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The Journal of
Heart and Lung
Transplantation

http://www.jhltonline.org

Deposition of C4d and C3d in cardiac transplants: A factor in the development of coronary artery vasculopathy

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KEYWORDS:
coronary artery vasculopathy;
antibody mediated rejection;
complement;
cardiac transplant;
myocardial biopsy

BACKGROUND: Coronary artery vasculopathy (CAV) is the major life-limiting factor in cardiac transplantation, after 1 year. Antibody-mediated rejection (AMR) has been associated with development of both acute and chronic rejection. We analyzed endomyocardial biopsies for pathologic markers of AMR (C4d and C3d), from the first 2 years post-transplantation, to determine complement deposition in relation to the development of CAV.

METHODS: A retrospective, matched-pair study was used. Group 1 subjects ($n = 26$) were CAV-negative at 8 years, and Group 2 ($n = 26$) had angiographically detectable CAV at 4 years. Biopsies from six time-points were studied (total = 282). Immunohistochemistry was performed for C4d, C3d and CD68. Biopsies were graded for rejection using ISHLT criteria.

RESULTS: Although CAV was not significantly associated with C4d deposition, it was associated with C3d deposition ($p = 0.043$). Only 4% of C4d and 5% of C3d biopsies were completely negative. Group 1 had 6 AMR-positive biopsies, with Group 2 having 8. There was no significant relationship between acute cellular rejection or AMR events and CAV.

CONCLUSIONS: This study demonstrates that complement deposition is a frequent occurrence in the first 2 years post-transplantation. Although acute rejection is a known risk factor for CAV, in this study the relationship was found not to be significant. No relationship was found with the development of CAV and histologic features of AMR, when assessed by C4d deposition alone. However, an association between C3d deposition and the development of CAV was determined in this study group, suggesting that complement activation may play a role in the pathogenesis of CAV.

J Heart Lung Transplant 2010;29:417–423

The major cause of mortality and morbidity in cardiac allograft patients at 1 year post-transplantation is chronic rejection, taking the form of coronary artery vasculopathy (CAV).[1,2] Although recent advances in immunosuppressive therapies have done much to improve first-year survival, and reduce the incidence of acute cellular rejection, they have done little to impact the onset of CAV.[1] The precise pathogenic mechanisms that contribute to development of CAV have not been completely elucidated. A number of risk factors have been identified and associated with its pathogenesis, including, but not limited to, metabolic factors, brain death, prolonged ischemic times, viral infections and incidence of acute rejection.[3–5] The latter has been the topic of much discussion with a number of studies demonstrating both strong and weak correlations with cellular

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1053-2498/10/$ -see front matter
doi:10.1016/j.healun.2009.12.018

rejection episodes and CAV.[6,7] Although the development of CAV almost certainly appears to be immune-mediated, the role of cellular rejection in its pathogenesis is unclear, especially as modern advances in immunosuppressive agents that significantly reduce cellular rejection episodes have done little to reduce the incidence and development of CAV.[8,9]

Recent studies have shown that, in addition to acute cellular rejection, grafts are susceptible to antibody-mediated rejection (AMR).[10,11] AMR is characterized by myocardial capillary injury, endothelial swelling and intravascular macrophage accumulation. Further, interstitial edema and hemorrhage can be present with neutrophils in and around capillaries. Immunohistochemistry study for C4d on capillary endothelium is also used to support the diagnosis of AMR.[10] Much of our current understanding of AMR has come initially from studies in renal transplantation and, more recently, cardiac transplantation.[12,13] Numerous renal studies have shown that activation of the complement system, demonstrated by intragraft deposition of C4d and C3d, is associated with the development of chronic allograft nephropathy.[12–14] Taken together, these data suggest that AMR and complement activation are associated with the development of chronic rejection; however, few studies have addressed this in the context of cardiac transplantation.[15–17] A single study by Poelzl et al,[18] who investigated a small group of patients, showed that AMR episodes were related to CAV development. They analyzed endomyocardial biopsies taken over the first year post-transplantation for expression of C4d. They demonstrated that cardiac transplant patients with at least two C4d-positive endomyocardial biopsies in the first year post-transplantation were significantly more susceptible to the development of CAV, as assessed by intravascular ultrasound (IVUS) at 1 year.

The aim of the present study was to investigate the deposition of membrane complement split fragments, C4d and C3d, in protocol endomyocardial biopsies taken over the first 2 years post-transplantation to determine whether the incidence of complement activation was associated with the development of CAV.

Methods

Ethics approval

Use of archived myocardial biopsy tissue for this project and access to patient information was granted ethics approval from Peterborough and Fenland Research Ethics Committee (04/Q0106/11).

Patient population and study design

Fifty-two patients evaluated from 1991 to 1997 were identified for this study. The CAV group consisted of 26 patients with irreversible CAV, as defined by angiography within 4 years of transplantation (age 49 ± 11 years, 4 females). A control group of patients ($n = 26$) were matched to cases with at least 8 years of CAV-free angiography (age 49 ± 8.5 years, 2 females), at an individual level, by transplant date to ensure that changes over time did not confound the comparison. In addition, at a group level, cases and controls were matched for pre-transplant diagnosis, age and cytomegalovirus (CMV) status. Despite this, in the CAV-positive group, more patients had a pre-transplant diagnosis of ischemic heart disease, an organ from an older donor, and a slightly longer ischemic time at transplantation, although the differences were not statistically significant (Table 1). However, case–control pairs did not differ with regard to gender or CMV status of both recipients and donors (Table 1).

Table 1 Summary of Case–Control Pairs

	No CAV ($n = 26$)	CAV ($n = 26$)	p-value
Recipient gender (male)	22 (85%)	24 (92%)	0.668
Donor gender (male)	19 (73%)	22 (85%)	0.499
Mean (SD) recipient age (y)	49 (11)	49 (9)	1.000
Mean (SD) donor age (y)	31 (12)	49 (9)	0.056
Recipient CMV-positive	15 (58%)	15 (58%)	1.000
Donor CMV-positive	9 (35%)	13 (50%)	0.400
Pre-Tx diagnosis IHD	13 (50%)	19 (73%)	0.153
Mean (SD) ischemia time (min)	185 (64)	196 (67)	0.520

Pairs were matched in respect to recipient gender and age, donor gender, CMV status of donor and recipient, pre-transplant diagnosis and ischemic times. CAV, coronary artery vasculopathy; CMV, cytomegalovirus; IHD, ischemic heart disease, SD, standard deviation; Tx, transplant.

All patients were on a triple immunosuppressive regimen consisting of cyclosporine, steroids and azathioprine.

Endomyocardial biopsies

Endomyocardial biopsies were taken as routine monitoring of patients' progression post-transplantation at six timepoints: 1, 4 and 8 weeks; 6 months; and 1 and 2 years post-transplantation.

At the time the biopsies were taken, they were fixed in 10% neutral-buffered formalin for 1 hour before being processed to paraffin wax. Standard serial sections for diagnostic use were taken and stained with hematoxylin–eosin. The biopsies were graded for acute rejection using the 1990 classification of the International Society for Heart and Lung Transplantation (ISHLT).[19] For this study, biopsy hematoxylin–eosin stains were regraded for AMR using the ISHLT's 2005 guidelines,[10] looking for myocardial capillary injury with endothelial-cell swelling and intravascular macrophage accumulation. Interstitial edema or hemorrhage can also be present with neutrophils in and around capillaries. If one or more of these features could be seen throughout the biopsy, then it was considered potentially AMR-positive and, in a clinical setting, staining for the complement markers and macrophage marker CD68 would be performed.