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Assessing the potential of angiotensin II type 1 receptor and donor specific anti-endothelial cell antibodies to predict long-term kidney graft outcome

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ABSTRACT

Endothelial cell antigens have been reported as potential targets for antibodies in the context of organ transplantation, leading to increased risk for graft failure. Serum samples from 142 consecutive living donor kidney recipients were tested for the presence of antibodies to angiotensin II – type 1 receptor (AT1R), donor endothelial cells, and donor HLA. Graft survival was monitored for five years post-transplant, and secondary outcomes, including biopsy-proven rejection, proteinuria, biopsy-proven vasculopathy, and renal function based on serum creatinine were also assessed for the first two to three years. AT1R antibody levels were positive (>17 U/mL) in 11.3%, 18.8% and 8.1% of patients pre-transplant, post-transplant and at time of indication biopsy, respectively. XM-ONE assay was positive in 17.6% of patients pre-transplant (7 IgG+; 15 IgM+; 3 IgG+/IgM+). Overall, 4 patients experienced antibody-mediated rejection (AMR), 31 borderline cellular rejection (BCR), 19 cellular rejection (CR) and 3 mixed AMR and CR within the first 24 months. While pre-existing and de novo donor-specific HLA antibodies were associated with graft failure and many secondary outcomes, no statistical association was found for either anti-endothelial or anti-AT1R antibodies, indicating that these tests may not be the best predictors of graft outcome in living donor renal transplantation.

1. Introduction

Antibodies specific for donor human leukocyte antigen (HLA) molecules are a known risk factor in renal transplantation. Since the development of the first lymphocytotoxic crossmatch by Patel and Teraski in 1969 [1], the techniques available to identify patients with pre-existing antibodies to donor HLA have increased in their complexity and sensitivity, allowing for better identification of patients at risk for antibody-mediated rejection (AMR). However, several studies have suggested that AMR can occur even in the absence of HLA mismatching. Within a large cohort of HLA-identical sibling renal transplants, Opelz and colleagues observed that an increase in the level of recipient HLA sensitization led to worse long-term outcomes, despite the lack of HLA mismatches [2]. Since this time, a number of non-HLA antigens have been identified that may be targeted by the humoral response during graft rejection.

Endothelial cells, which line the vasculature of the transplanted organ, are a potential target, as these are the first cells to come in contact with host immune cells following organ reperfusion [3–5]. The challenge of testing *ex vivo* for donor-specific antibodies to

Abbreviations: AT1R, angiotensin II type-1 receptor; HLA, human leukocyte antigen; AMR, antibody-mediated rejection; CR, cellular rejection; BCR, borderline cellular rejection; AECAs, anti-endothelial cell antibodies; SOC, standard of care; DSA, donor-specific antibody; ELISA, enzyme-linked immunosorbent assay; FSGS, focal segmental glomerulosclerosis; MICA/MICB, major histocompatibility complex class I chain related antigen A and B; UA, urinalysis.

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endothelial cell targets was addressed by the identification of Tie-2 receptor-expressing endothelial precursor cells in the peripheral blood [6]. Using a commercially available assay in which these precursors are isolated and used as targets in a flow cytometric crossmatch with recipient serum, Breimer and colleagues demonstrated that patients with donor-specific anti-endothelial cell antibodies (AECAs) were at a higher risk of developing graft rejection, even when negative by conventional lymphocyte crossmatch [7]. Other groups have also noted a higher rate of rejection in patients with donor-specific AECAs [8,9]; however, a study from our center found no correlation between a positive pre-transplant XM-One assay and overall graft outcome in living donor renal transplant recipients [10]. Another potential endothelial target is the angiotensin II type 1 receptor (AT1R), which is expressed on endothelial cells in many organs and mediates actions of the renin-angiotensin system, including vasoconstriction, cellular proliferation, and vascular remodeling [11,12]. Antibodies targeting this receptor were first identified in pregnant women with pre-eclampsia [13], and several recent studies have suggested that these agonistic antibodies may be a risk factor for kidney and heart allograft rejection and graft loss [14–18].

Given the potential for these non-HLA antibodies to mediate graft rejection, the aim of our current study was to assess the predictive ability of assays for anti-AT1R antibodies and anti-endothelial antibodies in risk stratification for living donor kidney transplant recipients, prospectively followed for 5 years.

2. Materials and methods

2.1. Patient population

150 consecutive living donor kidney recipients transplanted between January 2010 and December 2010 at the Comprehensive Transplant Center of Northwestern University were enrolled in this study, for which IRB approval was obtained. 8 patients were excluded due to either lack of sufficient Tie-2 positive pre-cursor endothelial cells to perform the XM-One assay or instance of acute graft failure within 48 h of transplant due to a surgically-related non-functioning graft. 88/142 patients received our institution's standard of care (SOC) induction protocol, comprised of alemtuzumab with rapid corticosteroid withdrawal and tacrolimus and mycophenylate maintenance. The remaining 54 patients were subjected to various immunosuppressive protocols including SOC without alemtuzumab, desensitization, an investigational agent trial, and an HLA-identical donor/recipient trial. Patient demographics are summarized in Table 1. Physicians were blinded to all anti-endothelial antibody testing results and patients were treated post-transplant as dictated by our institutional SOC or by a method dictated by enrollment in other clinical trials.

2.2. HLA testing

Intermediate to high-resolution HLA-typing was performed for both patients and donors at the HLA-A, -B, -C, -DRB1, -DQA1, -DQB1 loci, as well as -DPA1, and -DPB1 if the recipients were positive for anti-HLA-DP antibodies (LABType, One Lambda, Canoga Park, CA). Recipient serum was tested for the presence and strength of donor-specific HLA antibodies (HLA-DSA) by Luminex-based single antigen bead analysis (LABScreen, One Lambda). Our laboratory uses a rough cutoff of 1000 MFI to determine antibody positivity, as well as manual analysis of reaction patterns to adjust the cutoff higher or lower in the case of high background or shared epitope binding. The presence of HLA-DSA was assessed pre-transplant, at the time of final crossmatch, and at the time of 3- and 12-month protocol biopsy, as well as any indication post-transplant biopsies.

Table 1
Patient demographics of living donor kidney transplant recipients.

	SOC (n = 88)	Total Cohort (n = 142)
Gender		
Male	51 (58.0%)	81 (57.0%)
Female	37 (42.0%)	61 (43.0%)
Age		
	47.3 ± 14.2	47.2 ± 14.3
Race		
Caucasian	54 (61.3%)	79 (55.6%)
African American	17 (19.3%)	33 (23.2%)
Hispanic	12 (13.6%)	22 (15.5%)
Asian	5 (5.7%)	7 (4.9%)
Middle Eastern	0 (0%)	1 (0.7%)
BMI		
	28.8+/−6.7	28.2+/−6.3
Underlying disease[a]		
Hypertension	56 (63.6%)	74 (52.1%)
Diabetes mellitus	33 (37.5%)	45 (31.7%)
Polycystic Disease	12 (13.6%)	15 (10.6%)
FSGS[b]	3 (3.4%)	8 (5.6%)
IgA Nephropathy	6 (6.8%)	8 (5.6%)
Membranous Nephropathy	3 (3.4%)	4 (2.9%)
Systemic Lupus	2 (2.3%)	5 (3.5%)
Class I PRA		
0% (Not Sensitized)	46 (52.3%)	67 (47.2%)
1–80% (Sensitized)	38 (43.2%)	63 (44.4%)
>80% (Highly Sensitized)	4 (4.5%)	8 (5.6%)
Class II PRA		
0% (Not Sensitized)	68 (77.3%)	104 (73.2%)
1–80% (Sensitized)	17 (19.3%)	30 (21.1%)
>80% (Highly Sensitized)	3 (3.4%)	8 (5.6%)

[a] Both primary and secondary diagnoses were included in underlying disease and therefore will overlap in some categories.
[b] FSGS – Focal Segmental Glomerulosclerosis.

2.3. AT1R testing

Recipient serum was tested for the presence of anti-AT1R antibodies using the commercially available AT1R ELISA kit (One Lambda). AT1R ELISA was performed on pre-transplant samples, as well as on samples obtained at the time of 3- and 12-month protocol biopsy or indication biopsy. Normal ranges for AT1R ELISA were confirmed by testing sera from 20 HLA non-sensitized male controls with no known medical conditions (Supplementary Fig. 1). Cut-off values were determined based on HLA non-sensitized male control results, previously published work [15,19] and manufacturer's recommendations (Negative <17 U/mL, Positive ≥17 U/mL, unless otherwise noted).

2.4. XM-ONE testing

The presence of donor-specific anti-endothelial antibodies in recipient serum was determined using the commercially available XM-ONE assay (AbSorber AB, Stockholm, Sweden). The XM-ONE assay was performed using enriched donor precursor endothelial cells and recipient serum obtained for final lymphocyte flow-cytometric crossmatch. XM-ONE was performed in parallel with final lymphocyte crossmatch. The presence of both donor-specific IgG and/or IgM anti-endothelial antibodies was assessed. XM-ONE cut-off values were assigned as >64 channels for both IgG and IgM as previously described [10]. All positive donor-specific XM-ONE assays were followed up with autologous recipient XM-ONE assay.

2.5. MICA testing

Recipient serum was tested for the presence of anti-MICA antibodies using the commercially available LABScreen MICA assay