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Cytotoxic antibodies monitoring in kidney transplantation – their clinical relevance and challenges

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Abstract

Introduction: The key of the successful renal transplantation is the ability to identify the best immunological match between donor and recipient considering the possibility of rejection phenomenon. The aim was to identify class I and/or class II cytotoxic antibodies in renal-transplanted patients in order to assess the immunological potential for prevention of subclinical or acute rejection episodes. *Patients and Methods*: We have evaluated ninety-two patients who had kidney transplantation in 2010 in Fundeni Clinical Institute, Bucharest, Romania, concerning HLA matching and anti-HLA immunization status. For HLA genotyping were used molecular biology methods – PCR-SSP (Invitrogen, USA). For cytotoxic antibodies, the methods used were ELISA (GTI Diagnostics, USA) and Luminex (One Lambda, USA). Crossmatch tests between donor cells and recipient serum were performed by ELISA (GTI Diagnostics, USA). Rejection diagnosis was supported by renal biopsy. *Results*: In the 20 presensitized cases, the rate of acute rejection was 30% while in the 72 unsensitized cases the rejection was 19.4%. The incidence of acute rejection was higher in anti-HLA class I presensitized patients compared with anti-HLA class II (20% and 14.3%, respectively) but there was no significant difference compared to pre-transplant unsensitized patients (19.4%). Sequential post-transplantation monitoring of anti-HLA antibodies has shown in pre-transplant sensitized patients group a constantly increasing of PRA value, while in the pre-transplant unsensitized patients group, 32% developed *de novo* cytotoxic antibodies. *Conclusions*: More sensitive and specific methods to detect anti-HLA antibodies before transplantation and sequential post-transplantation monitoring of these antibodies would be useful to identify patients who are at higher risk for allograft failure.

Keywords: anti-HLA sensitization, renal transplant, acute rejection.

Introduction

The key of the successful renal transplantation is the ability to identify the best immunological match between donor and recipient considering the possibility of rejection phenomenon. HLA typing plays an important role in renal transplantation and the correctly identification of HLA alleles is one of the most complex problems in molecular diagnosis. Patients may develop alloantibodies against foreign HLA molecules acquired by pregnancy, transfusion or previous allografts [1].

Renal transplantation in sensitized patients remains a significant challenge worldwide. Panel reactive antibody (PRA), produced against HLA pose a major risk factor for increased incidence of hyperacute/acute graft rejection and graft dysfunction which leads to reducing graft survival. However, sensitized candidates should not be eliminated from transplant waiting lists since the use of desensitization therapy (plasmapheresis, immuno-absorbtion or intravenous immunoglobulin) and of the newer immunosuppressive agents are usually adopted in presensitized recipients in order to ensure a successful transplant [2–5].

The aim of our work was to identify class I and/or class II cytotoxic antibodies in renal-transplanted patients in order to assess the immunological potential for prevention of subclinical or acute rejection episodes.

Patients and Methods

We have evaluated ninety-two patients (43 men and 49 women) who had kidney transplantation in 2010 in Fundeni Clinical Institute, Bucharest. The age range was 23–51 years. The study design consists into two groups according to pre-transplant anti-HLA immunization status: first group have included unsensitized patients and the second group sensitized recipients. The prophylactic induction immunosuppressive therapy consisted of 40 mg interleukin-2 receptor antibodies (Basiliximab) administered in two doses, 20 mg within two hours prior to transplantation surgery and other 20 mg four days post-transplantation in combination with corticosteroids. Triple therapy with cyclosporine A (CsA), mycophenolate mofetil (MMF), and prednisolone (Pred) was adopted as the immunosuppressive maintenance protocol for five recipients following the transplantation. Tacrolimus (Prograf, FK506) was given to 84 recipients in combination with MMF and Pred and three patients

who had 100% HLA compatibility with donors received only MMF and Pred. Follow-up period was at least one year post kidney transplantation.

Pre-transplant, for all patients and donors, HLA low-resolution genotyping for A, B and DRB1 loci, cytotoxic antibodies screening/identification and crossmatch tests were performed at Centre for Immunogenetics and Virology, Fundeni Clinical Institute, Bucharest.

For HLA genotyping were used molecular biology methods: PCR with sequence-specific primers (AllSet™ Gold HLA ABDR Low Res, Invitrogen, USA). The specificity of HLA alleles is determined directly by the primers. After amplification, the amplicons are viewed by agarose gel electrophoresis and the specific pattern is analyzed using a software in order to get an alleles assignment.

For cytotoxic antibodies, the methods used were ELISA (GTI Diagnostics, USA) and Luminex (Lab Screen® Mixed, LabScreen® PRA, LabScreen® Single Antigen, One Lambda, USA). Crossmatch tests between donor cells and recipient serum were performed by ELISA (GTI Diagnostics, USA).

ELISA is a qualitative solid phase enzyme linked immunosorbent assay, which is based on antigen–antibody reaction. In this case, the antigens are purified HLA class I or class II glycoproteins that will be incubated with patient serum, allowing antibodies, if present, to bind. Unbound antibodies are washed away. The bound antibodies are highlighted by adding a conjugate (anti-human IgG reagent labeled with alkaline phosphatase) and a substrate PNPP (*p*-nitrophenyl phosphate). Enzyme action on substrate develops a color reaction whose intensity is measured using a spectrophotometer and 405 nm wavelength. The optical density for each sample is compared to a cutoff value represented by double of the average value of the negative control wells optical densities.

The Luminex technology is also based on antigen–antibody reaction but, in this case, the purified HLA class I or class II glycoproteins are anchored to the surfaces of uniquely fluorescent microscopic beads. Every microsphere is accurately classified to its own subset, based on its fluorescent signature. The beads are incubated with a small volume of patient serum and then, after a washing step to remove unbound antibodies, anti-human IgG antibodies conjugated to phycoerythrin are added. After another incubation, the samples are diluted and analyzed on the Luminex instrument. The excitation system in the Luminex analyzer uses two solid-state lasers. A reporter laser excites fluorescent molecules (phycoerythrin) bound to cytotoxic antibodies at the microsphere surface, and a classification laser excites fluorochromes embedded in the microsphere. These fluorescent signals are discriminated with selective emission filters and are converted into intensity units by a digital signal processor. The signal intensity from each bead is compared to the signal intensity of a negative control beads.

Results

In both groups, in most cases, HLA matching for A, B and DRB1 loci was 50% (*i.e.*, three HLA-mismatch). In seven cases, all with cadaveric donor, we found four HLA-mismatch (4MM) and other 11 pairs had two mismatch (2MM). In all these cases, the HLA-DRB1 allele compatibility was at least 50%. HLA compatibility was 100% (0MM) only in three pairs. In all these three cases, it was the living related donor. Donor-recipient pairs were brothers, in one case even twins.

Pre-transplant, using ELISA method, the cytotoxic antibodies screening was positive in seven patients and negative in all donors and the other 85 patients, noting that in 12 patients, the optical density value read by spectrophotometer was close to the cutoff value. When patients sera were reviewed in terms of cytotoxic antibodies using Luminex method, in seven of the 12 cases with the cutoff value, were obtained positive results. In addition, they have detected anti-HLA antibodies in another six patients. A total of 20 patients were presensitized, five with cadaveric donor and 15 with living donor (Table 1). All crossmatch tests prior to transplantation were negative. This was the mandatory condition for transplantation.

Table 1 – ***HLA matching and donor source in the two groups***

HLA-mismatch	Non-sensitized recipients (n=72)		Sensitized recipients (n=20)		Total
	LD	*CD*	*LD*	*CD*	
0 MM	2	0	1	0	3
2 MM	6	1	3	1	11
3 MM	37	19	11	4	71
4 MM	0	7	0	0	7
Total	45	27	15	5	92

LD – living donor; CD – cadaveric donor; MM – mismatch.

The PRA values were variable, between 7 and 28%. Next step was to determine the specificity of these antibodies. Half of the cases (10 patients) had only anti-HLA class I antibodies, in seven patients the antibodies' targets were HLA class II antigens, and the rest (three patients) were sensitized for both, class I and class II. The most frequent specificities for class I were A2, A23, A24, A25, A30, A32, B7, B27, B8, B51, B55, B57, Cw2, Cw4, Cw5 and for class II DR1, DR4, DR7, DR11, DQ2, DQ5, DQ7.

In the 20 presensitized cases, the rate of acute rejection was 30% (six recipients). In the 72 unsensitized cases, the rejection was 19.4% (14 patients). In addition, the number of patients who had more than two acute rejection episodes, in the first six months, was higher in sensitized recipients compared with unsensitized recipients, 66.7% *vs.* 50% (Figure 1).

The incidence of rejection was slightly higher but not statistically significant in the patients who had cadaveric donor to those with living donor, 26.7% and 21.4%, respectively.

The incidence of acute rejection, in both groups, was higher in recipients with three and four HLA-mismatches (15/71 cases and 4/7 cases) (Figure 2).

Only one of the 11 patients with 2MM presented, in the first two months post-transplantation, two acute rejection episodes who had a favorably outcome under high doses corticosteroids therapy.

Rejection diagnosis was supported by renal biopsy and revealed various interstitial inflammatory infiltrates,

tubulitis, arteritis, often associated with signs of chronic damages like tubular atrophy and interstitial fibrosis (Figure 3).

In the pre-transplant sensitized patients group, 14 of them (70%) presented during follow-up period a constantly increasing of PRA value while in the pre-transplant unsensitized patients group, 32% (23 patients) developed *de novo* cytotoxic antibodies. One year post-transplantation, the highest PRA value was 52%. The most post-transplant cytotoxic antibodies were anti-HLA class I, 46% (17/37 pts.) class I alone and 32.4% (12/37pts.) both classes I and II. Donor specific antibodies (DSA) were identified in 15 recipients, MICA antibodies in five cases and in other two recipients, we have found the both types of antibodies.

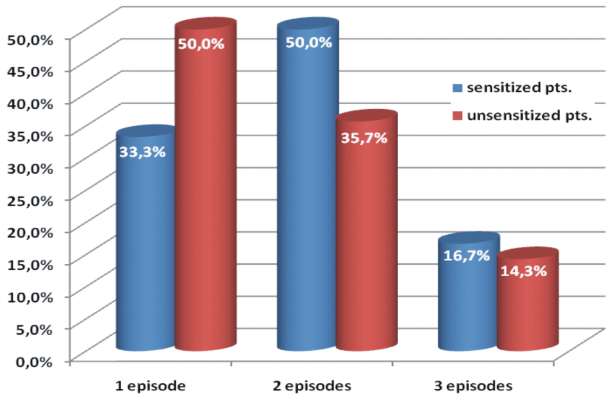

Figure 1 – ***The incidence and the number of acute rejection episodes in the two groups.***

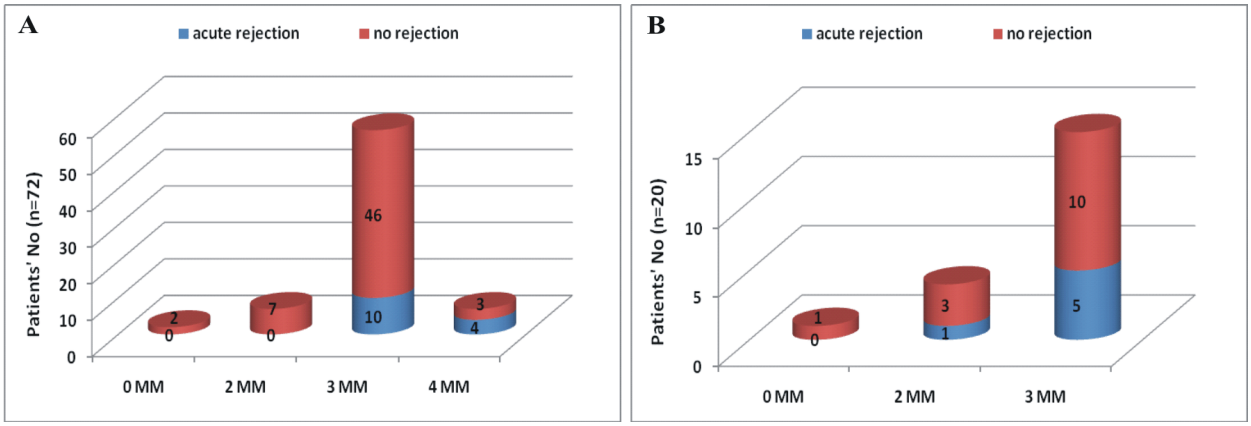

Figure 2 – ***Frequency of acute rejection episodes in the two groups according to number of HLA-mismatches. (A) Unsensitized patients (n=72); (B) Presensitized patients (n=20).***

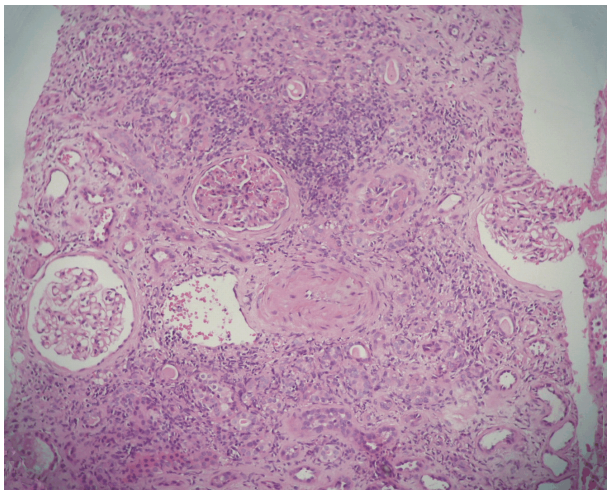

Figure 3 – ***Renal biopsy. Acute rejection (BANFF IIA), interstitial inflammatory infiltrate, tubulitis, glomerular artery, tubular atrophy, mild glomerular infiltrate (HE stain, ob. ×20).***

Discussion

It is widely accepted that pre-transplant positive PRAs are related to increased incidence of hyper-acute/acute rejection, chronic rejection, and early/latent graft loss [6–8]. In this study, clinical following-up was recorded at 12 months. Our results overlap those in literature, with a higher incidence of acute rejection in presensitized patients *vs.* unsensitized recipients (30% *vs.* 19.4%). In addition, the number of patient who had more than two acute rejection episodes, in the first six months, was higher in sensitized recipients compared with unsensitized recipients, 66.7% *vs.* 50%.

The individual importance of anti-HLA class I and class II antibodies to graft rejection is incompletely understood. A large-scale multi-center clinical study illustrated that graft survival rate at two or three years was decreased in recipients with both anti-HLA class I and class II antibodies whereas isolated reactivity has not clinical consequences [7–9]. Detection and ability to correctly identify these antibodies before transplantation is an important step in determining immunological risk for recipient.

HLA class I antigens can be identified on nucleated cells, including on the endothelia of small renal vessels. Anti-HLA-I IgG antibodies can injure the small vascular endothelia of the graft and induce serious rejections such as hyperacute rejection [10]. HLA class II antigens are mainly expressed by immune cells. It is previously accepted that anti-HLA-II antibodies have a relatively minor impact on the early graft outcome, despite a few cases reported with a higher rejection rate and hyper-acute rejection occurrence due to HLA class II antibodies [11, 12]. On the other hand, several recently reported cases have shown that anti-DP antibodies are a potential risk factor for graft dysfunction and failure. HLA-DP mismatch between donor and recipient does not influence graft function at the first kidney transplant but has a negative impact in case of a retransplant [13–15]. HLA-DR antigens were also expressed on the renal micro-vascular endothelia as the target antigen [16, 17].

The incidence of acute rejection was higher in anti-HLA class I presensitized patients compared with anti-HLA class II (20% and 14.3%, respectively) but there was no significant difference compared to pre-transplant unsensitized patients (19.4%).

The frequency of anti-HLA antibodies detected after kidney transplantation is extremely variable, ranging between 1.6 and 60% [18–26]. This variability among studies is the result of multiple factors, including the type of assays used (*e.g.*, less sensitive techniques such as complement-dependent cytotoxicity [CDC] cross-match assays, compared with more sensitive methods such as FlowPRA or Flow Specific Beads [Luminex]) or variable times of sample collection.

In our study, the sequential post-transplantation monitoring of anti-HLA antibodies in serum was performed in one month, three months, six months and one-year post-transplant, using Luminex technique. In the pre-transplant sensitized patients group, 14 of them (70%) presented during follow-up period a constantly increasing of PRA value, but only six of them developed rejection signs. The donor specific antibodies (DSA) were identified in four recipients and MICA antibodies in two cases. The remaining patients in Group 2 (30%) had fluctuating PRA values (around pre-transplant values) and only in one case, we identified DSA. In the pre-transplant unsensitized patients group, 32% (23 patients) developed *de novo* cytotoxic antibodies and 12 of them have had acute rejection. DSA were identified in 11 cases, anti-MICA antibodies in three cases and in other two recipients, we have found the both antibodies. One-year post-transplantation, the highest PRA value in this group was 52%. According to other studies, the most post-transplant cytotoxic antibodies were anti-HLA class I, 46% (17/37 patients) class I alone and 32.4% (12/37 patients) both classes I and II.

The impact of HLA matching on graft rejection and survival rate remains controversial [27–32]. Results showed that the significance of HLA matching decreased while the results improved with the new immuno-suppressant drugs. Although, some studies have not found a correlation between HLA mismatch degree and rejection episodes rate, in our study we observed that the incidence of acute rejection, in both groups, was higher in recipients with three and four HLA-mismatches (15/71 cases and 4/7 cases). Only one of the 11 patients with 2MM presented, in the first two months post-transplantation, two acute rejection episodes who had a favorably outcome under high doses corticosteroids therapy. In this case was about a female young patient (34 years), belonging presensitized patients group (PRA 21%). Although, the patient was transfused four months before transplantation (2 units packed red blood cells), the most likely causes of anti-HLA antibodies were the two-births and the high number (6) of abortions in her medical history.

Conclusions

The introduction of more sensitive and specific methods to detect anti-HLA antibodies before transplantation might better discriminate between immunologically low- and high-risk kidney transplant recipients. Therefore, sequential post-transplantation monitoring of anti-HLA antibodies in serum would be useful to identify patients who are at higher risk for allograft failure. The control of alloimmune humoral responses with appropriate immunomodulatory drug regimens play an important role, particularly in the current era of "immunosuppression minimization" strategies.

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