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Use of complement binding assays to assess the efficacy of antibody mediated rejection therapy and prediction of graft survival in kidney transplantation

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ABSTRACT

Background: The Luminex® single antigen bead assay (SAB) is the method of choice for monitoring the treatment for antibody-mediated rejection (AMR). A ⩾50% reduction of the dominant donor-specific antibody (IgG-DSA) mean fluorescence intensity (MFI) has been associated with improved kidney allograft survival, and C1q-fixing DSA activity is associated with poor outcomes in patients with AMR. We aimed to investigate if C1q-DSA can be used as a reliable predictor of response to therapy and allograft survival in patients with biopsy-proven AMR.

Methods: We tested pre- and post-treatment sera of 30 kidney transplant patients receiving plasmapheresis and low-dose IVIG for biopsy-proven AMR. IgG-DSA and C1q-DSA MFI were measured and correlated with graft loss or survival. Patients were classified as nonresponders (NR) when treatment resulted in <50% reduction in MFI of IgG-DSA and/or C1q-DSA was detectable following therapy.

Results: Differences in the percentage of patients deemed NR depended upon the end-point criterion (73% by reduction in IgG-DSA MFI vs. 50% by persistent C1q-DSA activity). None of the seven patients with <50% reduction of IgG-DSA but non-detectable C1q-DSA-fixing activity after therapy experienced graft loss, suggesting that C1q-DSA activity may better correlate with response. Reduction of C1q-DSA activity predicted graft survival better than IgG-DSA in the univariate Cox analysis (20.1% vs. 5.9% in NR; log-rank P-value = 0.0147).

Conclusions: A rapid reduction of DSA concentration below the threshold required for complement activation is associated with better graft survival, and C1q-DSA is a better predictor of outcomes than IgG-DSA MFI reduction.

1. Introduction

After the pivotal work of Patel and Terasaki in 1969, a negative crossmatch reaction became an essential prerequisite to proceeding with kidney transplantation [1]. When complement-dependent cytotoxicity or flow cytometry crossmatch were the only available technologies to assess for the presence of antibodies against the donor (DSA) in the recipient's sera, the involvement of the histocompatibility laboratory was significantly limited during the post-transplant stage. The need for viable donor cells, interference from immunosuppressant agents, and the low specificity of the available methods limited the post-transplant monitoring of *anti*-HLA antibodies.

These limitations have been overcome by recent technological advances that use recombinant HLA molecules for the detection of antibodies by highly sensitive methods such as Luminex® [2].

Abbreviations: AMR, antibody-mediated rejection; DSA, donor-specific antibody; HEL, high expression loci; IVIG, intravenous immunoglobulin; LEL, low expression loci; MFI, mean fluorescent intensity; NR, nonresponders; R, responders; SAB, single antigen bead Luminex® assay.

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Currently, post-transplant DSA monitoring using single antigen beads (SAB) has been incorporated into clinical practice and has complemented histological findings in the identification of injury from antibody-mediated rejection (AMR) [3].

Although the U.S. Food and Drug Administration has not officially approved SAB as a quantitative method, many studies have demonstrated a strong association between SAB-measured antibody strength and graft survival. Numerous reports have demonstrated that the risk of post-transplant AMR increases proportionally with the peak mean fluorescence intensity (MFI) value of the dominant preformed DSA in pre-transplant sera [4]. The same has been observed with *de novo* DSA, where higher titer and strength measured in MFI units correlate with a markedly higher incidence of graft loss [5].

On the basis of these studies, Luminex® SAB has become the method of choice to monitor the efficacy of AMR treatment and to predict the risk of graft failure. A $\geqslant$50% reduction in MFI or titer of the dominant IgG-DSA after treatment for AMR is the accepted indicator of better kidney allograft survival [6,7]. However, we and other groups have shown that in some patients, the strength of IgG-DSA frequently remains unaffected by antibody removal techniques, especially DSA with class II specificities [8,9].

This apparent lack of reduction in antibody strength in response to plasma exchange can result from diverse factors that affect the quantification of DSA by MFI, such as assay saturation due to high antibody concentration. Steric hindrance (or the "prozone" effect) in which macromolecules present in the sera interfere with DSA binding to their antigenic target, reduces assay sensitivity. These experimental hurdles are usually overcome through sera dilution or removal or inactivation of serum macromolecules (i.e., IgM and autologous complement) through heat, chemical, or physical inactivation. Elimination of these hurdles allows for better DSA binding to their cognate antigen and more accurate assessment of DSA strength manifested as an increase in DSA strength usually observed after plasmapheresis [10].

Another limitation of the SAB assay is its inability to distinguish antibody subclasses capable of fixing the complement cascade (i.e., IgG1 and IgG3) from those that do not (i.e., IgG2 and IgG4). Since complement fixation by DSA is an important mechanism for injury in AMR, incorporation of the C1q binding assay provides relevant information about the biological activity of DSA and can be used as predictor of graft survival [11].

Taking into account these limitations of IgG-DSA, we sought to investigate if C1q-DSA activity is a better predictor of therapy response and allograft survival after AMR treatment than a 50% reduction in the strength of the dominant IgG-DSA.

2. Materials and methods

2.1. Patient selection

For this study, we identified 30 kidney transplant recipients diagnosed with and treated for acute AMR between September 2010 and August 2012 at the University of Michigan Transplant Center. These patients had received their transplants between March 2000 and July 2011.

All patients were treated with triple immunosuppression that included a calcineurin inhibitor (CNI), mycophenolate mofetil, and prednisone. Patients transplanted before and after January 1, 2011 received cyclosporine or tacrolimus, respectively, as the CNI of choice.

2.2. AMR diagnosis and treatment

Diagnosis of AMR was defined as graft dysfunction indicated by an increase in serum creatinine $\geqslant$15% above baseline, evidence of histological antibody injury in the renal allograft biopsy, and the detection of *anti*-HLA DSA in the recipient's sera by SAB methods. Although some patients in our cohort had histological evidence of antibody mediated injury but negative C4d staining, they were treated similarly as other C4d positive patients. C4d negative ABMR was recently formally recognized in the Banff 2013 criteria [3]. Patients received six sessions of plasmapheresis every other day, followed by IVIG 100 mg/kg after sessions 1–5, and 500 mg/kg after session 6 for the treatment of AMR, Due to the interference of a high dose IVIG with single antigen assay, samples were collected after the plasmapheresis and before the infusion of IVIG. Concurrent cellular rejection was treated with pulse corticosteroids or rabbit *anti*-thymocyte globulin (Thymoglobulin®, Genzyme, Cambridge, MA, USA).

2.3. Graft and patient outcomes

Patients were followed until December 2014. Return to dialysis or death were used as indicators of graft failure and used as endpoints for the survival analysis. Follow-up biopsy was not available on every patient.

2.4. Donor-Specific antibody IgG and C1q detection

The identification and measurement of IgG-DSA was performed utilizing a Luminex® bead array, where each bead is coated with a single recombinant HLA molecule (LABScreen®, One Lambda Inc., Thermo Fisher Scientific Inc., Canoga Park, CA). Three different lots of LABScreen® reagents were used during the study period: Class I-LS1A04NC 005/006/007 and the Class II-LS2A01 007/008/009. The pre-treatment sample was obtained at the time of the biopsy and the post-treatment sample was obtained after the completion of AMR treatment as described above. All pre- and post-treatment sera were heat-treated (56 °C for 30 min) and tested following the manufacturer's protocol. Our laboratory uses heat inactivation on undiluted samples to eliminate interfering macromolecules, such as IgM or autologous complement (incorrectly known as prozone effect). Anti HLA-antibodies were assigned based on patterns of reactivity, background level and individual bead performance. The strength of the IgG-DSA is measured in the semi-quantitative unit mean fluorescence intensity (MFI), using a cut-off of 700 MFI. This cut off was selected by comparison with our flow cytometric crossmatch. Antibodies in this range are capable to produce a slight median channel shift, most likely when these antibodies are directed to HLA antigens with higher expression on the lymphocytes surface.

Retrospectively, all sera samples were tested with the C1q binding assay (C1qScreen™, One Lambda Inc., Canoga Park, CA) following the manufacturer's protocol. The same SAB array for IgG-DSA was used for the C1q-DSA binding assay. Results were documented as positive or negative using an MFI cut-off of 500 MFI, and the MFI value for each C1q-DSA was collected for continuous variable analysis. We decided to use this cut-off to compare our work with previous published work that correlate the C1q reaction with AMR [12].

2.5. Prediction of treatment outcomes

To evaluate the performance of IgG-DSA MFI in outcome prediction, we used an MFI cut-off of $\geqslant$50% reduction. The selection of this cut-off was based on previous publications by Everly et al., which correlated reduction of DSA-IgG MFI with AMR treatment outcome [6]. Patients classified as responders (R) showed a $\geqslant$50% reduction of IgG-DSA MFI after treatment, and those with <50% MFI reduction were classified as nonresponders (NR). For outcome prediction analysis using C1q-DSA, we also divided patients