Use of Fresh Decellularized Allografts for Pulmonary Valve Replacement May Reduce the Reoperation Rate in Children and Young Adults

Early Report

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Background—Degeneration of xenografts or homografts is a major cause for reoperation in young patients after pulmonary valve replacement. We present the early results of fresh decellularized pulmonary homografts (DPH) implantation compared with glutaraldehyde-fixed bovine jugular vein (BJV) and cryopreserved homografts (CH).

Methods and Results—Thirty-eight patients with DPH in pulmonary position were consecutively evaluated during the follow-up (up to 5 years) including medical examination, echocardiography, and MRI. These patients were matched according to age and pathology and compared with BJV (n=38) and CH (n=38) recipients. In contrast to BJV and CH groups, echocardiography revealed no increase of transvalvular gradient, cusp thickening, or aneurysmatic dilatation in DPH patients. Over time, DPH valve annulus diameters converge toward normal z-values. Five-year freedom from explantation was 100% for DPH and 86±8% and 88±7% for BJV and CH conduits, respectively. Additionally, MRI investigations in 17 DPH patients with follow-up time >2 years were compared with MRI data of 20 BJV recipients. Both patient groups (DPH and BJV) were at comparable ages (mean, 12.7±6.1 versus 13.0±3.0 years) and have comparable follow-up time (3.7±1.0 versus 2.7±0.9 years). In DPH patients, the mean transvalvular gradient was significantly (P=0.001) lower (11 mm Hg) compared with the BJV group (23.2 mm Hg). Regurgitation fraction was 14±3% and 4±5% in DPH and BJV groups, respectively. In 3 DPH recipients, moderate regurgitation was documented after surgery and remained unchanged in follow-up.

Conclusions—In contrast to conventional homografts and xenografts, decellularized fresh allograft valves showed improved freedom from explantation, provided low gradients in follow-up, and exhibited adaptive growth. (Circulation. 2011;124[Suppl 1]:S115–S123.)

Key Words: valves ■ surgery ■ heart defects ■ congenital

Because of the higher thromboembolic risk in mechanical prosthesis and the lifetime need for anticoagulation, xenograft and allograft valves are usually preferred for right ventricular outflow reconstruction in children and young adults. However, implantation of these valves in pediatric patients often results in graft degeneration and failure. Degradation of allografts and xenografts is usually attributed to high immunologic competence in children and young adults and leads to repeated operations.

Heart valve tissue engineering represents an upcoming alternative source to create viable, nonimmunogenic, and biologically active grafts. There is strong evidence that elimination of immunogenic cells from the valvular matrix using different decellularization protocols significantly decreases immunologic responses in valve recipients. In our previous preclinical study, we showed that once implanted, these decellularized scaffolds undergo extensive remodeling in vivo by repopulation with autologous cells. Moreover, these valves did not degenerate and provided excellent hemodynamics in the sheep model.

In the present study, we report our clinical midterm results on implantation of fresh decellularized pulmonary homografts (DPH) compared with glutaraldehyde-fixed bovine jugular vein (BJV) and cryopreserved homografts (CH) in children and young adults.

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Methods

Patients
The present study represents a 2-center experience of DPH implantation in children and young adults. Eighteen patients from the Cardiac Surgery Center, Chisinau, Moldova, and 20 patients from Hannover Medical School, Hannover, Germany, operated on during the period of 2005 to 2010, were enrolled in regular follow-up investigations. All patients were investigated after surgery, at 6 and 12 months, and then every 12 months, including clinical and functional examinations (echocardiography, MRI). For control groups, 38 patients of 147 BJV recipients and 38 of 213 CH recipients were selected to match the patients from the investigation group, according to age at implantation and pathology (Table 1). The matched CH recipients were a subset of homograft patient cohort from a previously reported study. All patients were operated on in the authors’ institution, and all patients/parents agreed to the procedure and gave written informed consent. In Moldova, the Ethics Committee of the Ministry of Health approved this study, and in Germany, the institutional Ethics Committee of Hannover Medical School was informed on every patient, elected and operated on using the DPH valve.

Valve Preparation
Human pulmonary valve allografts were harvested under sterile conditions from cadavers and transplant patients (“domino” hearts). Donors were mostly adults. Valve donors were tested for transmissible diseases (AIDS, hepatitis, syphilis, tuberculosis). Warm ischemic time was up to 6 hours. The decellularization process has been previously described. Briefly, pulmonary allografts were treated under shaking conditions with a solution of 0.5% sodium deoxycholate (Sigma) and 0.5% sodium dodecylsulfate (Carl Roth) for 36 hours at room temperature. Homografts were washed with NaCl 0.9% solution and stored at 4°C (up to 3 weeks) until implantation. Surgery was performed after individual control of valve sterility. Both institutions used exactly the same decellularization protocol and homograft selection criteria.

Surgical Implantation
Each patient was admitted to the hospital on short notice once the appropriate homograft was available. Operations were performed under general combined intravenous anesthesia through a median sternotomy, using cardiopulmonary bypass with standard bicaval and aortic cannulation and mild hypothermia (32° to 34°C), using intermittent cold crystalloid or blood cardioplegia. In all cases, the right ventricular outflow tract (RVOT) was reconstructed with an appropriate homograft. Operations were performed after individual control of valve sterility. Both institutions used exactly the same decellularization protocol and homograft selection criteria.

Clinical Evaluation
Clinical follow-up included a regular physical examination of the patients (physical status, measurements of body height and weight and systemic blood pressure, ECG, and New York Heart Association classification).

Transthoracic Echocardiography
Echocardiographic evaluation (M-mode, 2-dimensional, color flow, pulsatil, and continuous wave Doppler) was performed by the same institutional cardiologist using Vivid-3 Pro (GE Vingmed, Horten, Norway) and Philips IE 33 (Philips Medical Systems, Best, The Netherlands) as previously described.

Magnetic Resonance Imaging
Seventeen DPH patients with the longest follow-up (>2 years) underwent MRI investigation, and their results were compared with MRI data of 20 BJV patients. Patients were well comparable regarding age and follow-up time since implantation (Figure 1) as well as diagnoses (Table 2). Cardiac MRI was performed on standard 1.5-T MRI systems (Magnetom Avanto, Siemens Medical Solutions, Erlangen, Germany) by modern vector ECG-gated balanced gradient-echo sequences (SSFP) for volumetric analysis, morphology of the RVOT, phase-contrast flow measurements, and contrast-enhanced angiography, following the protocol of the CMR project of the German Competence Network for Congenital Heart Defects published on the network’s website (www.kompetenznetz-ahf.de/en/research/mri). Standard volumetric analysis for end-diastolic and end-systolic ventricular size, ventricular mass, and flow measure-
Table 2. MRI Subgroup Description

<table>
<thead>
<tr>
<th>Diagnoses</th>
<th>BJV</th>
<th>DPH</th>
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<tbody>
<tr>
<td>PA/IVS</td>
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<td>1</td>
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<tr>
<td>PA+VSD</td>
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<td>0</td>
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<tr>
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<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>17</td>
</tr>
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Mean age at implantation, y: 13.0 ± 3.0 (BJV), 12.7 ± 6.1 (DPH).
Mean interval implantation MRI, y: 2.7 ± 0.9 (BJV), 3.7 ± 1.0 (DPH).

Type of previous procedures:
- None: 1, 9
- Pulmonary artery conduit: 3, 3
- Catheter-based intervention: 3, 0
- Open valvulotomy: 0, 1
- Intracardiac repair, as RVOT patch: 13, 4

Conduit diameter, mm:
- 12–19: 4, 2
- 20–23: 16, 11
- 24–26: 0, 4

BJV indicates glutaraldehyde-fixed bovine jugular vein; DPH, decellularized pulmonary homograft; PA + VSD, pulmonary atresia with ventricular septal defect; PV/PS, pulmonary stenosis and/or insufficiency; TAC, truncus arteriosus communis; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; and RVOT, right ventricular outflow tract.

**Statistical Analyses**
Summaries of numeric data are given as means and standard deviation. For comparisons of data with a skew distribution, we used the Mann-Whitney U test for nonpaired samples. Analysis of time-related events as freedom from explantation or arriving at degeneration threshold values is shown according to Kaplan-Meier, including numbers at risk at 0, 2, 4, and 5 years, as well as freedom-from-event rates at 5 years, with their 95% confidence limits.

Valve Annulus Size Development
Annuus size development was documented by echocardiography in 17 DPH patients with a follow-up more than 2 years. The graph was constructed by calculating z-values of implanted conduits (diameter at valvular level) according to a thesis of W. Berdau (Medical University of Kiel, Germany, 1989) and presented in Figure 2. The size development of each individual conduit is shown in relation to the expected size and growth (absolute diameter increase related to the previous examination) and is marked with a black triangle over the measurement symbol. Interpolation lines (Lowess fit) ease the assignment of the symbols to single patients.

Conduit Valve Degeneration
Degeneration was defined as maximal peak gradient over any part of the conduit of 50 mm Hg or more and/or at least moderate insufficiency.

Results
Intraoperative DPH Valve Handling
DPH valves showed robust mechanical properties for surgical implantation and provided enough resistance and stability for suture lines. There were no cases of graft rupture or dislodgment documented in these patients.
Postoperative Clinical Results

In the study group (DPH patients), the follow-up was from 0 to 5.1 years (mean, 2.1 ± 1.7 years); total observation time was 76.5 years (Table 1). The patients from all 3 groups were mostly children and young adults, and the mean age of implantation did not differ significantly. In all groups, most of the patients were previously operated for tetralogy of Fallot repair with RVOT patch enlargement (Table 1). Regarding the role of previously implanted homografts as potential sensitizing agents for the second homograft, we found that 4 of the 7 homograft recipients who had a previous homograft had degeneration within the observation period, whereas 18 of the 31 homograft recipients without prior homograft implantation had a degeneration. Kaplan–Meier evaluation of the role of previous homograft implantation for homograft recipients showed a probability value of 0.81 for this factor.

One patient (18 years old) with DPH valve died in the late postoperative period; however, his death was non-valve-related. Eleven months after surgery, this patient was urgently referred to our hospital with severe cardiac arrhythmias and suspicion of mesenterial infarction. Echocardiography examination revealed normal pulmonary valve function, low transvalvular gradient (peak 16.3 mm Hg), and unchanged trivial/mild valve insufficiency but severely altered global cardiac function under atrial tachyarrhythmia. The patient died of acutely developed ventricular tachycardia and fibrillation. The autopsy revealed a normal appearance of the pulmonary valve graft with absence of any macroscopic signs of graft dilatation, stenosis, or degeneration. Microscopic examination (hematoxylin and eosin staining) of the valve tissue showed a partial repopulation of decellularized graft tissue with autologous cells. The pulmonary arterial wall was repopulated with almost homogenously distributed interstitial cells, whereas the leaflets were repopulated only partially and predominantly in the proximal leaflet segments (Figure 4A and 4B). Von Kossa staining revealed no signs of calcification in arterial wall and pulmonary cusp tissue (Figure 4C). Further analyses characterizing cell types or more specific rejection markers were not performed because of strong formalin (10%) fixation of the explanted tissue.

Pulmonary Valve Graft Performance

DPH valves showed no signs of cusp thickening or reduction of cusps mobility, as well as no signs of relevant conduit stenosis (corresponding to a peak gradient of ≥50 mm Hg) or dilatation during the entire follow-up.

Valvular Graft Stenosis

Postoperative echocardiographic data at discharge showed low-pressure transvalvular pressure gradients in all 3 groups. In contrast to both CH and DPH groups, BJV patients had significant gradients, and the freedom from gradient >49 mm Hg curve decreased to 75.8% at 5-year follow-up (Figure 5A). Moderate gradient development (>24 mm Hg) is shown for all 3 conduit types in Figure 5B. The development as illustrated by the Kaplan–Meier curve shows the same tendencies as for higher gradients curve development. Echocardiography data represented as box plots confirm that transvalvular gradient in the DPH group remained low and had not increased during the entire follow-up (Figure 6). Almost the same graft performance was observed in the CH group; however, 1 patient had a significant gradient at 3 years after implantation.
Valvular Graft Insufficiency
Freedom from moderate insufficiency was 84% from the beginning in the CPH group, which did not progress up to 5 years of follow-up. In the case of BJV, valves were more competent in the beginning but progressively developed valve insufficiency in 10.1% of grafts at 1-year and 22.3% at 5-year follow-up. Freedom from moderate insufficiency decreased constantly in CH patients and reached the range of $52\pm11\%$ at 5 years (Figure 5C).

Valvular Graft Degeneration and Freedom From Reoperation
Freedom from operative reintervention in patients after RVOT reconstruction using the DPH valve was 100% at
Figure 4. A and B, Bright-field microscopy of hematoxylin and eosin staining of explanted decellularized pulmonary homograft (DPH) valve tissue. A, Pulmonary wall; B, pulmonary leaflet. Arrows indicate repopulation of valve scaffold with autologous cells. C, von Kossa staining of pulmonary wall and leaflet. Lack of black dots indicate absence of tissue calcification areas.

5-year follow-up. In contrast, freedom from reintervention curve drops to $85.9 \pm 7.8\%$ in BJV patients and to $87.6 \pm 6.8\%$ in CH patients at 5 years (Figure 3). The reason for BJV explantations was valve dysfunction caused by foreign body reaction and caused by graft endocarditis. Homografts were explanted for severe regurgitations and dysfunction of calcified valves. Figure 5 depicts the state of patients as observed in the described intervals after implantation; additionally, the fraction free from explantation (calculated by Kaplan–Meier) is shown. Below this curve, we point out the remaining patients with degeneration signs among “healthy” patients. A comparison of the conduit types shows a similar rate of explantations for CH and BJV but more patients with degenerated valves in the CH group (Figure 3).

**MRI Data on Valvular Graft Degeneration**

To refine the possible midterm graft degeneration in our study group, 17 DPH patients with the longest follow-up $\geq 2$ years were additionally examined, using cardiac MRI. These data were analyzed and compared retrospectively with MRI data of 20 BJV patients. The selected patient subgroups are described in Table 2. The results showed normal mean gradient values in DPH patients (11 mm Hg). Whereas mean age of the patients (12.7 \pm 6.1 versus 13.0 \pm 3.0 years) and follow-up time (3.7 \pm 1.0 versus 2.7 \pm 0.9 years) was comparable in both groups, the mean transvalvular gradient was significantly lower in the DPH group (11 mm Hg) compared with the BJV group (23.2 mm Hg, $P=0.002$) (Figure 1).

On the other hand, a tendency of an increased regurgitation fraction has been documented in patients with DPH in contrast to BJV patients. However, this was based only on 3 patients with DPH that exhibited moderate pulmonary regurgitation (PR) leading to only moderate ventricular dilatation (patient 1: PR, 32.9%, RV end-diastolic volume index, 129.6 mL/m$^2$; patient 2: PR, 32.5%, RV end-diastolic volume index, 96.3 mL/m$^2$; patient 3: PR, 32.5%, RV end-diastolic volume index, 118.1 mL/m$^2$). In all other DPH patients, regurgitation was trivial to mild (mean PR, 4.7%; range, 0% to 20.9%).

**Anulus Size Development**

The majority of valve donors were adults and, because of this, most of the DPH valves were implanted oversized (Figure 2). Especially in younger children, oversizing appears in this figure as descending lines: patients grow, but DPH valve annulus remain unchanged. Some oversized DPH valves even decrease in diameter. In both situations $z$-values of oversized DHV grafts normalize. When conduit size begins to come close to the normal range, growth keeps the conduits within the green zone. Horizontal lines within the green zone show constant and adequate growth. Until now, no conduit required exchange for outgrowth; those implanted within the normal range kept their $z$-value and their competence, despite growth of the patients (Figure 2). Moreover, inappropriate conduit dilatation was not observed.

**Discussion**

Glutaraldehyde-fixed xenografts including porcine and bovine tissue valves are successfully used for pulmonary valve replacement.$^{10}$ However, the limited durability of xenografts is caused by cell debris fixation, which leads to progressive tissue degeneration including cusp mineralization and structural damage.$^{12}$ Cryopreserved valve homografts have been the principal conduits used over decades for RVOT reconstruction.$^{13}$ These valves proved to be resistant to infection and provide excellent initial physiological hemodynamic properties. However, in the majority of reports, all patients have some conduit valve regurgitation and, additionally, the homografts calcify.$^{14}$ Accelerated graft degeneration has been observed especially in children and young adults as the result of high immunologic competence in these patients.$^{15}$ Decellularized xenograft valves were introduced as an alternative valve substitute for RVOT reconstruction. Goldstein et al$^{16}$ stated that the elimination of cells from the matrix reduces graft immunogenicity, and subsequent matrix reseeding with autologous cells provides matrix remodeling.$^{16}$ Unfortunately, clinical implementation of these valves resulted in dramatic outcomes.$^{17}$ It has been demonstrated that porcine extracellular matrix is immunogenic and can induce immunologic responses.$^{18}$ Alternatively, our preclinical studies in the sheep model showed excellent hemodynamic performance of decellularized allograft valve scaffolds.$^{5,19}$ We report our clinical follow-up of decellularized homograft implantation in children and young adults. These results were compared retrospectively with mostly xenograft and allograft types of valves in pediatric cardiac surgery. We chose to match patients “manually” in our relatively small study groups instead of relying on the propensity score–matching algorithm, because by this approach we could better specify and weigh the importance of factors that are known to
influence degeneration. As repeatedly stated in the literature, the age of implantation represents one of the most important predictor factors for graft degeneration and has been used in our study as an essential criteria for matching.2,20

Although not free from signs of dysfunction (3 patients with relevant regurgitation rates were seen), our decellularized homografts provided superior performance compared with cryopreserved homografts and gluteraldehyde-fixed “Contegra” xenografts. Freedom from degeneration was higher in DPH valves. These grafts did not have significant gradients, in contrast to BJV grafts and cryopreserved homografts. However, a higher rate of valve insufficiency has been documented in DPH valves compared with xenografts. Individual MRI examination revealed important valve regur-
and 79% at 8 years. Konuma et al23 studied in a pediatric population SynerGraft decellularized valve versus conventional homografts performance and revealed no difference in terms of regurgitation, stenosis, or reintervention. Moreover, Yacoub et al25 showed superior clinical results for DPH in neonates and infants.

During the last 2 years, there were several reports on the usage of SynerGraft decellularized cryopreserved allografts in children and adults. Bechtel et al21 described significantly higher pressure gradients in decellularized grafts than across conventional allografts and concluded that for Ross procedure, SynerGraft allograft valves did not provide any major advantage over conventional allografts. Burch et al22 described freedom from reintervention for SynerGraft decellularized valves in pediatric patients of about 85% at 5 years and 79% at 8 years. Konuma et al23 studied in a pediatric population SynerGraft decellularized valve versus conventional homografts performance and revealed no difference with regard to regurgitation, stenosis, or reintervention.

In our series, DPH valves provide better performance and intervention-free survival compared with cryopreserved homografts and BJV xenografts. In contrast to SynerGraft valves, we used fresh decellularized valves that were stored up to 3 weeks before implantation at 4°C, and the patient was admitted to the hospital at a short notice. According to Narine et al,24 cryological conservation appears to be detrimental for decellularized grafts, because structural properties of the scaffold tissue are significantly affected by cryopreservation. Moreover, Yacoub et al25 showed superior clinical results for “homovital” valve allografts (fresh homografts stored at 4°C prior implantation) compared with cryopreserved homografts. In our study group, we had 1 late (11 months), non–valve-related postoperative death. Histological analyses revealed almost complete repopulation of the arterial wall, partial repopulation of the cusps with autologous cells, and absence of any signs of calcification. Our preclinical results in a sheep model showed that infiltration of inflammatory cells in decellularized valves is absent in contrast to conventional allografts, which are invaded by cells stained for typical leukocyte marker. In contrast, decellularized valve conduits become repopulated by endothelial and interstitial cells that positively responded to endothelial nitric oxide synthase, von Willebrand factor, and α-smooth muscle actin stainings.19

In our previous study, we described clinical application of tissue-engineered heart valves using autologous progenitor cells, which were preseeded on trypsin-decellularized allograft valves.3 In the present study, we used our new method of detergent treatment for decellularization.7 By using this method of decellularization, we are able to produce more stable constructs with mechanical properties comparable to native valves.26 Moreover, after treatment with detergent, the basement membrane remained well preserved on the surface of the valve scaffold, thus facilitating graft reendothelialization in vivo.

Interestingly, DPH valves provided a tendency to adapt to somatic development of the patients. In case of oversizing, these valves remain unchanged or even decrease in annulus size and tend toward normal values of the patients. These valves continue to function normally without developing a gradient over the conduit. Moreover, they dilate together with physiological growth of the patient. To claim physiological annulus size development, the observed growth tendencies require further evaluation over longer follow-up. Definitive growth assessment can be expected after implantation of DPH in neonates and infants.

Limitations
We are aware of the limitations given by the relatively small study groups, which cannot be totally overcome by any statistical method. The matching process we performed does not produce perfectly comparable patient groups, but we followed reasonable clinical criteria to select corresponding patients.

Although the patient subgroups for MRI studies did not perfectly match (Table 2), the differences are not advantageous for the DPH group (longer time since implantation), but the most important factor (age at implantation) was well balanced (Figure 1).

This is an early observational report on the use of fresh decellularized homografts for pulmonary valve replacement in congenital heart disease. The comparison with other conduits is given to show the promising character of the results obtained with the new substitute. This study is not conceptualized or intended as a proof of superiority. This work remains to be done with more patients and longer observation times.

Conclusions
At present, decellularized fresh pulmonary homograft valves showed very promising early results concerning durability (no development of relevant gradients, rare insufficiency observations, no explantations up to 5 years). The initial evidence for adaptive growth in the decellularized pulmonary homograft valves was not reported previously in any other type of conduit.
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Disclosures
None.

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