

The Occurrence of Postoperative Pulmonary Homograft Stenosis in Adult Patients Undergoing the Ross Procedure

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Background and aim of the study: The Ross procedure employs an autologous pulmonary valve to replace the aortic valve, but requires pulmonary homograft replacement. Concerns regarding long-term homograft function may limit the adoption of this technique. Herein, the incidence of, and factors leading to, stenosis of the homograft were examined.

Methods: Data were collected from 131 patients (32 females, 99 males) who underwent a Ross procedure between July 1994 and December 2003. Complete follow up data were collected from 113 of 125 (90.4%) living patients. Donor valve information, including storage time, was supplied by the graft manufacturers. Data were analyzed using chi-square tests, *t*-test and logistic regression.

Results: The mean patient follow up was 703 ± 574 days (median 599 days; range: 2 to 2,408 days). Echocardiographic stenosis had occurred in 14 patients (12.4%). Four patients (3.2%) required homograft replacement, and two required balloon valvuloplasty. There was no significant difference in graft vendor, recipient, donor age or blood type match

between stenotic and non-stenotic recipients. Donor valve size was appropriate for the recipients, and greater than predicted by recipient body surface area (BSA). Donor valves that developed stenosis had a shorter storage time after processing (160 ± 100 versus 249 ± 223 days; $p = 0.03$). Male donor valves became stenotic in 9.9% (7/71) of male recipients, but in none of 20 females. Female donor valves became stenotic in 27.3% (3/11) of male recipients, and in 28.6% (2/7) females. Logistic regression showed donor gender to be a significant predictor for stenosis ($p = 0.007$; odds ratio 14.1 for female/male donors; 95% CI 2.1-96.4).

Conclusion: Donor valves which developed stenosis had a shorter mean cryopreservation time than those that did not develop stenosis. In addition, female donor homografts appeared to develop stenosis at a greater rate, independent of patient age, graft size to BSA match, and blood type.

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Donald Ross first described aortic valve replacement using a pulmonary autograft in 1967. Subsequently, critics of the Ross procedure have pointed out that a two-valve operation for single valve disease is technically difficult and increases the chance of reoperation. Wider adoption of the procedure has thus been limited by the perceived technical difficulty of the autograft transfer and by the findings of early pulmonary homograft stenosis. Several large series, however, have demonstrated low operative mortalities and achieved rates of

survival and freedom from autograft reoperation that were equal or superior to those achieved with other tissue and mechanical substitutes (1-4). The autograft demonstrates growth potential in children, a low resistance to infection, and obviates the need for anticoagulation (2,5). Further refinements in the technical implantation of the autograft with the adoption of autograft transfer as a total root and stabilization of both the annular and sinotubular junction with either felt or pericardium has resulted in much more reproducible outcomes (6,7). Further refinements in patient selection and re-examination of the use of the pulmonary autograft in the subcoronary position in some patients can be expected to further improve operative results.

Problems with pulmonary homograft replacement and early stenosis have been refractory to clinical investigation, however. Various studies have shown that homograft size, cryopreservation time and donor

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age may all be important factors in the longevity of the pulmonary homograft (8-11). All of these studies have focused on stenosis being a cellular rejection phenomenon that is correlated with donor cell viability in the homograft (8,12). Most studies, however, have included infants, children and adults in their analyses, making comparisons difficult. These heterogeneous series include varying degrees of associated congenital abnormalities and pulmonary vascular resistance (10,13,14). The homograft cannot be expected to perform identically in these disparate populations. Additionally, published series have employed homografts with varying degrees of cell viability. Homovital, beating heart donors, and cryopreserved homografts have all been used, making comparisons between series difficult (1,8,11).

The Ross procedure was introduced at the present authors' institution in 1994 for young and middle-aged adults. Cryopreserved pulmonary homografts were used in all pulmonary outflow tract reconstructions. Among this homogeneous population, donor, recipient, and preservation measurements and characteristics that might predict homograft stenosis, were examined.

Clinical material and methods

Patient population

Between July 1994 and December 2003, Ross procedures were performed by one surgeon at a single institution in 131 consecutive young adult patients (99 females; 32 males; mean age 42.0 ± 12.1 years; median age 41 years; range 18 to 65 years). The mean follow up was 703 ± 574 days (median 599 days; range: 2 to 2,408 days). There were three operative deaths (one heparin-induced thrombocytopenia on day 1, one pulmonary embolism on day 8, and one sudden cardiac death on day 14) and three late deaths (one chronic obstructive pulmonary disease at six years, one lymphoma at two

years, and one automobile accident at one year). Thirteen patients were lost to follow up. All others were followed yearly with transthoracic echocardiography (TTE) and clinical examination. The patient who died six years after surgery had been followed regularly until near his time of death, and these data were retained in the study. Follow up data were available from 113 (90.4%) patients who were currently alive.

Operative technique

The Ross procedure was performed in all cases as a total root. The aortic annulus was sized appropriate to the patient's body surface area (BSA) (Elkins). When necessary, a circumferential aortic annuloplasty of 3-0 Prolene was performed. The annular suture line comprised 4-0 Prolene interrupted sutures tied over a double-layer pericardial buttress. The neosinotubular junction was sized to the autograft sinotubular junction and reinforced with a double-layer pericardial buttress. When there was an aortic autograft mismatch, or when the aorta size was ≥ 4 cm, a Dacron interposition graft was used.

The pulmonary homograft was sewn distally to the pulmonary artery bifurcation with two running 5-0 Prolene sutures, tied twice. Proximally, a posterior running 4-0 Prolene suture was reinforced at the septal perforator with a strip of pericardium. This was tied to an anterior running 4-0 Prolene suture to complete the pulmonary homograft/right ventricular anastomosis.

Echocardiographic data

Serial TTE measurements were performed at one month postoperatively and yearly thereafter by the referring cardiologist. Echocardiography was available on 113 patients, with a mean follow up of 22.4 ± 18.6 months (median 19.5 months). Measurements were performed in standardized fashion with a 2.5-MHz ultrasound transducer and standard longitudinal and cross-sectional views; all data were recorded on video-

Table I: Homograft donor variables and cryopreservation time (data retrieved from homograft data banks).

Variable	CryoLife® (n = 48)	LifeNet® (n = 82)	p-value
Donor gender male*	39 (83.0)	66 (84.6)	NS
Donor gender female*	8 (17.0)	12 (15.4)	
Donor age (years)+	37.5 ± 11.6 (median 37)	40.0 ± 12.1 (median 41.5)	NS
Cryopreservation time (days)+	171 ± 232	276 ± 206	0.01
Donor valve size (mm)+	26.5 ± 1.8 (median 26.5)	28.9 ± 1.1 (median 26.5)	<0.001

*Donor gender data were unavailable for one CryoLife implant, and four LifeNet implants.

+Values are mean \pm SD.

Values in parentheses are percentages.

NS: Not significant.

tape. Maximum velocities across the pulmonary valve were calculated using a continuous-wave Doppler imaging transducer. Pressure gradients were determined using the Bernoulli equation. In order to assess pulmonary homograft regurgitation, pulse and continuous-wave and color flow Doppler were performed with regurgitation graded from 0 to 3, based on the length and width of the regurgitant jet in the parasternal short-axis view. All videotapes and echocardiography reports were independently reviewed and repeated if any necessary information was found to be missing.

Homograft characteristics

A total of 131 pulmonary homografts was used, of which 82 (63.1%) were supplied by LifeNet and 48 (36.9%) by CryoLife; manufacturer data were unavailable for one patient. Homograft donor variables and cryopreservation time were collected from the homograft data banks; data are listed in Table I. Only the cryopreservation time was included since cold ischemic time was not available. The cryopreservation protocols were followed as per the respective homograft manufacturers.

Definitions

Homograft stenosis was defined as a mean trans-homograft gradient ≥ 20 mmHg, with or without symptoms. Homograft failure was defined as a trans-homograft gradient ≥ 40 mmHg, or right ventricular failure or the presence of grade 3 to 4+ tricuspid regurgitation with any pulmonary stenosis.

Statistical analysis

Patient follow up data were extracted from charts and entered into an Excel spreadsheet. The data were then loaded into a SAS (v. 9.1.3; SAS Institute, Cary, NC, USA) dataset and combined with the data record for the patient from the Society of Thoracic Surgeons certified database. Statistical analyses for categorical variables used chi-squared statistics or Fisher's Exact test when analyzing tables with cells having small counts (usually < 5). Continuous variables were compared using *t*-tests. Predictive factors were obtained using logistic regression for the occurrence of stenosis as the dependent variable.

Results

There were 14 cases of pulmonary homograft stenosis (12.4%). Four patients (3.5%) developed homograft failure and required replacement of the homograft; all four patients had a Medtronic freestyle heterograft inserted in the pulmonary position. Two homografts (one of which was later replaced) required balloon

valvuloplasty (see Fig. 1) in order to dilate the stenosis.

There was no statistical difference between the size of the homograft valves which became stenotic and those that did not. In female patients, the mean valve diameter was 27.4 ± 2.0 mm for those not developing stenosis and 26.0 ± 1.4 mm for those who developed stenosis ($p = \text{NS}$). In males, the diameter was 28.3 ± 1.8 mm for non-stenotic cases and 28.3 ± 0.8 for those developing stenosis ($p = \text{NS}$). In order to ensure that one group was not receiving undersized valves, Elkin's (15) formula was used to calculate an expected valve size based on the patient's BSA. In all cases, the actual implant valve was slightly larger than the predicted size. In female recipients this was $108 \pm 9\%$ for those without stenosis and $105 \pm 5\%$ for those who developed stenosis ($p = \text{NS}$), whilst in male recipients the ratios were $111 \pm 8\%$ and $108 \pm 5\%$ in non-stenotic and stenotic valves, respectively.

The cryopreservation time and recipient age were examined using univariate analysis. The cryopreservation time for valves developing stenosis was statistically different from that for non-stenotic valves. Stenotic valves were stored for 160 ± 100 days, and non-stenotic valves for 249 ± 223 days ($p = 0.03$). The mean recipient age was similar in both groups: 42.6 ± 11.8 years for those patients without stenotic homografts, and 41.0 ± 14.1 years for those in which stenosis developed ($p = \text{NS}$). The mean donor age for grafts developing stenosis was 34.8 ± 11.6 years, while that for non-stenotic grafts was 38.9 ± 12.1 years ($p = \text{NS}$).

A logistic regression for stenosis occurrence was developed using patient age, recipient gender, donor age, donor gender, graft manufacturer, cryopreservation time, and blood type matching between donor and recipient as independent predictors.

Donor gender was the only statistically significant factor ($p = 0.007$). The odds ratio was 14.1 (95% CI 2.1-



Figure 1: Patient requiring balloon valvuloplasty. Left: the balloon shows the extent of the stenosis. Right: post-dilatation, the balloon shows the return of profile.

Table II: Analysis of parameters measured in the study cohort.

Parameter	Stenotic (%)	p-value
Male gender	7.7	0.03
Female gender	27.8	
Blood type match	13.8	NS
Blood type mismatch	6.4	
Male donor/female recipient	0	0.01
Female donor/female recipient	28.6	
Female donor/male recipient	27.3	NS
Male donor/male recipient	9.9	
CryoLife	16.2	NS
LifeNet	10.5	

NS: Not significant.

96.4) for a female-donor valve becoming stenotic compared to a male-donor valve. With regard to graft manufacturer, CryoLife grafts showed a higher probability of developing stenosis than LifeNet grafts ($p = 0.08$), most likely due to the shorter cryopreservation time used for the former graft. By using the limited data set, this model was highly discriminatory, with a c-statistic of 0.86.

Discussion

With more frequent use of the Ross procedure during the past two decades, the early development of pulmonary homograft stenosis has become recognized as a significant clinical problem. Most clinical series have included a heterogeneous population of infants, children and adults, and this has made comparisons between studies difficult. Nevertheless, two areas of consensus have emerged that center on either technical or immunologic issues for the development of pulmonary stenosis.

The technical insertion of a pulmonary homograft is simple and employs two end-to-end anastomoses in a low-pressure system. Anatomical sites of postoperative obstruction have been found at all levels of the homograft conduit. Moidl et al. (16) found obstruction to occur predominantly at the level of the valve leaflets, while Ward et al. (17) noted predominantly supravalvular obstruction. Carr-White et al. (10) and Raanani et al. (8) found obstruction at all levels of the homograft. In the present series, both echocardiographic findings and operative specimens demonstrated stenosis of the right ventricle to homograft muscle cuff in all patients. Balloon dilatation in two patients and predilatation angiograms also showed stenosis located subvalvularly at the muscle cuff (Fig. 1).

Although it is unlikely that the surgical technique causes pulmonary homograft stenosis, several studies have shown clearly that pulmonary homografts performed better than aortic homografts in the pulmonary position (13). The most important technical factor influencing the long-term performance of the pulmonary homograft, however, is homograft size. Tweddell et al. (18), Forbess et al. (19) and Feier et al. (11) all found homograft diameter to be an independent predictor of stenosis particularly in children, but also in adults. In most centers with large clinical series the pulmonary homograft is now over-sized for both pulmonary annulus and BSA. In the present series, both stenotic ($107.4 \pm 5.3\%$ of predicted) and non-stenotic ($110.2 \pm 8.2\%$ of predicted) grafts were oversized, and size was not predictive of pulmonary homograft stenosis (20). In summary, a careful surgical technique, avoidance of aortic homografts in the pulmonary position, and the oversizing of pulmonary homografts should avoid the technical issues associated with pulmonary homograft stenosis.

Unfortunately, the main cause of pulmonary homograft stenosis does not appear to be a technically correctable problem but rather an immunologically mediated rejection phenomenon. Cryopreserved donor cells can induce lymphocyte proliferation, and antibodies against donor human lymphocyte antigens (HLA) have been found in recipients shortly after implantation (21,22). There is no clear evidence, however, that the level of antibody titer or the rate of rise of antibody correlates with stenosis of the homograft (11,21). Whilst HLA and ABO mismatch may be important in children - and particularly when aortic homografts are used (23,24) - the results of other studies have not confirmed this (11,25). In the present series, among the 80 patients in whom ABO and Rhesus factor data were available, mismatch was not associated with homograft stenosis. HLA testing was not carried out.

Indirect evidence has accumulated that high donor homograft cell viability correlates with earlier pulmonary homograft failure. Early studies conducted by Ross (1), using antibiotic-treated, sterilized, refrigerated, non-living valves demonstrated a low incidence of pulmonary homograft stenosis, whilst a recent report by Feier et al. (11), in which beating heart donors were utilized with short cold ischemic times and greater cell viability, demonstrated a high rate of pulmonary homograft stenosis. Other reports utilizing predominantly cryopreserved homografts with varying degrees of viability have correlated longer cryopreservation time with less pulmonary homograft stenosis (8,14). The results of the present series suggested that a longer cryopreservation time might be associated with less postoperative pulmonary homograft stenosis.

The most surprising finding among the present patients, however, was the striking difference in performance between homografts from female and male donors. Whilst 27.3% of female-donor/male-recipient resulted in stenosis, only 9.9% of male-donor/male-recipient became stenotic. Moreover, 28.6% of female-donor/female-recipient became stenotic, but no male-donor/female-recipient developed stenosis. Perhaps this should not be so surprising. Several cardiac transplant studies have shown a gender-associated risk ratio of 1.13 for female donor organs in male recipients, whereas male donor hearts performed equally well in female and male recipients (26-28). In liver transplantation, Kahn and others reported a 3.7-fold higher risk of graft failure when female donor livers were transplanted into male recipients (29-31). The survival of cadaveric renal transplants is lower when the donor is female, and this effect is more pronounced in younger patients (aged <45 years), suggesting that hormonal influences might play a role in this rejection (32-34). Although female kidneys contains more HLA antigens, HLA matching does not account for the observed gender-related effect. In the present series, logistic regression analysis demonstrated only donor gender as a significant predictor for stenosis, with an odds ratio of 14.1 ($p = 0.007$; 95% CI 2.1-96.4). Whilst it is not yet certain, it appears that size cannot account for most of the transplant gender differences; in the present study all grafts were oversized using Elkins' (20) criteria, and there was no significant size difference between stenotic and non-stenotic valves.

This study had significant limitations, namely that it was retrospective in nature and did not involve a core echocardiography laboratory. Moreover, the sample size for female donor grafts was small, and a longer follow up was needed in the most recently operated patients. Consequently, only a multi-center collaborative follow up study will determine whether donor gender is as important as it appeared to be in the present series.

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Meeting discussion

DR. CHARLES YANKAH (Berlin, Germany): During the 1990s we performed studies with children who had a right ventricular outflow tract reconstruction with homografts, and obtained similar results to yours. The pulmonary stenosis was considered to be due to two different factors: first, whether the homograft was viable or not, because a viable homograft is being implanted into a patient who is most likely histoincompatible with the homograft; and second, if the homograft was non-viable and being implanted into a viable tissue. Either factor might cause stenosis. Did you determine whether your homografts were viable, or not, before implanting?

DR. WILLIAM H. RYAN (Dallas, Texas, USA): No, we did not do that. Your point is very interesting, because in most studies it seems that the less cellularity associated with the homograft, the less likely it is to develop stenosis. In other words, antibiotic-treated, refrigerated donor homografts are much less likely to develop stenosis than valves with very short, warm ischemic times that are rapidly frozen and used quickly. This is a common thread throughout all previous studies performed in this area. Extrapolating from that, it seems that less cell viability correlates with less early homograft stenosis.

DR. YANKAH: How did you implant your distal anastomosis? Also, how wide was the anastomotic area, because this might have an effect on the development of early or late stenosis?

DR. RYAN: We had no stenosis in the distal suture line in any patient - even in those with a mild gradient the stenosis was always proximal. When we explanted the pulmonary homografts there was never any stenosis in the body or distal portion of the homograft at the anastomosis. But we did use part of the bifurcation so that the distal anastomosis was slightly spatulated.

DR. HIKARU MATSUDA (Osaka, Japan): Do you mean that the cause of the stenosis may be partly related to a technical issue? Is the proximal part more likely to have the pressure gradient, or is it the distal part?

DR. RYAN: The muscle cuff is actually oversized for

the valve - the stenotic and non-stenotic valves were all oversized. There was no valve that was undersized by body surface area. The theory in most studies is that this is an early, immunologically mediated rejection phenomenon. Some people think it occurs throughout the body, and some at the valve leaflets. Our experience was that it occurred at the muscle cuff.

DR. MATSUDA: You referred to a shorter period of storage causing a higher pressure gradient. How do you explain that?

DR. RYAN: With a univariate analysis, the cryopreservation time appeared to be a significant factor. But logistically, female gender of the donor overwhelmed any cryopreservation time effect.

DR. CLAUDIA SCHMIDTKE (Luebeck, Germany): Is it not simply that the size of the homograft from a female is smaller than that from a male? We compared differences between men and women receiving homografts, and found the pressure gradient to be lower in females independently of the donor. But this was probably because the homograft was larger.

DR. RYAN: When we adjusted for homograft size, every homograft we used was oversized by body surface area, and there was no statistical significance.

DR. SCHMIDTKE: What do mean by oversized?

DR. RYAN: The graft was upsized by 2-3 mm, based on body surface area. This study was small, but all grafts being upsized did not seem to be a factor. The study also involved only adults - there were no chil-

dren - and only cryopreserved homografts. In other studies of children and infants the data are much less clear. One issue here is that the populations need to be separated to examine this problem more closely.

DR. ROBERTO FAVALORO (Buenos Aires, Argentina): Did you consider the recipient's age? We investigated this two years ago and found that age less than 40 years, in a population aged from 16 to 70 years, was related to homograft stenosis.

DR. RYAN: We did examine age, but we could not identify any correlation between donor or recipient age and rejection.

DR. F DA COSTA (Curitiba, Brazil): When using homografts in the Ross operation, if we extend the proximal side with an autologous pericardial or bovine pericardial patch, and release the tension, then stenosis seems to occur much less often. Using this method, we had only one patient with a gradient more than 40 mmHg, and their follow up now is at four or five years. Can you comment on that?

DR. RYAN: We simply sewed directly in all of our patients - they were all operated on in exactly the same way. Did you use the pericardial patch in adults, children, or a mixture of both?

DR. DA COSTA: The patients had a mean age of 25 years.

DR. RYAN: I think in younger patients it may be more important to do that. I don't know that it is an issue in adults.