Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Acceptable Donor-Specific Antibody Levels Before and After Desensitization Therapy in Living Donor Kidney Transplantation

Ji Won In [1, 2], Minjeong Nam [2], Eun Youn Rho [2], Sue Shin [2], Yun Ji Hong [2], Kyoung Un Park [2], Eun Young Song [2]

[1] *Departments of Laboratory Medicine, Seoul Medical Center, Seoul, Korea*
[2] *Departments of Laboratory Medicine, Seoul National University College of Medicine, Seoul, Korea*

SUMMARY

Background: **Plasmapheresis (PP) is commonly used for desensitization in highly sensitized patients with donor-specific antibodies (DSA) in living donor kidney transplantation. We analyzed the impact of DSA levels before and after desensitization on renal allograft outcome.**
Methods: **Twenty-three patients who underwent desensitization with PP, intravenous immunoglobulin (IVIG), and rituximab before kidney transplantation in Seoul National University Hospital from August 2006 to August 2016 were enrolled. The association of median fluorescent intensity (MFI) value of DSA with graft outcome was analyzed.**
Results: **The frequency of positive HLA class II DSA after desensitization was lower in patients without antibody-mediated rejection (AMR) compared to those with AMR (p = 0.006). The cutoff value of MFI sum of HLA class II DSA after desensitization for predicting AMR was 2,122 with 63% sensitivity and 94% specificity. The frequency of moderate HLA class II DSA (MFI 5,000 - 10,000) after desensitization was significantly higher in patients with graft loss compared to those without graft loss (p = 0.02).**
Conclusions: **Weak HLA class II DSA after desensitization including PP, IVIG, and rituximab was related to AMR and moderate levels of HLA class II DSA after desensitization was related to graft loss in living donor kidney transplantation.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190706)

Correspondence:
Eun Young Song, MD, PhD
Department of Laboratory Medicine
Seoul National University College of Medicine
Daehak-ro 101, Jongno-gu
03080 Seoul
Korea
Phone: +82 2-2072-0197
Fax: +82 2-747-0359
Email: eysong1@snu.ac.kr

Manuscript accepted July 12, 2019

KEY WORDS

graft rejection, kidney transplantation, plasmapheresis

INTRODUCTION

The presence of donor specific HLA antibodies (DSAs) pre-transplantation are known to be associated with antibody-mediated rejection (AMR) and allograft loss [1, 2]. Desensitization including plasmapheresis (PP), low-dose intravenous immunoglobulin (IVIG), and rituximab has been used to decrease DSA in highly sensitized patients for living donor kidney transplantation [3-6]. However, the acceptable level of DSA before and after desensitization including PP, IVIG, and rituximab in living donor kidney transplantation is not well defined

yet. The aim of this study was to evaluate the association of DSA levels before and after desensitization therapy including PP, IVIG, and rituximab with renal allograft outcome.

MATERIALS AND METHODS

Subjects

A total of 23 patients who were treated with desensitization therapy with PP, IVIG, and rituximab before kidney transplantation from living donors in Seoul National University Hospital from August 2006 to August 2016 were included. The desensitization protocol was as follows; Tacrolimus (0.05 mg/kg twice daily) and mycophenolate mofetil (MMF, 750 mg twice daily) were administered two days before the first PP. PP was performed three times a week using 4% albumin and/or fresh frozen plasma, replacing 1 to 1.5 plasma volumes at a time. The number of PP procedures for each patient was from 3 to 11 (5.9 ± 2.5). After each PP, intravenous immunoglobulin (IVIG, 100 mg/kg) was given. Rituximab (375 mg/m^2 of body surface area, i.v.) was given three days before the first PP and pre-transplantation one day. Transplantation was performed when the flow cytometric HLA crossmatch test was converted to negative. For induction therapy, basiliximab (4 mg i.v. on day 0 and day 4) was administered. The immunosuppression with tacrolimus, MMF, and prednisolone were administered during the post-transplantation period. This study was approved by Institutional Review Board of Seoul National University Hospital (IRB No. 1904-123-102).

HLA DNA typing

DNA samples of patients and donors were extracted from peripheral blood by using QuickGene DNA whole blood kit (Fujifilm, Tokyo, Japan) or LaboPass Genomic DNA Extraction Kit (COSMO, Seoul, Korea). HLA-A, -B, and -DR typing was performed using LIFECODES HLA sequence specific oligonucleotide (SSO) Typing Kits (Immucor Transplant Diagnostics, Stamford, CT, USA), according to the manufacturer's recommendations. HLA-DQ typing of donors was performed when DQ antibodies were detected to determine reactivity to donor HLA-DQ.

HLA crossmatch test

Complement dependent cytotoxic crossmatch was performed following the method by National Institutes of Health and enhanced technique with anti-human globulin [7]. Flowcytometric crossmatch (FCXM) was performed as previously described with pronase treatment [8]. Peridinin chlorophyll protein (PerCP)-mouse anti-human CD3 and phycoerythrin (PE)-mouse anti-human CD19 (all from BD Bioscience, San Jose, CA, USA) were used for staining T and B lymphocytes, respectively. FITC-mouse anti-human IgG (Jackson Immunoresearch, West Grove, PA, USA) antibodies were added for detection of bound antibodies. FACSCalibur (BD Biosciences, NJ, USA) was used for flowcytometric analysis. Positive crossmatch result was defined as the MFI (median fluorescent intensity) ratio (test sample median MFI value to normal human AB type serum MFI value) of more than 2.0 for T cells and more than 1.5 for B cells.

Anti-HLA antibody assay

Anti-HLA antibody assay was performed by LIFECODES LSA Class I and II Single Antigen (Immucor, Stamford, CT, USA), according to the recommendations of the manufacturer. In brief, 10 μL of serum and 40 μL of single antigen beads of HLA class I or class II were added to microplate wells. Incubation of the microplate for 30 minutes was performed at room temperature in the dark. After the microplate wells were washed, 50 μL of secondary PE-conjugated goat anti-human IgG antibody was added, followed by incubation at room temperature for 30 minutes in the dark. Samples were analyzed with a Luminex 200TM system (Luminex, Austin, TX, USA). The cutoff value of MFI to define the positive reaction was 500. The intensity of MFI values of DSA were classified as strong (MFI ≥ 10,000), moderate (5,000 - 9,999), weak (1,000 - 4,999), and very weak (500 - 999). For quantitative comparison, MFI of a negative result (< 500) was regarded as 500. DSA levels were defined as the sum of MFI values of each DSA: anti-HLA class I DSA (anti-HLA-A+anti-HLA-B), anti-HLA class II DSA (anti-HLA-DR, anti-HLA-DR51/52/53, and anti-HLA-DQ), and total DSA (anti-HLA class I + anti-HLA class II).

Monitoring and treatment of rejection

Protocol biopsy was performed at about 12 days and 1 year post-transplantation. In addition, all patients who were suspected of rejection by abnormal laboratory tests underwent a kidney biopsy. The biopsy results were described by the Banff 2013 classification [9]. Patients with T-cell mediated rejection (TMR) were given methylprednisolone pulse therapy for 3 days (1g/day). AMR was treated with PP, IVIG (100 mg/kg), and rituximab (375 mg/m^2). The median follow-up months was 64.2 ± 37.9 (median ± SE).

Statistical methods

The median ± standard error (SE) was calculated for quantitative variables. Comparison of categorical variables between the two groups was analyzed using Fisher's exact test or the chi-square test. For analysis of continuous variables, Mann-Whitney *U* test was applied. ROC curve analysis of each parameter (DSA class I, class II, and total DSA) for predicting TMR or AMR was performed. A p-value < 0.05 was considered significant. Statistical software used was SPSS, version 21.0 (IBM, Armonk, NY, USA).

Table 1. Association of donor-specific HLA antibody before desensitization (baseline) with graft rejection.

DSA	AR (n = 11) n (%)	No AR (n = 12) n (%)	p	AMR (n = 8) n (%)	No AMR (n = 15) n (%)	p	TMR (n = 5) n (%)	No TMR (n = 18) n (%)	p
Class I/II	11 (100.0)	10 (83.3)	ns	8 (100.0)	14 (93.3)	ns	4 (80.0)	17 (94.4)	ns
Class I	5 (45.5)	8 (66.7)	ns	3 (37.5)	10 (66.7)	ns	2 (40.0)	11 (61.1)	ns
A	4 (36.4)	3 (25.0)	ns	3 (37.5)	4 (26.7)	ns	2 (40.0)	5 (27.8)	ns
B	2 (18.2)	6 (50.0)	ns	1 (12.5)	7 (46.7)	ns	1 (20.0)	7 (38.9)	ns
Class II	8 (72.7)	5 (41.7)	ns	7 (87.5)	6 (40.0)	ns	4 (80.0)	9 (50.0)	ns
DR *	5 (45.5)	2 (16.7)	ns	5 (62.5)	2 (13.3)	0.026	3 (60.0)	4 (22.2)	ns
DR51/52/53	3 (27.3)	3 (25.0)	ns	3 (37.5)	3 (20.0)	ns	0 (0.0)	6 (33.3)	ns
DQ	5 (45.5)	2 (16.7)	ns	4 (50.0)	2 (13.3)	ns	2 (40.0)	5 (27.8)	ns

AR - all rejections, AMR - antibody mediated rejection, TMR - T cell mediated rejection, ns - not significant. * - HLA DR specificities excluding DR51/52/53.

Table 2. Association of donor-specific HLA antibody after desensitization (at the time of transplantation) with graft rejection.

DSA	AR (n = 11) n (%)	No AR (n = 12) n (%)	p	AMR (n = 8) n (%)	No AMR (n = 15) n (%)	p	TMR (n = 5) n (%)	No TMR (n = 18) n (%)	p
Class I/II	11 (100.0)	5 (41.7)	0.037	8 (100.0)	8 (53.3)	ns	5 (100.0)	11 (61.1)	ns
Class I	5 (45.5)	3 (25.0)	ns	3 (37.5)	5 (33.3)	ns	2 (40.0)	6 (33.3)	ns
A	3 (27.3)	0 (0.0)	ns	2 (25.0)	1 (7.1)	ns	1 (20.0)	2 (11.1)	ns
B	2 (18.2)	3 (25.0)	ns	1 (12.5)	4 (26.7)	ns	1 (20.0)	4 (22.2)	ns
Class II	8 (72.7)	2 (16.7)	0.012	7 (87.5)	3 (20.0)	0.006	4 (80.0)	6 (33.3)	ns
DR *	5 (45.5)	0 (0.0)	0.014	6 (75.0)	0 (0.0)	< 0.001	3 (60.0)	3 (16.7)	ns
DR51/52/53	3 (27.3)	2 (16.7)	ns	3 (37.5)	1 (66.7)	ns	0 (0.0)	5 (27.8)	ns
DQ	5 (45.5)	1 (8.3)	ns	3 (37.5)	2 (13.3)	ns	1 (20.0)	4 (22.2)	ns

AR - all rejections, AMR - antibody mediated rejection, TMR - T cell mediated rejection, ns - not significant. * - HLA DR specificities excluding DR51/52/53.

RESULTS

Among 23 patients, after desensitization, class I DSA was positive in 8 patients and class II DSA was positive in 10 patients. Eight patients had acute AMR (three combined with TMR), one patient had chronic AMR, and two patients had only TMR.

Regarding baseline DSA (before desensitization), positive rate of baseline HLA-DR DSA was significantly lower in patients without AMR compared to those with AMR (13.3% vs. 62.5%, p = 0.026) (Table 1). After desensitization, class I DSA did not affect occurrence of allograft rejection. However, frequency of class II DSA was significantly lower in patients without all rejections (AR) compared to those with AR (16.7% vs. 72.7%, p = 0.012) (Table 2). Positive rate of class II DSA after desensitization was also significantly lower in patients without AMR compared to those with AMR (20.0% vs. 87.5%, p = 0.006), however, it was not associated with TMR. Among class II DSA, frequency of HLA-DR DSA was significantly lower in patients without AR compared to those with AR (0.0% vs. 45.5%, p = 0.014). Frequency of HLA-DR DSA was also significantly lower in patients without AMR compared to those with AMR (0.0% vs. 75.0%, p < 0.001). Positive rates of HLA-DR51/52/53 or HLA-DQ DSA were not associated with AMR (Table 2).

For the survival of the allograft, the frequency of strong HLA class II DSA (MFI > 10,000) before desensitization and moderate intensity HLA class II DSA (MFI 5,000 - 10,000) after desensitization were significantly higher in patients with graft loss (n = 2) compared to

Table 3. Median MFI values of DSA according to occurrence of AMR.

	AMR (n = 8)	No AMR (n = 15)	p
Baseline (before desensitization)			
Class I + II	**9,352 ± 2,014**	**2,953 ± 2,361**	**ns**
Class I	**500 ± 839**	**966 ± 1,064**	**ns**
Class II	**7,535 ± 1,669**	**500 ± 2,233**	**0.013**
HLA-DR	**2,587 ± 1,261**	**500 ± 81**	**0.023**
DR51/52/53/DQ	**2,980 ± 1,428**	**500 ± 492**	**ns**
After desensitization			
Class I + II	**4,247 ± 1,101**	**504 ± 701**	**0.023**
Class I	**500 ± 136**	**500 ± 131**	**ns**
Class II	**3,508 ± 1,022**	**500 ± 715**	**0.005**
HLA-DR	**893 ± 679**	**500 ± 0**	**0.013**
DR51/52/53/DQ	**1,439 ± 688**	**500 ± 99**	**ns**

MFI - median fluorescence intensity, AMR - antibody mediated rejection, ns - not significant.

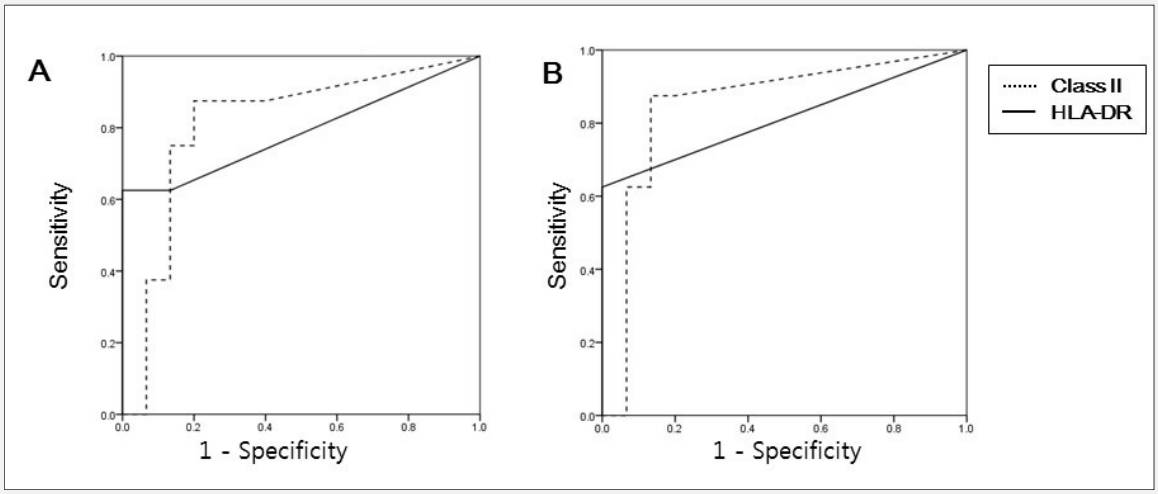

Figure 1. ROC analysis of median fluorescence intensity (MFI) value of donor specific HLA antibody (DSA) for antibody mediated rejection (AMR).

A: MFI value of baseline DSA before desensitization for predicting AMR. B: MFI value of DSA after desensitization for AMR. Cutoff value of MFI sum of baseline HLA class II DSA was 5,743 with 75% sensitivity and 97% specificity. Cutoff value of MFI sum of baseline HLA-DR DSA was 1,999 with 63% sensitivity and 100% specificity. Cutoff value of MFI sum of HLA class II DSA after desensitization was 2,122 with 63% sensitivity and 94% specificity. The cutoff value of HLA-DR DSA after desensitization was 577 with 63% sensitivity and 100% specificity.

those without graft loss (n = 16) (100.0% vs. 6.3%, p = 0.02 for both).

The MFI sum of baseline class II DSA was lower in patients without AMR than in patients with AMR (median ± SE, 500 ± 2,233 vs. 7,535 ± 1,669, p = 0.013) (Table 3). Among class II DSA, the MFI sum of baseline HLA-DR DSA was lower in patients without AMR compared to those with AMR (median ± SE, 500 ± 81

vs. 2,587 ± 1,261, p = 0.023). The MFI sum of baseline HLA-DR51/52/53 and HLA-DQ DSA was not associated with AMR (Table 3).

HLA class II DSA and HLA-DR DSA levels before and after desensitization were evaluated by ROC curve analysis (Figure 1). For predicting AMR, a cutoff value of MFI sum of baseline HLA class II DSA was 5,743 with 75% sensitivity and 97% specificity (area under the curve, AUC 0.813, p = 0.015) (Figure 1, panel A). The cutoff value of MFI sum of baseline HLA-DR DSA was 1,999 with 63% sensitivity and 100% specificity (AUC 0.787, p = 0.026). The cutoff value of MFI sum of HLA class II DSA after desensitization was 2,122 with 63% sensitivity and 94% specificity (AUC 0.850, p = 0.007) (Figure 1, panel B). The cutoff value of HLA-DR DSA after desensitization was 577 with 63% sensitivity and 100% specificity (AUC 0.813, p = 0.015). Class II DSA or HLA-DR DSA did not predict occurrence of TMR in ROC analysis (data not shown).

DISCUSSION

Transplantation from living donors after desensitization in sensitized patients has been performed successfully with desensitization protocols including IVIG, PP, and rituximab [3,6] PP is effective in lowering DSA levels over a short-term period; however, the association between DSA levels before or after desensitization and graft outcome is still not clear.

Regarding baseline DSA levels before desensitization, the high levels of DSA (MFI >10,000) were associated with the development of AMR and lower graft survival [10]. Another study reported that the baseline strong DSA (MFI > 10,000) was associated with AMR but not with graft survival [5]. In our study, the presence and level of baseline class II DSA before desensitization affected the occurrence of AMR and the presence of strong class II DSA (MFI > 10,000) was associated with allograft loss. Based on this study results, we may consider finding other possible donors rather than performing desensitization therapy if class II DSA is strongly positive.

With regards to association of DSA level after desensitization and graft outcome, Keven et al. reported that mean MFI level of DSA was 2,753 in 5 patients treated with PP and IVIG, and though acute rejection rate was increased, there was no graft failure during 17 months follow-up [4]. In 4 pediatric patients with DSA levels of negative to MFI value of 4,672 after desensitization, there was no occurrence of acute rejection and the graft function was stable for 1 to 4.5 years of follow-up [6]. In our study, there was also strong association with low levels of class II DSA and AMR. However, in patients with low levels of class II DSA, with immediate therapy for AMR, the graft function was stable in long term follow-up. Therefore, kidney transplantation after desensitization with close monitoring of occurrence of AMR and immediate therapy for AMR can be reasonable choice for moderately (MFI < 10,000 at baseline) sensitized patients.

In our study, using an MFI cutoff value of 2,122 for class II DSA after desensitization, the sensitivity and specificity for predicting AMR were 63% and 94%, respectively. With a cutoff value of MFI 577 of HLA-DR DSA, the sensitivity and specificity for predicting AMR were 63.0% and 100.0%, respectively. The level of HLA-DR DSA which can predict occurrence of AMR was very low (MFI value of 577) in our study. Wu et al. also reported that there was significant association between weak DSA of MFI > 500 and AMR in 15 living donor transplantations with pretransplant DSA positivity (HR = 7.9, p = 0.001) [11]. In many studies, it has also been reported that HLA class II DSAs are more strongly associated with AMR than class I antibodies [12-14]. HLA class I antigens are expressed not only on the endothelium of peritubular and glomerular capillaries but also on larger vessels of kidneys [15]. HLA class I antibodies with sub-saturating concentration cause resistance towards complement mediated lysis and result in accommodation [16]. Whereas, HLA-DR antigens are expressed only on the endothelium of peritubular and glomerular capillaries [15], it can therefore be postulated that even lower levels of HLA-DR DSA can damage the endothelium of peritubular and glomerular capillaries and cause AMR.

In our study, DR51/52/53 or HLA-DQ DSA was not associated with occurrence of AMR. The expression level of HLA-DR51/52/53 or HLA-DQ is lower than that of HLA-DR in normal renal vascular endothelial cells, although HLA-DQ expression is increased by cytokines such as IFN-gamma which is related to the vessel injury [17]. Therefore, we can assume that HLA-DQ DSA can be relatively tolerable in living donor transplantation, because the damage of the donor kidney can be less in living donor transplantation (short cold ischemic time and relatively good general condition of living donors compared to deceased donors). We should perform further studies to confirm our hypothesis.

In addition, MFI values of DSA among different laboratories are not standardized yet. It can partially contribute to the difference of level of DSA which impacts renal allograft outcome from different transplantation centers. The variations are due to the lack of standardization in Luminex assays for MFI values, variable density of HLA antigens on single antigen beads, lot-to-lot variation, differences between vendors, and difference in the calculation of MFI intensity of DSA (peak vs. sum of DSA, low resolution vs. high resolution HLA typing) [18,19]. Further efforts are needed to standardize the methods for detecting and determining DSAs that have clinical significance.

CONCLUSION

Though there are some limitations to our study (small number of patients and heterogeneity in number of plas-

mapheresis for desensitization), we revealed that low levels of class II DSA, especially HLA-DR DSA after desensitization including PP, IVIG, and rituximab was associated with AMR in living donor kidney transplantation, however, it did not affect long term graft outcome. Pretransplant measurement of DSA by Luminex single antigen bead assays before and after desensitization can be useful to predict the occurrence of AMR and allograft loss in living donor kidney transplantation.

Acknowledgment:
This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF), funded by the Ministry of Education, Science, and Technology (grant no. 2015R1D1A1A01060641)

Declaration of Interest:
No potential conflicts of interest relevant to this article were reported.

References:

1. Amico P, Honger G, Mayr M, Steiger J, Hopfer H, Schaub S. Clinical relevance of pretransplant donor-specific HLA antibodies detected by single-antigen flow-beads. Transplantation 2009; 87:1681-8 (PMID: 19502960).

2. Eng HS, Bennett G, Tsiopelas E, et al. Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex predict late graft loss. Am J Transplant 2008;8:2335-42 (PMID: 18782289).

3. Gloor JM, DeGoey SR, Pineda AA, et al. Overcoming a positive crossmatch in living-donor kidney transplantation. Am J Transplant 2003;3:1017-23 (PMID: 12859539).

4. Keven K, Sengul S, Celebi ZK, et al. Kidney transplantation in immunologically high-risk patients. Transplant Proc 2013;45: 919-22 (PMID: 23622587).

5. Chung BH, Choi BS, Oh EJ, et al. Clinical impact of the baseline donor-specific anti-human leukocyte antigen antibody measured by Luminex single antigen assay in living donor kidney transplant recipients after desensitization therapy. Transpl Int 2014;27:49-59 (PMID: 24118413).

6. Pirojsakul K, Desai D, Lacelle C, Seikaly MG. Management of sensitized pediatric patients prior to renal transplantation. Pediatr Nephrol 2016;31:1691-8 (PMID: 26801944).

7. Amy B. Hahn, Geoffrey A. Land, Rosemarie M. Strothman, editors. ASHI Laboratory Manual, 4th Ed. Mt. Laurel: ASHI; 2000, vol I, I.C.1.1

8. Vaidya S, Cooper TY, Avandsalehi J, et al. Improved flow cytometric detection of HLA alloantibodies using pronase: potential implications in renal transplantation. Transplantation 2001;71: 422-8 (PMID: 11233905).

9. Haas M, Sis B, Racusen LC, et al. Banff 2013 Meeting Report: Inclusion of C4d-Negative Antibody-Mediated Rejection and Antibody-Associated Arterial Lesions. Am J Transplant 2014;14: 272-83 (PMID: 24472190).

10. Gloor JM, Winters JL, Cornell LD, et al. Baseline donor-specific antibody levels and outcomes in positive crossmatch kidney transplantation. Am J Transplant 2010;10:582-9 (PMID: 20121740).

11. Wu P, Jin J, Everly MJ, Lin C, Terasaki PI, Chen J. Impact of alloantibody strength in crossmatch negative DSA positive kidney transplantation. Clin Biochem 2013;46:1389-93 (PMID: 23726814).

12. Pollinger HS, Stegall MD, Gloor JM, et al. Kidney transplantation in patients with antibodies against donor HLA class II. Am J Transplant 2007;7:857-63 (PMID: 17295642).

13. Issa N, Cosio FG, Gloor JM, et al. Transplant glomerulopathy: risk and prognosis related to anti-human leukocyte antigen class II antibody levels. Transplantation 2008;86:681-5 (PMID: 18791449).

14. Campos EF, Tedesco-Silva H, Machado PG, Franco M, Medina-Pestana JO, Gerbase-DeLima M. Post-transplant anti-HLA class II antibodies as risk factor for late kidney allograft failure. Am J Transplant 2006;6:2316-20 (PMID: 16925566).

15. Muczynski KA, Cotner T, Anderson SK. Unusual expression of human lymphocyte antigen class II in normal renal microvascular endothelium. Kidney Int 2001;59:488-97 (PMID: 11168931).

16. Narayanan K, Jaramillo A, Phelan DL, Mohanakumar T. Pre-exposure to sub-saturating concentrations of HLA class I antibodies confers resistance to endothelial cells against antibody complement-mediated lysis by regulating Bad through the phosphatidylinositol 3-kinase/Akt pathway. Eur J Immunol 2004;34:2303-12 (PMID: 15259028).

17. Muczynski KA, Ekle DM, Coder DM, Anderson SK. Normal human kidney HLA-DR-expressing renal microvascular endothelial cells: characterization, isolation, and regulation of MHC class II expression. J Am Soc Nephrol 2003;14:1336-48 (PMID: 12707403).

18. Warner PR, Youngs D, Nelson KA. HLA density on microparticles versus antibody detection sensitivity: implications for crossmatch prediction. Hum Immunol 2007;68(S):S28 https://www.researchgate.net/publication/247021374_25-P_HLA_density_on_microparticles_versus_antibody_detection_sensitivity_Implications_for_crossmatch_prediction.

19. Reed EF, Rao P, Zhang Z, et al. Comprehensive Assessment and Standardization of Solid Phase Multiplex-Bead Arrays for the Detection of Antibodies to HLA-drilling down on key sources of variation. Am J Transplant 2013;13:3050-1 (PMID: 24103158).