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Angiotensin II Type 1 receptor antibodies are associated with inflammatory cytokines and poor clinical outcomes in pediatric kidney transplantation

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Abstract

Angiotensin II type 1 receptor (AT1R)-antibody has been linked to poor allograft outcomes in adult kidney transplantation. However, its clinical consequences in children are unknown. To study this, we examined the relationship of AT1R-antibody with clinical outcomes, biopsy findings, inflammatory cytokines, and HLA donor specific antibodies (DSA) in a cohort of pediatric renal transplant recipients. Sixty-five patients were longitudinally monitored for AT1R-antibody, HLA DSA, IL-8, TNF-α, IL-1β, IFN-γ, IL-17, and IL-6, renal dysfunction, hypertension, rejection, and allograft loss during the first two years post transplantation. AT1R-antibody was positive in 38 of the 65 of children but was not associated with HLA DSA. AT1R-antibody was associated with renal allograft loss (odds ratio of 13.1 (95% confidence interval (1.48 – 1728)), the presence of glomerulitis or arteritis, and significantly higher TNF-α, IL-1β, and IL-8 levels, but not rejection or hypertension. AT1R-antibody was associated with significantly greater declines in eGFR in patients both with and without rejection. Furthermore, in patients without rejection, AT1R-antibody was a significant risk factor for worsening eGFR over the two year follow-up period. Thus, AT1R-antibody is associated with vascular inflammation in the allograft, progressive decline in eGFR, and allograft loss. AT1R-antibody and inflammatory cytokines may identify those at risk for renal vascular inflammation and lead to early biopsy and intervention in pediatric kidney transplantation.

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Keywords

Pediatric Nephrology; Inflammation; Endothelium; Cytokines; Angiotensin

Introduction

Antibody mediated rejection (AMR) remains a significant barrier to successful long-term outcomes in kidney transplantation.[1–3] The role of alloantibody responses against Human Leukocyte Antigens (HLA) in mediating AMR has been a primary focus in transplantation.[2, 4, 5] However, non-HLA autoantibodies have gained importance for their involvement in AMR.[6–8] Moreover, the interplay between alloantibody and autoantibody responses is becoming important to our understanding and management of AMR. Antibodies to various non-HLA targets,[6, 7] such as major-histocompatibility-complex class I-related chain A,[9–14] endothelin type A receptor,[15, 16] perlecan,[17] collagen-IV,[18] fibronectin,[18] and angiotensin II type 1 receptor antibody (AT_1R-Ab)[19–24] have been associated with poor allograft outcomes in renal transplantation. Evidence for routine testing, however, remains insufficient.[8]

AT_1R-Ab, in particular, is becoming recognized for its association with vascular injury and allograft failure in adult kidney transplant patients.[19–24] Dragun and colleagues were the first to report the association of AT_1R-Ab with acute AMR, endarteritis, and severe hypertension in kidney transplant recipients.[19] Since that time, AT_1R-Ab has also been linked to AMR in the absence of hypertension, allograft loss, acute cellular rejection (ACR), and decreased renal function in patients without this classical presentation,[21–25] suggesting multiple clinical phenotypes exist for AT_1R-Ab mediated allograft injury.

AT_1R-Ab can directly injure endothelial and vascular smooth muscle cells, leading to elevated transcription factors associated with pro-inflammatory responses.[26] Therefore, peripheral blood markers of inflammation such as cytokines might enhance our understanding of the effects of AT_1R-Ab in clinical transplantation. Additionally, the relationship between allograft injury due solely to AT_1R-Ab versus that due to the combination of AT_1R-Ab and HLA DSA is unclear. Certain studies have found that the pathogenesis of AT_1R-Ab-induced injury is independent of HLA DSA[24] while others have found that AT_1R-Ab and HLA DSA together portend inferior clinical outcomes.[22]

Recently, high AT_1R-Ab levels have been shown to be more common in pediatric than adult renal transplant recipients.[27, 28] However, the clinical significance and impact of elevated AT_1R-Ab levels on allograft outcomes in pediatric patients remains unclear. Therefore, we examined the relationship of AT_1R-Ab with clinical outcomes including allograft loss, biopsy findings, and renal function in a longitudinal cohort of pediatric renal transplant recipients. In addition, we investigated the association of AT_1R-Ab with HLA DSA and a panel of inflammatory cytokines.

Results

Prevalence and Clinical Characteristics

The prevalence of AT_1R-Ab >17 U/mL in our pediatric cohort was 58% (38/65) at any time pre-transplant to 2 years post-transplant. The cutoff of 17 U/mL was initially chosen based on the literature[20, 29]. Furthermore, we generated an area under the curve (AUC) for AT_1R-Ab and clinical outcomes which confirmed a cut-off of 17 U/ml was within 6% of the optimal threshold in our pediatric cohort (Table S1). In AT_1R-Ab positive patients, AT_1R-Ab was present prior to transplantation (i.e., preformed) in 39% (15/38), *de novo* after transplant in 45% (17/38), and undetermined in 16% (6/38) due to the lack of pre-transplant sera. Of the patients who developed *de novo* AT_1R-Ab, 59% (10/17) did so in the first 6 months, 35% (6/17) between 6–12 months, and 6% (1/17) between 12–24 months post-transplant. Figure 1 shows the longitudinal comparison of AT_1R-Ab levels in AT_1R-Ab positive (Figure 1a) versus negative (Figure 1b) patients during the first 2 years post-transplantation. Patients who were AT_1R-Ab positive at any time during the monitoring period generally remained positive throughout. All patients who were positive pre-transplant continued to be AT_1R-Ab positive at some time post-transplant.

There were no significant differences in demographic or baseline clinical characteristics between AT_1R-Ab positive (defined as >17 units/mL at any time point) and negative patients (Table 1). Table 2 compares immunological characteristics and therapy between the two groups. There were no differences in pre-transplant sensitization risk factors including HLA mismatch, panel reactive antibody (PRA), and history of prior transplantation. There was an association between AT_1R-Ab and anti-thymocyte globulin (ATG) induction (p=0.037). Four of six patients given ATG induction were sensitized with PRA Class I or II >30% and two were given ATG as part of a rapid steroid withdrawal protocol. The development of HLA DSA was notably not a risk factor for AT_1R-Ab (p>0.99). Additional immunomodulatory treatments beyond our standard immunosuppression protocol (Table 2) were given for delayed graft function, acute rejection or disease recurrence. Overall, patients in the AT_1R-Ab positive group received more total treatment days of augmented immunomodulation (p=0.010) and plasmapheresis (p=0.002). Using a mixed-effects longitudinal regression model, we found no temporal association between AT_1R-Ab and the development of HLA DSA, acute rejection, or immunomodulatory treatments including plasmapheresis (data not shown).

AT_1R-Ab and Allograft Loss

AT_1R-Ab positive status within the first 2 years post-transplant was associated with renal allograft loss (p=0.036) (Figure 2). Seven patients experienced allograft loss: 1 between 0–6 months, 3 between 6–12 months, and 3 between 12–24 months. Five of 7 patients had a positive AT_1R-Ab at the time of allograft failure. The remaining 2 patients were AT_1R-Ab positive at the time of treatment resistant ACR episodes with vascular involvement and were treated with rituximab and/or bortezomib plus plasmapheresis. The AT_1R-Ab became negative after treatment; however, these two patients subsequently developed progressive fibrosis and allograft failure.

We conducted univariate and multivariable analysis to further assess risk factors for allograft loss (Table S2). We limited the number of variables in our model given the small number of events and excluded potential intermediate outcomes. On univariate analysis, AT_1R-Ab positive status (OR 95% CI of 13.1 (1.48 – 1728.44), p=0.036), deceased donor transplant (p=0.038), mean HLA mismatch (p=0.005), and physician assessed non-adherence (p=0.008) were associated with renal allograft loss (Table S2). As significant collinearity existed between donor type and HLA mismatch, only HLA mismatch was included in the final model (see Statistical Methods). AT_1R-Ab positive status remained associated with allograft loss (OR 95% CI of 9.24 (0.51–168.38), p=0.061) after accounting for mean HLA mismatch and physician assessed non-adherence in the multivariable model (Table S2).

AT_1R-Ab and Biopsy Findings

AT_1R-Ab was not associated with ACR, C4d negative AMR, or C4d positive AMR (Table S3). AT_1R-Ab was not associated with elevated acute interstitial or tubular inflammation scores. A combination score reflecting the presence of vascular inflammation represented by either glomerulitis or arteritis was statistically significant (p=0.037). AT_1R-Ab was not associated with acute peritubular capillaritis or other combination acute vascular inflammation scores. AT_1R-Ab was not associated with chronic change scores or degree of interstitial fibrosis and tubular atrophy (Table S3).

AT_1R-Ab and Renal Function

Patients with AT_1R-Ab demonstrated larger declines in eGFR over the first 2 years post-transplant compared to those without AT_1R-Ab (p=0.013) (Figure 3a). Because AT_1R-Ab may exert direct effects on the allograft endothelium outside the context of biopsy proven acute rejection, we investigated AT_1R-Ab as a risk factor for worsening renal function in patients both with and without rejection. In both groups, patients with AT_1R-Ab had a greater median decline in eGFR than those without AT_1R-Ab (p=0.003) (Figure 3b). Furthermore, we longitudinally analyzed eGFR in patients without rejection and found that patients with AT_1R-Ab had significantly greater decline in eGFR over the first 2 years post-transplant (p=0.032, Figure 3c).

AT1R-Ab and Cytokine Analysis

AT_1R-Ab was associated with vascular inflammation and a decline in allograft function that was not mediated by acute rejection. Therefore, a panel of serum cytokines associated with activation of the AT_1R in hypertension, scleroderma, and preeclampsia[30–35] was measured post-transplantation to assess the activity of AT_1R mediated inflammatory pathways in kidney transplant patients. Although these cytokines have been associated with AT_1R activation in other disease states, it remained unclear which of the 6 cytokines were important in kidney transplantation, therefore, they were analyzed individually. Patients positive for AT_1R-Ab had higher TNF-α (p=0.043), IL-1β (p=0.045), and IL-8 (p=0.016) levels (Figure 4). There was no association between AT_1R-Ab status and IFN-γ, IL-17, or IL-6 levels. We further investigated the temporal relationship between these cytokines and AT_1R-Ab positivity in blood samples over the 2 year period. The blood sample analysis was based on the AT_1R-Ab status (>17 units/mL considered positive) and cytokine levels in individual blood samples controlled for patient-level random effects. In this longitudinal

analysis, TNF-α, IL-1β, and IL-8 were all significantly elevated in AT_1R-Ab positive versus negative blood samples (Figure S1 a–c, p-values 0.037, 0.005, and 0.004 respectively). A similar analysis did not reveal any differences in IFN-γ, IL-17, or IL-6 levels.

Discussion

In the largest, comprehensive pediatric cohort to date, we longitudinally examined the association of AT_1R-Ab with clinical outcomes, development of HLA DSA, biopsy findings, and inflammatory cytokines. Consistent with previous studies, AT_1R-Ab was highly prevalent in pediatric renal transplant recipients.[27, 28] Notably, we are the first to show the detrimental association of AT_1R-Ab on allograft survival and function in children, separate from the effects of HLA DSA. Furthermore, we found a significant correlation between AT_1R-Ab, arteritis or glomerulitis on renal biopsy, and elevated serum TNF-α, IL-1β, and IL-8 levels, which may represent vascular inflammation. Taken together, our data suggests that renal transplant recipients with AT_1R-Ab may manifest a pattern of vascular injury as evidenced by renal biopsy findings, decrement in renal function, increased incidence of allograft failure, and elevated serum cytokines.

The lack of a standardized cutoff for determining AT_1R-Ab positivity presents a challenge for comparing and generalizing studies; many adult studies have found lower levels of AT_1R-Ab (>9,[21, 36, 37]10,[24, 38] and 15[22] units/mL) to be associated with poor renal transplant outcomes. A prior pediatric study[27] showed that pediatric patients with stable allograft function have higher levels of AT_1R-Ab compared to adults. To address this question, we constructed an AT_1R-Ab AUC that confirms 17 U/mL to be the optimal threshold to predict poor transplant outcomes including allograft loss, eGFR decline, and glomerulitis/arteritis in a pediatric population. Our study supports the use of the 17 U/mL AT_1R-Ab cutoff in children to avoid excessive false positive values and may guide future studies.

Pre-transplant AT_1R-Ab was present in 23% (15/65) of the total population, which is within the range of 17–33% that has been described in adults using similar cutoffs,[20, 22] but higher than the 0% reported in a pediatric study of 20 patients.[28] Importantly, 26% (17/65) of our cohort developed *de novo* AT_1R-Ab. By contrast, the largest analogous study in adult kidney transplant recipients found only 3% of patients with *de novo* AT_1R-Ab.[22] The higher prevalence of AT_1R-Ab found in children compared to adults is most likely multifactorial. Inflammatory events at the time of transplant, such as ischemia reperfusion injury, may trigger non-HLA antibody formation.[6] Younger patients may be at higher risk of ischemic injury[39, 40] secondary to surgical and hemodynamic challenges related to their size. Additionally, children are more susceptible to post-transplant infections.[41] Activation of inflammatory pathways by infectious agents, particularly Parvovirus,[42] have been implicated in the development of AT_1R-Ab formation. Unfortunately, we did not routinely measure Parvovirus. Although we did not find an association between CMV, EBV, and BK viremia and AT_1R-Ab, we may have been underpowered to detect a difference and this requires further investigation in a larger cohort.

Consistent with other studies, the presence of HLA DSA was not associated with AT_1R-Ab in our cohort. Giral et al reported no association between pre-transplant AT_1R-Ab and HLA

DSA in adult recipients.[24] Furthermore, Hesemann et al found no association between the development of HLA DSA with de novo AT_1R-Ab in 8 children.[28] In contrast, Cuevas and colleagues reported an association between high levels of pre-transplant AT_1R-Ab and the formation of de novo HLA DSA,[23] and Taniguchi and colleagues found that patients with both HLA DSA and AT_1R-Ab had inferior allograft survival.[22] These conflicting results support the need for continued investigation to understand the relationship of auto- and allo-immune responses in kidney transplantation.

Surprisingly, ATG induction was the only significant risk factor we found for the presence of AT_1R-Ab in the first 2 years post-transplant. Patients receiving ATG induction were more likely to be sensitized against HLA antibodies which could lead to subsequent autoimmune responses and development of AT_1R-Ab.[6] ATG may also deplete T regulatory cells[43, 44] thereby promoting autoimmunity. However, the association of AT_1R-Ab with ATG induction was unexpected since cell depletion diminishes antibody production by reducing CD4 helper T cells. Furthermore, we, like others,[21, 22, 24] have not found an association between pre-transplant PRA and AT_1R-Ab. ATG induction has been associated with lower risk of *de novo* HLA DSA in sensitized patients[45] and a recent study reported a potential benefit of using an ATG induction protocol in AT_1R-Ab positive patients.[46] Therefore, our results must be interpreted with caution.

Perhaps the most important findings of our study were the associations of AT_1R-Ab with poor clinical outcomes. We are the first to show an association between AT_1R-Ab and allograft loss in children. Heseman et al found no association between AT_1R-Ab and rejection in 29 pediatric patients.[28] Bjerre et all showed that AT_1R-Ab levels are higher in stable pediatric kidney transplant patients when compared to adults, however, history of rejection events in the pediatric group were too rare to be analyzed.[27] Furthermore, AT_1R-Ab was correlated with greater declines in renal function in our cohort, even in the absence of rejection, which has also been reported in adults.[21, 23] Our longitudinal analysis, demonstrating significant progressive decline in eGFR in patients without rejection, suggests a sustained, long-term impact of AT_1R-Ab. This finding is particularly compelling as percent change in eGFR has been shown to predict long term allograft outcomes.[47] Given the high prevalence of AT_1R-Ab in pediatric kidney transplant recipients shown in our study and by others,[27, 28] a better understanding of this relationship is essential to their clinical management.

We performed a detailed examination of sequential biopsies and analyzed serum cytokines associated with AT_1R activation found in other disease states such as scleroderma and preeclampsia.[30–35] We showed that patients with AT_1R-Ab were more likely to have glomerulitis or arteritis on biopsy which is consistent with other studies reporting associations between AT_1R-Ab and vascular inflammation.[21, 48] Furthermore, we are the first to report that kidney transplant patients with AT_1R-Ab have higher TNF-α, IL-1β, and IL-8 levels. These elevations occur simultaneously with AT_1R-Ab positivity. Angiotensin II has been shown to stimulate TNF-α production in glomerular endothelial cells in rats.[49] Endothelial cells can also produce IL-1β and IL-8, which are known to promote leukocyte chemotaxis and artherogenesis.[35, 50–52] Taken together, our data support an association between AT_1R-Ab and renovascular inflammation, which is consistent with previous

work.[19, 48] It appears that this process can worsen functional outcomes in the absence of rejection. Thus, we hypothesize that AT_1R-Ab can activate the endothelium[19] and mediate vascular inflammation leading to progressive decline in renal function and eventual allograft loss. This hypothesis requires testing in prospective mechanistic studies. Additionally, validating the use of AT_1R-Ab and cytokine testing to identify patients at highest risk for poor allograft outcomes may be warranted.

There are important limitations of our study. Although some studies have shown an association between AT_1R-Ab and hypertension,[19, 21, 24] our study and others[23, 28] have not. Furthermore, this retrospective examination shows association and not causation. Nevertheless, our findings are hypothesis generating for prospective mechanistic studies. We also cannot rule out the potential of unmeasured confounders or confounders with limited events, such as other non-HLA antibodies, recurrent disease, medication toxicity, or infection. Our study only examined patients for the first 2 years post-transplantation, which may not allow sufficient time for chronic change to develop on biopsy. Our small sample size makes negative findings more difficult to interpret and restricted our ability to do extensive multivariable and subgroup analyses. A larger cohort is required to validate the clinical impact of AT_1R-Ab, importance of pre-transplant versus post-transplant development of AT_1R-Ab, interaction of AT_1R-Ab with both HLA and other non-HLA antibodies, and the role of cytokines as a predictor of AT_1R-Ab mediated allograft injury. No treatment effects were observed in our study, however, this may be secondary to the timing of sample collection. Success with the use of angiotensin receptor blockade (ARB), plasmapheresis, IVIG, and ATG in the treatment[19, 53] and peri-operative prevention[46] of AT_1R-Ab mediated AMR have been reported. Randomized controlled trials are warranted to examine the efficacy of these treatments for AT_1R-Ab mediated AMR and the potential role of ARBs in preventing subacute decline in renal function.

In conclusion, our comprehensive observational study demonstrates an association between AT_1R-Ab and allograft loss, decline in renal function, and vascular inflammation in a cohort of pediatric renal transplant recipients. Our data suggests cytokines may be useful to identify renal vascular inflammation in conjunction with renal biopsy and AT_1R-Ab testing, leading to early intervention with ARB therapy and potential attenuation of AT_1R-Ab mediated allograft injury.[46] Our study highlights the clinical consequences of AT_1R-Ab in pediatric kidney transplant recipients and supports the use of an AT_1R-Ab cut-off of 17 U/mL in children.

Methods

Patients and Study Design

In this retrospective study, 65 pediatric kidney transplant patients were monitored for 2 years post-transplant. From August 2005 to November 2014, 83 patients were enrolled in the UCLA Pediatric Kidney Transplant Immune Monitoring Study, and 18 patients were excluded from analysis secondary to missing > 1 study sample at the below time points. This study was approved by the UCLA Institutional Review Board (#11-002375) and conforms with the 1964 Helsinki declaration and its later amendments or comparable ethical standards and the Principles of the Declaration of Istanbul. Informed consent and when appropriate

patient assent was obtained for all patients. Blood samples were obtained pre-transplant and at 6, 12, and 24 months post-transplant and during episodes of kidney transplant rejection. In longitudinal analyses, blood samples were grouped by periods within 3 months of the time point. Demographic and clinical data including age, race, ethnicity, etiology of ESRD, transplant type (deceased/living donor), sensitization history, time on dialysis, delayed graft function (defined as dialysis in the first week post-transplant), immunosuppression regimen, cytomegalovirus, Epstein Barr virus, and BK Virus levels, medication nonadherence, and blood pressure were collected. Non-adherence by staff report was defined as physician and/or clinic staff documentation of patient report, undetectable drug levels, or missed appointments.[4] Patients were monitored for rejection, allograft loss, and decrease in eGFR as determined by the updated Schwartz equation[54] in patients <18 years old at the time of transplant and by the abbreviated Modification of Diet in Renal Disease (MDRD) equation[55] in patients ≥18 at the time of transplant. Hypertension was determined by age, sex, and height percentile[56] for children <18 years old and based on American Society of Hypertension and the International Society of Hypertension guidelines for patients ≥18.[57] Additional approaches for eGFR are shown in Table S4. Study data were collected and managed using a secure REDCap (Research Electronic Data Capture) electronic data capture tools hosted at UCLA.[58] Of the 65 patients, 54 patients had complete 2 year follow up, 7 patients suffered graft loss, and 4 patients transferred care to a different institution. No patients died during the study period. A total of 9.1 patient-years of follow up were analyzed.

Clinical Protocols and Biopsy Evaluation

UCLA immunosuppressive regimen for pediatric transplant recipients included induction with either ATG for PRA ≥ 30%, delayed graft function, or rapid-steroid withdrawal protocol or anti-CD25 monoclonal antibody for those with PRA<30%. Maintenance immunosuppression consisted of steroid free or steroid based immunosuppression, a calcineurin inhibitor, and an anti-metabolite. Acute and chronic rejection were treated with previously described protocols.[59]

Patients underwent protocol biopsies at 6, 12, and 24 months post-transplantation or for clinical indication. Biopsies were evaluated based on the 2013 Banff Criteria.[60] Two patients in the AT_1R-Ab positive group and one patient in the AT_1R-Ab negative group were not included in the biopsy score data as they had missing values.

Antibody Testing

HLA typing of recipient and donor was performed using molecular methods as previously described.[59] HLA antibodies were detected using a Luminex single antigen bead (SAB) assay (Immucor, Stanford, CT) and quantified by mean fluorescence intensity (MFI). Antibodies were considered positive when MFI was ≥1000 for HLA-A, -B, -DR, -DQ, and ≥2000 for HLA-C and –DP.[61] AT_1R-Ab was measured by enzyme-linked immunosorbent based assay (One Lambda, Canoga Park, CA). Sera were diluted 1:100, tested in duplicate and AT_1R-Ab concentrations were determined by a standard curve. AT_1R-Ab IgG >17 units/ml was considered positive.[29, 62] AT_1R-Abs exceeding the limit of the standard curve were further diluted and re-tested to determine concentration.

Cytokine Testing

Cytokines were selected based on a literature review of cytokines that have been associated with activation of the AT_1R[30–35] and measured in serial post-transplant samples to avoid effects of dialysis and end-stage renal disease. A custom magnetic bead kit including IL-8, TNF-α, IL-1β, IFN-γ, IL-17, and IL-6 (EMD Millipore, Darmstadt, Germany) was used per manufacturer's instructions. Fluorescence was quantified using a Luminex 200TM instrument. For the patient level analysis of cytokines, the median of the post-transplant values of each cytokine across samples was used as representative. Prior to statistical analysis, cytokines were transformed using the log(x+1) transformation.

Statistical Methods

A receiver operating characteristic analysis was performed to validate the AT_1R-Ab >17 U/mL cutoff suggested by the literature. This was done by generating thresholds to predict various clinical outcomes and comparing these thresholds to an AT_1R-Ab >17 U/mL cutoff. The best threshold for each clinical outcome was determined using the Youden Index.[63] Categorical variables were compared between groups using Fisher's exact test. Continuous variables were compared between groups using either the Wilcoxon Rank Sum test or a t-test based on the distribution of the data. To assess the relationship of clinical factors with the outcome of allograft loss, we constructed univariate logistic regression models. Variables that had a p-value on univariate analysis below 0.10 were included in a multivariable model. Due to small number of events of allograft loss, we used Firth's penalized likelihood approach to fit the logistic regression models. To address concerns about collinearity between donor type and HLA mismatch, separate models were fit to using these variables and the model with the highest c-statistic is presented. An additional longitudinal mixed-effect regression model was constructed to assess the effect of AT_1R-Ab positivity over time on various clinical characteristics (i.e., viremia, rejection, HLA antibodies). Separate models were used for each characteristic with each model including a random slope and random intercept for time from transplant. Mixed effects regression models were used to evaluate the effect of AT_1R-Ab positivity on eGFR and cytokine levels over time. The model for longitudinal cytokine analysis was controlled for patient level random effects. The regression coefficients, represented by "β" are reported. A p- value below 0.05 was considered statistically significant and all tests were two-sided. The R Statistical Computing Environment was used for analysis (R Core Team; Vienna, Austria).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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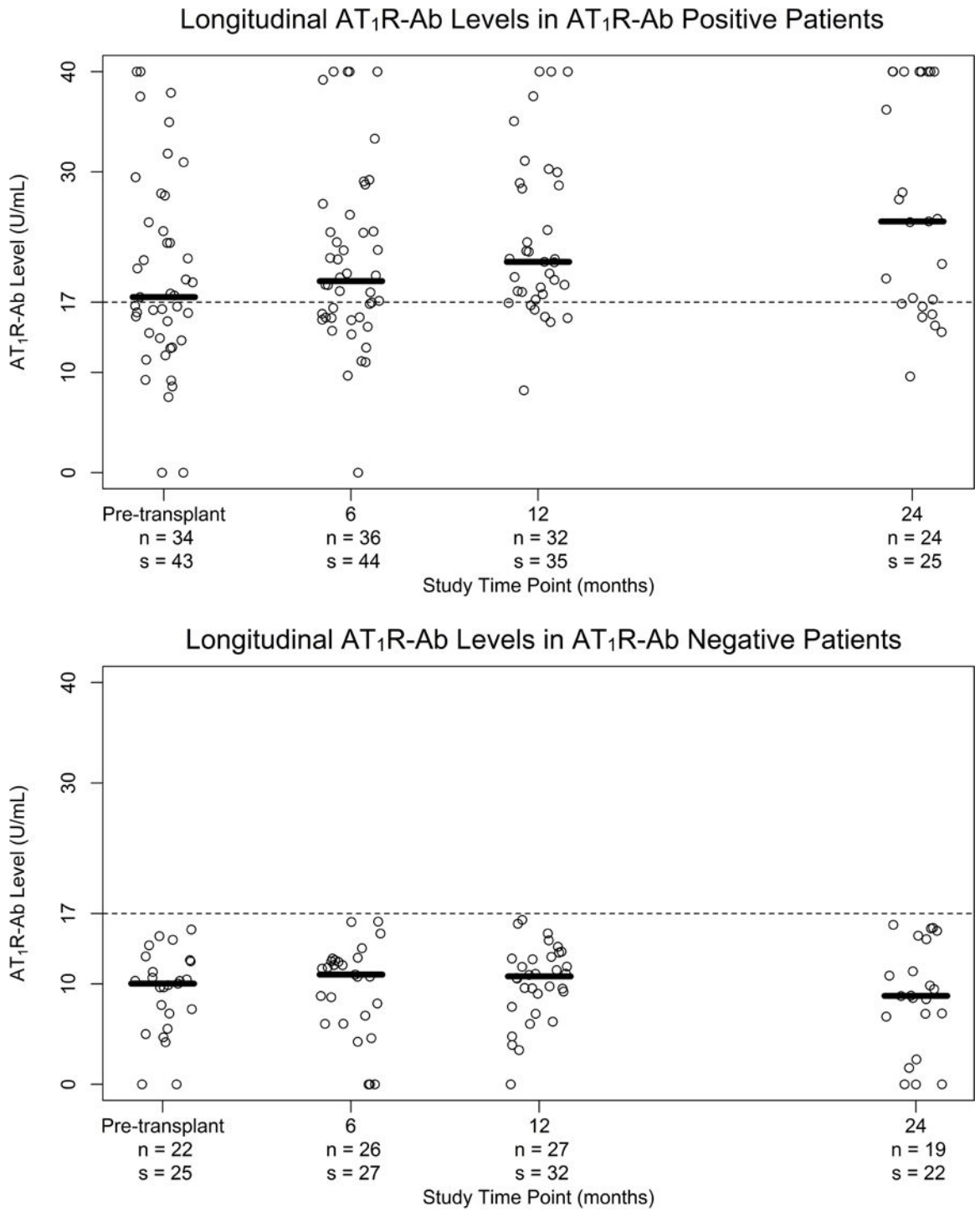


Figure 1. Comparison of AT_1R-Ab Levels over 2 year Follow-Up Period

in a) patients positive for AT_1R-Ab at any time point and b) patients who were negative for AT_1R-Ab at all time points. Each plot point represents an individual patient blood sample taken pre-transplant and at 6, 12, and 24 months after transplantation. The median AT_1R-Ab level at a given time point is represented by the bar in relation to the AT_1R-Ab positive threshold of >17 units/mL (dashed line). n= number patients s= number of samples

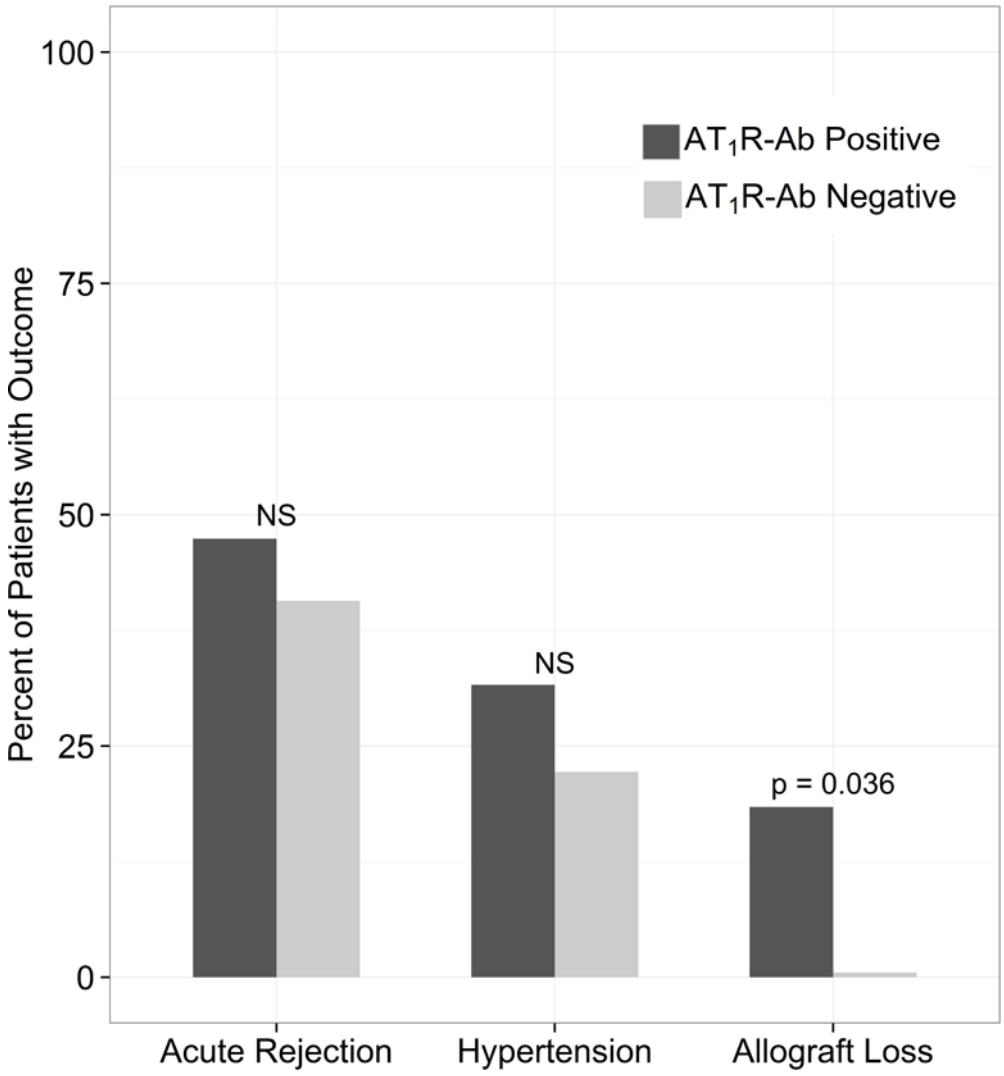
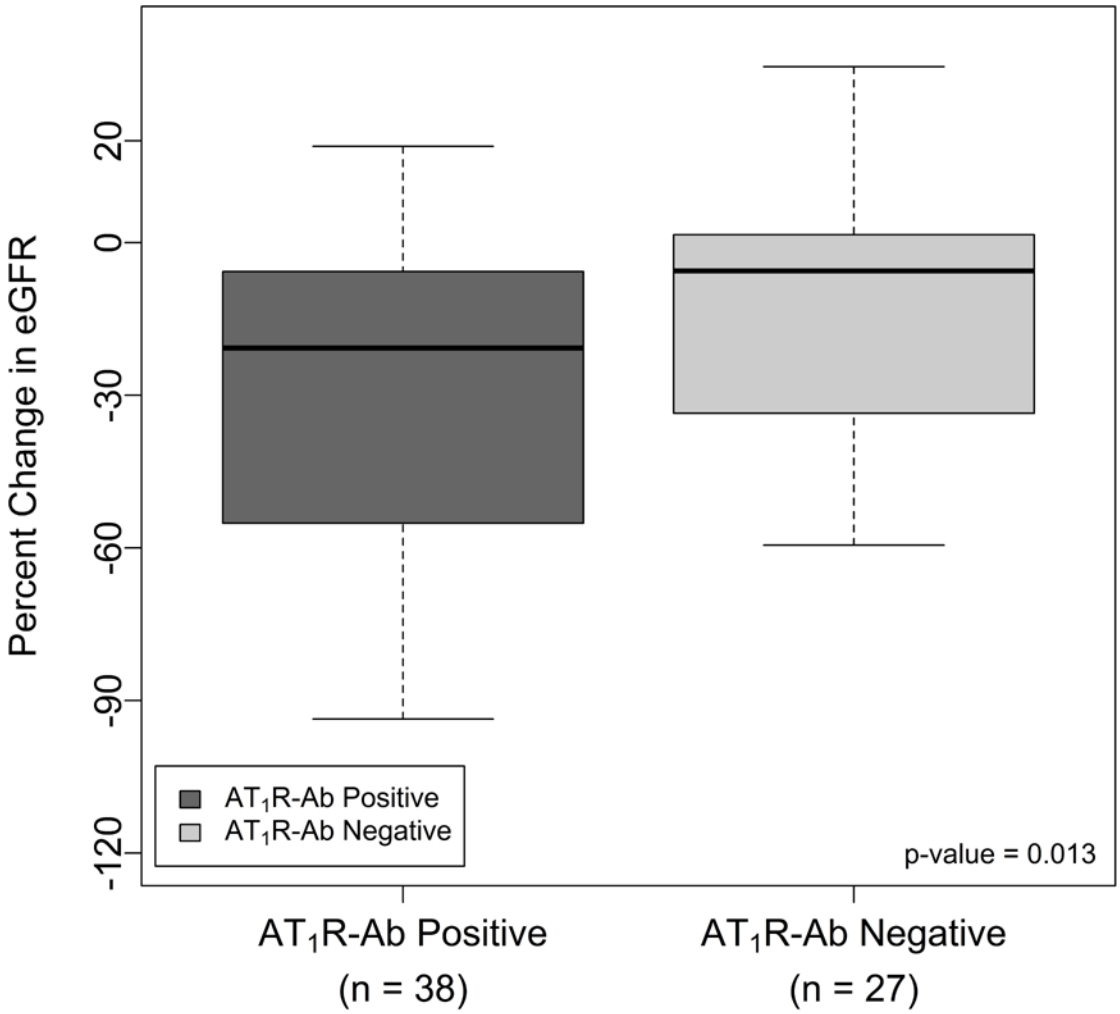


Figure 2. Clinical Outcomes by AT_1R-Ab Status

AT_1R-Ab Positive status was defined as having AT_1R-Ab >17 units/mL at any time point from pre-transplant through the first 2 years post-transplant. AT_1R-Ab positive status was associated with allograft loss but not acute rejection or hypertension. Of the 65 patients, 29 had acute rejection, 18 had hypertension, and 7 had allograft loss.



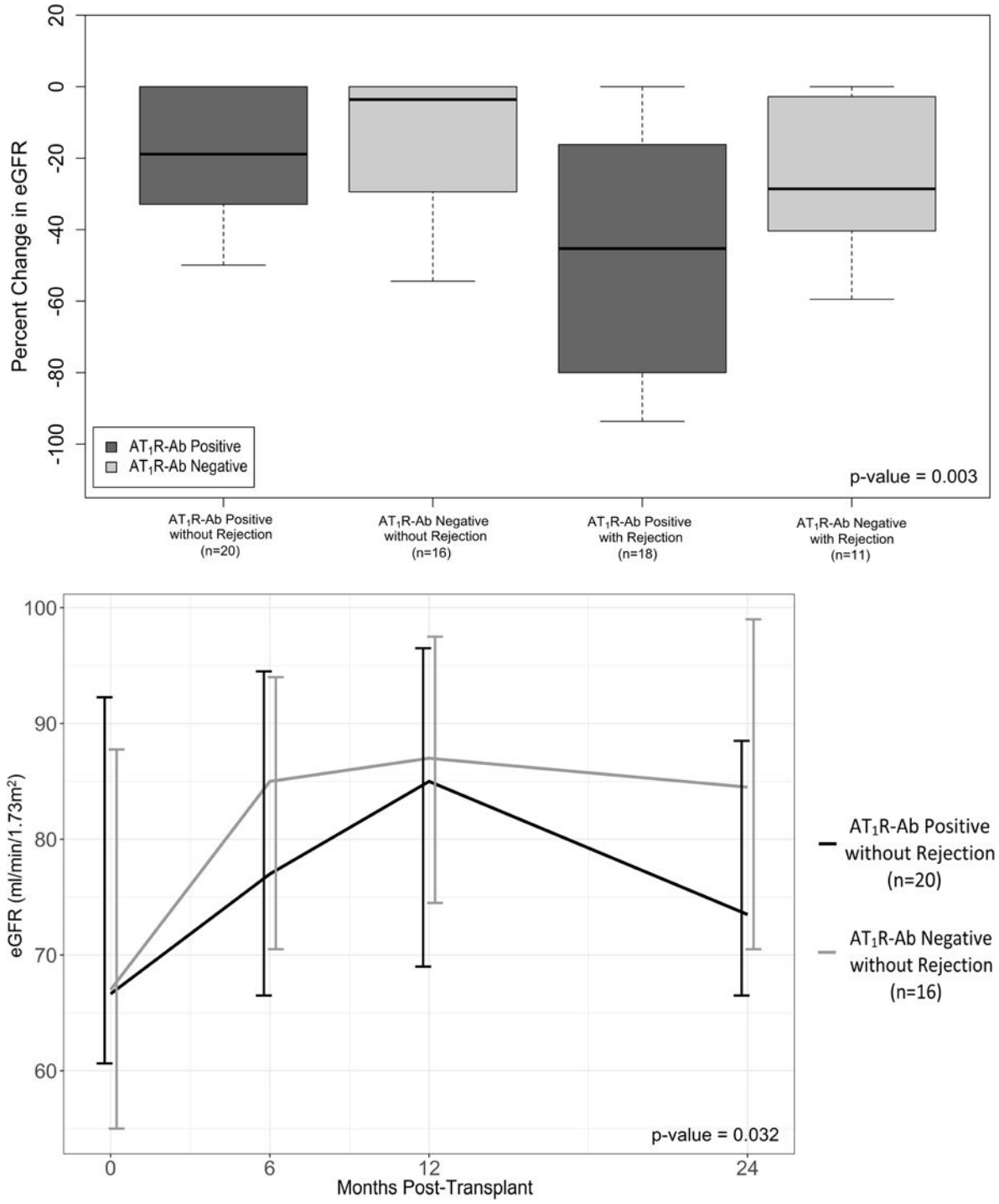


Figure 3. Relationship between Renal Function and AT_1R-Ab

Median percent change in eGFR by a) AT_1R-Ab status and b) AT_1R-Ab and Rejection Status shown, p-value for 4 group comparison. Percent change in eGFR was taken from hospital discharge to lowest eGFR value during the 2 year follow up period. c) The impact of AT_1R-Ab status on eGFR over time in patients without rejection assessed by a mixed-effects regression model. Median eGFR and interquartile range at each time point post-

transplant is shown. All patients in this analysis had developed AT_1R-Ab by the 12 month time-point. Notably, patients with AT_1R-Ab, in the absence of rejection, had progressive decline in renal function in contrast to patients who remained free from AT_1R-Ab development over the 2 year period. $\beta = 0.85$ (0.00 – 1.69).

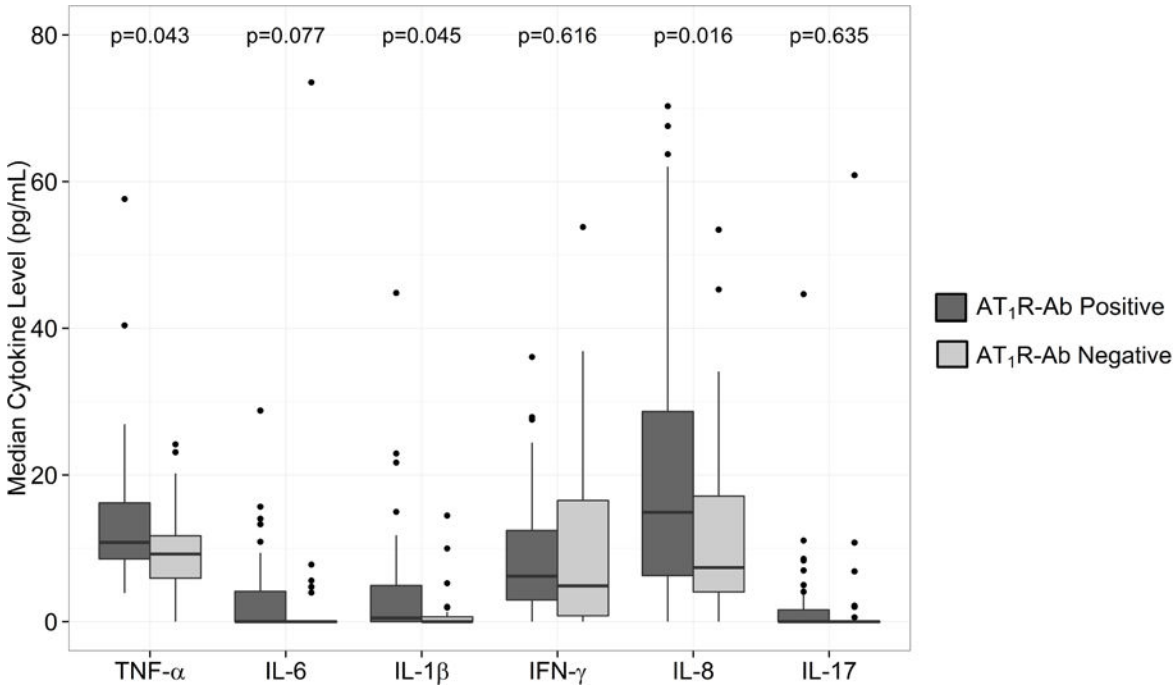


Figure 4. Cytokine Levels in Patients by AT_1R-Ab Status

Comparison of cytokine levels in patients with (n=38) and without AT_1R-Ab (n=27) in the first 2 years post-transplant. For each patient, the median of all the post-transplant values for each cytokine over the 2 year period was used as representative.

Table 1
Demographics and Clinical Characteristics

Comparison of demographic and clinical characteristics between AT_1R-Ab positive and negative patients. IQR, interquartile range; ESRD, end stage renal disease; FSGS, focal segmental glomerulosclerosis; PKD, polycystic kidney disease; EBV, Epstein-Barr virus; CMV, cytomegalovirus; BKV, BK virus

	AT_1R-Ab Positive (n=38)	AT_1R-Ab Negative (n=27)	p-value
Age, median (IQR)	14.8 (12.8–17.6)	16.4 (13.3–18.4)	0.369
Male Sex	23 (60.5%)	16 (59.3%)	>0.99
Hispanic Ethnicity	18 (47.4%)	18 (66.7%)	0.138
Race			0.243
White	28 (73.7%)	19 (70.4%)	
Asian	2 (5.3%)	2 (7.4%)	
Black	4 (10.5%)	0 (0%)	
Other	4 (10.5%)	6 (22.2%)	
Dialysis Prior to Transplant	32 (84.2%)	19 (70.4%)	0.227
Years on Dialysis, median (IQR)	2.4 (0.8–3.1)	1.7 (1.1–2.5)	0.355
EBV Immune	28 (73.7%)	20 (74.1%)	>0.99
CMV Immune	24 (63.2%)	14 (51.9%)	0.446
Deceased Donor	26 (68.4%)	14 (51.9%)	0.204
Etiology of ESRD			0.931
Dysplasia	5 (13.2%)	4 (14.8%)	
FSGS	6 (15.8%)	3 (11.1%)	
Glomerulonephritis	5 (13.2%)	3 (11.1%)	
IgA nephropathy	0 (0%)	1 (3.7%)	
Obstructive Uropathy	9 (23.7%)	7 (25.9%)	
PKD	2 (5.3%)	0 (0%)	
Other	5 (13.2%)	5 (18.5%)	
Unknown	6 (15.8%)	4 (14.8%)	
Cold Ischemia Time (hours), median (IQR)	11.1 (1–13.8)	6.3 (1–15)	0.447
Delayed Graft Function	3 (7.9%)	1 (3.7%)	0.636
EBV, CMV, or BK Viremia	18 (52.9%)	11 (44%)	0.601
Physician Assessed Non - Adherence	11 (28.9%)	5 (18.5%)	0.393

Table 2
Immunological Characteristics and Therapy

Comparison of sensitization status and immunomodulatory treatments between AT_1R-Ab positive and negative patients. HLA, human leukocyte antigen; SD, standard deviation; PRA, panel reactive antibody; ATG, anti-thymocyte globulin; IL-2, interleukin-2; DSA, donor specific antibody; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; IQR, interquartile range; IVIG, intravenous immunoglobulin

	AT_1R-Ab Positive (n=38)	AT_1R-Ab Negative (n=27)	p-value
HLA Mismatch, mean (SD)	1.2 (0.6)	1.2 (0.5)	0.730
Primary Transplant	33 (86.8%)	26 (96.3%)	0.388
Baseline PRA Class I > 20%	3 (7.9%)	1 (3.7%)	0.636
Baseline PRA Class II > 20%	6 (15.8%)	1 (3.7%)	0.224
ATG Induction (vs. IL - 2 Inhibitor)	6 (15.8%)	0 (0%)	0.037
Steroids Based Immunosuppression	17 (44.7%)	14 (51.9%)	0.621
Presence of HLA DSA in the First 2 Years Post-Transplant			>0.99
Negative	27 (71.1%)	19 (70.4%)	
Class I Only	3 (7.9%)	2 (7.4%)	
Class II Only	7 (18.4%)	5 (18.5%)	
Class I and Class II	1 (2.6%)	1 (3.7%)	
ACE Inhibitor Treatment	10 (26.3%)	3 (11.1%)	0.209
ARB Treatment	1 (2.6%)	0 (0%)	>0.99
Immunomodulatory Treatment Days, median (IQR)	6 (0–21.5)	0 (0–3)	0.010
Any Immunomodulatory Treatment	23 (60.5%)	12 (44.4%)	0.219
Immunomodulatory Treatment by Medication			
High Dose Steroids	15 (39.5%)	8 (29.6%)	0.444
IVIG	11 (28.9%)	4 (14.8%)	0.239
ATG	15 (39.5%)	5 (18.5%)	0.102
Rituximab	6 (15.8%)	1 (3.7%)	0.224
Plasmapheresis	11 (28.9%)	0 (0%)	0.002
Bortezomib	2 (5.3%)	0 (0%)	0.507