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The choice of immunosuppressive therapy depending on the level of anti-HLA antibodies in kidney transplantation

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Seeking to develop immunosuppression regimens that would take into account the patient's level of sensitization to the antigens of the main histocompatibility complex, we studied 123 patients after kidney transplantation. Depending on the choice of immunosuppressive therapy, two groups were formed. The study group included 55 patients who received the immunosuppression regimen adapted to their HLA sensitization level. In the comparison group, 68 patients received baseline immunosuppression, including calcineurin inhibitors, mycophenolic acid preparations, and corticosteroids. Anti-HLA antibody detection was performed by assessing the mean fluorescence intensity (MFI) on the Luminex platform when patient's placing on the transplant waiting list. It was found that highly HLA-sensitized recipients should receive anti-thymocyte polyclonal antibodies with or without plasmapheresis immediately after surgery in order to prevent the rejection reaction. The moderately HLA-sensitized

patients should receive the baseline immunosuppression in combination with monoclonal antibodies (simulect); the polyclonal antibodies should be administered only if necessary (in decreased diuresis rate, increased the level of creatinine in the blood, etc.). In unsensitized patients, the baseline immunosuppression is enough to induce tolerance. Thus, the administration of immunosuppressive therapy adapted to the preexisting HLA-sensitization level can significantly improve the treatment outcomes in kidney transplant recipients in the post-transplant period.

Keywords: HLA Antibodies, kidney transplantation, immunosuppression therapy

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CRF – chronic renal failure

MHC – specific major histocompatibility complex

MFI – mean fluorescence intensity

Currently, transplantation uses a wide arsenal of efficient immunosuppressive drugs, which has led to an obvious improvement in graft survival both in the early and late postoperative periods. On the other hand, excessive suppression of the recipient's immunity is associated with a significant risk of developing cancer, opportunistic infections, and metabolic disorders. Thus, one of the key tasks of modern transplantology is the search for an ideal immunosuppressive therapy, which would induce a stable tolerance of the immune system in relation to the transplanted organ while

maintaining a sufficient response to any other antigen. In this connection, the presence of markers enabling the selection of the immunosuppressant spectrum necessary for a particular recipient is important, as well as the assessment of the therapy efficacy.

In kidney transplantation, one of the criteria for selecting the immunosuppression regimen is the preexisting sensitization of the recipient for the major histocompatibility complex antigens. It is known that anti-HLA antibodies (Abs) may be present in 30% of recipients on the waiting list [1-3]. They are a key factor in the development of super-acute, acute and chronic rejection of the graft and have a negative impact on its function and the early post-transplant course [4]. In this regard, when preparing a recipient for organ transplantation, researchers recommend that the desensitization therapy be used [5-7], including plasmapheresis sessions and the administration of monoclonal antibodies against B lymphocytes. At the same time, the postoperative management of sensitized patients, the issues of choosing an optimal immunosuppression scheme remain insufficiently studied.

Many pharmaceutical companies are trying to develop an optimal immunosuppression drug that would displayed the perfect balance of efficiency and safety. Over the recent two decades, many immunosuppressants have been developed, some of them are now widely used in a variety of immunosuppression protocols for organ transplantation. An important achievement was the implementation of chimeric monoclonal Abs, basiliximab and daclizumab, into clinical practice of transplantation in Russia. Their action is directed against the alpha-chain of the interleukin-2 receptor (antigen CD25) expressed on the surface of T lymphocytes in response to the stimulation with antigens. The use of anti-CD25 monoclonal

antibodies as an induction together with a triple-therapy immunosuppression regimen (calcineurin inhibitors, mycophenolate mofetil, and corticosteroids) made it possible to reduce the incidence of acute kidney graft rejection from 40-35% to 18.28-14.5% [8]. The important characteristics of these drugs included their excellent tolerability by patients and the absence of serious side effects. So, if in 2007 the induction with monoclonal antibodies was used in N.V. Sklifosovsky Research Institute in 60% of patients, then in 2010 it was used in as much as in 94% of recipients. Such a good immunological effect of the acute rejection prevention in unsensitized recipients allowed surgeons to avoid using polyclonal Abs in most cases. The polyclonal Abs, anti-thymocyte immunoglobulin (ATGAM, thymoglobulin) whose action is directed against the activation of T cells (CD2, CD3, CD8, CD11a, CD25, HLA Dr and HLA I class) still remain one of the main agents to treat acute graft rejection. However, severe adverse side effects do not allow their use in a wide range of patients.

Thus, despite the advances in modern transplantation and pharmacology and the availability of highly efficient immunosuppressants, the further development of therapy protocols is required that would ensure the suppression of the immune system response to an allogeneic organ and preserve the protective function against infectious agents and neoplasms, taking into account the patient's sensitization level to donor-specific major histocompatibility complex (MHC) antigens.

The study objective was to assess the possibility of achieving immunosuppression in patients with different sensitization levels to MHC antigens.

Material and methods

The study included 123 patients (58 men and 68 women) who underwent kidney transplantation in the Sklifosovsky Institute for Emergency Medicine in the period from 2011 to 2016. The mean age of the patients at the time of transplantation was 41.0 ± 9.4 years old ($x \pm \sigma$), the incompatibility degree (HLA mismatch) was from 4 to 6. The chronic renal failure (CRF) requiring the kidney transplantation had developed as a result of chronic glomerulonephritis, pyelonephritis, polycystic kidney disease, systemic diseases or type I diabetes mellitus.

The patients were allocated into two groups, depending on the choice of immunosuppressive therapy.

The study group included 55 patients who received the immunosuppression regimen adopted to their HLA-sensitization level. The patients (n=24) having high levels of anti-HLA Abs received, besides the basic immunosuppression, the induction with monoclonal Abs (n=9), those combined with plasmapheresis (n=2), polyclonal Abs (n=5), and polyclonal Abs in combination with plasmapheresis sessions (n=8) from the first post-transplant day. Patients having moderate levels of Abs (n=16) received, besides basic immunosuppression, the induction with monoclonal antibodies (n=7), those in combination with plasmapheresis sessions (n=2); polyclonal Abs were administered to 5 recipients and in combination with plasmapheresis to 2. The recipients unsensitized to MHC antigens (n = 15) received the induction with monoclonal antibodies (n=2 patients) and with polyclonal antibodies (1 patient), the other 12 patients received baseline immunosuppression only, including calcineurin inhibitors, mycophenolic acid agents, and corticosteroids.

In the comparison group, 68 patients received baseline immunosuppression. The immunosuppression was adjusted, including the administration of mono- and polyclonal Abs, plasmapheresis, etc., only in case of clinical signs of complications occurring in the early postoperative period.

The anti-HLA antibodies were assayed at the time of recipient's placing on the transplant waiting list, and then, in the early postoperative period on the 7th-21st day, considering the clinical course. The detection of anti-HLA antibodies was made by means of a multiplex assay on the Luminex platform using LabScreen kits (One Lambda, USA). The reactivity of each serum was assessed by the fluorescence signal from each microsphere after the correction of the nonspecific binding on the microsphere with negative control. If the mean fluorescence intensity (MFI) did not exceed 500 units, the result was assessed as negative; at MFI values of 500-3000 a.u., the moderate HLA-sensitization was recorded; the values over 3000 a.u. were interpreted as a high sensitization level.

Statistical analysis of the data was performed using the Statistica 10 software package (StatSoft, Inc., USA). The groups were compared using Fisher's exact test. The threshold level of significance was assumed equal to 0.05.

Results and discussion

The HLA-sensitization levels in the patients of the study group and the comparison group were as follows: a high sensitization level in 24 and 11 patients, the moderate sensitization in 16 and 19, no Abs in 15 and 38 patients, respectively. Thus, the HLA-sensitized patients prevailed in the study group (72.7% versus 44.1% in the comparison group) and,

accordingly, had a higher risk of transplant rejection reaction in the post-transplant period.

The choice of immunosuppression in patients of the study group was based on the preexisting HLA-sensitization level. The effect of the immunosuppressive therapy was assessed with regard to the development of the transplant rejection reaction (Table 1-3). The rejection crisis was diagnosed on the basis of clinical data and confirmed by a morphological study of the biopsy material. Table 1 presents the results of the treatment in 24 recipients with a high preexisting level of anti-HLA antibodies. The acute rejection was diagnosed in 8 patients (33.3%), 4 of them received monoclonal antibodies only, other 2 received monoclonal antibodies in combination with plasmapheresis sessions; polyclonal Abs and polyclonal Abs in combination with plasmapheresis sessions were prescribed to 1 patient each. An uncontrollable rejection leading to the renal graft loss was observed only in 2 recipients: one of them received monoclonal anti-CD25-Abs, the other one received monoclonal anti-CD25-Abs in combination with plasmapheresis sessions. In highly HLA-sensitized recipients, the statistical analysis of treatment outcomes in early post-transplant period was performed among the patients who received monoclonal anti-CD25-Abs (n=11) and polyclonal anti-thymocyte Abs (n=13) to induce tolerance. The incidence of acute rejection was found to be 3.5 times lower in the patients who received polyclonal AT immediately after surgery compared to the patients receiving monoclonal antibodies, and the difference was statistically significant ($p < 0.05$, Fisher's exact test).

Table 1. Immunosuppression therapy regimens and treatment outcomes in the highly HLA-sensitized kidney transplant recipients of the study group

Immunosuppression therapy (IST)		Number of recipients	Acute rejection	Number of recipients	Acute rejection
Monoclonal anti-CD25-Abs + baseline IST		9	4 (44.4%)	11	6 * (54.5%)
Monoclonal anti-CD25-Abs + baseline IST	+ plasmapheresis	2	2 (100%)		
Polyclonal Abs + baseline IST		5	1 (20%)	13	2 (15.4%)
Polyclonal Abs + baseline IST	+ plasmapheresis	8	1 (12.5%)		
TOTAL				24	8 (33.3%)

* Statistically significant differences ($p < 0.05$).

Among the study group patients with a moderate level of anti-HLA Abs (n=16), an acute rejection crisis developed in 3 recipients receiving the induction with monoclonal antibodies only (Table 2). As seen from the table, no statistically significant differences in the kidney transplantation outcomes between the patients receiving polyclonal Abs (n=7) immediately after surgery and the compared patients receiving monoclonal anti-CD25-Abs (n=9) were observed ($p>0.05$).

Table 2. Immunosuppression therapy regimens and treatment outcomes in the moderately HLA-sensitized kidney transplant recipients of the study group

Immunosuppression therapy (IST)		Number of recipients	Acute rejection	Number of recipients	Acute rejection
Monoclonal anti-CD25 Abs + baseline IST		7	3 (42.8%)	9	3 (33.3%)
Monoclonal anti-CD25 Abs + baseline IST	+ plasmapheresis	2	0		
Polyclonal AT + baseline IST		5	0	7th	0
Polyclonal Abs + baseline IST	+ plasmapheresis	2	0		
TOTAL				16	3 (18.8%)

Among the cases of no prior HLA-sensitization (n = 15), the rejection crisis was diagnosed in only one recipient of the study group who had not received any induction either with monoclonal or polyclonal antibodies (Table 3). There was no statistically significant difference in the incidence of rejection crisis between the patients who received the baseline immunosuppression and those who received the immunological tolerance induction with mono- or polyclonal Abs ($p > 0.05$).

Table 3. Immunosuppression therapy regimens and treatment outcomes in the unsensitized kidney transplant recipients of the study group

Immunosuppression therapy (IST)	Number of recipients	Acute rejection
Monoclonal anti-CD25 Abs + baseline IST	2	0
Polyclonal anti-thymocyte AT + baseline IST	1	0
Baseline IST	12	1 (8.3%)
TOTAL	15	1 (6.7%)

The comparative analysis of the treatment outcomes in the study group patients in whom the immunosuppressive therapy was administered considering the anti-HLA Ab levels versus the comparison group is presented in Table 4. The therapy efficacy was assessed with consideration of the graft rejection development. As seen from the table, the immunosuppression chosen as adapted to the preexisting HLA-sensitization level, allowed the overall incidence of acute rejection to be reduced by 13.4% (1.6 times). Meanwhile, the incidence of acute rejection in the sensitized patients of the study group was lower by 32.5% (2.2 times) than in the sensitized recipients of the comparison group and the difference was statistically significant ($p < 0.05$).

Table 4. The treatment outcomes in the patients receiving immunosuppression therapy adapted to their preexisting HLA-sensitization levels

Patient groups		Number of patients	Graft rejection
The study group: Immunosuppression chosen as adapted to anti-HLA Abs detected	Highly or moderately HLA-sensitized recipients	40	**11 (27.5%)** *
	Unsensitized recipients	15	1 (6.7%)
	TOTAL	55	12 (21.8%)
Comparison group	Highly or moderately HLA-sensitized recipients	30	**18 (60%)**
	Unsensitized recipients	38	6 (15.8%)
	TOTAL	68	24 (35.2%)

* The difference in the incidence of graft rejection between the study group and the comparison group is statistically significant ($p < 0.05$).

It is known that a pretransplant HLA-sensitization of the recipient is associated a high risk of the renal graft acute rejection [1-3]. In this connection, a therapy aimed at reducing anti-HLA-Abs level is recommended when preparing a recipient for organ transplantation [5, 6]. Meanwhile, statistically significant difference discovered by us in the incidence of allograft rejection between the HLA-sensitized and unsensitized patients calls the necessity of finding reasonable immunosuppression options that would help to achieve tolerance with minimized adverse reactions.

We obtained the data demonstrating that in highly HLA-sensitized recipients (MFI over 3000 a.u.), the induction of immunological tolerance by using monoclonal antibodies and their combination with plasmapheresis

sessions is ineffective in preventing the rejection reaction. Such patients are recommended anti-thymocite polyclonal Abs to be administered immediately after surgery, which allows a statistically significant decrease in the incidence of the rejection crisis. Plasmapheresis sessions aimed at patient's desensitization, should be performed before transplantation and in the early post-transplant period.

Since there were no statistically significant differences in the incidence of graft rejection between the moderately HLA-sensitized patients (MFI 500-3000 a.u.) receiving either monoclonal antibodies or polyclonal antibodies, it is sufficient to prescribe them baseline immunosuppression in the postoperative period combined with the use of monoclonal anti-CD25-Abs (simulect). Polyclonal antithymocyte Abs (ATGAM, Thymoglobulin) should be included in the management of immune therapy of the patients with a decreased diuresis rate, increased creatinine, and other signs of the graft rejection reaction.

In unsensitized patients (with MFI under 500 a.u. screened by the moment of kidney transplantation), statistically significant differences in the treatment results were found neither between the recipients of the study group taking different immunosuppression regimens, nor between the study group and the comparison group. That is, the causes of acute rejection in such patients are not related to the difference in the immunosuppression therapy regimens used. In this case, the baseline immunosuppression therapy, including calcineurine inhibitors (tacrolimus, cyclosporin), mycophenolic acid agents (myfortic, cellcept), and corticosteroids (prednisolone) is sufficient to prevent the development of graft rejection.

Thus, the obtained study results in general suggest promising the differentiated approach to the choice of the post-transplant

immunosuppression regimen in kidney transplant recipients based on the anti-HLA Abs screening results. The immunosuppression therapy regimen adapted to the preexisting HLA-sensitization significantly improves the treatment outcomes in kidney graft recipients in the post-transplant period.

Conclusions

1. The immunosuppression therapy for kidney transplant recipients chosen accordingly the preexisting HLA-sensitization level, and administered from the first day after transplantation significantly reduces the incidence of rejection from 60% to 27.5%.

2. Patients with high anti-HLA Ab levels (MFI over 3000 a.u.) should receive, besides the basic/baseline immunosuppression, the polyclonal antibodies in the early post-transplant period for the induction of immunological tolerance, which significantly reduces the incidence of rejection from 54.5% (in patients who received monoclonal anti-CD25-Abs) to 15.4%.

3. The administration of monoclonal anti-CD-25-Abs in combination with baseline immunosuppressive therapy is sufficient for tolerance induction in moderately HLA-sensitized patients (with MFI from 500 to 3000 a.u.)

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