Ross Operation with decelularized pulmonary allografts: medium-term results

Operação de Ross com homoenxertos valvares decelularizados: resultados de médio prazo

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Abstract

Objective: To evaluate the medium-term results (4 years) of decelularized allografts during Ross Operation.

Method: From January 2003 to February 2007, 68 patients underwent Ross Operation with decelularized allografts. Forty eight were male and the mean age was 30.3±11.2 years. Decelularization was done with deoxicolic acid (DOA) in 35 cases and with sodium dodecylsulfate (SDS) in 33. For comparison of the gradients, 68 patients with cryopreserved allografts and matched for age were selected. All patients had a control echo before hospital discharge and annually thereafter. In addition, eight patients had MRI studies. In two patients, samples of the conduit wall were analyzed by histological analysis.

Results: There was one (1.4%) early death. In the late follow-up, there were two reoperations for endocarditis and one late death. The early gradients varied between 4 - 29 mmHg (m= 10.3± 5.5mmHg) and exhibited an increase to 16.5±12.2 mmHg (min= 4, max= 45) at 24 months postoperatively. There were no significant differences when compared to the cryopreserved group. There was, however, a tendency towards lesser gradients in the SDS decelularized group after 12 months. Histological analysis revealed partial reendothelization and progressive repopulation of the tunica media ~~media wall~~ with autogenous cells. There was no progressive pulmonary insufficiency. The MRI results showed a lesser tendency to shrinkage in the decelularized conduits.

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Conclusions: **The use of decelularized allografts was safe and with good medium-term results up to 4 years. There was a tendency to lower late gradients in the SDS decelularized allografts after 12 months.**

Descriptors: **Transplantation, homologous. Tissue engineering. Aortic valve. Heart valves.**

Resumo

Objetivo: **Avaliar os resultados de médio prazo do uso de homoenxertos decelularizados na Operação de Ross.**

Métodos: **Entre janeiro de 2003 e fevereiro de 2007, 68 pacientes foram submetidos à Operação de Ross com homoenxertos decelularizados. Quarenta e oito pacientes eram do sexo masculino, com idade media de 30,3±11,2 anos. A decelularização foi feita com Ácido Deoxicólico (DOA), em 35 casos, e com Dodecilsulfato de Sódio (SDS), em 33. Para a comparação dos gradientes, foram selecionados 68 pacientes pareados pela idade, e que usaram homoenxertos criopreservados. Todos os pacientes realizaram ecocardiograma antes da alta e estão sendo avaliados anualmente. Oito pacientes tiveram controle por ressonância nuclear. Em dois pacientes reoperados, foi possível fazer análise histológica de um segmento do conduto pulmonar.**

Resultados: **Houve um (1,4%) óbito imediato. Na evolução tardia, houve duas reoperações e um óbito. Os gradientes imediatos variaram de 4 a 29mmHg (m=10,3±5,5), e apresentaram elevação para 16,5± 12,2mmHg (min= 4, max = 45) aos 24 meses. Quando comparados com o grupo criopreservado, não houve diferenças significativas. Entretanto, houve tendência a melhores resultados em homoenxertos decelularizados com SDS após 12 meses de evolução. A análise histológica revelou reendotelização e repovoamento parcial da camada média com células autógenas. Não houve insuficiência pulmonar progressiva. Os dados de ressonância magnética demonstraram menor tendência de retração dos condutos decelularizados.**

Conclusões: **O uso de homoenxertos decelularizados foi seguro, com bons resultados até quatro anos de evolução. Houve tendência a menores gradientes tardios nos homoenxertos decelularizados com SDS após 12 meses.**

Descritores: **Transplante homólogo. Engenharia tissular. Valva aórtica. Valvas cardíacas.**

INTRODUCTION

Many experts consider the Ross operation as the operation of choice in infants, children, adolescents, and young adults [1-4]. Pulmonary autograft presents several characteristics of an ideal valve surrogate, such as durability, physiologic hemodynamic performance, and despicable incidence of thromboembolism, disease resistance, and growth potential when used in children. However, the procedure is often discussed by the complexity of the operation technique, which is limited by valve homograft availability by involving double valve replacement in patients with only one valve lesion [1,3].

Medium- and long-term results with the Ross operation have demonstrated a late survival nearly comparable to the normal population and that the majority of the patients present with a normal functional capacity, being free of medications and with an excellent quality of life. On the other hand, problems related to the pulmonary autograft and/or to the homograft used to reconstruct the right ventricular outflow tract (RVOT) may result in functional restraints and requires reoperations [1,5,6].

On our 12-year experience with a large consecutive series with approximately 300 operations, the late gradient occurrence in the cryopreserved homograft implanted in the right ventricular outflow tract have been a most often cause of failure to obtain a "perfect outcome" after the surgical procedure [7]. Similar findings were reported in other series [8,9].

The mechanisms involved in the dysfunction of the homografts implanted in the right ventricular outflow tract are still not completely understood [9,10]. Experimental and clinical data have unmistakably demonstrated that the homografts trigger humoral and cellular immune response

accounting for tissue degeneration and destruction [11,12]. In attempt to lessen or even to suppress the antigenic stimulus some groups have suggested the use decellularized homografts. The experimental findings with this new technology have been very promising, however, the clinical outcomes in humans are precursory and poorly known [13,16].

Our early clinical experience concerning the use of decellularized homografts in the Ross operation demonstrated a significant decreased immune response and adequate hemodynamic performance up to an 18-month development [17]. At the present study, we perform an assessment of the medium-term results up to a 4-year development (mean time = 23 months) in comparison with a paired historical group, which received cryopreserved homografts. Refinements in the decellularization technology and its implications are also addressed.

METHODS

Patients

Between January 2003 and February 2007, 128 patients underwent aortic valve replacement through pulmonary autograft at the Cardiac Surgery Services of Aliança Santa Casa – PUCPR and Ecoville Hospital. Of the 128 patients, in 68 the right ventricular outflow tract reconstruction was performed with decellularized pulmonary homografts and they constituted the study group (G Desc). This group was divided into two subgroups according to the decellularization methodology.

In the first 35 cases, the decellularization of the grafts was performed using deoxicolic acid (DOA), 1%, and ethanol, 80% (AutoTissue®) (G Desc-DOA), and in the 33 subsequent cases with sodium dodecylsulfate (SDS), 0.1%. The technology was developed at PUCPR (G Desc-SDS). Forty-eight patients were male, age ranging from 9 to 56 years (mean = 30.3±11.2 years). Fourteen patients were under 20 years of age.

The most frequent valve disease etiology was rheumatic aortic valve disease in 34 (50%) of the cases; the first five patients presented with an important mitral valve dysfunction associated. Two patients presented with bacterial endocarditis affecting the native valve or valve prosthesis, and two had aneurysm of the ascending aorta. Seven patients underwent reoperation.

In order to compare the late homograft valve function results, 68 patients were selected, from our Ross Operation databases, which underwent surgery between May 1995 and December 2006. These patients were sequentially paired by sex and age, which composed the control group (G Cryo), and had their right ventricular outflow tract reconstructed with cryopreserved pulmonary homografts.

Some clinical data and complementary examinations of both groups are listed in Table 1.

Table 1. Clinical dada of the groups submitted to The Ross Operation.

Data	Dec G (n%)	Cryo G (n%)
Age	9 – 56	10-53
	(30 ±11)	(31±10)
Gender Male	48 (70.6)	52 (76.4)
Female	20 (29.4)	16 (23.6)
VHD		
AS	18 (26.4)	18 (26.4)
AI	33 (48.5)	25 (36.7)
DAD	16 (23.5)	20 (29.4)
PD	1 (1.6)	5 (7.3)
Ethiology		
Rheumatic	34 (50)	33 (48.5)
Congenital	26 (38.2)	29 (42.6)
Degenerative	5 (7.3)	3 (4.5)
Endocarditis	2 (2.9)	3 (4.5)
Functional Class		
I	38 (55.8)	16 (23.5)
II	16 (23.5)	31 (45.6)
III	11 (16.1)	19 (27.9)
IV	3 (4.6)	2 (2.9)
Operation Primary		
Reoperation	61 (89.7)	57 (83.8)
Diameter (mm)	7 (10.3)	11 (16.2)
	22-30	20-31
	25.1±1.9	25±2.2

Dec G– Decelularized Group; Cryo G – Cryopreserved Group; AS – Aortic Stenosis; AI – Aortic Insufficiency; DAD – Double Aortic Disease; n – number; % -percentual VHD - Valve Heart Disease; AS - Aortic Stenosis; AI - Aortic Insufficiency; PD - Prosthesis Dysfunction

Valve Homografts

All the homografts were provided by the Human Heart Valve Bank of the Hospital de Caridade da Irmandade da Santa Casa de Misericórdia de Curitiba. The details of the acquiring, processing, and distribution of tissues or organs for transplantation were closely detailed elsewhere [18]. In brief, the grafts were decontaminated with RPMI 1640 a nutrient solution, with low antibiotics concentrations cefoxitin (240 µg/mL), lincomycin (120 µg/mL), vancomycin (50 µg/mL), and polymyxin B (100 µg/mL) over 24 to 48 hours at 4°C. The freezing process was made in a RPMI 1640 solution, 10% dimethyl sulphoxide and 10% of fetal bovine serum in cryopreservation equipment (Planer, model KRIO 10-16 Series III and temperature controller model K10-12, Sunbury-on-Thames, UK) with tissue freezing at a speed of -1 °C/min until the temperature was -80°C. At the end of

the freezing process, the grafts were transferred to storage freezers (Sanyo, model ultra-low temperature freezer -152ºC - MDF-1155ATN or Custom Biogenic Systems, model storage unit S-1500 B, Osaka-Japan) at a temperature of liquid nitrogen vapor (-150ºC).

Patients who received cryopreserved homografts, the grafts were thawed rapidly just before the implantation using saline solution at temperatures ranging from 42 to 50ºC, followed by gradual dilution of the cryoprotector solution with RPMI 1640 and 10% fetal bovine serum.

Patients who received decellularized homografts, the grafts were thawed approximately 15 to 30 days before the procedure and submitted to the decellularization chemical process. In the first 35 cases, the grafts were decellularized in a 1% deoxycholic acid solution over 24 hours under continuous stirring in electromagnetic shakers. Then, the grafts were placed in 80% ethanol over 3 to 6 hours and harvested in a nutrient solution with antibiotics until transplantation time. The aforementioned technology was released by AutoTissue Ltda. In the 33 subsequent cases, the grafts were decellularized with a proprietary technology developed at PUCPR, which is basically composed of 1% SDS solution, also under continuous stirring in electromagnetic shakers.

Operative technique

All the surgeries were performed by the same surgeon (F.C.) and the operative technique was fully described in previous publications [19]. In short, the surgeries were performed under standard cardiopulmonary bypass (CPB) conditions with the use of moderate systemic hypothermia of 30 to 32ºC and myocardial protection was achieved with a continuous cold cardioplegic intermittent sanguineous solution direct to the coronary ostia. The aortic cross-clamping time averaged 92 ± 19 minutes (range from 64 (min) to 134 (max) minutes) and cardiopulmonary bypass time averaged 108 ± 26 minutes (range from 50 (min) to 154m (max) minutes).

The most employed technique was the complete aortic root replacement and in some selected cases the mini-root technique was employed with latero-lateral anastomosis between the native coronary ostia and the pulmonary autograft. Regardless the technique employed, the proximal anastomosis was always intra-annular, in a way that the native aortic annulus could provide support for the pulmonary autograft. Any discrepancies between proximal e/or distal diameters of the pulmonary autograft and the aortic annulus were properly compensated with specific surgical maneuvers.

Right ventricular outflow tract reconstruction was routinely done with continuous propylene 4-0 sutures for both proximal and distal anastomosis. In all the cases, an wide resection of the valve homograft residual musculature,

leaving only a residual border of 2 to 3 mm, which were sufficient to allow its proximal anastomosis into the right ventricular outflow tract. The homograft diameters employed in the Cryo Group ranged from 20 t0 31 mm (mean 25 ± 2.2 mm), and in the Desc Group, ranged from 22 to 30 mm (mean 25.1 ± 1.9 mm).

Postoperative evaluation

Anticoagulants were not given to any patient and either cardiotonic or heart failure drug prescriptions were done at the referral physician's discretion. The observation of postoperative complications was performed according to well-established guidelines [20].

All the patients had a mono and a two-dimensional transthoracic echocardiography with Doppler prior to hospital discharge. Patients were advised to repeat this examination at 6 and 12 months after surgery and thereafter, yearly. Transvalvular gradients in the right ventricular outflow tract homograft were calculated by the modified Bernoulli's equation based on the flow rates through the valves. The degree of valve insufficiency was estimated by the maximal length and area of the regurgitant jet at the level of the ventricular outflow tract according to Perry and graded as absent, trivial, light, moderate, or severe [21].

On late echocardiographic assessment of the pulmonary gradients only the examinations performed through echocardiography at our utility were taken into consideration because they were carried out orderly by a single operator. To compare the late function of pulmonary homografts, the maximum prompt gradients available were joined together on account of the postoperative time allowing the direct comparison between both groups.

Late postoperative clinical data survey and the attainment of follow-up echocardiograms were carried out by three in-hospital physicians through a guided approach. In the ongoing study group, six patients were lost to follow-up. The 62 remaining patients had a follow-up period ranging from 1 to 50 months (mean = 23.4 months).

A total of 8 patients in both groups (2 patients in the Desc Group and 6 in Cryo Group) that are part of a prospective study comparing homografts in the right ventricular outflow tract had nuclear magnetic resonance scan studies and the preliminary results are presented here.

Histological assessment

Reoperation was required in two patients who (see results) had the segments of the previously implanted homograft anterior wall available for microscope and immunohistochemistry studies. Hematoxylin and eosin staining, Weigert staining, and Picrosirius stain, and Factor VIII and alpha actin immunohistochemistry studies were performed.

Statistical analysis

Mann-Whitney nonparametric test for independent measures to compare the evolution of the gradients and the differences between both groups were used. We considered $p<0.05$ as significant.

RESULTS

There was only one immediate death (1.6%) due to low cardiac output syndrome. In the late postoperative, reoperations was required in two patients. On patient who underwent the Ross operation for aortic valve active endocarditis had a good immediate outcome, however, went back to hospital after 6 month with a clinical picture of mitral valve bacterial endocarditis. He underwent mitral valve repair and, at present, he is asymptomatic. During the reoperation, a small ellipsis was removed from the distal anastomosis region of the previously implanted pulmonary homograft for histological assessment.

In the second case, three years after outcome a pulmonary-aortic-mitral bacterial endocarditis occurred leading to the patient's death postoperatively presenting a clinical picture of low cardiac output and multiple organ failure. On necropsy, an apparently healthy segment of the homograft arterial wall was also histologically examined. Valve cuspides were completely destroyed by infection and it was not possible to microscopically analyze it. This was the only late death of this series.

The consistency of the decellularized homografts was very satisfactory during surgery, with good homeostasis in the suture lines, despite the absence of its adventitia layer, which was removed during the decellularization process.

The gradients measured in the immediate postoperative were very low, with maximum instantaneous gradients generally below 10 mmHg. When they were observed from a serial view, there was a discrete raise of the gradients over time. This was the most evident phenomenon between 6 and 18 months after surgery postoperatively (Figure 1 and Table 2). At 24 month of evolutionary course, the mean maximum instantaneous gradient was 16.3±12.2 mmHg. There were no reoperations due to valvular homograft stenosis in the Desc Group. However, two patients in this group, despite being asymptomatic, presented maximum instantaneous gradients superior to 40 mmHg. These patients are still under clinical monitoring.

The clinical outcome in the cryopreserved homografts had the same pattern observed in the decellularized ones (Figure 1). When considering the Desc Group as a whole, there were no statistically significant differences related to the Cryo Group in all periods observed (Figure 1 and Table 2). However, a patient from Cryo Group presented a gradient superior to 60 mmHg and required reoperation for homograft dysfunction.
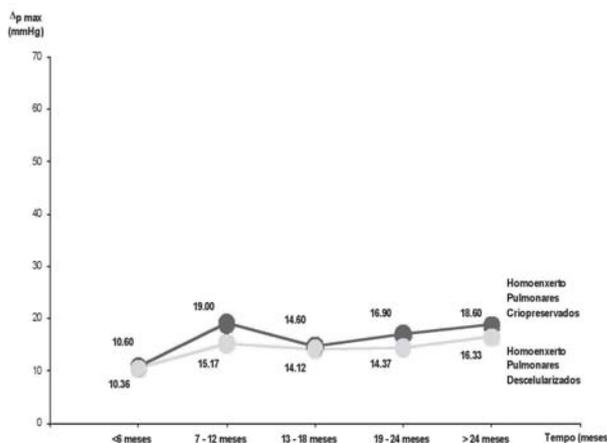

Fig. 1 – *Maximum instantaneous gradients obtained in different periods after decellularized and cryopreserved homografts implantation*

Table 2.   Gradients in the homografts over time.

Time	Dec G (mmHg)	Crio G (mmHg)	p-value
< 6 months	4 – 29 (10.3 ±5.5)	3 – 31 (10.6 ±7.4)	0.539012
7 – 12 months	5 – 45 (15.1 ±10)	4 – 63 (19±13.3)	0.288845
13 – 18 months	3 – 52 (16.8 ±14.1)	7 – 38 (14.6±10.1)	0.979483
19 – 24 months	5 – 28 (14.3 ±10.3)	3 – 30 (16.9±9.1)	0.565703
> 24 months	8 – 45 (16.5 ±12.2)	4 – 55 (18.6±12.5)	0.149668

Dec G – Decelularized Group; Cryo G – Cryopreserved Group

The closely analysis of the group of the decellularized homografts presented differences according to the technology employed. Contrary to the cryopreserved homografts, or the decellularized homografts using deoxicolic acid (DOA), it was not observed any gradient elevation over time until 18 months after the outcome in patients with decellularized homografts using sodium dodecylsulfate (SDS). When analyzed from the statistical standpoint of view, there was a trend ($p<0.08$) to low gradients in the Dsec Group using sodium dodecylsulfate (SDS). Probably, a large number of cases at 12 month after the outcome will confirm this trend (Figure 2 and Table 3).
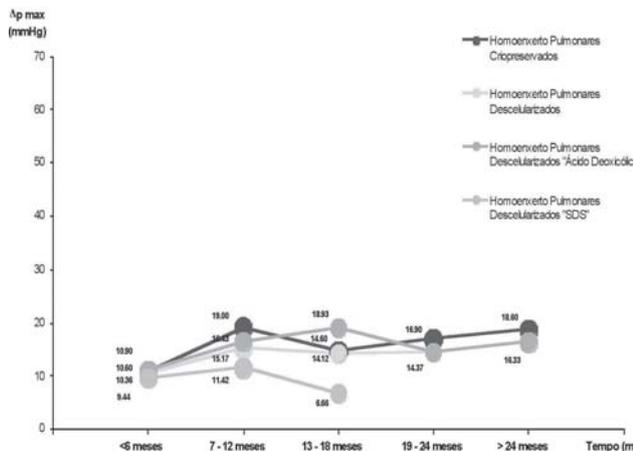
Fig. 2 – Maximum instantaneous gradients obtained in different frame times in cryopreserved, decellularized, decellularized using SDS, and decellularized using DOA pulmonary homografts

Table 3. Gradients in decellularized valvular homografts over time.

Time	SDS Group	DOA Grop	p-value
<6 months	4 – 20 (9.4 ±4.1)	4 - 29 (10.9 ±6.2)	0.611379
7–12 months	6-22 (11.4 ±7)	5 – 45 (16.4 ±10.7)	0.232574
13–18 months	3 – 9 (6.6 ±3.2)	5 – 52 (18.9 ±14.6)	0.085832
19–24 months		5 – 28 (14.3 ±7.8)	
>24 months		8 – 45 (16.3 ±12.2)	

SDS – Sodium dodecyl sulfate; DOA – Deoxycholic acid

The histological study showed that the conduit arterial walls were well preserved; a periadventitial inflammatory reaction was occurring with the migration of autogenous cells into the vessel's tunica media. The elastic fibers (Weigert) were also unimpaired with a minimum degree of fragmentation, while the collagenous fibers preserved their normal undulation framework. By the immunohistochemistry analysis, we could observe the conduit partial reendothelization showed with factor VIII staining, graft tunica media repopulation by cell-like fibroblasts that stain positively with alpha actin, thus suggesting their differentiation into myofibroblasts (Figure 4).

Fig. 3 – Decellularized homograft postoperative control using nuclear magnetic resonance

Patients from Desc Group did not presented any evidence of progressive valve insufficiency. All the grafts were competent, or with minimum insufficiency. As in the cryopreserved homografts, two patients had valve insufficiency quantified as moderate.

Magnetic resonance image (MRI) assessment is still very much preliminary. In none of the cases studied we could not possibly observe a remarkable conduit retraction and the valve cuspides mobility were normal (Figure 3).
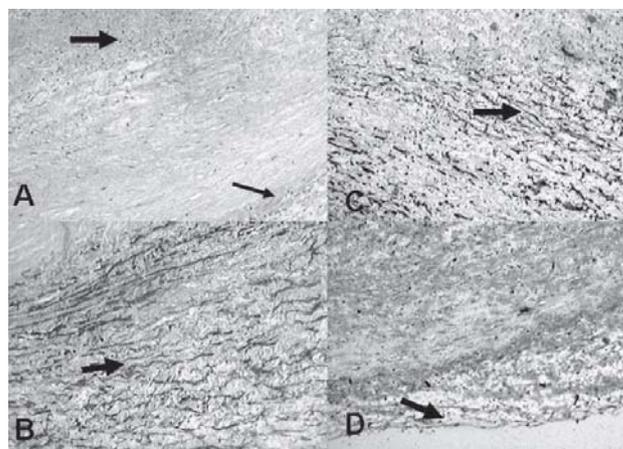

Fig. 4 – Explanted and decellularized valvular homograft histological analysis with 6 months of outcome. A- HE staining showing the periadventitial inflammatory reaction (thin arrow) and the partial repopulation of tunica media (thick arrow). B- Weigert staining showing the preservation of elastic fibers. C- Alfa-actin showing the differentiation in the tunica media myofibroblasts. D- Factor VIII showing discrete intimal hyerplasia with focal reendothelization

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DISCUSSION

The hemodynamic performance of the pulmonary autograft in aortic position is physiologic being superior to all the others types of valve prosthesis currently available. For this reason, several authors have considered the Ross Operation as the operation of choice for young patients requiring aortic valve repair [1-3]. Nevertheless, in order to compare the postoperative functional capacity with that of normal individuals, the homograft function used for reconstruction of the right ventricular outflow tract must also be normal in the long-term [9,10].

Despite the relatively low incidence of significant dysfunctions requiring right ventricular outflow tract reoperations even after a 20-year follow-up, the occurrence of light-to-moderate pulmonary stenoses has also been often reported [6,8]. In Carr-White et al. [9] experience with 144 consecutive patients undergoing the Ross operation, 17% of the right ventricular outflow tract homografts developed maximum gradients over 30 mmHg, even though the reoperation was necessary in 3% of the cases only [9]. Likewise, in our 10-year experience with 227 cases, 85% were free of pulmonary gradients over 40 mmHg and those requiring reoperations were only 3%.

Despite several studies showing that the implantation of valvular homografts triggers cellular and humoral immune response from the receptor, the correlation between the response intensity with tissue degeneration is not well defined [11,12]. The rejection definition comprises not only the presence of humoral and cellular immune activity (antigenicity), but also some functional consequence of the immune reaction to the implanted tissue (immunogenicity). Mitchell et al. [22] did not achieve to demonstrate signs of tissue injury by immune process in explanted homografts, and a possible explanation would be that the valvular homograft interstitial cells may cause immune tolerance by T-cell anergy. On the other hand, more recent studies have suggested a direct relationship between the immune reaction and the tissue degeneration. Dignan et al. [11] demonstrated that the presence of the human leukocyte antigen (HLA) antibody class II was associated to the earliest stenosis or insufficiency occurrence of aortic homografts in adult patients. In a study by Baskett et al. [12] the right ventricular outflow tract homograft dysfunctions in children was more marked in the presence of human leukocyte antigen-DR (HLD-DR) mismatch.

More recently, the decellularization technology of valvular homografts has been proposed as a solution to reduce or even suppress the immune response. Several technologies have been described with conflicting outcomes [14,16,19,23,24].

The experimental studies by Elkins et al. [14] using decellularized grafts with hypo- and hypertonic solutions demonstrated not only a reduced antigenicity, but they also observed that the grafts were partially repopulated by host cells, which had fibroblasts morphology and actively synthesized new collagenous fibers. Presumably, these "living" grafts would have a better durability and more favorable late outcomes.

Some clinical homograft reports treated with SynerGraft process (Cryolife Inc, Kennesaw, GA) demonstrated a significant antigenicity reduction. More recently, Bechtel et al. [15] demonstrated that, despite the lower immune response, SynerGraft-processed homografts had the same biological behavior when compared to the cryopreserved homografts. Using echocardiography and magnetic resonance, it was demonstrated that the SynerGraft-processed homografts also exhibited retraction of the conduit arterial wall with elevations in the pressure gradients comparable to the cryopreserved control group. Nevertheless, in the experience carrien out by Tavakkol et al. [16] the SynerGraft-processed homografts implanted in the right ventricular outflow tract of children with congenital heart defects had both lower gradient elevations and valve insufficiency than did the cryopreserved homografts after a 16-month follow-up.

Dohmen et al. [25] demonstrated excellent experimental outcomes with decellularized homografts with deoxicolic acid (DOA). The clinical outcomes reported by Konertz et al. [24] with heterografts therefore decellularized (Matrix P valve - Auto Tissue Ltd) were remarkable. There were no gradient pressures rises in 50 patients up to 2 years.

Our initial experience with decellularized homografts using DOA have demonstrated a significant reduced immune response and a better hemodynamic performance when compared to cryopreserved homografts up to 18 months [17]. The present study provides a more long-term assessment with a greater number of patients. Despite using similar technology to that of Konertz et al. [24] our 35 decellularized homografts using DOA had some gradients rise; two cases had maximum instantaneous gradient over 40 mmHg.

Despite being a trend to lower late gradients than cryopreserved homografts this difference was not statistically significant.

In our experience, DOA did not allow a complete decellularization in all the grafts. There have been residual cell persistence in some grafts. This fact may at least explain in some cases the gradients rise and are under closely investigation at the Heart Valve Bank.

The change of decellularization method using SDS was due to the extensive experimental investigation that in our experience was not more effective for a complete and reliable decellularization of the grafts [26]. In the animal model, decellularized tissues using SDS were better incorporated and more completely repopulated that the ones

decellularized using DOA. In the present study, we could not yet observe a late rise in homografts decellularized in that manner, however, the clinical follow-up is still reduced and does not allow us to draw more definite conclusions.

The biological study in two cases has confirmed that the decellularized grafts in humans are partially repopulated by autogenous cells. The focal reendothelization, presence of cell-like myofibroblasts associated to a god extracellular matriz preservation, with integrity of the elastic and collagenous fibers have demonstrated a more appropriate behavior of the decellularized grafts in relation to the ones cryopreserved. Recent literature reports support our remarks.

CONCLUSIONS

The 4-year experience with decellularized homografts was satisfactory, demonstrating that its use was safe and with no complications due to the new technology introduced. The homografts remained all competent and despite the late pressure gradients being somewhat inferior to those of the cryopreserved, the differences were not significant. There has been a trend to lower gradients in decellularized homografts using SDS after 12 months. The decellularizarion using SDS is probably superior, however, to drawn more definite conclusions a longer follow-up period is still needed.

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