Calcification of homograft valves in the pulmonary circulation — is it device or donation related?

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Abstract

Objective: Homograft valved conduits are used in the reconstruction of right ventricular outflow tract (RVOT), and calcification is a recognised phenomenon in these devices. The purpose of this study was to assess the effect of type (pulmonary and aortic) and mode of harvest of these cryopreserved homografts (cadaveric and beating heart) on the incidence of calcification of these conduits when used in the pulmonary circulation. Methods: A retrospective study was carried out on 60 patients with congenital heart defects who underwent reconstruction of RVOT using cryopreserved homograft valved conduits. The homografts were harvested from two different groups of donors; beating heart donors and cadaveric donors. The period of study was from 1st January 1990 to 31st December 2000. There were 34 males and 26 females, and the median age was 75 months. The 30-day mortality was 10 (16.7%). The 50 survivors were followed-up 3–108 months (median 36 months). Twenty-four had aortic homografts and 26 pulmonary homografts. Twenty-four devices were from cadaveric donors and 26 from beating heart donors.

Results: There were 10 (20%) calcified devices, all aortic in origin. In a logistic regression analysis, aortic homografts were significant risk factor for calcification (P = 0.0006). However, source of harvest was not significantly related to the incidence of calcification (P = 0.6).

Conclusion: Cryopreserved pulmonary homografts placed in the right side of the heart are less likely to undergo calcification. Homografts harvested from beating heart donors do not appear to reduce the incidence of calcification.

Keywords: Homograft; Calcification; Pulmonary circulation; Beating heart

1. Introduction

Ross and Somerville first reported the use of aortic homograft valve for correction of pulmonary atresia in 1966 [1]. This technique made reconstruction of many congenital heart defects possible. However one of the early complications encountered was calcific stenosis of these conduits resulting in reoperation [2,3]. In 1976, Moodie [4] reported an incident of 14% stenosis in his series. These valves were sterilised by high-intensity radiation, and this method of preparation seemed to result in high incidence of calcification and obstruction [5]. Cryopreservation of valved conduits results in favourable cell viability and preservation of biomechanical parameters [6,7].

The type of homograft, aortic or pulmonary, and the site of placement, right versus left ventricular outflow tract may affect the long-term function and outcome of these devices. In our unit, we have used cryopreserved homograft conduits from two groups of donors. The first group were from cadaveric donors and the second group of homografts were harvested from beating heart donors. This study was undertaken to see if there is any difference in the incidence of calcification between aortic and pulmonary homografts placed in the right ventricular outflow tract (RVOT), and what effect if any the mode of harvest has on the rate of calcification of these homografts.

2. Methods

Between 1st January 1990 and 31st December 2000, 60 patients underwent insertion of cryopreserved homograft valved conduits for reconstruction of RVOT. The hospital and out-patient records of all the patients were studied retrospectively. There were 34 boys and 26 girls. The median age at operation was 75 months (range 1–182). The weight ranged from 2.8 to 45.5 kg (mean of 22.4, SD 14.3). Six patients had a previously placed conduit (other than a homograft). The types of cardiac defects are shown in Table 1. Thirty-nine patients had undergone at least one procedure (mostly shunt operations) before the definitive operation.
was carried out. Calcification was detected on chest roentgenograms and echocardiograms.

2.1. Homograft donors and preservation method

The conduits were harvested from two groups of donors, with group one being cadaveric donors and group two beating heart donors. Both sets of devices were cryopreserved in the same manner. However the cadaveric group had rifampicin added to the cryopreserved solution.

Our preservation method is as follows. After harvesting, aortic and pulmonary valves are each placed in 100 ml of M199 medium plus antibiotics (50 iu/ml Penicillin, 50 mcg/ml Streptomycin, 10 mcg/ml Amphotericin B), and are incubated at 37°C for 24 h. During this 24-h sterilisation period, tissue culture is performed (mitral or tricuspid valves can be used) to determine the viability of leaflet tissue. The viability is assessed by determining the rate at which the valve metabolises the glucose in the tissue culture medium. Samples of the arterial wall are used from each homograft to test for any resistant organisms persisting after the 24-h sterilisation period. The homografts are rinsed in cold RPMI 1640 culture medium (supplied by Bio Sciences Ltd., Ireland). They are then transferred into a sterile cryopreservation pack containing 30 ml of dimethylesulfoxide freezing solution consisting of 10 ml of dimethylesulfoxide vapour phase. This pack is then sealed hermetically using heat sealer, and then packed into a second cryopreservation pack, and preserved using the KRYO 10 Ser.III controlled rate freezer to ~190°C. The valves are stored in liquid nitrogen in its vapour phase.

2.2. Statistical analysis

Statistical analysis was performed using JMP, Version 3.2.5 (SAS Institute). Logistic regression analysis was carried out using Log Xact (Cytel Software Corporation, MA, USA).

Continuous variables were described using mean ± SD. Student’s t-test was used to compare continuous variables, Mann–Whitney Rank Sum test was used to compare medians; where normality test failed. Non-continuous variables were compared using chi-squared test or Fisher Exact test as appropriate.

Logistic regression analysis was performed using Exact tests with both types and source of homografts as factors and controlling for follow-up time.

3. Results

Ten patients (16.7%) died in the hospital or within 30 days of the operation, two deaths were related to haemorrhage, six died of low output syndrome and one patient could not be weaned from the CPB and one had bronchopneumonia. There were no late deaths. There were no conduit failures requiring re-operation. The follow-up period ranged from 3 to 108 months (median 36 months).

From the 50 survivors, 24 patients had aortic homografts and 26 had pulmonary homografts. Twenty-four of the devices were obtained from cadaveric donors and 26 were from beating heart donors. Calcification was detected in 10 homografts, (20.0%). Patients’ age, sex, conduit size and ABO compatibility were not related to the incidence of calcification. In addition, there was no significant difference in the recipient characteristics of cadaveric and beating heart donor homografts. All the calcified devices were aortic in origin. The incidence of calcification in the aortic homografts was significantly higher when compared to pulmonary homografts ($P < 0.0001$) (Fig. 1). Of particular interest is the comparison of cadaveric donor homografts versus beating heart donor homografts. Eight of the cadaveric (33.0%) and two of beating heart donor devices (8.0%) showed evidence of calcification.

The median follow-up period for pulmonary and aortic conduits were 28.5 and 37.5 months, respectively ($P = 0.6$, Mann–Whitney Rank Sum test). However, the cadaveric devices were followed up for a significantly longer period than the beating heart devices (median: 48 months and 24 months, respectively, $P < 0.001$). To control for the follow-up time, we carried out a logistic regression analysis which

### Table 1

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of patients</th>
</tr>
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<tbody>
<tr>
<td>Pulmonary atresia</td>
<td>14</td>
</tr>
<tr>
<td>Tetrology of Fallot</td>
<td>9</td>
</tr>
<tr>
<td>Tricuspid atresia</td>
<td>7</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
<td>9</td>
</tr>
<tr>
<td>Transposition (complex)</td>
<td>10</td>
</tr>
<tr>
<td>Others</td>
<td>11</td>
</tr>
</tbody>
</table>

* Others: double outflow right ventricle, congenital arterial stenosis, post-arterial switch RVOTO, post-Fontan conduit stenosis.
showed that the type of homograft was again significantly related ($P = 0.0006$). However, the source of conduit was not significantly related to the incidence of calcification ($P = 0.6$).

4. Discussion

Extracardiac valved conduits have made total reconstruction of many complex congenital heart defects possible. Since 1966, when Ross and Somerville reported the use of homograft aortic valve for correction of pulmonary atresia, there has been a search to find the ideal conduit and the most appropriate method of preservation. The use of gamma radiation for sterilising aortic homografts proved to result in early calcific stenosis. McGoon reported a reoperation incidence of 28% after 5 years, and 59% after 10 years [5]. Schaff et al. also reported obstruction rate of 59% after 10 years in aortic homografts sterilised by irradiation [8].

Today cryopreserved homograft conduits and fresh antibiotic preserved conduits have gained widespread acceptance. Actuarial freedom from reoperation for obstruction in patients receiving cryopreserved or fresh homografts implanted in the right side of the heart is reported to be 94% at 3.5 years [9]. Kay and Ross reported on their experience with antibiotic sterilised homografts, the actuarial probability of conduit obstruction requiring reoperation was 13% at 10 years [10]. Fontan et al. reported similar excellent results [11].

There has been a considerable debate on the choice of conduit for RVOT reconstruction. Non-valved synthetic conduits, porcine-valved conduits, aortic homografts and pulmonary homografts have been tried. Dacron tube grafts may develop obstruction due to neointimal hyperplasia. A considerable proportion of patients who have xenograft conduit implanted in the RVOT will need reoperation at 10 years. Kirklin reported 59% freedom from replacement of xenograft (or irradiated allograft) at 10 years [9]. Richard et al. reported actuarial freedom from xenograft conduits replacement of 81% at 5 years and zero at 10 years [12].

Aortic homografts have been used extensively for RVOT reconstruction, although the high incidence of calcification in earlier series were likely to be due to irradiation methods of preservation. It now appears that when implanted in the right side of the heart, cryopreserved pulmonary homografts have a better performance than their cryopreserved aortic counterparts. Eguchi and Asano [13] in a dog model of RVOT reconstruction discovered that neither calcification nor degeneration occurred in the homograft pulmonary arterial wall as long as 16 months after implantation, while aortic homograft showed marked calcification during the same period. Albert et al. [14] reported similar survival for cryopreserved aortic and pulmonary homografts in the pulmonary position in humans. However, pulmonary homografts showed better valvular mechanics.

Our results support the work of those authors [14,15], who have concluded that pulmonary homograft is the conduits of choice for the RVOT reconstruction. Although none of our patients required reoperation, all the calcified conduits in our series were aortic in origin.

Histological examination shows that calcification is localised mostly in the elastic structure and is related to the total amount of tissue calcium. The amount of pulmonary elastic tissue per unit of medial surface area in a histologic section is only 50–60% of that in the aorta. In addition, the average amount of biochemically detectable calcium in the pulmonary tissue is half of that in the aortic specimens [16].

In our series, the incidence of calcification in beating heart donor homografts was lower than the cadaveric ones. However, this difference was not statistically significant. Although our follow-up period for the cadaveric conduits was longer, all the calcifications appeared within 18 months of implantation and despite extended follow-up, no new calcification occurred.

Theoretically one might expect reduced incidence of calcification in the beating heart harvested conduits because one might achieve a better cellular viability with these devices. In cadaveric homografts, 80% of fibroblasts are viable after 11 h. The percentage of viable cells decreases progressively to 65% with an interval of 48 h after death of the donor [16]. Segments of pulmonary valves obtained from beating heart donor valves had a higher initial viability than non-beating-heart donor valves [17]. It has been shown that increased cell viability in cryopreserved heart valves correlates with improved clinical performance [18].

In conclusion, we have shown excellent results using cryopreserved homograft valved conduits for RVOT reconstruction. Our intermediate-term survival and freedom from reoperation has encouraged us to continue implanting these devices. In our experience, pulmonary homografts have significantly lower incidence of calcification than aortic ones when placed in the right side of the heart. Harvesting these devices from beating heart donors does not reduce the incidence of calcification significantly.

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References