Single antigen flow beads for identification of human leukocyte antigen antibody specificities in hypersensitized patients with chronic renal failure

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Abstract

Aims of this study were to identify class I and class II antibodies in highly sensitized patients by flow cytometry single antigen bead (FC-SAB) assay and to evaluate according to donor HLA type in order to increase their kidney transplantation chance.

Material and methods: We analyzed 60 hypersensitive patients of 351 individuals, who applied to our laboratory for Pra test in November 2013-December 2014. Flow cytometric Pra screening and single antigen bead commercial kits were used for these analyses.

Results: In our study group, 19 (31.7\%) of these patients were male while 41 (68.3\%) patients were female. The most common acceptable antigens were A*02 (10.11\%), HLA-A*23 (10.11\%), HLA-B*38 (8.79\%) and HLA-DRB1*03 (7.83\%) in hypersensitive patients. The highest antibody reactivity on SAB was observed against HLA-A*25, HLA-B*45, HLA-DRB1*04 and HLA-DRB1*08 antigens.

Conclusions: The determination of these acceptable and unacceptable antigens may increase their transplantation chance. Pre-transplant HLA antibody identifications provide prognostic information with respect to the determination of patients who are at increased risk of graft loss.

Key words: flow cytometry, DSA, single antigen bead, HLA.

Introduction

Anti-human leucocyte antigen (anti-HLA) antibodies produced by patients via blood transfusions, transplantation history and pregnancies are called panel reactive antibody (PRA) \cite{1}. The individuals with \( \geq 85\% \) PRA ratio are referred to hypersensitized. The obvious solution for the transplantation of these hypersensitized patients is to find an HLA-identical donor. However, it is almost impossible since HLA system has extensive polymorphisms. There are two ways to carry out transplantation of these patients: i) removal of the antibodies that cause the positive crossmatch by various methods used in clinics, ii) definition of the holes in their antibody repertoire to enhance selection of crossmatch-negative HLA mismatched donors \cite{2}.

One-third of the patients in United Network for Organ Sharing (UNOS) waiting list are sensitive to HLAs. When the PRA results and waiting duration of the patients on the list were evaluated, it was found that the patients with < 30\% PRA had kidney transplantation after 493 days, while the patients with \( \geq 30\% \) PRA waited an average of 1047 days for the transplantation \cite{3}. According to these data, it was considered that higher PRA ratio increased the waiting duration in the list. Thus, it decreased the transplantation chance of sensitized (\( \geq 30\% \) PRA) patients.

Patel and Terasaki \cite{4} demonstrated the impact of complement-dependent lymphocytotoxic cross-match (CDC-XM) test in identifying immunologic risk in renal transplantation. This became the gold standard method for graft allocation and it is still used before transplantations. However, it is well-known that it cannot identify specifically the preexisting donor-specific HLA antibodies (HLA-DSA) any more (i.e. HLA-A*24). Recently, HLA antibody detection techniques have become more sensitive and specific with solid-phase assays \cite{5}. One of these tests is based on analyzing of patient sera with the beads covered by a single HLA antigen (single antigen bead-SAB) \cite{6}. The clinical significance of the specific antibodies detect-
ed by these more sensitive techniques has not been fully evaluated for graft survival and definition of acceptable grafts [5, 7].

Flow cytometry-based solid phase assays (flow-beads) have at least similar sensitivity for the detection of specific HLA as flow cytomter crossmatch (FCXM) [8]. Therefore, HLA-DSAs can be determined without performing a FCXM test by comparing the HLA antibody specificities of the recipient with HLA-typing of the donor (i.e. virtual XM). The absence or presence of DSA can be decided by virtual XM and it may become invaluable tool for the evaluation of organ allocation and pretransplant risk level [9].

HLA-DSAs produced in recipients are specific to the epitopes of HLA antigens in donors. Thus, these epitopes and determination of acceptable antigens should be considered during the assessment of donor-recipient compatibility. One of the important assessment strategies is use of Acceptable Mismatch program that is used in Eurotransplant. In this strategy, selection of specific panel cells is used during the screening of the serum for each hypersensitized patient and this specific panel cells have only one HLA-A or HLA-B mismatch with the patient. Accordingly, the HLA antigens that the patient can accept may be found by these acceptable mismatches [12].

The aims of the study were to identify class I and class II antibodies in highly sensitized patients by flow cytometry single antigen bead (FC-SAB) assay and to evaluate according to donor HLA type in order to increase their kidney transplantation chance.

Material and methods

Patients

In this study, 60 hypersensitive patients of 351 individuals who applied to our laboratory for PRA test in November 2013-December 2014, were included. The patients with > 85% PRA reactivity were accepted as hypersensitive and tested by FC-SAB. HLA typing of hypersensitive patients were performed by molecular method. Nineteen (31.7%) of these patients were male while 41 (68.3%) patients were female. Thirty-three (55%), 17 (28%) and 10 (17%) patients were analyzed for Class I, II and both of them by FC-SAB method, respectively. The study was approved by the Committee on Medical Ethics of the Izmir Katip Celebi University Faculty of Medicine.

HLA typing

Some of the patients were typed for HLA-A, -B and -DR loci by Luminex technology using commercial sequence-specific oligonucleotide (SSO) kits (Gen-Probe, Stanford, USA). The manufacturer’s instructions were followed during the procedure. Some of the patients were typed for HLA-A, -B and -DR by serological methods. Some of them were also analyzed in other centers.

Antibody detection

Flow cytometric PRA screening and single antigen bead commercial kits (One Lambda, Inc.) were used for these analyses according to the protocol recommended by the manufacturer. These beads are used in order to research antibodies against common HLA antigens in our population. 20 µl of patient serum was incubated with 5 µl of class I- and class II-coated microparticles for 30 minutes and after incubation the tubes were washed two times by adding 1 ml wash buffer and centrifuging at 1900 rpm for 10 minutes. After the washing step, 100 µl fluorescein isothiocyanate-conjugated anti-human IgG (FITC-anti-Ig; Fc fragment specific, Dako, Glostrup, Denmark) was added into the tubes and incubated at room temperature for 30 minutes in the dark. After incubation the washing steps were repeated as mentioned above. The fluorescence of the samples was then assessed by flow cytometer instrument. Positive samples were analyzed for single antigen specific antibodies by using single antigen beads coated with a single HLA antigen. Fluorescence of 5000 events was analyzed on a flow cytometer instrument (BD Facs Calibur, USA).

SAB analysis was based on excitation and emission of the beads at 488 nm and 580 nm, respectively. This fluorescence was collected on the FL2 channel on a flow cytometer. The class I bead contained four different groups of beads each containing nine different beads and having a unique FL2 channel shift. Thus, these beads could be separated from each other by the FL2 channel of a flow cytometer. However, the positive reactions of HLA antibodies were detected by the FL1 channel of a cytometer. The main population of beads was gated on the SSC vs. FSC dot blot and FL2 vs. FL1 dot plot was obtained on the gated beads. Gates were set for each bead population that had reacted with the negative control serum on the FL2 vs. FL1 dot plot. The same gates were also used to analyze all the testing sera on their FL2 vs. FL1 dot plot. The reaction was accepted as positive by a shift of the beads to the right of the gate. The channel shift of the beads was then scored and the scores ≥ 4 were accepted as positive.

Statistical method

We analyzed HLA allele frequencies in our donor population for hematopoietic stem cell transplantation (HSCT) (2975 donors) and our study group (60 patients) in order to investigate relation between our SAB results and the frequency of HLA alleles.

The comparisons of antigen proportions (as percentages) between the patients groups and the given population proportions were performed using Z tests and any resulting value such as Z ≥ 1.96 was considered as statistically significant.
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For comparing proportions, the computation formula given below was utilized:

$$Z = \frac{(\hat{p}_1 - \hat{p}_2) - 0}{\sqrt{\hat{p}(1-\hat{p}) \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}}$$

were \( \hat{p} = \frac{n_1 + n_2}{N_1 + N_2} \) and \( p < 0.05 \) if \( Z \geq 1.96 \)

Results

Three hundred and fifty one patients applied to our laboratory for PRA test in November 2013-December 2014. One hundred and twelve (32%) of these patients were PRA positive (in terms of Class I and/or Class II). Thirty-four (9.5%) of these patients were class I positive and class II negative, while 33 (9.5%) were class I negative and class II positive. Forty-five (13.0%) of these patients were positive for both of the classes. Among these patients, 60 hypersensitive individuals (with > 85% PRA) were analyzed by FC-SAB method. The characteristics (Table 1) and FC-SAB results of the patients (Table 2) were summarized in tables.

When 60 hypersensitive patients were evaluated in terms of alloimmunization rates, 78%, 40% and 38.3% were found to have blood transfusion, pregnancy and rejection history, respectively.

According to our FC-SAB results, the most common acceptable antigens were HLA-A*02 (10.11%), HLA-A*23 (10.11%), HLA-B*38 (8.79%), HLA-DRB1*03 (7.83%). The highest unacceptable antigens on SAB was observed against HLA-A*25, HLA-B*45, HLA-DRB1*04 and HLA-DRB1*08.

The most common alleles were HLA-A*02 (22%), HLA-B*35 (20.88%) and HLA-DRB1*11 (19.75%) in HSCT-donor population. In addition, HLA-A*02, HLA-B*35 and HLA-DRB1*11 frequencies in our study group were 35%, 30% and 30%, respectively. HLA-A*25, HLA-B*45, HLA-DRB1*04 and HLA-DRB1*08 frequencies in HSCT-donor population were 0.80%, 0.21%, 14.45% and 1.89%, respectively (Table 3).

Discussion

Kidney transplantation is the good option for the patients with end-stage renal disease [13]. The significance of anti-HLA antibodies in kidney transplantation, especially HLA-DSA, is non-negligible because these antibodies may lead to graft failure after transplantation. In recent years, various methods have been developed to determine these antibodies in order to extend the graft survival. However, even solid-phase PRA screening and specific assays have been insufficient for the identification of the HLA-DSA specifically (i.e. HLA-A24) until recently.

Hypersensitive patients may develop antibodies against a large variety of HLA antigens because of different alloimmunization ways such as pregnancy, blood transfusion, and transplantation. Therefore, these patients have the least chance to receive a suitable organ by standard procedures of the various organ exchange organizations. If special preventive actions are not taken, the number of these patients in the waiting list will increase due to their long waiting duration [14].

In our study group, 23 (38.3%) hypersensitive patients had transplantation history. These patients may become sensitized against the mismatched HLA antigens of the rejected organ. The sensitization incidence after a failed transplant depends on the number of HLA mismatches of the donor. According to Doxiadis et al., it can vary between 20% (0-1 mismatches) and 46-52% (5-6 mismatches). Reduction of the HLA mismatches in previous transplants will reduce the incidence of sensitization [2]. However, we did not match functional epitopes of patients and donors.

We also investigated the relation between antibody reactivity on SAB and HLA allele frequencies. We observed that the highest antibody reactivity was against rare alleles [HLA-A*25 (0.80%), HLA-B*45 (0.21%), HLA-DRB1*04 (14.45%) and HLA-DRB1*08 (1.89%)]. It was found that the relation between antibody reactivity and HLA allele frequencies was statistically significant because individuals would produce antibodies against rare HLA antigens rather than common antigens since they would also have the common HLA antigens (\( p < 0.001 \)). The only exception in our study was HLA-DRB1*04. It was one of the antigens against which the highest antibody reactivity was observed, although it has > 10% frequency in HSCT-donor population. This may be due to allele differences in HSCT-donor population.

Some of our patients seemed as they produced auto-antibodies (Table 2). However, we considered that the results might be due to allele differences or the alterations in three dimensional structure of the antigen during denaturation for the preparation of the beads [15].

In this study, we focused on the method for increasing the chance of hypersensitive patients to find a crossmatch negative donor. It is difficult to determine HLA antibody specificities in highly sensitized patients because these sera include antibodies to many HLA antigens. Moreover, the FC-SAB provides a tool to identify each single antigen reaction against the antibodies in the serum [8]. FC-SAB method can also eliminate undefined results that had been wrongly assigned by computer based programs in specific PRA method. Recently, single antigen bead technology and acceptable mismatch (AM) programs have been used to determine HLA antigens which are suitable for transplantation of hypersensitive patients. Acceptable mismatch programs increase the probability of highly sensitized patients to receive a suitable organ. It was revealed that approximately 60% of the hypersensitive patients would be transplanted within two years after inclusion in the AM
Table 1. Characteristics of patients

| Patient No | Age | Gender | Number of blood transfusion (Unit) | Tx A C P |
|------------|-----|--------|-----------------------------------|---------|
| 1          | 44  | F      | 0 0 0 1 6                         |         |
| 2          | 40  | M      | 2 1 N/A N/A N/A                   |         |
| 3          | 67  | F      | 2 0 0 0 5                         |         |
| 4          | 63  | M      | 8 0 N/A N/A N/A                   |         |
| 5          | 42  | M      | 1 1 N/A N/A N/A                   |         |
| 6          | 75  | F      | 1 1 0 0 8                         |         |
| 7          | 35  | M      | 2 0 N/A N/A N/A                   |         |
| 8          | 37  | M      | 5 1 N/A N/A N/A                   |         |
| 9          | 49  | F      | 5 1 1 0 4                         |         |
| 10         | 16  | F      | 1 1 0 0 0                         |         |
| 11         | 25  | M      | 6 1 N/A N/A N/A                   |         |
| 12         | 30  | M      | 1 1 N/A N/A N/A                   |         |
| 13         | 43  | F      | 3 0 1 1 3                         |         |
| 14         | 37  | F      | 1 0 0 0 3                         |         |
| 15         | 38  | F      | 1 1 0 1 1                         |         |
| 16         | 46  | F      | 1 1 0 0 0                         |         |
| 17         | 57  | F      | 0 0 0 0 0                         |         |
| 18         | 52  | F      | 5 0 1 0 4                         |         |
| 19         | 58  | F      | 2 0 3 0 2                         |         |
| 20         | 31  | F      | 1 1 0 0 0                         |         |
| 21         | 57  | F      | 1 0 1 1 6                         |         |
| 22         | 62  | M      | 3 1 N/A N/A N/A                   |         |
| 23         | 59  | F      | 4 0 0 0 0                         |         |
| 24         | 34  | F      | 0 1 0 0 0                         |         |
| 25         | 58  | F      | 3 0 3 2 10                        |         |
| 26         | 39  | F      | 2 0 2 0 3                         |         |
| 27         | 51  | F      | 0 0 0 1 0                         |         |
| 28         | 54  | F      | 3 1 0 0 1                         |         |
| 29         | 46  | M      | 1 0 N/A N/A N/A                   |         |
| 30         | 40  | F      | 1 0 0 0 1                         |         |
| 31         | 33  | F      | 0 0 1 0 1                         |         |
| 32         | 51  | M      | 1 0 N/A N/A N/A                   |         |
| 33         | 64  | F      | 6 0 0 0 0                         |         |
| 34         | 64  | F      | 2 0 0 0 1                         |         |
| 35         | 54  | F      | 0 0 1 1 1                         |         |
| 36         | 50  | F      | 3 0 0 0 0                         |         |
| 37         | 42  | F      | 0 0 1 0 0                         |         |
| 38         | 59  | F      | 1 1 0 0 0                         |         |
| 39         | 21  | F      | 2 0 0 0 0                         |         |
| 40         | 65  | M      | 2 0 N/A N/A N/A                   |         |
| 41         | 28  | M      | 1 0 N/A N/A N/A                   |         |
| 42         | 45  | F      | 1 0 1 1 1                         |         |
| 43         | 64  | F      | 3 0 0 1 8                         |         |
| 44         | 62  | M      | 1 0 N/A N/A N/A                   |         |
| 45         | 60  | M      | 1 0 N/A N/A N/A                   |         |
| 46         | 22  | F      | 1 1 0 0 0                         |         |
| 47         | 46  | F      | 1 1 0 0 0                         |         |
| 48         | 35  | F      | 0 0 1 2                           |         |
| 49         | 40  | F      | 1 0 0 1 2                         |         |
| 50         | 45  | F      | 2 0 0 0 3                         |         |
| 51         | 43  | M      | 4 1 N/A N/A N/A                   |         |
| 52         | 34  | F      | 1 0 0 1 1                         |         |
| 53         | 51  | F      | 3 0 0 0 1                         |         |
| 54         | 54  | F      | 0 1 0 0 0                         |         |
| 55         | 25  | F      | 3 1 0 0 0                         |         |
| 56         | 4   | M      | 1 1 N/A N/A N/A                   |         |
| 57         | 37  | F      | 2 1 0 0 0                         |         |
| 58         | 44  | M      | 70-80 N/A N/A N/A                 |         |
| 59         | 65  | M      | 2 0 N/A N/A N/A                   |         |
| 60         | 35  | M      | 0 1 N/A N/A N/A                   |         |

Tx – transplantation; M – male; F – female; A – abortus; C – curettage; P – pregnancy; N/A – not applicable
Table 2. Comparison of HLA types, PRA and SAB results of the patients

| Patient No | HLA types of the patients | PRA% | SAB Results |
|------------|---------------------------|------|-------------|
|            |                           |      | CI          | CII          |
| 1          | A*26, A*68, B*55, –, DRB1*13, – | 94   | 100 | A*01, A*03, A*29, A*30, A*26, A*68, A*11, A*34, A*33, B*40, B*07, B*55, DRB1*13, DRB1*12, DRB1*03 |
| 2          | A*02, A*68, B*27, B*53, DRB1*11, – | 100  | 100 | A*02, A*68, A*24, B*51, B*13, B*44, B*38, B*57, B*53, DRB1*11, DRB1*08, DRB1*13, DRB1*15, DRB1*16, DRB1*03, DRB1*01, DRB1*04 |
| 3          | A*21, A*02, B*37, –, DRB1*01, DRB1*10 | 100  | 100 | A*01, A*02, B*37, DRB1*01, DRB1*01, DRB1*10 |
| 4          | A23(19), A32(19), B52(5), B35, DRB1*15, DRB1*16 | 100  | Neg | A*23, A*32, B*52, B*35, B*51, B*18, B*15, B*45, B*14 |
| 5          | A*02, B*35, B*51, DRB1*09, DRB1*14 | 100  | 96.67 | B*18, B*15, B*44, B*52, B*14, DRB1*09, DRB1*10, DRB1*14, DRB1*17, DRB1*03 |
| 6          | A2, A24, B8, B61, DR03, DR15 | 94   | Neg | A*02, A*24, B*08, B*40 |
| 7          | A*24, A*29, B*35, –, DRB1*15, DRB1*16 | Neg | 96.67 | DRB1*01, DRB1*10, DRB1*15, DRB1*16 |
| 8          | A1, A3, B7, B60, DRB1*10, DRB1*12 | 100  | 100 | A*01, A*03, B*07, B*40, DRB1*01, DRB1*07, DRB1*10, DRB1*12, DRB1*16, DRB1*09, DRB1*15 |
| 9          | A*11, A*68, B*35, –, DRB1*01, DRB1*13 | 100  | 100 | A*11, A*68, B*35, DRB1*01, DRB1*01, DRB1*12, DRB1*13, DRB1*03 |
| 10         | A1, A32(19), B38(16), DRB1*13, DRB1*14 | 100  | 86.67 | A*01, A*32, B*38, DRB1*07, DRB1*04, DRB1*13, DRB1*14, DRB1*15, DRB1*16, DRB1*03, DRB1*01, DRB1*09, DRB1*15 |
| 11         | A*02, A*03, B*55, DRB1*04, DRB1*14 | 98   | Neg | A*02, A*03, B*13, B*18, B*35, B*15, B*40, B*8, B*14, B*55 |
| 12         | A1, A2, B49(21), B35, DRB1*11, DRB1*13 | Neg | 96.67 | DRB1*01, DRB1*04, DRB1*10, DRB1*15 |
| 13         | A*01, A*11, B*52, B*53, DRB1*07, DRB1*10 | Neg | 93.33 | DRB1*07, DRB1*10, DRB1*52, DRB1*53, |
| 14         | A*02, A*03, B*18, B*44, DRB1*01, DRB1*04 | Neg | 93.33 | DRB1*01, DRB1*04, DRB1*10, DRB1*15 |
| 15         | A*11, A*34, B*18, B*35, DRB1*13, DRB1*14 | 92   | Neg | A*03, A*11, A*23, A*24, B*51, B*52, B*18, B*35, B*15, B*38, B*07, B*08, B*14, B*55 |
| 16         | A*03, A*32, B*08, –, DRB1*03, DRB1*11 | 92   | Neg | A*33, A*02, A*24, A*32, A*23, A*68, B*18, B*40, B*44, B*38, B*52, B*27, B*08, B*14 |
| 17         | A26, A30, B51, B13, DRB1*04, DRB1*11 | 100  | Neg | A*26, A*30, A*33, A*49, B*51, B*52, B*13, B*38 |
| 18         | A1, A11, B44, B49, DRB1*03, DRB1*13 | 96   | Neg | A*01, A*11, A*23, A*30, A*31, B*13, B*44, B*49, B*57 |
| 19         | A3, B35, B52, DRB1*13, DRB1*14 | 94   | Neg | A*03, B*49, B*51, B*13, B*18, B*35, B*15, B*38, B*57, B*14 |
| 20         | A*23, A*26, B*35, B*50, DRB1*04, DRB1*13 | 100  | Neg | A*01, A*02, A*25, A*26, A*23, A*50, A*51, B*49, B*35 |
| 21         | A*02, A*32, B*27, B*41, DRB1*04, DRB1*13 | 100  | Neg | A*01, A*02, A*23, A*32, B*27, B*40 |
| 22         | A24(9), A30(19), B13, B18, DR7, DR53 | 97   | 97 | DRB1*02, DRB1*07 |
| 23         | A2, A24, B51, B35, DRB1*03, DRB1*13 | Neg | 90 | DRB1*01, DRB1*10, DRB1*13, DRB1*15, DRB1*16, DRB1*03, DRB1*09 |
| 24         | A29(19), A30(19), B13, B77(15), DRB1*11, DRB1*14 | 100  | Neg | FM |
| Case | Genotypes | Alleles | MHC Class I | MHC Class II |
|------|------------|---------|-------------|-------------|
| 25   | A24, B35, B55, DRB1*04, DRB1*13 | 100 | 96 | DRB1*11, DRB1*03, DRB1*13, DRB1*14, DRB1*09, DRB1*04 |
| 26   | A2, A32(19), B18, B35, DRB1*11 | 96 | 93.33 | A*02, A*29, A*68, A*32, A*33, B*51, B*18, B*35, B*15, B*45, B*44, B*38, B*57, B*52, B*14, DRB1*08, DRB1*11 |
| 27   | A11, A24, B49, B51, DRB1*08, DRB1*11 | Neg | 100 | DRB1*08, DRB1*11, DRB1*13, DRB1*16, DRB1*03, DRB1*15 |
| 28   | A24, A26, B27, B40, DRB1*04, DRB1*11 | 88 | Neg | A*25, A*26, A*23, A*24, A*32, B*18, B*40, B*38, B*57, B*27, B*18, B*14 |
| 29   | A*26, A*29, B*38, B*51, DRB1*03, DRB1*13 | Neg | 93.33 | DRB1*08, DRB1*11, DRB1*13, DRB1*14, DRB1*15, DRB1*16, DRB1*03, DRB1*01, DRB1*12 |
| 30   | A*02, A*03, B*51, DRB1*04, DRB1*14 | 100 | Neg | A*02, A*03, A*25, A*29, A*30, A*26, A*68, A*11, A*34, A*52, A*33, B*51 |
| 31   | A*66, A*68, B*18, B*41, DRB1*11, DRB1*13 | 94 | Neg | A*66, A*30, A*34, A*33, A*31, B*18, B*41, B*07, B*14, B*55 |
| 32   | A*23, A*24, B*40, B*49, DRB1*11, DRB1*15 | 98 | Neg | A*02, A*03, A*26, A*68, A*11, A*34, A*24, A*33, A*31, A*23, B*08, B*38, B*15, B*44, B*51 |
| 33   | A*02, A*03, B*44, B*51, DRB1*07, DRB1*10 | Neg | 96.67 | DRB1*07, DRB1*10, DRB1*01,09, DRB4*01 |
| 34   | A*31, A*32, B*14, B*15, DRB1*01, DRB1*13 | 100 | Neg | A*29, A*30, A*31, A*32, A*33, B*15, B*14 |
| 35   | A*24, A*26, B*35, B*58, DRB1*01, DRB1*13 | 96 | Neg | A*01, A*25, A*26, A*11, A*23, A*24, B*35, B*40, B*45, B*07 |
| 36   | A*02, A*03, B*51, DRB1*08, DRB1*15 | 100 | Neg | A*02, A*03, A*25, A*29, A*30, A*68, A*11, A*34, A*52, A*33, B*51 |
| 37   | A*23, B*08, B*49, DRB1*11, DRB1*13 | 96 | Neg | A*01, A*23, B*49, B*13, B*38, B*52, B*27 |
| 38   | A*11, A*24, B*51, B*57, DRB1*04, DRB1*07 | 98 | Neg | A*01, A*02, A*03, A*11, A*26, A*68, A*33, A*31, A*23, A*25, A*29, A*32, B*51, B*57 |
| 39   | A*23, A*24, B*40, B*49, DRB1*04, DRB1*11 | 96 | Neg | A*02, A*25, A*29, A*30, A*68, A*11, A*34, A*32, A*33, A*31, B*51, B*18, B*38, B*14, B*08 |
| 40   | A2, A24, B35, B50, DRB1*04, DRB1*07 | Neg | 90 | DRB1*07, DRB1*01, DRB1*04 |
| 41   | A*01, A*24, B35, B50, DRB1*04, DRB1*07 | 100 | Neg | A*01, B*13, B*44, B*57 |
| 42   | A*02, A*38, B*44, B*46, DRB1*11, DRB1*12 | 100 | Neg | A*02, A*68 |
| 43   | A69(28), A30(19), B51(5), B35, DRB1*04, DRB1*11 | 96 | Neg | A*69, A*02, A*29, A*30, A*68, A*32, A*33, A*31, B*49, B*51, B*35, B*38, B*55 |
| 44   | A*01, A*03, B*08, B*11, B*15, B*42, B*44, B*49 | 100 | Neg | A*01, B*08 |
| 45   | A*33, A*69, B*49, B*51, DRB1*04, DRB1*11 | 92 | Neg | A*33, A*02, A*24, A*32, A*23, A*68, B*18, B*40, B*44, B*38, B*52, B*27, B*08, B*14 |
| 46   | A*02, A*32, B*44, B*55, DRB1*03, DRB1*11 | 97 | Neg | DRB1*03 |
| 47   | A*03, A*23, B*14, B*51, DRB1*01, DRB1*14 | 98 | Neg | A*03, A*23, A*30, B*49, B*14, B*51, B*35, B*38, DRB1*01, DRB1*04, DRB1*14, DRB1*03, DRB1*09, DRB1*12, DRB1*15 |

**Table 2. Cont.**

| Case | Genotypes | Alleles | MHC Class I | MHC Class II |
|------|------------|---------|-------------|-------------|
| 48   | A*33, A*69, B*49, B*51, DRB1*04, DRB1*11 | 92 | Neg | A*33, A*02, A*24, A*32, A*23, A*68, B*18, B*40, B*44, B*38, B*52, B*27, B*08, B*14 |
| 49   | A*02, A*32, B*44, B*55, DRB1*03, DRB1*11 | 97 | Neg | DRB1*03 |
| 50   | A*03, A*23, B*14, B*51, DRB1*01, DRB1*14 | 98 | Neg | A*03, A*23, A*30, B*49, B*14, B*51, B*35, B*38, DRB1*01, DRB1*04, DRB1*14, DRB1*03, DRB1*09, DRB1*12, DRB1*15 |
Single antigen flow beads for identification of human leukocyte antigen antibody specificities in hypersensitized patients with chronic renal failure

| Table 2. Cont. | A2, A31(19), B18, B49(21), DR13(6), DR14(6) | 86 | 95 | A*02, A*31, A*33, A*23, B*51, B*52, B*18, B*15, B*40, B*14, B*55, DRB1*04, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*03 |
|----------------|---------------------------------------------|-----|-----|-----------------------------------------------------------------|
| 52             | A2, A31, B18, B49, DR13, DR14              | Neg | 92 | DRB1*08, DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*03 |
| 53             | A3, A24, B38, B44, DRB1*04, DRB1*07       | 94  | 90 | DRB1*07, DRB1*04, DRB1*15 |
| 54             | A25(10), A26, B18, B35, DR10, DR7         | Neg | 90 | DRB1*01, DRB1*07, DRB1*09, DRB1*10 |
| 55             | A24(9), A31(19), B7, B13, DR4, DR53       | Neg | 80 | DRB1*11, DRB1*12, DRB1*13, DRB1*14, DRB1*15, DRB1*16, DRB1*03 |
| 56             | A*11, A*26, B*18, B*55, DRB1*11, DRB1*13 | Neg | 98 | DRB1*11, DRB1*12, DRB1*13, DRB1*03, DRB1*01, DRB1*15 |
| 57             | A*11 A*26 B*18 B*55 DRB1*11 DRB1*13     | 100 | Neg | B*15, B*35 |
| 58             | A*24, A*30, B*35, B*53, DRB1*03, DRB1*11 | 98  | Neg | A*23, B*44, B*49, B*51/52, B*18, B*15 |
| 59             | A*03, A*68, B*07, B*51, DRB1*07, –        | Neg | 95 | DRB1*07, DRB1*12, DRB1*03, DRB1*15 |
| 60             | A3, A69, B8, B35, DRB1*03, DRB1*04       | 92  | Neg | A*02, A*03, A*68, A*34, A*69, A*33, A*31 |

Table 3. The relation between unacceptable antigens in the patient group and population antigen frequencies

| Unacceptable antigens (%) | Population antigen (%) | Z-value | p     |
|---------------------------|------------------------|---------|-------|
| HLA-A*25                  | 61.60                  | 0.8     | 33.289 | < 0.001 |
| HLA-B*45                  | 65                     | 0.21    | 41.000 | < 0.001 |
| HLA-DRB1*04               | 41.6                   | 14.45   | 5.833  | < 0.001 |
| DRB1*08                   | 41.6                   | 1.89    | 18.874 | < 0.001 |

program [16]. However, hypersensitive patients only with extra points in the standard Eurotransplant allocation program have about a 20% chance for transplantation within the same time period [14].

While the patients in the acceptable mismatch program will receive an organ more quickly, some studies suggest that graft survival of hypersensitive patients is lower than the other patients [17]. However, this is not the case for patients transplanted via the acceptable mismatch program. In recent studies, it was observed that the hypersensitized patients also have short term (two years) graft survival similar to the unsensitized patients [14]. In contrast, they revealed that other sensitized patients had indeed a significantly poorer graft survival. They suggested that graft survival of all sensitized patients could be increased by AM programs. Moreover, not only the short term but also the long term graft survival in patients transplanted from a suitable donor via the acceptable mismatch program was excellent [16, 18].

Recently, similar programs based on Eurotransplant acceptable mismatch program have been implemented in France, Italy, and Greece, whereas implementation is in progress in Scandia transplant, Switzerland, and Canada [18]. We expect that in the near future our country will implement a similar approach. The FC-SAB results of 60 patients waiting for transplantation will attribute to the pre-transplantation assessments and most probably the transplantation chance of these patients will be increased.

In conclusion, identification of SAB and the matching strategies that depend on both epitope sharing of mismatch antigens and related antibody production are very important to achieve transplantation. Thus, the transplantation chance of some patients may increase 25-50%. If we look from the viewpoint of these results, we can prevent negative impacts on their psychology by avoiding the calls of the patients to each testing for transplantation from deceased donors.

The authors declare no conflict of interest.
The authors thank the staff of Tissue Typing Laboratory of Izmir Tepecik Education and Research Hospital for their continuous efforts in performing of the tests. This study was supported by Scientific Research Project Foundation of Izmir Katip Celebi University (Project No: 2013-1-TSBP-11).
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