August, 2019

Angiotensin II Type I Receptor Agonistic Autoantibodies are Associated with Poor Allograft Survival in Liver Retransplantation.

Qingyong Xu, *Western University*

Vivian C. McAlister, *Western University*

Steve Leckie, *Western University*

Anton Skaro, *Western University*

Paul Marotta, *The University of Western Ontario*

**Take a deep dive into the
business of transplantation!**

**Liver Transplant
Financial Bootcamp
&
Kidney Transplant
Financial Bootcamp**

Co-located for 2019!

**Loews Chicago
O'Hare Hotel**

**September
19-20,
2019**

**Register at
ASTS.org/bootcamps**

DR QINGYONG XU (Orcid ID : 0000-0003-0579-1924)

Article type : Brief Communication

Angiotensin II Type I Receptor Agonistic Autoantibodies are Associated with Poor Allograft Survival in Liver Retransplantation

Qingyong Xu^{1,2}, Vivian C. McAlister², Steve Leckie¹, Andrew A. House², Anton Skaro², Paul Marotta²

¹Department of Pathology and Lab Medicine, London Health Science Centre, London, ON, Canada

²Multiorgan Transplant Program, London Health Sciences Centre, London, ON, Canada.

Correspondence

Qingyong Xu

Email: Qingyong.Xu@lhsc.on.ca

Presented at the American Transplant Congress, Boston, 4 Jun 2019

www.clinicaltrials.gov NCT03815864

University of Western Ontario Research Ethics Board protocol #106961

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ajt.15571

This article is protected by copyright. All rights reserved.

Abbreviations:

AIH: Autoimmune hepatitis

ARB: Angiotensin II type I receptor blockers (ARB)

AT1R: Angiotensin II type I receptor

AT1R-AA: Angiotensin II type I receptor agonistic autoantibodies

DSA: Donor-specific antibodies

HLA: Human Leucocyte Antigen

MELD: Model for End-Stage Liver Disease

MFI: Median fluorescence intensity

PBC: Primary biliary cirrhosis

PNF: Primary nonfunction

PSC: Primary sclerosing cholangitis

Abstract

Angiotensin II type I receptor (AT1R) agonistic autoantibodies (AT1R-AA) are detrimental to kidney transplantation. Early studies suggested a similar negative effect in primary liver transplantation. Here, we studied AT1R-AA in a retrospective cohort of 94 patients who received a second liver transplant to determine their prevalence and effects. The concentrations of preformed AT1R-AA before transplantation were higher ($p=0.019$) in the 48 patients who lost their liver grafts than in the 46 patients whose grafts survived. About half (48/94, 51.1%) of the patients were positive for AT1R-AA >17 U/ml before the second liver transplantation. In 22 (23.4%) patients, strong positive AT1R-AA (defined as >40 U/ml) were detected, of whom 16 (72.7%) patients lost their grafts. Based on Kaplan-Meier analysis, patients with strong positive AT1R-AA had significantly worse graft survival than those with

AT1R-AA<40U/ml ($p=0.035$). In multivariate Cox models that included confounders such as sex and age, either AT1R-AA>40U/ml [HR=1.999 (1.085-3.682), $p=0.026$] or increased concentrations of AT1R-AA [HR=1.003 (1.001-1.006) per incremental U/ml, $p=0.019$] were significantly associated with elevated risk for graft loss. In conclusion, our data indicate that there is a high prevalence of AT1R-AA in candidates for second liver transplantation and that their presence is associated with inferior long-term outcomes of the second graft.

Introduction

In 2005, Dragun and colleagues reported that angiotensin II type I receptor (AT1R) agonistic autoantibodies (AT1R-AA) were present in 50% of kidney recipients with refractory vascular rejection (1). All patients positive for AT1R-AA had malignant hypertension, and 25% had seizures. In an animal model, they demonstrated reversal of this effect by an AT1R antagonist, losartan (1). Since then, multiple studies have shown reduced kidney graft survival with AT1R-AA, but the effects on rejection and malignant hypertension are less certain (2-5). Similar negative effects have been reported for heart, lung, and intestine transplants, especially when donor-specific antibodies (DSA) were also present (6-8). O'Leary and colleagues found AT1R-AA positive liver recipients to be at a higher risk for vascular rejection and graft fibrosis with evidence of complement deposition close to liver sinusoidal capillaries and stellate cell activation (9).

Preformed AT1R-AA were reported to be positive in 17-25% of patients before kidney transplantation (3, 10-12) and were usually more prevalent in kidney retransplant patients (3, 11). We have speculated previously that low prevalence makes it difficult to see the effects of DSA on liver transplantation and that candidates for re-transplantation may make a better test cohort because of higher prevalence of DSA (13). Believing the same might be true for

AT1R-AA in liver transplantation, we studied a continuous cohort of second liver transplants to determine the association of preoperative AT1R-AA with the survival of the second liver graft.

Methods

We reviewed all patients who received a second liver-alone transplant (n=131) at the University Hospital of London Health Sciences Centre (LHSC), London, Ontario, Canada (1991-2018). Ninety-four patients with available preoperative sera, donor human leukocyte antigen (HLA) typing, and clinical data were included for analysis. All second liver grafts were from ABO-compatible deceased donors after brain death. Crossmatches were not performed prospectively before retransplantation. Neither DSA nor HLA-matching were used for organ allocation or immunosuppression management. In this retrospective cohort, no patient underwent a desensitization protocol to reduce preformed DSA or AT1R-AA. OKT3 inductions followed by cyclosporine / azathioprine / corticosteroid maintenance immunosuppression were used between 1991 and 1994 in 12 patients. After that, all retransplantations were performed without induction therapy and were maintained with a tacrolimus / mycophenolate / corticosteroid immunosuppression protocol (n=82). No patient was weaned off immunosuppression during the study period. This study was approved by the institutional ethics review committee (Protocol #106961) and registered with www.clinicaltrials.gov (NCT03815864)

DSA to the second liver transplant were determined based on a previously described method (13, 14). Briefly, banked preoperative sera were screened with Luminex penal reactive antibody beads. Positive samples were tested using class I (Lot #11) and class II (Lot# 12) Luminex single antigen beads (One Lambda, Canoga Park CA) using the guideline of median fluorescence intensity (MFI) > 1000. Higher MFI thresholds were used for alternative

analysis, as specifically indicated. AT1R-AA were tested on pretransplantation sera using commercially available ELISA kit (Lot #30, One Lambda, Canoga Park, CA) according to vendor-provided protocol (1, 9). For samples with a concentration of AT1R-AA higher than 40 U/ml, accurate measurements were achieved by dilution. Model for End-Stage Liver Disease Sodium (MELD-Na) scores were calculated retrospectively using an online tool (http://meld.otdcalculators.ca/ctr2_en.html) based on pretransplantation data on all patients with exception of those who received a second liver transplant during the period 1991-1998 (n=22), for whom complete data were not available (15).

Graft survival was defined as the interval from the date of second liver transplantation to the date of either the third transplantation, patient death, or the last follow-up, whichever came first. Patient survival was calculated from the date of second liver transplantation to the date of patient death or the date of last follow-up, whichever came first. Baseline characteristics were compared between the groups of patients using a Chi-squared test or Fisher's exact test for categorical or binary variables. For continuous variables, a two-tailed T-test or nonparametric Mann-Whitney test was used. Patient and graft survival curves were plotted using the Kaplan-Meier method and analyzed for statistical significance using the log-rank test. P-values <0.05 were considered to be statistically significant. Univariate and multivariate Cox regression were used to calculate hazard ratios. Only variables with p-values <0.05 in the univariate analyses were included in multivariate models. All statistical analyses were performed using IBM SPSS version 25.

Results

During a follow-up period of up to 26 years, 48/94 (51.1%) of the second liver grafts were lost, and 41/94 (43.6%) patients died. Concentrations of pretransplantation AT1R-AA were found to be significantly higher ($p=0.019$, Mann-Whitney Test) in the 48 patients who lost their grafts [median=19.63 (IQR 14.95, 72.20) U/ml] compared to the 46 patients with functioning grafts [median=16.12 (IQR 11.50, 21.21) U/ml] (Figure 1). When the vendor-recommended threshold of > 17 U/ml was used to define positivity, about half ($n=48$, 51.1%) of the patients were considered to be positive for AT1R-AA before the second liver transplantation. However, according to Cox regression (Table 1), patients with AT1R-AA > 17 U/ml were not at a significantly higher risk for graft loss than patients with AT1R-AA < 17 U/ml [HR=1.464 (0.816 - 2.629), $p=0.201$]. Interestingly, AT1R-AA was found to be quantitatively associated with a higher risk of graft loss [HR=1.003 (1.000 - 1.006) per incremental U/ml, $p=0.040$]. Neither the vendor-recommended cutoff for “at risk” (10 U/ml) nor a higher cutoff of 20 U/ml was able to identify patients with a significantly higher risk for graft loss. The maximum detection limit of the standard ELISA assay without additional sample dilutions is 40 U/ml. This level was used, for convenience, as the threshold for strongly positive AT1R-AA. Strong positive AT1R-AA were found in nearly a quarter ($n=22$, 23.4%) of patients before second liver transplantation. The rates of graft loss were much higher in patients with AT1R-AA > 40 U/ml (16/22, 72.7%) than in patients with AT1R-AA < 40 U/ml (32/72, 44.4%; $p=0.02$) (Table 2). Patients with strong positive AT1R-AA > 40 U/ml had a significantly higher risk for graft loss [HR=1.888 (1.035 - 3.445), $p=0.038$] than patients with AT1R-AA < 40 U/ml (Table 1).

Most demographic and baseline characteristics were similar for patients with AT1R-AA > 40 U/ml versus < 40 U/ml (Table 2). Importantly, pretransplantation MELD-Na scores, the prevalence of Class I or Class II DSA, and immunosuppression regimens were not

significantly different between the two groups. The reasons for losing the first liver graft were significantly different ($p=0.026$), as there were more instances of graft rejection (31.8% versus 19.4%), more recurrences of primary disease (36.4% versus 15.3%), and less observations of primary nonfunction (PNF) (0% versus 22.2%) in patients with AT1R-AA > 40 U/ml than in patients with AT1R-AA < 40 U/ml. Primary liver disease profiles were slightly different ($p=0.065$) in the two groups, notably with three times more primary sclerosing cholangitis (PSC) (45.5% versus 15.3%) and less autoimmune hepatitis (AIH) (0% versus 11.1%) in patients with AT1R-AA > 40 U/ml compared to those with < 40 U/ml. In Kaplan-Meier analysis, patients with AT1R-AA > 40 U/ml were found to have significantly worse graft survival ($p=0.035$, Figure 2A) than patients with AT1R-AA < 40 U/ml. Although it was not statistically significant ($p=0.12$, Figure 2B), patient survival was also worse for recipients with AT1R-AA > 40 U/ml than those with AT1R-AA < 40 U/ml.

Several recipient and donor characteristics that may contribute to liver graft loss were analyzed in univariate Cox regression model (Table 3). Male patients had a significantly higher risk for graft loss [HR=2.233 (1.186 - 4.206), $p=0.013$] than female patients. Age of either patients [HR=1.018 (1.001 - 1.034) per incremental year, $p=0.032$] or donors [HR=1.018 (1.003 - 1.033) per incremental year, $p=0.015$] was found to be associated with a higher risk for graft loss. Primary liver diseases, immunosuppression, and length of survival of the first liver transplant, were not significantly associated with graft loss. Thirty-four (36.2%) patients were found to have positive preformed DSA, of which 19 (55.9%) patients had lost their liver allografts. However, neither all DSA, class I DSA, nor class II DSA were associated with higher risks of losing second liver transplants according to univariate Cox regressions (Table 3). Furthermore, No significant contributions to graft loss were found with locus-specific DSA, DSA determined with higher positive thresholds (5000, 10000, 20000 or

40000), sum MFI for all DSA as continuous variables, or with combinations of DSA and AT1R-AA (Supplemental Table 1).

In multivariate Cox regression adjusting risk for age, gender and cold ischemia time (Table 4), patients with AT1R-AA > 40 U/ml were significantly associated with a higher risk for graft loss than those with AT1R-AA < 40 U/ml [HR=1.999 (1.085 - 3.682), p=0.026]. Notably, male gender [HR=2.255 (1.162 - 4.376), p=0.016] and donor age [HR=1.019 (1.004 - 1.034) per incremental year, p=0.016] were still significant risk factors for graft loss. When the same confounders were tested in multivariate analysis for patient death, a strong but statistically insignificant trend towards worse patient survival was found for patients with AT1R-AA > 40 U/ml [HR=1.879 (0.961 - 3.675), p=0.065]. In a separate multivariate model in which AT1R-AA > 40 U/ml was replaced with the concentration of AT1R-AA (Supplemental Table 2), patients with increased concentrations of AT1R-AA [HR=1.003 (1.001 - 1.006) per incremental U/ml, p=0.019] were also found to have a higher risk of graft failure.

Discussion

Liver retransplantation is not as well studied as primary liver transplantation because of smaller numbers and poorer graft survival (16). We found that a high prevalence DSA (36.2%) and AT1R-AA (84% >10 U/ml, 51.1% >17 U/ml, 23.4% >40 U/ml) in patients undergoing a second liver transplantation. These features make recipients of liver retransplants a good cohort to study the effects of AT1R-AA and DSA. Unlike some other reports (17, 18), we did not find inferior graft survival in retransplant recipients with preformed DSA, regardless of their class, loci, and strength. Recipients with strong preformed AT1R-AA (> 40 U/ml) had a substantial risk for graft loss. This finding is

concordant with other reports (9, 19). We found the detrimental effect of AT1R-AA on liver retransplant survival to be independent of DSA, which is different from other reports on AT1R-AA in primary kidney (3, 5, 10), liver (9) and lung (7) allografts. While in other studies, vendor-recommended 10 U/ml or 17 U/ml were used as positive thresholds in cohorts of primarily first grafts, we performed dilutions for samples that reached the maximum detection limit of the kit so that the effects of the high-titer AT1R-AA could be investigated. In our cohort, we detected levels of AT1R-AA up to 448 U/ml. The risks of graft loss were found to increase by 0.3% per incremental U/ml in the concentration of AT1R-AA (Table 1 and Supplemental Table 2). The detrimental effects of AT1R-AA on liver transplants were concentration-dependent, which is consistent with how this agnostic antibody exerts its effect by activating AT1R (1). AT1R-AA were frequently found in patients with malignancy hypertension (1) or systemic sclerosis (20).

The AT1R-AA were discovered in 1999 by Wallukat and colleagues in women with pre-eclampsia (21). The mechanism of inducing AT1R-AA in pregnancy was thought to be the posttranslational modifications resulting in a neoantigen (22). In organ transplantation, recipients might lose immune tolerance to modified self-antigens. An example is the development of autoimmunity to collagen type V in lung transplantation (23). We found that retransplant liver patients with strong preformed AT1R-AA often lost their first liver grafts due to recurrence of the primary disease or graft rejection but not to primary nonfunction. AT1R-AA, which likely develop slowly after transplantation, may contribute to recurrence of native disease or the development of chronic rejection by causing relative ischemia. In our cohort, strong AT1R-AA were common in patients with PSC (45.5%) but not in those with AIH (0%). A much larger cohort would be required to verify and understand the prevalence of AT1R-AA in specific liver diseases.

Xia and Kellems (22) summarized the possible mechanisms by which AT1R-AA affect the placenta and retard intrauterine growth of the fetus. These mechanisms include increased production of procoagulants, inflammatory, and anti-angiogenic factors. Transplanted grafts may be injured in a similar manner. Elevated inflammatory cytokines have been reported in pediatric renal recipients who were positive for AT1R-AA but not DSA (24). AT1R activation could promote proliferation of smooth muscle cells and collagen synthesis in fibroblasts (20). Similar processes may be responsible for the fibrosis of liver (9, 19) and kidney (4) allografts. In kidney allografts, pathological features of damage caused by AT1R-AA were usually lack of C4d positivity (25). In AT1R-AA positive liver allograft biopsies, C4d patterns differed from those seen in DSA positive biopsies (9). Therefore, there are sufficient mechanisms for the detrimental effects of AT1R-AA to be complement-independent and to possibly affect not only the grafts but the hosts as well. On the other hand, these provocative stimuli may aggravate acute vascular rejection (1, 26) and increase the risk of developing *de novo* DSA and AMR (2, 7, 12) after transplantation. We did not routinely collect posttransplantation sera, therefore could not examine the relationship of preformed AT1R-AA and the development of *de novo* DSA.

This study is limited by a lack of longitudinal post-transplantation data regarding AT1R-AA, DSA, liver enzymes and detailed clinical outcomes. We also acknowledge that our exploratory results need to be confirmed in larger cohorts and in primary liver transplantation. We do not advocate for modifying organ allocation protocols according to preformed AT1R-AA. Attempts have been made to eliminate AT1R-AA in kidney transplant recipients with refractory rejection using antibody-removal regimens such as plasmapheresis and rituximab, with (27) or without intravenous immune globulin (1). Bortezomib was added to the protocols to treat aggressive rejection in renal transplant recipients when both DSA and AT1R-AA were positive (28). However, the agonistic action of AT1R-AA opens the

possibility for novel treatments. ARB, which are currently used treat hypertension, may block the detrimental effects of AT1R-AA (1). Theoretically they may reduce the risk of *de-novo* development of AT1R-AA and DSA after transplantation, if given prophylactically. The ARB, candesartan, has been successfully used in combination with antibody-reduction therapy to manage kidney patients before transplantation (29) and to treat rejection in recipients who are positive for AT1R-AA (1, 30). Data from this report and others regarding AT1R-AA may be sufficient to justify a clinical trial of ARB in liver transplantation.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

Data Availability Statement

Anonymized subject level data and statistical codes are available.

Figure Legends

Figure 1. Pretransplantation levels of AT1R-AA (U/ml) were higher in patients who lost their second liver transplants [median=19.63 (IQR 14.95 to 72.20)] than in those with functioning grafts [median=16.12 (IQR 11.50 to 21.21)], $p=0.019$, Mann-Whitney test.

Figure 2. Kaplan–Meier survival curves for graft (panel A) and patient (panel B) survival, comparing patients with levels of AT1R-AA < 40 U/ml and patients with levels of AT1R-AA > 40 U/ml.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

References:

1. Dragun D, Muller DN, Brasen JH, Fritsche L, Nieminen-Kelha M, Dechend R, et al. Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med*. 2005;352(6):558-69.
2. Fichtner A, Susal C, Schroder C, Hocker B, Rieger S, Waldherr R, et al. Association of angiotensin II type 1 receptor antibodies with graft histology, function and survival in paediatric renal transplant recipients. *Nephrol Dial Transplant*. 2018;33(6):1065-72.
3. Taniguchi M, Rebellato LM, Cai J, Hopfield J, Briley KP, Haisch CE, et al. Higher risk of kidney graft failure in the presence of anti-angiotensin II type-1 receptor antibodies. *Am J Transplant*. 2013;13(10):2577-89.
4. González-Almada A, Arreola-Guerra JM, López-Sánchez JA, Cuevas E, Vilatoba M, Contreras AG, et al. Pretransplant angiotensin ii type 1-receptor antibodies point to an increase in renal graft sub-intimal fibrosis in living- donor kidney transplant recipients. *Transpl Immunol*. 2018.
5. Giral M, Foucher Y, Dufay A, Van Huyen JP, Renaudin K, Moreau A, et al. Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss. *Am J Transplant*. 2013;13(10):2567-76.
6. Reinsmoen NL. Role of angiotensin II type 1 receptor-activating antibodies in solid organ transplantation. *Hum Immunol*. 2013;74(11):1474-7.
7. Reinsmoen NL, Mirocha J, Ensor CR, Marrari M, Chaux G, Levine DJ, et al. A 3-Center Study Reveals New Insights Into the Impact of Non-HLA Antibodies on Lung Transplantation Outcome. *Transplantation*. 2017;101(6):1215-21.
8. Gerlach UA, Lachmann N, Ranucci G, Sawitzki B, Schoenemann C, Pratschke J, et al. Non-HLA Antibodies May Accelerate Immune Responses After Intestinal and Multivisceral Transplantation. *Transplantation*. 2017;101(1):141-9.
9. O'Leary JG, Demetris AJ, Philippe A, Freeman R, Cai J, Heidecke H, et al. Non-HLA Antibodies Impact on C4d Staining, Stellate Cell Activation and Fibrosis in Liver Allografts. *Transplantation*. 2017;101(10):2399-409.
10. Pearl MH, Zhang Q, Palma Diaz MF, Grotts J, Rossetti M, Elashoff D, et al. Angiotensin II Type 1 receptor antibodies are associated with inflammatory cytokines and poor clinical outcomes in pediatric kidney transplantation. *Kidney Int*. 2018;93(1):260-9.
11. Philogene MC, Zhou S, Lonze BE, Bagnasco S, Alasfar S, Montgomery RA, et al. Pre-transplant Screening for Non-HLA Antibodies: Who should be Tested? *Hum Immunol*. 2018;79(4):195-202.
12. Gareau AJ, Wiebe C, Pochinco D, Gibson IW, Ho J, Rush DN, et al. Pre-transplant AT1R antibodies correlate with early allograft rejection. *Transpl Immunol*. 2018;46:29-35.
13. Xu Q, Shrum B, Leckie S, Skaro A, McAlister VC. The impact of alloantibodies directed against the second donor on long-term outcomes of repeat liver transplantation. *Hepatobiliary Surgery and Nutrition*. 2019;8(3):6.
14. McCaughan J, Xu Q, Tinckam K. Detecting donor-specific antibodies: the importance of sorting the wheat from the chaff. *Hepatobiliary Surg Nutr*. 2019;8(1):37-52.
15. Kim WR, Biggins SW, Kremers WK, Wiesner RH, Kamath PS, Benson JT, et al. Hyponatremia and mortality among patients on the liver-transplant waiting list. *N Engl J Med*. 2008;359(10):1018-26.
16. Kim WR, Lake JR, Smith JM, Skeans MA, Schladt DP, Edwards EB, et al. OPTN/SRTR 2015 Annual Data Report: Liver. *Am J Transplant*. 2017;17 Suppl 1:174-251.
17. O'Leary JG, Kaneku H, Banuelos N, Jennings LW, Klintmalm GB, Terasaki PI. Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. *Am J Transplant*. 2015;15(4):1003-13.

18. O'Leary JG, Kaneku H, Jennings LW, Bañuelos N, Susskind BM, Terasaki PI, et al. Preformed class II donor-specific antibodies are associated with an increased risk of early rejection after liver transplantation. *Liver Transpl*. 2013;19(9):973-80.
19. Ohe H, Uchida Y, Yoshizawa A, Hirao H, Taniguchi M, Maruya E, et al. Association of anti-human leukocyte antigen and anti-angiotensin II type 1 receptor antibodies with liver allograft fibrosis after immunosuppression withdrawal. *Transplantation*. 2014;98(10):1105-11.
20. Riemekasten G, Philippe A, Nather M, Slowinski T, Muller DN, Heidecke H, et al. Involvement of functional autoantibodies against vascular receptors in systemic sclerosis. *Ann Rheum Dis*. 2011;70(3):530-6.
21. Wallukat G, Homuth V, Fischer T, Lindschau C, Horstkamp B, Jupner A, et al. Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J Clin Invest*. 1999;103(7):945-52.
22. Xia Y, Kellems RE. Angiotensin receptor agonistic autoantibodies and hypertension: preeclampsia and beyond. *Circ Res*. 2013;113(1):78-87.
23. Burlingham WJ, Love RB, Jankowska-Gan E, Haynes LD, Xu Q, Bobadilla JL, et al. IL-17-dependent cellular immunity to collagen type V predisposes to obliterative bronchiolitis in human lung transplants. *J Clin Invest*. 2007;117(11):3498-506.
24. Pearl MH, Grotts J, Rossetti M, Zhang Q, Gjertson DW, Weng P, et al. Cytokine Profiles Associated With Angiotensin II Type 1 Receptor Antibodies. *Kidney Int Rep*. 2019;4(4):541-50.
25. Lefaucheur C, Viglietti D, Bouatou Y, Philippe A, Pievani D, Aubert O, et al. Non-HLA agonistic anti-angiotensin II type 1 receptor antibodies induce a distinctive phenotype of antibody-mediated rejection in kidney transplant recipients. *Kidney Int*. 2019.
26. Delville M, Lamarthee B, Pagie S, See SB, Rabant M, Burger C, et al. Early Acute Microvascular Kidney Transplant Rejection in the Absence of Anti-HLA Antibodies Is Associated with Preformed IgG Antibodies against Diverse Glomerular Endothelial Cell Antigens. *J Am Soc Nephrol*. 2019;30(4):692-709.
27. Wiwattanathum P, Ingsathit A, Thammanichanond D, Worawichawong S. Successful Treatment of Anti-angiotensin II Type 1 Receptor Antibody-Associated Rejection in Kidney Transplantation: A Case Report. *Transplant Proc*. 2018;50(3):877-80.
28. Iesari S, Lai Q, Favi E, Pisani F. Bortezomib-Containing Multimodality Treatment for Antibody-Mediated Rejection with Anti-HLA and Anti-AT1R Antibodies after Kidney Transplantation. *Yonsei Med J*. 2017;58(3):679-81.
29. Carroll RP, Riceman M, Hope CM, Zeng A, Deayton S, Bennett GD, et al. Angiotensin II type-1 receptor antibody (AT1Rab) associated humoral rejection and the effect of peri operative plasma exchange and candesartan. *Hum Immunol*. 2016;77(12):1154-8.
30. Jobert A, Rao N, Deayton S, Bennett GD, Brealey J, Nolan J, et al. Angiotensin II type 1 receptor antibody precipitating acute vascular rejection in kidney transplantation. *Nephrology (Carlton)*. 2015;20 Suppl 1:10-2.

Figure 1.

Table 1. Univariate Cox regressions for graft loss and AT1R-AA

	Total (n=94)	Graft functioning (n=46)	Graft lost (n=48)	HR(95% CI)	<i>p-val</i>
AT1R-AA (U/mL)- Median (IQR)		16.12 (11.50,21.21)	19.63 (14.95,72.20)	1.003 (1.000 - 1.006)	0.040
AT1R-AA >10 U/ml	79(84.0%)	38 (82.6%)	41 (85.4%)	0.934 (0.416 - 2.096)	0.868
AT1R-AA >17 U/ml	48(51.1%)	18 (39.1%)	30 (62.5%)	1.464 (0.816 - 2.629)	0.201
AT1R-AA >20 U/ml	36(38.3%)	13 (28.3%)	23 (47.9%)	1.509 (0.856 - 1.659)	0.155
AT1R-AA>40 U/ml	22(23.4%)	6 (13.0%)	16 (33.3%)	1.888 (1.035 -3.445)	0.038

P-values and hazard ratios (HR) were calculated using univariate Cox regression; negative counterparts were used as references for binomial variables. P-values <0.05 are highlighted in bold.

Table 2. Baseline characteristics and outcomes according to levels of preformed AT1R-AA

Variables	< 40 U/ml (n=72)	> 40 U/ml (n=22)	<i>p-val</i> ²
Patient Characteristics			
Recipient Age - Mean $\pm$ SD	39.1 $\pm$ 19.3	33.2 $\pm$ 14.9	0.190
Recipient Male gender - n (%)	46 (63.9%)	13 (59.1%)	0.684
MELD Score - Mean $\pm$ SD ¹	24.4 $\pm$ 9.3	24.7 $\pm$ 9.1	0.919
Class I DSA Positive >1000 – n (%)	12 (16.7%)	3 (13.6%)	0.734
Class II DSA Positive >1000 – n (%)	18 (25.0%)	9 (40.9%)	0.149
Immunosuppression			1.000
No induction/Tac/MMF	63 (87.5%)	19 (86.4%)	
OKT3/CsA/AZA	9 (12.5%)	3 (13.6%)	
Primary liver disease			0.065
Viral	18 (25.0%)	3 (13.6%)	
PSC	11 (15.3%)	10 (45.5%)	
PBC	4 (5.6%)	1 (4.5%)	
Autoimmune Hepatitis	8 (11.1%)	0	
Alcoholic cirrhosis	3 (4.2%)	2 (9.1%)	
Cryptogenic / steatohepatitis	7 (9.7%)	2 (9.1%)	
Other	21 (29.2%)	4 (18.2%)	
Survival of 1st Graft (Years)			
- Mean $\pm$ SD	2.8 $\pm$ 4.4	4.5 $\pm$ 5.5	0.141
Reasons for loss of initial graft			0.026
PNF	16 (22.2%)	0	
Rejection	14 (19.4%)	7 (31.8%)	
Hepatic Artery Thrombosis	16 (22.2%)	5 (22.7%)	
Recurrence	11 (15.3%)	8 (36.4%)	
Other	15 (22.8%)	2 (9.1%)	
Donor Characteristics			
Donor Age - Mean $\pm$ SD	39.9 $\pm$ 19.0	37.7 $\pm$ 20.0	0.630
CIT of 2nd Graft (Hour) - Mean $\pm$ SD	7.6 $\pm$ 2.5	7.6 $\pm$ 3.0	0.953
Year of 2nd Transplant - Mean $\pm$ SD	2004.7 $\pm$ 8.3	2003.4 $\pm$ 7.6	0.512
Outcomes			
Graft loss – n (%)	32 (44.4%)	16 (72.7%)	0.020
Patient death – n (%)	28 (38.9%)	13 (59.1%)	0.094

PBC - primary biliary cirrhosis; PSC - primary sclerosing cholangitis; PNF - primary nonfunction; CIT – cold ischemia time

¹ 22 cases missing MELD scores (11 with graft loss, 11 with graft functioning)

² Binomial/categorical variables were compared using Chi-square test or Fisher's exact test for counts < 5; continuous variables were compared using Student's T-test. P-values <0.05 are highlighted in bold.

Figure 2.A

Figure 2B.

Table 3. Confounders associated with graft survival based on univariate Cox regression.

Variables	Graft functioning (n=46)	Graft lost (n=48)	HR(95% CI)	<i>p-val</i>
Patient Characteristics				
Recipient Age - Mean $\pm$ SD	39.9 $\pm$ 20.5	41.7 $\pm$ 16.8	1.018 (1.001 - 1.034)	0.032
Recipient Male gender - n (%)	25 (54.3%)	34 (70.8%)	2.233 (1.186 - 4.206)	0.013
Survival of 1st Graft (Years) - Mean $\pm$ SD	2.4 $\pm$ 3.4	4.0 $\pm$ 5.6	1.058 (0.997 - 1.122)	0.063
Immunosuppression				
No induction/Tac/MMF	41 (89.1%)	41 (85.4%)	1	
OKT3/CsA/AZA	5 (10.9%)	7 (14.6%)	0.999 (0.446 - 2.240)	0.998
Primary Diseases				
Viral	11 (23.9%)	10 (20.8)	1	
PSC	9 (19.6%)	12 (25.0%)	0.814 (0.348 - 1.906)	0.636
PBC	2 (4.3%)	3 (6.3%)	0.893 (0.242 - 3.293)	0.864
Autoimmune Hepatitis	2 (4.3%)	6 (12.5%)	1.260 (0.457 - 3.473)	0.656
Alcoholic cirrhosis	1 (2.2%)	4 (8.3%)	1.834 (0.573 - 5.865)	0.307
Cryptogenic/Steatohepatitis	5 (10.9%)	4 (8.3%)	0.582 (0.181 - 1.870)	0.363
Other	16 (34.8%)	9 (18.8%)	0.433 (0.173 - 1.082)	0.073
Donor Characteristics				
Donor Age - Mean $\pm$ SD	35.3 $\pm$ 19.1	43.3 $\pm$ 18.7	1.018 (1.003 - 1.033)	0.015
CIT (Hour) - Mean $\pm$ SD	7.9 $\pm$ 2.6	7.3 $\pm$ 2.6	0.889 (0.793 - 0.997)	0.045
DSA Characteristics				
Any DSA Positive >1000	15 (32.6%)	19 (39.6%)	1.037 (0.581 - 1.850)	0.903
Class I DSA Positive >1000	8 (17.4%)	7 (14.6%)	0.813 (0.365 - 1.813)	0.613
Class II DSA Positive >1000	10 (21.7%)	17 (35.4%)	1.257 (0.695 - 2.272)	0.449

PBC: Primary biliary cirrhosis, PSC: Primary sclerosing cholangitis; CIT: Cold ischemia time; P-values and hazard ratios (HR) were calculated using univariate Cox regression. P-values <0.05 are highlighted in bold.

Table 4. Multivariate Cox regression of graft and patient survival

Variables	Graft Loss		Patient Death	
	HR (95% CI)	<i>p-val</i>	HR (95% CI)	<i>p-val</i>
Male Gender	2.255 (1.162 - 4.376)	0.016	2.030 (0.986 - 4.183)	0.055
Recipient Age, Year	1.009 (0.991 - 1.027)	0.309	1.020 (1.000 - 1.041)	0.056
Donor Age, Year	1.019 (1.004 - 1.034)	0.016	1.015 (0.999 - 1.032)	0.065
CIT, Hour	0.927 (0.826 - 1.039)	0.192	0.946 (0.839 - 1.067)	0.366
AT1R-AA>40 U/ml	1.999 (1.085 - 3.682)	0.026	1.879 (0.961 - 3.675)	0.065

CIT: Cold ischemia time; P-values and hazard ratios (HR) were calculated using multivariate Cox regression. P-values <0.05 are highlighted in bold.