A Significant Role for Anti–Human Leukocyte Antigen Antibodies and Antibody-Mediated Rejection in the Biopsy-for-Cause Population

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ABSTRACT

The role of anti–human leukocyte antigen (HLA) antibodies and antibody-mediated rejection is well known, but our comprehension and the preventive measures we take seem to be insufficient. One of the major causes of premature renal transplant loss is recepients' immunologic hyperactivity to donors' antigens. Monitoring of humoral alloreactivity gives hope for early diagnosis and adequate therapy. The goal of our analysis was the assessment of the influence of anti-HLA antibodies on the function and survival of transplants. In our study we included 60 consecutive renal transplant recipients who had a renal transplant biopsy-for-cause performed due to insufficiency. Transplant biopsies were performed between the 7th day and 12th year (median, 2 years) after transplantation. Anti-HLA antibodies were present in 20 patients (33%). The patients were divided into 2 groups according the presence of anti-HLA antibodies. In a 12-month observation, 10/20 (50%) patients in the anti-HLA(+) group returned to dialysis in contrast with 7/40 (17.5%; $P = .014$) in the anti-HLA(−) group. Also, 8/10 (80%) of the anti-HLA(+) patients who lost the transplant had anti-HLA Abs class II and only 2/10 (20%) had anti-HLA Abs class I. Anti-HLA antibodies were specific to a donor (donor-specific antibodies [DSA]) in 8/10 (80%) of the patients who lost the transplant. Anti-HLA antibodies appeared de novo in 50% of patients who lost the transplant. Nonadherence was suspected in 50% of patients. Acute humoral rejection occurred in 1 patient. Also, 8/10 (90%) developed chronic active humoral rejection. Our study revealed that graft loss in the renal transplant biopsy-for-cause population with the presence of anti-HLA Abs during a 12-month observation reached 50%. Nonadherence in these patients was very high and amounted to 50%. Monitoring of renal transplant recipients and individualization of therapy considering humoral activity should prolong renal graft survival.

THE ROLE of anti–human leukocyte antigen (HLA) antibodies and antibody-mediated rejection (AMR) is well known, but our comprehension and the preventive measures we take seem to be insufficient [1–4]. Numerous studies have shown that circulating antibodies against HLA are associated with accelerated renal transplant failure but, on the other hand, many patients with these antibodies have good graft function, which decreases our cautiousness or causes underestimation [5].

According to the Banff classification, the definition of AMR relies on 3 features: allograft pathology, peritubular C4d deposition, and serological anti-HLA donor-specific

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antibodies (DSA) [6,7]. The diagnostic criteria of antibody-mediated lesions have been extended lately to renal microcirculation deterioration [7,8], transplant glomerulopathy [9], de novo transplant microangiopathy [10], and accelerated arteriosclerosis [11]. The C4d deposition in peritubular capillary was established as a specific but not sensitive marker for all of these injuries. It seems that nearly 50% of AMR was misclassified [12–14] with the biopsy findings and clinical phenotypes diagnosed as "chronic allograft nephropathy" or calcineurin inhibitor (CNI) toxicity [15–19]. As a consequence, patients with a biopsy for clinical indications may have AMR due to donor-specific HLA antibodies, usually anti class II [20], either C4d-positive or C4d-negative [4].

It gives rise to the question why immunosuppression is unsuccessful in preventing the activity of anti-HLA, DSA, and AMR lesions. The first reason is nonadherence, which is difficult to distinguish until the rejection, which is often resistant to therapy [21,22]. The second is under-immunosuppression used to avoid CNI-induced toxicity, which appears as the nonspecific hyalinosis and fibrosis in late biopsy specimens [15]. It may stimulate clinicians to minimize immunosuppression, which is a mistaken action in case of anti-HLA activity [19].

The knowledge of the exact impact of the antibodies on renal insufficiency might help to prolong transplant survival. Thus, in our prospective study, we decided to investigate the role of anti-HLA antibodies in patients with renal transplant insufficiency to understand their significance in graft loss.

MATERIAL AND METHODS

In our study we included 60 consecutive renal transplant recipients who had a renal transplant biopsy for cause performed due to insufficiency. Renal transplants were allocated on the basis of ABO compatibility with a negative cross-match before transplantation.

On the day of biopsy, blood was collected for detection of HLA antibodies. All serum samples were kept frozen at −80°C until assayed. Humoral immunization was evaluated using the Flow-PRA Screening Test (One Lambda, Canoga Park, Calif, United States) to detect the presence and class of the anti-HLA antibodies. The determination of the panel-reactive antibodies using beads coated with purified HLA antigens was performed according to the manufacturer's instructions. The beads consisted of a pool of 30 different bead preparations, coated with either HLA class I antigens (HLA-A, HLA-B) or class II antigens (HLA-DR, HLA-DQ) from different cell lines. The cut-off value for positive values was set at 5% both in class I and II.

We analyzed characteristics of the patients according to anti-HLA presence (Table 1). The immunosuppression consisted of the following: cyclosporine or tacrolimus, mycophenolate mofetil/acid, azathioprine, and steroids (Table 2).

The diagnosis of acute rejection (AR) was based on Banff criteria. Immunohistochemistry method was used to verify C4d depositions. In case of AR, the recipients received steroids. Plasmapheresis and intravenous immunoglobulin were considered in patients with AMR.

There was no statistically significant difference considering recipients' age or gender, time on dialysis before transplantation, cause of chronic renal failure, number of presensitized patients, number of HLA mismatches, donor gender, and cold ischemia time between the groups. The anti-HLA–positive group was younger (Table 1).

The ethics commission of the Wroclaw Medical University approved all study protocols and informed consent was obtained from all patients.

Table 1. Patient Population Characteristics

	Anti-HLA(+) n = 20	Anti-HLA(−) n = 40	*P*
Recipients age (y)	41.5 ± 14	37.5 ± 16	NS
Male gender, n (%)	31 (77.5%)	12 (60%)	NS
Time on dialysis before transplantation (d)	929 ± 632	941 ± 823	NS
Cause of chronic renal failure			
Chronic glomerulonephritis	5	14	NS
Diabetic nephropathy	2	2	
Hypertonic nephropathy	1	2	
Polycystic kidney disease	5	7	
Pyeloneohritis	5	8	
Others	2	7	
First transplant	18	36	NS
Retransplant	2	4	NS
No. of presensitized patients	7/20	5/40	NS
No. of presensitized patients			
PRA <10%	1	1	NS
PRA 10–50%	4	3	NS
PRA >50%	2	1	NS
No. of HLA mismatches	3.7 ± 1.1	3.8 ± 1.0	NS
Donor gender (%)			
Female	32	36	NS
Male	68	64	NS
Donor age (y)	35 ± 15	48 ± 13	.009
CIT (h)	20.8 ± 9.5	23.7 ± 6.8	NS

Abbreviations: NS, not significant; PRA, panel-reactive antibodies; CIT, cold ischemia time.

Statistics

Statistica version 10 was used for statistical analysis. Continuous data were presented as the mean ± standard error of the mean (SEM). The comparison between the groups was performed using a Student *t* test and the Mann-Whitney *U* test for metric variables, whereas the chi-square test was used to identify a connection between AR and the presence of antibodies. Univariate and multivariate logistic regression analyses were performed to evaluate the association of chronic rejection risk factors with anti-HLA antibodies. Factors associated with graft failure were analyzed using Cox proportional hazard analysis (univariate and multivariate analysis; Table 3). Cox model was performed to show the graft survival in the anti-HLA–positive and–negative group. The Fisher

Table 2. Initial Immunosuppression

	Anti-HLA(+) n = 20	Anti-HLA(−) n = 40	*P*
TAC-MMF/MPA+S	9	20	NS
CsA-MMF/MPA+S	6	17	NS
CsA-AZA+S	2	3	NS
Simulect+TAC-MMF/MPA+S	3	0	NS

Abbreviations: TAC, tacrolimus; CsA, cyclosporine; MMF, mycophenolate mofetil; MPA, mycophenolic acid; AZA, azathioprine; S, steroids.