Early and late failure of tissue-engineered pulmonary valve conduits used for right ventricular outflow tract reconstruction in patients with congenital heart disease

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Abstract

OBJECTIVES: To identify factors associated with the surgical outcome in patients undergoing right ventricular outflow tract reconstruction (RVOTR) using decellularized tissue-engineered pulmonary valve conduits (TEPvC) and to study their safety and longevity.

METHODS: From April 2006 to April 2010, 93 patients underwent either palliative or corrective RVOTR using Matrix P (37) and Matrix P Plus (56) xenogenic decellularized TEPvC (size range 11–27 mm). Median age and weight at operation were 20 (0.16–290) months and 10.15 (2.65–86) kg respectively. Primary and redo surgery occurred in 40 and 60% of cases, respectively. Eighty-eight patients (94.6%) received conduit implantation in the framework of corrective surgery, whereas in 5 (5.4%) a palliative procedure was undertaken. Follow-up was complete in 91% of patients, with a median duration of 12 months (range: 2 days–51 months). Data analysis included diagnosis, type of surgery (palliative vs. corrective) and age at surgery. Predetermined primary outcomes were represented by conduit failure or dysfunction.

RESULTS: Two patients with Matrix P and two with Matrix P Plus died in the early post-operatively phase (4.3%). None of the deaths were conduit-related. One patient died at conduit replacement. Thirty-three patients (35.5%) experienced conduit failure whereas conduit dysfunction occurred in 27 patients (29%). Two-year freedom from conduit failure and dysfunction was 60.2% (95% CI: 50.1–69.6) and 77.4% (95% CI: 67.9–84.7), respectively. Reasons for failure were conduit stenosis in 20 cases (61%), pseudoaneurysm in 3 (9%), conduit dilatation (>50% of original diameter) in 2 (6%), stenosis of distal anastomosis involving pulmonary bifurcation in 6 (18%) and allograft dissection in 2 (6%). Histological examination showed inflammatory giant-type cells in the presence of a poor autologous cell seeding in all explanted specimens. Univariate and multivariate analyses showed an association between age at surgery ≤ 1 year and conduit dysfunction (adjusted HR: 2.29; 95% CI: 1.01–5.20, P = 0.04).

CONCLUSIONS: Compared with the other conduit for RVOTR Matrix conduits showed a high incidence of failure. Our results suggest that the use of Matrix conduits for RVOTR should be considered with caution.

Keywords: Right ventricular outflow tract reconstruction • Tissue-engineered pulmonary conduit • Decellularized valve

INTRODUCTION

Right ventricular outflow tract reconstruction (RVOTR) is one of the most common procedures in congenital heart surgery, often requiring conduit implantation. Pulmonary homografts, initially introduced in 1966 by Ross and Somerville [1], are still generally considered the best devices for RVOTR [2, 3], due to the technical ease of implantation and improved homeostasis [4, 5], good haemodynamic profile, low incidence of thromboembolism and optimal resistance to infection [6].

Principal drawbacks of homografts are the lack of growth with patient development, progressive calcification and limited availability. Moreover, an immunological reaction can be triggered by cellular elements that remain in the homograft's matrix and may cause early homograft failure [7, 8]. New technologies in tissue engineering have currently led to the development of decellularized pulmonary valve conduits [9–12] acting as matrix scaffolds for in vivo autologous seeding and reconstitution of viable tissue [13–15]. Decellularization of pulmonary valve substitutes is believed to eliminate immunogenicity, allow post-operative colonization with viable autologous vascular cells and improve conduit durability by providing a performance comparable with the native pulmonary valve [16].

We sought to identify factors associated with the surgical outcome in patients undergoing RVOTR using decellularized...
tissue-engineered pulmonary valve conduits (TEPvC) and to test their safety and longevity.

MATERIAL AND METHODS

Study design

We retrospectively reviewed medical records, angiographic studies, surgical reports, discharge summaries and clinical follow-up reports of all consecutive patients undergoing xenogenic Matrix P and Matrix P Plus (AutoTissue GmbH, Berlin, Germany) TEPvC implantation from April 2006 to April 2010 for either palliative or corrective RVOTR at our institution. Approval from the Institutional Scientific Board of the Bambino Gesù Children’s Hospital was obtained. Implanted conduits were categorized into five groups according to the size: neonatal (11–12 mm), infant (13–15 mm), children (17–19 mm), intermediate (21–23 mm) and adult (25–27 mm). We collected sex, age and weight at surgery, diagnosis, previous operations, cardiopulmonary bypass and aortic cross-clamping time as well as instrumental evaluation of conduit dimension and function, interventional procedures and rate of reoperations. Data obtained from histological and immunohistochemical staining of explanted conduits were also collected. The primary outcome variables were conduit failure and dysfunction. Conduit failure was defined as any conduit-related condition leading to death or conduit replacement. Conduit dysfunction was defined as a moderate to severe stenosis or a moderate to severe conduit valve incompetence detected at follow-up. Moderate to severe conduit stenosis was defined echographically as the presence of a peak instantaneous pressure gradient ≥40 mmHg at any level of the conduit. Moderate to severe conduit incompetence was defined echographically as the presence of a regurgitant jet of more than 70% of the pulmonary annular width associated with colour-Doppler flow reversal from pulmonary arterial branches. Follow-up information was available for 91% of the 93 patients. Median follow-up time was 12 months (range: 2 days–51 months).

Surgical technique

The surgical technique varied based on the type of conduits and the type of surgical procedures. Matrix P conduits were implanted in the same way as pulmonary homografts: the distal anastomosis was performed with an everting 6-0 polypropylene running suture, whereas proximal anastomosis was performed directly on the posterior aspect and with the interposition of a heterologous pericardial hood on the anterior aspect running a 5-0 polypropylene running suture, whereas proximal anastomosis was accomplished by means of the 5-0 polypropylene running suture after adequate trimming of the pericardial reinforcement layer of the conduit upstream the valve. The choice of conduit size was based on patient’s anatomy, age, body surface and matched pulmonary arterial diameter. In the case of a palliative procedure or a Ross operation, conduit implantation was performed under moderate hypothermic cardiopulmonary bypass and cardiopulmonary cardiac arrest while normothermic cardiopulmonary bypass on the beating heart was used for corrective surgery.

Statistical analysis

STATA software, Version 11.1 data analysis and statistical software (StataCorp LP, College Station, TX, USA), was used for statistical analysis. Continuous variables are reported as the median (range) and as number (proportion) for categorical variables. ANOVA techniques were used for continuous variables; χ²/Fisher’s exact tests were used for categorical variables as appropriate. Survival curves for freedom from valve failure and valve dysfunction were obtained by the use of the Kaplan–Meier method, and comparisons were performed with the log-rank test. The continuous variables examined included age and weight at treatment, which were dichotomized at a cut-off of 1 year and 3 kg, respectively, to explore the effect of younger age and low body weight on outcomes. A further dichotomization of age variable at 10 years was also performed. Additional multivariate survival analyses for the separate end points of conduit failure or dysfunction were performed by means of Cox proportional hazards, multiple regression models. The selection of independent variables for the model was based on statistical significance at univariable testing. Covariates with a value of P < 0.2 were included in the multivariable analysis. All tests were two-sided.

RESULTS

Ninety-three patients received RVOTR by TEPvC implantation at our institution during the study period. There were 47 males and 46 females. Median age and weight at operation were 20 (0.16–290) months and 10.15 (2.65–86) kg, respectively. We used Matrix P conduits in 37 patients and Matrix P plus conduits in 56 patients. Eighty were first implants and 13 were conduit replacements. Patient characteristics including the conduit size and the type are reported in Table 1. There were 37 (40%) first implants and 56 (60%) re-do implants. Eighty-eight patients (94.6%) received conduit implantation in the framework of corrective surgery, whereas 5 (5.4%) underwent a palliative procedure. Preoperative diagnosis and distribution of type of surgery (either palliative or corrective) and median age at operation for each diagnostic group are reported in Table 2. For Matrix P and Matrix P plus implantation, median cardiopulmonary bypass time was 235 (107–745) min and 276 (95–547) min, respectively. In patients who underwent conduit surgery by means of cardiopulmonary cardiac arrest (73 patients, 78%), median aortic cross-clamping duration was 136 (6–389) min for Matrix P (32 patients, 44%) and 86.5 (13–300) for Matrix P plus implantation (41 patients, 56%).

Treatments and outcomes

Four (4.3%) patients died in the early post-operative period. There were no conduit-related deaths. One patient with diagnosis of pulmonary atresia and ventricular septal defect died of ischaemic myocardial dysfunction as a consequence of a lesion of the left coronary artery. One patient with tetralogy of Fallot and absent pulmonary valve developed multi-organ failure following prolonged extracorporeal membrane oxygenation support. Two patients died of pulmonary haemorrhage, one with pulmonary atresia, ventricular septal defect and major aortopulmonary collateral arteries and one with truncus arteriosus and interrupted aortic arch. One patient died at conduit replacement. It was a 23-month infant with repaired truncus arteriosus and obstructed conduit who died of cerebral
embolism. For the entire study population, late survival was 95.7% at 48 months (Fig. 1). Thirty-three (35.5%) patients experienced conduit failure. Two-year freedom from conduit failure and dysfunction was 60.2% (95% CI: 50.1–69.6) (Fig. 2) and 77.4% (95% CI: 67.9–84.7), respectively. Reasons for failure were conduit stenosis in 20 cases (61%), pseudoaneurysm in 3 (9%), conduit dilatation (>50% of original diameter) in 2 (6%), stenosis of distal anastomosis involving pulmonary bifurcation in 6 (18%) and allograft dissection in 2 (6%, Fig. 3). Reintervention included conduit replacement in 25 cases (27%); reintervention was associated in 3 (2%) patients with pseudoaneurysm resection and in 10 (9%) patients with pulmonary arterial confluence reconstruction. Devices used for conduit replacement included a new Matrix P plus conduit in 11 cases (44%), a cryopreserved homograft conduit in 12 (48%) and a Contegra conduit (Medtronic, Minneapolis, MN, USA) in 2 (8%). Four (4%) patients underwent percutaneous conduit dilation, three (3%) percutaneous conduit stenting and one (1%) underwent Melody valve implantation (Medtronic). In all patients undergoing conduit replacement, histological examination of the explanted xenograft specimen showed inflammatory giant-type cells in the presence of a poor autologous cell seeding (Fig. 4). In particular, 19 (70%) patients developed moderate to severe stenosis at any level of the conduit, whereas in 8 (30%) patients moderate to severe conduit valve incompetence was detected. Univariate and multivariate analyses failed to identify factors associated with conduit failure. Conduit type (Matrix P vs. Matrix P plus) and age at surgery ≤10 years were not associated with conduit failure or dysfunction (Supplementary Figs S1 and S2). An association between conduit dysfunction and age ≤1 year at surgery was detected both at univariate and multivariate levels (adjusted HR: 2.29; 95% CI: 1.01–5.20, P = 0.04; Table 3, Fig. 5).

Table 1: Patient characteristics according to diagnosis and the type of surgery

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N (%)</th>
<th>Type of surgery palliative/ corrective</th>
<th>Procedure</th>
<th>N</th>
<th>Age at surgery (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA + VSD + MAPCAs</td>
<td>24 (25.8)</td>
<td>4/20</td>
<td>Unifocalization (VSD open)</td>
<td>4</td>
<td>17.5 (10–50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unifocalization (VSD closed)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Truncus arteriosus</td>
<td>19 (20.4)</td>
<td>0/19</td>
<td></td>
<td>7 (1–18)</td>
</tr>
<tr>
<td></td>
<td>TOF/DORV + PS</td>
<td>14 (15)</td>
<td>0/14</td>
<td></td>
<td>40.5 (24–65)</td>
</tr>
<tr>
<td></td>
<td>PA + VSD</td>
<td>12 (12.9)</td>
<td>1/11</td>
<td></td>
<td>9.5 (0.88–18.5)</td>
</tr>
<tr>
<td></td>
<td>AS ± LVOTO</td>
<td>10 (10.8)</td>
<td>0/10</td>
<td></td>
<td>57.5 (20–221)</td>
</tr>
<tr>
<td></td>
<td>TGA + VSD + PS</td>
<td>6 (6.5)</td>
<td>0/6</td>
<td></td>
<td>74.5 (16–204)</td>
</tr>
<tr>
<td></td>
<td>TOF + APV</td>
<td>6 (6.5)</td>
<td>0/6</td>
<td></td>
<td>99 (52–136)</td>
</tr>
<tr>
<td></td>
<td>CAV + TOF/DORV + PS</td>
<td>2 (2.1)</td>
<td>0/2</td>
<td></td>
<td>73.5 (50–97)</td>
</tr>
<tr>
<td>Total</td>
<td>93 (100)</td>
<td>5/88</td>
<td></td>
<td>93</td>
<td>20 (8–67)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as the median (range). AS: aortic stenosis; APV: absent pulmonary valve; CAV: atrioventricular canal; DORV: double outlet right ventricle; MAPCA: major aorto-pulmonary collateral arteries; LVOTO: left ventricular outflow tract obstruction; PA: pulmonary atresia; PS: pulmonary stenosis; TGA: transposition of great arteries; TOF: tetralogy of Fallot; VSD: ventricular septal defect.

Table 2: Patient characteristics according to the conduit type and the size group

<table>
<thead>
<tr>
<th>Conduit size group</th>
<th>Matrix P [N (%)]</th>
<th>Age (months)</th>
<th>Weight (kg)</th>
<th>Matrix P, plus [N (%)]</th>
<th>Age (months)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal (11–12 mm)</td>
<td>8 (8.6)</td>
<td>1 (0.16–13)</td>
<td>3.55 (3–7.5)</td>
<td>19 (20.4)</td>
<td>7 (0.63–142)</td>
<td>5.2 (2.6–30)</td>
</tr>
<tr>
<td>Infant (13–16 mm)</td>
<td>9 (9.6)</td>
<td>17 (0.3–44)</td>
<td>8 (3–13.4)</td>
<td>17 (18.2)</td>
<td>17.5 (5–59)</td>
<td>9 (5.6–15)</td>
</tr>
<tr>
<td>Children (17–20 mm)</td>
<td>12 (12.9)</td>
<td>26 (7–97)</td>
<td>11.5 (6–20)</td>
<td>9 (9.7)</td>
<td>60.5 (36–204)</td>
<td>18.5 (11–43)</td>
</tr>
<tr>
<td>Intermediate (21–23 mm)</td>
<td>7 (7.5)</td>
<td>96 (16–289)</td>
<td>28 (13.5–77)</td>
<td>6 (6.4)</td>
<td>184.5 (102–265)</td>
<td>53.5 (34–77)</td>
</tr>
<tr>
<td>Adult (24–27 mm)</td>
<td>1 (1.2)</td>
<td>136</td>
<td>40</td>
<td>5 (5.3)</td>
<td>210 (187–290)</td>
<td>64 (35–86)</td>
</tr>
<tr>
<td>Total</td>
<td>37 (40)</td>
<td>20 (0.16–289)</td>
<td>10 (3–77)</td>
<td>56 (60)</td>
<td>21 (0.63–290)</td>
<td>10 (2.6–86)</td>
</tr>
</tbody>
</table>

Continuous variables are presented as the median (range).
**DISCUSSION**

RVOTR with conduit implantation is a frequent procedure in congenital heart surgery. In spite of the overall good performance of cryopreserved homografts used as pulmonary valve substitutes [1–3], the need for a valid alternative led engineering research to develop new forms of tissue valve conduits potentially able to overcome drawbacks and limited durability of existing devices. Tissue-engineered heart valves are living tissue offering potential for growth, do not require any use of anticoagulant therapy, stimulate a limited antigenic immunological response and therefore may potentially represent a possible innovative solution [7, 10]. Among different types of engineered tissues and xenogenic TEPVc in particular, decellularized scaffolds constitute the object of our study. The main purpose of decellularization is to remove all cells and cellular components from the tissue by maintaining intact the main structural elements of the extracellular matrix only. Decellularization provides a suitable environment for cell migration and enables tissue reorganization [7, 10] therefore limiting the immunological responses of the body. In our study, we analysed the performance of two specific types of xenogenic TEPVc conduits. Matrix P conduits are decellularized porcine pulmonary valves that have been processed with a patented special procedure from AutoTissue GmbH; this procedure is able to destroy and remove all living cells (fibroblasts, endothelial cells, bacteria, viruses, fungi and mycoplasm) and keep only fibres of collagen and elastin [9, 10]. The Matrix P valve contains residual myocardium at the site of the proximal anastomosis and pulmonary arterial tissue at the site of the distal anastomosis. On the other hand, Matrix P plus conduits are manufactured as a combination of the Matrix P porcine heart valve and a glutaraldehyde-fixed equine pericardial patch. Matrix conduits undergo a specific procedure of sterilization that reduces DNA and RNA tissue content to minimal levels precluding the transmission of porcine
endogenous retrovirus and promoting the repopulation of autologous cells by preserving the extracellular matrix [10, 15]. The histological examination of all explanted conduits from our population showed a limited cell repopulation and the presence of giant-type inflammatory cells: these data strongly support the hypothesis of a possible immunological response similar to foreign body-type reaction [17]. Such reaction might be advocated as the main cause of the rapid intimal peel formation at the site of the distal anastomosis, responsible for stenosis and subsequent failure of the device. Moreover, the presence of inflammatory response might have led to the development of a thick external fibrous sheet around the graft contributing to produce conduit stenosis.

Simon et al. [12] reported that the absence of in vivo new repopulation cells in another type of TEPV, Synegraft, may be advocated as a cause of graft failure. Ruffer et al. [16] reported a different possible cause of failure of Matrix conduits (toxicological, immunological, haemodynamic and conduit-related) and also suggested that the conduit wall matrix proteins may be responsible for an immunogenic-like reaction resulting in graft stenosis. Cicha et al. [18] reported that early obstruction of TEPV is related to fibrosis and massive neointimal formation around the porcine valve wall due to an immunogenic foreign body-like reaction and described a limited repopulation of new cells, as we demonstrated in our histological specimens. In two patients from our study population, the cause of conduit failure was allograft dissection related to the disruption of the suturing material of the xenograft to the surrounding pericardial patch allowing blood flow in the thickness of the conduit wall, therefore creating the conditions for the development of a possible subsequent pseudoaneurysm. This type of conduit dissection has been described elsewhere [16]. Furthermore, pseudoaneurysm formation occurred in further three cases from our series.

Our study showed an association between younger age and conduit dysfunction which could be explained both by the natural tendency towards overgrowth of neonates and their high immunological activity [4] which could represent the cause of a reaction against a non-completely decellularized graft. An incomplete graft decellularization could also account for a severe foreign body-type reaction directed towards the valve leaflets [19], causing complete destruction of this structures and fibrotic degradation of xenograft tissue. Our data do not support the recent observation made by Konertz et al. [20], who reported satisfying short- and mid-term results with Matrix conduits used for RVOTR, comparable with those obtained with the use of other conduits. Our data show that the longevity of Matrix conduits is lower than the longevity of other types of similar devices. Breymann et al. [21] described a better 27-month durability of Contegra conduits compared with homografts, with a 100% freedom from explantation for Contegras compared with 86% for homografts. Schoenhoff et al. [22] reported an 82% 80-month freedom from explantation for Contegras. Prior at al. [23] recently described a 10-year freedom from reintervention for up to 90% of patients that received Contegras conduits, with a maximum of 95% for larger size implants (diameter ≥16 mm). Yang et al. [24] reported a 79% 3-year freedom from dysfunction together with an 89% 5-year freedom from explantation for size-reduced cryopreserved homografts used for RVOTR. Meyns et al. [3] described a 5- and a 10-year freedom from explantation of 94 and 82%, respectively, for cryopreserved homografts, their span being determined by graft size and the non-anatomic position, whereas immunological variables, like blood group incompatibility, implantation of a second homograft and short warm ischaemic time were not significant. Finally, Tweddell et al. [25] analysed specific risk factors for reduced longevity of homograft valves used for RVOTR and showed an overall 74% 5-year freedom from failure while identifying specific variables affecting the conduit outcome. Compared with other conduit types, our data suggest a much worse performance of Matrix conduits when used for RVOTR.

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**Table 3: Risk factors for conduit failure and dysfunction: results of univariable and multivariable analysis (P/C stands for palliative/corrective)**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Unadjusted Failure (P-value)</th>
<th>Dysfunction (P-value)</th>
<th>Adjusted Failure*</th>
<th>Dysfunction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgery (&gt;1 year vs. ≤1 year)</td>
<td>0.09</td>
<td>0.01</td>
<td>1.67 (0.69–3.96), 0.25</td>
<td>2.29 (1.01–5.20), 0.04</td>
</tr>
<tr>
<td>Age at surgery (&gt;10 years vs. ≤10 years)</td>
<td>0.61</td>
<td>0.82</td>
<td>0.90</td>
<td>0.77</td>
</tr>
<tr>
<td>P/C procedure</td>
<td>0.32</td>
<td>0.81</td>
<td>0.44</td>
<td>0.63</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>0.11</td>
<td>0.39</td>
<td>0.11</td>
<td>0.39</td>
</tr>
<tr>
<td>Conduit type (Matrix P vs. Matrix P plus)</td>
<td>0.44</td>
<td>0.63</td>
<td>0.44</td>
<td>0.63</td>
</tr>
<tr>
<td>Conduit size</td>
<td>0.07</td>
<td>0.41</td>
<td>0.07</td>
<td>0.41</td>
</tr>
<tr>
<td>Reintervention</td>
<td>0.90</td>
<td>0.77</td>
<td>1.16 (0.98–1.38), 0.08</td>
<td>2.29 (1.01–5.20), 0.04</td>
</tr>
<tr>
<td>Weight &lt;3 kg</td>
<td>0.24</td>
<td>0.10</td>
<td>0.24</td>
<td>0.10</td>
</tr>
</tbody>
</table>

*Values are the hazard ratio (95% confidence interval), P-value.
Moreover, the fact that the conduit type was not associated with conduit failure and dysfunction indicates that both Matrix P and Matrix P plus performances are equally unsatisfactory.

Limitations of the study

This is a non-randomized single-centre retrospective study in which a device was analysed in the absence of a control group. Follow-up was incomplete and particularly short for the most recent implants. This might have led to an underestimation of the real rate of conduit failure and dysfunction.

CONCLUSION

Our study showed a more rapid conduit failure for Matrix-type conduits with no difference between Matrix P and Matrix P plus compared with other available conduits as well as a higher incidence of conduit dysfunction for younger patients. Further studies are warranted to explore the long-term outcomes of Matrix-type conduits. Nonetheless, our findings suggest that the use of Matrix-decellularized TEPVc for RVOTR has to be considered with caution.

ADDENDUM

During the analysis of our series, a new generation Matrix P plus N conduit was introduced by the manufacturer as an improvement of the Matrix P plus pulmonary valve conduit. Its main declared peculiarity is the glutaraldehyde-free processing technology used to construct it, and a decellularized external reinforcement pericardial layer. The analysis of our data on previous generation Matrix conduits makes us cautious towards this new generation device as well, as no changes on the decellularization technology or xenograft-to-pericardium suturing material compared with Matrix P plus conduits were introduced. Further studies are needed to provide definitive insights on the effectiveness of these new generation decellularized TEPVc.

SUPPLEMENTARY MATERIAL

Supplementary material is available at EJCTS online.

Conflict of interest: none declared.

REFERENCES