

The Effect of Bortezomib on Antibody-Mediated Rejection after Kidney Transplantation

Juhan Lee^{1,2}, Beom Seok Kim^{1,3}, Yongjung Park⁴, Jae Geun Lee^{1,2},
Beom Jin Lim⁵, Hyeon Joo Jeong⁵, Yu Seun Kim^{1,2}, and Kyu Ha Huh^{1,2}

¹The Research Institute for Transplantation, Yonsei University College of Medicine, Seoul; Departments of ²Surgery, ³Internal Medicine (Nephrology), ⁴Laboratory Medicine, and ⁵Pathology, Yonsei University College of Medicine, Seoul, Korea.

Purpose: Recently, bortezomib has been used to treat antibody-mediated rejection (AMR) refractory to conventional treatment such as plasmapheresis, intravenous immunoglobulin, and rituximab. The authors aimed to describe their experiences when bortezomib was used to treat refractory AMR.

Materials and Methods: Eleven refractory AMR episodes treated with bortezomib were included in this study. The patients received one or two cycles of bortezomib (1.3 mg/m²) on days 1, 4, 8, and 11.

Results: Bortezomib effectively reduced antibodies against various targets, including human leukocyte antigen (HLA) class I and II, ABO blood group antigen, and angiotensin II type 1 receptor. Antibodies were depleted or reduced significantly in eight AMR episodes. Overall, there was a significant improvement in the mean estimated glomerular filtration rate (eGFR) at 3 months after therapy (36.91±22.15 mL/min/1.73 m²) versus eGFR at time of AMR diagnosis (17.00±9.25 mL/min/1.73 m²; *p*=0.007). All six early-onset AMR episodes (within 6 months post-transplantation) showed full recovery of allograft function. Additionally, three of the five late-onset AMR episodes (>6 months post-transplantation) showed improved allograft function.

Conclusion: Anti-humoral treatment based on bortezomib might be an effective strategy against refractory AMR caused by various types of antibodies. Notably, this treatment could be more effective in early-onset AMR than in late-onset AMR.

Key Words: Angiotensin II type 1 receptor antibodies, antibody-mediated rejection, bortezomib, HLA antibodies, kidney transplantation

INTRODUCTION

Despite major advances in transplant medicine, antibody-mediated rejection (AMR) remains one of the major barriers to successful long-term outcomes.¹ Traditional treatments for AMR, such as plasmapheresis (PP), intravenous immunoglobulin (IVIg), and rituximab have provided suboptimal results. Furthermore, these strategies do not deplete plasma cells (B-lympho-

cyte lineage cells) that produce antibodies.² Recent studies have demonstrated that the proteasome inhibitor bortezomib reduces antibodies by depleting antibody-producing plasma cells; thus, bortezomib has been effectively used to treat AMR episodes refractory to traditional AMR therapies.³⁻⁷

Bortezomib is a proteasome inhibitor that induces the apoptosis of plasma cells and was approved by the Food and Drug Administration for the treatment of multiple myeloma in 2008.⁸ Recently, it has been used to reduce HLA antibodies either before transplantation or as treatment for AMR. Studies on bortezomib in the transplant field have focused on donor-specific anti-HLA antibodies (DSHA).^{3-5,9,10} However, in view of its action mechanism as a proteasome inhibitor, its effect may not be limited to anti-HLA antibodies,^{3,4} and in fact, bortezomib has been used to treat disorders other than multiple myeloma.^{11,12} Therefore, the aim of this study was to investigate the effect of bortezomib on AMR caused by various types of antibodies.

Received: March 4, 2015 **Revised:** April 13, 2015

Accepted: May 28, 2015

Corresponding author: Kyu Ha Huh, MD, PhD, Department of Surgery, Yonsei University College of Medicine, 50-1 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea.
Tel: 82-2-2228-2138, Fax: 82-2-313-8289, E-mail: khuh@yuhs.ac

•The authors have no financial conflicts of interest.

© Copyright: Yonsei University College of Medicine 2015

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

MATERIALS AND METHODS

Subjects

This retrospective study was conducted on ten consecutive patients diagnosed with AMR and treated with bortezomib from November 2011 to April 2014. The study was approved by the Institutional Review Board of Severance Hospital, Yonsei University Health System (4-2014-0968).

Diagnosis and treatment of AMR

Patients presented with the defining features of AMR, as described by the Banff 2011 meeting report,¹³ and all ten were refractory to conventional treatment involving PP, IVIg (200 mg/kg administered after each PP treatment), and rituximab (single dose, 375 mg/m²). Bortezomib was administered after conventional treatment had failed. Each bortezomib cycle consisted of four doses of 1.3 mg/m², which was reduced to 1.0 mg/m² in three patients depending on toxicities, and was administered on days 1, 4, 8, and 11. The second cycle of bortezomib was decided on case by case after weighing up the advantages and disadvantages in accordance with each patient's clinical status (antibodies, serum creatinine, and side effects). Early-onset AMR was defined as an occurrence ≤6 months after kidney transplantation, and late-onset AMR was defined as an occurrence at >6 months post-transplantation.

Renal function evaluation

Initial renal function was assessed at the time of AMR diagnosis based on serum creatinine and the estimated glomerular filtration rate (eGFR, calculated using the Modification of Diet in Renal Disease formula). Responses to therapy were also assessed using serum creatinine and eGFR at 3 months after treatment. We evaluated the most recent renal function data and follow-up duration.

Detection and characterization of antibodies

DSHA were identified using a single antigen bead assay that utilized the multiplex flow-bead microarray method (Lifecodes LSA class I and II; Gen-Probe Transplant diagnostics, Inc., Stamford, CT, USA). The presence and antigen specificities of Abs to HLA-A, -B, -DR, and -DQ were determined. Results are expressed as mean fluorescence intensities (MFI). A normalized value of >1000 MFI was considered positive for DSHA. A C1q binding assay was performed on all available sera of recipients with DSHA (C1qScreen™, One Lambda, CA, USA). Anti-ABO antibody titers were measured using standard serological techniques.¹⁴ Anti-angiotensin II type 1 receptor (AT1R) antibodies were retrospectively evaluated in cases of biopsy-proven AMR without DSHA. Levels of anti-AT1R antibodies (U/mL) were quantified using AT1R assay kits (One Lambda, CA, USA), which utilize the enzyme-linked immunosorbent assay principle.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Wilcoxon's signed-rank test was used to compare differences before and after treatment. *p* values of <0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Demographic data, including immunologic risk factors are presented in Table 1. A total of ten patients received bortezomib for AMR. Two patients underwent deceased-donor kidney transplantation and eight underwent living-donor kidney transplantation. Five recipients had pre-formed DSHA. Of these five patients, four received preoperative desensitization, including PP, IVIg, and rituximab due to a positive crossmatch or ABO incompatibility. The remaining patient received one dose of rituximab prior to transplantation. One patient diagnosed with biopsy-proven AMR in the absence of DSHA was pre-sensitized against AT1R.

Five episodes of AMR developed early (≤6 months post-transplantation), and four developed late (>6 months post-transplantation). One patient experienced recurrent AMR (at 1 and 27 months post-transplantation).

Histologic data

Histologic data are shown in Table 2. All rejection episodes manifested as pure AMR, that is, not as AMR mixed with acute cellular rejection. C4d status and biopsy scoring are presented according to the Banff 2011 classification. One of six early-onset AMR cases and four of five late-onset AMR cases exhibited transplant glomerulopathy (cg≥1).

Clinical outcomes after bortezomib treatment

Treatment outcomes are shown in Table 3 and Fig. 1. Six episodes of AMR occurred within 6 months of transplantation. Patient B (refer to Table 3 for patient details) had anti-B antibody and class II HLA antibody. His initial anti-B isoagglutinin titer was low (IgM/IgG 1:32/1:16); however, this increased to 1:64/1:256 after transplantation despite postoperative PP with IVIg treatment. Patient F had no DSHA yet had a high anti-AT1R antibody titer at the time of biopsy. Four patients received a standard dose of bortezomib (1.3 mg/m²), and two patients (A and F) received a reduced dose (1.0 mg/m²) due to toxicities. In all early-onset AMR cases renal function fully recovered after bortezomib treatment. The DSHA of two patients (A and B) disappeared completely after treatment; however, although the DSHA of three patients (C, D, and E) declined, it was not eradicated. Patient F showed a decline of anti-AT1R antibody after treatment.

Five episodes of late-onset AMR were included in this study. Patient A experienced recurrent AMR with class I and II HLA

antibodies. She received one cycle of bortezomib and recovered well. Three patients (G, H, and I) had class II HLA antibodies, and one patient (J) had class I HLA antibodies. They received one to two cycles of bortezomib. Renal function recovered in two patients (H and I) after bortezomib treatment; however, their antibody titers increased. Patient G received a reduced dose (1.0 mg/m²) due to thrombocytopenia.

Overall, a significant improvement in the mean eGFR was observed at 3 months after therapy (36.91±22.15 mL/min/1.73 m²) versus the mean eGFR at the time of diagnosis (17.00±9.25 mL/min/1.73 m²; *p*=0.007).

Bortezomib-related toxicities (thrombocytopenia and peripheral neuropathy) were all transient and responded to con-

servative management.

DISCUSSION

Traditional AMR therapeutic strategies have focused on antibody removal and B-cell depletion while not directly focusing on plasma cell depletion. However, bortezomib is a proteasome inhibitor that induces the apoptosis of plasma cells, which are the sole source of antibody production.^{2,5} Patients in this series experienced substantial rejection episodes refractory to PP, IVIg, and rituximab. Bortezomib was effective against various antibody targets, including HLA class I and II, ABO antigen,

Table 1. Baseline Characteristics of Patients

Patient ID	Sex/age	Donor type (relation)	HLA mismatches	Preoperative immunologic condition			Preoperative desensitization	AMR after KT (months)
				Cross match (CDC)	PRA (class I/II, %)	Antibodies*		
A	F/25	LD (parent)	3	T 1:2 (AHG) B 1:4	100/40	A2 (9618), B13 (4928)	PP+IVIg, RIT	1/27
B	F/68	LD (offspring)	2	Negative	16/60	DR8 (4981) Anti-B IgM/IgG (1:32/1:16)	PP+IVIg, RIT	1
C	F/45	LD (spouse)	6	T 1:2 (AHG) B 1:4	10/50	B52 (594), DR15 (2827)	PP+IVIg, RIT	0
D	M/21	DD	3	Negative	98/13	DQ9 (10485)	RIT	1
E	M/55	LD (offspring)	3	B 1:16	16/60	B51 (1334), DQ7 (523)	PP+IVIg, RIT	0
F	M/55	DD	3	Negative	0/33	No DSHA AT1R Ab >50 U/mL	No	0
G	F/38	LD (exchange donor)	4	Negative	N/A	N/A	No	120
H	M/28	LD (sibling)	2	Negative	N/A	N/A	No	152
I	F/46	LD (sibling)	2	Negative	N/A	N/A	No	113
J	F/32	LD (others)	2	Negative	N/A	N/A	No	262

LD, living donor; DD, deceased donor; CDC, complement-dependent cytotoxicity; AHG, anti-human globulin; PRA, panel reactive antibodies; AMR, antibody-mediated rejection; PP, plasmapheresis; IVIg, intravenous immunoglobulin; RIT, rituximab; KT, kidney transplantation; DSHA, donor specific anti-HLA antibodies; AT1R Ab, angiotensin II type 1 receptor antibody; N/A, not available.

*Data were expressed as mean fluorescence intensities (MFI).

Table 2. Pathologic Data of Patients

Patient ID	Time from transplant to Bx	C4d grade	Combined pathology	Banff biopsy scoring										
				(g)	(t)	(i)	(v)	(cg)	(ct)	(ci)	(cv)	(mm)	(ah)	(ptc)
A	1	C4d 0	Arteriosclerosis, moderate	0	0	0	0	0	1	1	0	0	0	1
B	1	C4d 0	TG	0	0	1	0	2	0	0	0	0	0	2
C	0	C4d 3	Acute tubular injury	0	0	0	0	0	0	0	0	0	0	0
D	1	C4d 3	Acute tubular injury	1	1	0	0	0	0	0	0	0	0	2
E	0	C4d 3	Diffuse intratubular microcalcification	2	0	0	0	0	1	0	0	0	0	1
F	0	C4d 0		1	0	0	0	0	0	0	0	0	0	2
A	27	C4d 3		1	1	0	1	2	1	0	0	0	0	2
G	120	C4d 3		0	0	1	0	0	1	1	1	0	0	3
H	152	C4d 0		3	0	1	0	3	2	1	0	0	3	1
I	113	C4d 3		1	1	0	0	2	2	1	3	3	3	2
J	262	C4d 2	TG with FSGS	2	0	1	0	3	3	3	2	1	1	1

Bx, biopsy; TG, transplant glomerulopathy; FSGS, focal segmental glomerulosclerosis.

Table 3. Changes of Renal Allograft Function and Antibody Levels after Bortezomib Treatment

Patient ID	At AMR diagnosis			Bortezomib		At 3 months after bortezomib			Last follow up			
	Antibodies*	C1q	Cr	eGFR	Cycle	Dose reduction	Antibodies*	Cr	eGFR	Cr	eGFR	Duration (months)
A	A2 (14589), B13 (1365)	N/A	4.87	11	1st	Peripheral	No DSHA	4.23	13			
	A2 (6738), DR12 (1571)	(+)	3.06	18	2nd	neuropathy	N/A	1.93	31	2.09	32	10
B	Anti-B IgM/IgG (1:64/1:256) DR8 (16389)	N/A	5.63	8	1		Anti-B IgM/IgG (1:16/1:64) No DSHA	0.95	58	0.93	59	36
C	B52 (1063), DR15 (12466)	N/A	4.47	11	1		A30 (1068), DR15 (7809)	1.04	57	0.89	68	28
D	B27 (12878), DQ9 (17209)	(+)	2.31	36	1		DQ9 (11404)	1.24	74	1.37	65	22
E	B51 (4136)	(-)	2.32	29	1	Thrombocytopenia	B51 (2182)	1.24	61	0.86	90	11
F	DSHA (-), AT1R Ab >50 U/mL	N/A	8.52	7	1		AT1R Ab 11.7 U/mL	2.01	35	1.72	41	10
G	DR13 (14274), DQ6 (5148)	(-)	4.26	11	2	Thrombocytopenia	DR13 (8943), DQ6 (1306)	4.44	11	4.12	12	9
H	DR7 (2118), DQ2 (17258)	(+)	3.37	20	2		DR7 (2109), DQ2 (21065)	2.55	28	2.46	29	9
I	DSHA (-), Donor CREG Ab-DR13 (11372)	(-)	2.22	23	2		Donor CREG Ab-DR13 (17475)	1.97	26	2.02	25	7
J	B13 (9177)	(-)	3.7	13	1		B13 (2583), DQ5 (3338)	3.95	12	4.16	11	7

AMR, antibody-mediated rejection; Cr, creatinine; eGFR, estimated glomerular filtration rate; DSHA, donor specific anti-HLA antibodies; N/A, not available; AT1R Ab, angiotensin II type 1 receptor antibody; CREG, cross reactive group.

*Data were expressed as mean fluorescence intensities.

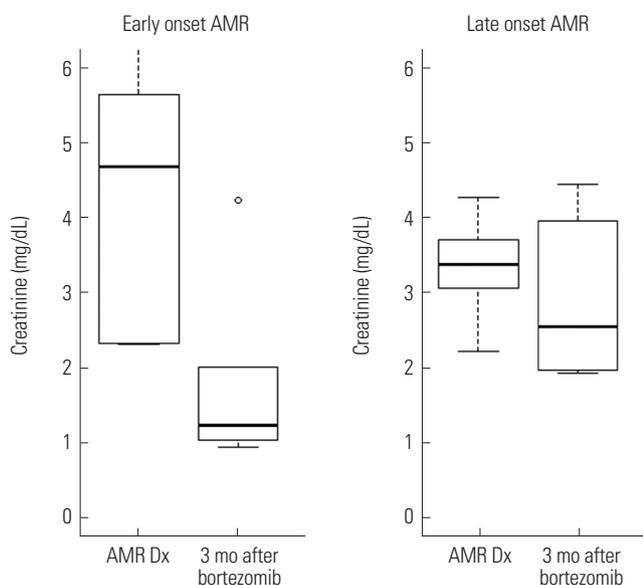


Fig. 1. Changes of renal allograft function after bortezomib treatment according to AMR onset time. AMR, antibody-mediated rejection; Dx, diagnosis; mo, months.

and angiotensin receptor. However, the antibody-removing effect of bortezomib was not sustained long-term. Furthermore, the treatment effect of bortezomib was more evident in early-onset AMR than in late-onset AMR.

In this case series, bortezomib effectively reduced antibodies against various targets, including HLA class I, HLA class II, ABO blood group antigen, and AT1R. The majority of studies conducted to date have only reported on the effectiveness of bortezomib against anti-HLA antibodies.^{4,5,9} Only a small number of case reports have described the effect of bortezomib on anti-ABO antibody-mediated AMR.¹⁵ In the present study, AMR

caused by anti-AT1R antibodies was included, and treatment outcomes were satisfactory. To the best of our knowledge, no previous studies have been conducted on the use of bortezomib for the treatment of AMR caused by anti-AT1R antibodies.

The effects of bortezomib on immune response are complex. Endoplasmic reticulum stress and caspase induction are considered the primary mechanisms by which bortezomib eliminates plasma cells. The inhibition of nuclear factor-kappa B activity plays a central role in the anti-humoral activity of bortezomib. In addition, it has been shown to cause apoptosis and cell-cycle arrest.^{3,4} With such a wide range of actions, bortezomib may also have an effect on various kinds of antibodies.

In view of costs and side-effects, the selection of the correct treatment target is important. We analyzed DSHA class (HLA class I or II) and complement binding capacity. In the present study, preformed antibodies were observed in most early onset cases, and antibodies in late onset AMR were predominantly directed at HLA class II. These distributions concur with those already described.^{2,5,10} Several studies reported that bortezomib has different effects on HLA class I and II antibodies.¹⁰ Bortezomib reduces HLA class I-restricted antigen presentation by reducing cell-surface HLA class I expression. However, in the present study, most DSHAs were reduced after bortezomib treatment regardless of DSHA class. Recently, the complement-binding capacity of DSHA was found to be an important factor of graft injury.¹⁶ In the present study, we performed C1q binding assays to assess the complement binding capacity of DSHA retrospectively and found that bortezomib treatment was effective in AMR with or without C1q-fixing DSHA. However, caution is required in generalizing this result due to the small numbers of patients involved. Nevertheless, we experienced satisfactory treatment outcomes regardless of DSHA class or the comple-

ment-binding capacity of DSHA.

The time between kidney transplantation and AMR onset impacts the treatment effect of bortezomib. In the present study, we divided our study group into early-onset AMR and late-onset AMR. The six early onset episodes experienced full recovery from AMR after one cycle of bortezomib treatment. However, late-onset AMR demonstrated a poorer response. Three of five patients with late-onset AMR showed improved renal allograft function, while the other two did not respond to bortezomib treatment. Prior studies have also reported different responses for early- and late-onset AMR.^{5,9} Two explanations have been proposed. First, early-onset AMR is likely to be detected early in its course, whereas late-onset AMR is more likely to cause irreversible damage due to exposure to harmful antibodies for a longer time.^{5,17} In fact, most of our late-onset AMR patients demonstrated transplant glomerulopathy. Second, early- and late-onset AMR may differ in terms of plasma cell characteristics.⁵ Future studies are required on this topic.

Despite its wide range of action, the duration of the anti-humoral response to bortezomib was not sustained. We found that certain patients experienced *de novo* antibody formation or antibody rebound after bortezomib treatment. According to previous studies, antibody titer after transplantation represents dynamic change and continues at varying levels thereafter.^{13,18} Accordingly, given that antibodies can reoccur, regular monitoring of antibody titers is required after bortezomib-based anti-humoral treatment.

Several limitations of our study require consideration. First, the study was limited by a small cohort size, as only eleven episodes were included. A larger, randomized controlled trial with a long-term follow-up is required. Second, given that patients received bortezomib after conventional treatments (PP+IVIg+rituximab), it was difficult to assess the contribution of bortezomib to overall efficacy. Third, in the absence of accepted guidelines for the evaluation of therapeutic response in the setting of AMR, we evaluated renal function and antibody levels at 3 months after bortezomib treatment.

In conclusion, anti-humoral treatment based on bortezomib might be an alternative strategy against refractory AMR. Despite the small cohort size, our data suggested therapeutic effectiveness against a wide range of antibodies, including HLA antibodies, ABO blood group antibodies, and anti-AT1R antibodies. Finally, bortezomib treatment could be more effective in early-onset AMR than in late-onset AMR.

ACKNOWLEDGEMENTS

This study was supported by a faculty research grant of Yonsei University College of Medicine for 2013 (6-2013-0042).

REFERENCES

1. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al.

Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012;12:388-99.

2. Djamali A, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. *Am J Transplant* 2014;14:255-71.

3. Ejaz NS, Alloway RR, Halleck F, Dürr M, Budde K, Woodle ES. Review of bortezomib treatment of antibody-mediated rejection in renal transplantation. *Antioxid Redox Signal* 2014;21:2401-18.

4. Walsh RC, Alloway RR, Girnita AL, Woodle ES. Proteasome inhibitor-based therapy for antibody-mediated rejection. *Kidney Int* 2012;81:1067-74.

5. Walsh RC, Brailey P, Girnita A, Alloway RR, Shields AR, Wall GE, et al. Early and late acute antibody-mediated rejection differ immunologically and in response to proteasome inhibition. *Transplantation* 2011;91:1218-26.

6. Yang KS, Jeon H, Park Y, Jo IH, Kim JI, Moon IS, et al. Use of bortezomib as anti-humoral therapy in kidney transplantation. *J Korean Med Sci* 2014;29:648-51.

7. Kim MG, Kim YJ, Kwon HY, Park HC, Koo TY, Jeong JC, et al. Outcomes of combination therapy for chronic antibody-mediated rejection in renal transplantation. *Nephrology (Carlton)* 2013;18:820-6.

8. Kumar SK, Rajkumar SV, Dispenzieri A, Lacy MQ, Hayman SR, Buadi FK, et al. Improved survival in multiple myeloma and the impact of novel therapies. *Blood* 2008;111:2516-20.

9. Gupta G, Abu Jawdeh BG, Racusen LC, Bhasin B, Arend LJ, Trollinger B, et al. Late antibody-mediated rejection in renal allografts: outcome after conventional and novel therapies. *Transplantation* 2014;97:1240-6.

10. Philogene MC, Sikorski P, Montgomery RA, Leffell MS, Zachary AA. Differential effect of bortezomib on HLA class I and class II antibody. *Transplantation* 2014;98:660-5.

11. Gertz MA. Immunoglobulin light chain amyloidosis: 2014 update on diagnosis, prognosis, and treatment. *Am J Hematol* 2014;89:1132-40.

12. Neubert K, Meister S, Moser K, Weisel F, Maseda D, Amann K, et al. The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. *Nat Med* 2008;14:748-55.

13. Mengel M, Sis B, Haas M, Colvin RB, Halloran PE, Racusen LC, et al. Banff 2011 Meeting report: new concepts in antibody-mediated rejection. *Am J Transplant* 2012;12:563-70.

14. Yoo S, Lee EY, Huh KH, Kim MS, Kim YS, Kim HO. Role of plasma exchange in ABO-incompatible kidney transplantation. *Ann Lab Med* 2012;32:283-8.

15. Westphal S, Hansson S, Stelin G, Holgersson J, Mjörnstedt L, Fridman S. Successful treatment of severe ABO antibody-mediated rejection using bortezomib: a case report. *Transplant Proc* 2013;45:1213-5.

16. Loupy A, Lefaucheur C, Vernerey D, Prugger C, Duong van Huyen JP, Mooney N, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med* 2013;369:1215-26.

17. Gloor JM, Sethi S, Stegall MD, Park WD, Moore SB, DeGoey S, et al. Transplant glomerulopathy: subclinical incidence and association with alloantibody. *Am J Transplant* 2007;7:2124-32.

18. Loupy A, Hill GS, Suberbielle C, Charron D, Anglicheau D, Zuber J, et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). *Am J Transplant* 2011;11:56-65.