

## PROSPECTIVE HLA-DP TYPING OF A CADAVERIC ORGAN DONOR FOR KIDNEY TRANSPLANTATION – A CASE REPORT

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**Abstract:** In this paper, we present the case of an HLA-DP immunized patient in the process of kidney re-transplantation for which potential pre-transplantation HLA-DP donor-specific antibodies were detected. For that reason, for the first time in our laboratory practice, prospective cadaveric donor HLA-DP typing was performed during the process of kidney allocation. This case, together with other international study reports and protocols of some transplantation programs, points to the importance of introducing pre-transplant HLA-DP typing in cadaveric kidney transplantation in Croatia.

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### INTRODUCTION

HLA matching provides numerous benefits in organ transplantation, including a better graft function, fewer rejection episodes, longer graft survival, and the possibility of reduced immunosuppression. As a member of Eurotransplant (ET) - an international organization which coordinates organ donation procedures in Austria, Belgium, Croatia, Germany, Hungary, Luxembourg, the Netherlands and Slovenia - Croatia must follow the ET requirements for patient and donor HLA typing and HLA matching,<sup>1</sup> as well as for defining the antibody profile of each recipient. The kidney allocation procedure requires recipient and organ donor typing for HLA-A, -B, -C, -DRB1, and -DQB1 loci. The immunological profile of each recipient allows for the listing of antibody specificities and unacceptable antigens for these five HLA loci, while the HLA matching/mismatching (MM) strategy is based on HLA-A, -B antigen broad level and antigen split level for HLA-DR.

The role of class I (HLA-A, -B) and class II (HLA-DR, -DQ) HLA antibodies in acute or chronic antibody-mediated rejection (AMR) of transplants is well known and proven by numerous studies, while the presence of antibodies directed against HLA-C, -DQA1 and especially -HLA-DP is considered to confer a low risk of AMR due to the low expression of these HLA molecules on renal endothelial cells.<sup>2, 3</sup> However, numerous recent studies offer accumulative evidence that preformed HLA-DP donor-specific antibodies (DSA) in immunized patients without any other HLA DSAs have an impact on the incidence of AMR, especially in a re-transplant setting.<sup>2, 4, 5</sup> The development of solid phase immunoassays and multiplexed bead testing on the Luminex platform enabled the detection of anti-HLA antibodies for all class I and class II specificities, including DPA1 and DPB1 specificities. This development has consequently led the transplant professionals' community to consider the need to type organ donors

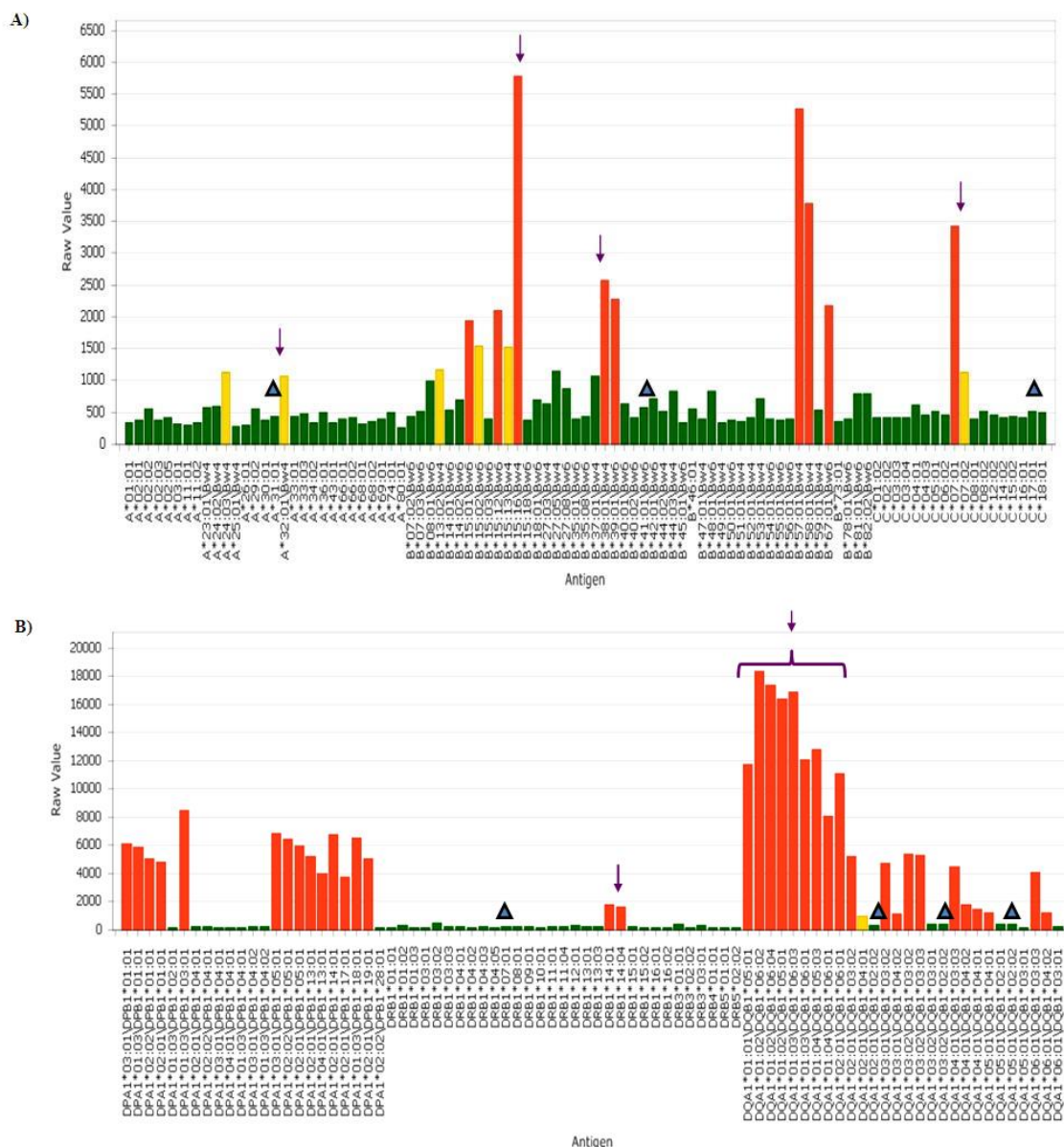
for all HLA loci against which antibodies in a potential transplant recipient have been detected.

As a result, mandatory HLA-DP typing along with standard HLA-A, -B, -C, -DR, and -DQB typing, was introduced in many transplant centers. The United Network for Organ Sharing (UNOS) has implemented HLA-DP typing as mandatory on all deceased kidney donors in the United States since 2016 in order to permit transplant centers to specify unacceptable donor HLA-DP antigens, and thus exclude a recipient with listed unacceptable antigens from a cross-match procedure with a donor carrying those antigens.<sup>6</sup> In light of the results obtained by all recent studies that point to HLA-DP as a clinically relevant locus in

cadaveric kidney (re)transplantation, HLA-DP typing and matching should be implemented in the allocation procedure.

## CASE REPORT

In this paper, we present the first prospective HLA-DP typing of a cadaveric organ donor in a kidney allocation procedure to an HLA-DP-sensitized patient. The case of a female patient, born in 1992, who was diagnosed with focal segmental glomerulosclerosis (FSGS) at age 9 is presented. The patient started with a



**Figure 1.** Luminex single antigen beads test results showing the presence of HLA class I antibodies (A) and the presence of HLA class II antibodies (B) in the patient sera before the second cadaveric kidney transplantation. The arrows point to the positive donor-specific antibodies (DSA) revealed to all mismatched HLA alleles between patient and donor after first transplantation in 2007. The triangles mark HLA antibody specificities of mismatched HLA alleles between the patient and a second potential cadaveric donor.

Green - negative reaction; yellow - weak positive reaction; red - positive reaction

hemodialysis protocol at University Hospital Centre cadaveric kidney transplant. Shortly after the transplantation, the underlying disease FSGS reappeared, causing graft rejection in 2010. The patient returned to the chronic hemodialysis program and in 2011 returned to the waiting list. Since then, according to the ET protocol, she has been quarterly tested for the presence and specificity of HLA antibodies in sera (screening) using the complement-dependent cytotoxicity (CDC) test and once a year by the Luminex method using the commercial Immucor's LIFECODES LSA class I and class II Single Antigens test (SA1 and SA2; Immucor Transplant Diagnostics Inc., Stamford, Connecticut). The Luminex SA1 and SA2 test results (Figure 1) showed the presence of anti-HLA class I antibodies (specificity HLA-A, -B, -C), as well the presence of anti-HLA class II antibodies (specificity HLA-DR, -DQ and -DP). The patient's antibody profile included DSAs against all MM alleles with the first cadaveric donor (A32, B38, B63, Cw7, Cw12, DR14, DQ5, DQ6; ABCDRDQ MM 12212). As the first donor was not typed for HLA-DP, and the donor material was not available for retyping, it can only be speculated that some of the HLA-DP antibodies detected (DP1, DP3, DP5, DP13, DP14, DP17, and DP19) are also DSAs. HLA-A, -B, -C, -DR, -DQ antibodies with median fluorescent intensity (MFI) > 1000 have been listed in the patient's immunological profile as unacceptable antigens, while HLA-DP sensitization was noted.

The patient received a kidney offer from a cadaveric donor from the Netherlands and a chance for a second transplantation in July 2017. The donor/patient ABCDRDQ MM was 11111, with Luminex SA1, SA2 testing results negative for all HLA-A, -B, -C, -DR, -DQ MM antigens (Figure 1a, 1b; triangles). The

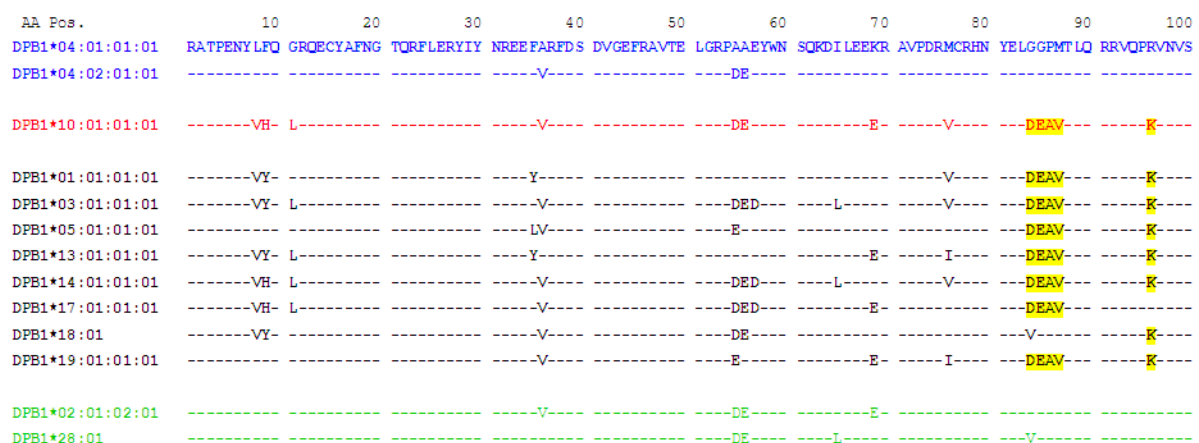
Zagreb at age 14. After a year, in 2007, she received a HLA-DP typing of the donor was unknown and pre-transplantation HLA-DP DSAs could not be excluded. Therefore, for the first time in our laboratory practice, the prospective HLA-DP typing of the cadaveric donor was performed by a Polymerase Chain Reaction - Sequence Specific Primer (PCR-SSP) method (Olerup SSP AB, Sweden). The typing result was HLA-DPB1\*04:01, \*10:01, revealing the HLA-DPB1\*10:01 as MM allele with the patient.

Luminex testing revealed that the patient has HLA-DP antibodies specific for 84DEAV and 96K epitopes which are shared by different HLA-DP antigens, including the MM HLA-DPB1\*10:01 allele (Figure 2). It has to be noted that the HLA-DP10 antibody itself was not reported in the patient's HLA-DP antibody profile since the test does not include a bead for its detection.

Since the patient was negative for DSAs against ABCDRDQ MM antigens, the reactions for epitope HLA-DP MM antigens were below MFI 10000, and the CDC cross-match was negative, the transplantation was performed.

## DISCUSSION

In this paper, we report the first case of a prospective cadaveric donor HLA-DP typing during the process of kidney allocation in our laboratory practice. The kidney was allocated to an HLA-DP immunized patient in the process of kidney re-transplantation. The results showed that the patient and the potential donor were mismatched for HLA-DP at the allelic level, but the allele-specific antibody could not be detected due to a limitation of the used test (no DP10 bead).



**Figure 2.** The amino acid sequence alignments of the patient's HLA-DPB1 alleles (blue letters) with mismatched HLA-DPB1 allele of the 2nd donor (red letters), other HLA-DPB1 alleles against which the presence of antibodies in the patients sera was detected (black letters) and HLA-DPB1 alleles with negative results in the Luminex single-antigen bead testing (green letters). Mismatched HLA-DPB1\*10 allele belongs to the group of HLA-DPB alleles with immunizing epitopes 84DEAV and/or 96K (yellow marked), which are absent in the alleles with negative bead reactions, including the patient's own alleles. These motifs can be considered responsible for the HLA-DP antibodies detected in the patient's sera by Luminex. The alignment was made according to the data from the IPD-IMGT/HLA Database ([www.ebi.ac.uk/ipd/imgt/hla](http://www.ebi.ac.uk/ipd/imgt/hla)).

Subsequent epitope analyses revealed that the patient's HLA-DP antibody profile includes antibodies specific for the 84DEAV and 96K immunogenic HLA-DP epitopes. The donor's mismatched HLA-DPB1\*10 allele codes an HLA-DP antigen which belongs to the group of antigens carrying these epitopes, thus possessing two epitopes different from patient's HLA-DPB1 alleles, which can potentially induce an antibody response, leading to an HLA-DP donor-specific epitope positive transplantation.

The interpretation of Luminex single-antigen tests requires the knowledge and understanding of HLA molecular structure, polymorphism, and serologic reactivity with epitopes shared among different HLA antigens, as well as the pattern of HLA antibodies reactivity with epitopes. The polymorphism of HLA-DP alleles is concentrated in six hypervariable regions (HVR) of exon 2 of the  $\beta$ 1 domain (residues 8-11, 32-36, 55-57, 65-69, 76 and 84-87; designated A-F) and combinations of amino acids in the six HVRs characterize the different HLA-DP alleles.<sup>2, 7</sup> The study by Simmons et al. has proven that antibodies against HLA-DP recognize epitopes rather than single antigens, so solid phase testing with single antigen beads can provide information regarding the reactivity of individual alleles or shared epitopes on multiple alleles, although not every allele is represented.<sup>6</sup> According to research, HLA-DP DSA can induce AMR soon after transplantation; therefore, it is important to detect any MM HLA-DP epitope in order to prevent the development of AMR by pre-transplantation desensitization and careful monitoring of DSA levels. Thauinat et al. concluded in their study that HLA-DP typing should be performed for regrafts and female patients, and that is more significant to match the epitopes which cause antibody response than to match the antigen alone.<sup>8</sup> All these data emphasize the importance of introducing pre-transplant HLA-DP typing in cadaveric kidney transplantation in Croatia. The completeness of the donor's HLA typing is in direct proportion to our ability to produce a precise analysis of the pre-transplant or/and post-transplant DSA presence, and thus contribute to better patient treatment and transplantation outcome.

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