Algorithm to Manage Highly Sensitized Kidney Transplant Recipients in Poland

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ABSTRACT

Objective. Programs for immunized transplant recipients are essential to achieve graft survivals comparable to those of non-immunized recipients. The threshold in Poland is a PRA by the complement-dependent cytotoxicity (CDC) method greater than 50%, which includes approximately 3.8% of the patients. At the same time the United Network for Organ Sharing there recipients represent approximately 16% of the waiting list in the United Network for Organ Sharing (UNOS). The underestimation of the immunized group in Poland may be due to differences in laboratory techniques to assess alloantibodies.

Materials and methods. This study investigated 55 potential recipients with a PRA by CDC $>$ 50%. We used the following algorithm to assess their immunization: Luminex screening test for an HLA antibody; specificity assessed with Luminex Single Antigen, vPRA (evaluation of immunization of the patient); and analysis of acceptable HLA incompatibilities (HLAMatchmaker).

Results. All recipients were positive class I anti-HLA antibodies and 94.5% were positive for class II. For the groups of subjects with PRA-CDC from 50% to 79% versus those greater than 80%, the average values of PRA-CDC were 62.2% and 89.5%, respectively. The virtual PRA results for these groups were 95.7% and 97.2%, respectively. In addition, anti-HLA-Cw, anti-DQ and anti-DP antibodies were detected in 77%, 84%, and 51% of recipients, respectively. Immunized recipients reported to the next transplant were characterized by the antibodies against mismatch only in 68%. For all potential recipients, additional acceptable non-compliance was determined with HLAMatchmaker: 152 specificity for locus A and 252 for locus B.

Conclusions. Evaluation of immunization status of recipient candidates should be routinely performed using tests to assess class and specificity as well as level of alloantibodies to enable determination of a safe potential donor. As a routine test, PRA-CDC underestimates the number of highly immunized patients. Exclusion from the list of patients with repeated non-compliance is a simplification, which reduces their chance for transplantation.

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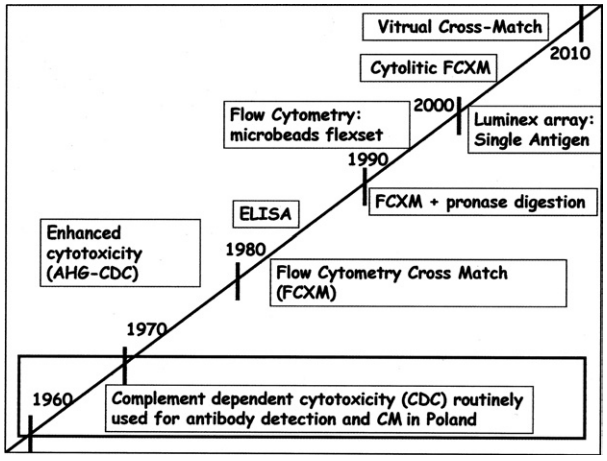

Fig 1. Evolution of the diagnostic methods for immune humoral response in the routine practice, based on Gebel and Ray.[1]

HIGHLY immunized recipients suffer a higher risk of antibody mediated rejection (AMR). Before the introduction of programs for immunized recipients, graft survival in this group was significantly worse, even without AMR. Along with the evolution of transplant diagnostic techniques to assess the humoral immune response, it may be possible to precisely assess which of the anti-HLA antibodies may be harmful to the recipient, thereby increasing the chance for an immunized patient to achieve a successful transplantation (Fig 1).[1]

Currently, a number of programs have been introduced for highly immunized recipients.[2]: The Emory University, the United Network for Organ Sharing (UNOS),[3] Acceptable Mismatch Program in Eurotransplant (ET-AMP)[4,5,6] with subsequent assessment of immunogenicity with the use of HLAMatchmaker software,[7–10] and guidelines of the British Transplantation Society.[11] All of these programs seek graft survival among highly immunized recipients comparable to that in other patients without increasing the risk for complications of immunosuppressive drugs. Introduction of these programs has increased access to transplantation for immunized recipients: almost 60% of them underwent the procedure within 2 years after the introduction of the ET-AMP. According to previous rules (KAS-ET), only 20% of these patients would have been a recipient.[6] This advance is mainly because of the increased sensitivity of tests to assess the specificity of anti-HLA antibodies. Among 14 transplant centers testing 6620 recipients, there was a correlation of the findings that were analyzed by enzyme-linked immunosorbent assay (ELISA) and of luminex single antigen tests for unacceptable pairs with flow cytometry cross-matches (FCXM). Unacceptable HLA-antigens by the Luminex single antigen technique correctly predicted the result of FCXM at 93.5% for T cells and 97.8% for B cells. Intermediate results were obtained with ELISA, which predicted the results of FCXM in 86.1% for T lymphocytes and 91% for Blymphocytes.[12] These results have become a platform for the routine use of the so-called virtual cross-match (VCM), which is an algorithm to match a kidney to an immunized patient defined as a calculated PRA (CPRA) greater than 80% based on a database generated among reported anti-HLA antibodies toward particular donor HLA specificities in a given population.[13] Moreover, the algorithm can be used for national allocation rules seeking to mitigate the need for cross matching against the donor.[11] In UNOS, as in Poland, the HL typing is based on loci A, B, and DR, which is a problem because a large proportion of recipients display antibodies against the HLA-Cw, DRB3, DRB4, DRB5, DQ, and DP loci. These antibodies are mainly responsible for positive FCXM in the absence of an unacceptable HLA-A, B, DR match.[14]

IMPACT OF DONOR-SPECIFIC ANTI-HLA ANTIBODIES

Although highly sensitive tests for solid phase diagnostic algorithms have been used routinely for approximately 8 years, their impact on transplant success, especially when present in low titers, is still controversial,[15–17] particularly, the cut-off values for highly sensitive tests such as single antigen positivity and the definition of an unacceptable HLA incompatibility are problematic as evidenced by recent reports.[18–20] A retrospective study of 402 transplant recipients over an 8-year interval by Lefaucheur et al[21] identified differences in graft survival and incidence of AMR in relation to the mean fluorescent intensity (MFI) level: of detected antibodies showing donor-specific antibody (DSA) specificity. Among the DSA positive recipients, graft survival at 5 years was 18% shorter and after 8 years, increased to 22%. Provided that the antibody was not DSA-specific the immunization alone did not constitute a risk factor for worse graft survival. The AMR which occurred among 40% of subjects was preceded by the presence of DSA, but even in the absence of AMR, graft survival was shorter among the DSA positive group at 69.5% versus 84.4%. The predictive value for AMR was strongly associated with the level of DSA, expressed as MFI: a value $>$ 3000 MFI was associated with significantly greater more incidences of AMR and shortened graft survival.

Comparing the number of immunized recipients in Poland to worldwide data, revealed a significant difference: in Poland approximately 1.3% of recipients qualified as complement-dependent cytotoxicity (CDC) PRA $>$ 80%, whereas in the UNOS database it is 15.8% of recipients (Table 1).[22] In the United Kingdom, immunized patients (PRA $>$ 10%) represented 41% of the waiting list[11] with 52% of re-transplant recipients classified as highly immunized (CPRA $>$ 85%).[23] The underestimation of the immunized group in Poland is due to the type of laboratory techniques to assess alloantibodies. In Eurotransplant (ET) countries and in the United States (UNOS), solid phase tests assays (SPA), such as Luminex or ELISA, are used to identify the type of anti-HLA antibodies, which are then entered into a computer program. CPRA in the UNOS[24] or

Table 1. Aspect of the Method of Evaluation of Immunization: PRA-CDC Versus cPRA (UNOS) According to Gupta and Sinnott[22]

Recipient	Poland PRA-CDC	cPRA (UNOS)
Expectant recipient	~1450	~80,000 (66% non-immunized)
PRA > 80%	1.31% (n = 19) 16% (232 ?)	15.8% (12,800)
PRA > 95%	0.48% (n = 7) 10% (145 ?)	10% (8,000)

Abbreviations: PRA-CDC cPRA UNOS, United Network for Organ Sharing

virtual PRA (vPRA) in ETs are calculated in relation to the population frequency of each HLA specificity as determined in large groups.[25] vPRA is based on the 4000 donors identified in ET countries: for example, if there is completely acceptable donor for certain recipient having vPRA > 80%, the transplantation is mandatory. It can be expected that with determination of the antibody specificity more than 200 cases in Poland would be deemed to be highly immunized recipients (vPRA > 80%).

In Poland, the serologic CDC- PRA technique is used with T cells isolated from 30 potential donors. This method detects only high titers antibodies, although it can neither detect anti-HLA class II antibodies nor identify the type of alloantibody (Fig 1, Table 1). In addition, assessment of immunization by the CDC-PRA shows a lack of reproducibility, which is determined by the lymphocyte panel. Comparative studies have shown the variability to be as high as 90%.[26] Transplantation of highly immunized recipients (CDC-PRA > 80%) is mandatory when there is a negative cross match with the donor. Recipients with CDC-PRA values of 50% to 79% receive extra points for the allocation. Transplantation is performed only when the CDC crossmatch with T-lymphocytes is negative. Futhermore, many transplant centers do not routinely perform FCXM. Some centers increase the sensitivity of the test by performing CDC-CM in addition using a pool of B cells isolated from donor lymph nodes; however, it is not a mandatory procedure. Thus, without knowledge of the alloantibody specificity, it is impossible to identify recipients who receive well-matched organs and to decide the intensity of the initial and the maintenance immunosuppression.

METHODS

Examined Material

Serum samples were obtained from 55 recipients (30 women and 25 men) who were scheduled for a kidney transplantation between November 15th and December 15th, 2010 who had confirmed CDC-PRA values greater than 50% (data from daily reports from the server of the national waiting list). Recipients were divided into two groups depending on PRA values: from 50% to 79% (n = 36) versus 80% to 100% (n = 19).

Evaluation of Antibody Class and Specificity

We assessed immunization using a multiplex screening test (Luminex 200, Fusion software HLA) to detect the presence and class of the anti-HLA antibodies (LABScreen Mixed, One Lambda, USA). Then as a screening test we identified the class specificity of the antibodies (LABScreen Single Antigen, One Lambda, USA). The procedure was performed according to the manufacturer's specifications. In 96-well plates we mixed 5 μL of microspheres with 20 μL serum. The plates were placed on a rotor (200 rpm) for incubation in the dark for 30 minutes thereafter, the plates were washed three times with 200 μL buffer before addition of 100 μL of anti-human immunoglobulin G conjugated with PE. After standing for 30 minutes in the dark and a double washing, the material was suspended in 80 μL of wash buffer for analysis in the Luminex 200 apparatus. The calculation was performed using HLA-Fusion software according to the formula BASELINE. The cut-off value for positive values was set at 1000 MFI: an MFI Range, 1000 to 3000 was adopted as a low and a level greater than 3000 MFIs as a high level of HLA antibodies.

Verification of the Level of Immunization: The Use of VPRA

For recipients with CDC-PRA > 50%, specificity of the antibodies was examined in the locus HLA-A, B, DR. Data were entered into the vPRA available at www.etrl.org (Eurotransplant Reference Laboratory) to compare the results of the CDC-PRA and the vPRA.

HLAMatchmaker: Identification of Additional, Not Immunogenic to Recipients of HLA Incompatibility

HLA class I antigens were tested using the ABC Eplet Matching Macro v2.1q and HLA class II antigens, using the DR DQ DP Matching Eplet v2.1. We evaluated the most frequent alleles in European population because immunogenicity can be compared only at the level of allelic resolution (4-digit system), because of the need to analyze the protein conformation to assess inconsistent eplets (mmEp).

RESULTS

The Level and Type of HLA-Immunization

All recipients were positive for anti-HLA Class I antibodies. There were 94.5% positive results for HLA class II that were undetectable in the PRA-CDC. In addition, 77% of the recipients had anti-HLA-Cw antibodies, 84% anti-HLA-DQ, and 51% for anti-HLA-DP, including 1 person (1.8%) who had only HLA class II antibodies with anti-DP and DQ specificities.

Comparing the level of immunization determined by PRA-CDC and the vPRA, there was a noticeable underestimate of the number of immunized recipients in Poland: namely, the groups of PRA-CDC 50% to 79% (mean value = 62 ± 10%) whereas the vPRA was 96 ± 10%. Importantly, vPRA was performed using calculations of HLA-A, B, and DR only (Table 2).

Transplant Recipients Reported to the Next Transplantation—Immunization of Previous HLA Discrepancies

Sixty-seven percent (37/55) of the study group for second transplantation had a PRA greater than 50%. In this group 8.1% of recipients (3/37) did not show antibodies against any incompatibilities from the previous donor HLA (mis-

match) [MM]. Moreover, despite the high level of immunization only 68.13% were detected among all HLA-mismatch DSA-specificity alloantibodies.

Additional, Acceptable Non-immunogenic Mismatches: HLAMatchmaker

Using Eurotransplant HLAMatchmaker software, it was possible to specify acceptable HLA non-immunogenic mmEp 0: mismatch eplets. Among the 152 additional antigens of the HLA locus A, 259 HLA-B, and 98 HLA-DR. On average, for each recipient, there was an additional 2.7, 4.7 and 1.8, respectively, acceptable MM in HLA-A, B, and DR loci.

DISCUSSION

By changing the allocation system in late 2009, there was a significant increase in access to transplantation for recipients immunized by more than 50% by PRA-CDC namely, from 15.7% in 2009 to 30.5% in 2010).[27] However, failure to identify the specificity of alloantibodies hampers the possibility to assess the risk for highly immunized transplant recipients. Measurement of immunization using CDC PRA significantly underestimates the number of immunized recipients in Poland. A study of 55 recipients with CDC PRA $>$ 50%, according to vPRA assessment showed the whole group to be greater than 95%. Another problem is the access for re-transplant recipients. Under the current rules, they cannot be transplanted with a repeat HLA-mismatch; however, a large proportion of them do not possess anti-HLA antibodies specifically those whose cause of graft loss was a relapse or surgical complication and in addition those who show a different antibody specificity. Even among the re-transplant immunized group as there were only 68% of antibodies against previous incompatibilities thus, the assumption avoids an erroneous repeated HLA mismatch was 32% unduly limiting the chances for a transplantation.

Herein we have proposed an algorithm to evaluate immunization (Fig 2), including the identification of the type of antibodies. HLA-A, B, and DR specificities are then introduced into the vPRA to establish the level of immunization among 4000 potential donors in Europe. We would provide adequate sensitivity studies with knowledge of the real level of immunization, allowing standardization of testing and preventing fluctuations associated with the PRA CDC method. Other benefits for the patients on waiting list include: improved CDC secure access to the graft by vPRA scoring with extra points for recipients whose PRA is currently classified by as low-immunized; determination of acceptable HLA incompatibilities for each recipient, regardless of their immunization and increased chance for re-transplant recipients who show an absence of antibodies against the previous HLA incompatibility.

Table 2. The Comparison of Results of the PRA-CDC and the Virtual PRA Estimated Based on the Result of Luminex Single Antigen

	PRA-CDC (Average +/− SD)	Virtual PRA (Average +/− SD)
PRA-CDC (n = 36)		
50% to 79%	62.2 +/− 9.5	95.7 +/− 9.9
PRA-CDC (n = 19)		
80% to 100%	89.5 +/− 8.3	97.2 +/− 4.7

Abbreviation: PRA-CDC,

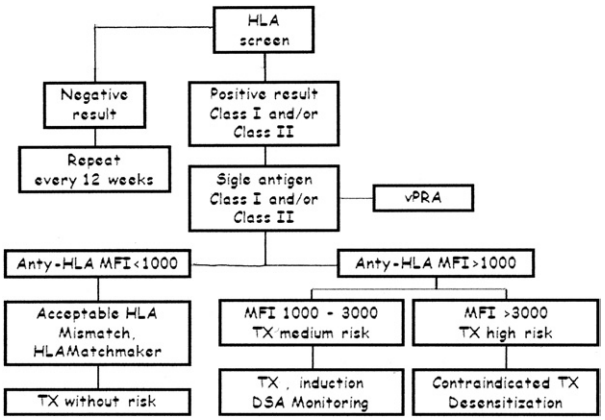

Fig 2. The proposed algorithm for the evaluation of immunization.

The proposed algorithm to select donors for immunized recipients is based on assumptions already in use in ET and UNOS systems. Tangible benefits from the application algorithm are: the possibility to make decisions on the basis of transplantation studies, namely, a real knowledge of risk factors; adequate treatment for acute rejection episodes of the restrictive; and to prolong graft survival. The algorithm identifies highly immunized subjects previously classified by the routine CDC-PRA as poorly or not immunized. If this categorization is true, it is possible to apply a reduced immunosuppression protocol, thus reducing drug toxicity. For highly immunized subjects (vPRA $>$ 85%), the algorithm evaluates the risks of rejection, allowing adaptation of the treatment protocol based on monitoring of the response to transplantation.

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