

# Long-Term Cardiac Allograft Valves after Heart Transplant are Functionally and Structurally Preserved, in Contrast to Homografts and Bioprostheses

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**Background and aim of the study:** Homograft valves undergo degenerative changes over time, which finally lead to functional deterioration. Immunological events are believed to play a pivotal role in this process. To further evaluate this hypothesis, the valvular morphology and function, as well as comorbidities predisposing to deteriorative processes, were evaluated in patients who had undergone heart transplant more than 10 years previously.

**Methods:** In a consecutive cohort of 146 patients (125 males, 21 females; mean age at transplant  $43.8 \pm 11.2$  years), heart and valve function were assessed by color Doppler echocardiography at a mean of  $5,306 \pm 987$  days after heart transplant. Evaluated parameters included chamber dimensions, cardiac function, valvular morphology/function, and concomitant diseases.

**Results:** Atrial and ventricular dimensions were within normal ranges for the left atrium (LA;  $n = 7$ ), right atrium (RA;  $n = 7$ ), left ventricle (LV;  $n = 143$ ) and right ventricle (RV;  $n = 119$ ). Slight enlargements occurred in the LA ( $n = 138$ ), RA ( $n = 137$ ), LV ( $n = 1$ ) and RV ( $n = 11$ ), while significant enlargements were seen in the LA ( $n = 1$ ), RA ( $n = 2$ ), LV ( $n = 2$ ), and RV

( $n = 16$ ). With regard to cardiac function, the ejection fraction (EF) was  $63.9 \pm 4.9\%$ , left ventricular isovolumic relaxation time (IVRT)  $85.04 \pm 14.64$  ms, fractional shortening (FS)  $34 \pm 12\%$ , and pulmonary artery systolic pressure (PASP)  $29.81 \pm 6.4$  mmHg. Valvular regurgitation (grade  $\geq 2$ ) was present in 34 patients (31 tricuspid valves, three mitral valves). No patients presented with aortic valve regurgitation. Concomitant conditions with a potential impact on calcium balance/valvular deterioration included immunologic/chronic inflammatory diseases ( $n = 6$ ), malignancies ( $n = 12$ ), kidney ( $n = 41$ ), cardiovascular system ( $n = 39$ ) and thyroid/parathyroid ( $n = 12$ ).

**Conclusion:** During the long term after heart transplant, heart valves were characterized by normal morphology and function in the majority of cases. Although most patients presented with concomitant conditions strongly predisposing for valvular deterioration/calcification, sole immunosuppressive/anti-inflammatory therapy appears to prevent these processes in heart transplant patients.

Human allograft prostheses represent the best choice for heart valve replacement in some, mostly elderly, patients. Today, no commercially available mechanical heart valve prosthesis is able to meet the functional properties comparable to those of human allografts or other biological heart valve prostheses. However, although novel harvesting, preservation and storage techniques lead to better function and viability, with better long-term results (1), a substantial number of these prostheses degenerate and need to be replaced after 10-12 years (2).

In a recent study (3) on the impact of inflammation

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and ischemia on the process of valvular degeneration, it was hypothesized that, apart from damage caused by storage and surgical manipulation, which potentially might lead to endothelial deterioration and thus increase susceptibility to hemodynamic endothelial damage, inflammatory reactions play a pivotal or even leading role in homograft valve deterioration (4).

Based on the fact that the receipt of immunosuppressive - and thus anti-inflammatory - therapy is one major difference between cardiac transplant recipients and patients who undergo human allograft heart valve replacement, the study aim was to investigate cardiac and heart valve morphology and function in patients who had undergone cardiac transplant. As allogeneic heart valve deterioration progresses over time, cardiac transplant recipients were evaluated more than 10 years after organ transplantation.

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## Clinical material and methods

### Patients

Between 1984 and 2005, almost 800 patients underwent orthotopic heart transplant at Hannover Medical School. Among these recipients, 146 patients (125 males, 21 females) were transplanted more than 10 years previously (10-year survival rate 53%) and are currently alive. Due to severe graft vasculopathy, nine of these patients (mean age  $51.1 \pm 5.1$  years; range: 42 to 61 years) required re-transplantation. These patients were evaluated at a follow up visit to the authors' heart-transplant outpatient unit.

The mean age of the patient cohort at the time of transplant was  $43.8 \pm 11.2$  years (range: 6 to 64 years).

The mean follow up period was 5,306 days (~14.5 years) (range: 3,651 days (~10 years) to 7,395 days (~20.2 years)). Underlying or concomitant diseases, which may have affected either directly or indirectly the calcium balance and thus, had an impact on valvular deterioration, are listed in Table I.

### Post-transplant care

#### Immunosuppressive therapy

Surgical procedures were performed using the Shumway bi-atrial anastomosis technique (5). Immunosuppressive therapy was conducted using a triple-drug regimen of cyclosporine A, prednisolone, and azathioprine (in 1998/1999 azathioprine was

Table I: Diagnoses leading to transplant and concomitant diseases.

Diagnoses leading to transplant		Diagnoses with potential impact on calcium balance/valve deterioration			
Condition	No. of patients	Organ/system	Condition	No. of patients	
CCM	82	Kidney	IDDM	11 (7.5)	
FCCM	10		NIDDM	4 (2.7)	
ICM	45	Cardiovascular system	Nephrosclerosis	1 (0.7)	
Myocardial fibrosis	1		Glomerulonephritis	1 (0.7)	
Severe aortic valve insufficiency	1		Renal insufficiency	24 (16.4)	
Combined aortic- and mitral valve vitium	1		Hemodialysis	10 (6.8)	
HCM	4		Graft vasculopathy	32 (21.9)	
Adriamycin-induced cardiomyopathy	1		Peripheral artery disease	7 (4.8)	
Right ventricular dysplasia	1		Malignancies	Hodgkin lymphoma	1 (0.7)
				Non-Hodgkin lymphoma	2 (1.4)
				Renal carcinoma	2 (1.4)
				Prostatic carcinoma	4 (2.7)
		Kaposi's sarcoma		1 (0.7)	
		Malignant carcinoid		1 (0.7)	
		Plasmacytoma		1 (0.7)	
		Immunologic and chronic inflammatory diseases		Rheumatic diseases	1 (0.7)
				Colitis ulcerosa	1 (0.7)
				Muscular dystrophy	1 (0.7)
		Thyroid/parathyroid	COPD	3 (2.1)	
			Adenoma	1 (0.7)	
			Struma nodosa	3 (2.1)	
			Multifocal autonomy	1 (0.7)	
			Radioiodine therapy	2 (1.4)	
			Osteoporosis	5 (3.4)	

Values in parentheses are percentages.

CCM: Congestive cardiomyopathy; COPD: Chronic obstructive pulmonary disease; FCCM: Family-related congestive cardiomyopathy; HCM: Hypertrophic cardiomyopathy; ICM: Ischemic cardiomyopathy; IDDM: Insulin-dependent diabetes mellitus; NIDDM: Non-insulin-dependent diabetes mellitus.

replaced by 1 g mycophenolatmofetil, two- to three times daily, due to high rates of malignancy). Target levels for cyclosporine A were 200-250 µg/l during the first year, 150-200 µg/l during the second year, and 100-150 µg/l thereafter (translated into monoclonal assay).

### Blood pressure control

For blood pressure control, patients were treated with ACE-inhibitors, beta-blockers, calcium-channel antagonists, peripheral vasodilators, or combinations of these drugs. Examination intervals were at least three months beyond the first year, and comprised electrocardiography, echocardiography and coronary angiography conducted on an annual basis.

### Echocardiography

For echocardiographic evaluation patients were placed in a left lateral position and examined using a Hewlett-Packard Sonos 7500 ultrasound imaging system fitted with a 2 to 5-MHz transducer (Hamburg, Germany). M-mode recordings in the parasternal long axis view were used to measure atrial and ventricular dimensions. Atrial measurements were obtained from the donor portion of the atria. The ejection fraction (EF), fractional shortening (FS), left ventricular isovolumic relaxation time (IVRT), pulmonary artery systolic pressure (PASP), in addition to valvular competence and morphology, were assessed by color Doppler, continuous-wave and pulsed-wave echocardiography.

### Statistical analysis

Statistical analyses were performed using SPSS (Statistical Package for Social Sciences) for Windows,

Version 12.0 (SPSS Inc., Chicago, IL, USA). Continuous values were presented as mean ± SD, minimum and maximum.

## Results

### Cardiac morphology

Among the patient group (n = 146), the left and right atrial dimensions were found to be either slightly (left 144 (98.6%); right 143 (97.6%)) or significantly enlarged (left 2 (1.4%); right 3 (2.1%)) in most cases, whereas the left and right ventricular dimensions in the majority of patients were within the normal range (left 140 (95.8%); right 116 (79.9%)). With regard to the morphology of the three directly investigated heart valves (aortic, mitral and tricuspid), echocardiography revealed flimsy, normal-shaped aortic and mitral valve leaflets, without matrix thickening, thrombotic vegetations or other signs of endocarditis. In contrast, although similar in thickness and without apparent thrombus formations, the tricuspid valves, in association with enlarged right atrial dimensions, appeared somewhat disarranged in most cases.

### Cardiac function

Echocardiographic quantification of the left ventricular EF (mean 63.9 ± 4.9%) demonstrated normal graft function. Systolic function was completely normal (EF >60%) in all but six patients, where values were between 35-45% (n = 2) and 50% (n = 4). In these patients severe allograft vasculopathy was present. The mean FS of 34 ± 12% was observed to be within the reference range. The mean left ventricular IVRT was 85 ± 12 ms.

Functional competence of the aortic, mitral and tri-

Table II: Long-term valvular competence.

Grade	Aortic valve	Mitral valve	Tricuspid valve
<i>Regurgitation</i>			
0	121 (82.9)	28 (19.2)	6 (4.1)
0-1	16 (10.9)	89 (60.9)	56 (38.3)
1	9 (6.2)	19 (13.0)	37 (25.3)
1-2	0 (0)	6 (4.2)	16 (10.9)
2	0 (0)	2 (1.5)	8 (5.6)
2-3	0 (0)	0 (0)	9 (6.2)
3	0 (0)	1 (0.6)	10 (6.8)
3-4	0 (0)	1 (0.6)	4 (2.8)
<i>Stenosis</i>			
0	146 (100)	146 (100)	146 (100)
>0	0	0	0

Values in parentheses are percentages of all patients evaluated.

Table III: Aortic root dimensions and pulmonary artery systolic pressure.

Parameter	Value	No. of patients	Mean ± SD	Range (min/max)
Aortic root diameter (cm)*	2.0-4.0	3 (2)	3.13 ± 0.45	1.9/4.2
Aortic root area (cm <sup>2</sup> ) <sup>+</sup>	3.1-12.6	6 (4.1)	7.84 ± 2.18	2.7/14
PASP (mmHg) <sup>‡</sup>	>30	47 (32.2)	29.81 ± 6.4	13/49

Values in parentheses are percentages of all patients evaluated.

\*Normal value >4.0 cm.

<sup>+</sup>Normal value >12.0 cm<sup>2</sup>.

<sup>‡</sup>Normal value >30 mmHg.

PASP: Pulmonary artery systolic pressure (an indirect parameter of pulmonary valve competence).

cuspid valves was rated based on a score from 0 to 4 (equal to trivial, mild, medium and severe, respectively). Pulmonary valve competence and regurgitation were indirectly assessed by examining the PASP. The aortic root diameter and area were also obtained. Echocardiographic examination of valvular competence revealed regurgitation (insufficiency) depending on the valve location, but no stenoses. Regurgitation of grades 1 and ≥1-2 was identified in 27.2% of the mitral valves and 36.2% of the tricuspid valves (Table II), while a PASP ≥30 mmHg was observed in 47 (32.2%) of the pulmonary valves (Table III). The aortic valves leaflets did not demonstrate any severe regurgitation; moreover, the aortic root diameter and area were within normal ranges in most cases.

Following transplantation, only nine patients who presented with severe tricuspid valve regurgitation had to undergo further surgical intervention. In two of these cases tricuspid valve replacement was performed with a biological prosthesis, and in six cases with a mechanical valve. The tricuspid valve was repaired in one patient.

## Discussion

The 'optimal' biological heart valve prosthesis and human allograft valve should exhibit durability, lack of immunogenicity, anti-thrombogenicity, resistance to infection, an ability to grow, adequate hemodynamic performance, and should not cause trauma to the blood components (6). Currently, all commercially available biological prostheses and human allograft heart valves undergo progressive degeneration, calcification, and finally loss of function. Although limited viability and function restricts the use of these primary optimal prostheses to a selected (mostly elderly) patient cohort, many questions regarding the underlying pathophysiological mechanisms of degeneration remain unresolved. Recently, the impact of inflammation and ischemia on the degeneration of xenogeneic

and allogeneic valve conduits was evaluated (4), in addition to the expression of immunologically relevant endothelial adhesion molecules on the deterioration of heart valve prostheses and those obtained from chronically rejected cardiac grafts (7). According to observations obtained from these studies, it was concluded that immunological mechanisms, which involved inflammatory reactions originating from the antigenic potential of donor-specific endothelial cells (8-13), might be responsible for biological heart valve degeneration. This hypothesis was supported by the observation that decellularized allografts - and therefore also heart valves without any cellular antigen-presenting component - demonstrated an important reduction in immunogenic response when compared to cryopreserved allograft valves used in the Ross procedure (14-18).

In contrast, heart valve prostheses originating from transplanted, chronically rejected cardiac grafts showed a continuous, smooth endothelial layer with no evidence of internal or external structural deterioration. Thus, heart valves native to transplanted grafts - at least in the histological evaluation - seemed resistant to structural deterioration. Neither the level of HLA mismatch, nor antibody status or ABO compatibility, were found to have any significant impact on homograft valve function (19,20), which further underscored the immunological impact of interstitial allogeneic cells as well as the modulating influence of immunosuppressive therapy in this unique setting.

During recent years many studies have reported on biological and homograft heart valve degeneration. However, most of these referred to observation times of less than 10 years' follow up. In order to evaluate the long-term in-vivo performance of 'valvular allografts' embedded in (and thus native to) transplanted hearts, cardiac and heart valve morphology and function was assessed more than 10 years after cardiac graft transplant. A considerable number of patients suffering from concomitant conditions and complications of

immunosuppression, such as renal failure, hemodialysis therapy or graft vasculopathy with a strong impact on the risk of soft-tissue degeneration and/or calcification, were identified. Moreover, cyclosporine-induced arterial hypertension (21) and a progressive interstitial myocardial fibrosis induced by ongoing cytokine activation (22,23) further promote potential damage. However, echocardiographic assessment revealed well-preserved left heart valve function and morphology in most cases. Endocarditis, as might be expected due to immunosuppressive therapy, was not observed in the present study, and appears rare among heart transplant recipients in general (24-26). Although severe tricuspid regurgitation could be observed in a considerable number of patients, this finding relates to right atrial enlargement due to a geometric mismatch between donor and recipient atrial size, an inadequate dilated pericardial cavity (27), biopsy-induced leaflet injury, and pacemaker leads (28,29). Thus, structural rather than other (perhaps immunological/inflammatory) reactions are the causative mechanism of this observation.

When considering the frequency of bioprosthetic regurgitation/degeneration in the long term after aortic valve replacement, the present findings suggest that immune/inflammatory mechanisms play a leading role in bioprosthetic/allograft deterioration. The strong and life-long immunosuppressive (and thus anti-inflammatory) drug regimen may protect these patients from valvular deterioration. Attempts to improve the long-term performance of biological heart valve prostheses/allografts using several different approaches seem conceivable. In addition to tissue-engineering concepts to create viable, non-immunogenic and competent bioartificial heart valve prostheses (31,32), the administration of low-dose immunosuppression might be an alternative approach. However, since cyclosporine A and azathioprine are associated with severe side effects, which do not justify their use in patients with aortic valve replacement only, an attenuated drug regimen needs to be defined. Some authors have suggested that donor endothelial cells, and therefore the main targets of immunological reactions, are replaced by recipient cells over time (31,32).

This could imply that somehow an anti-inflammatory therapy - perhaps well-regulated cortisone - could be reduced or even abandoned after a certain time. This may facilitate 'engraftment' of the transplant valve and prevent it from triggering degenerative cascades.

*In conclusion*, to the present authors' knowledge this is the first study to report on the long-term function of cardiac and heart valve morphology and function,

more than 10 years after heart transplant in a large patient cohort. These data show that heart transplant results in normal cardiac and heart valve function, even more than 10 years postoperatively. Although tricuspid valve competence is frequently jeopardized due to donor-recipient size mismatches and repeated biopsies, and despite concomitant diseases in this unique patient cohort, no signs of immunological heart valve deterioration were found. This leaves a void for suggestions pertaining to the mechanism of biological/allograft heart valve degeneration. Although transplant recipients exhibit a considerable concomitant and drug-related risk profile, no aortic regurgitation or structural damage could be observed. Immunosuppressive/anti-inflammatory therapy against biological heart valve deterioration indicates that immunological/inflammatory mechanisms might play the leading role in such an event.

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