,67,71,72] Furthermore, the learning curve with subcoronary techniques suggest that early in the surgeon's allograft experience, root replacement may allow more reproducible results.

In all cases, preoperative echocardiography is performed to estimate the diameter of the aortic annulus. This is especially important if the supply of allograft valves is limited and allografts must be obtained from outside the institution. A median sternotomy is performed and the patient placed on standard cardiopulmonary bypass. An aortotomy is made, the native valve excised, and the aortic ring measured with prosthetic valve sizers or a Hegar dilator to confirm the annular diameter. An appropriately sized allograft is then thawed. It is trimmed appropriately according to the chosen method of insertion, thinning the septal myocardium and partially or completely removing the anterior leaflet of the mitral valve.

The subcoronary 120° rotation method was initially described by Barratt-Boyes.[73] Aortic wall tissue is removed from all three sinuses leaving 3–4 mm margin of tissue for suturing. The valve is rotated 120° counter clockwise to avoid remaining graft muscle from opposing the recipient's ventricular septal muscle. The valve is inverted and the lower edge is sutured to the left ventricular outflow tract with continuous or interrupted 4–0 polypropylene suture. The valve is then everted back to its normal position and the upper margins are sutured to the recipient's aortic sinus with continuous 4–0 polypropylene.

Ross[3] initially described the subcoronary intact non-coronary sinus method. Aortic tissue is removed from only the left and right sinuses leaving the non-coronary sinus intact. This ensures a more reliable valve implantation as the anatomical relationship between two commissures is maintained, leaving only one commissure to be accurately positioned. The allograft is positioned in its anatomical position and the lower edge is secured to the outflow tract as described above. The upper edge is sutured to the recipient aorta and the aortotomy may be attached to the allograft adventitia during closure to obliterate the space behind the allograft sinus.

In the root replacement technique, the allograft is thinned of septal myocardium and the anterior mitral leaflet removed, preserving all three sinuses and a length of ascending aorta. The allograft coronary ostia are excised. The recipient's aortic valve and sinuses are removed, leaving buttons of aortic sinuses around the coronary ostia. Some surgeons choose to insert a pericardial collar between the lower margin of the allograft and left ventricular outflow tract, incorporating this into the lower suture line. Although not essential it may improve haemostasis and provide additional support. The recipient's coronary buttons are sutured to the corresponding openings in the allograft with continuous 5–0 polypropylene. The upper end of the allograft is then attached to the recipient ascending aorta with an end-to-end anastomosis using continuous 4–0 polypropylene sutures.

In the aortic root inclusion technique the recipient's aortic valve is removed leaving the aortic sinus and coronary ostia intact. The allograft is trimmed as for the root replacement method, except a shorter length of ascending aorta is retained. The allograft is placed anatomically within the recipient's aortic root and its lower and upper margins are secured with continuous 4–0 polypropylene sutures.

Buttons are removed from the allograft wall to allow access to the native coronary ostia. The allograft wall is then secured to the native aortic wall around the coronary ostia with 5–0 polypropylene sutures.

Outcomes

Reported early mortality rates (30-day mortality) of 1.1–8.0% are low and comparable to that of xenograft or mechanical valve replacements (Table 2). Early deaths are unrelated to valve failure or dysfunction. Leading cause of early death seems to be non-valvular cardiac failure.[1,2]

Subgroup analysis within series reveals that cardiac surgery has become safer over the years, with early mortality rates dropping accordingly. O'Brien et al.[2] reported an early mortality rate of 3% overall, 8.9% between 1969 and 1975, and 1.13% between 1985 and 1998. Subgroup analysis reveals an expected lower early mortality in low risk subgroups, such as patients who undergo primary allograft AVR with no other concomitant procedures. In Kirklin's series of 178 patients, a relatively high overall early mortality rate of 8.0% was noted. However, in this series patients undergoing primary isolated AAVR ($n = 92$) had an early mortality of only 1%. Hence, for the majority of AAVR patients today who undergo a primary isolated procedure, a low early mortality rate of less than 4% can be expected.

Overall survival is consistently reported in AAVR series (Table 2). Survival is usually determined by non-valve patient-related factors and is not indicative of valve function.[74] Hence, its usefulness is limited and it cannot be used to make meaningful comparisons between different valves.

Direct comparisons of allograft valve failure between series are difficult as they differ in patient selection, methods of sterilisation and preservation, and surgical techniques. Furthermore, several studies report overall results on combinations of preservation and operative techniques, with different numbers of patients in each group. Follow-up duration is also variable. Outcomes for valve failure are reported in several different ways. Valve-related deaths,[19,69] reoperation for structural deterioration,[2] reoperation for any cause,[2,32] reoperation for valve-related causes,[69] redo AVR,[1] one primary tissue failure,[1,19] degenerative valve failure,[32] structural valve degeneration[32] and significant incompetence[23] are all used. Careful examination of their exact definitions is required to determine if comparisons can be made between series. For instance, "primary tissue failure" is most commonly defined as valve dysfunction requiring reoperation or causing death. Langley et al.[75] uses "valve failure" instead, while Lund et al.[1] uses this term to include survivors with clinical, echocardiographic and angiographic evidence of moderate to severe valve failure (otherwise most commonly referred to as "structural valve degeneration"). Internationally agreed guidelines for reporting morbidity and mortality after cardiac valvular surgery now exist.[76] We encourage authors to strictly adhere to these guidelines as this will allow meaningful comparisons to be made between bioprosthetic valve series.

The main reason for valve failure requiring repeat AVR is aortic regurgitation secondary to structural degeneration. Infective endocarditis and early failure due to technical errors are minor causes of valve failure. Freedom from repeat aortic valve replacement for any cause at 5, 10, 15, 20 and 25 years are 93–98, 79–92, 55–76, 35–50 and 24–31%, respectively (Table 3). This demonstrates that the valve has a high risk of failure requiring reoperation beyond 15 years. Hence, series that fail to reach significant numbers of patients past 15 years should be viewed with caution as they may not show the true failure rate of the allograft valve.

There is evidence that the viable allograft (homovital and cryopreserved) is superior to the non-viable antibiotic sterilised 4° stored allograft. The Harefield Hospital series of Lund and Yacoub reported freedom from primary tissue failure at 10 and 15 years of 71 and 71% for the homovital allograft compared to 61 and 32% for the antibiotic sterilised allograft ($P < 0.5$). They also showed a survival benefit with 77% survival in patients receiving the homovital allograft and 46% survival in the non-viable allograft group ($P < 0.5$).[1] O'Brien's Brisbane series had been reporting an impressive superiority in favour of the viable cryopreserved valve over the non-viable antibiotic sterilised 4 °C stored allograft.[77,78] However, a more recent report with follow-up to 29 years showed that the superiority was evident only within the second decade, there being no difference by 20 years.[2]

Special Considerations

Table 4 shows factors that have been associated with allograft valve failure (increased structural degeneration and redo AVR) in published series of long-term results. Three factors are discussed.

A younger recipient age has been associated with higher valve failure and repeat AVR rates due to accelerated valve degeneration in many long-term series.[1,16,23,62,68] The mode of failure is usually leaflet and annular calcification leading to aortic stenosis. O'Brien et al.[2] noted a 10-year freedom from reoperation for structural deterioration of only 47% in the under-20 age group compared to 94% in the over-60 group. Yacoub et al.[32] identified a recipient age of less than 30 as a risk factor for late structural degeneration on multivariate analysis. A similar phenomenon, to a much greater extent, has long been observed in the structural failure of xenograft valves.[79,80] The reason for this is unclear. Most authors postulate an immunologic basis such as a heightened immunologic response in the young.[81,82] Alternatively, early allograft valve failure in the young may be due to growth of the native heart and aorta. The optimal valve replacement conduit in children and young adult may be the pulmonary autograft,[83] with its potential for life-long autograft growth. Although an age cut-off between the indication for an aortic allograft and pulmonary autograft has not been defined, it is likely to be about 20–30 years of age. The aortic allograft is nevertheless a good alternative in the younger patient due to its slow mode of failure and low risk of reoperation.[84]

Table 2. *Early Mortality and Overall Survival for Allograft Aortic Valve Replacement*

Series	Era	Number of Patients	Preservation[a]	Operative Method[b]	30-Day Mortality (%)	Overall Survival (%)[c]
Harefield[1]	1969–1993	618	AB (479), CP (12), HV (127)	SC (551), RR (67)	5.0	84/67/48/35/26
Brisbane[2]	1969–1998	1022	AB (124), CP (898)	SC (635), RR (352), CI (35)	3.0	90/77/60/42/19
	1969–1975	*124*	AB	*SC*	8.9	
	1975–1995	*546*	CP	*SC* (*511*), *CI* (*35*)	2.9	
	1985–1998	*352*	CP	*RR*	1.1	
Salt Lake City[69]	1985–1996	117	CP	SC (106), RR (12)	3.4	76% at 10 years
Southampton[75]	1973–1994	249	AB	SC	2.4	–/78/66/54/–
Southampton[86]	1973–1983	200	AB	SC	1.5	–/81/68/58/–
Birmingham[85]	1981–1991	178	CP	SC (155), RR (23)	8.0	87/85 at 8 years
London19	1964–1986	555	AB (337), CP (218)	SC		52% at 20 years
Auckland[23]	1968–1974	252	AB	SC	6.0	77/57/38 at 14 years
Rotterdam[68]	1987–2001	267	CP	SC (95), RR (180)	5.5	87/73 at 9 years

Series subgroups shown in italics.
[a]AB, four degrees antibiotic sterilised; CP, cryopreserved; HV, homovital.
[b]SC, subcoronary; RR, root replacement; CI, cylinder implantation.
[c]Actuarial rates at 5/10/15/20/25 years, unless stated otherwise.

Table 3. *Long-term Valve-related Outcome after Allograft Aortic Valve Replacement*

Series	Era	Number of Patients	Mean or Median Age (Years) ± S.D.	Preservation[a]	Operative Method[b]	Maximum/ Mean Follow-up (Years)	Freedom from Repeat AVR, Any Cause (%)[c]	Freedom from Valve-related Mortality (%)[c]	Freedom from Structural Valve Failure (%)[c,d]	Freedom from Primary Tissue Failure (%)[c,e]
Harefield[1]	1969–1993	618	51 ± 16		RR (67), SC (551)	27.1/10.1	–/81/55/35/31	–	–	–/62/34/18/14[f]
	1980–1993	*127*	–	*HV*	–	*17.1/–*	–	–	–	*–/71/71/–/–*
	1969–1993	*491*	–	*AB (479), CP (12)*	–	–	–	–	–	*–/61/32/–/–*
Harefield[32]	1980–1993	275	45.8 ± 19	HV	RR (128), SC (147)	14/4.8	98/91/–/–/–	–	94/89/–/–/–	–
Brisbane[2]	1969–1998	1022	–			29/7.3		–	–	–
	1969–1975	*124*	*47.2*	*AB*	*SC*	–	*98/84/58/50/24*	–	–	–
	1975–1995	*546*	*48.7*	*CP*	*SC (511), CI (35)*	–	*96/87/76/48/–*	–	–	–
	1985–1998	*352*	*43.1*	*CP*	*RR*	–	*96/83/–/–/–*	–	–	–
Southampton[86]	1973–1983	200	50.0 ± 14.0	AB	SC	24/15.6	–/86/70/39/–	–/98/95/95/–	–/81/62/31/–	–/81/60/32/–
Southampton[75]	1973–1994	249	51.4 ± 14.9	AB	SC	21/12.4	–/88/72/50/–	–/98/95/94/–	–/86/64/42/–	–/84/60/39/–
Salt Lake City[69]	1985–1996	117	45.6	CP	SC (106), RR (12)	11/4.6	–/92/–/–/–	93% at 10 years	–	–
London[19]	1964–1986	555	40.5 ± 14.0	FD, AB, CP	SC	21/–	–	67% at 20 years	–	–/57/–/12/–
Rotterdam[68]	1987–2001	267	46.1 ± 16	CP (268), HV (7)	SC (95), RR (180)	13.8/4.8	90/77 (9 years)	–	–	–
Auckland[23]	1968–1974	252	51.1	AB	SC	16.5/10.8	93/79/–/–/–	–	95/78/–/–/–	–
Birmingham[85]	1981–1991	178	46	AB	SC (155), RR (23)	9.2/4.0	98/86 (8 years)	–	94/85 at 8 years	–

Series subgroups shown in italics.
[a]AB, antibiotic sterilised and stored in culture medium at 4 °C; CP, cryopreserved; HV, homovital; FD, freeze-dried.
[b]RR, root replacement; SC, subcoronary; CI, cylinder implantation.
[c]Freedom from event at 5/10/15/20/25 years.
[d]Defined as moderate or severe valve malfunction discovered at clinical examination, echocardiography, reoperation or post-mortem examination. Patients with endocarditis and technical failures excluded.
[e]Defined as valve dysfunction, either requiring reoperation or causing death (unless otherwise stated).
[f]Defined as significant regurgitation (at least grade 3/4) or stenosis (=35 mmHg) at angiography or Doppler echocardiography, discovered at redo AVR or post-mortem examination, or when following clinical criteria fulfilled: diastolic murmur, wide pulse pressure (>50 mmHg), increased cardiothoracic index (>0.5), and increasing left ventricular hypertrophy and strain pattern electrocardiogram, all in the absence of infectious endocarditis.

Table 4. *Factors Predicting Valve Failure or Repeat Aortic Valve Replacement in Patients Undergoing Allograft Aortic Valve Replacements*

Factors	Authors
Rising donor age	Lund et al.,[1] Barratt-Boyes et al.,[23] Takkenberg et al.[68]
Younger patient age	Yacoub et al.,[32] Langley et al.,[75] Barratt-Boyes et al.,[23] Kirklin et al.,[85] Takkenberg et al.,[68] Willems et al.,[62] O'Brien et al.[16]
Rising donor minus patient age	Lund et al.[1]
Rising harvest time (death to harvest)	Penta et al.[87]
Rising implantation time (harvest to implantation)	Lund et al.[1]
Large aortic root diameter	Barratt-Boyes et al.,[23] Takkenberg et al.[68]
Aortic root tailoring before subcoronary implantation	Lund et al.,[1] Langley et al.[75]
Operation for endocarditis	Yacoub et al.[32]
Previous xenograft valve	Yacoub et al.[32]
Diabetes mellitus	Lund et al.[1]
Marfan's syndrome	Lund et al.[1]
Operation for infective endocarditis	Yacoub et al.[32]
Operation for pure aortic regurgitation	Yacoub[32]
AV block grade 1–3	Lund et al.[1]
Previous myocardial infarction	Lund et al.[1]

Various donor factors have also been identified as predictive of long-term valve failure. Rising allograft donor age has been reported as a significant factor by several authors.[1,23,32,68] Yacoub identified donor age over 55 years as significant, whilst Barratt-Boyes et al.[23] showed freedom from significant incompetence of 94% at 10 years when donor age was less than 20 years and 64% when donor age was over 50 years. Lund identified donor age greater than 65 as an independent determinant for tissue failure with an odds ratio of 1.75. Conversely, Langley's[75] and Kirklin's[85] groups limited allografts to donors up to 50 and 55–60 years, respectively. Neither series could identify donor age as a significant predictive factor for valve failure. The increasing failure rates with rising donor age are presumably due to age-related structural changes in the valve connective tissue or cellular components. Most authors agree on a donor age cut-off of 50–55 years as being significant and would prefer to implant a younger allograft. However, on this point, supply problems are a great limiting factor in many centres forcing surgeons to accept the older donor valve on occasion.

The increased failure rate seen with aortic root tailoring could reflect disruption of the geometry and structural integrity of the aortic root and sinotubular complex. Alternatively, it could reflect the poor outcome of the group of patients with annulo-aortic ectasia, aortic root dilatation or distortion, whose underlying disease is not treated in the subcoronary, as opposed to the root replacement, method of implantation. This has led most authors to advocate root replacement rather than aortic root tailoring in this group of patients.[20,66,70]

Conclusion

The allograft aortic valve is now known to be an excellent valve replacement conduit out to 15–20 years. In the hands of an experienced tissue-valve surgeon AAVR has low operative and reoperative mortality rates. It offers the adult patient excellent haemodynamic function, close to no thromboembolic and endocarditis risk, and most importantly, freedom from the complications of anticoagulation. Particularly in the active adult the freedom of lifestyle afforded by this is immeasurable. Therefore, AAVR is indicated in younger patients requiring tissue aortic valve replacement when anticoagulation is not desired or contra-indicated. It also has significant advantages over other valve choices in the setting of native or prosthetic valve endocarditis. Future improvements in outcomes can be expected with a greater understanding of the technical aspects for successful implantation, immunology of the allograft, improvement in the supply of fresh allografts and refinement of the sterilizations and preservation process.

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Factors influencing late allograft valve failure

Cheng-Hon Yap and Michael Yii

Allograft valves are a valuable valve replacement substitute in the surgical management of heart valve disease. It remains the valve substitute of choice in the reconstruction of the right ventricular outflow tract in children with congenital heart disease and in the Ross procedure. However, its durability remains suboptimal, particularly in children. This article reviews the mechanisms and factors implicated in late allograft dysfunction, with a focus on the evidence for an immunological cause for allograft failure. Unravelling the mechanisms of allograft valve failure may allow modification of the allograft to improve its long-term durability.

Key Words: allograft, aortic valve, heart valve surgery, immunology, pulmonary valve

Tissue valves used in heart valve replacement surgery are limited by finite durability. Amongst tissue valves, allograft valves continue to hold a place in valve replacement surgery with proven durability up to 15–20 years (1–3). Additionally, they show near-perfect haemodynamics (4, 5) and may have benefits in the setting of endocarditis (6, 7). Most of the evidence favouring the use of allograft valves in the literature is based on the extensive experience with aortic valve allografts in the aortic position and pulmonary valved conduits used for the reconstruction of the right ventricular outflow tract. This article reviews the mechanisms and factors implicated in late allograft dysfunction, with a focus on the evidence for an immunological cause for allograft failure.

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FACTORS INFLUENCING LATE ALLOGRAFT VALVE FAILURE

Identifiable causes of allograft failure, such as endocarditis and technical error at time of implantation, are responsible for only a minority of allograft valve failures. The majority of allograft failures occur in the absence of the above causes. The cause of such valve failure is not clearly understood. It is likely to be complex and multifactorial, with an interaction between donor factors, recipient factors, technical factors, valve procurement and processing factors, and a host immune response towards the allograft. The interaction and magnitude of factors such as mechanical stress, ischaemia, chemical insults and/or immunological destruction is likely to be highly variable. Nevertheless, it is vital that the role of these factors be identified and understood. This would allow modification of important variables, such as better patient selection, host response, technical/operative and valve processing variables, in order to improve long-term results of allograft valve implantation.

Donor factors

The quality of the donor valve, although never formally assessed in the literature, is an important factor. Most tissue valve banks have strict criteria in assessing allografts for quality. The Donor Tissue Bank of Victoria, Australia, for instance, adopts a system assessing for tears, lacerations, fenestrations, contusions, atheroma or calcific deposits in the allograft valve or conduit. Allografts with major structural anomalies or pathological lesions are not implanted as they could lead to valve failure. Even minor lesions may cause undue mechanical stress within the valve–root complex of the implanted allograft, leading to accelerated failure over time.

Increasing donor allograft age has been reported as a significant factor by several authors (1, 8–11). Yacoub et al. in their series of homovital aortic allografts identified donor age over 55 years as significant (9). Barratt-Boyes et al. showed freedom from significant valve incompetence of 94% at 10 years when donor age was less than 20 years and 64% when donor age

was over 50 years (8). Lund et al. identified donor age greater than 65 as an independent determinant for tissue failure with an odds ratio of 1.75 (1). In view of this, most valve banks exclude valves from donors greater than 50–60 years of age. Langley's (12) and Kirklin's (13) groups limited allografts to donors up to 50 years and 55–60 years, respectively. Consequently, neither series could identify donor age as a significant predictive factor for valve failure. The increasing failure rates with rising donor age are presumably due to age-related structural changes in the valve connective tissue or cellular components.

In contrast, Gerestein et al. in their analysis of 316 allografts used for right ventricular outflow tract (RVOT) reconstruction identified donor age less than 30 years as a strong risk factor (hazard ratio 3.5) for valve-related reoperation (14). This finding likely reflects the strong association between young patient age at operation, need for a small-sized allograft and hence young allograft donor age. It is probable that young patient age at operation and small allograft are independent risk factors, whereas younger donor age is not.

It is possible that the donor to recipient age relationship may more accurately predict valve survival than either the donor or recipient age taken in isolation. Lund et al. showed in their large aortic allograft valve series that rising donor age minus recipient age may be the more important factor, rather than either donor or recipient age alone, in accurately predicting valve dysfunction (1). In conclusion, most authors agree on a donor age cut-off of 50–55 years as being significant and would prefer to implant a younger allograft. However, supply problems are a great limiting factor in many centres forcing surgeons to accept older donor valves on occasion.

Host factors

A consistent finding in most series of allografts in the aortic or pulmonary position is that long-term allograft durability falls with younger age at operation. This relationship of age at operation and allograft durability is linear. In long-term series of aortic allograft replacement, a younger recipient age has consistently been associated with allograft failure and need for allograft valve reoperation due to accelerated valve degeneration (1, 2, 8, 10, 15). O'Brien et al. noted a 10-year freedom from reoperation for structural deterioration of only 47% in the under-20 age group compared to 94% in the over-60 group (2). A similar age trend is seen in allografts implanted in the RVOT (14, 16–22). Forbess et al. showed that 5-year survival of the allograft in the RVOT was 25% for recipients less than 1 year old, 61% for those between 1 and 10 years, and 81% for those older than 10 years (23).

A similar phenomenon, to a greater extent, has long been observed in the structural failure of xenograft valves (24, 25). The reason for this age-related degeneration is unclear. Most authors suggest an immunologic basis for this, such as a heightened immunologic response in the young (21, 26, 27). However, convincing evidence for this is lacking. Alternatively, early allograft valve failure in the young may be due to outgrowth of the implanted allograft, with progressive growth of the native heart and aorta or pulmonary artery. A third factor is the increased haemodynamic demands placed on the allograft by younger patients who are both more active and have higher metabolic needs.

Smaller allograft size has frequently been found to be associated with valve failure in studies of RVOT allografts (14, 17, 19, 20, 23). Only one large series of RVOT allografts did not find conduit size as predictive of failure (28). Two speculative explanations for this finding are possible. Firstly, all implanted allografts undergo a similar destructive process but in smaller allografts the resultant haemodynamic impact is greater, in the form of a higher transvalvular gradient or greater valve incompetence. Secondly, in younger patients smaller valves are more rapidly outgrown with increasing body size. The size of the heart and pulmonary artery increases out of proportion to the implanted allograft, resulting in either progressive valvular incompetence due to annular dilatation and failure of cusp coaptation, or conduit stenosis. This is particularly evident in infants, who generally triple their size within 18 months. As a consequence, most surgeons prefer to implant an oversized allograft conduit in the RVOT.

Allografts implanted in the RVOT in an anatomical position occur in patients undergoing the Ross procedure. In contrast, extra-anatomic implantation occurs in patients requiring RVOT reconstruction for complex congenital heart disease. Allograft survival is greater in the former compared to the latter (14, 22, 23, 29). This is probably because greater haemodynamic stress is placed on the allograft in patients with congenital heart disease, due to high pulmonary vascular resistance and abnormal pulmonary vascular anatomy (18, 30, 31). It could also be related to other technical factors inherent to the non-anatomical placement of the allograft such as utilization of non-allograft material for conduit extension and sternal compression of the allograft (21).

Method of insertion

There are four surgical techniques for implantation: subcoronary 120-deg rotation, subcoronary intact non-coronary sinus, aortic root replacement or aortic root inclusion (miniroot or cylinder). It remains unclear as to which is the best technique with each technique having potential advantages and disadvantages. It is likely that no single technique is applicable to all types of aortic

valve and root disease (32). In general, current evidence is in favour of the root replacement technique. Some have suggested root replacements, compared to subcoronary implantation, have a reduced incidence of redo aortic valve replacement (AVR), valve failure and reoperation for technical reasons (2, 10), as well as improved survival (1). Echocardiographic follow-up studies have shown a greater incidence of aortic allograft regurgitation and higher transvalvular gradients in the subcoronary compared to the root replacement groups at various institutions (11, 15, 33, 34). Lower incidence of pulmonary autograft valve reoperation and dysfunction has also been reported with the use of the root replacement technique (35). Furthermore, the learning curve with subcoronary techniques suggests that early in the tissue valve surgeon's experience, root replacement may allow more reproducible results (15, 36, 37).

Valve procurement, processing and storage factors

Current valve procurement, processing and storage methods are intended to maximize cellular and connective tissue viability of allografts. Hence, ischaemic times (duration between donor death and cryopreservation, or in the case of homovital and antibiotic-preserved valves, duration between death and implantation) and the use of potentially damaging chemical compounds are minimized.

In relation to ischaemic times, conflicting results are found in the literature. Dignan et al. (38) and Baskett et al. (17) found short duration (<16 h) between allograft procurement and cryopreservation, and shorter antibiotic preservation times prior to cryopreservation, respectively, were predictive of valve failure. On the basis of this one may conclude that when valve ischaemic time is greater, cryopreserved valve allografts may be less antigenic due to loss of histocompatibility antigen expression or cell death. Hence, once implanted these valves would be less subject to immunological damage. In both studies the total ischaemic time (duration between death and cryopreservation) is not clear so interpretation of the results is difficult.

Tweddell et al., in their study of 220 cryopreserved aortic and pulmonary allografts used in the reconstruction of the RVOT found warm ischaemic time (time from donor death to organ procurement) in excess of 5.5 h to be associated with allograft failure (20). Yacoub's group has found greater warm ischaemic time (39) and duration between valve harvest and implantation (1) as predictive of valve failure. Given that longer ischaemic times reduce cellular viability (29, 40) and possibly connective tissue matrix integrity, long-term allograft durability may be thus reduced.

In conclusion, there may be an optimal duration of allograft ischaemia, which would maximize the long-term durability of the implanted allograft. The duration should be long enough to reduce the detrimental expression of elements responsible for allograft antigenicity, for instance human leukocyte antigen (HLA) expression by endothelial cells or dendritic antigen-presenting cells, whilst preserving the elements conducive to long-term durability such as the connective-tissue matrix and fibroblast cell viability.

Previous sterilization techniques such freeze-drying, irradiation, chemical preservation with beta-propiolactone, buffered formaldehyde and ethylene oxide are no longer in use because they were implicated in early valve failure due to cusp rupture. More recently, the use of amphotericin B has also been identified as deleterious to allograft viability (40). Most centres have thus avoided the use of amphotericin B in their sterilization procedure. Currently, most allograft valves implanted are of the cryopreserved variety. Cryopreservation maintains greater than 50% of cellular viability, provided the duration between death and cryopreservation is kept relatively short (<24–48 h) (29). Although difficult to prove, preserved viability with cryopreservation and the use of viable "homovital" valves is believed to be the basis for the improved durability of currently used allograft valves (2, 9).

INFLUENCE OF IMMUNE SYSTEM ON VALVE FAILURE

Despite an abundance of evidence that allograft valves are immunogenic and that a host immune response to implanted valves exists, the clinical relevance of this remains unclear. Hence, several clinically important questions remain unresolved: Will immunosuppression of allograft recipients postoperatively improve allograft valve survival? Is there an advantage to prospective preoperative HLA matching of donors and recipients? What modifications can we make to the sterilization and preservation process to improve the clinical durability of allograft valves? In examining the myriad of evidence for a role of the host immune system in allograft valve failure, perhaps the strongest evidence is a simple observation made by Donald Ross, the pioneer of allograft and autograft valve surgery: that the pulmonary autograft shows improved durability over the aortic allograft in the aortic position (80% (3) vs 50% (1, 2) valve survival at 20 years) stands alone as strong evidence for a role of the immune system in mediating allograft valve failure (3).

Valve antigenicity and viability

Currently used allografts fall into one of three groups:

1. Homovital or fresh valves are harvested using sterile technique from heart transplant recipients or

brain-dead multi-organ donors and kept in nutrient medium (e.g. Medium 199) containing extremely low doses of antibiotics. They are stored at 4°C and used at the earliest opportunity. They are considered viable if used within 4–8 days. Valve viability is defined as viability of fibroblasts at time of implantation.

2. Cryopreserved valves are obtained from either heart transplant recipients, brain-dead multi-organ donors or, as is most often the case, routine autopsies. They are sterilized in an antibiotic and nutrient medium solution for a variable amount of time, usually between 6 and 48 h, then cryopreserved and stored in liquid nitrogen. They are seen as viable if cryopreserved within 2–3 days of donor death (41).
3. Antibiotic-sterilized valves stored at 4°C are usually harvested from routine autopsies and kept in an antibiotic and nutrient medium until used at a later date. They are considered non-viable.

The aortic valve consists of endothelial cells and fibroblasts that produce and remodel the valve matrix of collagen, elastin and mucopolysaccharides. These cells maintain the haemodynamically perfect structure of the valve and ensure its non-thrombogenicity. Current sterilization and preservation techniques aim to ensure the cellular and molecular matrix remain intact and functional at implantation, thereby allowing for greatest valve durability (42).

Although the subject of controversy, valve cellular viability is believed by most surgeons to ensure greatest valve durability and provides the rationale for the current use of homovital (9) and cryopreserved (43) valves. It remains unclear as to what constitutes valve viability. Most studies have been centred on culturing fibroblasts as preservation of these cells is felt to be a marker of the integrity of the extracellular matrix. However, as Albertucci points out, "cell viability at implantation does not necessarily mean the valve will remain viable", and "lack of viability does not necessarily mean the valve will not remain functional" (44).

To validate their cryopreservation technique, O'Brien et al. (41) and Angell et al. (45) performed studies showing their cryopreserved valves were viable at implantation. O'Brien et al. cultured fibroblasts from cryopreserved valves on thawing, indicating fibroblast viability at time of implantation, and on valve explantation 2 months to 10 years after implantation. These explanted valves have shown variable but largely preserved leaflet cellularity (46). Furthermore, chromosomal studies on an explanted valve from a female patient at 9 years post-allograft aortic valve replacement (AAVR) suggested that persisting cells were from the male donor (43). O'Brien et al. concluded that the superior durability seen in cryopreserved over antibiotic sterilized valves in their series is due to ongoing leaflet donor cell viability many years post-implantation (43).

Others have not been able to consistently replicate these findings. Several studies on explanted allograft valves have shown reduced to absent leaflet cellularity, absent endothelial cells and altered gross architecture of valves (47–49). Such studies suggest that cryopreserved valves become largely acellular post-implantation due to loss of donor cellularity, but retain their connective-tissue framework that alone maintains the structural basis of function. *In situ* hybridization (50) and chromosomal (51) studies have shown leaflet cellularity to be of host origin. This has led some authors to believe that host fibroblast ingrowth after implantation, not persisting donor cells, is responsible for the leaflet cellularity in explanted valves and is responsible for preserving the structure and function of implanted valves. The lack of leaflet cellularity and evidence of host cell invasion both indicate that cellular viability at time of implantation may not be important for the long-term durability of the allograft valve. Hence, the issue of valve viability and durability remains unresolved.

Retaining cellular viability of the allograft has the disadvantage of preserving the antigenic endothelium, fibroblasts and dendritic cells of the valve, thereby potentially inducing a destructive recipient immunologic response. This may be akin to the major role played by HLA mismatch and HLA-specific antibodies in rejection of cardiac transplants (52–55).

Host-immune response

Given that valvular elements are antigenic, it is not surprising that allograft valves are capable of triggering both cell-mediated and humoral immune responses that are donor-specific. *In vitro* studies by Hoekstra et al. showed that fresh and cryopreserved allograft valve leaflets stimulated human peripheral blood lymphocytes in a lymphocyte proliferation assay (56, 57). Hoekstra et al. then studied T lymphocytes in explanted allograft valves and showed cytotoxicity was directed against donor-specific class I and II antigens (58). Yacoub's group also showed that endothelial cells and fibroblasts of cardiac valves were immunogenic, as they each stimulated peripheral blood mononuclear cells when co-cultured *in vitro* (59). *In vivo* studies in allograft valve and conduit recipients have consistently shown humoral immune responses directed against donor-specific HLA class I and II antigens (52, 60–63). Furthermore, this response remained positive for up to 15 years after allograft implantation, suggesting that the presence of allograft material leads to a persistent and prolonged antibody response (64). In summary, recipients of currently used viable allograft valves show donor-specific humoral and cell-mediated immune responses,

which may play a role in the subsequent dysfunction of allograft valves.

As previously mentioned, some studies have linked short times between death and cryopreservation (i.e. ischaemic time) with long-term allograft dysfunction (38, 65). This would be consistent with the theory of immune-mediated valve dysfunction as it is known that allograft viability (29) and immunogenicity (66) decrease with increasing ischaemic times. This suggests that a clinically significant role for immune-mediated allograft dysfunction exists and that this role is modifiable by defining and applying the optimal duration of allograft ischaemia.

In Stark et al.'s series of 405 allografts implanted in the RVOT for congenital heart disease it was observed that patients receiving second or subsequent allografts had higher allograft failure rates than those receiving their first allografts (28). It is likely that this is caused by an enhanced immune response to the allograft in a patient sensitized by previous allografts. Hence, young patients receiving a second or subsequent allograft may benefit from careful HLA matching or immunosuppression.

In contrast to human studies, animal studies have shown good direct evidence for immune mediated allograft valve destruction. Moustapha et al. showed that both fresh and cryopreserved cardiac allograft valves in the rat model undergo changes that are characteristic of cell-mediated rejection and lead to structural valve failure. This is in contrast to the syngeneic valves that were used as controls in their study which showed no cellular infiltration and remained structurally preserved (67). Legare et al. showed that in a rat model of allograft valve transplantation the immune destruction is T cell mediated. In their study T-cell-deficient rats who received allograft valves showed none of the cellular infiltration or valvular structural deterioration seen in the immune competent rats (68). They also showed that immune-modulatory measures, such as cyclosporin therapy and anti-integrin monoclonal antibodies are effective in preventing allograft valve structural failure in the rat model (69).

Despite substantial evidence as described above, until recently the magnitude and clinical significance of a destructive immune response in allograft valve degeneration was unclear as no direct association with functional outcomes in humans had been established. Such direct evidence can only be found by showing that the performance of allograft valves is associated with donor-recipient matching of the major transplantation antigen systems, namely the ABO and HLA in humans. This is akin to the well-established role of ABO and HLA matching in solid organ and bone marrow transplantation (70–73).

ABO blood group compatibility

The A and B histo-blood group antigens are coded on the ABO locus on chromosome 9. This locus codes for two glycotransferase enzymes which transfer a terminal carbohydrate unit to a core H chain, giving either A or B antigenic properties to the cell membrane. The H chain is a transmembrane saccharide chain that is part of a glycolipid or glycoprotein complex present on the surface of most nucleated cells (74). The addition of N-acetylgalactosamine to the H chain as the terminal carbohydrate confers blood group A specificity, and the addition of D-galactose confers group B specificity. In humans, A and B antigens are widely distributed on the surface of almost all cells, principally on vascular endothelium, epithelium, primary sensory neurons, bone, cornea, skin, red blood cells and platelets. They are also found within the cells, such as within Golgi apparatus, secretory granules and cell nuclei. Furthermore, these antigens are secreted in plasma and secretions, such as saliva and urine (75). The importance of the ABO system in transplantation is that humans have antibodies against those ABO antigens absent in their own tissue. These antibodies can then induce an immune rejection process against transplanted tissue expressing foreign A or B antigens. Hence, ABO matching is usually performed in solid organ transplants (i.e. kidney, heart and liver) and in bone marrow transplantation (75).

The issue of ABO incompatibility in allograft valves is unsettled. Some authors (19, 76) have advocated ABO matching of allografts based on endothelial cell ABO antigen expression (77) and allograft heart transplantation experience (78). In studies on paediatric recipients of allograft valved conduit for reconstruction of the RVOT, ABO blood group mismatch was found to be predictive for allograft failure in two series (17, 79). Notably, both were series of paediatric valve allograft recipients. However, the vast majority of other series of adult and paediatric allograft replacement in the pulmonary and aortic positions have not identified either ABO or Rheuses incompatibility as a significant factor in predicting allograft dysfunction (9, 11, 14, 19, 21, 26, 28, 38, 80–82). There may be several reasons for this. Firstly, it may be that cardiac valvular endothelium, unlike other vascular endothelium, does not express blood group antigens. Kadner et al., using antibodies to ABO antigens, showed that fresh and cryopreserved allograft valvular endothelium does not express ABO antigens in contrast to microvascular cardiac endothelium (83). Secondly, most allografts may be non-ABO immunogenic, due to pre-implantation endothelial cell loss from prolonged warm ischaemic times, and the sterilization and preservation process. Finally, it may be that as predicted, allograft

valvular endothelium is rejected and destroyed post-implantation in ABO-incompatible cases. However, this may not translate to a difference in valvular function, as unlike solid organ transplants, intact endothelium in the transplanted allograft valve may not be vital to its long-term function. In support of this final possibility, Fischlein et al. showed that all cryopreserved allografts valves provoked an immunologic reaction postoperatively, which was more intense in ABO-incompatible, compared to ABO-compatible allografts. However, the significance of the detected immunologic reaction was unclear as it was reversible without immunosuppression after an average of 3 days (84). In conclusion, the need to prospectively ABO match donor and recipients of allograft valves is unresolved and requires further study.

Human leukocyte antigen compatibility

The HLA molecules are encoded on chromosome 6 in a region known as the major histocompatibility complex (MHC), which spans over 4 megabases. The MHC contains the six major HLA loci which encode structurally similar molecules, known as HLA class I and II molecules based on their distribution, structure, source of peptide antigen and the class of responding T cells. These molecules function as receptors for peptide fragments (i.e. antigens) that are displayed on the cell surface where they are recognized by appropriate T cells. By facilitating antigen presentation to helper and cytotoxic T cells they regulate specific immunity and play a vital role in determining rejection of tissue between genetically distinct individuals (85, 86).

There are approximately 20 class I HLA genes, 3 of which, HLA-A, -B and -C, are the main players in regulating antigen presentation. The three main class II HLA genes are HLA-DR, -DQ and -DP. Class I molecules are expressed on most nucleated cells and interact with antigen-specific T cells of the CD8 phenotype, also known as cytotoxic T cells. In contrast, class II molecules are only expressed by specialized antigen-presenting cells such as B cells, dendritic cells and macrophages. The class II molecules specifically interact with the antigen-specific T cells of the CD4 phenotype. These are the helper T cells which play a central role in the immune system by augmenting macrophage function and promoting the proliferation and differentiation of B cells and cytotoxic T cells (86).

The importance of HLA matching in bone marrow and certain solid organ transplants, such as kidney and heart transplantation, is well established. In the international multi-centre Collaborative Transplant Study a highly significant advantage of HLA matching on patient and graft survival rates was found in the analysis of kidney transplants. Ten-year post-transplantation graft survival rates were 17% lower in first cadaver kidney transplants with a complete mismatch (6 HLA-A + -B + -DR mismatches) compared to those with no mismatch (70). The effect was similarly strong in first heart transplants where at 3 years graft survival was 84% in those with zero or one mismatch and 71% in those with six mismatches (53). The accepted mechanism behind HLA-mismatch-related rejection of allogeneic tissue is a host T cell response triggered by recognition of non-self class II HLA antigens on antigen-presenting and endothelial cells present in transplanted tissue. This is followed by an amplification phase against class I and II non-self antigens present on most transplanted cells, with a resultant destructive donor-specific cell-mediated and humoral response against graft cells possessing non-self HLA antigens (86). There is good evidence for this mechanism applying in allograft valve transplantation in a rat model. As mentioned above, Legare et al. found that T cell nude mice did not show immune cell infiltration and valve destruction after allograft valve implantation (68).

Fresh allograft valves are antigenic in that valvular endothelial and dendritic cells, and possibly fibroblasts, within allograft valves express HLA class I and II antigens (66, 76). Accounting for the fact that the preservation and storage process may reduce this antigenicity (as described above) by down-regulating HLA antigen expression or by reducing cellular viability, what then is the role of HLA compatibility in the contemporary use of allograft valves? Several studies have attempted to answer this question with inconsistent results. Two studies by Smith et al. (87) and Bechtel et al. (88) have shown no association between HLA mismatch and valve performance. In contrast, two studies by Baskett et al. (17) and Dignan et al. (38) have linked HLA mismatch with poorer valve performance.

Baskett et al. studied 47 paediatric allograft valve recipients who had reconstructions of the RVOT, with a mean age of 5 years. They found that HLA-DR mismatch was associated with echocardiographic valve failure by multivariate analysis, after a mean follow-up period of 4.6 years (maximum 11 years). It was unclear in their paper as to whether HLA class 1 antigen matching also was analysed (17). Dignan et al. studied 162, mainly adult, recipients of cryopreserved aortic allografts with a maximum follow-up of 12 years. They found that HLA-A, -B and -DR were not associated with echocardiographic allograft failure in the overall analysis of the entire cohort. However, when the subset of 92 patients with at least 5 years follow-up was analysed separately HLA-DR (but not HLA-A or -B) mismatch was associated with allograft valve failure by bivariate analysis. It is likely that the two studies that failed to reveal an association between HLA match and valve performance did so because of the bias of small numbers (88), short follow-up (88), older recipient cohort and long durations between donor death and

cryopreservation or implantation (87) (in the case of homovital valves).

In conclusion, there is emerging clinical evidence supporting a role for HLA-DR, but not HLA class 1, antigen matching in determining long-term allograft valve performance. This is supported by *in vitro* tissue culture studies by Hoekstra et al., which show that HLA-DR matching between responder T-lymphocytes and stimulatory allograft valve pieces and allograft valve endothelial cells, reduces the intensity of T-lymphocyte stimulation (56, 57).

Use of immunosuppression

Studies in the rat model have shown that cyclosporin therapy may decrease immune cellular infiltration and preserve structural integrity of implanted allograft valves (69, 89). The optimal duration of cyclosporin therapy required to arrest allograft valve destruction is unclear. These studies suggest that a 14- or 28-day course is effective whereas a 7-day course is insufficient. Long courses of immunosuppression are undesirable in the clinical setting due to the risks of infection and malignancy. These risks would have to be balanced against that of accelerated valve failure.

Clinical efforts have been made to use immunosuppressive therapy for the prevention of allograft rejection in paediatric patients (26). However, there is no convincing evidence that it works, and many have abandoned such therapy for fear of deleterious side effects. Nevertheless, some groups currently use routine immunosuppression in younger (90) and other selected patients deemed at high risk for allograft valve rejection (17, 89).

FUTURE DIRECTIONS

Allograft valves and conduits will remain a useful valve replacement conduit in patients requiring tissue valves, particularly in those undergoing the Ross procedure, those requiring RVOT reconstruction due to congenital heart disease and patients with infective endocarditis. This will remain so until newer xenograft or tissue-engineered valve prostheses show proven durability with long-term follow-up. Modifications to reduce allograft antigenicity, such as the Synergraft™ (Cryolife Inc., Kennesaw, GA, USA) decellularization process, may extend the durability of the allograft (91). Over 1000 Synergraft™ pulmonary allografts have been utilized in the last 2.5 years within the Ross Registry series, with good short-term results (92). Tissue-engineering methods such as *in vitro* seeding of decellularized allografts with fibroblasts or endothelial cells also show promise (93).

HLA matching studies of allografts with large numbers of paediatric patients and longer follow-up beyond 5 years are required to put to rest the question of the clinical significance of immunological destruction. Improvement in our understanding of the mechanisms of allograft valve failure may allow modification of the allograft or of management of allograft recipients to improve its long-term durability.

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Anti-HLA Antibodies after Cryopreserved Allograft Valve Implantation does not Predict Valve Dysfunction at Three-Year Follow Up

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Background and aim of the study: **As the cause of allograft heart valve degeneration is poorly understood, the study aim was to investigate the host antibody response to allograft valve implantation.**

Methods: **Sera were obtained from 92 recipients of allograft heart valves (61 pulmonary, 31 aortic). Sera were tested for anti-HLA class I antibodies by ELISA and complement-dependent cytotoxicity (CDC) methods, and anti-HLA class II antibodies by ELISA. Specificities of recipient anti-HLA class I antibodies were defined by standard CDC testing against a panel of T lymphocytes from 80 blood donors. Donor valve HLA typing was performed on stored donor DNA samples using molecular methods. The presence of donor-specific anti-HLA class I antibodies was hence defined in recipient sera. The presence of anti-HLA antibodies and donor-specific anti-HLA class I antibodies were correlated with function of allograft valves at the most recent echocardiographic follow up.**

Results: **At a mean of 3.0 years (range: 0.3-5.4 years) after allograft implantation, 96% (87/92) and 82% (75/92) of patients were positive for anti-HLA class I and II antibodies, respectively, by ELISA testing. Some 68% (61/90) of patients were positive for anti-HLA class I antibody (PRA >5%) by CDC testing. PRA levels decreased with greater postoperative interval (r = -0.31, p = 0.003). In 68 recipients where donor HLA type was defined, 54% (37/68) of patients had antibodies specific to at least one donor HLA class I antigen. In 87 patients with a recent echocardiographic examination available for analysis (at a mean of 3.5 ± 1.6 years postoperatively), there was no association between valve dysfunction and antibody status.**

Conclusion: **Anti-HLA class I and II antibodies were detected by ELISA methods in most patients after allograft implantation extending to 5.4 years. The clinical significance of these findings is unclear, as no correlation was found between the prevalence of anti-HLA antibody and echocardiographic parameters of valve dysfunction at a mean of 3.5 years follow up.**

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Tissue valves used in heart valve replacement surgery are limited by finite durability. However, among tissue valves allografts continue to hold a place in valve replacement surgery, with proven durability up to 15-20 years (1-3). Allograft valves are therefore a valuable valve replacement substitute in the surgical management of heart valve disease, and they remain the valve substitute of choice in reconstruction of the right ventricular outflow tract (RVOT) in children with congenital heart disease, and in the Ross procedure. Allograft valves are also used for aortic valve replacement (AVR), particularly in the setting of endocarditis. Nonetheless, allograft durability remains suboptimal, particularly in children and young adults. The causes of allograft heart valve degeneration are poorly understood, though a variety of factors have been associated with valve degeneration, including recipient and donor age, valve size, warm ischemia time, and immunological factors (4). There is increasing evidence that a valve-specific host immune response might be responsible for allograft valve degeneration (5-9). The aim of the present study was to investigate the long-term host antibody response to allograft valves by performing a cross-sectional study of allograft valve recipients in the Melbourne region of Australia.

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Clinical material and methods

Patient population

A total of 92 patients (28 females, 64 males; mean age 45 ± 14 years; range: 18 to 72 years at the time of surgery) was included in the study. Allograft valve replacements were performed by five surgeons at eight institutions in Melbourne, Australia. Sixty-one patients received pulmonary allografts in the pulmonary position, and 31 received aortic valve allografts implanted in the aortic position. The primary operation type was AVR in 31 patients, Ross procedure in 51, and pulmonary allograft reconstruction of the RVOT in 10. At least one concomitant procedure was performed in 58 patients (63%); these included reduction of the aortic valve annulus (n = 38), tailoring of aneurysmal ascending aorta (n = 11), replacement of aneurysmal ascending aorta (n = 10), coronary artery bypass grafting (n = 5), repair of main pulmonary artery (n = 4), RVOT enlargement (n = 2), mitral valve repair (n = 2), AVR (n = 2), left ventricular outflow tract myomectomy (n = 1), pulmonary valve annulus enlargement (n = 1), pulmonary valve annulus reduction (n = 1), tricuspid valve repair (n = 1), patent ductus arteriosus closure (n = 1), and patent foramen ovale closure (n = 1).

Valve disease was predominantly stenosis in 37 patients (40%), regurgitation in 42 (46%), and mixed in 13 (14%). The etiology of valve disease was predominantly congenital unicuspid or bicuspid aortic valve disease. This was the sole etiology in 70 patients (76%); the remaining patients had various other valve etiologies, including congenital heart disease (n = 6), active endocarditis (n = 4), previous endocarditis (n = 3), degenerative RVOT conduit or valve prosthesis (n = 4), degenerative aortic valve disease (n = 3), degenerative aortic valve prosthesis (n = 1), and rheumatic valve disease (n = 1). Eighteen patients (20%) had undergone at least one previous cardiac operation.

Allografts had been implanted in four patients previously. Two patients had each received a single aortic valve allograft previously, one patient had received two aortic allograft valves implanted previously for recurrent endocarditis, and one had previously received a pulmonary valve allograft for a Ross procedure.

Study design

All adult patients (those aged >18 years at the time of surgery) who received an allograft heart valve supplied by the Donor Tissue Bank of Victoria (DTBV), Australia, between 1st June 1998 and 31st March 2003, operated on by five participating surgeons, were considered for the study. Patients who failed to provide their informed consent to participate were excluded. The study was carried out in accordance with National Health & Medical Research Council of Australia guidelines following approval from the Ethics Committee of St. Vincent's Hospital Melbourne and the Victorian Institute of Forensic Medicine in March 2003.

Allograft valve harvest and preservation

Allograft valves from the DTBV were obtained from routine coronial cadavers. The donor age limit was 55 years, and hearts were obtained within 24 h of donor death. For valves used in this study, the mean donor age was 36.6 ± 12.5 years (range: 14 to 55 years). Upon retrieval, the heart valves were incubated at 37ºC for 6-8 h in a solution containing nutrient media and antibiotics (penicillin 30 µg/ml; streptomycin 50 µg/ml). The valve was then frozen to -40ºC in a controlled rate liquid nitrogen freezer and stored in the vapor phase of liquid nitrogen until use. The mean valve ischemic time (interval between death and cryopreservation) was 30.5 ± 5.2 h (range: 20.2 to 47.6 h).

Antibody and donor HLA testing

Recipient blood samples were obtained between May 2003 and April 2004 for antibody testing. Patient serum was tested for immunoglobulin G (IgG) antibodies against HLA class I antigens using a commercially available qualitative solid-phase enzyme-linked immunosorbent assay (ELISA) (QUIKSCREEN®; GTI Diagnostics, Waukesha, WI, USA) (10). Patient serum was added to microwells coated with affinity-purified HLA class I (HLA-A, B and C) glycoproteins, obtained from platelets of White, Black and Hispanic blood donors, allowing antibody, if present, to bind. Reagents were added according to the manufacturer's instructions, and the optical density (OD) of the color that developed was measured. Results showing OD values equal to or greater than two-fold the value obtained for the mean of the negative controls were regarded as positive results. Patient serum was tested for IgG antibodies against HLA class II antigens using a qualitative solid-phase ELISA (B-SCREEN®; GTI Diagnostics) (11). The testing procedure was identical to that described for QUIKSCREEN® above, with the exception that the HLA class II glycoproteins were obtained from Epstein-Barr virus-transformed B lymphocyte cell lines; these were carefully chosen to provide a wide range of HLA class II antigens.

Patient serum was also tested for antibodies against HLA class I antigens using a standard complement-dependent microlymphocytotoxicity (CDC) technique (12). Recipient sera were tested against a panel of T lymphocytes from 80 blood donors of known HLA-type. The panel covered all known HLA class I specificities. A positive reaction was defined as >10% dead cells per well. The panel reactive antibody (PRA) level is the number of wells against which the patient's

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serum reacted, expressed as a percentage of the total number of wells tested. Recipients were assigned as having antibodies against HLA class I antigens if the PRA was >5%. Anti-HLA class I antibody specificities were assigned by an experienced laboratory scientist, blinded to the donor HLA type, if the PRA was >5%.

DNA was extracted from donor cadaveric blood by conventional salting-out methods (13). Donor HLA class I typing was performed by polymerase chain reaction (PCR) amplification and sequencing methods (14), using in-house PCR primers, Big Dye terminator chemistry and an ABI 3730 48 capillary sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Donor HLA class II typing was performed by PCR amplification sequence-specific oligonucleotide (SSO) typing methods. PCR amplification of exon 2 was performed, followed by detection of hypervariable regions by a set of oligo-probes specific for sequence differences.

Echocardiography

Echocardiography was performed in participating patients as part of their routine follow up, as requested by their treating cardiologist or cardiothoracic surgeon. In the absence of symptoms, routine echocardiography was performed every one to two years, depending on individual surgeon or physician preferences. The outcome measured was the valve function at most recent postoperative echocardiographic study with regards to trans-allograft valve mean gradient in mmHg (continuous data) and degree of allograft valve regurgitation (categorical data: trivial, mild, moderate, or severe). The degree of allograft valve dysfunction on echocardiography was further categorically classified as follows: (i) nil dysfunction: nil or trivial valvular regurgitation, and mean gradient ≤10 mmHg; (ii) mild dysfunction: mild valvular regurgitation, or mean gradient 11-30 mmHg; (iii) moderate dysfunction: moderate valvular regurgitation, or mean gradient 31-50 mmHg; and (iv) severe dysfunction: severe valvular regurgitation, or mean gradient >50 mmHg.

Data analysis

Data analysis was performed using SPSS version 12.0 (SPSS, Chicago, IL, USA). Data were presented as mean ± SD. Continuous data were compared using the Mann-Whitney log-rank test, while categorical data were compared using the chi-square test. Correlation between continuous variables (PRA and postoperative duration) was assessed by calculating the Pearson correlation coefficient (r). Correlation between categorical and continuous variables (antibody status and postoperative duration) was assessed with the Spearman's rho statistic. A p-value <0.05 was considered to be statistically significant.

Results

Blood samples were drawn between 0.3 and 5.4 years after allograft implantation. The mean duration between allograft implantation and antibody testing was 3.0 ± 1.4 years. By ELISA testing, 96% (87/92) of patients were positive for anti-HLA class I antibody, and 82% (75/92) were positive for anti-HLA class II antibody. Testing for anti-HLA class I status by the CDC method was performed in 90 patients. 68% (61/90) of patients were positive for anti-HLA class I antibody by CDC. There was no significant correlation between the postoperative interval and anti-HLA class I and II antibody positivity by ELISA or CDC. However, there was a significant correlation between postoperative duration and PRA level ($r = -0.31$, $p = 0.003$).

Specificities of anti-HLA class I antibody were determined by CDC in 90 patients. Corresponding donor HLA specificities were available in 68 cases (76%). The remaining 22 donor HLA types were unknown because of failure to extract suitable DNA for typing. The results are summarized in Figure 1. Of the 68 patients, 19 (28%) were anti-HLA class I antibody-negative (PRA ≤5%). Of the remaining 49 patients, 37 had antibodies specific to one or more donor HLA class I antigen. The other 12 patients, who were antibody-positive, had non-donor-specific anti-HLA class I antibodies. Anti-HLA class I antibodies in six of these 12 patients were considered multi-specific, precluding the determination of specificity (PRA 91-99%).

Echocardiography data were available from 87 patients at a mean of 3.54 ± 1.58 years (range: 0.52 to 7.08 years) postoperatively. The 'mean valve gradient' was 8.67 ± 5.93 mmHg (range: 1 to 31 mmHg). Valve regurgitation was either nil or trivial in 64 patients (74%), mild in 20 (23%), and moderate in three (3%). No patient had severe regurgitation. Categorically, valve dysfunction was absent in 49 patients (56%), mild in 33 (38%), and moderate in five (6%).

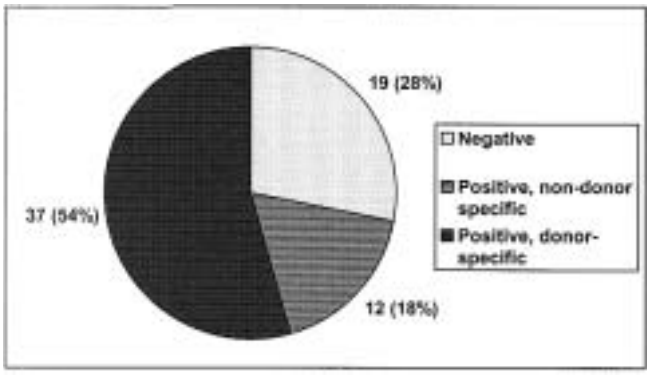


Figure 1: Anti-HLA class I antibody status by microlymphocytotoxicity testing.

No significant difference was observed in mean valve gradient (log-rank test), frequency of mild to moderate regurgitation (chi-square test), or frequency of mild to moderate valvular dysfunction (chi-square test), according to anti-HLA class I or II status by CDC or ELISA testing, or according to donor-specific anti-HLA class I antibody status.

Discussion

In this cross-sectional study of allograft recipients which extended to five years postoperatively, 96% and 82% of recipients were found to have anti-HLA class I and II antibodies, respectively, by ELISA testing. In a similar study conducted by Dignan and coworkers (5), anti-HLA class I antibodies were found in 83% of recipients, and class II antibodies in 61%, at a mean of 6.5 years postoperatively, using a different commercially available ELISA test. Other groups have utilized microlymphocytotoxicity methods and found anti-HLA class I antibodies in 54 to 100% of patients (6,8,15-17). Anti-HLA class II antibodies have been found in 19 to 77% of recipients using a variety of testing methods (6,7). The difference in frequency of antibodies found in these studies was likely due to varying valve preservation methods (17), ischemic time, postoperative interval (18), and the method of testing (6). The present high prevalence rates of anti-HLA class I and II antibodies may be due to the relatively short postoperative interval, short valve ischemic time, and high sensitivity of the ELISA method used. To the present authors' knowledge, the QUIKSCREEN and B-SCREEN qualitative ELISA kits used in this study have not been previously used in allograft valve studies, though they have been widely used in the setting of solid organ transplants, and have proven to have high sensitivity compared to other methods (19,20).

In the present study, 76% of recipients positive for anti-HLA class I antibodies by CDC had detectable donor-specific antibodies. This was consistent with the findings of previous studies which reported 81 to 100% of HLA-antibodies to be donor-specific (7,8,15-17). Six other recipients in the present study had broad panel reactivity precluding HLA specificity designation. It is probable that these patients also had donor-specific antibodies. It is therefore most likely that the allograft valve was the cause of the formation of anti-HLA antibodies in recipients, as the allograft material provides a long-term source of foreign HLA antigens. It is worth considering that patients may form antibodies to HLA antigens in two other settings: blood transfusion and fetal-maternal transfusion. The rate of blood product usage or multi-parity was not considered in the present study, although previous studies of allograft valve recipients found blood transfusions not to be significant in the formation of anti-HLA antibodies (17,21). Blood transfusion in those patients not pre-sensitized is also known to produce very low rates of antibody formation - only 2.7% in one study (22). Further evidence that allografts are the source of anti-HLA antibodies was derived from studies of patients receiving decellularized allograft material; two such studies showed a reduced antibody response in those receiving decellularized allografts (23,24).

In the present study no correlation was found between anti-HLA antibodies and the echocardiographic evidence of valve dysfunction. To date, only a single study conducted by Dignan et al. has shown the presence of anti-HLA antibodies to be associated with greater valve dysfunction (6). In that study, an association was found between aortic valve allograft structural deterioration and a high titer of anti-HLA class II antibodies detected by ELISA. Significantly, no link was found between valve dysfunction and antibodies when either CDC or flow cytometry methods were used.

The present study cohort included patients within the adult age range; indeed, 16% of the patients were aged >60 years. It is known that patients aged over 60 years have improved allograft durability compared to younger patients (1,2). However, it is unlikely that any detectable immune response would be of significant clinical relevance in those aged over 60 years as the long-term durability of allografts in these patients is very good.

The chance of a type II error in the present study was very real given the small size of the dataset. Hence, based on the study findings it cannot be concluded that the formation of anti-HLA antibodies is clinically unimportant - only that they could not be shown as a significant factor related to valve dysfunction at a mean echocardiographic examination interval of 3.5 years after allograft implantation. At present it remains unclear whether valve-specific antibodies are a causative factor in allograft valve degeneration, and larger studies with longer follow up and younger patients are required to clarify this issue. Alternatively, an improved durability of decellularized allograft valves over conventional allografts may resolve this question.

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