Published in final edited form as:

Am J Cardiol. 2018 November 15; 122(10): 1707–1711. doi:10.1016/j.amjcard.2018.07.048.

Usefulness of Asymmetric Dimethylarginine to Predict Outcomes After Heart Transplantation

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Abstract

Asymmetric dimethylarginine (ADMA) is a key mediator of vascular homeostasis and an independent predictor of the development of accelerated cardiac allograft vasculopathy (CAV) after heart transplantation. However, its association with clinical outcomes in heart transplant recipients has not been described. Plasma levels of ADMA were assayed within 8 weeks following transplantation (baseline) using a competitive enzyme-linked immunosorbent assay. The primary endpoint was the composite of non-fatal myocardial infarction, percutaneous coronary intervention (PCI), re-transplantation, or death at 5-year follow-up. Kaplan–Meier curves were generated to assess the association between baseline ADMA levels (stratified at 0.70 μ M, a previously established cutoff) and cumulative event-free survival. Multivariate Cox regression was performed to adjust for other candidate predictors. Among 69 heart transplant recipients at Stanford, the primary endpoint occurred in 11 patients (16%)—4 PCI, 1 re-transplant, and 6 deaths—during 5-years follow-up. Patients with baseline ADMA $\geq$ 0.70 μ M had lower cumulative 5-year event-free survival (77% vs. 93%, $p=0.059$). In multivariate Cox analysis, baseline ADMA was the only significant predictor of the primary endpoint [Hazard ratio (HR)=1.33, 95% confidence interval (CI): 1.03–1.72 per 0.1 μ M; $p=0.031$]. This association remained significant even after restricting the endpoint to death or re-transplantation (HR=1.48, 95% CI: 1.12–1.97 per 0.1 μ M; $p=0.006$). In conclusion, elevated baseline plasma levels of ADMA independently predicted 5-year clinical outcomes after heart transplantation, suggesting that ADMA has potential prognostic value in the heart transplant arena.

Keywords

Heart transplantation; asymmetric dimethylarginine; mortality; outcomes

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Introduction

Cardiac allograft vasculopathy (CAV)—a complex fibroproliferative process affecting the entire coronary tree—is a principal cause of adverse outcomes following heart transplantation.¹ Despite advances in CAV treatment over the past few decades, contemporary worldwide registry data indicate that CAV remains a major limitation to long-term graft survival.² Identification of a noninvasive prognostic biomarker for CAV and related clinical outcomes may lead to less frequent invasive monitoring and earlier, more potent immunosuppression in select patients. One potential candidate is asymmetric dimethylarginine (ADMA), an endogenous inhibitor of endothelial nitric oxide synthase, and hence, a key mediator of vascular function.³ We have previously reported that higher levels of ADMA soon after transplant are associated with abnormal epicardial function and the development of accelerated CAV at 1-year in heart transplant recipients, both of which are known predictors of 5-year graft failure (i.e. death or re-transplantation).^{4–8} However, to the best of our knowledge, the relationship between ADMA and clinical outcomes after heart transplantation has not been described.

Methods

This retrospective cohort study included heart transplant recipients from a prospective, National Institutes of Health-sponsored study conducted by our group at Stanford University from 2002 to 2004 that evaluated the effect of cytomegalovirus on the development of CAV (1 PO1-A150153). In the present study, we included all patients who had a documented baseline ADMA level (within 8 weeks after transplant). The study protocol was approved by the Stanford University Institutional Review Board and informed consent was obtained from each patient prior to enrollment.

All patients received a routine immunosuppressive regimen including induction therapy (daclizumab or antithymocyte globulin), corticosteroids (tapered over 8 months if free of acute rejection), a calcineurin inhibitor (tacrolimus or cyclosporine), and cell-cycle inhibitor (mycophenolate mofetil). The proliferation signal inhibitor sirolimus was also a standard component of the initial post-transplant protocol at Stanford because it had been shown to slow CAV progression, but increased wound dehiscence complications relegated its use to only cases of accelerated CAV beginning in 2004. Of note, sirolimus exposure is associated with lower ADMA levels, presumably due reduced immune-mediated oxidative stress.^{5,9} However, infectious prophylaxis included co-trimoxazole for pneumocystis jiroveci and valganciclovir for cytomegalovirus (CMV) prophylaxis. Aspirin and statins were given for prevention of CAV.

Blood samples for ADMA were obtained during the routine baseline invasive coronary evaluation following transplantation. They were immediately centrifuged at 4°C, and plasma was then stored at –80°C. Plasma ADMA levels were measured using a competitive enzyme-linked immunosorbent assay described previously (DLD Diagnostika GmbH, Hamburg, Germany).¹⁰

Descriptive statistics are expressed mean±standard deviation or frequency (percentage). Differences between groups were tested using non-parametric Wilcoxon Rank-Sum or Fisher's exact tests, as appropriate. The primary endpoint was the composite of non-fatal myocardial infarction, percutaneous coronary intervention (PCI), re-transplantation, or death at 5-year follow-up. The secondary endpoint was 5-year graft failure (i.e. death or re-transplantation). We chose the 5-year time point because 1) it has been used in prior landmark heart transplant studies, 2) it is a reasonable cutoff for outcomes related to accelerated CAV, and 3) clinical event data for the original study was only systematically collected for 5-years of follow-up. Kaplan–Meier curves stratified by ADMA were built to analyze time-to-event data. An ADMA concentration of 0.70 µM was used to dichotomize the cohort because this cutoff has previously been found to provide the greatest diagnostic accuracy in identifying patients with accelerated CAV.⁵ Relevant candidate covariates (demographic, cardiovascular, and transplant-related factors listed in Table 1) were tested for their ability to predict the primary endpoint using univariate Cox regression. Covariates with a p-value <0.20 were included in the multivariate Cox regression models to identify independent predictors. A p-value <0.05 was considered statistically significant.

Results

A total of 112 heart transplant recipients were enrolled in the original trial; the initial 78 patients were enrolled in an ADMA-based substudy, 69 of whom had a documented baseline ADMA level and were included in the current study; the final 34 study participants did not have ADMA levels drawn. With respect to the 9 patients without baseline ADMA data: 3 died prior to baseline investigation, 2 did not undergo baseline investigation due renal insufficiency, and 4 withdrew from the study prior to baseline investigation. The only significant difference in baseline clinical characteristics between those included and excluded from the study was sirolimus use during the first year after transplant (61% vs. 25%, p<0.001).

Overall, the mean age of the cohort was 45.7±16.7 years, 75% were male, 29% developed significant rejection during the first year post-transplant, which was defined as cellular rejection 2R or antibody-mediated rejection with graft dysfunction that resulted in acute escalation of immunosuppression, and 61% took sirolimus during the first year. Thirty patients (43%) had a baseline ADMA <0.70 µM and 39 patients (57%) had a baseline ADMA ≥0.70 µM. Notably, patients in the high ADMA cohort had significantly less CMV serology mismatch, less exposure to sirolimus (known to lower ADMA levels), and older donor hearts than those in the low ADMA cohort (Table 1). Consistent with prior studies, those with higher ADMA also had more donor-related atherosclerosis on baseline intravascular ultrasound investigation [2.32 µM (1.51–3.47) vs. 1.54 µM (0.99–2.43), p=0.047].

The primary endpoint occurred in 11 patients (16%) during the 5-year follow-up period (0 myocardial infarctions, 4 PCI, 1 re-transplant, and 6 deaths); 9 (82%) of these events occurred among patients with a baseline ADMA ≥0.70 µM (Figure 1). None of the patients had multiple events, and so the total number of events among the entire cohort was also 11. Patients with a baseline ADMA level ≥0.70 µM had a 16% absolute and 17% relative risk

reduction in cumulative 5-year event-free survival rate compared to those with a baseline ADMA level $<0.70 \mu\text{M}$, though this risk difference narrowly missed statistical significance (77% vs. 93%, $p=0.059$; Figure 2). In univariate Cox regression analysis, higher baseline ADMA (continuous variable), significant rejection during the first year after transplant, BMI, and donor age were associated ($p<0.20$) with the primary endpoint and included in the multivariate model. Following multivariate adjustment, higher baseline ADMA was the only remaining independent predictor (Hazard ratio (HR) = 1.33, 95% confidence interval (CI): 1.03–1.72 per $0.1 \mu\text{M}$; $p=0.031$) (Table 2). This significant association persisted even after restricting the endpoint to graft failure (i.e. death or re-transplantation) (HR=1.48, 95% CI: 1.12–1.97 per $0.1 \mu\text{M}$; $p=0.006$).

Discussion

The main findings of this retrospective cohort study in heart transplant recipients were: 1) patients with a baseline level of plasma ADMA $>0.70 \mu\text{M}$ had a 16% absolute and 17% relative risk reduction in cumulative event-free survival for the primary endpoint of non-fatal myocardial infarction, PCI, re-transplantation, and all-cause death at 5-years follow-up, and 2) increased baseline ADMA was the only independent predictor of this primary endpoint as well as graft failure alone (i.e. death and re-transplantation). Taken together, the clinical implication of these data is that baseline ADMA has prognostic value in heart transplant recipients.

Despite recent advances in the clinical care of heart transplant recipients, CAV remains a leading impediment to long-term graft survival in the contemporary era.² In fact, prior landmark studies found that accelerated CAV detected within the first year after transplant predicts graft failure (re-transplantation and/or death).^{6,7} ADMA—a surrogate biomarker for nitric oxide-mediated vascular dysfunction—has been implicated in the pathogenesis of CAV (Figure 3). Chronic immune and inflammatory activation inherent to the post-transplant state result in a large burden of oxidative stress following heart transplantation, which in turn impairs dimethylarginine dimethylaminohydrolase (DDAH), the enzyme responsible for metabolizing ADMA.¹¹ Indeed, animal models of heart transplantation have shown that DDAH underexpression increases levels of ADMA and CAV.¹² Our group has previously reported that ADMA is an independent predictor of accelerated CAV and lower FFR values early after transplant in heart transplant patients.^{5,4} Collectively, the data to-date imply that ADMA may be a clinically relevant, prognostic tool in the heart transplant arena, but the relationship between ADMA and clinical outcomes in this setting has not been studied.

In the present study, we observed that higher baseline plasma ADMA (continuous variable) is a strong independent predictor of clinical outcomes at 5-years follow-up, including graft failure alone. Unlike in non-transplant patients in whom ADMA primarily accumulates in the presence of oxidative stress generated by cardiovascular risk factors,^{13–16} there are additional immune and inflammatory-mediated factors that yield oxidative stress and affect ADMA levels in all heart transplant recipients.^{1,17} These factors likely attenuate the difference in ADMA levels between recipients with and without adverse cardiovascular outcomes, thus making our findings particularly noteworthy. Of note, dichotomizing ADMA

($>0.70 \mu\text{M}$) modestly weakened its predictive ability, and the difference in cumulative 5-year event-free survival rate among patients with baseline ADMA $>0.70 \mu\text{M}$ compared to those with baseline ADMA $<0.70 \mu\text{M}$ also did not reach statistical significance. However, the absolute risk difference was sizeable (16%), suggesting that our relatively small study may have simply been underpowered, particularly given that dichotomization fundamentally reduces statistical power. Larger prospective studies are warranted to confirm our findings and provide further clarity.

This study has a few important limitations. The retrospective study design created selection bias—only 62% of the original cohort had documented baseline ADMA levels and were included in the present study, resulting in a significant imbalance in sirolimus use between those included and not included in the study. Furthermore, the broad use of sirolimus in this study somewhat limits the generalizability of the findings since sirolimus is typically not considered first-line therapy in the contemporary era. The retrospective nature also substantially limited the sample size. Of note, the ADMA data we recently published from a more recent trial used a different assay for measuring plasma ADMA (high performance liquid chromatography), and so we unfortunately could not combine the two cohorts.⁴ Nonetheless, the sample size of the current study is comparable to those of other similar single-center heart transplant studies. Lastly, we do not have serial ADMA data to assess if changes in ADMA over time predict outcomes.

In summary, an elevated level of plasma ADMA early after heart transplantation is a strong independent predictor of clinical outcomes including graft failure at 5-years follow-up among heart transplant recipients. Future prospective studies are needed to determine if patients with an elevated baseline ADMA benefit from more frequent screening and earlier, more intensive immunosuppression upfront.

Acknowledgements

This study was funded by the NIH: 1 PO1-A150153 (HV).

WFF receives research support from Abbott Vascular, Acist Medical, Edwards LifeSciences, CathWorks and Medtronic and has a consulting relationship with HeartFlow. The other authors report that they have no relationships with industry relevant to the contents of this manuscript to disclose.

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Figure 1. 5-Year Clinical Outcomes Stratified by Baseline ADMA. Over 5-year follow-up, heart transplant patients with a baseline plasma ADMA <0.70 μM had 2 adverse events while those with a baseline plasma ADMA ≥0.70 μM had 9 adverse events.

Figure 2.

Association of Baseline ADMA with 5-Year Clinical Outcomes. Heart transplant recipients with a baseline plasma ADMA $\geq$ 0.70 μ M demonstrated 16% lower late event-free survival at 5-year follow-up compared to those with baseline ADMA <0.70 μ M (77% vs. 93%, p=0.059). ADMA, asymmetric dimethylarginine

Figure 3. Mechanistic Role of the ADMA in Adverse Clinical Outcomes Following Heart Transplantation ADMA, asymmetric dimethylarginine; CAV, cardiac allograft vasculopathy; DDAH, dimethylarginine dimethylaminohydrolase; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; O_2^- , oxidative stress; HT, heart transplantation.

Table 1:**Baseline Clinical Factors ADMA (μM)**

Characteristics	Total Cohort (n=69)	<0.70 (n=30)	0.70 (n=39)	P- value
Recipient age (years)	45.7 $\pm$ 16.7	43.6 $\pm$ 18.1	47.5 $\pm$ 15.3	0.39
Recipient sex (male)	52 (75%)	20 (67%)	32 (82%)	0.14
Donor age (years)	31.2 $\pm$ 14.1	27.3 $\pm$ 12.3	34.2 $\pm$ 14.8	0.045
Donor sex (male)	50 (72%)	19 (63%)	31 (80%)	0.14
Cold ischemic time (minutes)	211.7 $\pm$ 50.1	214.0 $\pm$ 58.5	210.0 $\pm$ 43.3	0.95
Cytomegalovirus serology mismatch *	19 (28%)	12 (40%)	7 (18%)	0.044
Diabetes mellitus	8 (12%)	1 (3%)	7 (18%)	0.062
Body mass index (kg/m^2)	26.7 $\pm$ 6.1	25.3 $\pm$ 5.7	27.8 $\pm$ 6.3	0.095
Dyslipidemia	16 (23%)	6 (20%)	10 (26%)	0.59
Hypertension	10 (14%)	4 (13%)	6 (15%)	0.81
Statin at 1 year	65 (94%)	28 (93%)	37 (95%)	0.79
Significant rejection during first year [†]	20 (29%)	8 (27%)	12 (31%)	0.71
Sirolimus use during first year	42 (61%)	25 (83%)	17 (44%)	0.001

Data are presented as mean $\pm$ standard deviation or numbers (percentage) as appropriate.

* Cytomegalovirus serology mismatch denotes donor+/recipient-status.

[†]Significant rejection was defined as cellular rejection 2R or antibody-mediated rejection with graft dysfunction that resulted in acute escalation of immunosuppression.

Table 2.**Predictors of 5-Year Clinical Outcomes**

Candidate Covariate	Univariate Analysis Hazard Ratio (95% Confidence Interval), P- Value	Multivariate Regression Hazard Ratio (95% Confidence Interval), P- Value
Asymmetric dimethylarginine (per 0.1 μM increase) *	1.40 (1.09–1.79), p=0.007	1.33 (1.03–1.72), p=0.031
Asymmetric dimethylarginine 0.70 μM *	3.92 (0.85–18.2), p=0.081	3.92 (0.85–18.2), p=0.081
Significant rejection during first year	2.29 (0.70–7.49), p=0.17	2.22 (0.64–7.70), p=0.21
Body mass index	1.06 (0.97–1.16), p=0.17	1.02 (0.92–1.13), p=0.74
Donor age (years)	1.03 (0.99–1.08), p=0.14	1.03 (0.98–1.07), p=0.27

Covariates listed are those with a $p < 0.20$ in univariate Cox regression that were then included in the multivariate model.

* Separate regression models were run for ADMA as a continuous and dichotomous variable.