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EXPEDITED REVIEWS

Multicenter Intravascular Ultrasound Validation Study Among Heart Transplant Recipients

Outcomes After Five Years

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OBJECTIVES We sought to assess the validity of first-year intravascular ultrasound (IVUS) data as a surrogate marker for long-term outcome after heart transplantation.

BACKGROUND Cardiac allograft vasculopathy (CAV) is a major impediment to long-term graft survival. Intravascular ultrasound is more sensitive than coronary angiography and detects intimal thickening (early CAV) in the coronary arteries of the donor heart. Single-center studies have suggested first-year IVUS results might be a surrogate marker for long-term outcome.

METHODS First-year IVUS results and subsequent five-year clinical follow-up data were reviewed in 125 heart transplant recipients from five institutions. The IVUS tapes (at baseline and one year) were re-analyzed at a core IVUS laboratory. The change in maximal intimal thickness (MIT) from baseline to one year was recorded for several matched sites in the same coronary artery. Patients were classified into two groups: those with ≥0.5 mm in the MIT in any matched site (group 1) and those with MIT <0.5 mm (group 2).

RESULTS Group 1 patients compared with group 2 patients had a higher incidence of death or graft loss (D/GL, 20.8% vs. 5.9%; $p = 0.007$), had more nonfatal major adverse cardiac events and/or D/GL (45.8% vs. 16.8%; $p = 0.003$), and had more findings of newly occurring angiographic luminal irregularities (65.2% vs. 32.6%, $p = 0.004$).

CONCLUSIONS This multicenter study suggests that progression of intimal thickening ≥0.5 mm in the first year after transplantation appears to be a reliable surrogate marker for subsequent mortality, nonfatal major adverse cardiac events, and development of angiographic CAV through five years after heart transplantation. (J Am Coll Cardiol 2005;45:1532–7) © 2005 by the American College of Cardiology Foundation

Cardiac allograft vasculopathy (CAV) is an accelerated form of intimal hyperplasia that occurs in the coronary arteries of the transplanted heart. The precise mechanisms are unknown, but both immune and nonimmune mechanisms have been implicated. The disease represents the major impediment to long-term graft survival. To date, treatment options have been limited due to the often diffuse nature of the disease, precluding the routine use of percutaneous intervention or coronary artery bypass grafting. Symptomatically, CAV may lead to congestive heart failure, angina (as a result of allograft reinnervation), and sudden death.

The processes of CAV begin in the donor even before transplantation. Perioperative and early postoperative events continue to play a pivotal role in the up-regulation of the immune system, which causes chronic vascular insults. It appears that the events of the first year after transplantation (e.g., ischemia, rejection, and infection) may determine the recipient's immune response to the donor heart and the subsequent development of CAV. As of yet, there is no clear surrogate marker for the development of CAV. However, recent work has suggested that the first-year intravascular ultrasound (IVUS) data might provide this information (1–3).

Intravascular ultrasound is an invasive procedure that detects thickening in the walls of the coronary arteries. Because the IVUS catheter provides a sonar image of intimal and media thickness, IVUS is more sensitive than coronary angiography, which only outlines the lumen with contrast dye. The coronary angiogram may appear normal, whereas IVUS reveals significant amounts of atherosclerosis (or intimal thickening) (1,4). Intravascular ultrasound is performed during the patient's angiogram (four to six weeks after transplantation and again at one year after transplantation). There have been single-center studies (1–3) suggesting that the first-year IVUS results might predict

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Abbreviations and Acronyms

CAD	= coronary artery disease
CAV	= cardiac allograft vasculopathy
CMH	= Cochran-Mantel-Haenszel
CMV	= cytomegalovirus
D/GL	= death and/or graft loss
IA	= intimal area
IVUS	= intravascular ultrasound
MIT	= maximal intimal thickness
NF-MACE	= nonfatal major adverse cardiac events

long-term outcome; however, this has not been established in a large multicenter study. Therefore, this multicenter study examines whether there are IVUS variables at one year after transplantation, which can be used to predict death and/or graft loss (D/GL) and nonfatal major adverse cardiac events (NF-MACE) in the subsequent five years after transplantation.

METHODS

Patients were included in the study if they received a primary heart transplant before December 31, 1997, and survived at least one year. Patients who had two IVUS examinations performed (at four to six weeks after transplantation and at one year after transplantation) from five heart transplant centers with IVUS experience were included in the study. Outcome data during five years were abstracted from medical records. Institutional review board approval was obtained at each participating center.

The IVUS tapes (baseline and one-year follow-up) from each patient were sent to a core laboratory (University of California at Los Angeles) for analysis. The IVUS tapes were digitized, and quantitative ultrasound measurements were made using the Indec computer system (Mountain View, California). Approximately three to five matched cross sections, predominantly in the left anterior descending coronary artery, from baseline to one-year follow-up, were studied. The IVUS cross sections were matched by using identifiable landmarks in the images, such as bifurcations or arterial calcification, or external landmarks, such as coronary veins or pericardium. In addition, the one-year IVUS studies were obtained with an angiographic roadmap of where the initial IVUS study was performed along the length of the vessel. The IVUS system (CVIS Corp., Sunnyvale, California) used was 20 MHz, and a slow, manual pullback was performed at 1 mm/s from the mid-distal portion of the study vessel, where an easily identifiable landmark was visible (i.e., branchpoint). The following items were measured for each patient: maximal intimal thickness (MIT), intimal area (IA), and vessel area, defined as the border of the external elastic membrane. Percent area stenosis was then calculated as IA/external elastic membrane. The IVUS data were reviewed to determine the delta change (comparing baseline to one year) in intimal thickness. This method accounted for preexisting donor coronary artery disease (CAD) in the dataset.

The patients were classified into two groups according to their MIT (increase from baseline to one year): group 1 had vasculopathy (largest MIT change $\geq$0.5 mm); and group 2 had no vasculopathy (largest MIT change $<$0.5 mm).

The medical records of these patients were reviewed to assess the five-year outcome. This included graft survival, NF-MACE (defined as acute myocardial infarction, congestive heart failure, need for percutaneous cardiac intervention, coronary artery bypass grafting, cardiac defibrillator placement, cerebral vascular accident, peripheral vascular disease), and angiographic CAD (any new luminal irregularity and new stenosis $\geq$50%).

Statistical analysis. In this retrospective exploratory study, the study sample were all patients at the participating centers who had the required IVUS data and five-year follow-up outcome data.

The main predictor variable chosen was the largest change in MIT from baseline to one year. The progression of intimal thickening of $\geq$0.5 mm was the primary focus of the investigation and was used to define the presence of vasculopathy. Other predictor variables of interest are the largest change in IA and the largest change in percentage area stenosis. This study examines whether these predictor variables at one year after transplantation can be used to predict NF-MACE or D/GL in the first five years after transplantation.

Time to inclusion of patients with and without vasculopathy into each group was compared using Kaplan-Meier survival methods and the log-rank test. Percentages of patients in each group with and without vasculopathy were compared using the Cochran-Mantel-Haenszel (CMH) general association test, stratified by center. The effects of vasculopathy, correcting for possible covariates, were examined using Cox regression models. Summary statistics of data on demographics, IVUS data, outcome data, laboratory values, and treatment were calculated and compared using the Fisher exact test (categorical data), the CMH test (categorical variables stratified by center), and the van Elteren test (continuous variables stratified by center) (5).

RESULTS

A total of 125 primary heart transplant recipients (University of California at Los Angeles [n = 49], Cleveland Clinic Foundation [n = 44], Stanford University [n = 11], Ochsner Clinic Foundation [n = 6], and Emory University [n = 15]) transplanted before December 31, 1997, who had survived at least one year and had two IVUS examinations performed (at baseline [four to six weeks after transplantation] and one year after transplantation) were reviewed. There were 24 patients with MIT progression $\geq$0.5 mm (group 1) and 101 patients with MIT progression $<$0.5 mm (group 2). The characteristics of the two study groups were comparable, except for donor age, which was higher in

Table 1. Patient Demographics (n = 125)

Characteristics	Group 2 (MIT change <0.5 mm) (N = 101)	Group 1 (MIT change ≥0.5 mm) (N = 24)
Median age in yrs (range)		
Patient	54 (25–71)	56 (33–66)
Donor*	28 (13–67)	38 (13–62)
Male gender	82 (81%)	21 (88%)
Patients with most recent PRA >10%	6 (7%)	1 (5%)
Patients with CMV mismatch (D+/R−)	13 (13%)	2 (8%)
Pre-transplant CAD	50 (50%)	17 (71%)
Pre-transplant LVAD	12 (12%)	5 (21%)
Mean (SD)		
Cold ischemia time in h	2.7 (0.1)	2.7 (0.8)
First-year triglycerides (mg/dl)†	168 (85)	257 (177)

*p = 0.028, Cochran-Mantel-Haenszel test, stratified by centers. †p = 0.025, van Elteren test, stratified by centers.
CAD = coronary artery disease; CMV = cytomegalovirus; D+ = donor positive; LVAD = left ventricular assist device; MIT = maximal intimal thickness; PRA = panel reactive antibodies; R− = recipient negative; SD = standard deviation.

group 1 (37 ± 12 years vs. 30 ± 13 years; p = 0.028) (Table 1). Between group 1 and group 2 patients, there was comparable use of cyclosporine immunosuppression (100% vs. 95%), azathioprine immunosuppression (96% vs. 96%), cytolytic induction (8% vs. 27%), and use of statins (25% vs. 41%). Total cholesterol, low- and high-density lipoprotein cholesterol levels, as well as the incidence of diabetes, were similar between groups. However, triglyceride levels were significantly higher in group 1 versus group 2 patients (168 ± 85 mg/dl vs. 257 ± 177 mg/dl; p = 0.025).

Group 1 compared with group 2 patients had a 3.5-fold higher incidence of D/GL (20.8% vs. 5.9%, p = 0.025 by the log-rank test, p = 0.007 by the CMH test) (Fig. 1). The causes of death in group 1 compared with group 2 patients were sudden death (4% vs. 1%), congestive heart failure (0% vs. 1%), rejection (0% vs. 2%), malignancy (4% vs. 0%), and other (13% vs. 1%).

There were more NF-MACE and/or D/GL in group 1 compared with group 2 patients (45.8% vs. 16.8%, p = 0.001 by the log-rank test, p = 0.003 by the CMH test) (Fig. 2). The incidence of NF-MACE alone was also higher in group 1 compared with group 2 patients (38% vs. 12%). The causes of NF-MACE in group 1 compared with group 2 patients were congestive heart failure (13% vs. 2%, p =

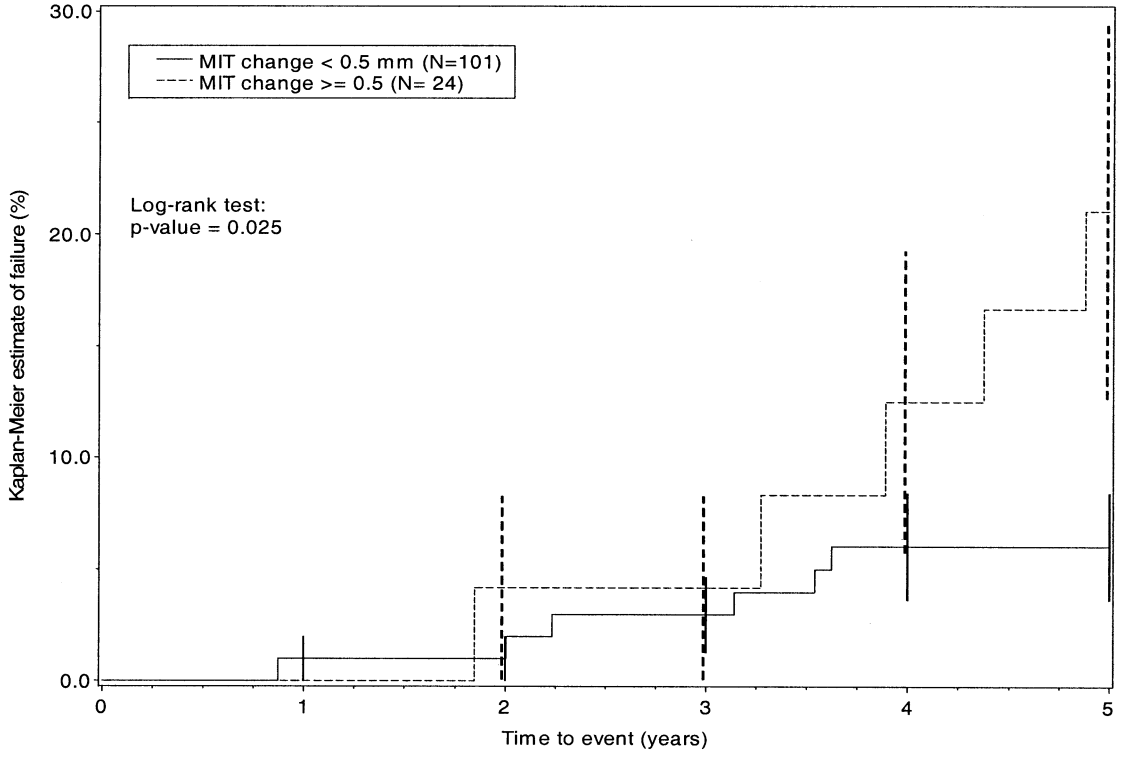

Figure 1. Kaplan-Meier curve of death and/or graft loss by maximal intimal thickness (MIT) classification. Group 1 = MIT change ≥0.5 mm; group 2 = MIT change <0.5 mm. **Vertical bars** = ± SE.

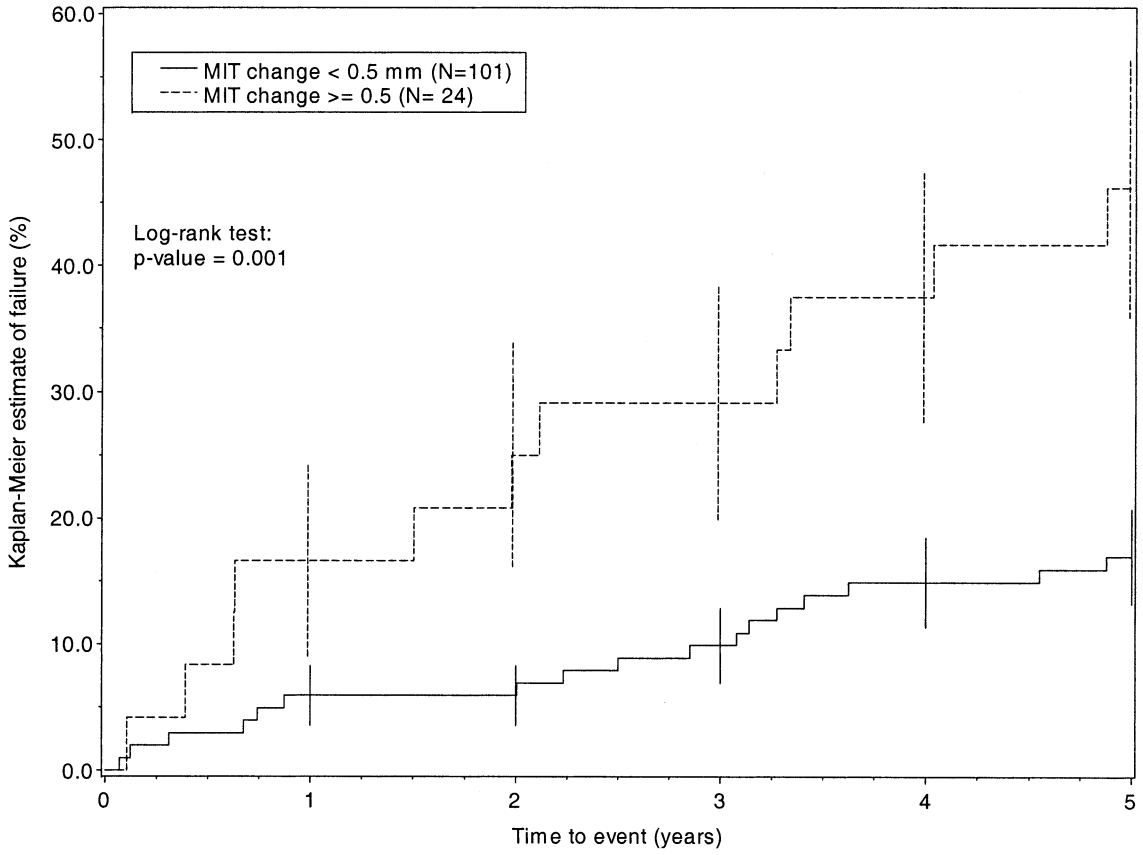

Figure 2. Kaplan-Meier curve of nonfatal major adverse cardiac events and death and/or graft loss by maximal intimal thickness (MIT) classification. Group 1 = MIT change ≥0.5 mm; group 2 = MIT change <0.5 mm. **Vertical bars** = ± SE.

0.048 by the Fisher exact test), percutaneous cardiac intervention (17% vs. 4%, p = 0.044 by the Fisher exact test), defibrillator (4% vs. 2%), cerebral vascular accident (4% vs. 1%), and peripheral vascular disease (13% vs. 3%).

Within the five-year follow-up period, there were more findings of newly occurring angiographic luminal irregularities in group 1 compared with group 2 patients (65.2% vs. 32.6%, p = 0.004). However, there was no significant difference between groups for new coronary lesion ≥50% stenosis (21% vs. 14%).

Other first-year IVUS measurements, including change in maximal IA (vasculopathy group ≥3.5 mm^2) and change in percent area stenosis (vasculopathy group ≥20%) were investigated as surrogate markers of outcome. Patients with a first-year change in maximal IA ≥3.5 mm^2 or a change in percent area stenosis ≥20% had a significantly higher five-year incidence of D/GL, NF-MACE and/or D/GL, and new angiographic luminal irregularities (Table 2).

Covariates that could influence outcome, including patient's age, gender, weight, cytomegalovirus (CMV) serol-

Table 2. First-Year Results for Baseline to 1 Year of IVUS Measurements and Subsequent 5-Year Outcome

IVUS Measurements	Death or Graft Loss	Nonfatal MACE and/or Death or Graft Loss	Any New Luminal Irregularity
First-year change in maximal intimal thickness from baseline			
<0.5 mm (group 2, N = 101)	6 (6%)	17 (17%)	31/95 (33%)
≥0.5 mm (group 1, N = 24)	5 (21%)	11 (46%)	15/23 (65%)
p value*	0.007	0.003	0.004
Log-rank p value (Survival curve)	0.025	0.001	—
First-year change in maximal intimal area from baseline			
<3.5 mm^2 (N = 107)	6 (6%)	20 (19%)	34/101 (34%)
≥3.5 mm^2 (N = 18)	5 (28%)	8 (44%)	12/17 (71%)
p value*	<0.001	0.023	0.011
First-year change in percent area of stenosis from baseline			
<20% (N = 108)	5 (5%)	18 (17%)	33/102 (32%)
≥20% (N = 17)	6 (35%)	10 (59%)	13/16 (81%)
p value*	<0.001	<0.001	<0.001

Note: the reduced N for new luminal irregularities are because some had baseline irregularities. *p value, Cochran-Mantel-Haenszel test stratified by center.

ogy, cold ischemic time, lipid levels, pretransplant CAD, donor age, and center effects, were examined, and only donor age, patient weight, and CMV serology were significant when added to the model in the presence of vasculopathy. When corrected for any covariates, vasculopathy remained a highly significant factor for D/GL, NF-MACE and/or D/GL, and new angiographic luminal irregularities (patients with change in MIT ≥0.5 mm having a two- to three-fold higher relative risk).

There was no difference in group 1 compared with group 2 patients in the percentage of patients experiencing any treated rejection (75.0% vs. 75.2%; $p = 0.916$) and acute rejection with ISHLT grade ≥3A or with hemodynamic compromise (70.8% vs. 63.4%; $p = 0.544$).

DISCUSSION

This multicenter IVUS study validates the use of first-year IVUS data as a surrogate marker for poor outcomes after heart transplantation. It appears that the events that occur in the donor heart in the first post-transplant year influence the recipient's immune response toward the donor heart. The more insults that occur to the donor coronary vasculature, especially early after transplantation, such as that which occurs with donor explosive brain death, recurrent low-grade cellular rejections (high biopsy score), donor specific antibody production (humoral rejection), and CMV infection, the greater the likelihood of developing CAV and subsequent decreased survival (6–10). The rapid progression in first-year intimal thickening as detected by IVUS appears to represent the cumulative effects of these adverse events that ultimately lead to a poor clinical outcomes.

Conventional risk factors, such as hypercholesterolemia, hypertriglyceridemia, and diabetes, have been reported to be associated with the development of CAV (11–13). The results of this study suggest that only higher one-year triglyceride levels were associated with greater first-year intimal thickening. From the patient demographics, it appears that older donors are associated with the development of greater intimal thickening in the first year after transplantation (and subsequent CAV), which has also been previously reported (14). This may possibly be due to preexisting intimal thickening and more endothelial cell dysfunction in the coronary arteries of the older donor heart, which might lead to subsequent poor outcomes.

Intravascular ultrasound has been recognized as a sensitive tool to assess the anatomy of the epicardial coronary arteries, including intimal and adventitial wall thickness. The procedure is performed at the time of the routinely scheduled angiogram and has been demonstrated to be safe and have reproducible findings (15–17). An early study performed within six to eight weeks after transplantation allows one to assess donor-transmitted, or "baseline," atherosclerosis. Serial follow-up imaging with careful site matching at one year provides important information on the development of CAV. It has been reported that the first-year IVUS results render the greatest amount of intimal thickening compared with the other early years after transplantation (18).

In CAV, MIT, defined as the greatest distance from the intimal leading edge to the external elastic membrane, has been shown to be a clinically useful measurement because of its high reproducibility and reported use in predicting outcome in transplant recipients. The threshold for an abnormal MIT in subjects with no clinical or angiographic evidence of significant CAD has been assessed by IVUS. Tuzcu et al. (19) performed IVUS in 262 heart transplant recipients (mean age 33.4 ± 13.2 years) at 30.9 ± 13.2 days after transplantation to assess coronary arteries in young subjects (donor hearts). In this study, extensive ultrasound imaging was performed in all three coronary arteries. From many sites that were analyzed for a given patient, the site with the smallest intimal thickness was taken as the representative of normal. All of the 262 subjects had at least one site with an intimal thickness <0.5 mm. Thus, 0.5 mm thickness was accepted as a threshold for the definition of atherosclerosis for all ages.

One of the first studies to report an association between outcome and IVUS data came from Mehra et al. (2) at the Ochsner Clinic. In this report of 74 patients, those with severe intimal thickening (>0.5 mm) had more events (death, myocardial infarction, and retransplantation), with approximately four years of follow-up. Rickenbacher et al. (3) reported an increased cardiac event rate in 145 patients with a mean intimal thickening of >0.3 mm. This study was a cross-sectional design performing IVUS on patients at 1 to 10 years after transplantation. During a mean follow-up time of 48 months, patients with a mean intimal thickness of >0.3 mm had significantly worse four-year overall survival (73% vs. 96%, $p = 0.005$) and cardiac survival (79% vs. 96%, $p = 0.005$). A mean intimal thickness by IVUS of >0.3 mm was associated with an inferior clinical outcome, regardless of the presence of angiographic CAV, and predicted the development of subsequent angiographic CAV. Both of these studies, however, did not have a baseline IVUS procedure performed early after transplantation, and therefore, preexisting donor disease may have been present.

One IVUS study assessed the change in intimal thickening from baseline to one year and compared these findings with long-term outcome. Kapadia et al. (1) reported the impact of rapidly progressive intimal thickening (>0.5 mm increase in intimal thickening) in the first year of transplantation in 100 transplant recipients. In 43 months of mean follow-up, patients with first-year rapidly progressive intimal thickening had more subsequent events (death, myocardial infarction, and heart failure) compared with patients with no evidence of rapidly progressive intimal thickening (25% vs. 11%). The results of the current multicenter study are consistent with this previous report. In addition, the current study correlates early intimal thickening to the subsequent development of angiographically detected CAV (any luminal irregularity).

The current study demonstrates consistency in other IVUS parameter data results. Not only was MIT predictive of poor outcome, but also the first-year change in maximal IA and the change in percent area stenosis. This is the first report of these latter two IVUS parameters being predictive of subsequent poor outcome after heart transplantation.

Conclusions. The results of this multicenter study suggest that progression of maximal intimal thickening ≥0.5 mm in the first year (change from baseline to one year) after transplantation appears to be a reliable surrogate marker for subsequent mortality, NF-MACE, and the development of angiographic CAV up to five years after heart transplantation. These data demonstrate the need to focus on new strategies during the first year after transplantation to alter CAV disease progression, as well as the need for IVUS to potentially become more of a standard in the field.

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