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Original Article

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Do Xenogeneic Anti-HLA-A3 Antibody Cause Antibody-Mediated Rejection in Kidney Transplant?

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ABSTRACT

Objective: Anti-thymocyte globulin-Fresenius is used for induction treatment in kidney transplantation. The antibody of rabbit originated against human leukocyte antigen A3 were demonstrated in the serum of patients who used anti-thymocyte globulin-Fresenius. We investigated whether anti-human leukocyte antigen A3 antibodies detected due to anti-thymocyte globulin administration had any effect on patient and allograft survival in short- and long-term follow-up.

Methods: Fifty-one patients who underwent kidney transplantation between 2004 and 2014 were included in the study. Twenty-nine patients who underwent transplantation from deceased donors received an induction therapy consisting of anti-thymocyte globulin-Fresenius. Antibodies against the human leukocyte antigen were identified using the LABScreen panel reactive antibody class I/II kits with the Luminex method. The graft function and loss, patient survival, and the presence of acute/chronic rejection were investigated.

Results: Anti-human leukocyte antigen A3 antibody was detected in 41.3% of the patients receiving anti-thymocyte globulin induction ($P = .001$). This antibody disappeared at 234.4 days posttransplant. No difference was found regarding pretransplant and posttransplant sensitization of the patients who had posttransplant anti-human leukocyte antigen A3 positivity. The anti-thymocyte globulin dose and administration period were similar for anti-human leukocyte antigen A3 antibody-positive and -negative patients ($P > .05$). There was no significant difference between groups in short-term, first year, and long-term results of serum creatinine, estimated glomerular filtration rate, and proteinuria values ($P > .05$).

Conclusion: We demonstrated that xenogeneic anti-human leukocyte antigen A3 antibody could be detected in posttransplant serum of patients receiving anti-thymocyte globulin induction independent of the dose and duration. The development of this antibody was independent of the exposure of the patient to pre- and posttransplant sensitizing event or the presence of human leukocyte antigen A3 in the allograft. While this study did not demonstrate the effect of xenogeneic anti-human leukocyte antigen A3 antibody on graft and patient survival, retrospective multicenter cohort studies are needed on this issue.

Keywords: Anti-thymocyte globulin-Fresenius, xenogeneic anti-HLA-A3 antibody, antibody-mediated rejection

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INTRODUCTION

Anti-thymocyte globulin-Fresenius (ATG-F) (Fresenius Biotech GmbH, Gräfelfing, Germany) is a polyclonal IgG antibody that is derived from rabbit immunized with the Jurkat cell line.[1] These polyclonal antibodies affect immunomodulatory and immunosuppressive functions by interacting with a large variety of molecules present on many different immune and nonimmune cells.[2,3] Anti-thymocyte globulin-Fresenius includes antibodies against various cell surface molecules, majority of which are against T cell lymphocyte cell surface molecules along with monocytes, neutrophils, thrombocytes, erythrocytes, and endothelial cells (EC).[4-6] The Jurkat cell line used for ATG preparations bears the antigens HLA-A3, 32; B7, 35 and xenogeneic therapeutic antibodies against human leukocyte antigen (HLA)

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were demonstrated in the serum of patients who used ATG-F.[2] While ATG-F is widely used in kidney transplantation and appears safe, it has been shown to cause C4d deposition in the graft and antibody-mediated graft damage in some rare case presentations.[7,8] The aim of the study was to investigate the effects of anti-HLA-A3 antibody on antibody-mediated rejection (AMR) occurrence and long-term graft and patient survival.

METHODS

Patient Group

In this retrospective study, we investigated 51 patients who underwent kidney transplantation between 2004 and 2014 at Istanbul Faculty of Medicine. Anti-thymocyte globulin was used as the induction regimen for 29 patients who underwent transplantation from deceased donors, and these patients were compared with 22 patients who did not receive induction therapy. The data from patients and donors regarding age, sex, previous treatments, number of HLA mismatch, the pretransplant panel reactive antibody (PRA), flow, and complement-dependent cytotoxicity (CDC) crossmatch (XM) results were obtained from the patient file records. The study protocol was approved by the Istanbul University Istanbul Faculty of Medicine Research Ethics Committee (2019/359) on March 15, 2019. Informed consent was obtained from the participants.

Induction and Maintenance Therapy

Calcineurin inhibitors (CNI), mycophenolate derivatives, and corticosteroids were used as the maintenance immunosuppression therapy for all patients. Patients who received ATG as the induction therapy were administered with a dose of 2.5 mg/kg ATG-F for 3-24 days. Anti-thymocyte globulin dose was adjusted according to leukocyte and thrombocyte levels of patients.

Identification of Anti-Human Leukocyte Antigen Antibody and Crossmatch

The HLA antibodies were identified using the LABScreen PRA class I/II (One Lambda, Canoga Park, Calif, USA) kits with the Luminex method. The threshold was a mean fluorescence intensity (MFI) >1000. Antibodies identification was performed using the serum samples taken in the routine controls on day 1, post transplant 3 times PRA 1st mean of 12.2 (7-62) days, PRA 2nd mean of 234.4 (14-360) days, and PRA 3rd mean 67.05 (15-141) months). Flow and complement-dependent cytotoxicity crossmatch results of all patients were found negative in the pretransplant period. Complement-dependent cytotoxicity and flow crossmatches were performed simultaneously together with PRA screening in all patients by using the HLA-A3 donor cells (obtained from the volunteered healthy individuals with HLA-A*03:01 antigen) and patients' serum.

Follow-Up After the Transplant

The study group investigated graft function and loss, patient survival, and for the presence of acute/chronic rejection retrospectively. The posttransplant sensitization of the patients was identified using the Luminex PRA, complement-dependent cytotoxicity, and flow crossmatch. The monitoring of the kidney functions was performed using serum creatinine, proteinuria, and estimated glomerular infiltration rate (eGFR) levels. Estimated glomerular infiltration rate was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.

Statistical Analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences version 21.0 (IBM SPSS Corp.; Armonk, NY, USA). The Kolmogorov–Smirnov test was performed for the assessment of the distribution. Data are described as mean values with standard deviation. For categorical data, Fisher's exact test or chi-square test was used. Continuous numerical data with normal distribution was analyzed by using the Student's *t*-test or Mann–Whitney *U*-test. Kaplan–Meier analysis was used for the calculation of graft and patient survival (log-rank). Univariate and multivariable stepwise Cox regression analyses were performed to determine the risk factors associated with graft loss and anti-HLA-A3. All *P*-values smaller than .05 were considered statistically significant.

MAIN POINTS

- Anti-thymocyte globulin includes antibodies against various cell surface molecules, the majority of which are against T cell lymphocyte cell surface molecules along with monocytes, neutrophils, thrombocytes, erythrocytes, and endothelial cells.
- The Jurkat cell line used for ATG preparations bears the antigens HLA-A3, 32; B7, 35 and xenogeneic therapeutic antibodies against HLA antigens were demonstrated in the serum of patients who used ATG.
- Anti-human leukocyte antigen A3 could be detected using the antibody identification tests in posttransplant patient serum independent of the dose and time duration of ATG administration.
- Anti-human leukocyte antigen A3 antibody had no association with the patient survival and graft dysfunction or rejection in short- and long-term follow-up.

RESULTS

Patient Characteristics

The pretransplant characteristics of the entire study group are presented in Table 1. Deceased donors were younger compared to living donors (41.76 ± 13.4 years, 36.86 ± 12.9 years; $P = .013$). However, no significant difference was detected regarding the pretransplant PRA (Table 1).

Anti-thymocyte Globulin and Panel Reactive Antibody

The posttransplant PRA results of all patients are presented in Table 2. While 18 out of 29 patients who received ATG were followed up for the long term, 11 patients were followed up for short term. We do not have data of 11 patients because of different reasons such as: 1 patient died due to respiratory failure

Table 1. Demographic Data of the Patients

Variables	All Subjects (n = 51) (100.0%)		
	Deceased Donors (n = 29) (56.9%)	Living Donors (n = 22) (43.1%)	*P*
Patients age (years, mean ± SD)	41.76 ± 13.4	36.86 ± 12.9	>.05
Donors age (years, mean ± SD)	42.90 ± 14.9	52.68 ± 11.4	***.013***
Patients' gender (male/female)	18/11	16/6	>.05
Donors' gender (male/female)	13/16	9/13	>.05
Months to follow-up (median) (minimum–maximum)	39.00 (1-141)	42.00 (12-72)	>.05
Maintenance treatment			
CNI/No CNI	28/1	16/6	***.034***
Tacrolimus/cylosporine	9/19	16/0	***<.01***
ATG/ATG + Simulect	17/12	0/0	
HLA MM >2	27/2	18/4	>.05
HLA haplotype match (yes/no)	10/19	20/2	***<.01***
Pretransplant sensitization (yes/no)	23/6	10/12	***.018***
Retransplantation	5/24	1/21	>.05
Pregnancy history	5/24	1/21	>.05
Transfusion history	21/8	9/13	***.043***
Pretransplant			
Anti-HLA class I (+/−)	2/27	1/21	>.05
Anti-HLA class II (+/−)	0	0	–
Anti-HLA-A3 ab	0	0	–

Ab, antibody; ATG, anti-thymocyte globulin; CNI, calcineurin inhibitors; HLA, human leukocyte antigen; HLA-A3, human leukocyte antigen A3; MM, mismatch; n, number of individuals.

Table 2. Panel Reactive Antibody and anti-HLA-A3 Production in ATG-Treated Transplant Patients

	ATG	No ATG	*P*
PRA 1st [mean 12.2 (7-62) days]	n = 29	n = 22	
Class I (+/−)	18/11	0/22	***<.01***
Class II (+/−)	3/26	1/21	.625
Anti-HLA-A3 (+/−)	12/17	0/22	***.001***
PRA 2nd [mean 234.4 (14-360) days]	n = 29	n = 22	
Class I (+/−)	8/21	0/22	***.007***
Class II (+/−)	6/23	1/21	.124
Anti-HLA-A3 (+/−)	1/28	0/22	.998
PRA 3rd [mean 67.05 (15-141) months]	n = 18	n = 17	
Class I (+/−)	2/16	0/17	.486
Class II (+/−)	3/15	1/16	.602
Anti-HLA-A3 (+/−)	0/17	0/17	–

ATG, anti-thymocyte globulin; HLA-A3, human leukocyte antigen A3; PRA, panel reactive antibody, n, number of individuals.

(day 22), and 3 patients underwent allograft explantation due to non-immunological reasons. 7 patients were lost to follow-up.

During the first PRA screening of patients, the positivity of PRA class I antibodies was found higher in the ATG group (*P* <.01). A3 antibody was detected in 12 out of 18 patients who had negative PRA before transplantation. In addition to A3 antibody positivity, another antibody was detected in 1 patient (*P* = .001). Along with A3 antibody positivity, we detected different antibodies in 6 patients during the first PRA screening (PRA 1st).

Eleven out of 12 patients' A3 antibody positivity disappeared at the second PRA screening time (PRA 2nd). Only 1 patient's A3 antibody positivity persisted. This patient also had other antibodies in addition to A3. The A3 antibody disappeared in all patients at the third PRA screening time (PRA 3rd).

Anti-Human Leukocyte Antigen A3 Antibody and Sensitization Events

No difference was found regarding pretransplant and posttransplant sensitization of the patients who had posttransplant anti-HLA-A3 positivity. While 5 patients with A3 antibody

positivity received blood transfusion in the early posttransplantation period, 7 patients received no transfusion. Two out of 5 patients received blood transfusion after the detection of HLA-A3 antibody.

Anti-Human Leukocyte Antigen A3 Antibody and Anti-thymocyte Globulin Dose

The presence of A3 antibody was found to be significantly related to ATG usage in accordance with the log-rank curve using the Kaplan–Meier analysis (P = .004).

The ATG dose and ATG administration period in patients with posttransplant anti-HLA-A3 antibody positivity were similar compared with HLA-A3 antibody negative patients. When we look at the donor HLA-A3 antigen status, we detected similar ATG dose and treatment duration in patients whose donors either carried A3 or did not carry A3 (Table 3).

Anti-Human Leukocyte Antigen A3 Antibody and Graft Outcome

Serum creatinine, eGFR, and proteinuria values were investigated for the evaluation of kidney functions in all patients. These results are summarized in Table 3. There was no significant difference between groups in short-term, first-year, and long-term results regarding the graft functions.

The posttransplant status of patients who developed anti-HLA-A3 antibody is summarized in Table 4. Pretransplant PRA and crossmatch tests of all patients were negative. Complement-dependent cytotoxicity and flow crossmatches were performed on all patients who received ATG after transplantation, using the cells which carried HLA-A*03:01 antigen simultaneously with the PRA screenings. B-flow crossmatch was found positive in only 2 patients who developed anti-HLA-A3 in PRA 1st screening after transplantation. The anti-HLA-A3 antibody titer (P7 MFI: 7850, P10 MFI: 7200) of these patients were found higher compared to others. All the crossmatches, which were performed simultaneously with the second PRA screening, were found negative. B-flow crossmatch positivity was detected in third PRA screening in only 1 patient (H19) who possessed new donor-specific class II HLA (DR12 MFI: 5860).

Two patients who received ATG and developed anti-HLA-A3 antibody died due to serious infections (1 and 21 months after

Table 3. Features of Patients Who Received ATG

	A3 Ab+ (n = 12) (41.3%) (Yes/No)	A3 Ab− (n = 17) (58.7%) (Yes/No)	*P*	Donor A3+ (n = 16) (55.1%) (Yes/No)	Donor A3− (n = 13) (44.9%) (Yes/No)	*P*
Pretransplant sensitization						
Retransplantation	0/12	5/12	.059	5/11	0/13	***.048***
Pregnancy	1/11	4/13	.370	4/12	1/12	.220
Transfusion	10/2	11/6	.408	12/4	9/4	.730
Early posttransplant sensitization						
Transfusion	5/7	5/12	.494	5/11	5/8	.714
Simulect use	4/8	8/9	.460	6/10	6/7	.716
ATG use						
Mean dose ± SD	91.17 ± 28.6	94.13 ± 25.1	.773	86.47 ± 23.5	100.23 ± 28.1	.169
Period of use (day) mean ± SD	12.17 ± 6.4	13.69 ± 6.4	.537	14.20 ± 6.5	11.69 ± 5.9	.301
Short-term kidney function						
14th day serum creatinine (mg/dL) ± SD	3.43 ± 1.1	3.03 ± 0.8	.763	3.69 ± 1.0	2.56 ± 0.6	.383
First-year serum creatinine (mg/dL) ± SD	3.51 ± 1.2	1.68 ± 0.3	.119	2.44 ± 0.8	2.44 ± 0.9	.999
eGFR (mL/min) ± SD	65.45 ± 49.3	87.73 ± 29.2	.162	83.62 ± 49.1	73.00 ± 28.7	.507
Long-term kidney function*	A3 Ab+ (n = 9)	A3 Ab− (n = 9)	*P*	Donor A3+ (n = 8)	Donor A3− (n = 10)	*P*
Last creatinine (mg/dL) ± SD	1.91 ± 0.30	1.71 ± 0.59	.782	1.58 ± 0.8	2.16 ± 1.1	.525
Last eGFR (mL/min) ± SD	71.3 ± 46.0	67.8 ± 29.7	.798	70.0 ± 35.1	70.7 ± 42.5	.999
Last proteinuria	0.07 ± 0.01	0.36 ± 0.08	.927	0.39 ± 0.2	0.07 ± 0.01	.189

Ab, antibody; eGFR, estimated glomerular infiltration rate; n, number of individuals; SD; standart deviation. *Patients who did not survive and were lost to follow-up were excluded from long-term kidney function.

Table 4. The Current Condition of the Transplant Patients in Whom the A3 Antibody Was Detected

Received ATG Patients	After Transplantation					Donor A3 Antigen (+)	Graft/ Patient Outcome	Last eGFR	Last Creatinine	Proteinuria	Follow-Up Time (Months)
	Number of Transfusion (Day)	First PRA Screening (MFI)	Days	Second PRA Screening (MFI)	Days						
P1	0	A3 (5600)	14	–	360	–	GDF	30	3.1	0.3	60
P3	2 (9 and 17)	A3 (2800)	62	–	360	–	SGF	138	0.6	0	64
P5	0	A3 (2500)	7	–	360	–	SGF	114	0.6	0	114
P7	8 (23, 29, 34, 43, 47, and 54)	A3 (7850)	7	+ (other Ab)	60	–	HD	5	9.4	8.7	2
P9	3 (24, 31, and 43)	A3 (1700)	14	–	360	–	EX	52	1.3	O	21
P10	0	A3 (7200)	14	–	360	–	HD	10	6.8	9.9	39
P11	0	A3 (1000)	7	–	90	–	HD	7	8.9	8.7	3
P12	0	A3 (1000)	7	–	90	–	SGF	96	0.9	0	51
P13	0	A3 (1000)	7	–	180	–	SGF	86	0.8	0	54
P17	0	A3 (1500)	6	–	21	+	GDF	57	1.6	0	102
P18	2 (0 and 4)	A3 (1500)	12	–	20	+	EX	8	12.9	4.5	1
P19	1 (3)	A3 (1500) + other	16	+ (A3 and other Ab)	360	+	HD	14	9.2	8.9	120

Ab, antibody; eGFR, estimated glomerular filtration rate; EX, exitus; GDF, graft dysfunction; HD, hemodialysis; MFI, mean fluorescence intensity; P, patient, PRA, panel reactive antibody; SGF, stable graft function.

transplantation). Graft dysfunction developed in 2 patients (eGFR 30-60 mL/min/1.73 m^2, grade III). Four patients lost grafts due to different reasons (3 of them nonimmunologic, 1 chronic AMR). Four patients had stable graft function.

In the group of patients (n = 9) who had ATG usage with no A3 antibody development, 1 graft dysfunction (grade IV) was detected, and 1 graft was lost due to chronic AMR. Other 7 patients had stable graft function. The posttransplant patient and allograft survival, and the outcomes are demonstrated in Table 5.

Multivariable Cox regression analyses demonstrated no association between allograft survival and recipient age, patient age, CNI usage, HLA-A3 positive graft, anti-HLA-A3 antibody, ATG dose, HLA match, sensitization (Table 6).

Table 5. Posttransplant Graft and Patient Outcomes

	ATG Group (n = 29)				
	First Year Following Patients (n = 29)		Long-Term Following Patients (n = 18)		
	n	%	n	%	*P*
Patient survival	26	89.7	16	88.9	.549
Graft lost	7	24.1	6	33.3	.521
SGF (grade I-II)	17	58.6	12	66.7	.759
GDF (grade III-IV)	5	17.2	3	16.7	.356
	Anti-HLA-A3 (yes/no) (12/17)		Anti-HLA-A3 (yes/no) (9/9)		*P*
Patient survival	10 (83.3%)	16 (94.1%)	8 (44.5%)	8 (44.5%)	.477
	P = .348		*P* = .999		
Graft lost	5 (41.7%)	2 (11.8%)	4 (44.4%)	2 (22.2%)	.793
	P = .064		*P* = .813		
SGF (grade I-II)	5 (41.7%)	12 (70.6%)	5 (27.8%)	7 (38.8%)	.694
	P = 147		*P* = .619		
GDF (grade III-IV)	2 (16.7%)	3 (25.0%)	2 (11.1%)	1 (5.6%)	.999
	P = .999		*P* = .813		

ATG, anti-thymocyte globulin; GDF, graft dysfunction; n, number of individuals; SGF, stable graft function.

DISCUSSION

Anti-thymocyte globulin is an agent that is used for induction treatment in kidney transplantation. Anti-thymocyte globulin—Fresenius and thymoglobulin contain different types of polyclonal antibodies of rabbit origin and ATGAM from the horse. Although ATG-F includes antibodies against various cell surface molecules including T lymphocytes as the majority, along with monocytes, neutrophils, thrombocytes, and erythrocytes, ATGAM has also been found to be binding to human smooth

Table 6. Risk Factor for Kidney Graft Survival

	Multivariate Regression		
Covariate	**HR**	**95% CI**	***P***
Donor HLA-A3 antigen +	1.317	0.145-11.993	.807
Haplotype	0.480	0.069-3.349	.459
Sensitization +	1.301	0.136-12.476	.820
Anti-HLA-A3 +	0.265	0.030-2.364	.234
Mean ATG use	0.990	0.950-1.032	.631
Calcineurin	1.787	0.850-2.043	.089
Donor's age	0.983	0.929-1.040	.298
Patient's age	0.971	0.917-1.428	.544

muscle and endothelial cells.[2-9] We demonstrated that xenoantibodies such as HLA-A3 could be detected using the antibody identification tests in posttransplant patient serum independent of the dose and time duration of ATG administration. However, due to the insufficient number of patients, it could not be concluded that anti-HLA-A3 antibody of rabbit origin cause cytotoxicity.

These xenoantibodies were demonstrated to trigger the AMR by inducing the complement activation and complement accumulation in the peritubular capillaries of the kidney allograft.[10-13] It was shown to include high numbers of antibodies against CD107a, which is a cytotoxic cell surface molecule in ATG-F.[2] It was stated that the interaction of this molecule with ATG deteriorated the attack ability of alloreactive cytotoxic T cells against the graft.[14] Xenoantibodies were demonstrated to induce the anti-glycan antibodies, and anti-*N*-glycolylneuraminic acid (Neu5Gc) immunoglobulin G molecules, which can activate the endothelial cells particularly in patients, and grafts.[15]

It was demonstrated that ATGs included HLA-specific antibodies and ATG-F preparation included high numbers of HLA class I antibodies, whereas HLA class II antibodies were only detected in thymoglobulin. The specificity of the anti-HLA antibodies may be predicted since ATG-F is produced from a single T cell line; however, the antibody specificity varied between the lots in thymoglobulin that is produced from human thymocytes.[2] Xenoantibodies may cause positive results with the interaction of antibody identification tests (cytotoxicity flow cytometry, solid phase), and it was reported that this positivity disappears after the termination of ATG use.[16,17]

Masson et al[18] specifically detected HLA-A3 antibody in Luminex™ testing after the use of ATG-F. Similarly, we detected HLA-A3 antibody in Luminex™ testing in 41.3% of the patients who underwent transplantation from a deceased donor and received ATG-F as the induction therapy in our study. The development of these antibodies was independent of the exposure of the patient to pre- and posttransplant sensitizing event or the presence of HLA-A3 antigen in the allograft. Therefore, these antibodies were anticipated to be rabbit xenoantibodies as demonstrated in previous studies. Our opinion is supported by the fact that the antibodies completely disappeared on average 234.4 days after discontinuing post-transplant ATG use. Though limited, xenoantibodies were detected to demonstrate complement dependent cytotoxicity against human cells in the previous studies.[16,18] The development of antibody against A3 from HLA antigens on Jurkat cells can be explained by the immunogenicity of the HLA-A3 antigen or its expression may be higher.[18] We could not demonstrate the effect on antibody levels on flow cross match positivity.

Anti-thymocyte globulin affects lymphocytes by the interaction of xenoantibodies with the antigens. The effect of ATG is evident from the interaction of xenoantibodies with the antigens located on the surface of target cells. Human leukocyte antigen-reactive antibodies in ATG were reported to have the ability to bind to the common epitopes shared by multiple HLA molecules, and the binding possibility of these antibodies to the host cells and tissues was higher.[19] Baldwin et al[8] demonstrated conducted with cardiac transplanted patients that ATGAM included antibodies that could block the polymorphic determinants of HLA and bound to capillaries, and myocytes in endomyocardial biopsies after transplantation. In addition, they reported that the prophylactic treatment using ATGAM was associated with horse IgG accumulation and complement activation of the biopsy material. Anti-thymocyte globulin therapy was demonstrated to result in C4d accumulation in kidney allografts and in antibody-mediated graft damage in the rare case presentations.[8,10]

Focosi and Boggi[20] suggested that the antibodies against HLA class I and II specific antigens in ATGs might include the donor-specific antibodies (DSA) which may have a role in the antibody mediated allograft rejection. However, another study reported that the rabbit ATG inhibited the DSA production and decreased the risk of antibody mediated rejection in kidney transplant patients who had pretransplant high titer DSA or had a potential role in decreasing the de novo DSA (dnDSA) in patients with moderate sensitivity.[21] Acute antibody mediated rejection was not detected in patients who had HLA-A3 antibody, with or without HLA-A3 antigen presence in their graft. Chronic AMR developed in month 120 in 1 patient who had HLA-A3 antigen in the allograft. Class II DSA (DR12 MFI: 5860) positivity was detected in the serum during the time of rejection; however, HLA-A3 antibody was not detected in that patient.

Some studies investigated the effects of ATG preparations on antirejection efficacy, rejection frequency, and graft survival. Shaw et al[22] described lower acute rejection frequency and better graft survival in patients who received thymoglobulin compared to the patients who received ATG. However, De Santo et al[23] prospectively demonstrated that both products were equivalent regarding the anti-rejection efficacy and graft

survival. However, ATG was safer for the patient. The patients who received ATG-F which was produced from a single T cell line and in whom the specificity of its antibodies could be anticipated were preferred in the study.

Some studies investigated the effect of ATG use in short- or long-term graft and patient survival. In a randomized study that had a long-term follow-up duration, the frequency of acute rejection was found lower and 1-year graft survival was found higher in patients who received ATG induction therapy, whereas no significant difference was detected in graft and patient survival 10 or 20 years after transplantation.[24,25] Induction therapy was found to reduce the acute rejection incidence, might increase short-term allograft survival and reduce the delayed graft function incidence.[26,27] In our study, the effect of ATG-F induction on kidney function, patient and allograft survival was unclear in the short and long term. Anti-thymocyte globulin induction was found to have no different effect on graft and patient survival, and graft function in long- and short-term follow-up.

Brokhof et al[28] found that the treatment of ATG, intravenous immunoglobulin, and plasmapheresis was associated with acute AMR and dnDSA. No statistically significant risk factor was found in the risk analysis for immunologic problems of kidney grafts in our study.

We mainly investigated whether the rabbit xenogeneic anti-HLA-A3 antibody detected due to ATG administration had any effect on kidney function, patient and allograft survival in short- and long-term follow-up. No direct effect of anti-HLA-A3 antibody was detected on patient survival. In the first year, 41.7% graft loss was observed in patients who developed anti-HLA-A3 antibody, and this rate was 44.4% in long-term follow-up.

Masson et al[18] reported that no adverse effects were detected in patients with HLA-A3 antigen, but it was unclear whether xenogeneic anti-HLA-A3 antibody caused tissue damage in the graft. In our study, we detected anti-HLA-A3 antibody in 3 patients who had HLA-A3 antigen in their grafts. Only 1 patient lost the graft due to class II DSA. The results of our study showed that xenogeneic anti-HLA-A3 antibody had no association with the patient survival, graft dysfunction or rejection in short- and long-term follow-up.

Our study has several limitations; The study period was short, there was a lack of a control group, and the patients were from a single center. Due to these limitations, we could not draw firm conclusions from this study; therefore, our findings should be confirmed by prospective cohort studies with a large population.

CONCLUSION

Studies have shown that humoral rejection of kidney allografts can be triggered by the passive transfer of xenogeneic antibodies. Therefore, regular immunological follow-up of patients with HLA-A3-positive grafts who received ATG-F induction therapy should be evaluated more carefully.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Istanbul Faculty of Medicine University (Date: 15.3.2019, Number: 2019/359).

Informed Consent: Written informed consent was obtained from the patients who participated in this study.

Peer-review: Externally peer-reviewed.

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