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Pre-transplant screening for non-HLA antibodies: Who should be Tested?

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ABSTRACT

Retrospective studies of angiotensin II type 1 receptor antibodies (AT1R-Ab) and anti-endothelial cell antibodies (AECA) have linked these antibodies to allograft injury. Because rising healthcare costs dictate judicious use of laboratory testing, we sought to define characteristics of kidney transplant recipients who may benefit from screening for non-HLA antibodies. Kidney recipients transplanted between 2011 and 2016 at Johns Hopkins, were evaluated for AT1R-Ab and AECA. Pre-transplant antibody levels were compared to clinical and biopsy indications of graft dysfunction. Biopsies were graded using the Banff 2009–2013 criteria. AT1R-Ab and AECA were detected using ELISA and endothelial cell crossmatches, respectively. AT1R-Ab levels were higher in patients who were positive for AECAs. Re-transplanted patients ($p < 0.0001$), males ($p = 0.008$) and those with FSGS ($p = 0.04$) and younger ($p = 0.04$) at time of transplantation were more likely to be positive for AT1R-Ab prior to transplantation. Recipients who were positive for AT1R-Ab prior to transplantation had increases in serum creatinine within 3 months post-transplantation ($p < 0.0001$) and developed abnormal biopsies earlier than did AT1R-Ab negative patients (126 days versus 368 days respectively; $p = 0.02$). Defining a clinical protocol to identify and preemptively treat patients at risk for acute rejection with detectable non-HLA antibodies is an important objective for the transplant community.

1. Introduction

Repeated injury to a transplanted kidney due to rejection is one significant predictor of graft loss [1–3]. The development of highly sensitive and specific assays for detecting and characterizing HLA antibodies has allowed recognition of the role of non-HLA antibodies in graft injury in patients negative for donor specific HLA antibody (HLA-DSA). Defining an appropriate clinical protocol to identify and preemptively treat kidney transplant recipients who are at risk for acute rejection with detectable non-HLA antibodies is an important objective for the transplant community. One such antibody, specific for angiotensin II type 1 receptor (AT1R-Ab), has been shown to be involved in poor transplant outcomes. Multiple single center retrospective studies have documented acute rejection and in some cases, graft loss associated with presence of AT1R-Abs [4–13].

Anti-endothelial cell antibodies (AECAs) detected in the endothelial cell crossmatch (ECXM) have also been identified in patients who developed severe antibody mediated rejection in the absence of HLA-DSA [14–16]. AT1R is expressed on endothelial cells [17,18] and we have shown that most patients who are positive for AT1R-Ab also have a positive ECXM [19].The ECXM also detects antibodies against targets other than AT1R [20]. Biopsy evaluations at our center have shown histological evidence of increased microcirculation inflammation in patients with positive AT1R-Ab and/or AECAs both in the presence or absence of HLA-DSA [14,19].

In 2013, two of the largest studies in kidney transplantation reported conflicting results on a correlation between pre-transplant AT1R-Ab and kidney allograft outcome. Giral et al. [21] reported an increased incidence of graft loss at 3 years in patients with a pre-transplant AT1R-Ab level > 10 units/ml. In contrast, Taniguchi et al.

Abbreviations: ABOi, ABO incompatible; AECA, anti endothelial cell antibody; AMR, antibody mediated rejection; AT1R, angiotensin II type 1 receptor; AT1R-Ab, anti-angiotensin II type 1 receptor antibody; HLA-DSA, donor specific HLA antibody; HLAi, HLA incompatible; ECXM, endothelial cell crossmatch; FCXM, flow cytometric crossmatch; FSGS, focal segmental glomerulosclerosis; MFI, mean fluorescent intensity; MPGN, membranoproliferative glomerulonephritis

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[22] did not find a correlation between pre-transplant AT1R-Ab and graft outcome but did find a significant association between post-transplant AT1R-Ab and graft failure. However, in the study by Taniguchi, 84% of patients who developed abnormal biopsies tested positive for AT1R-Ab prior to transplant and maintained the antibody post-transplant.

Transplant opportunities for sensitized patients have increased through desensitization treatment, kidney paired donation, and changes in the kidney allocation system [23]. The incidence of antibody mediated rejection in the presence of antibodies other than HLA was estimated to be between 10 and 40% [24], however, since centers are not testing for presence of non-HLA antibodies in patients who are positive for HLA antibodies, the true incidence of antibody mediated rejection associated with presence of non-HLA antibodies in highly sensitized patients is not known. In this study, we evaluate the value of pre-transplant assessment of AT1R-Ab and other AECAs in patients with increased immunological risks and explore the possibility that additional screening for non-HLA antibodies may provide an opportunity for pre-emptive intervention that could improve long term allograft survival.

2. methods

2.1. Study population

Pre-transplant AT1R-Ab test results were available for 170 recipients of a kidney transplant performed between May 1st, 2011 and July 1st, 2016, at the Johns Hopkins Comprehensive Transplant Center. We excluded 26 recipients whose serum date was > 2 months prior to their transplant date. The remaining 144 recipients had a specimen obtained < 50 days prior to transplantation. The study group included 3 categories of patients based on the time of pre-transplant serum evaluation and the reason for testing: group 1: 40 patients whose pre-transplant sera were tested retrospectively for AT1R-Ab and/or AECA to investigate allograft dysfunction that was not explained by HLA-DSA; group 2: 71 consecutive high risk patients transplanted with a living donor; and group 3: 33 consecutive highly sensitized kidney transplant recipients who received a deceased donor organ. An ECXM was performed within 30 days prior to transplantation for 74 of the 144 recipients. All data were obtained under an approved IRB protocol.

2.2. Pre-transplant and post-transplant treatment

Induction treatment consisted of Thymoglobulin® (1.5 mg/kg per day for 5 days), basiliximab (2 mg/kg), or alemtuzumab (30 mg single dose at time of transplantation). Desensitization for HLA and/or ABO incompatibility consisted of single-volume plasmapheresis, and either cytomegalovirus immune globulin (Cytogam®; 100 mg/kg) [25] or human immune globulin (Gamunex®-C; 100 mg/kg). The number of plasmapheresis treatments varied based on the level of HLA-DSA, and ABO antibody prior to transplantation. Recipients received rituximab prior to transplant if their current donor had HLA mismatches repeated from a previous donor and/or in cases where the patient was to receive a kidney that was ABO and HLA incompatible (n = 47). Maintenance treatment consisted of mycophenolate mofetil (2 g/day) and tacrolimus (serum level of 8–10 ng/ml). AMR was treated with plasmapheresis and immune globulin (n = 35), eculizumab (n = 5) and/or splenectomy (n = 5).

2.3. Laboratory evaluations

HLA typing, HLA antibody detection, ECXM and AT1R-Ab testing were performed as previously described [19]. Briefly, recipient and donor HLA-A, B, C, DQ, DR and DP typing were performed by reverse sequence specific oligonucleotide assay (One Lambda LABType®). HLA-DSA was identified using multianalyte bead based assays performed on the Luminex® platform (Immucor-Lifecodes, Stamford, CT and One Lambda, Canoga Park, CA). HLA antibody levels were assigned as cytotoxic positive crossmatch level (CDC +), flow cytometric crossmatch positive (FCXM +), and FCXM- Luminex + (Lum +) using MFI values of ≥10,000; 4000 to < 10,000; and 2000 to < 4000 MFI respectively for HLA-A, HLA-B, and HLA-DR, and MFI values of ≥20,000; ≥16,000; and ≥4000 respectively for HLA-C, HLA-DQ and HLA-DP. MFI values of 1000 or lower that lacked specificity patterns were considered negative. Cytotoxic crossmatch tests were performed prior to transplantation, with positively selected T and B lymphocyte targets [26]. FCXM tests were performed as previously described [27] and were acquired on BD FACSCanto II using FACSDIVA software (BD Bioscience, Franklin Lakes, NJ). Hypotonic dialysis was performed to remove IgM autoantibodies and IgG immune complexes [28] from sera and sera for crossmatch testing were back-dialyzed to achieve isotonicity.

Angiotensin II type 1 receptor antibody (AT1R-Ab) testing was performed using quantitative ELISA (One Lambda, ThermoFisher). Anti-endothelial cell antibodies (AECAs) of the IgG isotype were detected using a flow cytometric crossmatch test (ECXM) performed with angiopoietin receptor positive peripheral blood endothelial cell precursors (ECPs) (XM-ONE; Absorber AB, Stockholm, Sweden).

2.4. Biopsy interpretation

Allograft biopsy was performed either per protocol at 1, 3,6 and 12 months or for cause when serum creatinine increased ≥20% over baseline. Biopsies were graded using the Banff 2009–2013 criteria. Biopsy descriptions were extracted from the patient electronic records and were reviewed by a pathologist. The first biopsy for each recipient, with one or more of the characteristics described in Table 3 was labeled “Abnormal”. Patients who had sequential biopsies, up to the end of follow up that did not show any of the characteristics listed in Table 3 were considered to have “normal biopsies”.

2.5. Statistics

We examined the change in serum creatinine over time using a multilevel, mixed-effects generalized linear model with random effects at patient level. Because the distribution of serum creatinine is skewed to the right, we modeled the logarithmic form of serum creatinine. Our dependent variables were time (number of months since transplant) and AT1R-Ab status. We made spline terms of time period of 0 days – 1 week, 1 week – 1 month, 1 month – 3 months, and after 3 months since transplant. We performed both unadjusted and adjusted models. Covariates in the adjusted model included age at transplant, race, gender, pre-transplant treatment, presence of HLA-DSA prior to transplant and post-transplant treatment for rejection. Specifically, pre-transplant treatments (the use of plasmapheresis and IVIG and/or rituximab) were considered as having an effect on baseline serum creatinine levels and early post-transplant, so we adjusted for these treatments at baseline and within the first 7 days post-transplant. Regressions were performed using Stata 14.1/MP for Windows (College Station, Texas). Comparisons between groups were assessed by one way analysis of variance. Continuous variables were compared using Student’s t test and categorical variables were compared using the χ^2 test and/or Fisher’s exact test. Kaplan-Meier survival curves were generated using GraphPad Prism 6 software. The p-values for the Kaplan-Meier survival curve were determined using the Gehan-Breslow-Wilcoxon method and p-values < 0.05 were considered statistically significant.