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ORIGINAL ARTICLE

HLA ANTIGEN AND HLA EPLET MISMATCHES - IMPORTANT FACTORS FOR DEVELOPING DONOR-SPECIFIC ANTIBODIES AFTER KIDNEY TRANSPLANTATION

Lucija Jukic, Marija Burek Kamenaric

Abstract: Kidney donor-recipient mismatches (MM) in human leukocyte antigen (HLA) system can have the impact on graft survival as *de novo* formation of donor-specific HLA antibodies (DSA) post-transplant can increase the risk of acute and chronic rejections. Eplets, the smallest functional units of epitopes, are in recent years also being considered in recipient-donor matching. We have retrospectively analysed the relationship between MM at HLA-A, -B, -DRB1 and -DQB1 loci and the development of DSA in 47 kidney transplant recipients that were negative for the presence of HLA antibodies pre-transplant. A total of 19 patients (40%) developed DSA (DSA+) post-transplant, revealing sensitization to HLA class II MM antigens as the most prevalent (84% of DSA+ patients). MM at all HLA loci contributed to the development of HLA locus-specific antibodies, with the prevalence of HLA-DQ sensitization, as 41% of recipients with HLA-DQ MM developed DSA. HLAMatchmaker analysis for HLA-DR/DQ total eplet MM showed statistically significant difference between DSA- (N=33) and DSA+ recipients (N=14) for a total number of eplet MM (300 in DSA- vs 248 in DSA+; P=0.0004). These results emphasize the importance of HLA Class II matching in kidney transplantation.

Tissue Typing Centre, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb

Corresponding author:
Lucija Jukic
Tissue Typing Centre, Clinical Department for Transfusion Medicine and Transplantation Biology, University Hospital Centre Zagreb, Kišpatićeva 12, Zagreb, Croatia
e-mail: lucija.jukic@kbc-zagreb.hr

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INTRODUCTION

Kidney transplantation (Ktx) has been the main treatment for end-stage renal disease for more than half a century, but the main obstacle remains how to avoid immunological rejection that can lead to allograft dysfunction or even allograft loss.[1] Human leukocyte antigen (HLA) system plays the critical part of the host immune defence mechanism and for that reason, organ donor-recipient matching is done based on their HLA antigens. Mismatches (MM) in HLA system between the recipient and potential donor (HLA antigens present in donor but not the recipient) have the impact on graft survival and are usually leading to antibody-mediated rejection (AMR) which is still recognised as the leading cause of graft loss after Ktx despite the improvements in immunosuppressive treatments.[2]

Despite the improved and detailed pre-transplant immunological processing of the recipients through advances in HLA typing methods, HLA antibody screening methods and crossmatch testing methods, recipients that are not sensitised to HLA antigens (no HLA antibodies detected in their serum) before transplantation can still develop *de novo* donor-specific HLA antibodies (DSA) after transplantation1. *De novo* DSA develops in 15-25% of kidney transplant recipients (Ktr) within 5 years of transplantation and the risk of developing DSA is proportional to the number of HLA mismatched donor antigens. Formation of DSA post-transplant can increase the risk of acute and chronic rejections and is associated with 40% lower graft survival at 10 years post-transplant.[3] For this reason, carefully monitoring of HLA antibodies plays a crucial role in adjusting the post-transplant treatment.

Even though HLA matching has been the gold standard for evaluation of immunological risk after Ktx, recent progress in HLA protein analysis has made it possible to

evaluate differences more in detail by comparing epitopes.[4] HLA epitope matching is a novel strategy that may minimize DSA development. Structural epitopes comprise all amino acids of the HLA molecule involved in the binding to the HLA antibody, while functional epitope, also known as eplet, comprises the minimal amino acid configuration on the HLA molecule that is needed to induce an antibody response.[5] The HLA Epitope Registry (available via https://www.epregistry.com.br) has documented all theoretically defined HLA eplets, while only part of them are classified as antibody-verified due to experimentally verification based on different methods for validation. Although antibody-verified eplet MM correlate with DSA formation, there are still clinically relevant eplets which have not been antibody-verified yet.[6]

One of the commonly used computer-based algorithms for characterization of eplets is HLAMatchmaker.[7] The HLAMatchmaker algorithm defines the number of specific mismatched eplets either at each HLA class I (HLA-A, -B, -C) and class II (HLA-DRB1/3/4/5, -DQ, -DP) loci or as a cumulative number of eplets which are not shared with the recipient's HLA antigens. It has been shown that the quantity of mismatched eplets between donor and recipient HLA alleles correlate with DSA development, AMR and graft loss.[8] For that reason, HLAMatchmaker is becoming more and more relevant for matching donor and recipient to minimize DSA development.[9]

In this study, we have retrospectively analysed the possible association of MM at HLA-A, -B, -DRB1 and -DQB1 loci as well as the number of total eplet MM with the development of DSA in Ktr that were negative for the presence of any HLA antibodies before transplantation.

MATERIAL AND METHODS

Study cohort

The research includes a cohort of 47 Ktr (6 females and 41 males) that were transplanted in the period 2009-2018 at the University Hospital Centre Zagreb (UHC Zagreb) or Clinical Hospital Merkur.

The minimal inclusion criteria were availability of the HLA typing of recipient-donor pair and non-sensitisation at the time of Ktx defined by 0% pre-transplant calculated panel reactive antibody (cPRA). Exclusion criteria were repeated Ktx, prior pregnancies, combined renal and non-renal transplantation, sensitisation of the recipient at the time of Ktx and non-sensitized recipients with a follow-up time less than 5 years. Pregnancy as the exclusion criteria influenced female/male ratio in the study. Female Ktr make only 13% of the study cohort although the actual percentage of females in kidney transplantation programme including three Croatian transplantation centers (UHC Zagreb, Merkur Clinical Hospital, and UHC Osijek) in the period between 2009 and 2018 was 36%.

HLA and eplet mismatch identification

HLA-A, -B, -C, -DRB1, -DQB1 low resolution typing of the donors was performed by Polymerase Chain Reaction – Sequence Specific Primers (PCR-SSP) method (Olerup/CareDx, Stockholm, Sweden)[10] while Polymerase Chain Reaction – Sequence Specific Oligonucleotides (PCR-SSO) method (Immucor Transplant Diagnostics Inc., Stamford, CT, USA) was used for the low resolution typings of the recipients.[11] Among 47 recipient-donor pairs, complete HLA-A, -B, C, -DRB1, -DQB1 typing was available for 19 recipients and 42 donors while for 28 recipients and 5 donors HLA-DQB1 results were presumed based on linkage disequilibrium and population data (the most probable genotype for Caucasian population).[12]

Recipient-donor HLA antigen MM were defined as antigens present in donor but not in the recipient for all investigated loci at the level of split specificities (Table 1). Standard antigen MM levels for each locus were identified as 0 (perfect match, or no mismatched donor antigens), 1 (donor has one mismatched antigen) or 2 (both of donor's antigens are mismatched). In case of donor being a homozygous for a mismatched antigen (homologous chromosomes are presumed to code for identical antigens at that locus), it was calculated as 1 MM. The HLAMatchmaker analysis program (available via http://www.epitopes.net/downloads.html; ABC EpletMatching Program v4.0 and DRDQDP EpletMatching Program v3.1) was used to calculate number of eplet HLA MM for each recipient-donor pair at HLA class I (HLA-A, -B) and class II (HLA-DR, -DQ) loci. Total eplet MM were considered.

HLA antibody and DSA assignment

All Ktr sera were collected pre- and post-transplant and screened for the presence of HLA class I and HLA class II IgG antibodies using the Lifecodes class I and II SAB assay (Gen-Probe/Immucor, Stanford, CT, USA) according to the manufacturer's instructions.[13] Before testing all Ktr sera were treated with heating to inhibit prozone effect. The HLA antibody presence was detected using Luminex technology (Luminex Corporation, Austin, TX, USA) and specificities were evaluated in MATCH IT! Antibody software, v1.3.1. Positive reaction threshold was defined based on the laboratory's validation results together with the MFI threshold of ≥ 1.000 and the MFI/lowest rank antigen bead (MFI/LRA) ratio above the test-specific cut-off value.

For the final DSA assignment, both donor and recipient HLA-genotyping results were considered.

Table 1. The list of broad and associated split HLA antigens determined according to the HLA data dictionary from the IPD-IMGT/HLA database (available at https://hla.alleles.org/antigens/broads_splits.html)

HLA-A		HLA-B		HLA-B *(continued)*		HLA-DR		HLA-DQ	
broad	**split**	**broad**	**split**	**broad**	**split**	**broad**	**split**	**broad**	**split**
A1		B5	B51	B22	B54	DR1		DQ1	DQ5
A2			B52		B55	DR103			DQ6
A3		B7			B56	DR2	DR15	DQ2	
A9	A23	B8		B27			DR16	DQ3	DQ7
	A24	B12	B44	B35		DR3	DR17		DQ8
A10	A25		B45	B37			DR18		DQ9
	A26	B13		B40	B60	DR4		DQ4	
	A34	B14	B64		B61	DR5	DR11		
	A66		B65	B41			DR12		
A11		B15	B62	B42		DR6	DR13		
A19	A29		B63	B46			DR14		
	A30		B75	B47		DR7			
	A31		B76	B48		DR8			
	A32		B77	B53		DR9			
	A33	B16	B38	B59		DR10			
	A74		B39	B67					
A28	A68	B17	B57	B70	B71				
	A69		B58		B72				
A36		B18		B73					
A43		B21	B49	B78					
A80			B50	B81					

Statistical Analysis

Data were presented as absolute numbers and percentages for categorical variables and as median and interquartile range for continuous variables. The frequencies of HLA antigen MM and eplet MM between recipient-donor pair were obtained by direct counting. Pearson correlation coefficient was used to analyse the degree of association between HLA antigen MM and total number of eplet MM. The difference in means between the two groups was analysed by Student's t-test and ANOVA One-Way Analysis of Variance test. A two-tailed p-value <.05 was used as an indicator of statistical significance. All analyses were carried out using MedCalc software (version 19.2.6).

RESULTS

The mean follow-up time after transplantation was 76.8 months (range 7-134). At Ktx, all recipients were non-sensitised (negative for the presence of HLA antibodies).

The recipient-donor HLA antigen MM was evaluated. The highest number of HLA split antigen MM was observed in HLA class I loci where 39 (83%) of recipient-donor pairs had a MM at HLA-A locus and 43 (91%) at HLA-B locus. For HLA class II loci, 39 (83%) recipient-donor pairs had a MM at HLA-DR locus and 29 (62%) had a MM at HLA-DQ locus. Further, the recipient-donor pair HLA total eplet MM was evaluated. For HLA class I number of eplet MM was 405 with a median 9 (range 0-21) while for HLA class II number of eplet MM was 548 with a median 13 (range 0-27). Most eplet MM were found in HLA class II molecules and more specifically in the DRB1 molecule. Pearson correlations coefficient indicates strong positive association between the number of HLA antigen MM and number of total eplet MM both for HLA class I ($r = 0.5905$, $p<0.0001$) and class II ($r = 0.8139$, $p<0.0001$) (Figure 1). The number of antigen MM at the individual loci also correlated highly with the number of total eplet MM at each HLA antigen with Pearson correlations coefficient 0.6708 for HLA-A ($p<0.0001$), 0.5424 for HLA-B ($p<0.0001$), 0.7545 for HLA-DRB1 ($p<0.0001$) and 0.8277 for HLA-DQB1 ($p<0.0001$) (Figure 2).

Post-transplant sera samples obtained from the 47 Ktr were assessed for the presence of HLA antibodies. Frequency of HLA antibodies detected among 47 Ktr is shown in Figure 3a. A total of 19 (40%) recipients developed DSA (DSA+ group) during follow-up time, 7 (15%) recipients were positive for non-DSA HLA antibodies and remaining 21 (45%) recipients tested negative for HLA antibodies (DSA- group). Among DSA+ recipients (N=19), three patients (16%) developed only HLA class I antibodies, 4 patients (21%) developed both HLA class I and class II antibodies,

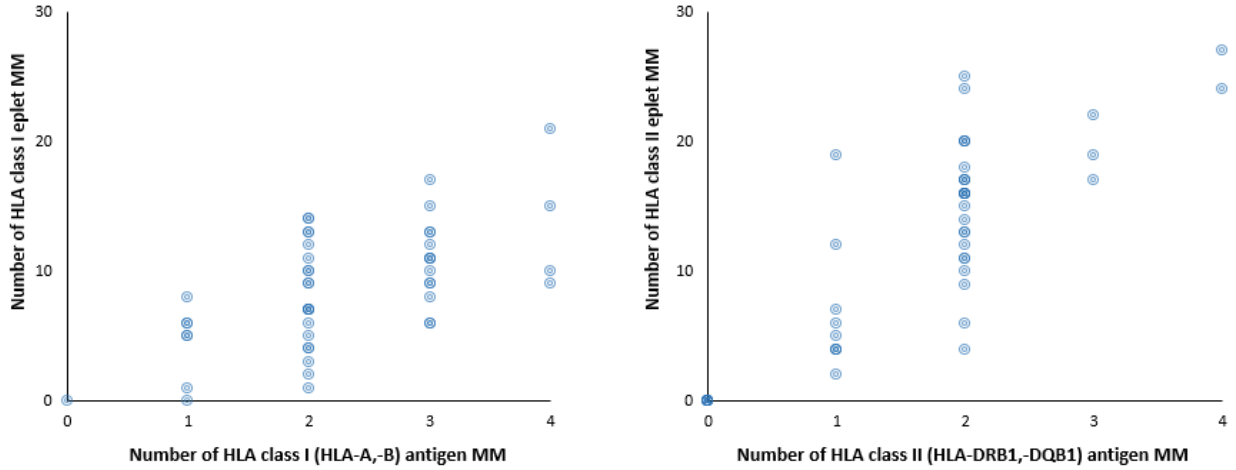

Figure 1. Positive correlation between total eplet mismatch (MM) load per number of HLA antigen mismatches for HLA class I (HLA-A and -B) and HLA class II (HLA-DR and -DQ) loci among kidney transplant recipients (N=47). By increasing the number of HLA antigen MM (both HLA class I and HLA class II), the number of HLA eplet MM increases proportionally.

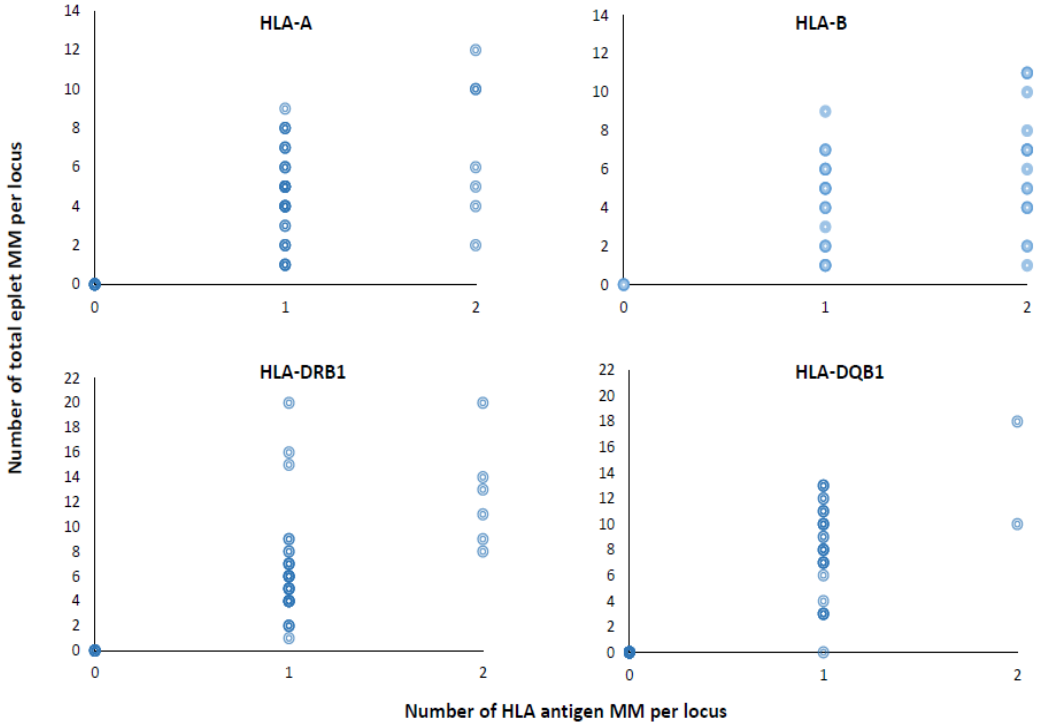

Figure 2. Scatter diagram of total eplet mismatch (MM) load per number of HLA antigen MM for HLA-A, B, DRB1, and DQB1 loci among kidney transplant recipients (N=47). Positive correlation between HLA antigen MM and total eplet MM is also seen at each individual locus.

while most of the recipients (12 patients, 63%) developed antibodies only against HLA class II (Figure 3b). All HLA loci MM contributed to the development of HLA locus-specific antibodies. However, HLA-DQ sensitization predominated, as 41% of recipients with HLA-DQ MM developed DSA, followed by 10%, 7% and 18% of recipients who developed DSA due to HLA-A, B and DR MM, respectively (Figure 4).

Analysis of the total number of HLA class I eplet MM and HLA class II eplet MM with development of DSA was also investigated. Graphical representation of data shows that an increasing number of eplet MM is in correlation with the higher DSA development rate, especially for HLA class II DSA (Figure 5). HLAMatchmaker analysis for HLA-DR/DQ eplet MM showed statistically significant difference (P=0.0004) between DSA- (N=33) and DSA+ recipients (N=14) for a total number of eplet MM (300 in DSA- vs 248 in DSA+) and for a median value of eplet MM (9 in DSA- vs 16.5 in DSA+).

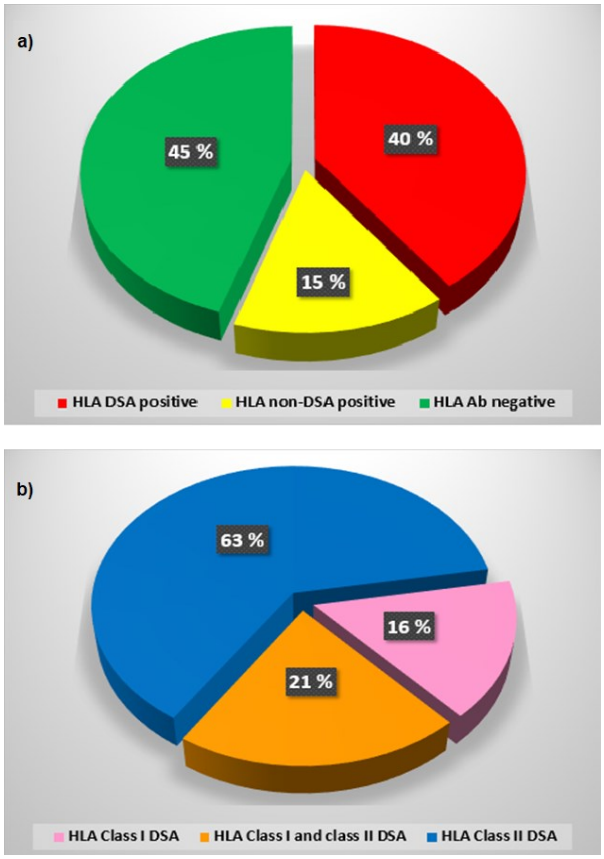

Figure 3. Post-transplant monitoring of HLA antibodies: a) frequency of HLA antibodies (Ab) detected among 47 kidney transplant recipients; b) distribution of defined HLA donor-specific antibodies (DSA) detected in the group of DSA positive kidney transplant recipients (N=19).

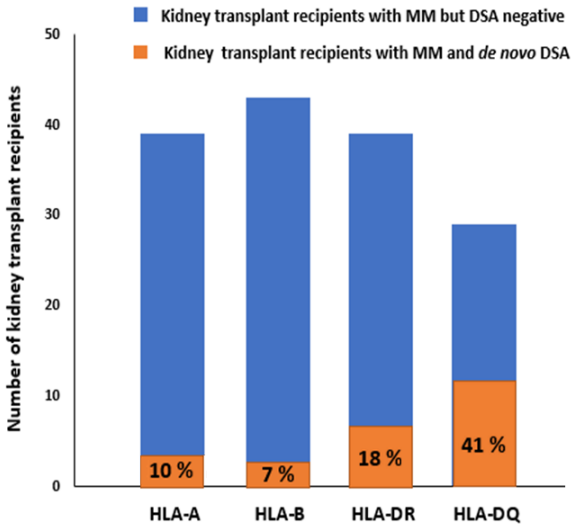

Figure 4. Distribution of *de novo* donor-specific HLA antibodies (DSA) in kidney transplant recipients with HLA mismatch (MM).

DISCUSSION

In this study, we have analysed sera from 47 recipients that received the first kidney transplant with low immunological risk and no pre-formed DSA. The goal was to screen for HLA DSA and to define which HLA loci MM contributed more to the development of DSA. Firstly, we observed more HLA split antigen MM in HLA class I than in HLA Class II between recipients and their donors in our study group, in contrast to the number of eplet MM, which was higher in HLA class II. This could be explained by the higher rate of polymorphism in HLA class I than HLA class II and by the fact that individual epitopes can be shared between different HLA alleles.[14] Still, positive correlation between the number of HLA class I and HLA class II antigen MM and HLA eplet MM was observed.

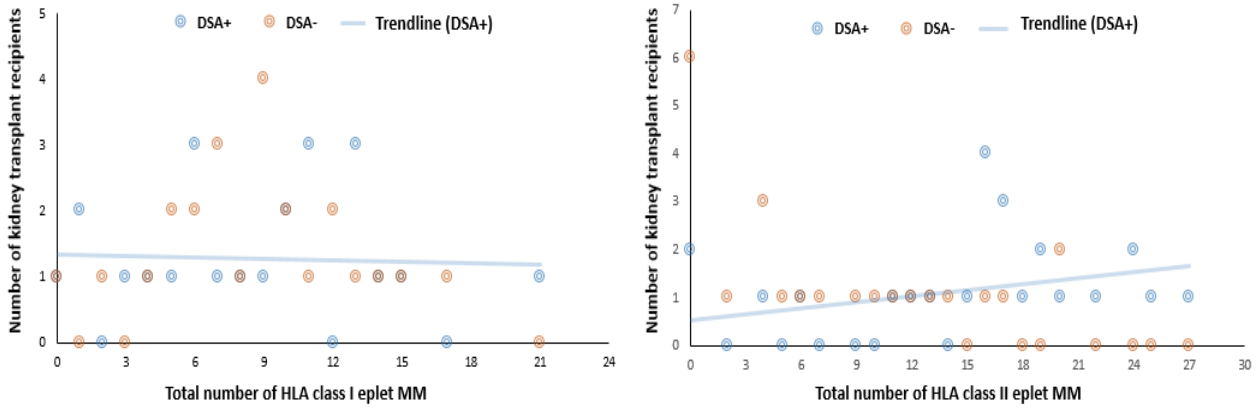

Figure 5. Relationship between the total number of HLA class I eplet mismatches (MM) and HLA class II eplet MM with development of *de novo* donor-specific HLA antibodies (DSA) among kidney transplant recipients (N=47). A stronger correlation was observed for HLA class II eplet MM where a higher number of eplet MM means a greater proportion of patients developing de novo DSA.

Secondly, we demonstrated that recipients of HLA class II incompatible grafts were at a higher risk for DSA development compared to the recipients of HLA class I incompatible grafts as 59% of HLA class II MM patients developed DSA in contrast to the 17% of DSA+ recipients within HLA class I MM group. This is in concordance with previous studies.[15, 16] Lee et al. showed that HLA-DQ DSA was the most detected type of DSA in a group comprised of previously non-sensitized patients and claim it could be explained by the high polymorphism of the genes which encode the DQ molecule. They also noticed that HLA-DQ was most accompanied by DSA against HLA-DR, which we also confirmed in our study. This is explained by the strong linkage disequilibrium between HLA-DQ and -DR locus.[16] Ntokou et al. also show that when there was HLA class II incompatibility, antibodies that appeared first in the circulation were HLA-DQ graft specific. They conclude that the high incidence of HLA-DQ antibodies is related to the high number of polymorphic epitopes that are expressed on both α and β chains of the HLA-DQ molecule unlike the HLA-DR antigens, which are polymorphic only in the β chain.[17]

Finally, we did the HLA eplet analysis using the HLAMatchmaker software and found that eplet MM in HLA class II were in stronger correlation with DSA formation than eplet MM in HLA class I. This is in accordance with the study of Senev et al. as they show that higher HLA-DQ eplet MM load is in positive correlation with an increased risk for the development of DSA and that MM in other loci seem to have a smaller effect.[18]

The limitation of our study is the small sample size, missing HLA-DQB1 typing results for some recipients and donors and typing at the low resolution level. Additionally, DSA were not monitored periodically, thus the time point of their occurrence cannot be determined. All these parameters are expected to be overcome in future, preferably by including our patient cohort in a large multicentre international study.

CONCLUSION

The results are pointing to the importance of HLA class II matching in Ktx as recipients of HLA class II MM grafts developed DSA more frequently than those receiving HLA class I incompatible grafts. The results also suggest that there is a positive correlation between antigen MM and eplet MM pointing to the HLA matching on the eplet level as a potential strategy to decrease formation of DSA and thus improve kidney allocation strategy.

Furthermore, the advance in the understanding of parameters that influence DSA formation can help clinicians to better calculate patient's immunologic risk and to guide clinical management.

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