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Utility of C4d immunostaining in the first year after pediatric and young adult heart transplantation

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BACKGROUND: C4d assessment of endomyocardial biopsies (EMBs) after heart transplantation (HTx) has been widely adopted to aid in the diagnosis of antibody-mediated rejection (AMR), yet it remains unclear whether or not to assess all patients routinely and with what frequency/duration. In this study we sought to evaluate the utility of routine C4d immunostaining in the first year after pediatric and young adult HTx.

METHODS: We reviewed pre-transplant alloantibody and clinical data, including serial EMB reports, on all 51 patients who received HTx at our center since we instituted routine C4d staining of all first-year EMBs. C4d was considered positive if diffuse capillary staining ($\geq 2^+$) was present. Rare/focal capillary staining or absence of staining was considered negative.

RESULTS: Twenty-six of 406 first-year EMBs (6%) were C4d$^+$ in 6 (12%) patients. Sixty-five percent of all C4d$^+$ EMBs occurred by 30 days post-transplant. Five of 6 patients had pre-transplant donor-specific antibody (DSA) $\geq$4,000 MFI. The sixth patient had neither pre-transplant anti-HLA antibodies nor a positive donor-specific cytotoxicity crossmatch (DSXM), but there was clinical concern for AMR. Among the entire cohort, 5 of 10 patients with pre-transplant DSA $\geq$4,000 MFI and/or a positive DSXM were C4d$^+$ compared with only 1 of 41 without (50% vs 2%; $p = 0.001$).

CONCLUSIONS: In the first year after HTx, C4d$^+$ occurred early and only in children and young adults with pre-transplant DSA or with clinical suspicion of AMR. Although our data suggest that assessment limited to the first 90 days post-transplant in patients with pre-transplant DSA $\geq$4,000 MFI may be appropriate in the absence of clinical concern for AMR, further research is needed to determine the optimum strategy for post-transplant surveillance.

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Although much has been learned in the last decade about risk factors for and adverse outcomes associated with antibody-mediated rejection (AMR) after heart transplantation, there is continued uncertainty about the appropriate work-up of endomyocardial biopsies (EMBs) for AMR.[1] The 2005 consensus report of the International Society for Heart and Lung Transplantation (ISHLT) recommended immunostaining for AMR not be performed routinely but should be reserved only for those EMBs showing hallmark pathologic features of AMR.[2] The difficulty with this approach is that classic histologic features of AMR are not readily apparent on all EMBs and interpretation is somewhat subjective.[3] The recent ISHLT working formulation for pathologic diagnosis of AMR recommends surveillance

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immunostaining be performed at 2 and 4 weeks after transplant and then at the time of serum alloantibody assessments (i.e., 1, 3, 6 and 12 months).[4] It also recommends that C4d assessment of EMBs be included, at a minimum, in the diagnostic panel, regardless of whether immunofluorescence (IF) or immunohistochemistry (IC) is utilized.

Since May 2007 we have prospectively utilized routine IC staining for C4d of all first-year EMBs. The purpose of the present study was to evaluate the utility of routine IC staining for C4d in the first year after pediatric and young adult heart transplantation.

Methods

After obtaining institutional review board approval, we reviewed the medical records of all patients who underwent heart transplantation at the Children's Hospital of Pittsburgh of UPMC, since we began routine assessment for C4d on all first-year EMBs. Patients who had at least one EMB through April 2011 were included in this study. Demographics, pre-transplant alloantibody testing, retrospective crossmatch results and clinical data were reviewed. Pre-transplant alloantibody assessment consisted of complement-dependent cytotoxicity (CDC) panel-reactive antibody (PRA) testing in all patients and an anti-HLA antibody profile using the Luminex platform (LABScreen; One Lambda, Canoga Park, California) in all but 2 patients, both of whom had CDC PRA 0% and negative enzyme-linked immunoassay (ELISA) anti-HLA antibody assessments. Clinical data included EMB reports and serial hemodynamics with temporally linked echocardiogram findings. Based on these data, we defined graft dysfunction as present if any of the following were observed: shortening fraction $<28\%$ or decrease in shortening fraction of $>12\%$ from prior echocardiogram; pulmonary artery wedge pressure >24 mm Hg; mixed venous oxygen saturation $<56\%$; or cardiac index <2.2 liters/min/m^2.

Data from EMB reports were abstracted by one investigator (Y.X.) who was blinded to the clinical information. Because we could not exclude that a pathology diagnosis of AMR was made solely on the basis of C4d$^+$ immunostaining, we did not record any mention of AMR from the EMB report. Instead the EMB reports were reviewed for acute cellular rejection (ACR) grade; the presence or absence of C4d immunostaining; and the presence or absence of histologic features that have been ascribed to cardiac AMR, which include endothelial activation, intracapillary neutrophils, intracapillary macrophages, intracapillary thrombi, hemorrhage and edema.[1,2,4–7] We then quantified an "AMR score" for each EMB by assigning 1 point to each histologic feature of cardiac AMR described and summing these values. We chose to weigh each feature equally because of the current uncertainty regarding the histologic findings of cardiac AMR, including the histologic features of "early" AMR and the significance of intravascular macrophages.[4] Also, because EMBs were interpreted during the clinical care of these patients between 2007 and 2011, the 2011 working formulation guidelines[4] were not available to be applied in real time.

Assessment for C4d was performed by paraffin IC as previously reported.[8] Diffuse brown-colored staining of capillary endothelial cells ($\geq 2^+$) was interpreted as positive C4d staining.[9] Staining of other vessels or serum was not considered positive.

All patients received thymoglobulin induction and corticosteroids during the first 5 to 7 days after transplantation. Tacrolimus was initiated on Days 2 to 4 post-operatively and patients were maintained on tacrolimus plus adjunctive therapy (either sirolimus or mycophenolate mofetil). In addition, patients with a positive donor-specific CDC crossmatch received daily plasmapheresis for 3 to 5 days after transplantation with continuation of plasmapheresis based on clinical status, alloantibody profile and EMB findings. These patients were also maintained on weaning doses of corticosteroids during the first year post-transplant. Surveillance EMBs were typically performed 6 to 8 times in the first year at 7 to 14 days and 1, 2, 4, 6 to 7, 9 to 10 and 12 months, with infants having slightly fewer EMBs.

Statistical analysis

Data are presented as mean $\pm$ standard deviation, median (range) or count (percent). Group comparisons were made using Wilcoxon's rank-sum test and the Fisher's exact test, as appropriate. The Kruskal–Wallis test was used to analyze associations of AMR score with graft dysfunction and C4d$^+$. Data were analyzed using STATA, version 10.1 (StataCorp LP, College Station, TX), and all comparisons were 2-sided with significance level of 0.05.

Results

Study cohort

Fifty-one patients met the inclusion criteria. Their median age at transplantation was 6.6 years (15 days to 20.4 years) and reasons for transplant were cardiomyopathy/myocarditis (59%), congenital heart disease (31%) and re-transplantation (10%). The cohort was 58% male, 68% white and 16% black. Median pre-transplant CDC PRA was 0% (range 0% to 89%) and 10 patients (20%) had a positive donor-specific CDC crossmatch ($n = 2$), pre-transplant donor-specific antibody (DSA) $\geq$4,000 mean fluorescent intensity (MFI) ($n = 3$) or both ($n = 5$). Four other patients (8%) had at least 1 pre-transplant DSA 1,000 to 3,999 MFI with a negative donor-specific CDC crossmatch. Two patients (4%) who did not have pre-transplant Luminex assessment had pre-transplant CDC PRA of 0% with negative ELISA anti-HLA antibody assessments, and negative donor-specific CDC crossmatches.

All but 2 recipients (96%) are alive as of the time of this report, with a median post-transplant follow-up of 2.9 years (range 1.0 to 4.9 years). Two infant recipients died at 27 and 67 days post-transplant having had 1 and 2 EMBs, respectively. One died of complications of adenovirus and the other of severe ACR or recurrent myocarditis. Neither was pre-sensitized, had a positive donor-specific CDC crossmatch, or had C4d$^+$ EMB.

Endomyocardial biopsies

A total of 406 first-year EMBs were assessed for C4d. The median number of first-year EMBs per patient was 8 (range 1 to 15). One first-year EMB among the cohort was inadvertently not stained for C4d. It was obtained on Day 161 post-transplant from a non-allosensitized male in whom all other first-year EMBs ($n = 6$) were C4d$^-$.