

Pathologic Findings in Pericardium and Native Valve Tissues after Aortic Valve-Sparing with Autologous Pericardial Leaflet Extension

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Background and aim of the study: Aortic valve repair with autologous pericardial leaflet extension is a valuable treatment option for aortic valve disease. The study aim was to examine and describe the histopathologic changes in native and pericardial extension leaflet tissues after this procedure.

Methods: The pathologic findings of nine patients (mean age 26.7 ± 2.9 years; range: 0-77 years) who underwent aortic valve repair with autologous leaflet extension were analyzed. The initial diagnosis included: bicuspid aortic valve (n = 4), truncus arteriosus (n = 3), ventricular septal defect (n = 1) and subaortic stenosis (n = 1). The pathologic endpoints of the study were fibrosis, calcification and myxomatous changes, based on a scale from 0 to 3.

Results: Fibrosis and calcification demonstrated similar grade results in the pericardial and native tis-

ues; no statistical difference was observed ($p = 0.261$ and $p = 0.999$, respectively). Myxomatous degeneration was greater in the native tissue ($p = 0.012$). Among the native tissue group, five patients were graded 1 and three graded 3 for myxomatous degeneration. Among the pericardial tissue patients, six were graded 0, and one each were graded 1, 2, or 3.

Conclusion: Following aortic valve repair with pericardial leaflet extension, both the pericardial and native valve tissue are susceptible to myxomatous degeneration, fibrosis, and calcification. Among the present patients, myxomatous degeneration was more often present in the native tissue, but there was no difference in calcification or fibrosis between the native and pericardial tissue groups.

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The first approach to repair aortic valves was developed by Taylor et al., in 1958 (1). Some 37 years later, in 1995, Carlos Duran described a technique using an autologous pericardial leaflet extension. The latter technique involved the intraoperative immersion of the pericardial tissue in glutaraldehyde (GA) solution for 9 min, followed by its use to increase the height of the leaflets and commissures, thus creating an additional area of coaptation (2-5).

In 2005, Nash et al. (6) showed that an absence of echocardiographic findings such as commissural thickening, cusp calcification and increased cusp thickness, was associated with better outcome after this surgery.

The study aim was to describe the histologic changes present in aortic valve tissues, and to determine if

there was any histopathologic difference between the autologous pericardium used for leaflet extension and the remaining native leaflet tissue subsequent to aortic valve repair, that might complement the findings of Nash et al.

Clinical material and methods

Patients

The pathologic findings of nine patients (eight males, one female; mean age 26.7 ± 2.9 years; range: 0-77 years) who underwent aortic valve repair with autologous leaflet extension were analyzed. The patient variables are listed in Table I.

The study was approved by the Institutional Review Board at the University of California, Los Angeles Medical Center.

Surgical technique

While in the supine position, each patient was administered a general endotracheal anesthesia, prepared with povidone-iodine (Betadine), and draped in normal fashion. Heparin was administered, and the

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Table I: Patient variables.

Patient no.	Native valve disease (years)	Age at valve repair (years)	Operation-reoperation interval (years)	Mode of valve failure
1	Truncus arteriosus	2.9	2.3	Severe aortic stenosis
2	Subaortic stenosis	77.1	4.5	Moderate aortic valve insufficiency
3	Bicuspid	6.5	3.8	Moderate aortic valve insufficiency
4	Bicuspid	16.2	4	Moderate aortic valve insufficiency
5	Bicuspid	28.8	6.5	Moderate aortic valve insufficiency
6	Bicuspid	37.4	4.3	Moderate aortic valve insufficiency
7	VSD	51.2	4.1	Narrowing of ascending aorta
8	Truncus arteriosus	0	1.8	Severe aortic stenosis
9	Truncus arteriosus	0	1.6	Moderate aortic insufficiency

VSD: Ventricular septal defect.

heart cannulated (Fr12 in the ascending aorta, Fr16 and 18, respectively, in the superior and inferior vena cavae). The patient was placed on cardiopulmonary bypass and the blood cooled to 18°C. The aorta was cross-clamped and cold blood cardioplegia infused into the aorta. Retrograde cardioplegia was administered via the coronary sinus at 10-min intervals during the procedure.

The main principle of aortic valve repair with leaflet extension is to preserve as much as possible the native leaflet, and to add a pericardial extension that is specially designated individually for each leaflet. Thus, the aim is to achieve good coaptation and normal valve function. The thickened edges of the valve were resected until the remaining tissue was relatively normal, after which the pericardium was trimmed to create the leaflet extensions.

The leaflet extension was sutured along the edge of the commissure to the aortic wall, in order to straighten the commissural structure. The height of the pericardial leaflet was measured in order to bring the extensions just below the sinotubular junction, where all extended cusps may naturally coapt at the center of the aorta.

Postoperatively, none of the patients had aortic insufficiency or stenosis grade >2, as proven by echocardiography.

Pathologic methods

The large majority of tissue from each leaflet was preserved for histologic examination. In all cases, staining was conducted with hematoxylin and eosin (H&E) and Masson's trichrome stain. Selected cases were stained by the von Kossa method to better demonstrate regions of calcification. All pathologic evaluations were performed by one cardiovascular pathologist.

Fibrosis is indicated by sparsely cellular dense connective tissue with fibers that stain dark pink with H&E stain, and dark blue with Masson's trichrome. Myxomatous degeneration is indicated by a sparsely cellular, loose extracellular matrix lacking dense fibers. Myxomatous tissue stains light blue with H&E stain and Masson's trichrome; these varied in distribution and severity, and were graded on a semi-quantitative scale from none (grade 0) to the most severe (grade 3). Grade 1 fibrosis and myxomatous degeneration was focal and mild, grade 3 was diffuse and marked, and grade 2 intermediate. For calcification, grade 1 consisted of minute microscopic focal calcifications observable only at high

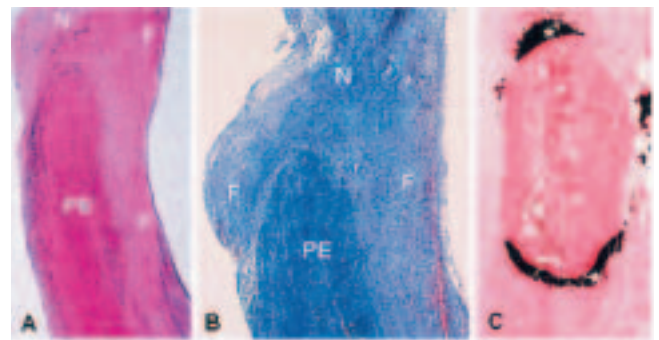


Figure 1: Representative photomicrographs of excised valves. A) H&E-stained section showing fibrosis (F) covering both the native (N) and pericardial extension (PE) portions of the valve (original magnification, $\times 40$). B) Trichrome-stained section showing fibrosis (F) over both the inflow and outflow surfaces of the leaflet at the anastomosis joining the native (N) and pericardial (PE) portions of the leaflet (original magnification, $\times 100$). C) von Kossa-stained section showing a nodule of calcification in the pericardial portion of the leaflet (asterisks). Much of the calcification (black staining) was removed by decalcification of the tissue to allow sectioning (original magnification, $\times 100$).

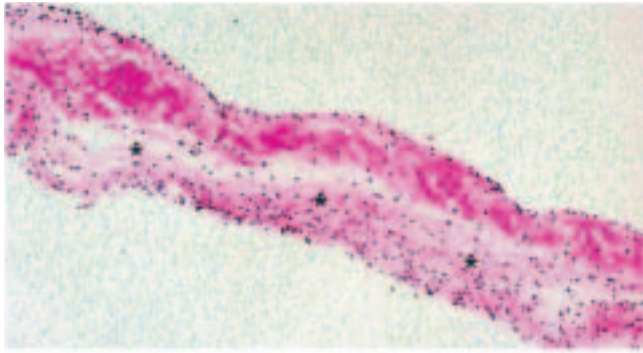


Figure 2: Photomicrograph showing myxomatous change (asterisks) in native leaflet tissue. H&E staining; original magnification, $\times 40$.

magnification ($\times 200$), grade 2 were larger multiple foci, and grade 3 were large nodular calcifications observable at low magnification ($\times 10$). Specimens with focal fibrosis covering the anastomotic sites were not included in the grading. The pericardial tissue was most likely normal at the time of valve repair, although the native valvular tissue may have had some pre-existing changes (though this could not be assessed) (Table 2). The native side was the patient's own valve leaflet, as opposed to the pericardial tissue. Inflow was the ventricular surface of the leaflet, and outflow the aortic surface.

Glutaraldehyde solution and anticoagulation

The GA solution was prepared under aseptic conditions (ultrafiltration) by the University of California, Los Angeles Medical Center pharmaceutical technology laboratory. All solutions were tested for sterility by culturing before use. The preparation consisted of sterile GA (0.625%) in phosphate buffer (pH 7.4) to which fluorescein 0.02% was added. Following the tanning process, and before creation of the leaflets, the

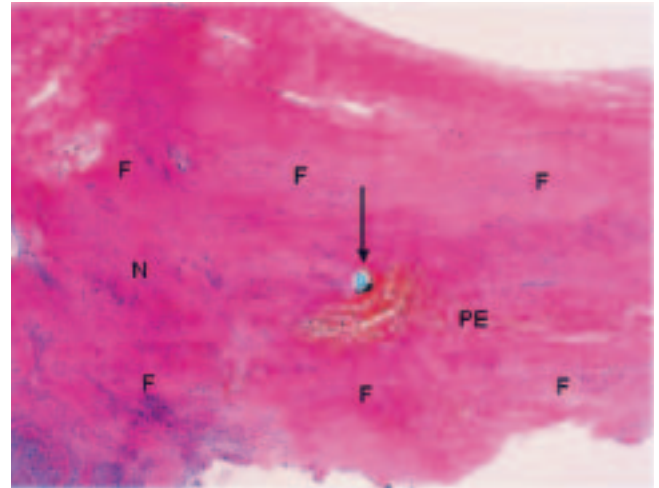


Figure 3: Photomicrograph showing the blue Prolene suture material (arrow) surrounded by marked fibrosis (F), at the junction of the native (N) and pericardial extension (PE) tissue. H&E staining; original magnification, $\times 100$.

pericardium was rinsed three times in sterile normal saline, each for 2 min.

Preoperatively, all patients were administered aspirin (100 mg per day), but none was anticoagulated.

Statistical analysis

All statistical analyses were performed using SPSS v13.0 (SPSS, Inc.). Continuous variables were reported as mean \pm SD, or percentage when appropriate. An analysis of dichotomous (ordinal) variables was performed with the chi-square test or Fisher's exact test, as required. Two-sided p-values were reported; a p-value < 0.05 was considered to be statistically significant. Any correlation between implantation and retrieval time and degenerative changes was assessed with the Spearman non-parametric test.

Table II: Echocardiographic and histological findings.

Patient no.	Echocardiographic findings			Leaflet histological findings					
	Commissural thickening	Cusp calcification	Cusp thickness	Pericardial			Native		
				Fibrosis	Calcification changes	Myxomatous	Fibrosis	Calcification changes	Myxomatous
1	Present	-	-	3	1	0	3	0	3
2	Present	-	Present	3	0	0	1	0	1
3	Present	Present	Present	3	0	2	3	3	2
4	Present	Present	Present	0	0	0	2	2	3
5	Present	Present	Present	3	3	3	3	0	3
6	Present	-	-	0	0	0	1	1	1
7	Present	-	Present	0	0	0	2	0	1
8	Present	Present	Present	2	0	0	3	0	1
9	-	Present	Present	3	3	1	3	0	1

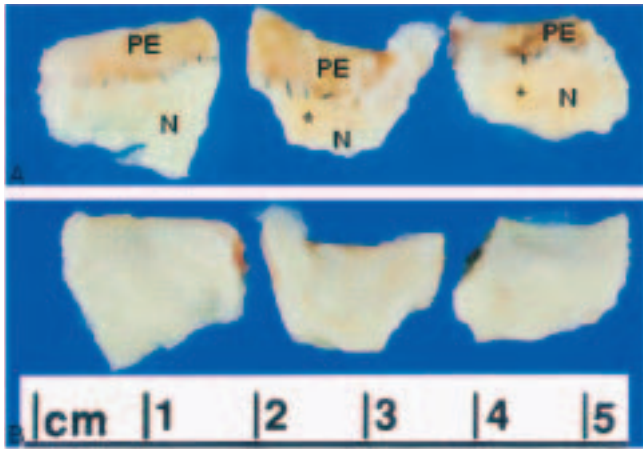


Figure 4: Gross examples of the inflow (A) and outflow (B) surfaces of an excised valve after pericardial extension. There is more fibrosis over the native tissue (N) than over the pericardial extension (PE), particularly on the inflow surface in this case. The calcifications (asterisks) appear grossly yellow.

Results

An initial pathologic examination revealed abnormalities in the tissue, with fibrosis, calcification, and myxomatous changes (Figs. 1-4). Both, fibrosis and calcification showed similar grade results in the pericardial and native tissues, with no statistical difference ($p = 0.261$ and $p = 0.999$, respectively). Myxomatous degeneration was greater in the native tissue ($p = 0.012$). Among the native tissue group, five patients were graded 1, and three graded 3, for myxomatous degeneration. Among the pericardial tissue patients, six were graded 0, and one each graded 1, 2, and 3. The histological findings of each patient are listed in Table 2.

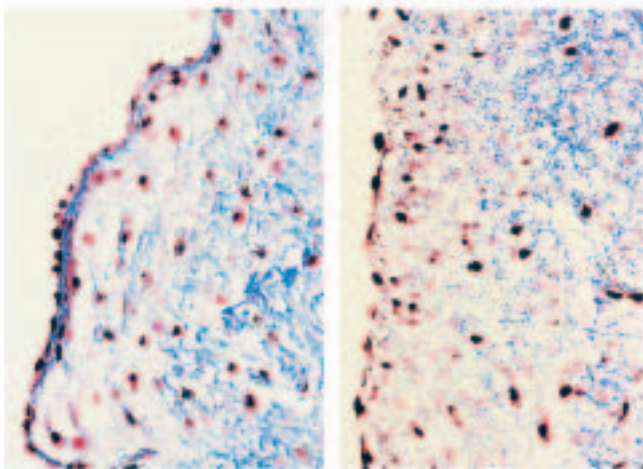


Figure 5: Trichrome staining, showing regions of re-endothelialization from two of the valves. Original magnifications: Left, $\times 200$; right, $\times 400$.

The correlation of each of the degenerative changes with time between implantation and retrieval was tested statistically (bivariate correlation Spearman test), and the correlation coefficient was found to be low ($r = 0.486$).

Connective tissue was seen to develop over the native leaflet and pericardial tissue and, for the most part, contained few nucleated stromal cells. However, in focal areas more cellular connective tissue elements were observed with features of smooth muscle cells and fibroblasts in trichrome-stained sections. None of the patients showed any regions of acute inflammation. Mild chronic inflammation was identified in some leaflets, usually around the suture sites and in regions of calcification. All leaflets demonstrated endothelialization of the leaflet surfaces (Fig. 5).

Discussion

Aortic valve repair with autologous leaflet extension consists of the intraoperative treatment of autologous pericardium with GA solution; the pericardium is subsequently used to increase the height of the leaflets and commissures, thus creating an additional area of coaptation.

Histologically, the pericardium is composed predominantly of compact collagen layers interspersed with elastin fibers. The abundance and orientation of these collagen fibers are responsible for the characteristic viscoelastic mechanical properties of the pericardium. The use of autologous pericardium without any treatment is associated with retraction and thickening of the tissue (7-9). This method of preserving the pericardium may influence these two factors. Treatment with GA solution endows the pericardium with a greater resistance to retraction and degeneration, and increases the ease of handling, while retaining its intrinsic tissue pliability (9). Previous studies have shown that 75% of the cross-linkages between collagen molecules were present after 15 min of immersion in 0.65% GA solution (10). This cross-linking of collagen modifies the progression of events that lead to thickening and shrinkage of the pericardium (7,10,11).

The effects of the GA solution on long-term calcification remain controversial. In 1988, Chachques et al., using scanning electron microscopy, reported an absence of calcification in the mesothelial cells of the pericardium (11). In contrast, Rocchini et al. reported an increased calcification in the pericardium previously treated with GA (12).

The correlation coefficient between the GA exposure time of pericardial and native tissues and the severity of the degenerative changes was low, and further studies should be conducted in order to elucidate this relationship.

Aortic valve repair can overcome some of the limitations of other techniques, for example by improving hemodynamic conditions, by avoiding anticoagulation therapy, and by allowing normal aortic annulus growth. Although several studies have been conducted to perfect the surgical techniques and to monitor the clinical outcomes, very few data exist relating to the comparative histopathologic changes of the native leaflet and pericardial extensions. Following aortic valve repair with autologous pericardial leaflet extension, both the pericardial and the native valve tissue are susceptible to degenerative changes, including myxomatous change, fibrosis, and calcification (13-15).

Study limitations

The primary limitation was that all specimens had focal fibrosis covering the anastomotic sites, but these regions were excluded from the grading scheme. While the pericardial tissue was most likely normal at the time of valve repair, the native valvular tissue may have shown pre-existing changes, though this could not be assessed. A second limitation was the relatively small number of valves available for histologic analysis. Further studies need to be performed to determine the relative role of various pathologic findings in the failure of valve-sparing pericardial leaflet extension, as well as in leaflet extension procedures utilizing other tissues.

In conclusion, the results of the present study showed that the native leaflet underwent a greater degree of myxomatous change than did the pericardial extension. Moreover, both fibrosis and calcification demonstrated similar grade results in the pericardial and native tissues. Given that changes in the pericardium appeared to be less intense than in the native tissue, a more extensive resection of the native leaflet and freehand autologous pericardium reconstruction might generate better results. These histologic changes might complement the echocardiographic findings reported by Nash et al. (6). While calcification was identified in both tissues, it is highly unlikely that the native pericardial tissue was calcified before being used to extend the valve leaflet.

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