Rate of Progression and Functional Significance of Aortic Root Calcification After Homograft Versus Freestyle Aortic Root Replacement

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Background—Calcification is an important limitation after aortic root replacement. The aims were to compare the long-term degree and rate of calcification of homografts versus Medtronic freestyle aortic roots to determine the functional consequences and predictive factors.

Methods and Results—One hundred sixty-six patients were prospectively randomized to undergo homograft versus freestyle total aortic root replacement. Of those, 98 patients underwent a total of 248 electron beam computed tomography studies at 0.5, 1, 1.5, 2, 3, and 8 years. All patients underwent yearly clinical and echocardiographic follow-up. Calcium scores were measured using Agatston scoring. Mixed effects models demonstrate significantly higher calcium scores in homograft roots than freestyle at 1.5 years (P=0.02), 2 years (P=0.02), and 3 years (P=0.01), with a trend at 1 year (P=0.06) and 8 years (P=0.1). Homograft calcification occurs significantly faster than in freestyle prostheses between 6 months and 3 years after surgery (P=0.02). Calcification occurs at a similar rate thereafter up to 8 years (P=0.3). At 8 years, freedom from aortic valve dysfunction was lower in homografts than freestyle roots (P=0.06). Freedom from reoperation was 93±4% in the homograft group versus 100±0% in the freestyle group at 8 years (P=0.01). On multivariate analysis, redo surgery (P<0.001), smoking (P<0.01), atrial fibrillation (P=0.001), family history of coronary artery disease (P<0.01), and a degenerative etiology (P=0.02) were predictive of higher calcium scores.

Conclusion—Homograft roots exhibit significantly higher calcium scores than freestyle roots because of faster early calcification. (Circulation. 2009;120[suppl 1]:S269–S275.)

Key Words: calcium ■ homografts ■ imaging ■ valves ■ xenografts

Previous studies have demonstrated that increased calcium content in native aortic valves correlates with poor long-term outcomes. Messika-Zeitoun et al showed that aortic valve calcification score measured by electron beam computed tomography (EBCT) is negatively correlated with event-free survival. Similarly, Rosenhek et al identified moderate-to-severe aortic valve calcification (based on echocardiographic assessment) as an independent predictor of long-term outcomes, with a risk ratio of 4.6 for death or valve replacement. Calcification remains one of the major problems after bioprosthetic aortic root replacement and is thought to impact long-term results after tissue valve replacement.

The degree of aortic valve and wall calcification can be accurately assessed by EBCT. We have previously described a method using contrast-enhanced EBCT to localize the cusps and accurately assess calcification at both valve and wall levels. A precise calcium score is attributed using a standardized evaluation method modified from the original Agatston scoring system described for coronary calcium quantification. We further validated these results by using in vivo and in vitro methods. We have shown low interobserver variability in calcium quantification, as well as a high correlation between calcium scores on preoperative EBCT and postoperative EBCT on the explanted valves (6% variability). Thus, EBCT represents an excellent clinical tool for the quantitative and longitudinal evaluation of aortic root calcification.

Aortic homografts have been used for several decades, offering excellent hemodynamics and good resistance to infection. However, concerns about their long-term durability and freedom from calcification remain. The Medtronic freestyle xenograft is a stentless porcine aortic root that was FDA-approved in the mid 1990s and has shown good hemodynamic and clinical results in large, retrospective series. Nevertheless, its propensity to calcify in vivo has not been addressed. Freestyle bioprostheses are pretreated with an anticalcification agent, alpha amino-oleic acid, which was shown to mitigate aortic cusp calcification in animal
models. The main advantage of these prostheses is their ready availability "off the shelf" in different sizes. Thus, resistance to calcification would represent a significant advantage over homografts, particularly in younger patients.

We have previously reported preliminary results showing a trend toward higher calcification in the homograft roots versus the freestyle. However, the postoperative follow-up was limited to 2 years and the number of subjects studied was low. Therefore, the aims of the current study were to examine a large cohort of prospectively randomized patients undergoing homograft versus freestyle aortic root replacement to determine: (1) the degree and rate of progression of root calcification up to 8 years after surgery; (2) the impact of root calcification on valvular and ventricular function, and the need for reoperation; and (3) potential determinants of increased calcification.

Materials and Methods

Patient Population

From 1997 to 2005, 166 patients were prospectively randomized to undergo total aortic root replacement using a homograft (n = 76) or a Medtronic freestyle aortic bioprosthesis (n = 90). Six patients from the homograft group crossed over to the other group in the operating room because of unavailability of a suitably sized homograft. Patients were analyzed according to allocated treatment (Figure 1).

An intent-to-treat analysis was additionally performed and showed no differences to the current results. All patients underwent total root replacement with reimplantation of the coronary arteries. Preoperative creatinine clearance was estimated for all patients using the Cockroft-Gault formula. Preoperative renal failure was defined as creatinine clearance \( \leq 50 \) mL/min. Exclusion criteria for EBCT scanning at each time point were estimated creatinine clearance \( \leq 50 \) mL/min, claustrophobia, and reduced mobility. The surgical technique and short-term operative results of this study have been previously reported. The study was approved by the internal review board and ethics committee and all patients consented to the study.

EBCT Studies

For logistical considerations, the entire cohort could not be included in this substudy. Therefore, the maximum number of patients that could be accommodated for EBCT examination was included, regardless of clinical status. Post hoc analysis comparing patients who underwent EBCT scanning or not at each time point showed no differences in terms of patient characteristics and intraoperative and postoperative results (data not shown). Of the 166 patients enrolled in the study, 136 were eligible to undergo EBCT scanning at time of first scanning (early death, n = 10; renal dysfunction, n = 20; Figure 1). A total of 98 patients underwent \( \geq 1 \) scans during the follow-up period (homograft: n = 42 patients [69% of eligible homograft population]; freestyle: n = 56 patients [75% of eligible freestyle population]). A total of 248 EBCT examinations were performed (homograft: n = 112 scans; freestyle: n = 146 scans). Scans were performed at 0.5, 1, 1.5, 2, 3, and 8 years after surgery (n = 24 patients at 8 years; 15 freestyle and 9 homografts). Because valve substitutes were considered to be free of calcium at the time of
implantation and for safety reasons, patients did not undergo scanning at baseline or at all individual time points. All examinations were performed using an Imatron C-100 scanner (Imatron, San Francisco, Calif). Images were recorded through the aortic root with a 100-ms acquisition time and 3-mm section thickness. Scanning was electrocardiographically triggered at 80% of the R-R interval on ECG. Twenty contiguous sections were obtained during a single breath-hold. All scans were read and calcium scores determined by 2 independent radiologists blinded to the study groups. Calcium scores were calculated using a modification of the Agatston score and expressed in Hounsfield units (HU). To account for the functional unity of the entire aortic root, calcium scores of the prosthesis aortic cusps and aortic wall were totalled. Accuracy of this evaluation technique, correlation with actual degree of calcification and interobserver reproducibility have been previously validated.

No adverse events related to EBCT scanning were observed.

Follow-Up

Patients were prospectively followed-up with yearly clinical and echocardiographic examinations. Functional status, the need for reoperation, and valve-related complications were recorded. Valvular and ventricular functions were echocardiographically assessed. Echocardiographic data presented in this study relate to the patients who underwent ≥1 EBCT study (n=98 patients). Data were analyzed over the study period using actuarial techniques or mixed effect models. Overall completeness of echocardiographic follow-up within 18 months of study closure; reoperation or death is 78% in both groups. At 8 years, completeness of echocardiographic follow-up in eligible patients was 94%.

Statistical Analysis

Data are expressed as mean±SD for continuous variables and analyzed using Student t test. Categorical variables are expressed as a number (percentage) and compared using χ2 test. To evaluate the progression of calcification over time, mixed effects models were used to account for the correlation between repeated follow-up calcification score measurements (the MIXED procedure in SAS software, version 9.1; SAS Institute, Cary, NC). A fully parameterized mixed effect model was built including a coefficient for each time point for each group. Group differences were assessed by contrasting the coefficients obtained for the 2 groups at each time point. Linear, square, cubic, and spline models were obtained and compared to the fully parameterized model using log likelihood ratios. A piecewise linear random effect model with a knot at 3 years was chosen based on post hoc analysis. The slopes of progression of calcification from 0 to 3 years and from 3 to 8 years of follow-up were compared between the 2 groups. For patients with missing calcification score measurement at a given time point, the outcome data were considered to be missing at random. All variables in Table 1, except for peripheral vascular disease (n=1) were considered for the univariate analysis, and those with a P<0.2 were included in the multivariate analyses. The effect of each variable was assumed to be constant over time. A stepwise backward elimination process was used and as for all other analyses; variables with a P<0.05 were considered statistically significant.

Statement of Responsibility

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Patient Characteristics

Patient characteristics and surgical data are presented in Table 1. Patients were comparable in terms of demographics, comorbidities, and intraoperative results. Characteristics of

<table>
<thead>
<tr>
<th>Variable</th>
<th>Homograft (n=76)</th>
<th>Freestyle (n=90)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>64.1±9.2</td>
<td>66.0±8.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>49/76 (64%)</td>
<td>61/90 (68%)</td>
<td>0.6</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.89±0.22</td>
<td>1.88±0.21</td>
<td>0.8</td>
</tr>
<tr>
<td>Creatinine clearance, mL/min</td>
<td>72.6 (29.5–138.2)</td>
<td>70.2 (24.9–128.8)</td>
<td>0.7</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>57.2±24.8</td>
<td>64.0±13.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Aortic stenosis</td>
<td>51/76 (67%)</td>
<td>63/90 (70%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Previous surgery</td>
<td>14/76 (18%)</td>
<td>16/90 (18%)</td>
<td>0.9</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDDM</td>
<td>5/76 (7%)</td>
<td>2/90 (2%)</td>
<td>0.3</td>
</tr>
<tr>
<td>NIDDM</td>
<td>6/76 (8%)</td>
<td>4/90 (4%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>5/76 (7%)</td>
<td>4/90 (4%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Renal failure*</td>
<td>9/76 (12%)</td>
<td>11/90 (12%)</td>
<td>0.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td>24/76 (32%)</td>
<td>19/90 (21%)</td>
<td>0.2</td>
</tr>
<tr>
<td>Previous CVA</td>
<td>1/76 (1%)</td>
<td>3/90 (3%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Previous TIA</td>
<td>8/76 (11%)</td>
<td>5/90 (6%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Previous syncope</td>
<td>3/76 (4%)</td>
<td>10/90 (11%)</td>
<td>0.1</td>
</tr>
<tr>
<td>PVD</td>
<td>1/76 (1%)</td>
<td>0/90 (0%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>1/76 (1%)</td>
<td>0/90 (0%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Treated</td>
<td>1/76 (1%)</td>
<td>2/90 (2%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Valve pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degenerative</td>
<td>51/76 (67%)</td>
<td>57/90 (63%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Congenital</td>
<td>17/76 (22)</td>
<td>23/90 (26)</td>
<td>0.7</td>
</tr>
<tr>
<td>Rheumatic</td>
<td>8/76 (11%)</td>
<td>10/90 (11)</td>
<td>1.0</td>
</tr>
<tr>
<td>Intraoperative data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-clamp time, min</td>
<td>90±30 [91 HU]</td>
<td>94±24 [100 HU]</td>
<td>0.8</td>
</tr>
<tr>
<td>Bypass time, min</td>
<td>131±37 [127 HU]</td>
<td>139±42 [138 HU]</td>
<td>0.2</td>
</tr>
<tr>
<td>Concomitant CABG</td>
<td>33/76 (44)</td>
<td>39/90 (43)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Table 1. Characteristics and Operative Data of Homograft (n=76) and Freestyle (n=90) Recipients

Patients undergoing EBCT examination at each time point were also comparable (data not presented).

Degree of Calcification and Rate of Progression

Figure 2 displays the mean calcium scores for both groups at each time point. Calcium scores were significantly higher in homografts than freestyle roots at 1.5 years (P=0.02), 2 years (P=0.02), and 3 years (P=0.01), with a trend at 1 year (P=0.06) and 8 years (P=0.1). Mean calcium scores in the freestyle group at 1, 2, 3, and 8 years were 366±60 HU, 538±100 HU, 709±151 HU, and 2544±498 HU versus 573±91 HU, 977±153 HU, 1381±227 HU, and 3781±591 HU for homograft roots.

The rate of progression of calcification was assessed using a spline model with a knot at 3 years. Homograft calcification...
occurs significantly faster than in freestyle prostheses between 6 months and 3 years after surgery \((P=0.02; \text{Figure } 3)\). Thereafter, the rate of progression becomes similar between both groups up to 8 years \((P=0.3)\).

**Correlation of Calcium Scores to Valvular and Ventricular Function**

**Valvular Function**

Of patients eligible for EBCT scanning at 8 years \((n=45)\), actuarial freedom from moderate to severe aortic insufficiency (grade 3 and 4) was 100±0% in the freestyle group versus 92±7% in the homograft group \((P=0.06; \text{Figure } 4A)\). In addition, freedom from an aortic valve gradient ≥30 mm Hg at 8 years was 100±0% in the freestyle group versus 79±20% in the homograft group \((P=0.06; \text{Figure } 4B)\).

**Ventricular Function**

Analyses were performed on patients undergoing EBCT examination \((n=98)\). Preoperative ventricular indices were similar between both groups. After aortic root replacement, there was a significant decrease in left ventricular end-diastolic dimensions in both groups \((P<0.01)\). There were no significant differences in left ventricular end-diastolic dimensions between both groups at all time points thereafter. Similarly, there was an increase in left ventricular ejection fraction in both groups that reached statistical significance 1 year after surgery \((P<0.01\text{ versus preoperative})\). Ejection fraction remained comparable between both groups throughout the study period. Left ventricular mass regression after surgery was similar in both groups, and remained comparable at all time points thereafter.

**Correlation of Calcium Scores to Symptomatic Status and the Need for Reoperation**

Actuarial freedom from NYHA class 3 to 4 at 8 years was 91±8% in the homograft group versus 96±4% in the freestyle group \((P=0.03)\). Four out of 42 patients in the homograft group required reoperation, whereas no patients in the freestyle group required reintervention. Freedom from the need for reoperation at 8 years was 93±4% in the homograft group versus 100±0% in the freestyle group at 8 years \((P=0.01)\).

Indications for reoperation and detailed calcium score measurements are presented in Table 2. Three of the 4 patients presented with severe aortic insufficiency, whereas the remaining patients had moderate aortic insufficiency with progressive left ventricular dilatation. Heavy calcification was observed in all 4 patients, accompanied by cusp tear in 3 of them. The valve was selectively replaced in all 4 patients, using a mechanical prosthesis in 2 cases and a tissue valve in the 2 others.
Determinants of Prosthetic Root Calcification

On multivariate analysis, reoperation (P<0.001), smoking (P<0.01), atrial fibrillation (P=0.001), a family history of coronary artery disease (P=0.01), and a degenerative etiology (P=0.02) are independent predictors of higher calcium scores (Table 3).

Discussion

The findings from this study show that: (1) homograft aortic roots are more prone to calcification than Medtronic freestyle roots, particularly in the first years after surgery; (2) this translates into increased valvular dysfunction and structural valve degeneration requiring reoperation; and (3) identifiable risk factors are predictive of increased calcification.

Analysis of the rate of progression of calcification reveals that differences in degree of calcification are attributable to a rapid early phase of calcium deposition in homografts, suggesting an early immune-mediated or inflammatory response elicited by the homograft tissue, with ensuing wall calcification. No explantations were performed during that early period in our cohort, precluding any histological analysis of the samples. Nevertheless, we and others have observed the presence of low-grade inflammatory or immune responses early after homograft implantation. Additionally, recipient-donor human leukocyte antigen mismatching appeared to correlate with late homograft degeneration. The faster rate of early calcification in homografts might be attributable to direct alloantigen recognition by recipient T cells of “living” donor cells on the surface of homografts. This could subsequently be followed by a slower rate of “rejection” mediated by indirect recognition pathways secondary to shedding of donor antigens from the graft, which is processed by recipient major histocompatibility complex molecules and presented as peptides. This represents a slow

Table 2. Indications and Calcium Scores Before Redo for Patients Requiring Reoperation in the Homograft Cohort (n=4)

<table>
<thead>
<tr>
<th>N</th>
<th>Pathological Observations</th>
<th>Years After Surgery</th>
<th>Surgical Indication</th>
<th>Calcium Score (HU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dense aortic wall Ca²⁺</td>
<td>7</td>
<td>Severe Al + LV Dilatation</td>
<td>235 (Q3) 290 (Q3) 345 (Q2)</td>
</tr>
<tr>
<td></td>
<td>Suture line Ca²⁺</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amorphous stroma on cusps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Cusp tear</td>
<td>5</td>
<td>Severe Al</td>
<td>357 (Q3)</td>
</tr>
<tr>
<td></td>
<td>Localized Ca²⁺</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Heavy cusp Ca²⁺</td>
<td>7</td>
<td>Moderate Al + LV Dilatation</td>
<td>139 (Q2) 293 (Q3) 1157 (Q3)</td>
</tr>
<tr>
<td></td>
<td>Heavy cusp Ca²⁺</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Heavy cusp Ca²⁺</td>
<td>8.5</td>
<td>Severe Al</td>
<td>18 (Q1) 26 (Q1) 883 (Q3)</td>
</tr>
</tbody>
</table>

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AI indicates aortic insufficiency; Ca²⁺, calcification; LV, left ventricle; Q, quartile.

No patients in the freestyle cohort required reoperation.
but persistent form of chronic rejection, which could be present in both homografts and xenografts.

The absence of a rapid early calcification phase in the freestyle prostheses could be attributed to the anticalcification treatment with alpha amino-oleic acid, which has been shown to significantly mitigate aortic cusp calcification in animal studies, an effect that was, however, not observed in the aortic wall. No studies have thus far correlated animal observations with human results. Alpha amino-oleic acid is a detergent that covalently binds to aldehyde groups in glutar-aldehyde-pretreated bioprostheses, thus diminishing calcium diffusion into the tissue and its binding to phosphate residues to form hydroxyapatite minerals. It is also thought to act as a surfactant replacing phosphate in phospholipid membranes, therefore reducing the number of nucleation sites for circulating calcium. However, concerns about the penetrance of alpha amino-oleic acid into aortic wall tissue and time-dependent leaching could limit its long-term efficacy. From our data, it appears that the rate of increase in calcification up to 8 years does not accelerate. Importantly, in this cohort of patients undergoing EBCT examination, the rates of valve dysfunction and reoperation were lower in the freestyle group. Nevertheless, continued close surveillance is mandatory, particularly after a recent report by Mohammadi et al of late cases of cusp tear with acute aortic insufficiency in a large cohort of subcoronary freestyle implants. The median time between implantation and explantation in that study was 8.7 years. Our policy has been to insert all freestyle valves as aortic roots. Although this is speculative, we believe that implanting a full root might decrease long-term stresses on the cusps, possibly decreasing the rate of cusp degeneration. However, this hypothesis needs to be properly addressed in a randomized study comparing both techniques.

Recent evidence into the pathophysiology of bioprosthetic calcification shows the presence of oxidized low-density lipoproteins and foam cells in explanted aortic valve bioprostheses, suggestive of atherosclerosis-like disease processes. Interestingly, peripheral vascular disease and a family history of coronary artery disease were independent predictors of increased root calcification in this study population, further supporting this hypothesis. The use of statins did not appear to affect the rate of calcification in the present study. However, statin use was neither uniform nor controlled. Their specific role on bioprosthetic calcification needs to be addressed in a separate study. Besides their lipid-lowering effect, the role of statins in reducing inflammation and in immune modulation has been reported, suggesting that rapid initiation of statins after surgery might affect early calcification.

Homograft calcification in this study is associated with an increased rate of valvular dysfunction and structural valve deterioration requiring reoperation. All surgically explanted homografts showed marked calcification of the cusps and the aortic wall and 3 of the 4 patients had calcium scores on EBCT in the third quartiles at each time point. Three-dimensional evaluation of valve calcification, which could be studied by EBCT, might determine the incidence and type of valve failure. All 4 patients undergoing reoperation in this series survived surgery. We believe that appropriate timing and attention to detail during redo surgery renders these operations safe.

Limitations
This study has several limitations. For safety reasons (radiation), each patient did not undergo EBCT scanning at baseline or at each point in the follow-up period. Mixed effect models were used to account for this study design. Patients undergoing >1 scan weigh more in the model prediction. Patients requiring a reoperation did not undergo EBCT scanning immediately before redo surgery, which was dictated by practical considerations. Cases of cusp tear have previously been reported in a large cohort of subcoronary freestyle implants at a median follow-up of 8.7 years. No such cases were observed in this cohort, possibly because of the shorter duration of follow-up. Continued patient surveillance is therefore mandatory to document and address this serious complication. Renal dysfunction is a well-known risk factor for tissue valve calcification. For safety reasons, patients with decreased estimated creatinine clearance were excluded to avoid contrast-associated renal injury. Therefore, the synergistic effect of renal dysfunction on homograft or xenograft calcification could not be evaluated in this study.

Conclusion
In conclusion, aortic root calcification is significantly higher after homograft root replacement than Medtronic freestyle up to 8 years after surgery. This is attributable to an early phase of rapid calcification in the first 3 years after homograft implantation, suggesting a possible role for inflammatory or immune-mediated mechanisms. This was paralleled by an increased incidence of valve dysfunction and reoperation. Predictive factors of increased calcification include atherosclerosis-related risk factors, suggesting a possible role in the pathophysiology of bioprosthetic aortic valve degeneration. Because of the low rate of events, a specific calcium score cut-off point predictive of late reoperations remains to be determined. Further follow-up is required to try predicting long-term homograft failure based on early calcium scores and to assess longer-term freestyle integrity.

Sources of Funding
Dr El-Hamamy is supported by a Fellowship Award from the Canadian Institutes for Health Research (CIHR) and by the Magdi Yacoub Institute (MYI). Dr Stevens is also supported by a Fellowship Award from the Canadian Institutes for Health Research (CIHR).

Disclosures
None.

References


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Circulation. 2009;120:S269-S275
doi: 10.1161/CIRCULATIONAHA.108.843748

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