HLA epitopes in kidney transplantation: from basic science to clinical application

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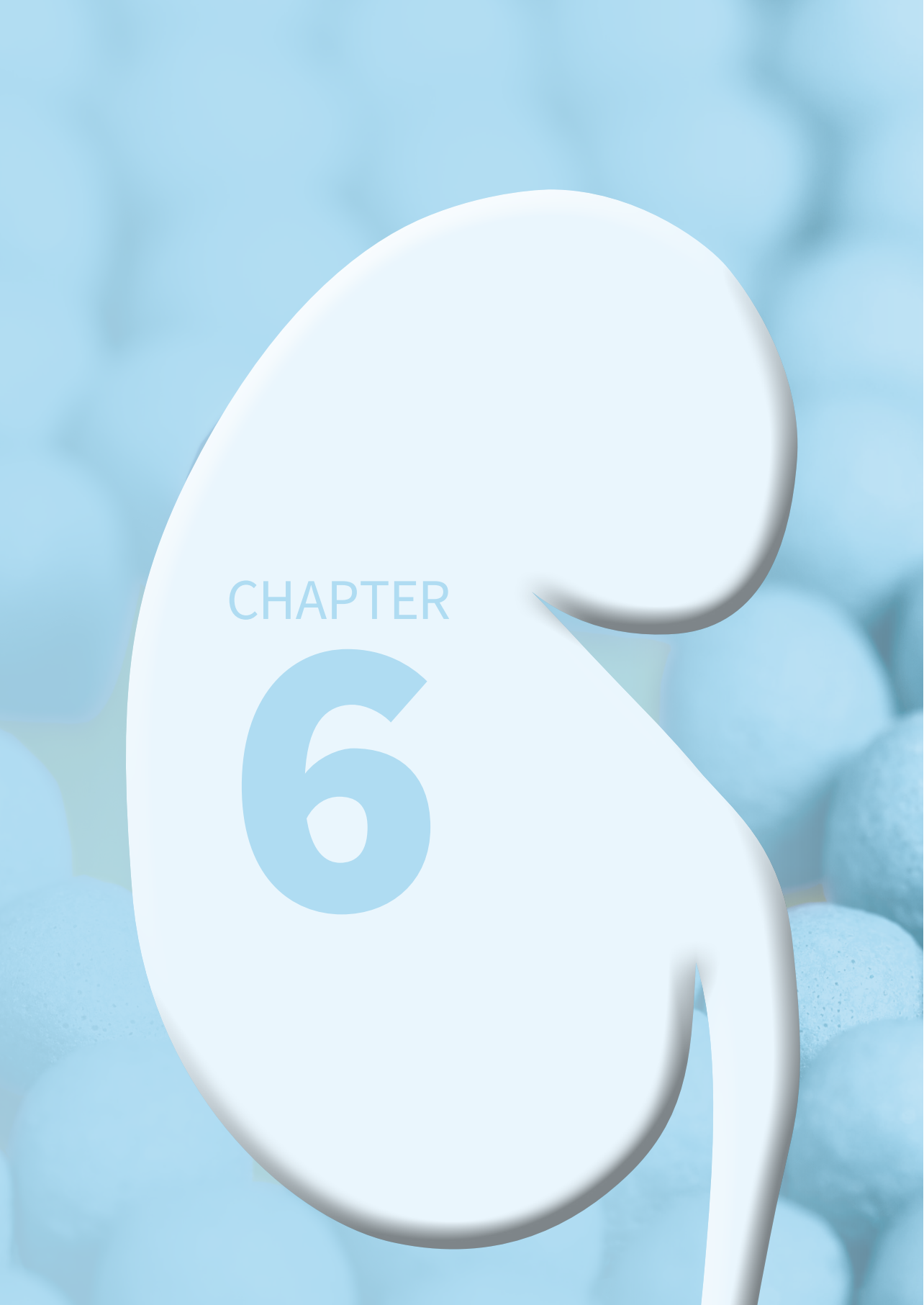
PART II

Clinical application of HLA epitopes in transplantation


CHAPTER
6

HLA solvent-accessible amino acid mismatch loads are associated with donor-specific antibody formation and graft failure after kidney transplantation

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ABSTRACT

HLA molecular mismatches as defined by eplet mismatch loads are associated with outcomes in solid organ transplantation. However, study results have been difficult to compare due to the changing definitions of HLA eplets. The HLA epitope mismatch algorithm (HLA-EMMA) determines solvent-accessible amino acid (saAA) mismatches between donor and recipient. Since the amino acid sequence of HLA alleles is a fixed entity, this approach will lead to more comparable results than HLA eplet analysis. Several studies have investigated the association between saAA mismatch loads and transplant outcomes. However, previous studies were small and were often based on low-resolution HLA typing. In the current study, a large cohort of over 900 kidney transplant recipients and donors with high resolution typing was investigated. Total saAA mismatch loads were associated with *de novo* donor-specific antibody formation and graft loss. In locus-specific analysis, HLA-DQ saAA mismatch load was correlated with the occurrence of anti-DQ donor-specific antibodies. Concluding, saAA mismatch analysis of kidney transplant recipients with HLA-EMMA provides a well-defined method for HLA molecular mismatch analysis.

1 INTRODUCTION

For decades, kidney transplantation has been the best treatment option for patients with end-stage renal disease. However, improvement in long-term graft and patient survival have decelerated since the last twenty years (1). Immunological rejection still is the main cause of graft loss, with antibody-mediated rejection being the dominant cause for late allograft failure (2, 3). Since the majority of kidney transplant recipients receives an HLA-mismatched graft, many patients are at risk for developing *de novo* donor-specific antibodies (dnDSA) directed against the mismatched HLA. dnDSA are induced upon interaction of recipient B cells with configurations of mismatched polymorphic amino acids on donor HLA, which are often referred to as eplets (4). HLA eplets have been theoretically defined based on the comparison of amino acid sequences of HLA alleles. Because these amino acid configurations are shared between different HLA alleles, HLA matching on the molecular level (i.e. eplets or amino acids) is likely to be a superior strategy to prevent dnDSA formation compared to matching on the antigen level. Indeed, several studies have shown that eplet mismatch load is associated with dnDSA formation, rejection and graft loss in kidney and other settings of solid-organ transplantation (5-10). However, because of changes of the definition of individual eplets, as well as the ongoing endeavor of experimental verification of clinically relevant eplets, the eplet repertoire has been subject to substantial change, which leads to difficulty in comparing studies (11).

Recently, the HLA epitope mismatch algorithm (HLA-EMMA) was developed. This freely available software determines HLA compatibility at the amino acid level by calculation of amino acid mismatches between recipient and donor. HLA-EMMA allows for assessment of the number of solvent-accessible amino acid (saAA) mismatches, which are the residues that can potentially interact with B cell receptors, and therefore induce an antibody response (12). Comparison of molecular mismatch analysis based on eplets, amino acids or electrostatic mismatch indicated that all three methods were significantly correlating with dnDSA formation (13). As amino acid sequences of HLA alleles are fixed entities, this type of immunogenicity analysis is more suitable for comparing different studies than eplets. Since the introduction of HLA-EMMA, several small studies have investigated the association between saAA mismatches and outcomes in cardiothoracic, liver, kidney and simultaneous pancreas/kidney transplant cohorts (14-18). The majority of these relatively small studies have assessed amino acid mismatches based on low or medium resolution HLA typed cells which have been translated to high resolution HLA typing. However, it has been shown that this method can be problematic when assigning dnDSA, which are the main outcome parameter in the majority of studies (19). Therefore, in the current study we used HLA-EMMA to analyze the association between saAA mismatches and dnDSA formation and graft loss in a large cohort of over 900 kidney transplant recipients with high resolution second field HLA typing.

2 METHODS

2.1 Patients

The cohort consists of all adult recipients of a single kidney transplant at University Hospitals Leuven between March 2004 and February 2013 and has been previously described elsewhere (20). Patients were treated with baseline immunosuppression primarily based on tacrolimus, mycophenolic acid and corticosteroids, with addition of induction therapy with basiliximab in higher-risk patients.

2.2 HLA genotyping and amino acid mismatch evaluation

Donor and recipient DNA samples were retrospectively genotyped at high resolution (second field) for 11 loci by next-generation sequencing (NGS). Genotyping of half of the donors was performed using the MIA FORA NGS FLEX 11 HLA Typing Kit (Immucor, Norcross, GA) on the MiSeq sequencing instrument (Illumina, San Diego, CA); the other half and all recipients were genotyped at a high-resolution level using the HiSeq sequencing system (Illumina). The high-resolution HLA genotypes were analyzed using the batch analysis option of HLA-EMMA version 1.04 (12). The total number of saAA mismatches were calculated for HLA class I and II separately, for each antigen separately, and for all loci together. Analysis of saAA mismatches was performed interlocus for HLA class I and intralocus for HLA class II.

2.3 Detection of donor-specific antibodies

Systematic monitoring for DSA was performed as previously described (20). In brief, pre- and post-transplant anti-HLA antibodies were monitored at day 0, at 3 months after transplantation, yearly after transplantation, and at time of an indication biopsy. All sera were screened using the Lifecodes LifeScreen Deluxe kit (Immucor) and, in case of positivity, specificity of HLA antibodies was assessed using Lifecodes Single Antigen Bead kits (Immucor). Antibodies against HLA-A, -B, -C, -DRB1, DRB345, -DQA1, -DQB1, -DPA1, and -DPB1 loci in the recipient sera were analyzed for donor specificity at the allelic level. The possible presence of DSA was indicated by a background corrected median fluorescence intensity value ≥ 500. For final DSA assignment, patients' sera reactivity was analyzed by taking into account both the donor and recipient HLA typing (21).

2.4 Statistical analysis

SAS software (version 9.4; SAS Institute, Cary, NC) and IBM SPSS Statistics version 25 (IBM Corp., Armonk, NY, USA) were used for statistical analyses. Patient characteristics were described by means and standard deviations for continuous variables and frequencies and percentages for categoric variables. The relationship of HLA saAA mismatch load with dnDSA formation and death-censored graft failure was statistically evaluated using univariable and multivariable Cox models. Patients were censored at the time of their last anti-HLA antibody measurement or at the experience of graft failure or at last clinical follow-up. All multivariable models were adjusted for donor and recipient sex, donor and recipient age, recipient race (white/nonwhite), recipient body mass index, donor type (living, brain death, or cardiac death), cold ischemia time, repeat

transplantation, and pre-transplant HLA antibodies (absence, non-DSA HLA antibodies, and DSA) to address confounding. Quantitative variables were included as continuous variables in the models. All tests were two-sided and P values < 0.05 were considered statistically significant.

Table 1. Main demographic, clinical characteristics, and follow-up of the study population (n=926).

Cohort Characteristics	Total (n=926)
Recipient demographics	
Female, n (%)	372 (40.2)
Age (yr), mean ± SD	53.8 ± 13.2
Donor demographics	
Female, n (%)	431 (46.5)
Age (yr), mean ± SD	47.9 ± 14.9
Living donor, n (%)	42 (4.54)
Cold ischemia time (h), mean ± SD	14.4 ± 5.50
Transplant characteristics	
HLA-A/B/DR split antigen mismatches (0–6), mean ± SD	2.70 ± 1.30
Total saAA mismatches, mean ± SD	45.4 ± 24.2
Total HLA class I saAA mismatches, mean ± SD	19.0 ± 10.9
Total HLA class II saAA mismatches, mean ± SD	26.5 ± 20.1
A saAA mismatches, mean ± SD	9.0 ± 7.9
B saAA mismatches, mean ± SD	5.4 ± 4.3
C saAA mismatches, mean ± SD	5.3 ± 4.4
DR saAA mismatches, mean ± SD	7.9 ± 8.4
DQ saAA mismatches, mean ± SD	13.6 ± 14.5
DP saAA mismatches, mean ± SD	5.1 ± 5.6
Pretransplant DSAs, n (%)	94 (10.2)
Post-transplant data	
Overall graft survival, %	57.7
Death-censored graft survival, %	82.5
dnDSAs, n (%)	43 (4.64)
HLA class I, n (%)	9/43 (20.9)
HLA class II, n (%)	32/43 (74.4)
HLA class I and II, n (%)	2/43 (4.7)

3 RESULTS

The main demographics and clinical characteristics of the study population have been previously described (20). In brief, 926 transplant pairs were evaluated, of which a total of 43 patients developed dnDSA during the median follow up of 7.5 years. dnDSA directed against HLA-DQ were most common (n=24). Mean saAA mismatch loads were 19 for Class I and 26.5 for Class II (Table 1). When considering the HLA loci separately, HLA-DQ had the highest number of saAA

mismatches (mean = 13.6). Cox analysis showed that total saAA mismatches and total class II saAA mismatches were independently associated with all dnDSA and HLA class II-specific dnDSA, respectively. Furthermore, HLA-DQ saAA mismatches correlated independently with HLA-DQ-specific dnDSA with an adjusted hazard ratio of 1.057 per single saAA mismatch (95% CI 1.029-1.085) (Table 2).

Table 2. Univariable and multivariable HRs for molecule-specific *de novo* DSA according to HLA solvent accessible amino acid mismatches in the complete cohort (n=926).

HLA saAA mismatches (HR per 10)	Patients at risk	Events	Univariable HR (95% CI)	p-value	Multivariable* HR (95% CI)	p-value
Total saAA MM	926	n=43	1.016 (1.003 – 1.028)	**0.01**	1.017 (1.004 – 1.030)	**0.01**
Total class I saAA MM	926	n=11	1.038 (0.985 – 1.094)	0.17	1.037 (0.982 – 1.095)	0.19
Total class II saAA MM	926	n=34	1.029 (1.013 – 1.045)	**0.0003**	1.031 (1.014 – 1.049)	**0.0003**
HLA-DQ saAA MM	926	n=24	1.050 (1.025 – 1.076)	**<0.0001**	1.057 (1.029 – 1.085)	**<0.0001**

HR, hazard ratio; saAA MM, solvent accessible amino acid mismatches
* Multivariable models were corrected for donor and recipient sex, donor and recipient age, recipient race, recipient body mass index, donor type, cold ischemia time, repeat transplantation, and pretransplant HLA antibodies (absence, non-DSA HLA antibodies, and DSA).

In a subsequent analysis, total saAA mismatches, HLA class I and HLA-B saAA mismatches were independently associated with death-censored graft loss (Table 3). Contrary to the previously performed eplet mismatch analysis, HLA-DQ saAA mismatches were only associated with death-censored graft loss with a borderline non-significant p-value of 0.07. When instead of death-censored graft loss the overall graft loss was analyzed in a multivariate model, HLA-DQ saAA mismatch did reach statistical significance (adjusted hazard ratio 1.008; 95% CI 1.001 – 1.015; p-value = 0.02).

Table 3. Univariable and multivariable HRs for death-censored graft loss according to HLA solvent accessible amino acid mismatches in the complete cohort (n=926).

HLA saAA mismatches (HR per 10)	Patients at risk	Events	Univariable HR (95% CI)	p-value	Multivariable* HR (95% CI)	p-value
Total saAA MM	926	162	1.008 (1.001 – 1.014)	**0.02**	1.008 (1.001 – 1.015)	**0.02**
Total class I saAA MM	926	162	1.018 (1.004 – 1.033)	**0.01**	1.019 (1.004 – 1.034)	**0.01**
HLA-A saAA MM	926	162	1.015 (0.995 – 1.034)	0.14	1.019 (1.000 – 1.040)	0.05
HLA-B saAA MM	926	162	1.052 (1.017 – 1.088)	**0.003**	1.040 (1.006 – 1.076)	**0.02**
HLA-C saAA MM	926	162	1.017 (0.983 – 1.054)	0.33	1.008 (0.972 – 1.046)	0.66
Total class II saAA MM	926	162	1.006 (0.998 – 1.013)	0.13	1.006 (0.998 – 1.014)	0.15
HLA-DRB saAA MM	926	162	1.007 (0.989 – 1.025)	0.50	1.006 (0.988 – 1.025)	0.51
HLA-DQ saAA MM	926	162	1.009 (0.999 – 1.019)	0.08	1.010 (0.999 – 1.020)	0.07
HLA-DP saAA MM	926	162	0.997 (0.970 – 1.025)	0.83	0.995 (0.967 – 1.023)	0.72

HR, hazard ratio; saAA MM, solvent accessible amino acid mismatches
* Multivariable models were corrected for donor and recipient sex, donor and recipient age, recipient race, recipient body mass index, donor type, cold ischemia time, repeat transplantation, and pretransplant HLA antibodies (absence, non-DSA HLA antibodies, and DSA).

4 DISCUSSION

This is the first study to investigate saAA mismatches as calculated by HLA-EMMA in a clinically well-defined large cohort of high resolution, second field HLA typed kidney transplant recipients with standardized post-transplant DSA monitoring. We demonstrate that HLA-DQ saAA mismatches correlated with HLA-DQ-specific dnDSA occurrence and total saAA and HLA-B mismatches correlated with death-censored graft loss. Notably, while HLA-DQ eplet mismatches were correlated with death-censored graft failure in the previous analysis of this cohort (20), HLA-DQ saAA mismatches just failed to reach statistical significance in our analysis ($p = 0.07$). However, when overall graft loss was analyzed, HLA-DQ saAA mismatches did correlate with graft survival. Since graft loss and death with functioning graft are competing risks, potentially affected by immunological damage or clinical decisions hereupon, analysis of overall graft loss can provide additional information on the role of molecular mismatch levels on clinical outcome.

Our results corroborate the earlier findings by Meneghini *et al.*, which showed that in a small population of 169 kidney transplant recipients HLA-EMMA scores predicted dnDSA formation, especially for HLA-DQ (18). Also in a cardiothoracic and a liver transplant cohort, HLA-EMMA derived saAA mismatches were associated with dnDSA, rejection and graft survival (16, 17). In a cohort of 113 simultaneous pancreas-kidney transplant recipients, an association between saAA mismatches and anti-HLA-DQ dnDSA was found, but this was not statistically significant (15). The lack of high resolution HLA typing and systematical detection of dnDSA, as well as the small study population might have influenced this result.

The outcomes in our study are in agreement with the previously published analysis of this cohort, which showed the association between antibody-verified HLA-DQ eplets and dnDSA occurrence, rejection and graft loss (20). The important difference between the two studies is that the previous analysis included only antibody-verified eplets, which are eplets that have proven to be able to interact with HLA-specific antibodies, while saAA mismatch analysis includes all solvent-accessible amino acid residues that could potentially be recognized by DSA. Hence, analysis with HLA-EMMA has lower specificity, since it includes residues that might not be clinically relevant, while analysis of antibody-verified eplets might dismiss clinically relevant eplets that have not been experimentally verified yet. Notably, hazard ratios for saAA mismatches are lower than for antibody-verified eplets, which is due to the higher number of possible amino acid mismatches compared to the number of eplets.

Strengths of this study are the large population of high resolution HLA-typed kidney transplant recipients and donors and systematic detection of HLA antibodies. Still, despite this large population, the association between saAA mismatches and dnDSA for other loci than HLA-DQ could not be assessed due to low occurrence of dnDSA and the subsequent lack of power.

Concluding, in this high-resolution typed cohort of kidney transplant recipients, total saAA mismatch loads were associated with dnDSA occurrence and graft loss. Furthermore, we demonstrated that HLA-DQ saAA mismatch load was correlated with the occurrence of anti-DQ dnDSA. Since there is an increasing number of studies investigating HLA molecular mismatches in transplantation, it is important that results can be compared and meta-analyses can be performed. Considering that amino acids sequences of HLA alleles are fixed, HLA-EMMA analysis provides a well-defined method to perform HLA molecular mismatch analysis that allows for comparison of study results.

Disclosures

The authors do not have any conflict of interest to disclose.

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