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Routine C4d immunohistochemistry in cardiac allografts: Long-term outcomes

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BACKGROUND: In the past decade, C4d has emerged as a potential marker for antibody-mediated rejection (AMR); however, evidence on its use as a prognostic tool has been controversial. Although the International Society for Heart and Lung Transplantation guideline recommends early routine surveillance of C4d in heart transplantation, there is no consensus on its value in the pathologic assessment of AMR. Herein we present a correlation analysis of C4d immunoreactivity in endomyocardial biopsies with clinical cardiac dysfunction, cellular rejection, human leukocyte antigen (HLA) status, cardiac allograft vasculopathy (CAV) and death.

METHODS: A total of 5,840 endomyocardial biopsies from 296 heart transplant recipients (January 2004 to December 2014) were stained prospectively for C4d. Strong, diffuse endothelial staining was considered positive. All patients had at least 1 year of follow-up. Positive C4d staining was present in 53 biopsies from 28 patients. Sixteen of 28 patients had clinically significant cardiac dysfunction at the time of positive biopsy. In C4d-positive patients, the mean panel-reactive antibody (PRA) level was 33%. Ten patients demonstrated a first C4d positivity within the first year post-transplant, whereas 18 patients had C4d positivity after 1 year post-transplant. At autopsy, all 11 C4d-positive patients examined demonstrated cardiac allograft vasculopathy (CAV) as the underlying cause of death. In contrast, only 2 of 8 (25%) C4d-negative patients had CAV at autopsy. In the surviving cohort, there was an angiographic diagnosis of higher-than-moderate CAV in 10 patients (3.8%).

RESULTS: C4d-positive patients contributed to 67% of the overall institutional mortality in heart transplant recipients. Late C4d positivity (>1 year post-transplant) demonstrated an even higher risk for developing CAV and poor prognosis than early C4d positivity (within 1 year). In the C4d-negative group with postmortem examination, 75% (6 of 8) deaths were due to non-cardiac causes.

CONCLUSIONS: Our findings show a positive association of C4d with CAV and death. We identified a prognostic role for C4d in heart transplantation warranting routine long-term detection of this marker in the pathologic evaluation of cardiac AMR.

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Over the past decade, the utility of immunohistochemical detection of C4d has gained importance as a tool for the pathologic diagnosis of antibody-mediated rejection (AMR) in heart transplantation. This is underscored by a comparison of the 2005 and 2011 consensus reports of the International Society for Heart and Lung Transplantation (ISHLT). The 2005 report did not advocate routine detection

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of C4d until pathologic hallmarks of AMR were present on hematoxylin-and-eosin (H&E) evaluation of endomyocardial biopsies (EMBs). However, the subjective nature of these findings and interobserver variability in the H&E interpretation for AMR rendered it necessary for modification of the ISHLT consensus report. The recent ISHLT Working Formulation for the pathologic diagnosis of AMR recommends surveillance immunostaining at 2 and 4 weeks after transplant and subsequently at the time of serum alloantibody assessments at 1, 3, 6 and 12 months post-transplantation. The ISHLT does not recommend either immunofluorescence (IF) or immunohistochemical (IHC) methods, leaving the staining method to each institution's discretion. In the 2013 ISHLT Working Formulation for the diagnosis of AMR, the choice is specifically between IHC C4d and CD68 (macrophage) staining and IF C4d and C3d staining.[1] For the diagnosis of pAMR (I^+), the presence of C4d staining is considered sufficient.

Despite these guidelines and an increase in our understanding of the risk factors and adverse outcomes associated with AMR after heart transplantation, there remains a lack of clarity regarding the schedule and utility of C4d in the appropriate work-up of EMBs for cardiac AMR. It has been proposed that the trigger for IHC staining >1 year from transplant should be clinical or serologic data in centers that do not routinely perform such testing. Since January 2004 we have prospectively immunostained all routine EMBs for C4d. Our aim was to evaluate the prospective utility of immunohistochemical detection of C4d in both adult and pediatric heart transplant recipients and to correlate with clinical cardiac dysfunction, cellular rejection, HLA status, cardiac allograft vasculopathy (CAV) and death.

Methods

Routine immunohistochemical analysis of C4d was performed on all diagnostic surveillance and symptomatic EMBs on all cardiac transplant recipients followed at the University of Chicago Medical Center over a 12-year period. Clinical data were collected from patients' electronic medical records. Patients with a heart transplant date between January 2004 and January 2014 and with at least 1 year of follow-up were included in this study. Prospective data were collected until January 2016, culminating the 12-year prospective analysis. Demographics, pre-transplant alloantibody testing and clinical cardiac dysfunction at the time of C4d positivity were all recorded. When available, donor-specific antibody testing results were recorded. The study was approved by the local institutional review board.

All patients received either anti-thymocyte globulin (Thymoglobulin; Genzyme Corp., Cambridge, MA) or an anti—interleukin-2 receptor antagonist (Simulect; Novartis Corp., East Hanover, NJ) induction, followed by triple drug—based immunosuppression with tacrolimus or cyclosporine, mycophenolate mofetil and tapering oral glucocorticoids, according to our institutional protocol. Adjunctive therapy (mammalian target-of-rapamycin [mTOR] inhibition) is used for clinical indications of CAV or renal insufficiency at the discretion of the treating provider.

Sensitized patients with a panel-reactive antibody (PRA) level of 80% received peri-operative plasmapheresis, and then again for 3 to 5 days after transplantation, based on retrospective crossmatch results and clinical status. This practice was common before the availability of virtual crossmatching. Surveillance EMBs were typically performed weekly for the first month, every 2 weeks for the second month and then monthly for the first year, with less frequent and annual biopsies thereafter. Infants and children have fewer EMBs per protocol.

Clinical data included serial hemodynamics and echocardiogram findings. Definition of graft dysfunction included either echocardiographic decline in left ventricular function to at least moderate dysfunction (ejection fraction [EF] $<40\%$), or an elevated pulmonary capillary wedge of left ventricular end-diastolic pressure of >22 mm Hg. The definition of clinical CAV was in accordance with the ISHLT 2010 guidelines for grading CAV.[2] A value of 2 (moderate) or greater was considered clinically relevant. The outcomes of graft dysfunction, cellular rejection, CAV and death or retransplant were recorded.

EMBs were graded for acute cellular rejection (ACR) according to the ISHLT guidelines (1990/2005).[3,4] Assessment for C4d was performed by paraffin immunohistochemistry, as reported previously.[5] Only strong, diffuse endothelial cell staining was interpreted as positive C4d (Figure 1). Strong, diffuse endothelial cell staining was defined as $>50\%$ capillaries with continous, circumferential 3^+ staining on a scale from 1 to 3, where 3 is the strongest. Serum and interstitial staining patterns were considered negative (Figure 2). CAV was assessed by angiography, at autopsy or at explant for retransplant. CAV was pathologically defined as significant intimal fibrosis of the coronary arteries (Figure 3).

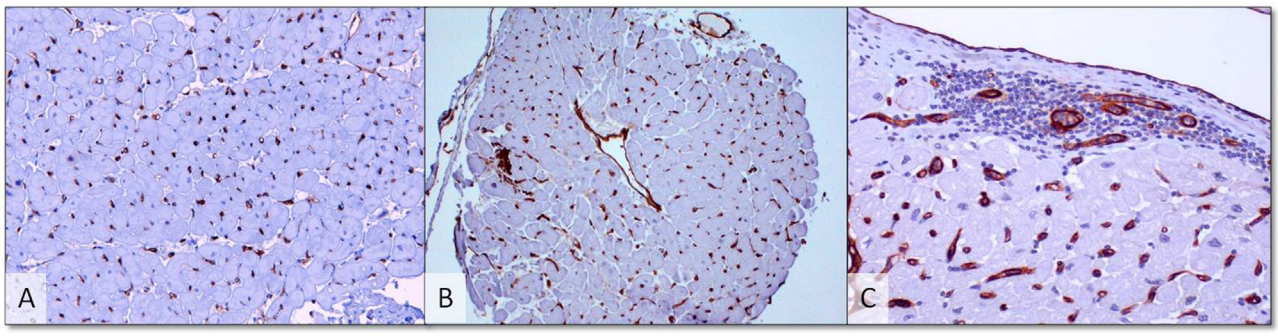

Figure 1 Positive immunohistochemical (IHC) staining for C4d. (A) Only strong, diffuse endothelial staining of all capillaries, as seen in this low power photomicrograph, is considered positive. (B) Strong endothelial staining is also seen in larger vessels. (C) High-power image shows strong endothelial staining of capillaries, including those present within the Quilty lesion, with staining of endocardium (endothelial cells only).