Feature Article

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 after HT (baseline) via a competitive enzyme-linked immunosorbent assay. Three-dimensional volumetric intravascular ultrasound of the left anterior descending artery was performed at baseline and at 1 year. Accelerated CAV (lumen volume loss) was defined with the 75th percentile as a cutoff. Patients were followed beyond the first year after HT for late death or retransplantation. A receiver operating characteristic (ROC) 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 ROC curve 0.69, 95% confidence interval [CI] 0.57−0.82; $P = .007$). In multivariate logistic regression, a higher baseline ET-1 concentration was independently associated with accelerated CAV (odds ratio [OR] 2.13, 95% CI 1.15−3.94; $P = .01$); this relationship persisted when ET-1 was dichotomized at 1.75 pg/mL (OR 4.88, 95% CI 1.69−14.10; $P = .003$). Eighteen deaths occurred during a median follow-up period of 3.99 (interquartile range 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; $P = .44$). However, ET-1 >1.75 pg/mL conferred a significantly lower cumulative event-free survival on Kaplan-Meier analysis ($P = .047$) and was independently associated with late mortality (HR 2.94, 95% CI 1.12−7.72; $P = .02$).

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. (*J Cardiac Fail 2019;25:97−104*)

Key Words: Endothelin-1, heart transplantation, cardiac allograft vasculopathy, mortality.

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Fifty years after the first human heart transplantation (HT), cardiac allograft vasculopathy (CAV) remains the nemesis of recipients' long-term survival.[1,2] CAV is a complex disease of the graft coronary arteries characterized by diffuse intimal hyperplasia and negative remodeling (ie, 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 (from 32% to 29% and from 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 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 proinflammatory effects throughout the vessel wall.[5,6] A series of seminal nonhuman 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 3-dimensional (3D) volumetric intravascular ultrasound (IVUS) and (2) clinical outcomes (late death or retransplantation) in a large HT cohort.

Methods

Study Population

This study included HT patients from 2 prospective National Institutes of Health–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 affect the development of CAV.[13] Patients were included in the present study if they had (1) undergone baseline (4–8 weeks after 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 retransplantation). All patients provided informed consents for the original parent trials, and the study protocols were approved by the Stanford Institutional Review Board on Human Subjects Research.

Post-transplantation Clinical Management

All HT recipients received the following standard pharmacologic regimen: (1) induction immunosuppressive therapy with the use of 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 *Pneumocistis jiroveci* prophylaxis, (5) valganciclovir for cytomegalovirus prophylaxis in the event of seropositive donor or recipient, and (6) aspirin and a statin as tolerated for prevention of CAV. The proliferation signal inhibitor sirolimus was given up front to all post-transplantation 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 owing to increased rates of wound dehiscence, reserving sirolimus only for use in cases of accelerated CAV. Patients were monitored for acute rejection with the use of 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 $\geq$2R.

ET-1 Measurements

At the baseline (4–8 weeks after 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 with the use of a previously described quantitative sandwich enzyme linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota).

IVUS Imaging Protocol

After diagnostic coronary angiography, unfractionated heparin (50–70 U/kg) was administered intravenously and nitroglycerin (200 μg) was administered via bolus injection through a 6-F 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, Massachusetts) 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]

Three-Dimensional Volumetric IVUS Analysis

IVUS measurements were carried out in a blinded fashion by the Stanford Cardiovascular Core Analysis Laboratory with the use of a validated quantitative IVUS analysis system (Echoplaque; Indec Systems, Santa Clara, California). Lumen, intimal, and vessel volumes were calculated with the use of the Simpson method and indexed as volume per length analyzed (mm^3/mm), with lumen volume loss (ie, 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 2-dimensional (2D) IVUS reported 20%–30% rates of accelerated disease at 1 year.[17,18] Therefore, we chose the 75th percentile of lumen volume loss as the cutoff to define accelerated CAV at 1 year after 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 n (%) or mean ± SD. Nonparametric 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 end points with the use of univariate logistic regression. As described earlier, ramipril use was not included as a candidate risk factor because prior data demonstrated that it does not affect the development CAV.[13] Risk factors with univariate $P < .10$ were included in multivariate forward stepwise logistic regression models to determine independent predictors. Time-to-event data were analyzed with the use of Kaplan−Meier curves stratified by ET-1. Cox proportional hazards regression models (multivariate models included risk factors with $P < .10$ 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 (ORs) and hazard ratios (HRs) with 95% confidence intervals (CIs), respectively. Statistical analyses were performed with the use of the SPSS system, version 21 (SPSS, Chicago, Illinois). A P value $<.05$ was considered to be 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 inclusion criteria for and were included in the present 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 (ie, clotted due to improper processing) for ET-1 testing. Sirolimus use was significantly lower among those included in the study compared with the excluded cohort (19% vs 52%; $P < .001$); 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 after HT, 92% of patients were tolerating statins, 32% had ≥1 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 up front per standard protocol at Stanford in 2002−2003.

Volumetric IVUS and ET-1 Data

Overall, from baseline to 1 year after HT, both lumen volume (12.66 ± 3.32 mm^3/mm to 10.97 ± 3.34 mm^3/mm; $P < .0001$) and vessel volume (15.29 ± 4.01 mm^3/mm to 14.09 ± 4.24 mm^3/mm; $P < .0001$) decreased significantly, whereas intimal volume (2.66 ± 1.37 mm^3/mm to 3.12 ± 1.73 mm^3/mm; $P < .0001$) increased significantly. Seventy-seven patients (86%) demonstrated CAV 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 mm^3/mm; accelerated negative remodeling: vessel volume loss <2.50 mm^3/mm; and accelerated intimal hyperplasia: intimal volume gain >0.91 mm^3/mm.

At 1 year after HT, patients who had developed accelerated CAV and accelerated negative remodeling had significantly higher baseline ET-1 levels than patients without accelerated disease, whereas there was no difference in ET-

Table 1. Baseline Clinical Characteristics

Characteristic	Overall Cohort (n = 90)	Endothelin-1 ≤1.75 pg/mL (n = 53)	Endothelin-1 >1.75 pg/mL (n = 37)	*P* Value
Age (y)	49.2 ± 15.3	47.6 ± 17.1	51.6 ± 12.3	.54
Male	64 (71%)	36 (68%)	28 (76%)	.43
Ischemic cardiomyopathy	20 (22%)	8 (15%)	12 (32%)	.053
Body mass index (kg/m^2)	25.8 ± 5.1	25.4 ± 5.4	26.3 ± 4.6	.33
Hyperlipidemia	21 (23%)	12 (23%)	9 (24%)	.80
Type 2 diabetes mellitus	12 (13%)	4 (8%)	8 (22%)	.06
Hypertension	36 (40%)	20 (38%)	16 (43%)	.60
Donor factors				
Age (y)	31.2 ± 12.5	31.4 ± 12.4	30.8 ± 12.8	.66
Male	64 (71%)	38 (72%)	26 (70%)	.88
Transplant factors				
Graft ischemia time (min)	222.7 ± 45.9	225.4 ± 46.9	218.7 ± 44.8	.20
Cytomegalovirus serology mismatch*	18 (20%)	13 (25%)	5 (14%)	.20
Statin use at 1 year	83 (92%)	49 (93%)	34 (92%)	.92
Sirolimus use during first year	17 (19%)	6 (11%)	11(30%)	.03
Significant rejection during first year†	29 (32%)	18 (34%)	11 (30%)	.67

Data are presented as mean ± SD or n (%).
*Cytomegalovirus serology mismatch denotes donor+/recipient− status.
†Significant rejection denotes cellular rejection ≥2R.

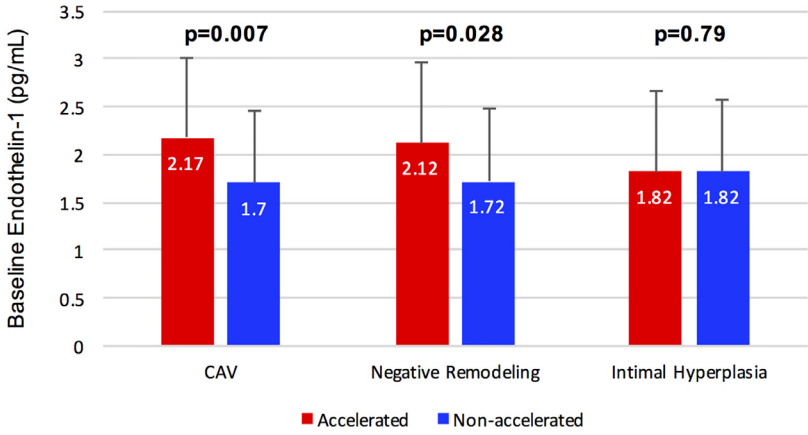

Fig. 1. Comparison of baseline ET-1 levels between HT patients with and without accelerated changes in intravascular ultrasound (IVUS) indexes. HT patients with accelerated CAV and negative remodeling had significantly increased baseline ET-1 levels compared with patients without accelerated disease. 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.

1 levels between patients with and without accelerated intimal hyperplasia (Fig. 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 (Fig. 2). The area under the ROC curve was 0.69 (95% CI 0.57−0.82; $P = .007$), 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 (Fig. 3).

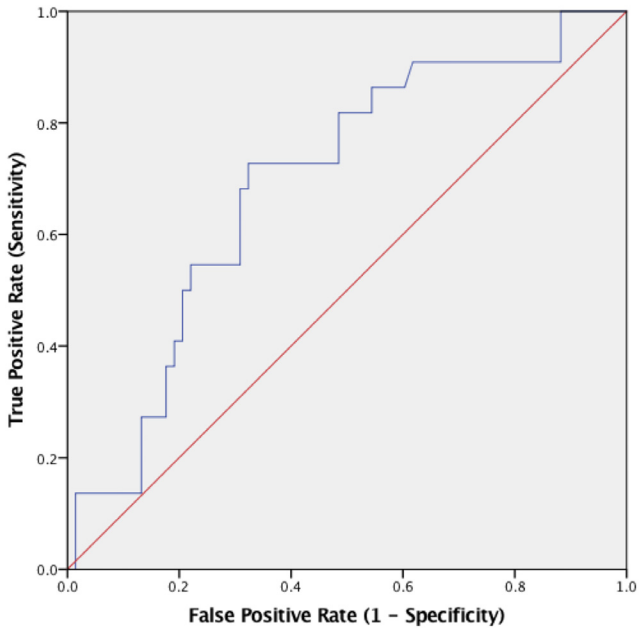

Fig. 2. Diagnostic accuracy of baseline ET-1 in identifying accelerated CAV. Area under the receiver operating characteristic curve is 0.69 (95% confidence interval 0.57−0.82); $P = .007$. A baseline ET-1 concentration of 1.75 pg/mL provides the greatest diagnostic accuracy (73% sensitivity, 68% specificity) to detect accelerated CAV. Abbreviations as in Fig. 1.

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%; $P = .03$; 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 ± 0.76 pg/mL vs 2.57 ± 0.69 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 after HT, although there was a trend toward reduced intimal volume gain (2.92 ± 38.52 mm^3/mm vs 21.57 ± 41.10 mm^3/mm; $P = .065$; 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 ($P < .10$) and included in the multivariable model. After adjustment, elevated baseline ET-1 (OR 4.88, 95% CI 1.69−14.10; $P = .003$) and statin use at 1 year (OR 2.13, 95% CI 1.15−3.94; $P = .01$) 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; $P = .04$). Of note, higher baseline ET-1 >1.75 pg/mL was associated with accelerated negative remodeling in univariable analysis (OR 2.64, 95% CI 0.99−7.08, $P = .05$), but this association lost statistical significance after multivariable adjustment (OR 2.63, 95% CI 0.94−7.34; $P = .07$) (Supplemental Table 3).

ET-1 and Clinical Outcomes

Major adverse events (18 late deaths, 0 retransplantations) occurred in 20% of patients over a median follow-up period of

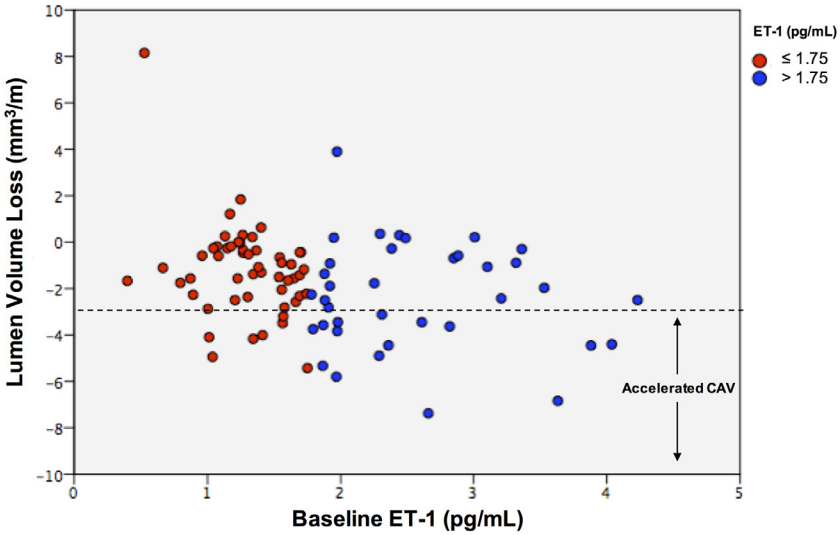

Fig. 3. Association of baseline ET-1 levels with lumen volume loss. Baseline ET-1 levels are categorized as high or low and are plotted against lumen volume loss at 1 year after HT. The dashed line represents the cutoff for accelerated CAV. Abbreviations as in Fig. 1.

4.0 (interquartile range 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 with those with baseline ET-1 ≤1.75 pg/mL (log-rank $P = .047$; Fig. 4). In univariate Cox regression analysis, higher baseline ET-1 (treated as a continuous variable) was not associated with the major adverse event end point (HR 1.22, 95% CI 0.72−2.05; $P = .44$), but ET-1 >1.75 pg/mL, age, hypertension, and significant rejection during the first year after HT were associated with the major adverse event end point ($P < .10$) and included in multivariate analysis. After adjustment, baseline ET-1 >1.75 pg/mL (HR 2.94, 95% CI 1.12−7.72; $P = .02$) and significant rejection during the first year after HT (HR 3.35, 95% CI 1.24−9.04; $P = .02$) remained independently associated (Table 3). Of note, baseline ET-1 and significant rejection were not significantly correlated ($r = 0.08$; $P = .44$).

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 with the use of 3D volumetric IVUS and appear to be

Table 2. Association Between Clinical Risk Factors and Accelerated CAV

	Univariate Analysis			Multivariate Analysis		
Factor	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Recipient age (y)	1.65	0.38−1.97	.50			
Recipient sex male	3.23	0.86−12.08	.08	2.10	0.47−9.37	.33
Ischemic cardiomyopathy	0.72	0.21−2.44	.60			
Body mass index (kg/m^2)	1.06	0.97−1.16	.19			
Hyperlipidemia	0.65	0.19−2.20	.49			
Type 2 diabetes mellitus	3.87	1.10−13.63	.03	2.22	0.53−9.25	.27
Hypertension	0.81	0.30−2.20	.68			
ET-1 >1.75 (pg/mL)[†]	4.48	1.59−12.56	.004	4.88	1.69−14.1	.003
ET-1 (per 0.1 pg/mL increase)[†]	1.98	1.10−3.58	.02	2.13	1.15−3.94	.01
Donor age (y)	0.97	0.93−1.01	.26			
Donor sex male	3.23	0.86−12.08	.08	3.74	0.94−14.8	.06
Graft ischemia time (min)	0.99	0.98−1.01	.96			
Cytomegalovirus serology mismatch[‡]	0.32	0.07−1.54	.16			
Statin use at 1 year	0.20	0.04−1.01	.05	0.17	0.03−0.95	.04
Sirolimus use during first year	1.94	0.62−6.06	.25			
Significant rejection during first year[‡]	2.15	0.79−5.79	.13			

CAV, cardiac allograft vasculopathy; CI, confidence interval; ET-1, endothelin-1; HR, hazard ratio.
[†]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.

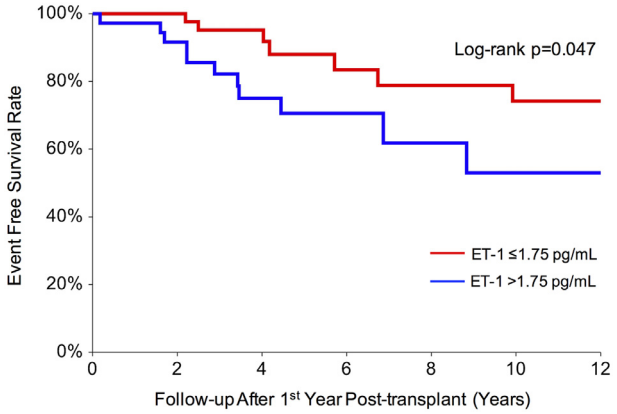

Fig. 4. Impact of ET-1 on late death and retransplantation. Kaplan-Meier analysis demonstrated significantly lower event-free survival among HT patients with baseline plasma ET-1 >1.75 pg/mL. Abbreviations as in Fig. 1.

associated with accelerated negative remodeling; and (3) baseline ET-1 levels >1.75 pg/mL 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 noninvasively identifying HT patients at risk for accelerated CAV.

Previous Data Support ET-1 as a CAV Mediator

A series of classic studies using rat models of HT established the biologic 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 nonselective endothelin receptor antagonist, significantly attenuated disease progression.[7] Simonson et al conducted similar studies with the use of 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 data suggested that ET-1 exerts effects throughout the graft coronary vessel wall and that reducing its bioactivity slows disease progression.

On the basis of that foundational nonhuman animal work, several groups have studied ET-1 in human HT patients. Ferri et al demonstrated that ET-1 expression on endomyocardial biopsy at 3 months after HT predicted the development of CAV on angiography at 2 years in 47 patients. In addition, 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 $P = .059$; HR 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 after HT) and found that those with advanced CAV (defined as ≥15% diameter stenosis) had a significantly greater vasomotor response compared with 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 after 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 nonhuman 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 used 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

Table 3. Association Between Clinical Risk Factors and Late Death or Retransplantation

	Univariate Analysis			Multivariate Analysis		
Factor	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Recipient age (y)	0.97	0.95–1.01	.09	0.98	0.95–1.01	.20
Recipient sex male	0.49	0.19–1.18	.14			
Ischemic cardiomyopathy	0.70	0.22–2.15	.53			
Body mass index (kg/m^2)	1.02	0.93–1.11	.68			
Hyperlipidemia	0.51	0.14–1.79	.29			
Type 2 diabetes mellitus	2.14	0.70–6.55	.18			
Hypertension	0.27	0.06–1.19	.08	0.35	0.07–1.59	.13
ET-1 > 1.75 (pg/mL)*	2.54	0.98–6.62	.05	2.94	1.12–7.72	.02
ET-1 (per 0.1 pg/mL increase)*	1.22	0.72–2.05	.44			
Donor age (y)	1.01	0.97–1.04	.62			
Donor sex male	0.62	0.23–1.65	.33			
Graft ischemia time (min)	1.0	0.99–1.01	.99			
Cytomegalovirus serology mismatch†	1.10	0.39–3.11	.85			
Statin use at 1 year	0.70	0.16–3.08	.64			
Sirolimus use during first year	1.99	0.77–5.17	.15			
Significant rejection during first year†	2.95	1.10–7.93	.03	3.35	1.24–9.04	.01

Abbreviations as in Table 2.
*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.

the vessel wall. Third, the analyses focused solely on intimal thickening and failed to assess negative remodeling, which is an integral component of CAV. Finally, short follow-up periods precluded evaluation of longer-term clinical outcome data.

ET-1, CAV, and Negative Remodeling

In the present 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 retransplantation) in a cohort of 90 HT recipients. We found that baseline ET-1 was independently associated with accelerated CAV at 1 year after 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, ~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 example, in the landmark study by Kobashigawa et al, which included 125 HT patients who received transplants in 1997 or earlier, cyclosporine was the backbone of immunosuppressive regimens and statin use was 38%.[17] In contrast, our cohort (who received transplants from 2002 to 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 anatomic 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 prespecified end points. Second, the retrospective design introduced selection bias; the significantly lower incidence of sirolimus use among those included in the study versus those 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 (eg, sirolimus use markedly decreased over time once per-protocol up-front 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 pancoronary analysis would provide data regarding distribution of disease, it would increase contrast use and procedural time, and it is not known whether it would provide additional prognostic benefit. Fifth, serial post-transplantation 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. Finally, endomyocardial biopsy specimens were not available for ET-1 staining.

Conclusion

In this retrospective study, plasma levels of ET-1 >1.75 pg/mL measured early after HT were independently associated with accelerated CAV as measured with the use of 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. These findings suggest that baseline ET-1 may delineate risk for accelerated CAV and provide durable prognostic value, although future prospective studies are required to externally validate these data.

Disclosures

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Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.cardfail.2018.12.001.

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