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## **Efficacy of Therapeutic Plasma Exchange on Angiotensin II Type 1 Receptor Antibodies on Two Kidney Transplant Recipients**

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**Running head: Efficacy of TPE on AT1R antibody removal**  
**Abstract**

**Background:** Angiotensin II type-1 receptor antibody (AT1RAb) has been reported to cause antibody mediated rejection (AMR) in kidney transplant recipients possibly by contraction of renal arteries. We here report two kidney transplant recipients with elevated AT1RAbs and negative HLA donor specific antibodies (DSA) and anti-major histocompatibility complex class I chain-related gene A (MICA) Abs who received TPE treatment followed by IVIG.

**Case 1:** 38-year-old patient received second kidney transplant for end stage renal disease (ESRD) with chronic rejection. Three years post-transplant, she developed AMR with AT1RAb level of >40 U/ml. She received 5 TPE and AT1RAb decreased by 20%, and biopsy showed improvement of AMR. She received another 3 TPE and AT1RAb decreased by 60%. Her Cr was stabilized at around 1.4 mg/dl.

**Case 2:** 24 -year-old patient received kidney transplant for ESRD with unclear etiology. Two weeks post-transplant, her Cr rose with AT1RAb level at 18 U/ml and biopsy showed possible AMR. She received 6 TPE treatments and AT1RAb decreased by 55% and biopsy showed improvement of AMR. She received weekly TPE for subsequently rising AT1RAb but TPE was discontinued because of unsuccessful decrease of AT1RAb. Her Cr was stabilized at around 1.7 ml/dl.

**Conclusion:** We reported two patients who received TPE treatments to decreased AT1RAbs. A course of TPE treatment successfully decreased AT1Rab. Histological improvement was observed quickly and Cr was also stabilized following the TPE treatment. Further study is necessary to determine the optimal use of TPE in renal transplant recipients with AT1RAbs.

**Key words:** therapeutic plasma exchange, kidney transplantation, antibody mediated rejection, angiotensin II type-1 receptor antibody

### **Introduction**

Some renal transplant recipients develop antibody mediated rejection (AMR) without detectable HLA donor specific antibodies (DSAs) in sera. In recent years, angiotensin II type-1 receptor antibody (AT1Rab) has been reported to be associated with kidney AMR, especially refractory AMR, possibly by contraction of renal arteries. Therapeutic plasma exchange (TPE) has been increasingly applied to reduce HLA DSA in kidney AMR, less is known regarding the role of TPE in AMR due to non-HLA Abs such as AT1RAB. Although there are several published reports describing the use of TPE in the treatment of AT1Rab associated AMR, the efficacy of TPE in reducing AT1Rab levels has not been reported.

Our institution has been implementing AT1Rab testing for the kidney transplant recipients with biopsy proven or suspicious for AMR on renal biopsy without HLA DSA, and a course of treatment with 5-6 TPE followed by IVIG has been applied on those patients as same as on patients with HLA DSA. We investigated AT1Rab and creatinine (Cr) levels before and after TPE treatments in those patients and report two patients here.

## **Methods and material**

### **Patients**

Renal transplant recipients with elevated AT1RAbs, negative HLA DSAs and negative anti-major histocompatibility complex class I chain-related gene A (MICA) Abs who received TPE treatments for histologically proven or suspicious for AMR between January 2014 and December 2016 were identified in our institutional renal transplant database. Among those patients, a total of 9 patients with elevated AT1RAb levels before TPE treatment were identified. Among them, 2 patients had positive HLA DSAs, 3 patients lack follow-up laboratory tests, and 2 patients had follow-up AT1RAb tests more than 3 months after the last TPE procedure. The remaining 2 patients' demographics, causes of kidney failure, types of donor, graft age at the first TPE procedure and angiotensin II receptor blockers (ARB) use are shown in Table 1. Of note, no patient received rituximab at the time of TPE treatment.

### **Angiotensin II type-1 receptor antibody (AT1RAb)**

Serum AT1RAb level was measured using ELISA test (One Lambda, Canoga Park, CA) and reported as high (>17 U/ml), intermediate (12-17 U/ml) and negative (<12 U/ml). Because of the lack of protocol to test AT1RAb level, AT1RAb levels were tested in various time points after TPE treatment. No patient was tested for AT1RAb prior to their transplantations and this test is currently unavailable for validation process at our institution.

## **Therapeutic Apheresis**

In our facility, renal transplant recipients with high DSA against HLAs or non-HLA Abs and/or biopsy proven AMR receive a course of TPE treatment (5-6 TPE followed by IVIG infusion each time). Additional TPE may be performed after the initial course based on the patient's response to the treatment or post-TPE antibody level (ex. AT1RAb, HLA DSA). TPE procedures were performed using either Cobe Spectra or Spectra Optia Apheresis System (TerumoBCT, Lakewood, CO), and one plasma volume was exchanged per procedure. Citrate dextrose solution A (ACD-A) was used as an anti-coagulant and 5% albumin was used as a replacement fluid.

## **Results**

### **CASE 1**

Patient A was a 42-year-old female with focal segmental glomerulosclerosis (FSGS) who received her first living related donor kidney transplant at age 22, and a second deceased donor transplant due to a chronic rejection of the transplanted kidney at age 38. Three years post-transplant, her Cr started to rise from 0.7 to 1.35 mg/dl and a biopsy showed Banff grade 2 AMR, grade 2A T-cell mediated rejection (TCMR) and grade 3 interstitial fibrosis and tubular atrophy (IFTA) (Table 2). Serum HLA DSA and anti-MICA Abs were negative, but high level AT1RAb was identified at >40 U/ml (Figure1 Case 1). She received 5 TPEs each followed by IVIG every other day over 9 days and started ARB treatment. The AT1RAb level measured 2 days after a course of TPE treatment decreased to 32 U/ml (20.0% reduction), and histology showed grade 0

AMR and 1B TCMR 26 days after the last TPE treatment. Over the course of the next month, her Cr slowly increased to 1.64 mg/dl, accompanied by a rise in AT1RAb (>40 U/ml). She was treated with 3 more TPE treatments every other day and her AT1RAb level decreased to 16 U/ml (60.0% reduction). Although AT1RAb level increased slightly to 20 U/ml, kidney biopsy still showed grade 0 AMR 3 months after the last TPE. Her Cr at approximately one year after the last TPE was at around 1.4 mg/dl, and her most recent Cr in January 2018 was 1.31 mg/dl. HLA DSA has been negative to date.

## **CASE 2**

Patient B was a 25-year-old female with renal failure due to unclear etiology who received a deceased donor kidney transplant at age 24. Her Cr started to rise 2 weeks post-transplant from 1.40 to 2.68 mg/dl (Figure 1 Case 2) without detectable HLA DSA and anti-MICA Abs. A biopsy showed Banff grade 0 AMR, grade 0 TCMR and grade 1 IFTA (Table 2), but had focally severe endothelial swelling and cell injury suggesting process for AMR. Her AT1RAb level was found to be elevated at 18 U/ml. She received 6 TPE each followed by IVIG every other day over 11 days and her AT1RAb level decreased to 8 U/ml (55.6 % reduction). Her Cr was improved to 1.98 mg/dl, and biopsy also showed improved arteriopathy 24 days after a course of TPE. However, she was started on weekly TPE treatment for the next 5 weeks because of rising AT1RAb (12 U/ml) two weeks after completing TPE. Her AT1RAb levels measured before her 5<sup>th</sup> weekly TPE treatment showed increase to 16 U/ml, therefore, TPE was discontinued. Kidney biopsy 25 days after the last TPE still showed grade 0 AMR. Clinically, her Cr remained stable

at around 1.7 ml/dl for 10 months after the last TPE. She had subsequent CMV infection, which caused mild deterioration of kidney function and her most recent Cr in March 2018 was 2.75 mg/dl. HLA DSA has been negative to date.

## Discussion

The majority of AMR in renal transplantation is attributed to DSAs to HLA antigens. In recent years, however, several non-HLA Abs have been linked to AMR including anti-endothelial cell Abs, MICA Abs, anti-AT1RAbs, anti-endothelin-1 type A receptor Abs, anti-poly-reactive Abs, anti-LG3 Abs and anti-vimentin Abs.<sup>3,4</sup>

AT1R mediates physiological actions of angiotensin II (ATII) in many organs including kidney. ATII promotes vasoconstriction, inflammation, thrombosis, and vascular remodeling<sup>5</sup>, and AT1R regulates arterial blood pressure, electrolyte and water balance, and renal function.<sup>6</sup> Therefore, hyper-activation of AT1R by AT1RAbs can cause hypertension and kidney dysfunction with vasoconstriction and thrombosis. AT1Rab can be autoimmune or alloimmune Ab. Preoperative positive AT1R using ELISA assay are found in 23% to 47.2% of the patients who received first or multiple kidney transplantations<sup>7-10</sup>, de novo development of AT1Rab after the transplantation is reported in 45% of the pediatric patients<sup>10</sup>, and 9.4% of the patients with rejection are reported to have AT1RAbs<sup>11</sup>. AT1Rab is also associated with steroid refractory vascular rejection or higher rate of late graft loss<sup>7,8,10,12-14</sup>. Banasik et al.<sup>8</sup> reported that patients

with AT1RAbs run a higher risk of graft failure independently of classical immunological risk factors, but not acute rejection or hypertension.

There are some reports in which TPE was used to treat AMR in kidney transplant recipients with AT1RAbs. Jobert et al. reported a case of AMR in a kidney transplant recipient with AT1RAb, who received 6 TPE procedures with 1.5 plasma volume exchange.<sup>15</sup> Fuss et al. used 3-11 TPE procedure to treat 6 kidney AMR patients with AT1RAbs.<sup>16</sup> Lee et al.<sup>17</sup> treated 11 cases of AT1RAb associated AMR with a combination of TPE and IVIG, sometimes followed by rituximab (9/12), anti-thymocyte globulin (5/12) or bortezomib (1/12). All patients experienced clinical improvement and/or resolution of AMR. None of these reports, however, measured AT1RAb level after TPE treatment or analyzed role and efficacy of TPE in reducing AT1RAb, and correlation with clinical outcomes. Our cases show that one course of 5-6 TPE treatment, followed by IVIG, successfully reduces AT1RAb level by 20.0 % and 55.6%, median 37.8%. This rate is similar to the reduction rates of HLA DSA in kidney transplant recipients with AMR that we previously reported elsewhere.<sup>18</sup> However, the effect of TPE was temporally and AT1RAb rebound and increase over the time were also observed, which indicates that follow-up of these patients is critical. Some of the cases in these reports received a combination of treatment with TPE and ARB, however, report for an effect of ARB only on renal AMR caused by AT1RAbs was not found at this time. It may be because transplant nephrologists have been reluctant to use ARB because of possible decreases in organ perfusion and filtration mentioned by Dragun et al.<sup>13</sup>.



The improvement of kidney function was also observed after the TPE treatment. Cr was stabilized without further increase. Therefore, it may be important to treat patients with biopsy proven or suspicious for AMR without HLA DSA even if AT1RAb testing is not available to prevent further deterioration of kidney function, especially when those patients were proven to have AT1RAbs which are thought to have a higher probability for late graft failure as mentioned above. In addition, there is a possibility that these patients had other unknown antibodies against donor kidney in addition to AT1RAb that could be also decreased by TPE treatment and medications, which might be taking some role in improvement of biopsy or kidney function.

The current Banff histologic classification for kidney AMR is based primarily on AMR resulting from HLA DSAs. Although the Banff classification is also used for non-HLA AMR, the mechanisms underlying renal damage and dysfunction may differ, which could impact biopsy results. Specifically, HLA Abs frequently cause tissue damage through complement cascade activation. As a result, biopsy results in HLA DSA-mediated AMR often shows C4d positivity on peritubular capillaries. In contrast, kidney failure and AMR due to AT1RAb are thought to be caused by renal artery vasoconstriction.<sup>19</sup> The reason that a negative C4d is a common finding in AT1RAb-mediated AMR<sup>15,16,20,21</sup> may be because complement may not be involved for kidney dysfunction. In addition, vasculitis, thrombosis and glomerulopathy may be common and the predominant histologic findings. Lim et al.<sup>22</sup> did not find an association between peritubular capillary C4d staining and AT1RAb, however, reported several cases with positive C4d staining. In our 2 patients, patient A had a weak C4d staining which disappeared

after TPE treatment and patient B had negative C4d staining on biopsies. Overall, the histological abnormalities were improved after a course of TPE treatment in both patients, which may suggest removing AT1RAbs by TPE and suppression of Ab production by medications may be able to decrease vasoconstriction quickly and to provide prevention of further damage on kidney.

In summary, we presented a first report on efficacy of TPE treatment followed by IVIG in reducing AT1RAb level in 2 patients. A course of TPE decreased AT1RAb by median 37.8% measured within 1 month after the last TPE. However, rebound and subsequent increase of AT1RAb was also observed, therefore follow-up of AT1RAb level is important when AT1RAb lab test is available. Histological improvement in AMR was observed after a course of TPE treatment and Cr did not show further deterioration of the kidney function in several months, which may suggest decreased level of AT1RAb by treatment with TPE followed by IVIG may be able to decrease vasoconstriction caused by AT1RAb and to prevent further deterioration of kidney function. A further study is needed to determine if TPE treatment is more favorable over ARB treatment, and optimal use of TPE in renal transplant patients with AT1RAbs.

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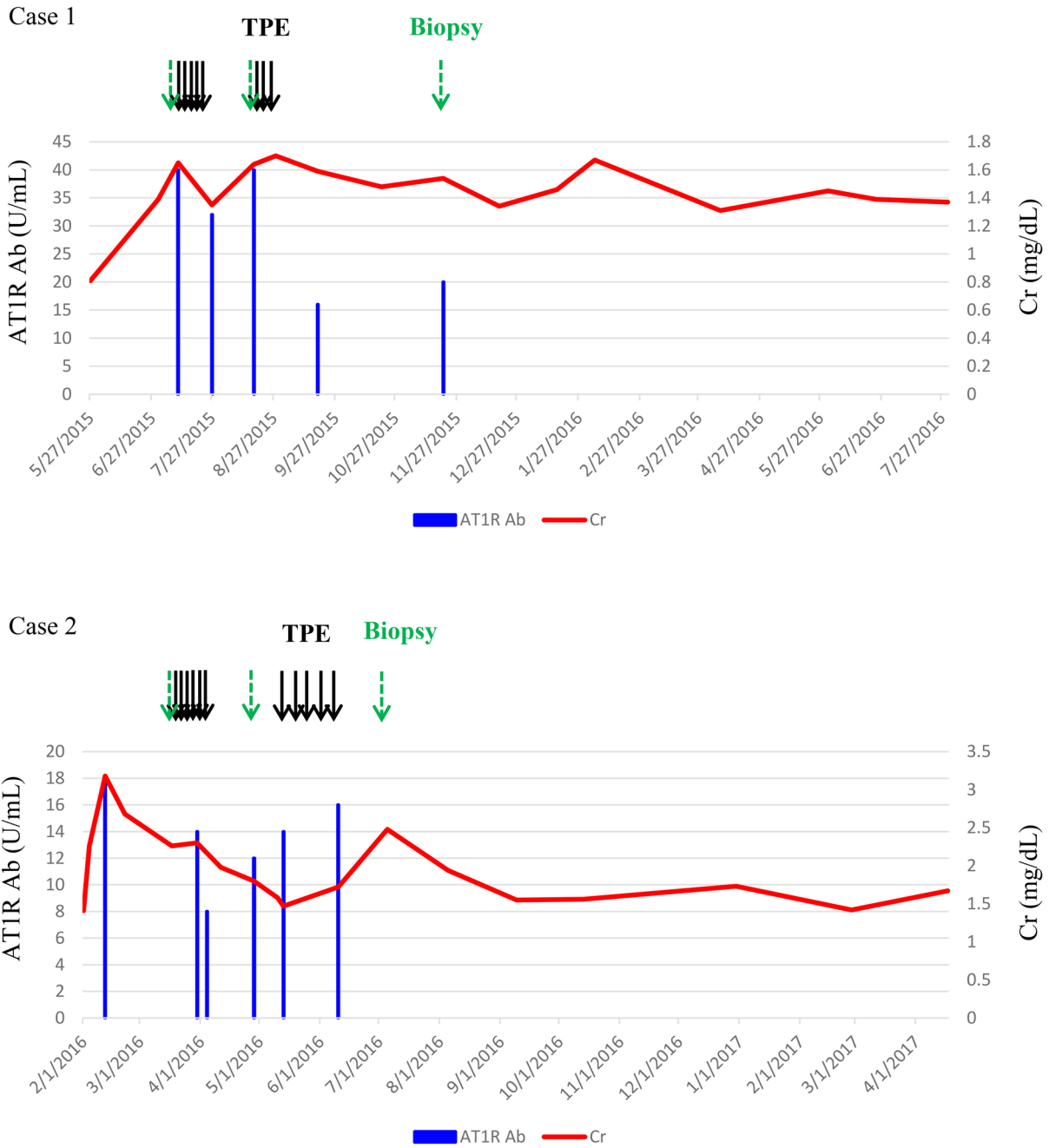
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Figure 1: AT1R Ab level, creatinine level and the timing of TPE and kidney biopsies



TPE: therapeutic plasma exchange, AT1R Ab: angiotensin II type 1 antibody, Cr: creatinine

Figure.tif



Table 1: Demographics and Angiotensin II Antagonist Use

<b>Patient</b>	<b>Gender</b>	<b>Race</b>	<b>Cause of kidney failure</b>	<b>Type of donor</b>	<b>Age at transplant</b>	<b>Age at 1<sup>st</sup> TPE</b>	<b>Graft age at 1<sup>st</sup> TPE (days)</b>	<b>ARB use</b>
A	female	AA	FSGS	Living related	22	-	-	-
			Chronic rejection	Deceased	38	42	1,098	Yes
B	female	AA	Unclear etiology	Deceased	24	25	61	No

FSGS: focal segmental glomerulosclerosis, AA: African American, HTN: hypertension, DM: diabetes mellitus, TPE: therapeutic plasma exchange, ARB: angiotensin II receptor blocker

Table 2: Kidney Biopsy Results

Patient	Renal Biopsy date	AMR Grade	TCMR Grade	IFTA Grade	Peritubular capillary C4d	Change regarding AMR
A	7/10/2015	2	2A	3	Focal, weak	Severe tubulitis and capillaritis
	8/14/2015	0	1B	3	negative	Reduction in interstitial inflammation, improvement in the capillaritis, persistent tubulitis
	11/20/2015	0	-	3	negative	No change compared to previous biopsy
B	3/18/2016	0	0	1	negative	Segmental glomerulitis and vasculopathy
	4/28/2016	0	0	2	negative	Increased interstitial fibrosis
	7/5/2016	0	0	1	negative	Focal glomerulopathy and interstitial fibrosis

AMR: antibody mediated rejection, TPE: therapeutic plasma exchange, ICMR: T-cell mediated rejection, IFTA: interstitial fibrosis and tubular atrophy