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Brief communication

The clinical significance of epitope mismatch load in kidney transplantation: A multicentre study

Liesbeth Daniëls[a,*], Maarten Naesens[d,e], Jean-Louis Bosmans[b], Daniel Abramowicz[b], Evi Nagler[c], Steven Van Laecke[c], Patrick Peeters[c], Dirk Kuypers[d], Marie-Paule Emonds[a,e]

[a] Histocompatibility and Immunogenetics Laboratory (HILA), Red Cross-Flanders, Mechelen, Belgium
[b] Department of Nephrology, Antwerp University Hospital, Edegem, Belgium
[c] Department of Nephrology, Ghent University Hospital, Ghent, Belgium
[d] Department of Nephrology and Renal Transplantation, University Hospitals Leuven, Leuven, Belgium
[e] Department of Microbiology and Immunology, KU Leuven, University of Leuven, Belgium

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ABSTRACT

Since the advent of kidney transplantation a key strategy for maximising graft survival by avoiding allorecognition has been to minimise HLA mismatching between donor and recipient. As HLA antibodies are now recognised as being specific for epitopes and donor-recipient HLA mismatch at the amino acid level can now be determined, HLA epitope mismatch load could be a better predictor for dnDSA development than classical HLA antigen mismatch calculation. This hypothesis has been investigated by other studies and the aim of our multicentre study was to confirm this observation in our population. Two algorithms, HLAMatchmaker and PIRCHE-II, were used to determine the HLA epitope mismatch load between donor and recipient. We have shown a significant association between the number of HLA epitope mismatches and the development of dnDSA and we have confirmed the earlier observations.

1. Introduction

Kidney transplantation is the treatment of choice for most patients suffering from end-stage renal disease (ESRD). HLA disparity between donor and recipient affects transplant immunity and consequently has an impact on graft outcome. One of the alloimmune mechanisms is the development of de novo donor specific antibodies (dnDSA), which represents a risk factor for humoral transplant rejection. This antibody-mediated rejection (ABMR) is a major cause of premature graft loss in kidney transplantation [1].

Currently, the Eurotransplant Kidney Allocation System (ETKAS) is based on HLA-A, -B broad and HLA-DR split antigen matching. Transplants with zero HLA-A, -B and -DR mismatches have superior outcomes compared with outcomes for grafts with one or more HLA mismatches. Nevertheless, a significant proportion of these transplants fail prematurely, possibly reflecting potential allorecognition of donor incompatibilities. This may be due to different alleles of HLA-A, -B and -DR loci or differences in antigens at other loci, such as HLA-C, -DQ and -DP. On the other hand, many HLA-mismatched grafts have excellent graft outcomes, suggesting that certain HLA mismatches may be permissible [2].

Another model of matching, considering cross-reacting groups (CREGs), may increase the probability of identifying more compatible kidneys. HLA antigens comprise multiple epitopes made of polymorphic amino acid residues and it is these structures and their conformation that determine antibody accessibility, recognition, and subsequent reactivity. CREGs share one or more epitopes and immunisation against an HLA antigen often results in antibodies that bind not only to the immunising antigens, but also to sets of structurally similar antigens. However, the true efficacy of CREG matching in cadaveric kidney transplantation is controversial [2,3].

Current methods using single antigen bead assays for “high resolution” antibody detection in combination with high resolution typing (or intermediate interpretation of low resolution data) allow better identification of B cell epitopes, initially based upon serologic cross-reactivity patterns (CREGs). A computer algorithm HLAMatchmaker developed by R. Duquesnoy defines the number and B cell functional epitope mismatches between donor and recipient, the so-called mismatched eplets or parts of the B cell epitopes that trigger an antibody response. It regards each mismatched HLA antigen as a three-dimensional structure of polymorphic amino acid configurations in antibody-accessible positions and the mismatches are determined by the

* Corresponding author.
E-mail address: liesbeth.daniels@rodekruis.be (L. Daniëls).

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recipient's HLA type, representing a repertoire of self-epitopes for which no antibodies can be made. It has been shown by several cohort studies that the number of eplet mismatches is associated with the development of dnDSA and premature graft loss [4].

Besides B cell epitopes, T cell epitopes may also play a role in antibody formation. DSA production occurs via the indirect allorecognition pathway in which foreign HLA is processed by the recipient's antigen-presenting cells and presented by HLA class II to CD4+ T cells, followed by B cell activation with antibody production. As such, HLA-derived T-cell epitopes, designated as PIRCHE-II (Predicted Indirectly ReCognizable HLA Epitopes presented by HLA class II molecules), also play a role in the generation of DSA. The group led by E. Spierings has shown that the PIRCHE-II score predicts the incidence of dnDSA and graft failure after kidney transplantation [5,6,7].

The aim of our multicentre study was to perform an independent confirmation of earlier observations that HLAMatchmaker and PIRCHE-II scores may be a better predictor for dnDSA formation than classical HLA antigen matching.

Here, we compared conventional HLA antigen mismatch assessment, with the algorithms HLAMatchmaker (www.epitopes.net) and PIRCHE-II (www.pirche.org) to predict dnDSA development [5,6,7,8].

2. Materials and methods

2.1. Patient population

This study was performed in the HLA laboratory of Red Cross Flanders (Histocompatibility and Immunogenetics Laboratory (HILA), Mechelen, Belgium). This is the only EFI accredited HLA laboratory in Flanders for organ transplantation and provides services for the kidney transplantation centres of the Antwerp University Hospital, the Ghent University Hospital and the University Hospitals Leuven.

We only included kidney transplant patients who were on the Eurotransplant list waiting for a second transplantation, and who had Luminex antibody testing performed in 2017. We specifically selected retransplant candidates, regardless of the cause of previous graft failure. Many retransplant candidates have HLA antibodies when back on the waiting list for a second graft, and these antibodies originate from HLA mismatching with the first kidney donor. This cohort selection increases the power of the analyses, because assessing the association between HLA mismatches and de novo antibody occurrence after transplantation in a cohort with first transplantations would lack power and require a very large data set.

We only included patients without pre-existing HLA antibodies prior to the first graft and who had complete HLA typing (HLA-A,-B,-C,-DR,-DQ) available for both the patient and the first kidney donor (35 deceased donors and 1 living related donor). Patients were transplanted between 1985 and 2016. Before 2008 pre-transplant screening was not performed with Luminex technology but with CDC (Complement-Dependent Cytotoxicity, 14 patients) or Enzyme-Linked-Immunosorbent assay (ELISA, 3 patients). Admittedly, CDC and ELISA are less sensitive than the Luminex Single Antigen test in detecting dnDSA. As CDC screening has a very low sensitivity, only men (12 patients), and women (2 patients) without a history of pregnancy, were included. A total of 36 patients were enrolled in the study (Table 1). Twenty-six patients (72%) developed dnDSA. For 33 patients DSA detection was performed at least 6 months after graft loss.

2.2. HLA genotyping and antibody testing

HLA-A,B,C,DR,DQB1 genotyping was performed with Immucor LIFECODES® HLA-SSO (sequence-specific oligonucleotides, intermediate resolution) or Olerup® HLA-SSP (sequence-specific primers, low resolution) kits. We used EpViX (www.epvix.com.br, version 2.4) to convert low resolution HLA typing (HLA-SSP) into the most likely high-resolution typing, based on common associations of HLA-B-C and HLA-DR-DQ and population frequency databases from NMDP and IMGT. HLA antibody evaluation of all patient samples was performed with Immucor LIFECODES® LifeScreen Deluxe kits. A positive screening for the presence of circulating HLA antibodies was followed by HLA antibody identification with LIFECODES® LSA (Luminex Single Antigen) kits. All tests were performed and interpreted according to the manufacturer's instructions. All serum samples were treated with EDTA to eliminate the prozone effect.

Table 1
Patient characteristics ($N = 36$).

Parameter	Median (range or percentage)
Age at time of first transplant	39 (4–71)
Female gender	13 (36%)
HLA antibody positivity prior to the first transplantation	0 (0%)
Time between transplantation and graft loss (years)	4 (0.01–24)
De novo DSA positivity, N (%)	26 (72%)
HLA class I, N (%)	15 (58%)
HLA class II, N (%)	24 (92%)
HLA class I + II, N (%)	13 (50%)
HLA-DQ, N (%)	21 (81%)
HLA antigen mismatches (A-B-DR)	3 (0–6)
HLA antigen mismatches (A-B-C-DR-DQ)	5 (0 − 10)
HLAMatchmaker score	28 (0–62)
PIRCHE-II score	48 (0 − 130)

2.3. HLAMatchmaker and PIRCHE-II analysis

We used HLAMatchmaker version 02 [8] to determine the number of non-self-B-cell-functional-epitope mismatches against the background of self-epitopes, and thus to calculate the number of eplet mismatches or the HLAMatchmaker score. The PIRCHE-II algorithm version 2.5 [6] was used to count the number of mismatched HLA-derived epitopes that are involved in indirect T cell alloimmune responses and that are predicted to be presented in the peptide binding groove of HLA class II molecules, and thus to calculate the T cell epitope mismatch load or PIRCHE-II score.

2.4. Statistics

The relationship between dnDSA and HLA mismatch load (HLA-A,B,DR split antigen mismatch load; HLA-A,B,C,DR,DQ split antigen mismatch load; HLAMatchmaker score; and PIRCHE-II score) was investigated using logistic regression analysis. Groups were compared using unpaired t tests. Data are presented as median and range or percentage, as appropriate. All analyses were performed with GraphPad Prism version 7.00, GraphPad Software, La Jolla California USA, www.graphpad.com.

3. Results

The study cohort comprised 36 renal transplant patients with a median age of 39 (range 4–71) at the time of their first transplant. Seventy-two percent of patients ($N = 26$) developed dnDSA after the first transplant with anti-HLA-DQ as the predominant dnDSA ($N = 21$, 81%). Recipients received a transplant with a median cumulative A-B-DR mismatch of 3 (range 0–6) and A-B-C-DR-DQ mismatch of 5 (range 0–10). HLAMatchmaker scores ranged from 0 to 62 for the overall cohort with a median of 28. PIRCHE-II scores ranged from 0 to 130 with a median of 48. Patient cohort characteristics and parameters are summarised in Table 1.

Fig. 1 shows the correlation of the number of HLA antigen mismatches, HLAMatchmaker and PIRCHE-II scores with the detection of dnDSA. The effect of HLA-A,B,DR antigen mismatch load was

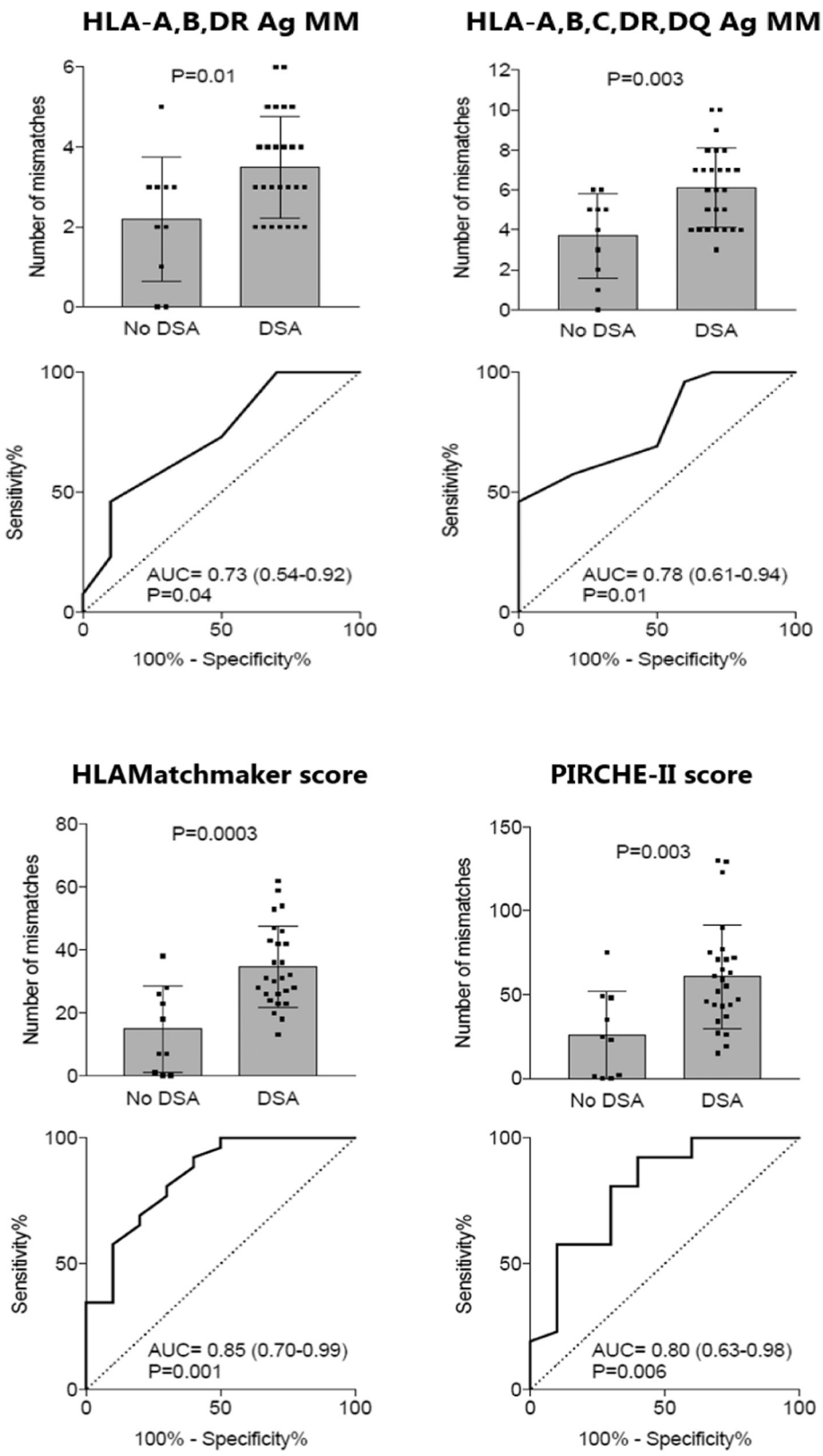

Fig. 1. Correlation of the number of classical HLA antigen (Ag) mismatches (MM), HLAMatchmaker and PIRCHE-II scores with dnDSA.

significant (AUC = 0.73, P = 0.01), but improved when HLA-C and HLA-DQ mismatch were added (AUC = 0.78, P = 0.003). PIRCHE-II scores (AUC = 0.80, P = 0.003) and HLAMatchmaker scores were the best predictors of dnDSA (AUC = 0.85, P = 0.003).

Given the dominance of anti-HLA-DQ antibodies in the de novo occurrence of HLA antibodies, we then focused on mismatches in the HLA-DQ locus. In this study no anti-HLA-DQA1 antibodies without anti-HLA-DQB1 DSA were detected. A wide variation in HLA-DQB1 HLAMatchmaker and PIRCHE-II scores were observed for the corresponding antigen mismatch (Fig. 2a). Fig. 2b shows the correlation of

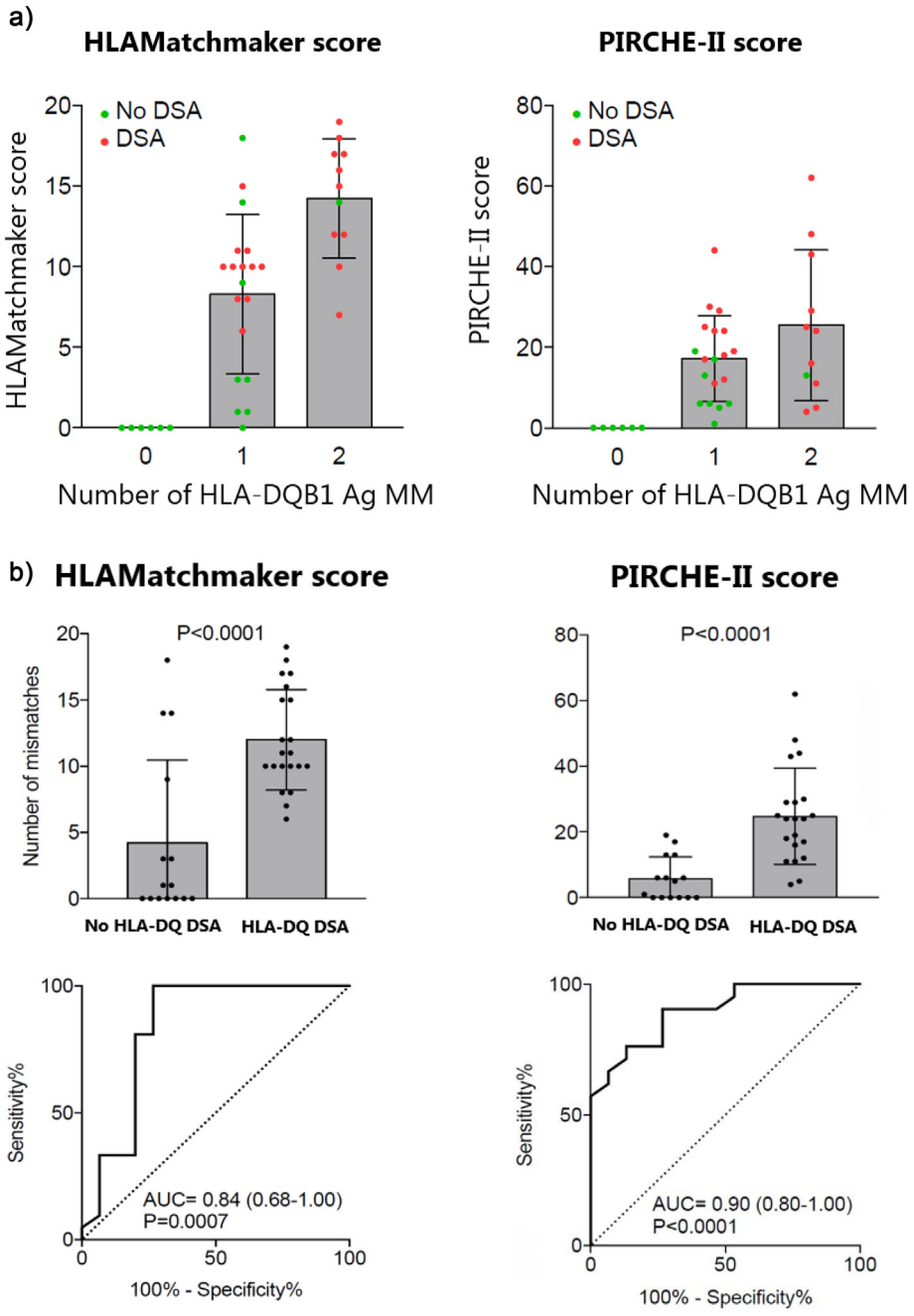

Fig. 2. Association between HLA-DQ mismatch and de novo anti-HLA-DQ antibodies.
2.a A wide variation in HLA-DQB1 HLAMatchmaker and PIRCHE-II scores for the corresponding antigen mismatch.
2.b Correlation of HLAMatchmaker and PIRCHE-II scores with de novo anti-HLA-DQ DSA.

HLA-DQB1 HLAMatchmaker and PIRCHE-II scores with the detection of de novo anti-HLA-DQ DSA. When the HLA-DQB1 HLAMatchmaker score was below 6, no de novo HLA-DQ antibodies were detected. In 84% of patients with an HLA-DQB1 mismatch load ≥6, de novo HLA-DQ DSA were present. As shown in Fig. 2a no upper limit could be defined; even up to an HLAMatchmaker score of 19 no dnDSA were observed.

For PIRCHE-II a stratification into low or high scores based on the first quartile and the third quartile of the HLA-DQB1 PIRCHE-II score could be performed. Below the first quartile de novo anti-HLA-DQ DSA was 0% and above the third quartile 100% in the group with one HLA-DQB1 antigen mismatch as shown in Fig. 2a.

4. Discussion

In this study, we described a significant association between the number of HLA epitope mismatches and the development of dnDSA. We showed that the epitope mismatch load was a better predictor than the classical HLA antigen mismatch calculation. HLA epitope mismatch assessment enhances the precision by quantifying the degree of similarity between donor/recipient HLA at the molecular level [9,10,11].

Because the majority of dnDSA in our study are anti-HLA-DQ antibodies and because DSAs directed against HLA-DQ are more frequently associated with antibody-mediated rejection and portend a poorer prognosis than DSAs against HLA class I [12,13], we also focused on the HLA-DQB1-specific epitope mismatch load. The number of these epitope mismatches was indeed highly predictive of HLA-DQ dnDSA

development post-transplant. We also confirmed, as shown in Fig. 2a, the observation of Lachman et al. [5] that the strongest impact of the PIRCHE-II score on differentiation into low- and elevated risk patients could be revealed for patients with one HLA-DQB1 mismatch.

Together with other studies illustrating the potential value of more detailed HLA mismatch analysis in kidney transplantation [4,5,7,9,10,11,12], our data suggest that the number of HLA epitope mismatches can be used as a risk assessment tool at time of transplantation and for post-transplantation follow-up, to evaluate those patients at the highest risk of HLA antibody formation. This could be especially valuable in young and paediatric kidney recipients, who will probably need more than one kidney allograft in their lifetime.

Although our study suggests that the total epitope mismatch load could be the determining factor in the risk of HLA antibody formation, it is clear that we lack data on the immunogenicity of the epitope mismatches. It could be that immunogenicity is not merely a quantitative issue, but that one or only a few epitope mismatches are sufficient to induce an antibody response. Maybe, the higher number of epitope mismatches only enhances the chance to include immunodominant epitopes [3]. High resolution level analysis of the antibody response is therefore required to further unravel the immunogenicity of epitopes in a given donor/recipient combination as well as a more detailed definition of the epitope mismatches. Although not analysed in this study, information on the ethnic background of patients versus donors is important when analysing epitopes and we acknowledge that this is a limitation of the study. It is known that individuals from different ethnic backgrounds, although having identical HLA antigens, can have a high prevalence of specific alleles that can be absent in other populations [4].

The strong association between the HLAMatchmaker and PIRCHE-II scores and the risk of dnDSA formation was observed despite the important heterogeneity in centre practice (e.g. of immunosuppression withdrawal and transplant nephrectomy), causes of graft failure and timing of the HLA antibody evaluation. This suggests that the HLA mismatch scores are indeed more important than the clinical intervention after graft loss in the development of dnDSA. Our limited study size does not allow this to be confirmed in multivariable analyses, and future research is warranted to evaluate the relative contribution of HLA mismatch scores and clinical interventions (transplant nephrectomy, immunosuppression withdrawal, etc.) to dnDSA formation.

In conclusion, we independently confirm earlier observations that HLAMatchmaker and PIRCHE-II scores are a better predictor for dnDSA formation after kidney transplantation than classical antigen mismatch analysis.

Disclosure

The authors of this manuscript have no conflict of interest to disclose as described by Transplant Immunology.

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