Corrected Panel-Reactive Antibody Positivity Rates for Hypersensitized Patients in Turkish Population With Calculated Panel-Reactive Antibody Software

S.T. Karadeniz[a,*], S.U. Akgul[b], Y. Ogret[b], H.S. Ciftci[b], A. Bayraktar[c], H. Bakkaloglu[c], Y. Caliskan[d], K. Yelekci[a], A. Turkmen[d], A.E. Aydin[c], F.S. Oguz[b], M. Carin[e], and F. Aydin[b]

[a]Calculated Biology and Bioinformatics Program, Department of Bioinformatics and Genetics, Kadir Has University, Istanbul, Turkey; [b]Department of Medical Biology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; [c]Department of General Surgery, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; [d]Department of Nephrology, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey; and [e]Tissue Typing Laboratories, Florence Nightingale Hospital, Istanbul, Turkey

ABSTRACT

Introduction. High rates of panel-reactive antibody (PRA) may decrease the chance of kidney transplantation and may result in long waiting periods before transplantation. The calculated PRA (cPRA) is performed based on unacceptable HLA antigens. These antigens are identified by a program that was created based on the antibodies that developed against the HLA antigens circulating in serum and on the risk of binding of these antibodies to antigens. The antigen profile of the population and antigen frequencies can be measured, and more realistic cPRA positivity rates may be obtained using this method.

Materials and Methods. We developed a program based on the HLA antigens of 494 blood donors in 2 European Federation for Immunogenetics–accredited Tissue Typing Laboratories in Turkey. Next-generation sequencing–based tissue typing (HLA-A, -B, -C, -DR, -DQ, 4 digits) of the samples was performed. The PRA screening test was performed on 380 patients who were waiting for organ transplant from a cadaver in Istanbul Faculty of Medicine. The single antigen bead assay testing was performed to identify the antibody profiles on 48 hypersensitized patients.

Results. The PRA testing results using the current methods were 44.6% ± 18.5%, and the cPRA rate was 86.2% ± 5.1%. The mean PRA positivity of the sensitized patients using the current methods was 44.6%; however, the rate was 86.2% using the cPRA.

Discussion. cPRA shows the rate of the rejected donors according to all unacceptable antigens. The need for a list of unacceptable antigens in place of the PRA positivity rate is a real change in the sensitization-dependent calculation as cPRA positivity rate.

Conclusion. In principal, implementation of cPRA will encourage many centers and laboratories to adopt a standard measurement of sensitization in Turkey. It will increase the chances of better donor match, particularly for hypersensitized patients, by the creation of an unacceptable mismatch program using cPRA software.

ALLOIMMUNIZATION remains a critical factor affecting the success of renal transplantation. Several recent studies evaluated the prevalence of HLA-specific antibodies and the clinical importance of these antibodies in acute allograft rejection. Antibodies that develop against

*Address correspondence to Sedat Tanju Karadeniz, Tissue Typing Laboratories, Florence Nightingale Hospital, Gayrettepe, Istanbul, Turkey. E-mail: stkaradeniz@gmail.com

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230 Park Avenue, New York, NY 10169

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Table 1. The 5 Most Frequent Alleles With 4-Digit Resolution

Frequency Rank	HLA-A*	HLA-B*	HLA-C*	HLA-DRB1*	HLA-DQB1*
Rank 1	02:01G (20.0%)	51:01G (11.3%)	04:01G (18.1%)	07:01G (9.5%)	03:01G (26.1%)
Rank 2	24:02G (13.8%)	35:01G (8.2%)	07:01G (11.5%)	11:04G (9.3%)	03:02G (9.2%)
Rank 3	01:01G (12.7%)	18:01G (6.8%)	12:03G (11.4%)	11:01G (9.2%)	05:02G (8.8%)
Rank 4	03:01G (9.2%)	35:03G (5.0%)	06:02G (9.4%)	03:01G (8.3%)	02:01G (8.3%)
Rank 5	11:01G (7.6%)	44:02G (4.9%)	07:02G (6.1%)	15:01G (6.5%)	02:02G (7.8%)

HLA after blood transfusions, pregnancies, and previous transplants are generally described as panel-reactive antibodies (PRA). High rates of PRA may decrease the chance of kidney transplantation and may result in long waiting periods before transplantation [1–3].

The PRA positivity rate is identified by considering the number of antigen groups and the total counts of antigens in the panel in current methods. The exact PRA positivity rate cannot be identified because the antigen counts in the panel do not reflect the antigen profile of the population.

The calculated PRA (cPRA) is performed based on unacceptable HLA antigens. These antigens are identified by software that was created based on the antibodies that developed against the HLA antigens circulating in serum and on the risk of binding of these antibodies to antigens [4].

The antigen profile of the population and antigen frequencies can be measured, and more realistic cPRA positivity rates may be obtained using this method. In 2009, the Organ Procurement and Transplantation Network that is governed by the United Network for Organ Sharing established the cPRA [4–6].

We developed software based on the HLA antigens of 494 blood donors in 2 European Federation for Immunogenetics–accredited Tissue Typing Laboratories in Turkey. Next-generation sequencing–based tissue typing (HLA-A, -B, -C, -DR, -DQ, 4 digits) was performed.

The cPRA positivity rate will provide more accurate criteria for selecting more appropriate donors for patients on the waiting list for organ transplants. cPRA implementation will improve the chance of transplantation of the proper organs for patients with high sensitization rates [6].

MATERIALS AND METHODS

We developed software based on the HLA antigens of 494 blood donors in 2 European Federation for Immunogenetics–accredited Tissue Typing Laboratories in Turkey. Next-generation sequencing–based (Omixon, Hungary; Illumina, USA, Miseq) tissue typing (HLA-A, -B, -C, -DR, -DQ, 4 digits) of the samples was performed.

PRA screening test was performed on 380 patients who were waiting for organ transplants from a cadaver in Istanbul Faculty of Medicine. The single antigen bead assay testing (One Lambda, USA) was performed to identify the antibody profiles on 48 hypersensitized patients.

The software calculates allele frequencies as follows for:

- each locus: HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1
- combinations of every 2 loci: HLA-A + HLA-B, HLA-A + HLA-C, HLA-A + HLA-DRB1, HLA-A + HLA-DQB1, HLA-B + HLA-C, HLA-B + HLA-DRB1, HLA-B + HLA-DQB1, HLA-C + HLA-DRB1, HLA-C + HLADQB1, HLA-DRB1, HLA-DQB1

Table 2. PRA% and cPRA% for the 5 of the Most Differed From Selected Antibodies and Unmatched Antigens

Patient No	PRA%		Results of SAB Antibodies	cPRA%
1	Class I	63	B47, A26, B59, B78, B63, A68, A69, B38, A80, B77, B18, B13, A2, B27, B44, B35, B49, B73, B45, B37, A1, B76, A33, A66, B52, B65, B64, B51, A43, B53, B75, B67, B42, B81, A34, B58, B57, A11, B54, B62, A24, B7, A32, A25, A23	78
	Class II	57	DR9, DR12, DR8, DQ5	
2	Class I	54	B81, B7, B27, B60, B42, B55, B61, B67, B48, B13, B56, B73, A66, A25, CW12, A26, B47, B82, A23, A34, A43, CW9, B49, B41, CW8, B54, B46, B50, B71, B62, CW10, CW16, CW7, CW1, CW15, A69, A68, B72, A31, CW14, A33, A32	85
	Class II	71	DR51, DR16, DR103, DR15, DR1, DR7, DR9, DR4, DR8, DR12, DR17, DR18, DR10, DR14, DR13, DR52, DQ8, DQ9, DQ2, DQ5, DQ6, DQ4	
3	Class I	53	A29, B67, B55, B42, A11, B76, B54, A80, B56, B7, B71, A2, B39, A23, B81, A69, B75, B82, A24, B8, B35, B62, B41, CW14, B78, B64, CW18, B45, B50, B61, B72, A68, A32, CW6, B18, CW15, B53, A33, CW2, CW9, CW4, B65, B60, B73	87
	Class II	54	DR17, DR13, DR8, DR18, DR11, DR14, DR11, DR12, DP9, DP20, DP14, DP6, DQ7, DP1, DP11, DP17, DP19, DQ9, DP13, DQ8, DP10, DR52, DR4	
4	Class I	38	A68, A69, B57, B58, A2, CW7, B7, A31, A66, A29, A32, A43, A33, A26, A74, A25, B73, B42, A24, B81, B67, B55, B56, A23, CW16, B82, CW17, B27	87
	Class II	18	DR51, DR15, DR11, DR16, DR8, DQ2, DQ7, DQ8, DQ9	
5	Class I	18	A25, A26, A1, A80, A36, B73, A66, A43, A34, A11, A3, A24, B76, B45, A32, A23, B44	94
	Class II	20	DQ7, DQ8, DQ9, DR4, DR5	

Abbreviations: cPRA, calculated panel-reactive antibody; PRA, panel-reactive antibody; SAB, single antigen bead assay.

- combinations of every 3 loci: HLA-A + HLA-B + HLA-C, HLA-A + HLA-B + HLA-DBR1, HLA-A + HLA-B + HLA-DQB1, HLA-A + HLA-C + HLA-DRB1, HLA-A + HLA-C + HLA-DQB1, HLA-B + HLA-C + HLA-DRB1, HLA-B + HLA-C + HLA-DQB1, HLA-C + HLA-DRB1 + HLA-DQB1
- combinations of every 4 loci: HLA-A + HLA-B + HLA-C + HLA-DRB1, HLA-A + HLA-B + HLA-C + HLA-DQB1, HLA-A + HLA-B + HLA-DRB1 + HLA-DQB1, HLA-A + HLA-C + HLA-DRB1 + HLA-DQB1, HLA-B + HLA-C + HLA-DRB1 + HLA-DQB1
- 5 loci: HLA-A + HLA-B + HLA-C + HLA-DRB1 + HLA-DQB1

As an example, the 5 most frequent alleles are listed in the Table 1 with 4-digit resolution.

Then, the software calculates all possible summed allele frequencies for unacceptable antigen combinations according to the identified antibody profile of the patient as follows:

- for each locus: S1 = AF(HLA-A) + AF(HLA-B) + AF(HLA-C) + AF(HLA-DRB1) + AF(HLA-DQB1)
- for combinations of 2 loci: S2 = AF(HLA-A + HLA-B) + AF(HLA-A + HLA-C) + … + AF(HLA-DRB1 + HLA-DQB1)
- for combinations of 3 loci: S3 = AF(HLA-A + HLA-B + HLA-C) + … + AF(HLA-C + HLA-DRB1 + HLA-DQB1)
- for combinations of 4 loci: S4 = AF(HLA-A + HLA-B + HLA-C + HLA-DRB1) + … + AF(HLA-B + HLA-C + HLA-DRB1 + HLA-DQB1)
- and for 5 loci: S5 = AF(HLA-A + HLA-B + HLA-C + HLA-DRB1 + HLA-DQB1)

Then the software calculates the cPRA by using the all summed allele frequencies according to the Hardy-Weinberg equilibrium: $cPRA = 1 - (1 - (S1 - S2 + S3 - S4 + S5))^2$. This calculated cPRA value shows the positivity for unmatched antigens in the Turkish population for the 494 donors counted. We calculated the cPRA for all 48 hypersensitized patients by using this software and by using the PRA positivity rates with single antigen bead assay test (Table 2).

RESULTS

The high-resolution HLA allele frequencies of 494 healthy donors are shown in Table 1. The PRA testing positivity rate using the current methods was 44.6% ± 18.5%, and the cPRA positivity rate was 86.2% ± 5.1%. The mean PRA positivity rate of the sensitized patients using the current method was 44.2%; however, the rate was 86.2% using the cPRA. The PRA% and the cPRA% rates of some patients are presented in Table 2.

DISCUSSION

The cPRA provides a significant change in measurement of the sensitization [4]. Transplant centers and laboratories report that PRA has wide ranges of sensitivity and specificity. In principal, implementation of cPRA will encourage many centers and laboratories to adopt a standard measurement of sensitization in Turkey.

The frequencies of HLA-A, -B, -C, -DR loci performed by Pingel et al with donor parentages from 17 different countries using DKMS (Deutsche Knochenmarkspenderdatei) donors were compatible with the results of our study [7].

The PRA positivity rate will be identified more accurately in the future after this method is transferred to an electronic environment and with the frequent use of transplant units and tissue typing laboratories. In addition, this will have a significant role in identification of the genetic structures of HLA antigens in the population. It will increase the chances of better donor match, particularly for hypersensitized patients, by the creation of an unacceptable mismatch program using cPRA software.

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