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Association of Endothelin-1 with Accelerated Cardiac Allograft Vasculopathy and Late Mortality Following Heart Transplantation

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Abstract

Background: Endothelin-1 (ET-1) has been implicated in the development of post-heart transplantation (HT) cardiac allograft vasculopathy (CAV), but has not been well-studied in humans.

Methods and Results: In 90 HT patients, plasma ET-1 was measured within 8 weeks of HT (baseline) via a competitive enzyme-linked immunosorbent assay. 3D volumetric intravascular ultrasound of the left anterior descending artery was performed at baseline and at 1 year. Accelerated CAV (lumen volume loss) was defined using the 75th percentile as a cutoff. Patients were followed beyond the first year post-HT for late death or re-transplantation. A receiver operative characteristic curve demonstrated that a baseline ET-1 concentration of 1.75 pg/mL provided the best accuracy for diagnosis of accelerated CAV at 1 year [area under the curve=0.69 (0.57-0.82), p=0.007]. In multivariate logistic regression, a higher baseline ET-1 concentration was independently associated with accelerated CAV [odds ratio (OR)=2.13, 95% confidence interval (CI): 1.15-3.94; p=0.01]; this relationship persisted when ET-1 was dichotomized at 1.75 pg/mL (OR=4.88, 95% CI: 1.69-14.10; p=0.003). Eighteen deaths occurred during a median follow-up period of 3.99 (2.51-9.95) years. Treated as a continuous variable, baseline ET-1 was not associated with late mortality in multivariate Cox regression [hazard ratio (HR)=1.22, 95% CI: 0.72-2.05; p=0.44]. However, ET-1 >1.75 pg/mL conferred a significantly lower cumulative event-free survival on Kaplan-Meier analysis (p=0.047), and was independently associated with late mortality (HR=2.94, 95% CI: 1.12-7.72; p=0.02).

Conclusions: Elevated ET-1 early after HT is an independent predictor of accelerated CAV and late mortality, suggesting that ET-1 has durable prognostic value in the HT arena.

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Keywords

Endothelin-1; Heart transplantation; Cardiac allograft vasculopathy; Mortality

Introduction

Fifty years after the first human heart transplant, cardiac allograft vasculopathy (CAV) remains the nemesis of recipient long-term survival (HT). (1,2) CAV is a complex disease of the graft coronary arteries characterized by diffuse intimal hyperplasia and negative remodeling (i.e. vessel shrinkage due to increased medial tone and adventitial fibrosis), dual processes that together lead to accelerated luminal obstruction of the entire coronary tree. (3,4) Despite advances over the past few decades to combat CAV, including the advent of statins and robust immunosuppressive agents, current international registry data demonstrate minimal improvement in its 5-year incidence and in patient survival over this time period (32% to 29% and 71% to 76%, respectively). (2) These sobering data highlight that the clinical care of HT recipients in the contemporary era is limited by the lack of effective treatment options to prevent and/or retard the development of CAV. There is a critical need to better ascertain the mechanisms of CAV in order to generate new therapies and improve outcomes.

Endothelin-1 (ET-1) is an important molecular regulator of vascular integrity that exerts potent vasoconstrictive, mitogenic, and pro-inflammatory effects throughout the vessel wall. (5,6) A series of seminal animal studies have provided robust causal evidence for ET-1 in the pathogenesis of CAV by elegantly showing that greater production of ET-1 leads to more CAV and that pharmacologically reducing ET-1 bioactivity markedly slowed disease progression. (7–9) Observational human studies have reported consistent findings, but are limited by small sample sizes, failure to evaluate vessel remodeling, less sensitive modalities of assessing the vessel wall, and lack of long-term clinical outcome data. (10–12) In the present study, we sought to address these limitations by investigating the association of ET-1 with 1) early changes in coronary architecture using contemporary 3D volumetric intravascular ultrasound (IVUS), and 2) clinical outcomes (late death or re-transplantation) in a large HT cohort.

Materials and Methods

Study Population.

This study included HT patients from 2 prospective NIH-sponsored trials at Stanford University from January 2002 to March 2014. The first trial examined the effect of cytomegalovirus on the development of CAV in 112 HT patients (1 PO1-A150153), and the second randomized placebo-controlled trial investigated the role of ramipril, an angiotensin converting enzyme inhibitor, on the progression of CAV in 66 HT patients (5 R01 HL093475-02). Of note, in the latter trial, treatment with ramipril did not impact the development of CAV.(13) Patients were included in the current study if they had 1) undergone baseline (4-8 weeks post-HT) and 1-year IVUS studies, and 2) adequate banked blood samples from their baseline visit to the cardiac catheterization laboratory available for

measuring plasma ET-1 concentrations. Although there were no exclusion criteria for the present study, patients with severe medical comorbidities or renal insufficiency did not undergo coronary angiography/IVUS in the original trials. Patients were followed beyond the first year after HT for major adverse events (late death or re-transplantation). All patients provided informed consent for the original parent trials and the study protocols were approved by the Stanford Institutional Review Board on Human Subjects Research.

Post-Transplant Clinical Management.

All HT recipients received the following standard pharmacologic regimen: 1) induction immunosuppressive therapy with daclizumab or antithymocyte globulin, 2) corticosteroids tapered over 8 months in the absence of acute rejection, 3) maintenance immunosuppression consisting of a calcineurin inhibitor (tacrolimus or cyclosporine) and a cell-cycle inhibitor (mycophenolate mofetil), 4) co-trimoxazole for pneumocystis jiroveci prophylaxis, 5) valganciclovir for cytomegalovirus prophylaxis in the event of seropositive donor or recipient status, and 6) aspirin and a statin as tolerated for prevention of CAV. The proliferation signal inhibitor sirolimus was given upfront to all post-transplant patients within the first week after HT at Stanford during the initial enrollment of the first trial (1 PO1-A150153) because of its reported association with lower incidence of early CAV. (14) However, this practice was halted in January 2004 due to increased rates of wound dehiscence, reserving sirolimus only for use in cases of accelerated CAV. Patients were monitored for acute rejection by routine surveillance endomyocardial biopsies for the first 6 months, and Allomap® peripheral gene expression testing thereafter in the absence of significant rejection, which was defined as acute cellular rejection 2R.

ET-1 Measurements.

At the baseline (4-8 weeks post-HT) visit to the cardiac catheterization laboratory, blood for ET-1 analysis was drawn after arterial access was obtained for diagnostic coronary angiography and IVUS. These blood specimens were initially centrifuged at 4°C and subsequently stored at -80°C. Plasma concentrations of ET-1 were assayed using a previously described Quantitative Sandwich Enzyme Linked Immunosorbent Assay (ELISA) (R&D Systems, Minneapolis, MN).

IVUS Imaging Protocol.

Following diagnostic coronary angiography, unfractionated heparin (50–70 U/kg) was administered intravenously and nitroglycerin (200 µg) was administered via bolus injection through a 6F guide catheter engaged in the left main coronary artery. Next, a 0.014-inch guide wire was positioned in the distal left anterior descending artery (LAD), after which a 40-MHz IVUS catheter (Galaxy with Atlantis SR Pro or OptiCross with iLab, Boston Scientific Corp., Marlborough, MA) was advanced over the wire to the mid to distal LAD. An automated pullback of the catheter was performed at 0.5 mm/s and images of the first 50 mm of the LAD were recorded and analyzed offline. (15)

3D Volumetric IVUS Analysis.

IVUS measurements were carried out in a blinded fashion by the Stanford Cardiovascular Core Analysis Laboratory using a validated quantitative IVUS analysis system (echoPlaque, Indec Systems, Santa Clara, California). Lumen, intimal, and vessel volumes were calculated using the Simpson method and indexed as volume per length analyzed (mm^3/mm), with lumen volume loss (i.e. CAV) equaling intimal volume gain plus vessel volume loss. (16) There are no established volumetric IVUS criteria for accelerated CAV, but previous studies using conventional 2D IVUS reported 20-30% rates of accelerated disease at 1-year. (17,18) Thus, we chose the 75th percentile of lumen volume loss as the cutoff to define accelerated CAV at 1-year post-HT in our cohort. We used the 75th percentile to similarly define the components of accelerated CAV—intimal hyperplasia (intimal volume gain) and negative remodeling (vessel volume loss).

Statistical Analysis.

Data are expressed as frequency (percentage) or mean $\pm$ standard deviation. Non-parametric Wilcoxon Rank-Sum or Rank-Sign tests were used to assess for differences between groups of variables, as appropriate. A receiver-operating characteristic (ROC) curve was generated to determine the baseline ET-1 concentration that provided the best diagnostic accuracy in identifying patients with accelerated CAV. ET-1 and relevant recipient and donor demographic, cardiovascular, and transplant-related factors were tested for their ability to predict the previously described IVUS endpoints using univariate logistic regression. As described earlier, ramipril use was not included as a candidate risk factor since prior data demonstrated that it does not affect the development CAV. (13) Risk factors with univariate p-value <0.10 were included in multivariate forward stepwise logistic regression models to determine independent predictors. Time-to-event data were analyzed using Kaplan-Meier curves stratified by ET-1. Cox proportional hazards regression models (multivariate models included risk factors with p-value <0.10 in univariate analysis) were constructed to identify those independently associated with major late adverse events. Logistic and Cox regression data are presented as odds ratios (OR) and hazard ratios (HR) with 95% confidence intervals (CI), respectively. Statistical analyses were performed with the SPSS system, version 21 (SPSS Inc., Chicago, IL). A p-value <0.05 was considered statistically significant.

Results

Baseline Clinical Characteristics.

A total of 178 HT patients were enrolled in the original 2 clinical trials at Stanford, 90 of whom met the previously described inclusion criteria and were included in the current study. Eighty-eight patients were excluded for the following reasons: 52 did not undergo baseline IVUS investigation, 23 did not undergo 1-year IVUS investigation, 7 had IVUS images that were unanalyzable, and 6 had inadequate blood samples (i.e. clotted due to improper processing) for ET-1 testing. Sirolimus use was significantly lower among those included in the study compared to the excluded cohort (19% vs. 52%, $p<0.001$); otherwise there were no significant differences in baseline clinical characteristics between the groups (Supplemental Table 1). The mean age was 49.2 ± 15.3 years, 71% were men, and 13% had type 2 diabetes mellitus (T2DM). At 1-year post-HT, 92% of patients were tolerating statins, 32% had one

or more episodes of significant acute rejection, and 19% were treated with sirolimus (Table 1). Of note, 94% of the patients who received sirolimus had it given upfront per standard protocol at Stanford in 2002-2003.

Volumetric IVUS and ET-1 Data.

Overall, from baseline to 1-year post-HT, both lumen volume ($12.66 \pm 3.32 \text{ mm}^3/\text{mm}$ to $10.97 \pm 3.34 \text{ mm}^3/\text{mm}$, $p < 0.0001$) and vessel volume ($15.29 \pm 4.01 \text{ mm}^3/\text{mm}$ to $14.09 \pm 4.24 \text{ mm}^3/\text{mm}$, $p < 0.0001$) decreased significantly, while intimal volume ($2.66 \pm 1.37 \text{ mm}^3/\text{mm}$ to $3.12 \pm 1.73 \text{ mm}^3/\text{mm}$, $p < 0.0001$) increased significantly. Seventy-seven (86%) patients demonstrated CAV as defined as any lumen volume loss. As described previously, we used the 75th percentile to establish the following cutoffs: accelerated CAV (lumen volume loss $> 2.93 \text{ mm}^3/\text{mm}$), accelerated negative remodeling (vessel volume loss $< 2.50 \text{ mm}^3/\text{mm}$), and accelerated intimal hyperplasia (intimal volume gain $> 0.91 \text{ mm}^3/\text{mm}$). At 1-year post-HT, patients who had developed accelerated CAV and accelerated negative remodeling had significantly higher baseline ET-1 levels than patients without accelerated disease, while there was no difference in ET-1 levels between patients with and without accelerated intimal hyperplasia (Figure 1). An ROC curve indicated that a baseline ET-1 concentration of 1.75 pg/mL provided the best accuracy for diagnosis of accelerated CAV at 1 year (Figure 2). The area under the ROC curve was 0.69 (0.57-0.82), $p = 0.007$, and the threshold of 1.75 pg/mL provided 73% sensitivity, 68% specificity, 42% positive predictive value, and 88% negative predictive value. The distribution of low versus high baseline ET-1 levels plotted against lumen volume loss highlights these test characteristics (Figure 3).

The baseline clinical characteristics were similar between the low and high ET-1 cohorts except for a higher rate of sirolimus use among patients with high baseline ET-1 (30% vs. 11%, $p = 0.03$) (Table 1). Specifically among patients with elevated baseline ET-1 levels, sirolimus use was not associated with significantly lower baseline ET-1 levels ($2.52 \pm 0.76 \text{ pg/mL}$ vs. $2.57 \pm 0.69 \text{ pg/mL}$). In addition, sirolimus use among the high ET-1 cohort did not result in lower rates of lumen or vessel volume loss at 1-year post-HT, though there was a trend toward reduced intimal volume gain ($2.92 \pm 38.52 \text{ mm}^3/\text{mm}$ vs. $21.57 \pm 41.10 \text{ mm}^3/\text{mm}$, $p = 0.065$) (Supplemental Table 2).

ET-1 and Accelerated CAV. In univariable logistic regression analyses, elevated baseline ET-1, recipient sex (male), T2DM, donor sex (male), and statin use at 1 year were associated with accelerated CAV ($p < 0.10$) and included in the multivariable model. Following adjustment, elevated baseline ET-1 (OR=4.88, 95% CI: 1.69-14.10; $p = 0.003$) and statin use at 1 year (OR=2.13, 95% CI: 1.15-3.94; $p = 0.01$) remained significantly associated with accelerated CAV (Table 2). The predictive ability of baseline ET-1 persisted when dichotomized at 1.75 pg/mL (OR=0.17, 95% CI: 0.03-0.95; $p = 0.04$). Of note, higher baseline ET-1 $> 1.75 \text{ pg/mL}$ was associated with accelerated negative remodeling in univariable analysis (OR 2.64, 95% CI: 0.99-7.08, $p = 0.05$), but this association lost statistical significance after multivariable adjustment (OR 2.63, 95% CI: 0.94-7.34, $p = 0.07$) (Supplemental Table 3).

ET-1 and Clinical Outcomes.

Major adverse events (18 late deaths, 0 re-transplants) occurred 20% of patients over a median follow-up period of 4.0 (2.5-10.0) years. These events were all deaths. Patients with baseline ET-1 >1.75 pg/mL had a significantly lower cumulative event-free survival rate compared to those with baseline ET-1 ≤1.75 pg/mL (log-rank $p=0.047$) (Figure 4). In univariate Cox regression analysis, higher baseline ET-1 (treated as a continuous variable) was not associated with the major adverse event endpoint (HR=1.22, 95% CI: 0.72-2.05; $p=0.44$), but ET-1 >1.75 pg/mL, age, hypertension, and significant rejection during the first year post-HT were associated with the major adverse event endpoint ($p<0.10$) and included in multivariate analysis. Following adjustment, baseline ET-1 >1.75 pg/mL (HR=2.94, 95% CI: 1.12-7.72; $p=0.02$) and significant rejection during the first year post-HT (HR=3.35, 95% CI: 1.24-9.04; $p=0.02$) remained independently associated (Table 3). Of note, baseline ET-1 and significant rejection were not significantly correlated ($r=0.08$; $p=0.44$).

Discussion

The principal findings of this retrospective study are as follows: 1) an ET-1 concentration of 1.75 pg/mL soon after HT confers the best accuracy for diagnosis of accelerated CAV at 1-year post-HT, 2) higher plasma concentrations of baseline ET-1 are independently associated with accelerated CAV as assessed by 3D volumetric IVUS and appear to be associated with accelerated negative remodeling, and 3) baseline ET-1 levels above the 1.75 pg/mL threshold are independently associated with late mortality in a HT population. These data imply that baseline ET-1 may have prognostic value and potential to be a viable biomarker for non-invasively identifying HT patients at risk for accelerated CAV.

Prior Data Support ET-1 as a CAV Mediator.

A series of classic animal studies using rat models of HT established the biological plausibility of ET-1 in the pathogenesis of CAV. Okada et al. observed strong ET-1 immunoreactivity throughout the entire coronary arterial wall in cases of severe CAV, and found that orally-administered bosentan, a non-selective endothelin receptor antagonist, significantly attenuated disease progression. (7) Simonson et al. conducted similar studies using phosphoramidon, an ET-1 converting enzyme inhibitor, and showed that it significantly reduced smooth muscle cell-derived intimal thickening, medial tone, adventitial fibrosis, and macrophage infiltration of the coronary vasculature, leading to significantly less CAV and improved survival. (8,9) Collectively, these animal data suggest that ET-1 exerts effects throughout the graft coronary vessel wall, and that reducing its bioactivity slows disease progression.

On the basis of this foundational animal work, several groups have studied ET-1 in HT patients. Ferri et al. demonstrated that ET-1 expression on endomyocardial biopsy at 3 months post-HT predicted the development of CAV on angiography at 2 years in 47 patients. Additionally, they observed that the ET-1 positive cohort tended to have worse survival over a mean follow-up period of 2.4 ± 0.3 years (log-rank $p=0.059$, hazard ratio not reported). (10) Larose et al. then measured angiographic epicardial dilation after intracoronary delivery of a selective endothelin receptor antagonist in 18 HT patients (mean 6 years post-HT) and found

that those with advanced CAV (defined as $\geq 15\%$ diameter stenosis) had a significantly greater vasomotor response compared to those without advanced disease, thereby providing the first mechanistic data in humans supporting a causal link between ET-1 and CAV. (11) More recently, Starling et al. measured plasma ET-1 and performed 2D IVUS within 2 months and at 1-year post-HT in 106 patients. They reported that changes in plasma ET-1 concentration but not baseline ET-1 (as a continuous variable) were associated with accelerated CAV (defined as change in maximal intimal thickness ≥ 0.5 mm). (12) Overall, the human data to date are consistent with the previously described animal data and support ET-1 as a mediator of CAV, but important limitations should be considered. First, the studies were primarily small and cross-sectional in design. Second, they utilized less sensitive techniques—coronary angiography or 2D IVUS—to examine coronary architecture, whereas contemporary 3D volumetric IVUS allows for greater accuracy in detecting smaller changes in the vessel wall. Third, the analyses focused solely on intimal thickening and failed to assess negative remodeling, which is an integral component of CAV. Lastly, short follow-up periods precluded evaluation of longer-term clinical outcome data.

ET-1, CAV, and Negative Remodeling.

In the current study, we built on this prior work and addressed several of the limitations by examining the association of ET-1 with 1) accelerated CAV and its components—intimal hyperplasia and negative remodeling—as measured by volumetric IVUS, and 2) hard outcomes (late death or re-transplantation) in a cohort of 90 HT recipients. We found that baseline ET-1 was independently associated with accelerated CAV at 1-year post-HT and that this association was primarily driven by negative remodeling as opposed to intimal hyperplasia. Specifically, patients with accelerated negative remodeling but not accelerated intimal hyperplasia had significantly elevated baseline ET-1 levels, and the association between baseline ET-1 >1.75 pg/mL and accelerated negative remodeling trended toward statistical significance. In addition, among our entire cohort, approximately 70% of lumen volume loss was attributable to negative remodeling and only 30% to intimal hyperplasia. Although ET-1 exerts effects in each layer of the vessel wall, one possible explanation for these findings is that the major shift in immunosuppressive regimens and ubiquitous use of statins over the past few decades have markedly reduced plaque growth. For instance, in the landmark study by Kobashigawa et al. which included 125 HT patients transplanted in 1997 or earlier, cyclosporine was the backbone of immunosuppressive regimens and statin use was 38%. (17) In contrast, our cohort (transplanted from 2002-2014) was on a tacrolimus-based regimen and 92% took statins. Our data only add to the growing body of literature highlighting the importance of negative modeling in the development of CAV. (16,19,20)

Future Directions.

Further invasive studies in the post-HT population are required to build on our work and further elucidate the role of ET-1 in the development of accelerated CAV. For example, exploring the association of ET-1 with indices of epicardial and microvascular coronary physiology would complement our current anatomical findings with important functional data. Moreover, examining the effect of oral endothelin receptor antagonism on vasomotor tone in patients with and without accelerated CAV would provide mechanistic data with possible clinical implications.

Study Limitations.

Our study has several limitations worth noting. First, the findings reflect retrospective analyses of prospectively collected data; these data will need to be externally validated in a prospective validation cohort with pre-specified endpoints. Second, the retrospective design introduced selection bias; the significantly lower incidence of sirolimus use among those included in the study versus excluded may have led to a higher overall burden of CAV in our selected cohort given the reported association of sirolimus with both lower ET-1 levels and reduced intimal hyperplasia. (14,21) Third, immunosuppressive regimens were not uniform because our cohort spanned a large time period (e.g. sirolimus use markedly decreased over time once per protocol upfront sirolimus was halted); however, we accounted for differences in sirolimus use in the multivariate analyses, and overall this era-driven heterogeneity provides real-world generalizability given the complex and nuanced nature of post-HT clinical care worldwide. Fourth, the structural assessment of CAV was confined to the LAD. Although a pan-coronary analysis would provide data regarding distribution of disease, it would increase contrast use and procedural time, and is not known whether it would provide additional prognostic benefit. Fifth, serial post-transplant banked blood samples were not available, precluding analyses of whether later ET-1 levels or changes in ET-1 concentration over time also predict accelerated CAV and/or clinical outcomes. Lastly, endomyocardial biopsy specimens were not available for ET-1 staining.

Conclusions

In this retrospective study, plasma levels of ET-1 >1.75 pg/mL measured early after HT was independently associated with accelerated CAV measured by volumetric IVUS and appeared to largely contribute to disease progression through negative remodeling. In addition, baseline ET-1 levels above this threshold were independently associated with late mortality. Taken together, these findings suggest that baseline ET-1 may delineate risk for accelerated CAV and provide durable prognostic value, though future prospective studies are required to externally validate these data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- An ET-1 level >1.75 pg/mL soon after HT independently predict accelerated CAV
- ET-1 appears to primarily contribute to disease progression via negative remodeling
- Baseline ET-1 >1.75 pg/mL is also an independent predictor of late mortality
- These data imply that ET-1 may have durable prognostic value for HT populations

Figure 1.

Comparison of Baseline ET-1 Levels Between HT Patients With and Without Accelerated Changes in IVUS Indices.

HT patients with accelerated CAV and negative remodeling had significantly increased baseline ET-1 levels compared to those patients without accelerated disease. There was no difference in baseline ET-1 levels between patients with and without accelerated intimal hyperplasia. Accelerated disease was defined using the 75th percentile as a cutoff.

CAV, cardiac allograft vasculopathy; ET-1, endothelin-1; HT, heart transplantation

Figure 2.

Diagnostic Accuracy of Baseline ET-1 in Identifying Accelerated CAV Area under the receiver operating characteristic curve is 0.69 (0.57-0.82), $p=0.007$. A baseline ET-1 concentration of 1.75 pg/ml provides the greatest diagnostic accuracy (73% sensitivity, 68% specificity) to detect accelerated CAV.

Abbreviations as in Figure 1.

Figure 3.

Association of Baseline ET-1 Levels with Lumen Volume Loss

Baseline ET-1 levels are categorized as high or low and are plotted against lumen volume loss at 1-year post-HT. The dashed line represents the cutoff for accelerated CAV.

CAV, cardiac allograft vasculopathy; ET-1, endothelin-1; HT, heart transplantation

Figure 4.

Impact of ET-1 on late death and re-transplantation

Kaplan-Meier analysis demonstrated significantly lower event-free survival among HT patients with baseline plasma ET-1 >1.75 pg/mL.

Abbreviations as in Figure 1.

Table 1.**Baseline Clinical Characteristics**

	Overall Cohort N=90	Endothelin-1 1.75 pg/mL N=53	Endothelin-1 >1.75 pg/mL N=37	p-value
Age (years)	49.2±15.3	47.6±17.1	51.6±12.3	0.54
Male	64 (71%)	36 (68%)	28 (76%)	0.43
Ischemic cardiomyopathy	20 (22%)	8 (15%)	12 (32%)	0.053
Body mass index (kg/m ²)	25.8±5.1	25.4±5.4	26.3±4.6	0.33
Hyperlipidemia	21 (23%)	12 (23%)	9 (24%)	0.80
Type 2 Diabetes Mellitus	12 (13%)	4 (8%)	8 (22%)	0.06
Hypertension	36 (40%)	20 (38%)	16 (43%)	0.60
Donor Factors				
Age (years)	31.2±12.5	31.4±12.4	30.8±12.8	0.66
Male	64 (71%)	38 (72%)	26 (70%)	0.88
Transplant Factors				
Graft ischemic time (minutes)	222.7±45.9	225.4±46.9	218.7±44.8	0.20
Cytomegalovirus serology mismatch [†]	18 (20%)	13 (25%)	5 (14%)	0.20
Statin use at 1 year	83 (92%)	49 (93%)	34 (92%)	0.92
Sirolimus use during first year	17 (19%)	6 (11%)	11 (30%)	0.03
Significant rejection during first year [‡]	29 (32%)	18 (34%)	11 (30%)	0.67

* Data are presented as mean±standard deviation or numbers (percentage) as appropriate

[†] Cytomegalovirus serology mismatch denotes donor+/recipient- status

[‡] Significant rejection denotes cellular rejection ≥2R

Table 2.

Association Between Clinical Risk Factors and Accelerated CAV

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Recipient age (years)	1.65	0.38-1.97	0.50			
Recipient sex (male)	3.23	0.86-12.08	0.08	2.10	0.47-9.37	0.33
Ischemic cardiomyopathy	0.72	0.21-2.44	0.60			
Body mass index (kg/m ²)	1.06	0.97-1.16	0.19			
Hyperlipidemia	0.65	0.19-2.20	0.49			
Type 2 Diabetes Mellitus	3.87	1.10-13.63	0.03	2.22	0.53-9.25	0.27
Hypertension	0.81	0.30-2.20	0.68			
ET-1 > 1.75 (pg/mL)	4.48	1.59-12.56	0.004	4.88	1.69-14.1	0.003
ET-1 (per 0.1 pg/mL increase)	1.98	1.10-3.58	0.02	2.13	1.15-3.94	0.01
Donor age (years)	0.97	0.93-1.01	0.26			
Donor sex (male)	3.23	0.86-12.08	0.08	3.74	0.94-14.8	0.06
Graft ischemic time (minutes)	0.99	0.98-1.01	0.96			
Cytomegalovirus serology mismatch	0.32	0.07-1.54	0.16			
Statin use at 1 year	0.20	0.04-1.01	0.05	0.17	0.03-0.95	0.04
Sirolimus use during first year	1.94	0.62-6.06	0.25			
Significant rejection during first year	2.15	0.79-5.79	0.13			

* Accelerated CAV defined as lumen volume loss >2.93 mm³/mm at 1-year post-transplant

† Separate regression models were run for ET-1 as a dichotomous and continuous variable

‡ Definitions of cytomegalovirus serology mismatch and significant rejection as in Table 1 CAV, cardiac allograft vasculopathy; CI, confidence interval; ET-1, endothelin-1; HR, hazard ratio

Table 3.

Association Between Clinical Risk Factors and Late Death or Re-transplantation

	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Recipient age (years)	0.97	0.95-1.01	0.09	0.98	0.95-1.01	0.20
Recipient sex (male)	0.49	0.19-1.18	0.14			
Ischemic cardiomyopathy	0.70	0.22-2.15	0.53			
Body mass index (kg/m ²)	1.02	0.93-1.11	0.68			
Hyperlipidemia	0.51	0.14-1.79	0.29			
Type 2 Diabetes Mellitus	2.14	0.70-6.55	0.18			
Hypertension	0.27	0.06-1.19	0.08	0.35	0.07-1.59	0.13
ET-1 > 1.75 (pg/mL)	2.54	0.98-6.62	0.05	2.94	1.12-7.72	0.02
ET-1 (per 0.1 pg/mL increase)	1.22	0.72-2.05	0.44			
Donor age (years)	1.01	0.97-1.04	0.62			
Donor sex (male)	0.62	0.23-1.65	0.33			
Graft ischemic time (minutes)	1.0	0.99-1.01	0.99			
CMV serology mismatch	1.10	0.39-3.11	0.85			
Statin use at 1 year	0.70	0.16-3.08	0.64			
Sirolimus use during first year	1.99	0.77-5.17	0.15			
Significant rejection during first year	2.95	1.10-7.93	0.03	3.35	1.24-9.04	0.01

* Separate regression models were run for ET-1 as a dichotomous and continuous variable

Abbreviations and definitions as in Table 2