Differences in Pathologic Features and Graft Outcomes of Rejection on Kidney Transplant

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ABSTRACT

Background. Rejection is still a barrier to long-term allograft survival, but there are not many reports of clinical outcomes according to rejection types. The purpose of this study was to investigate differences in pathologic features and graft outcomes of rejection on kidney transplant (KT).

Materials and Methods. We retrospectively analyzed 139 kidney transplant recipients diagnosed to rejection by allograft biopsy results between 2006 and 2018. We divided kidney transplant recipients into 3 groups as follows: T cell–mediated rejection (TCMR), antibody-mediated rejection, and mixed rejection. We investigated clinical characteristics, pathologic findings, death-censored graft survival rates, and patient survival rates among the 3 groups.

Results. Mean follow-up duration was 113.5 (SD, 80.6) months. The mixed rejection group was the youngest significantly. There were no significant differences of the proportion of sex, KT type, KT number, number of HLA mismatches, induction immunosuppressant, and maintenance immunosuppressant among the 3 groups. In pathologic findings, microvascular inflammation and C4d were significantly different among the 3 groups. Death-censored graft survival of mixed rejection was the least. In multivariate analysis, recipient age, TCMR, and positive C4d were the risk factors associated with graft failure. However, patient survival rates showed no significant differences among the 3 groups.

Conclusions. Our study showed that mixed rejection had poor prognosis in comparison with TCMR and antibody-mediated rejection groups, and TCMR and positive C4d were the most important risk factors for graft survival. Therefore, constant monitoring through allograft biopsy and early treatment for rejection are very important in post-transplant clinical outcomes.

REJECTION is still a barrier to improve long-term allograft survival and accounts for a high proportion of more than 50% of the graft failure [1]. To overcome this, the guideline to diagnose the rejection more precisely has been needed, and pathologic guidelines have been changed many times [2,3]. However, there are many controversies to diagnose and treat the rejection through the guidelines and not many reports of clinical outcomes according to rejection types, especially. The purpose of this study was to investigate differences in pathologic features and graft outcomes of the rejection after kidney transplant (KT).

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Table 1. Comparison of Clinical Parameters According to Rejection Type in Kidney Transplant

Variables	TCMR (n = 48)	AMR (n = 58)	Mixed Rejection (n = 33)	*P* Value
Donor age at KT, mean (SD), y	42.8 (11.7)	42.9 (14.3)	41.2 (10.8)	.81
Donor male sex, No. (%)	42 (87.5)	53 (91.4)	30 (90.9)	.77
Donor type, No. (%)				.17
Living donor	34 (70.8)	31 (53.4)	19 (57.6)	
Deceased donor	14 (29.2)	27 (46.6)	14 (42.4)	
Recipient age at KT, mean (SD), y	47.4 (11.6)	49.7 (12.3)	41.9 (12.2)	.01
Recipient male sex, No. (%)	28 (58.3)	39 (67.2)	20 (60.6)	.64
KT number				.77
First	42 (87.5)	53 (91.4)	30 (90.9)	
Second	6 (12.5)	5 (8.6)	3 (9.1)	
Dialysis vintage, mean (SD), mo	37.4 (41.6)	49.8 (49.4)	93 (200.7)	.07
HLA mismatch number, mean (SD)	3.7 (1.4)	3.6 (1.4)	3.5 (1.4)	.92
Induction immunosuppressant, No. (%)				.008
None	13 (31.7)	12 (21.1)	5 (15.2)	
Basiliximab	27 (65.9)	35 (61.4)	28 (84.8)	
Antithymocyte globulin	1 (2.4)	10 (17.5)	0	
Maintenance immunosuppressant, No. (%)				.74
Cyclosporine	12 (25.0)	11 (19.0)	6 (18.2)	
Tacrolimus	36 (75.0)	47 (81.0)	27 (81.8)	
Usage of MMF	44 (91.7)	54 (93.1)	31 (93.9)	> .99
PRA class I > 50%, No. (%)	1 (5.6)	6 (13.3)	3 (16.7)	.60
PRA class II > 50%, No. (%)	1 (5.6)	14 (31.8)	6 (33.3)	.07
Anti-HLA DSA, class I, No. (%)	2 (11.1)	9 (20.0)	3 (20.0)	.72
Anti-HLA DSA, class II, No. (%)	0	22 (48.9)	9 (60.0)	< .001

AMR, antibody-mediated rejection; KT, kidney transplant; MMF, mycophenolate mofetil; PRA, panel-reactive antibody; DSA, donor-specific antibody; TCMR, T cell–mediated rejection.

PATIENTS AND METHODS

Study Design

We retrospectively analyzed the 139 kidney transplant recipients (KTRs) diagnosed as having rejection through allograft biopsy results between December 2006 and July 2018. We divided the KTRs into 3 groups as follows: T cell–mediated rejection (TCMR), antibody-mediated rejection (AMR), and mixed rejection. We investigated the clinical characteristics, pathologic findings, and

Table 2. Comparison of Pathologic Findings of Allograft According to Rejection Type in Kidney Transplant

Variables	TCMR (n = 48)	AMR (n = 58)	Mixed Rejection (n = 33)	*P* Value
Glomerulitis (g score)				< .001
0–1	44 (91.7)	32 (55.2)	29 (90.6)	
2–3	4 (8.3)	26 (44.8)	3 (9.4)	
Peritubular capillaritis (ptc score)				< .001
0–1	42 (87.5)	23 (39.7)	13 (39.4)	
2–3	6 (12.5)	35 (60.3)	20 (60.6)	
Transplant glomerulopathy (cg score)				< .001
0	46 (95.8)	30 (51.7)	26 (78.8)	
≥ 1	2 (4.2)	28 (48.3)	7 (21.2)	
Arteritis (v score)				.495
0–1	45 (93.8)	55 (94.8)	33 (100)	
2–3	3 (6.3)	3 (5.2)	0	
Arterial intimal fibrosis (cv score)				.55
0–1	37 (77.1)	43 (74.1)	28 (84.8)	
2–3	11 (22.9)	15 (25.9)	5 (15.2)	
Microvascular inflammation (g + ptc score)				
> 1	18 (37.5)	54 (93.1)	32 (97.0)	< .001
IF/TA (ci + ct scores)				.49
0–1	8 (16.7)	13 (22.4)	5 (15.2)	
2–3	18 (37.5)	22 (37.9)	18 (54.5)	
≥ 4	22 (45.8)	23 (39.7)	10 (30.3)	
C4d	13 (27.1)	40 (69.0)	26 (78.8)	< .001

Values are expressed as No. (%).
Abbreviations: AMR, antibody-mediated rejection; IF/TA, interstitial fibrosis/tubular atrophy; TCMR, T cell–mediated rejection.

Table 3. Comparison of Clinical Outcomes According to Rejection Type in Kidney Transplant

Variables	TCMR (n = 48)	AMR (n = 58)	Mixed Rejection (n = 33)	*P* Value
Delayed graft function, No. (%)	0	2 (3.4)	1 (3.0)	.46
Causes of graft failure, No. (%)				.001
Acute rejection	11 (61.1)	1 (4.8)	9 (47.4)	
Chronic rejection	0	7 (33.3)	7 (36.8)	
Recurrent glomerulonephritis	1 (5.6)	2 (9.5)	0	
Infection	3 (16.7)	4 (19.0)	2 (10.5)	
Patient death with a graft function	1 (5.6)	4 (19.0)	1 (5.3)	
Others	2 (4.2)	3 (5.2)	0	
Patient death, No. (%)	4 (8.3)	8 (13.8)	4 (12.1)	.70
Cardiovascular disease	0	1 (12.5)	1 (25.0)	
Infection	3 (75.0)	5 (62.5)	3 (75.0)	
Others	1 (25.0)	2 (25.0)	0	
Time between KT and diagnosis of acute rejection, mean (SD), mo	61.4 (8.8)	79.9 (9.9)	43.4 (7.8)	.03
Time between diagnosis of acute rejection and graft failure, mean (SD), mo	26.7 (5.5)	21.2 (4.2)	30.5 (5.4)	.41

Abbreviations: AMR, antibody-mediated rejection; KT, kidney transplant; TCMR, T cell–mediated rejection.

clinical outcomes according to the rejection type by allograft biopsy results, death-censored allograft survival rates, and patient survival rates among the 3 groups.

The institutional review board of Keimyung University Dongsan Medical Center approved this study (2018–12–033).

Demographic and Clinical Data

We investigated donor and recipient ages at diagnosis of rejection, donor and recipient sex, donor type, frequency of KT, dialysis type before KT, dialysis vintage, causes of end-stage renal disease, number of HLA mismatches, immunosuppressant for induction and maintenance treatment, use of mycophenolate mofetil, panel-reactive antibody class I or II > 50%, positive donor-specific antibody (DSA) class I or II, and pathologic findings.

Statistical Analyses

Continuous variables were analyzed by the *t* test, and categorical variables were analyzed by the χ^2 or Fisher exact test. Graft and patient survivals were evaluated by the Kaplan-Meier analysis with log-rank test. Cox regression analysis was performed for risk factors of graft failure; *P* values less than .05 were considered statistically significant. Statistical analysis was performed using SPSS version 18.0 (IBM, Armonk, NY, United States).

RESULTS

Comparison of Baseline Characteristics Among TCMR, AMR, and Mixed Rejection Groups after KT

Mean follow-up duration was 113.5 (SD, 80.6) months. The numbers of KTRs with TCMR, AMR, and mixed rejection

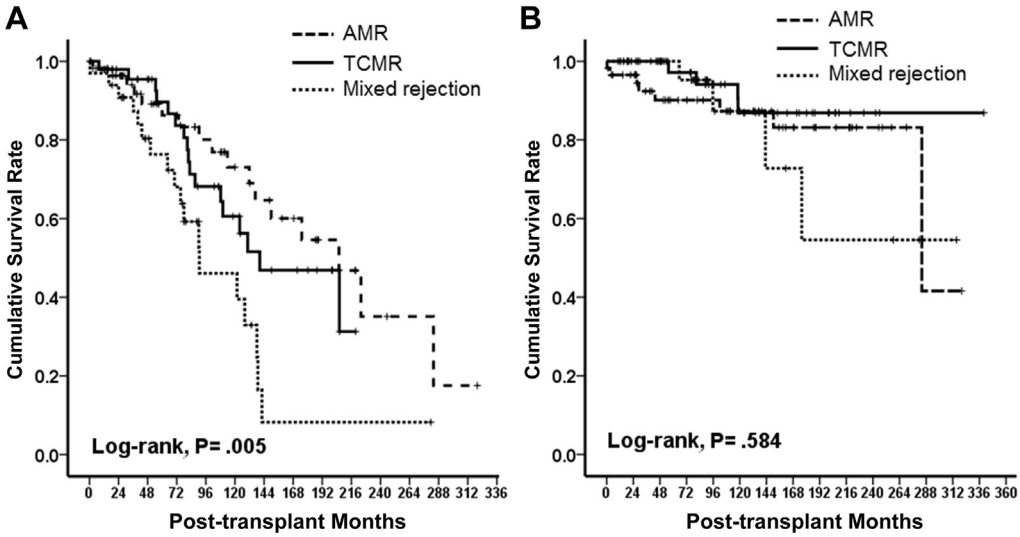

Fig 1. Death-censored graft survival rate **(A)** and patient survival rate **(B)** according to rejection types in kidney transplant. AMR, antibody-mediated rejection; TCMR, T cell–mediated rejection.

Table 4. Risk Factors Associated With Graft Failure in Kidney Transplant

Variables	Univariate			Multivariate		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Recipient male sex	0.575	0.330–1.000	.05	0.559	0.301–1.040	.07
Recipient age	0.966	0.943–0.989	.004	0.966	0.944–0.990	.005
Delayed recovery of graft function	4.157	0.995–17.359	.05	4.400	0.931–20.804	.06
T cell–mediated rejection	2.167	1.190–3.945	.01	2.713	1.375–5.353	.004
Antibody-mediated rejection	2.209	1.251–3.898	.006	1.664	0.900–3.076	.10
Mixed rejection	2.487	1.384–4.470	.002	0.469	0.152–1.442	.19
Positive C4d	2.234	1.217–4.102	.01	12.044	2.843–51.018	.001

Abbreviation: HR, hazard ratio.

were 48 (34.5%), 58 (41.7%), and 33 (23.7%), respectively. There was no significant difference of mean age of donor among the 3 groups, but mean age of recipient in the mixed rejection group was the youngest significantly ($P = .01$). There were also no significant differences of the proportion of sex, KT type, KT number, dialysis type before KT, causes of end-stage renal disease, number of HLA mismatches, induction and maintenance immunosuppressants, use of mycophenolate mofetil, and rate of panel-reactive antibody $> 50\%$ among the 3 groups. The rate of positive donor-specific antibody class II was significantly higher in the AMR and mixed groups ($P < .001$) (Table 1).

Comparison of Pathologic Findings of Allograft according to Rejection Type in KT

In the pathologic findings, microvascular inflammation (glomerulitis + peritubular capillaritis score > 1) and C4d was significantly different among the 3 groups, but there were no significant differences of arteritis, arterial intimal fibrosis, and interstitial fibrosis and tubular atrophy among the 3 groups (Table 2).

Comparison of Graft and Patient Survival Among TCMR, AMR, and Mixed Rejection Groups after KT and Risk Factors for Graft Failure

A total of 58 cases (41.7%) of graft failure developed, including 18 patients (37.5%) in the TCMR group, 21 patients (36.2) in the AMR group, and 19 patients (57.6%) in the mixed rejection group. The cause of graft rejection were as follows: acute rejection, 11 (61.1%), 1 (4.8%), 9 (47.4%); chronic rejection, 0, 7 (33.3%), 7 (36.8%); recurrent glomerulonephritis, 1 (5.6%), 2 (9.5%), 0; infection, 3 (16.7%), 4 (19.0%), 2 (10.5%); patient death with a graft function, 1 (5.6%), 4 (19.0%), 1 (5.3%); and others, 1 (4.2%), 3 (5.2%), 0 (Table 3).

A total of 16 patients (11.5%) died, including 4 patients (8.3%) in the TCMR group, 8 patients (13.8) in the AMR group, and 4 patients (12.1%) in the mixed rejection group. The causes of death were as follows: infection, 3 (75.0%), 5 (62.5%), 3 (75.0%); cardiovascular disease, 0, 1 (12.5%), 1 (25.0%); and others, 1 (25.0%), 2 (25.0%), 0 (Table 3).

In Kaplan-Meier curve, death-censored graft survival in the mixed rejection group was the least significantly ($P = .005$) (Fig 1A), and time between KT and diagnosis of biopsy-proven acute rejection (BPAR) in the mixed rejection group was also the shortest significantly ($P = .03$) (Table 3).

Patient survival rates showed no significant differences among the 3 groups (Fig 1B). In multivariate Cox regression analysis, recipient age, TCMR, and positive C4d were the risk factors associated with graft failure in KT after adjusting for the significant variables in the univariate analysis (hazard ratio, 0.966; 95% CI, 0.944–0.990; $P = .005$; hazard ratio, 2.713; 95% CI, 1.375–5.353; $P = .004$; hazard ratio, 12.044; 95% CI, 2.843–51.018; $P = .001$) (Table 4).

Comparison of Treatment Modalities According to Rejection Type in KT

In the treatment modalities, the proportion of steroid pulse therapy was significantly higher in the TCMR or mixed rejection ($P = .001$), but the proportions of rituximab, plasmapheresis, and intravenous immunoglobulin were significantly higher in the AMR and mixed rejection groups ($P < .001$) (Table 5).

DISCUSSION

In our study, the proportion of mixed rejection was the highest compared with other types of rejection. The mechanism and timing of the rejection differ, and thus the prognosis differs, but the time between KT and the diagnosis of each rejection is different. In particular, most of the TCMR occurred after a period of time rather than at the early period of KT. It was presumed that this was probably because of the irregular administration of immunosuppressive agents, which resulted in insufficient immunosuppression [4], or reduction of the dose of immunosuppressant for the treatment of infection, such as BK virus infection [5].

On the contrary, active AMR (AAMR) appears to have occurred mainly at the early stage of the KT, mainly by preformed donor-specific anti-HLA antibody [6,7]. Chronic AMR occurs when AAMR caused by preformed DSA is not treated completely, or AAMR caused by de novo DSA is progressed [8,9].

In the case of mixed rejection, maintenance immunosuppression could not be properly performed because of the adherence problem in the administration of immunosuppressant [9]. This is consistent with previous studies, which

Table 5. Comparison of Treatment Modalities According to Rejection Type in Kidney Transplant

Variables	TCMR (n = 48)	AMR (n = 58)	Mixed Rejection (n = 33)	*P* Value
Steroid pulse, No. (%)	25 (52.1)	18 (31.0)	24 (72.7)	.001
Antithymocyte globulin, No. (%)	0	0	1 (3.0)	.24
Rituximab, No. (%)	0	18 (31.0)	12 (36.4)	< .001
Plasmapheresis, No. (%)	0	19 (32.8)	11 (33.3)	< .001
Intravenous immunoglobulin, No. (%)	0	21 (36.2)	11 (33.3)	< .001

Abbreviations: AMR, antibody-mediated rejection; TCMR, T cell–mediated rejection.

showed the resulting early and late graft loss due to rejection [9–11].

Therefore, TCMR is also caused by lack of sufficient immunosuppression. Adherence to immunosuppressive drugs is important for stable immunosuppression. In addition, an immunosuppressant needs to be used more carefully because overimmunosuppression can result in the loss of immunosuppressant because of infection, such as BK or cytomegalovirus infection, and subsequent progression of weakened immunosuppression and TCMR progression.

In cases of AAMR by preformed DSA, removing the DSA through a sufficient desensitization is the method to prevent AAMR. To reduce AAMR by de novo DSA, continuous DSA monitoring and regular and serial protocol biopsy are needed [12,13].

Mixed rejection is considered to be the most advanced form of AMR and TCMR because the prognosis of mixed rejection is the worst. In our study, mixed rejection was the most common cause of graft failure in comparison with other rejections, and KTRs with TCMR had more graft failure than those with AMR because TCMR was the more acute rejection type. Therefore, it is important to prevent TCMR at the early stage after KT, to prevent the occurrence of mixed forms of TCMR and AMR by regular immunologic surveillance such as DSA and use of proper immunosuppressant, and to prevent chronic AMR by de novo DSA in the long-term for maintaining an effective allograft function.

Death-censored graft survival in the mixed rejection group was the least significantly, and time between KT and diagnosis of BPAR in the mixed rejection group was also the shortest significantly. Recipient age, TCMR, and positive C4d were the risk factors associated with graft failure in KT. This result suggests that post-transplant prognosis is worst when uncontrolled TCMR is accompanied by AMR with positive C4d and time between KT and diagnosis of BPAR is shorter.

In the treatment modalities, the proportion of steroid pulse therapy was significantly higher in the TCMR and mixed rejection groups. This suggests that the main treatment for acute rejection with the component of TCMR is a steroid pulse therapy. On the contrary, the proportions of rituximab, plasmapheresis, and intravenous immunoglobulin were significantly higher in the AMR and mixed rejection groups. This suggests that the main treatments for acute rejection with the component of AMR are rituximab, plasmapheresis, and intravenous immunoglobulin. Although mixed rejection was treated more aggressively in our study, the outcome after treatment was worse than other rejections. Therefore, we need to monitor the progression to the mixed rejection to maintain the allograft function for a long time.

In conclusion, our study showed that mixed rejection had poor prognosis compared with TCMR and AMR groups. Therefore, the prevention of the development of mixed rejection by TCMR at the early period after KT and chronic AMR by de novo DSA through constant monitoring of DSA and allograft biopsy is very important in post-transplant clinical outcomes.

REFERENCES

[1] Nankivell BJ, Alexander SI. Rejection of the kidney allograft. N Engl J Med 2010;363:1451–62.

[2] Loupy A, Haas M, Solez K, Racusen L, Glotz D, Seron D, et al. The Banff 2015 Kidney Meeting Report: current challenges in rejection classification and prospects for adopting molecular pathology. Am J Transplant 2017;17:28–41.

[3] Solar-Cafaggi D, Marino L, Uribe-Uribe N, Morales-Buenrostro LE. Antibody-mediated rejection in the Banff classifications of 2007 and 2017: a comparison of renal graft loss prediction capability. Transpl Immunol 2018;51:40–4.

[4] Ingulli E. Mechanism of cellular rejection in transplantation. Pediatr Nephrol 2010;25:61–74.

[5] Park WY, Kang SS, Jin K, Park SB, Choe M, Han S. Long-term prognosis of BK virus-associated nephropathy in kidney transplant recipients. Kidney Res Clin Pract 2018;37:167–73.

[6] Aubert O, Loupy A, Hidalgo L, Duong van Huyen JP, Higgins S, Viglietti D, et al. Antibody-mediated rejection due to preexisting versus de novo donor-specific antibodies in kidney allograft recipients. J Am Soc Nephrol 2017;28:1912–23.

[7] Zhang R. Donor-specific antibodies in kidney transplant recipients. Clin J Am Soc Nephrol 2018;13:182–92.

[8] Baldwin 3rd WM, Valujskikh A, Fairchild RL. Mechanisms of antibody-mediated acute and chronic rejection of kidney allografts. Curr Opin Organ Transplant 2016;21:7–14.

[9] Sellares 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.

[10] Scheel J, Reber S, Stoessel L, Waldmann E, Jank S, Eckardt KU, et al. Patient-reported non-adherence and immunosuppressant trough levels are associated with rejection after renal transplantation. BMC Nephrol 2017;18:107.

[11] Koo EH, Jang HR, Lee JE, Park JB, Kim SJ, Kim DJ, et al. The impact of early and late acute rejection on graft survival in renal transplantation. Kidney Res Clin Pract 2015;34:160–4.

[12] Sakai K, Oguchi H, Muramatsu M, Shishido S. Protocol graft biopsy in kidney transplantation. Nephrology (Carlton) 2018;23(Suppl. 2):38–44.

[13] Townamchai N, Safa K, Chandraker A. Immunologic monitoring in kidney transplant recipients. Kidney Res Clin Pract 2013;32:52–61.