Vascular Remodeling 1 Year After Cardiac Transplantation

Haiyan Li, MD, Koji Tanaka, MD, Ankush Chhabra, MD, Brandy Oeser, MPH, Jon A. Kobashigawa, MD, and Jonathan M. Tobis, MD

Background: The belief that vascular remodeling and intimal hyperplasia are causes of luminal narrowing in cardiac allograft vasculopathy (CAV) is controversial. This study evaluated the relationship of vascular remodeling and intimal hyperplasia to luminal narrowing 1 year after orthotopic heart transplantation.

Methods: Intravascular ultrasound imaging was performed on 190 cardiac transplant recipients at baseline and again 1 year after transplantation as part of a randomized trial of mycophenolate mofetil (MMF) and azathioprine (Aza). Studies 1 year apart were matched at 625 sites. All sites were classified into positive, non-significant and negative remodeling patterns, depending on a change of ±10% in external elastic membrane area. Of the 190 patients, 99 were randomized to receive MMF, and 91 to receive Aza.

Results: A total of 625 sites were observed. Of these, 52% had no remodeling, 25% exhibited vessel dilation, and 23% had vessel shrinkage in the presence of variable intimal growth (intimal area: 0.73 ± 1.70 mm², p < 0.0001; 1.23 ± 2.02 mm², p < 0.0001; and 0.20 ± 1.40 mm², p = 0.09, respectively). Sixty percent of the lumen loss was due to a decrease in external elastic membrane area and 40% to an increase in intimal area (p = 0.005). Compared with Aza-treated patients, the MMF-treated patients had a lower incidence of vessel shrinkage (17% vs 28%, p = 0.001), and a trend for smaller maximum intimal thickness (0.21 ± 0.25 mm vs 0.29 ± 0.31 mm, p = 0.052).

Conclusions: Positive remodeling is associated with intimal growth, but negative remodeling does not correlate with any specific change in intimal hyperplasia. Constrictive remodeling is more responsible than intimal hyperplasia for the luminal narrowing that occurs. MMF is more efficacious than azathioprine in preventing the development of CAV at 1 year, by reducing the degree and incidence of vessel shrinkage and the progression of intimal hyperplasia. J Heart Lung Transplant 2007;26:56–62. Copyright © 2007 by the International Society for Heart and Lung Transplantation.
either MMF or azathioprine (Aza). The trial was approved by the institutional review board of each participating center, and signed informed consent was obtained from all patients.

**IVUS Imaging Procedure**

Quantitative angiography and IVUS imaging were performed at 1 to 8 weeks and 12 months post-transplantation. After full anti-coagulation with heparin 100 U/kg, an 8F guide catheter was advanced over a guide-wire into the selected coronary artery. Patients received 0.4 mg of sub-lingual nitroglycerin and/or 200 µg of intracoronary nitroglycerin before advancing the IVUS catheter. A 30-MHz ultrasound transducer (4.3 Fr, CVIS, Sunnyvale, CA; 3.5 Fr, Hewlett-Packard, Palo Alto, CA) was inserted into a distal position of the selected vessel where the luminal diameter exceeded 2 mm. A manual slow (>30-second) pullback was performed from the distal position to the proximal coronary artery. The catheter location was recorded with cine-angiography. IVUS images were recorded on s-VHS videotape with voice annotation.

**IVUS Imaging Analysis**

The IVUS tapes were sent to a core laboratory that was blinded to patient treatment. The IVUS images were digitized using ECHOPLAQUE software (version 2.5, Indec Systems, Inc., CA). IVUS landmarks, such as side branches, calcification, pericardium and cardiac veins, were used in matching the sites. Two to four matched sites from the same artery were chosen using side-by-side comparison of the baseline and follow-up images. These sites included the left main, proximal, middle and distal sites of each coronary artery. Frames obtained during the diastolic phase of the cardiac cycle were selected for measurement. The frame with the most severe intimal thickening from each site was identified in the first-year IVUS study after OHT. These selected frames were matched with sites from the baseline IVUS study. Only sites that had clear matching identifiers were chosen for analysis. Luminal and vessel contours were drawn with the planimetry software on each cross-sectional view by manually tracing the border

<table>
<thead>
<tr>
<th>Parameter changes at Year 1</th>
<th>Expansive remodeling</th>
<th>No remodeling</th>
<th>Constrictive remodeling</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 159 sites)</td>
<td>(n = 325 sites)</td>
<td>(n = 141 sites)</td>
<td></td>
</tr>
<tr>
<td>MIT (mm)</td>
<td>0.13 ± 0.25</td>
<td>0.09 ± 0.21</td>
<td>0.04 ± 0.23</td>
</tr>
<tr>
<td>IA (mm²)</td>
<td>1.23 ± 2.03</td>
<td>0.73 ± 1.70</td>
<td>0.20 ± 1.40</td>
</tr>
<tr>
<td>EEM area (mm²)</td>
<td>2.91 ± 2.00</td>
<td>−0.06 ± 0.92</td>
<td>−3.31 ± 2.36</td>
</tr>
<tr>
<td>LA (mm²)</td>
<td>1.67 ± 2.54</td>
<td>−0.80 ± 1.91</td>
<td>−3.51 ± 2.75</td>
</tr>
<tr>
<td>IA/EEM area (%)</td>
<td>4.69 ± 8.88</td>
<td>3.71 ± 8.75</td>
<td>3.66 ± 9.65</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Significance (p-value) assessed 1-way ANOVA followed by multiple comparisons with the Bonferroni post hoc test. MIT, maximum intimal thickness; IA, intimal area; EEM area, external elastic membrane area; LA, lumen area; IA/EEM area, cross-sectional area stenosis.

\*p < 0.001, \*p = 0.009 for the comparison with expansive remodeling.

\*p < 0.001, \*p < 0.05 for the comparison with expansive remodeling.

\*p < 0.001, \*p < 0.05 for the comparison with no remodeling.
between the intima and the lumen, and the boundary between the media and adventitia. At each site, maximum intimal thickness (MIT), intimal area (IA), external elastic membrane area (EEM area) and lumen area (LA) were measured. Percent area stenosis was defined as $(\frac{IA}{EEM\ area}) \times 100\%$.

The reproducibility of measurement in terms of mean interobserver variability was $1.4 \pm 3.8\%$ for LA and $2.7 \pm 3.3\%$ for EEM area. Therefore, a change of $>10\%$ was chosen to represent a measurable difference of $>2$ standard deviations of interobserver variability. Based on the interobserver value, positive remodeling was defined as sites with an increase of $>10\%$ in EEM area from baseline to 12 months; negative remodeling was defined as sites with a decrease of $>10\%$ in EEM area; no significant remodeling was defined as an absolute change of $\leq 10\%$ in EEM area.

**Statistical Analysis**

The mean $\pm$ standard deviation was calculated for all numeric data. Comparisons between baseline and follow-up were determined by paired $t$-test. The data from different groups were compared using an independent $t$-test and 1-way analysis of variance (ANOVA). Differences among categoric variables were assessed using the chi-square or Fisher’s exact test. A linear regression was used to describe the correlation between measured variables. Two-sided $p < 0.05$ was considered statistically significant.

**RESULTS**

**Patient Enrollment**

A total of 190 de novo cardiac transplant recipients (155 men, 35 women, mean age 51.8 $\pm$ 9.4 years) were analyzed. IVUS imaging was performed on all recipients.

![Figure 1. An example of positive remodeling. EEM area: external elastic membrane area.](image1)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal area (mm²)</td>
<td>2.6</td>
<td>14.28</td>
</tr>
<tr>
<td>EEM area (mm²)</td>
<td>18.9</td>
<td>28.0</td>
</tr>
<tr>
<td>Lumen area (mm²)</td>
<td>16.3</td>
<td>13.7</td>
</tr>
</tbody>
</table>

![Figure 2. An example of negative remodeling. EEM area: external elastic membrane area.](image2)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal area (mm²)</td>
<td>1.26</td>
<td>2.13</td>
</tr>
<tr>
<td>EEM area (mm²)</td>
<td>15.95</td>
<td>10.70</td>
</tr>
<tr>
<td>Lumen area (mm²)</td>
<td>14.69</td>
<td>8.57</td>
</tr>
</tbody>
</table>
at 1.4 ± 0.6 months and again at 12.1 ± 0.7 months after OHT. A total of 625 sites from 190 arteries (mean 3.3 sites per artery) were matched from baseline to 12 months. The mean donor age was 28.6 ± 12.7 years (range 11 to 61 years). IVUS studies were performed on 154 left anterior descending arteries, 29 left circumflex arteries and 7 right coronary arteries. Of the 190 patients, 99 were assigned to receive MMF, and 91 to receive Aza. The patients in the two groups had similar demographic characteristics (Table 1).

Serial Changes in IVUS Parameters
By the first year after OHT, the coronary artery lumen area decreased by 5.7% (from 13.59 ± 6.40 mm² at baseline to 12.81 ± 5.90 mm², \( p < 0.0001 \)), but the average EEM area did not change significantly (from 15.47 ± 6.95 mm² at baseline to 15.43 ± 6.93 mm², \( p = 0.7 \)). The intimal area increased by 39.6% compared with baseline (2.61 ± 2.63 mm² vs 1.87 ± 1.92 mm², \( p < 0.0001 \)) and the maximum intimal thickness increased by 33.3% (from 0.27 ± 0.31 mm at baseline to 0.36 ± 0.37 mm, \( p < 0.0001 \)).

Vascular Remodeling Patterns
The 625 sites were classified into three vascular remodeling patterns depending on the change of ±10% for EEM area (Table 2). Of the 625 sites, 325 (52.0%) had no significant remodeling, 159 (25.4%) had vessel enlargement (positive remodeling; Figure 1), and 141 (22.6%) had vessel shrinkage (negative remodeling; Figure 2) in the presence of variable intimal growth. The average EEM area at baseline was smaller at sites with positive remodeling than at sites with negative remodeling (14.10 ± 5.87 mm² vs 17.11 ± 7.38 mm², \( p = 0.001 \)).

Intimal Hyperplasia and Vascular Remodeling
For the 625 sites, the change in EEM area correlated only mildly with the change in intimal area (\( r = 0.21, p < 0.0001 \)). Of the 625 sites, 345 (55.2%) had intimal growth of >10% from baseline to 12 months. The average increase in intimal area was 1.62 ± 1.88 mm², an increase of 97.6% over baseline. Of the 345 sites with intimal growth, 180 (52.2%) did not show significant remodeling, 120 (34.8%) exhibited compensatory vessel enlargement, and 45 (13.0%) had vessel constriction.

Lumen Loss or Gain
For the 625 sites, the change in lumen area correlated most closely with the change in EEM area (\( r = 0.81, p < 0.0001 \); Figure 3A), and mildly with the change in intimal area (\( r = -0.41, p < 0.0001 \); Figure 3B). Of the 625 sites, 227 (36.3%) showed a decrease of >10% in lumen area (lumen loss), 125 (20.0%) had an increase of >10% in lumen area (lumen gain), and 273 (43.7%) had no significant change (absolute percent change in lumen area ≤10%). In addition, 59.5% of lumen loss was due to a decrease in EEM area and 40.5% to an increase in intimal area (\( p = 0.005 \); Table 3). Lumen gain was caused primarily by an increase in EEM area, whereas intimal area did not change significantly.

Discordant Vascular Remodeling Patterns Within the Same Artery
Table 4 describes the relative incidence of remodeling patterns found within each artery. Only 24% (46 of 190) of the arteries had the same remodeling pattern at all sites measured (two to four sites per artery).

MMF and Aza
Of the 625 sites, 319 came from 99 MMF-treated patients and 306 came from 91 Aza-treated patients. At baseline IVUS study, there was no significant difference in MIT, intimal area, EEM area, lumen area or percent area stenosis between the MMF group and the Aza.
At Year 1 the MMF group than in the Aza group (57% vs 46%, incidence of no significant remodeling was higher in degree of vessel enlargement was higher in the MMF group than in the Aza group (17% vs 28%, negative remodeling was lower in the MMF group) 

However, there was no difference in incidence of positive remodeling between the two groups (26% vs 0.006). Compared with Aza-treated patients, the MMF-treated patients had a trend for smaller maximal change of elastic membrane area; LA, lumen area; IA/EEM area, cross-sectional area

The distribution in the two groups was different for three vascular remodeling patterns, defined as a change of 10% for EEM area. The incidence of negative remodeling was lower in the MMF group than in the Aza group (17% vs 28%, negative remodeling was lower in the MMF group than in the Aza group (57% vs 46%, 0.006). However, there was no difference in incidence of positive remodeling between the two groups (26% vs 25%). At those sites with positive remodeling, the degree of vessel enlargement was higher in the MMF group than in the Aza group (ΔEEM area: 3.36 ± 2.30 mm² vs 2.43 ± 1.47 mm², p = 0.003).

### DISCUSSION

This study has shown that 52% of all sites had no significant remodeling, 25% exhibited vessel enlargement, and 23% had vessel shrinkage in the presence of variable intimal thickening in the first year after OHT. Vessel compensatory enlargement is associated with intimal growth, but negative remodeling is not directly linked to any change in intimal hyperplasia at Year 1. Glagov et al. originally found from autopsy studies that human native coronary arteries enlarge in response to atheroma plaque area. Positive remodeling may be a compensatory mechanism in the early development of native coronary artery disease and CAV that prevents lumenal loss. This compensatory remodeling is generally inadequate to compensate for the effects of plaque growth in recipients.

The ability to undergo compensatory vessel enlargement in response to plaque formation is dependent on intact endothelial function. Coronary endothelial dysfunction has been found in the early post-transplantation period before the development of intimal thickening. Coronary endothelial dysfunction is an early marker for the development of intimal thickening and graft atherosclerosis.

The prevalence of epicardial endothelial dysfunction is 30% to 40% in patients during the first year post-OHT and persists at long-term follow-up. If compensatory positive remodeling is influenced by endothelial dysfunction in the first year after transplantation, this may explain why >50% of sites did not enlarge sufficiently in the presence of intimal growth. On the other hand, adventitial inflammation and subsequent fibrosis may inhibit vessel enlargement, and cause vessel shrinkage. Progression to symptomatic CAV is in part due to the lack of vessel compensatory dilation or even shrinkage in the setting of intimal proliferation.

The relative contribution of vascular remodeling and intimal hyperplasia as the cause for luminal narrowing in CAV is controversial. One study demonstrated that lumen loss in transplant coronary artery disease is a biphasic process involving early intimal thickening and late constrictive remodeling. In the present study we...
found that 20% of the sites had lumen enlargement of >10% from baseline, and 36% of the sites had lumen loss of >10% from baseline at Year 1. Lumen enlargement was associated primarily with an increase in EEM area. The contribution to lumen loss was due more to a decrease in EEM area than intimal thickening, which supports the finding by Pethig and colleagues. Thus, in CAV, the change in vessel remodeling is a major factor influencing the change in lumen area at the first year after OHT.

In addition, the discordant remodeling patterns within the same artery seen in 76% of the arteries is in agreement with previous studies. Endothelial function may not be uniformly disturbed after cardiac transplantation. Different vascular remodeling patterns could be observed in the same artery due to variable responses of the local vessel and the interaction of systemic immunologic processes with pre-existing pathology.

The randomized trial that formed the basis of this IVUS core laboratory data showed that MMF, an immunosuppressive agent that inhibits cellular proliferation, was more efficacious than Aza in reducing rejection episodes and improving survival among heart transplant recipients. The present study is an expansion of the IVUS core laboratory data showed that MMF, an immunosuppressive agent that inhibits cellular proliferation, was more efficacious than Aza in reducing rejection episodes and improving survival among heart transplant recipients. The present study is an expansion of the IVUS results from that clinical trial. The IVUS data demonstrate that the MMF-treated patients showed a trend for lower maximal MIT per patient compared with the Aza-treated patients (p-value was borderline, most likely due to small sample size). MMF also decreased the degree and incidence of vessel shrinkage. The mechanism of MMF in decreasing the development of CAV may be due to the inhibitory effects on both lymphocyte proliferation and smooth muscle cell proliferation. In addition, the benefit of MMF may partly contribute to the decrease in systemic inflammatory activity as indicated by reduced levels of highly sensitive C-reactive protein in MMF-treated patients.

Study Limitations

This is not a natural history study of CAV because the patients were treated with different medications. However, all studies of CAV are confounded by the multiple drugs these patients receive. All IVUS images were performed with manual pullback of the IVUS catheter because motorized pullback devices were not available during the study period. This could lead to difficulty in matching sites from the baseline and follow-up studies. However, the only sites chosen were those with physical characteristics that could be identified on both studies. In addition, automated pullback studies do not guarantee correspondence between sites based on the distance from reference markers. This study did not analyze the relation between vascular remodeling and clinical characteristics.

In conclusion, in CAV, the vascular remodeling pattern is the major factor influencing the change in lumen area at Year 1 after OHT. Vascular remodeling is a complex process determined by many factors, and the pathophysiologic mechanisms are not fully understood. In the first year after cardiac transplantation, vascular remodeling occurred in 48% of coronary artery sites and the majority of coronary arteries had discordant vascular remodeling patterns. When the lumen narrows, the contribution from the decrease in EEM area is greater than that of intimal thickening, whereas lumen enlargement results primarily from an increase in EEM area, and not from regression of baseline intima thickening.

Compensatory enlargement (positive remodeling) is associated with intimal growth, and vessel shrinkage (negative remodeling) is not directly linked to any change in intimal hyperplasia. Angiography alone cannot distinguish the mechanism of lumen narrowing and it requires IVUS cross-sectional imaging to clarify these issues. These results support the hypothesis that negative remodeling post-transplantation is due to the inflammatory process of CAV. Mycophenolate mofetil was more efficacious than azathioprine in preventing the development of CAV by reducing the degree and incidence of vessel shrinkage and the progression of intimal hyperplasia in the first year after cardiac transplantation.

The authors express their appreciation to the Hoffmann-La Roche Co. for making the clinical data available.

REFERENCES


