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**Which is the best predictor of *de novo* donor specific antibodies in a cohort of non-sensitized first kidney transplantation: antigenic, allelic, epitope or physiochemical HLA mismatches?**

**Running title:** Epitope and physiochemical HLA mismatches

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## **Abstract**

**Background:** Assessment of HLA matching by using high-resolution allele typing and knowledge of HLA molecule structure may lead to better prediction of *de novo* donor specific antibody (*dn*DSA) development.

**Methods:** We conducted a single-center cohort study among 150 non-sensitized first kidney transplant recipients to compare the association between antigenic (Ag), allelic (Al), eplet (Ep), amino acid (AAMS) HLA matching and electrostatic (EMS) and hydrophobic (HMS) mismatch scores and the development of *dn*DSA.

**Results:** After a mean follow-up time of  $49.3 \pm 17.7$  months, 18 patients (12%) developed *dn*DSA. The number of HLA mismatches (MM) was significantly associated with the development of *dn*DSA. The optimal threshold, determined by Harrell's C-index, varied according to the method (5 MM for Ag,  $p=0.006$ ; 6 for Al,  $p=0.009$ ; 22 for Ep,  $p=0.005$ ; 42 for AAMS,  $p=0.0007$ ; 45 for EMS,  $p=0.009$  and 44 for HMS,  $p=0.026$ ). C-indices were similar for all matching approaches, suggesting a similar prediction of *dn*DSA development.

**Conclusion:** In this cohort of low immunological risk transplant patients, the use of Al or Ep matching did not improve the prediction of *dn*DSA development in comparison with the traditional approach.

**Keywords:** HLA, kidney transplantation, epitope mismatches, physiochemical properties, donor specific antibody

## Introduction

While numerous factors influence long-term kidney graft survival, Human Leukocyte Antigen (HLA) matching is nevertheless of particular importance<sup>1</sup>. Recipients of a mismatched graft have an increased risk of developing donor-specific antibodies (DSA), which is an independent risk factor for acute and chronic antibody-mediated rejection (ABMR) and graft loss<sup>2</sup>. Furthermore, the development of DSA limits access to repeated transplantation due to long waiting list times in the case of highly sensitized patients. Due to the high polymorphism of HLA antigens, the chance of finding an unrelated fully matched kidney donor is very low. In France, more than ninety percent of transplant recipients in 2016 received grafts with one or more HLA mismatches based on HLA-A, -B, -DR and -DQ antigens<sup>3</sup>.

However, the probability of developing DSA or anti-HLA antibodies is not strictly proportional to the number of mismatched HLA antigens. Indeed, anti-HLA antibodies do not recognize whole HLA antigens but interact with a small set of amino acid residues in the structural epitope through six complementarity determining region (CDR) loops of the antibody<sup>4</sup>. Immunization by a single HLA antigen mismatch can result in the appearance of antibodies directed against numerous other HLA antigens due to the sharing of epitopes among different alleles. Conversely, the mismatched antigens may not necessarily present a "foreign" epitope and thus do not have the ability to induce the development of a DSA.

Several approaches have been described to determine the structural compatibility between donors and recipients. The available quaternary HLA molecule structures enabled Duquesnoy *et al.* to describe the eplet as a functional epitope bound by the CDR-H3 loop, which has a dominant role in determining antibody specificity<sup>5,6</sup>. The eplet is a cluster of polymorphic amino acids, either linear or discontinuous, within a 3-3.5 Å radius of an accessible sequence position with the ability of inducing an antibody response. For each mismatched donor HLA antigen, the latest version of HLA Matchmaker determines which eplet is non-self, compared with the repertoire of self-eplets of the recipients, resulting in a quantification of eplet mismatches. An important component of this algorithm is that antibodies cannot be induced against an eplet present on a self-HLA antigen.

More recently, Kosmoliaptsis *et al.* proposed that the relative antigenicity of an epitope could be predicted from the sequence of its amino acid residues and its physicochemical properties. They defined three physicochemical properties: the amino acid mismatch score (AAMS), the electrostatic mismatch score (EMS), and the hydrophobic mismatch score (HMS)<sup>7,8</sup>. Accordingly, the electrostatic difference between a non-self eplet and a self eplet may provide the trigger for B-cell activation.

Several studies have demonstrated significant correlations between eplet load (via HLA Matchmaker) of HLA mismatches and the development of DSA class I and class II as well as allograft outcomes<sup>9</sup>. However, to the best of our knowledge, only two recent studies, conducted by

Kosmoliaptsis *et al.*<sup>10</sup> and Wiebe *et al.*<sup>11</sup>, compared the two approaches provided by Duquesnoy *et al.* and Kosmoliaptsis *et al.*

In light of the above, the present study aimed to determine the best predictor of dnDSA development in non-sensitized first kidney transplant patients by estimating the associations between the number of antigenic mismatches (Ag MM), allelic mismatches (Al MM), Eplet mismatch score (Ep MM) as well as physiochemical properties of amino-acid sequences (via AAMS, EMS, and HMS) and the development of DSA class I and class II in a cohort of transplant recipients with low immunological risk.

## **Materials and Methods**

### ***Patient population and clinical data***

This single-center study included all patients aged >18 years, non-immunized, who received a first kidney transplantation from January 1, 2010 to December 31, 2014 in the Nancy University Hospital, Nancy, France. Patients were followed until January 1, 2017. All kidney transplantations (with living or deceased donor) were included. Recipients who had anti-HLA antibodies before transplantation, a kidney-pancreas transplant, an ABO-incompatible kidney transplant, other previous organ transplants or hematopoietic stem cell graft before kidney transplant were excluded (*Figure 1*). The transplantation allocation system followed the rules of the French national agency for organ assignment (Agence de la Biomédecine)<sup>12</sup>. Anonymized data were prospectively entered in a computerized database on day 0, at 3 months and 12 months after kidney transplantation, and annually thereafter.

Expanded criteria donors (ECD) were defined as: deceased donors aged 60 and older, and those aged over 50-59 years with at least two of the following three conditions: cerebrovascular cause of death, serum creatinine higher than 1.5 mg/dL (132.6  $\mu$ mol/L), or a history of hypertension. In our center, despite the absence of immunization, patients receive thymoglobulin as an induction treatment, in case of graft from an ECD, or in case of high-risk of delayed graft function (for example in the event of cardiac arrest with successful resuscitation). Otherwise, they receive either basiliximab, or no induction. Patients also received intravenous methylprednisolone intraoperatively, followed by tapering doses over the following days. Standard immunosuppression consisted of a calcineurin inhibitor (CNI), mycophenolate mofetil and prednisone.

### ***Renal biopsy***

In case of graft dysfunction, kidney allograft biopsies were performed and assessed by light microscopy and immunofluorescence. The graft biopsies were scored and graded by trained pathologists according to the international Banff criteria for kidney allograft transplantation<sup>13</sup>.

### ***HLA typing methods***

Recipient and living donor HLA typing was performed in the Histocompatibility Laboratory of Nancy on genomic DNA by a Luminex-based PCR-SSO typing method (LABType™ HD kit, One Lambda Inc., Canoga Park) according to the manufacturer's instructions. HLA typing of deceased donors was performed in each transplant center according to local practice. In Nancy, PCR-SSP typing methods were used (Micro SSP Generic HLA kit, One Lambda Inc., Canoga Park or Alow, Blow, DRBlow, DQlow kits, Olerup, Stockholm, Sweden).

Antigenic typing (serological nomenclature or the first set of digits of molecular HLA nomenclature) were converted into allelic typing (two first sets of digits of molecular HLA nomenclature) for the antigen with only one frequent allele in the European NMDP population (*Supplementary Table 1a and 1b*) while high definition typing was performed for the other HLA antigens (N= 31 HLA-A, 31 HLA-B, 81 HLA-DRB1 and 51 HLA-DQB1 high definition typing). Since HLA-C, -DRB3, -DRB4, -DRB5, -DQA1, -DPA1 and -DPB1 typing was not commonly performed during the study period and was not available for many included patients, focus was placed on HLA-A, HLA-B, HLA-DRB1, HLA-DQB1. For each locus, the number of Ag MM and AI MM mismatches was calculated using the serological nomenclature and the two first sets of digits of molecular HLA nomenclature, respectively.

### ***Eplet mismatch identification***

Eplet mismatch score (EpMM) was determined with the HLA Matchmaker program ([HLAMatchmaker](#), V02 for class I and V02.1 for class II) using the four-digit allele of each donor-recipient pair. This program analyzes donor and recipient HLA genotypes to generate a list of reactive eplets. The specific eplets found on the HLA molecules of the donor and the recipient are compared, within loci and across loci of the same class, leading to the calculation of the number of unique eplets found in the donor, but not in the recipient.

### ***Identification of amino-acid sequence and physicochemical disparities***

HLA immunogenicity of mismatches was analyzed by three properties, namely: amino acid mismatch score (AAMS), electrostatic mismatch score (EMS), and hydrophobic mismatch score (HMS), as described by Kosmoliaptsis *et al.*<sup>7,8</sup>. For each score, mismatched donor HLA was determined by performing inter- and intra-locus amino acid sequence comparisons between the donor and the recipient; using a freely available computer algorithm ([www.hlaimmunogenicity.org/download/Cambridge\\_HLA\\_Class\\_I\\_Immunogenicity\\_Algorithm.xls](http://www.hlaimmunogenicity.org/download/Cambridge_HLA_Class_I_Immunogenicity_Algorithm.xls)) and ([www.hlaimmunogenicity.org/download/Cambridge\\_HLA\\_Class\\_II\\_Immunogenicity\\_Algorithm.xls](http://www.hlaimmunogenicity.org/download/Cambridge_HLA_Class_II_Immunogenicity_Algorithm.xls)).

### ***Antibody monitoring***

Serum samples were collected at 0, 3, 6, 12 months and yearly thereafter, or at the time of biopsy when clinically indicated (presence of graft dysfunction or suspicion of rejection). Sera were screened for HLA-specific antibodies using solid-phase Luminex antibody-detection beads (LABScreen™ Mixed, One Lambda Inc., Canoga Park). Selected HLA-specific antibody-positive samples were analyzed using Luminex single-antigen class I and class II antibody-detection beads (LABScreen Single Antigen HLA Class I and Class II, One Lambda Inc Canoga Park). Specificities with mean fluorescence intensity (MFI) > 1000 were considered positive.

### ***Exposure to CNI***

Measurement of CNI (calcineurin inhibitor) exposure was identical to that described in Girerd et al.<sup>14</sup>. Briefly, blood concentrations of CNI were measured by the antibody-conjugated magnetic immunoassay (ACMIA) method using a Dimension® system (Siemens). Trough levels were measured at month 3, month 6, month 12, and annually thereafter. The mean absolute deviation percent (MAD%) formula was used as previously described by Shuker et al.<sup>15</sup>. The CNI Mean Absolute Deviation (%) was compared in the group of patients having developed dnDSA and those who did not during follow-up.

### ***Statistical analysis***

All analyses were performed using R software (the R foundation for Statistical Computing). The two-tailed significance level was set at  $p < 0.05$ . Continuous variables are described as means  $\pm$  standard deviation and median (interquartile range), and categorical variables as frequencies (percentages). Comparison of baseline characteristics and follow-up data according to development of dnDSA was carried out using the non-parametric Mann-Whitney test for continuous variables and Fisher's exact test for categorical variables. The number of epitope mismatches was compared among groups defined according the number of antigenic or allelic mismatches using the Kruskal-Wallis test. Survival analysis with dnDSA outcome was performed by the Kaplan-Meier method using the log-rank test for significance. Univariable and adjusted Cox proportional hazard models were used to assess the association between dnDSA development with antigenic, allelic and epitope mismatches and physiochemical scores. The adjustment variables included age, induction treatment and type of donor (living or deceased). Hazard ratios are presented with their 95% confidence intervals as HR (CI 95%). Harrell's C-index was used to determine the optimal threshold for each mismatch or score variables. The diagnostic performance of each mismatch variable dichotomized according to optimal threshold was assessed using Harrell's C-index with their respective confidence interval at 95%.

## Results

### *Baseline Characteristics of the Kidney Transplant Recipients and Donors (Table 1)*

A total of 398 transplantations were performed in our center between January 2010 and December 2014: 248 recipients were excluded (previous kidney (n=57), other organ (n=4) and HSC (n=1) transplant; ABO-incompatible kidney transplants (n=2), kidney-pancreas transplant (n=2), previously sensitized patients (n=182)). Hence, 150 non-sensitized first kidney transplantation recipients were eligible for the study (*Figure 1*).

Baseline characteristics of the included recipients and their donors are presented in *Table 1A*. The median recipient age was 52 years. Induction therapy with thymoglobulin (n=82) or basiliximab (n=58) was used in 93.4% patients. Standard immunosuppression therapy included a calcineurin inhibitor (tacrolimus or cyclosporine) and mycophenolate mofetil or azathioprine (n=3). Most patients also received low-dose prednisolone as long-term maintenance therapy.

### *dnDSA development and graft and patient outcomes during follow-up (Table 1)*

During a mean follow-up time of  $4.1 \pm 1.5$  years, 18 patients (12%) developed dnDSA (1 HLA class I dnDSA, 12 HLA class II dnDSA and 5 HLA class I and II dnDSA, *data not shown*). The proportion of living donors was similar in the two groups (33.3% in the groups with dnDSA vs. 37.9% in the group without dnDSA,  $p=0.22$ ). Of note, the proportion of diabetic patients was higher in the group who developed dnDSA, and mean BMI was significantly higher in this group. The proportion of patients who received thymoglobulin, basiliximab or no induction was similar between the two groups. Nevertheless, the majority of patients in the group who developed dnDSA were receiving cyclosporine (66.7%), whereas the majority of patients without dnDSA were receiving tacrolimus (63.6%). The CNI Mean Absolute Deviation (%) did not differ in the two groups ( $17.5 \pm 8.8$  vs.  $17.9 \pm 8.5$ ,  $p=0.71$ ).

Nine patients returned to dialysis during follow-up and 7 patients died with a functioning graft. Patients with dnDSA more frequently experienced graft rejection (55.6% vs. 24.2%,  $p=0.01$ ), and return to dialysis (22.2% vs. 3.8,  $p=0.013$ ). Of note, DSA was detected before the return to dialysis. No difference was observed with regard to patient survival.

### *Distribution of allelic, epitope and physiochemical mismatches within antigenic mismatch groups*

The use of the two first sets of digits of molecular HLA nomenclature to define allelic compatibility increased the number of antigenic mismatches of 11 donor-recipient pairs for class I and 53 for class II, mostly on the HLA-DR locus (*data not shown*).

As expected, the number of EpMM rose gradually with increasing number of AgMM and AIMM (*Figure 2*). In our cohort, the number of epitope mismatches varied between 0-31 for class I (vs. 0-4 with AgMM or AIMM) and 0-51 for class II (vs. 0-4 with AgMM or AIMM).



For the three physicochemical scores, AAMS, EMS and HSM, the range of values were respectively 0-61, 0-87 and 0-79 in class I, and 0-67, 0-85 and 0-63 in class II.

### ***Association between HLA mismatches and de novo DSA development***

The total number of MM (class I + class II) was significantly higher in the group of patients who developed dnDSA, irrespective of the method used (Table 2). When considering the total number of MM as a continuous variable, the latter was significantly associated with an increased risk of dnDSA development, even after adjustment for the recipient's age, induction treatment, and type of donor (living or deceased), irrespective of the method used (for AgMM-adjusted HR=1.66, p=0.004; for AlMM-adjusted HR=1.62, p=0.005; for EpMM-adjusted HR=1.04, p=0.01; for AAMS-adjusted HR=1.04, p=0.002; for EMS-adjusted HR= 1.02, p=0.004; for HSM-adjusted HR= 1.03, p=0.006) (Table 3). All approaches to evaluate compatibility (with or without adjustment) were significantly associated with the development of DSA during follow-up (which were mostly class II dnDSA) (*Supplementary Table 2*).

For each method, an optimal threshold of the sum of class I and class II mismatches was determined for the prediction of dnDSA development (5 for AgMM (p=0.006), 6 for AlMM(p=0.009), 22 for EpMM (p=0.005), 42 for AAMS (p=0.0007), 45 for EMS (p=0.009) and 44 for HSM (p=0.026)). The impact of a number of MM above the threshold value after adjustment for age, induction treatment and type of donor is presented in *Table 3*, with adjusted hazard ratios ranging from 3.22 to 6.85. *Figure 3* presents the probability of developing dnDSA when the number of MM was greater or lower than the optimal threshold value of the sum of class I and class II MM for each method.

### ***Which is the best predictor of de novo DSA development?***

The diagnostic performance of each mismatch variable (dichotomized according to the optimal threshold) was assessed using Harrell's C-index with their 95% confidence interval (*Table 4*). C-indices ranged from 64.1 to 70.4 (C-index of the sum of class I and II AAMS). Nevertheless, the standard antigenic approach yielded a C-index for dnDSA development prediction (C-index 68.0, p=0.0005) in a range similar to the allelic, epitope or physicochemical methods.

## Discussion

It is well established that the risk of allosensitization following kidney transplantation increases incrementally with the number of antigenic mismatches at individual HLA-A, -B, -C, -DR, and -DQ loci <sup>16</sup>. With the knowledge of HLA structure, new approaches such as epitope and physiochemical disparities have been described in order to take into account the differences in donor HLA immunogenicity according to the recipient's HLA type. The present study aimed to determine if one approach was superior to another in predicting dnDSA development in kidney transplantation.

In the present study conducted among homogenous cohort of non-sensitized first kidney transplant patients, the six different approaches (antigenic, allelic and epitope mismatches, as well as amino-acid sequence and physiochemical disparities (AAMS, EMS, HMS)) were compared for the assessment of HLA class I and class II immunogenicity. As detailed, our cohort was moderately well antigenically-matched with 61.3% and 32% of recipients presenting 3 or 4 antigenic mismatches for HLA Class I and II molecules, respectively. This observation is in accordance with the French allocation policy for deceased donor kidneys which promotes antigenic HLA-DR compatibility. At the allelic level, mismatching was greater than at the antigenic level with 65.3% of recipients presenting 3 or 4 allelic mismatches for HLA class I and 46.7% for HLA class II, particularly for HLA-DR. The results were expected and are explained by the presence, in the Caucasian population, of certain antigens (for example HLA-DRB1\*04, \*11, \*13 or HLA-DQB1\*06) with several high frequency alleles. Of note, numerous supplementary high definition HLA typing tests (N= 194) were performed herein in order to precisely define the allele rather than extrapolating the latter, which undoubtedly conferred an additional measure of robustness to our observations.

Although the AAMS (defined by Cambridge algorithm) and the number of epitope mismatches both reflect differences in amino sequence between donor and recipient, these two parameters do not yield the exact same information. The epitope mismatches only consider the number of polymorphic accessible amino acid residues at discontinuous positions of donor HLA that cluster together to form a potential epitope. In contrast, AAMS considers all amino acid differences between both donor and recipient HLA class I and class II molecules. Notwithstanding, these two parameters do not allow studying the physiochemical properties of the amino acid polymorphisms involved, while the specificity and affinity of antibody binding to target antigen is strongly influenced by electrostatic interactions; such interactions are determined by the number and polarity charges of amino acid chains. EMS and HMS, on the other hand, integrate information on the number of mismatched amino acids and the differences in electrostatic (EMS) and hydrophobic (HMS) charges between donor and recipient HLA class I and class II molecules <sup>7,8</sup>.

In our cohort, few patients developed de novo DSA (12%). This observation is in accordance with previous reports whereby Wiebe *et al.*<sup>17</sup> and Willecombe *et al.*<sup>18</sup> respectively observed 15% and 18% of dnDSA development. However, the low number of dnDSA of our cohort did not allow assessing the impact of HLA class I and HLA class II immunogenicity separately. For each of the six approaches, the significant total mismatch/score threshold on dnDSA onset was established, allowing a comparison between the different methods and illustrating the clinical applicability and relevance of these approaches.

As a result of the above analyses, a cut-off of 22 epitope mismatches was defined as a clinically relevant threshold for the risk of dnDSA development. Recent reports from Wiebe *et al.* focused on the development of DSA against HLA class II and the risk of graft loss according to the number of HLA mismatch eplets<sup>19,20</sup>. The optimal threshold for epitope mismatches on development of HLA Class II dnDSA was 10 for HLA-DR (including DRB1/3/4/5) and 17 for HLA-DQ (DQA1/B1). Such threshold was higher than in the present study and might be explained by the higher number of loci considered herein (HLA-DRB3/4/5, and HLA-DQA1 alleles in Wiebe *et al.* study). In effect, 17 eplets were defined on  $\alpha$  chain DQ (<http://epitopes.net>). Regarding class I immunogenicity, Silva *et al.* suggested a threshold of 6 HLA-B epitope mismatches for the risk of developing anti-HLA class I antibodies<sup>21</sup>. Overall, our epitope threshold is thus in keeping with previous reports.

Recently, Kosmoliaptsis *et al.* recorded the risk of allosensitization following a first renal transplant using three different approaches: eplet mismatches, AAMS and EMS<sup>10</sup>. The three approaches were independently associated with the risk of developing post-transplant HLA class I and class II specific antibodies. The authors furthermore did not demonstrate any advantage in using the eplet approach over enumerating the number of different amino-acids (AAMS) to assess the risk of DSA development. Wiebe *et al.* confirmed these results in a large cohort (N=596) evaluating the prediction capacity for the development of HLA-DR/DQ dnDSA<sup>11</sup>. Among the 6 approaches tested in the current study, the highest C-index was observed with the AAMS, albeit only marginally higher than that observed with the other techniques. Of note, the prediction capacity of the epitope approach was not strikingly higher. Importantly, in this low immunological risk cohort, the routine antigenic approach (with a threshold of 5 mismatches) was also very effective in predicting the development of dnDSA. However, our analyses were limited to the HLA-A, HLA-B, HLA-DRB1 and HLA-DQB1 loci, which are considered as the most crucial loci influencing transplant outcomes. The addition of HLA-C<sup>22</sup>, -DQA1<sup>23</sup>, -DPB1<sup>24</sup>, -DRB3/4/5<sup>25</sup> in future studies could help improve risk stratification.

The study of 11 loci is now easier with the marketing of fast typing kits that provide, within a few hours, a typing of all the loci (with notably HLA-C, HLA-DRB3, -DRB4, -DRB5, -DQA1, -DPA1 and -DPB1). However, to our knowledge, no fast HLA typing technique specifically aimed at HLA

typing of deceased donors is able to provide high-resolution typing (first two fields). For antigens that have a single allele with a very high frequency, regardless of the ethnic origin of the donor, it is possible to extrapolate the information obtained by these techniques as a result of 4 digits (first two fields). Conversely, it is not possible to extrapolate for antigens with numerous alleles with high and/or equivalent frequencies which may vary depending on the ethnic origin of the donor. However, the HLA typing resolution of deceased donors has increased with the latest versions of the fast typing kits. In living donor transplants, it is possible to determine the most compatible donor at the allelic, epitope and physiochemical level with high resolution typing techniques, such as NGS or PCR-SSO typing with high resolution kits, suitable for routine HLA typing in organ transplantation.

Certain limitations of this study should be acknowledged, the first of which being its observational single-center design. Secondly, protocol biopsies were not performed, nor was a biopsy systematically performed in instances of *de novo* DSA detection. Consequently, the number of antibody-mediated rejections observed in this study could have been underestimated. As a result, statistical analyses were only performed on the outcome of development of dnDSA. However, numerous study results have shown an association between DSA and hard evidence of injury <sup>26,27,28</sup>. Thirdly, certain factors that may contribute to *de novo* DSA development were not taken into account in this study, namely post-transplant pregnancies and transfusions. Maintenance immunosuppressive therapy (type and level of exposure) was also not taken into account in the multivariable analyses given that the CNI Mean Absolute Deviation (%) did not differ between the two groups (patient with or without dnDSA). However, the type of donor and the induction regimen were taken into account.

### **Clinical implications**

In all cases, when there is a choice to be made among several donors (in instances of living donor kidney transplant) or recipients (in instances of deceased donor transplant), we believe that it would be of interest to have all immunogenicity information. For instance, when two recipient-donor pairs have the same number of antigen mismatches, determining the number of epitope mismatches as well as the AMMS, EMS or HMS could help choose the best living donor or recipient in terms of immunological compatibility.

The main objective of all of these investigations is to minimize and anticipate the appearance of anti-HLA antibodies which could reduce the graft survival of the first graft <sup>26,27,28</sup>, but also decrease access to a second transplant. For immunized patients, the challenge is twofold. First, the graft should be as compatible as possible, similarly as in non-sensitized patients. Second, it should not contain epitopes against which the patient is already immunized. The epitope approach allows forbidding some epitopes, but also authorizes certain acceptable epitopes (which could have been forbidden by a traditional antigenic or allelic approach).

Ideally, as recently described by Wiebe *et al.*<sup>29</sup>, our capacity to predict the appearance of anti-HLA antibodies could allow adapting immunosuppressive treatments to each immunological risk and to minimize the risks of infections and neoplasia related to unnecessary over-immunosuppression. In this cohort of non-sensitized first graft recipients, we did not observe any single approach (antigenic, allelic, epitope, amino-acid or physicochemical disparities) to be more predictive than another. However, our results should be confirmed in a larger prospective cohort.

## **Conclusion**

HLA matching is one of the highlights of long-term research in the field of organ transplantation. To date, it remains unclear whether pre-transplantation epitope analysis or physicochemical mismatch analysis should be consistently incorporated into HLA-matching algorithms. In the present study including non-sensitized patients, a more physiochemically comprehensive approach including amino-acid and physicochemical disparities, as well as epitope and allelic analyses yielded similar prediction of the risk of DSA development as that observed with routine/classical approaches. Larger studies should confirm these results. Although no particular advantages of using each of these methods alone is demonstrated among non-sensitized patients with a low immunological risk, a prospective study should be conducted to assess whether implementing these various HLA mismatch algorithms as complementary tools would have an impact on the long-term prognosis of kidney transplant patients.

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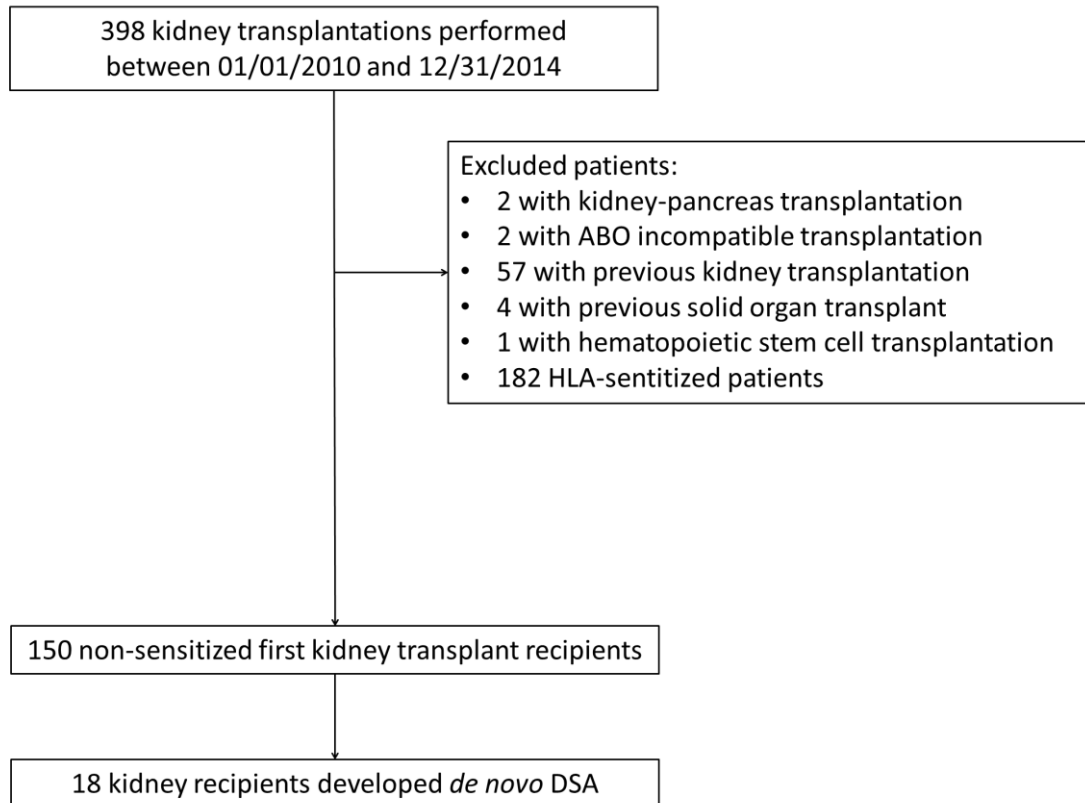
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## Figure Legends

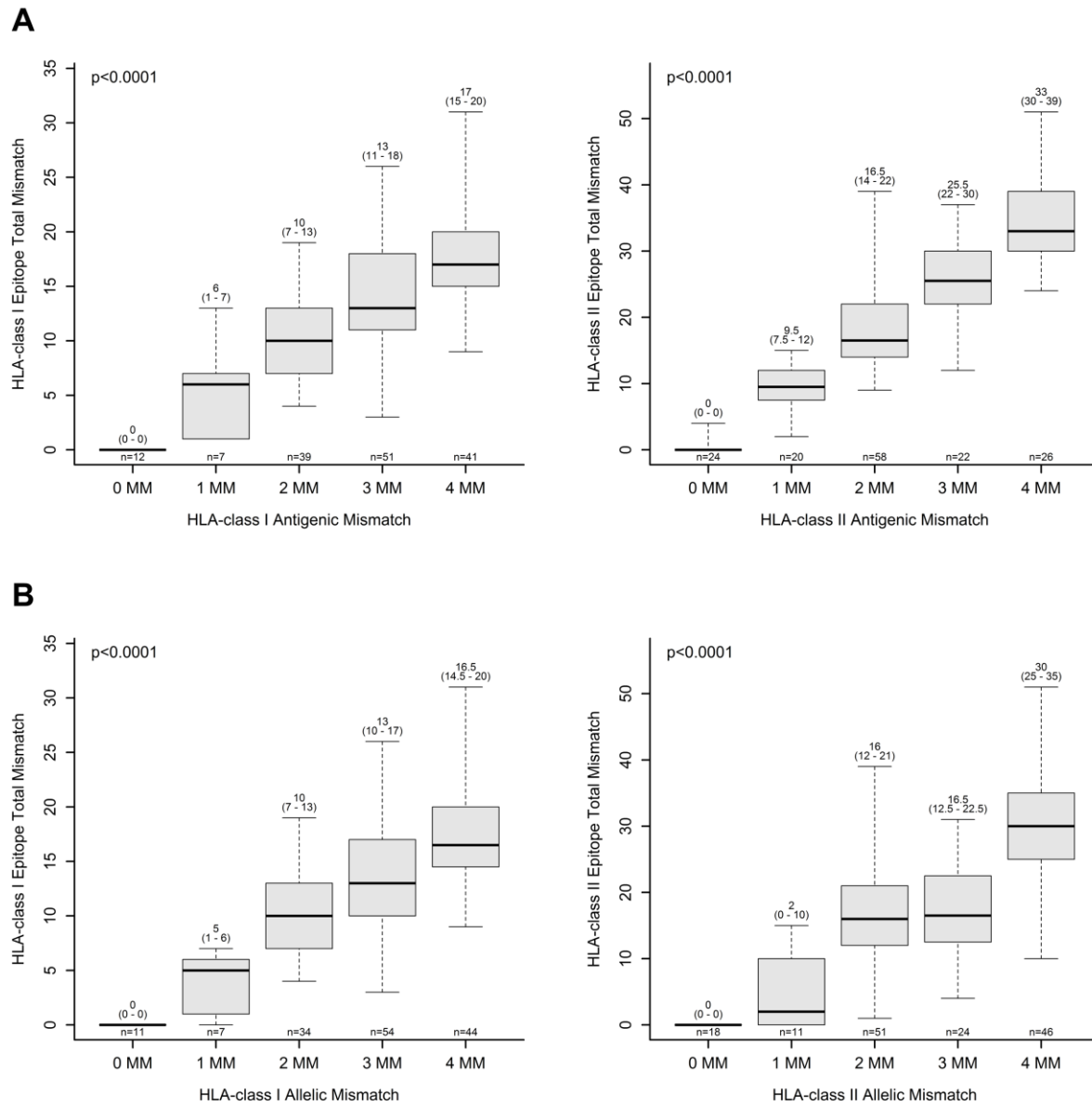
**Figure 1: Flow Chart**



**Figure 2: Distribution of epitope mismatches within antigenic (A) and allelic (B) mismatch groups.**

The band inside the box represents the median with the lower and upper portions representing the first and third quartiles, while the ends of the whiskers represent the minimum and maximum. Groups were compared using the Kruskal-Wallis test.

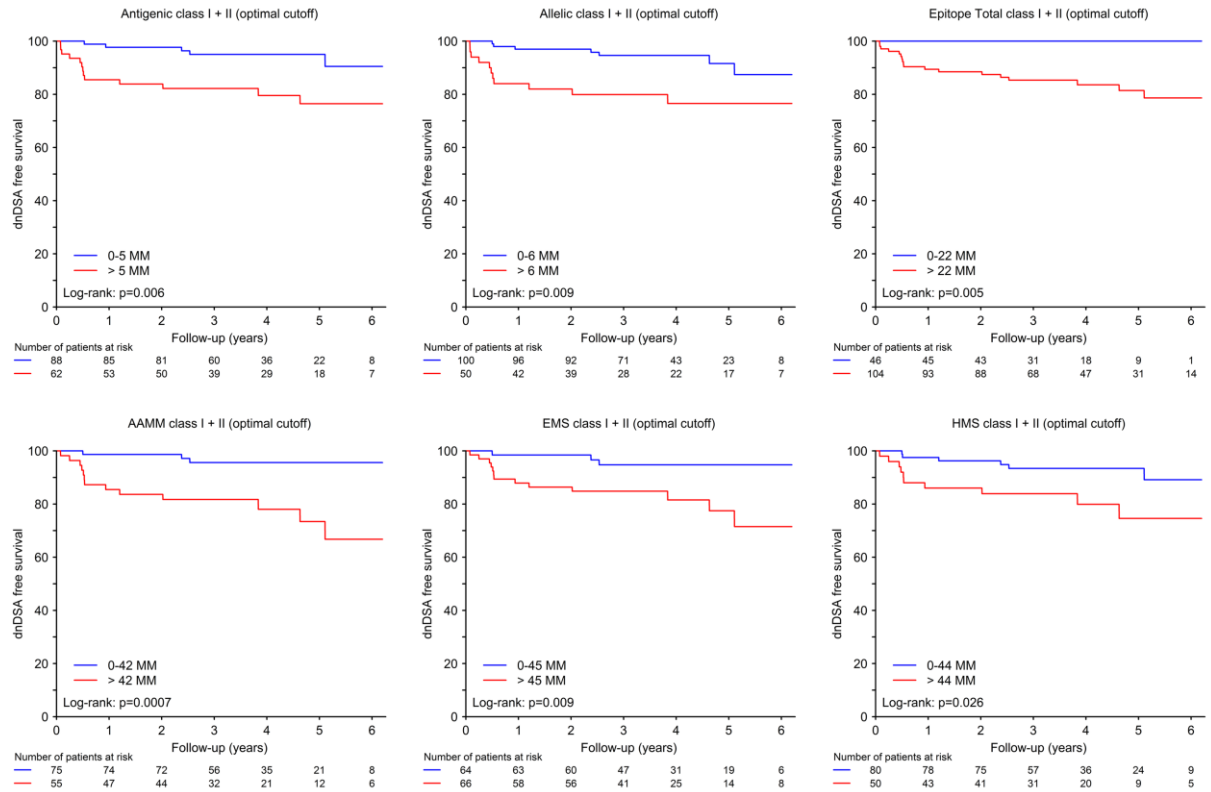
MM: mismatch(es).



**Figure 3: Kaplan-Meier survival curves for *de novo* DSA development according to each mismatch and score variable.**

Harrell's C-index was used to determine the optimal threshold for each mismatch and score variable.

*AAMM*: amino-acid mismatch; *dnDSA*: *de novo* donor specific antibody; *EMS*: electrostatic mismatch score; *HMS*: hydrophobic mismatch score;



**Table 1: Baseline characteristics and follow-up data of patients according to the development or not of *de novo* DSA during follow-up**

	<u>No DSA (n=132)</u>			<u>De novo DSA (n=18)</u>			<u>P-value</u>
	<u>N</u>	<u>Mean ± SD / n (%)</u>	<u>Median (Q1 - Q3)</u>	<u>N</u>	<u>Mean ± SD / n (%)</u>	<u>Median (Q1 - Q3)</u>	
<b><u>Demographics</u></b>							
<u>Age (years)</u>	<u>132</u>	<u>50.8 ± 14.7</u>	<u>53.0 (39.5 - 62.5)</u>	<u>18</u>	<u>45.1 ± 13.1</u>	<u>39.0 (35.0 - 59.0)</u>	<u>0.083</u>
<u>Gender</u>	<u>132</u>			<u>18</u>			<u>0.76</u>
<u>Male</u>		<u>103 (78.0 %)</u>			<u>15 (83.3 %)</u>		
<u>Female</u>		<u>29 (22.0 %)</u>			<u>3 (16.7 %)</u>		
<u>BMI (kg/m<sup>2</sup>)</u>	<u>132</u>	<u>25.5 ± 5.1</u>	<u>25.0 (21.5 - 29.0)</u>	<u>18</u>	<u>28.9 ± 4.6</u>	<u>30.0 (26.0 - 33.0)</u>	<b><u>0.006</u></b>
<b><u>Comorbidities</u></b>							
<u>Stroke</u>	<u>132</u>	<u>7 (5.3 %)</u>		<u>18</u>	<u>0 (0.0 %)</u>		<u>1.00</u>
<u>Diabetes</u>	<u>132</u>	<u>23 (17.4 %)</u>		<u>18</u>	<u>6 (33.3 %)</u>		<u>0.12</u>
<u>Diabetes</u>	<u>132</u>			<u>18</u>			<b><u>0.036</u></b>
<u>No history of diabetes</u>		<u>109 (82.6 %)</u>			<u>12 (66.7 %)</u>		
<u>Type I diabetes</u>		<u>4 (3.0 %)</u>			<u>3 (16.7 %)</u>		
<u>Type II diabetes</u>		<u>19 (14.4 %)</u>			<u>3 (16.7 %)</u>		
<u>History of coronary disease</u>	<u>132</u>	<u>15 (11.4 %)</u>		<u>18</u>	<u>0 (0.0 %)</u>		<u>0.22</u>
<u>Heart failure</u>	<u>132</u>	<u>22 (16.7 %)</u>		<u>18</u>	<u>2 (11.1 %)</u>		<u>0.74</u>
<u>Peripheral artery disease</u>	<u>132</u>	<u>14 (10.6 %)</u>		<u>18</u>	<u>2 (11.1 %)</u>		<u>1.00</u>
<u>Neoplasia</u>	<u>132</u>	<u>10 (7.6 %)</u>		<u>18</u>	<u>2 (11.1 %)</u>		<u>0.64</u>
<u>Causal nephropathy</u>	<u>132</u>			<u>18</u>			<u>0.29</u>
<u>Other</u>		<u>14 (10.6 %)</u>			<u>1 (5.6 %)</u>		
<u>Glomerulonephritis</u>		<u>38 (28.8 %)</u>			<u>7 (38.9 %)</u>		
<u>Toxic</u>		<u>1 (0.8 %)</u>			<u>0 (0.0 %)</u>		
<u>Diabetic nephropathy</u>		<u>14 (10.6 %)</u>			<u>6 (33.3 %)</u>		
<u>Vascular nephropathy</u>		<u>18 (13.6 %)</u>			<u>1 (5.6 %)</u>		
<u>Unknown</u>		<u>11 (8.3 %)</u>			<u>2 (11.1 %)</u>		
<u>Polycystic disease</u>		<u>19 (14.4 %)</u>			<u>0 (0.0 %)</u>		
<u>Nephrectomy</u>		<u>1 (0.8 %)</u>			<u>0 (0.0 %)</u>		
<u>Malformative uropathy</u>		<u>9 (6.8 %)</u>			<u>1 (5.6 %)</u>		
<u>Vasculitis</u>		<u>6 (4.5 %)</u>			<u>0 (0.0 %)</u>		
<u>Tubulo-interstitial</u>		<u>1 (0.8 %)</u>			<u>0 (0.0 %)</u>		
<u>Dialysis prior to transplantation</u>	<u>132</u>			<u>18</u>			<u>0.83</u>
<u>Non-dialyzed</u>		<u>15 (11.4 %)</u>			<u>2 (11.1 %)</u>		
<u>Peritoneal dialysis</u>		<u>16 (12.1 %)</u>			<u>3 (16.7 %)</u>		
<u>Hemodialysis</u>		<u>101 (76.5 %)</u>			<u>13 (72.2 %)</u>		
<u>Pre-transplant dialysis time (years)</u>	<u>117</u>	<u>2.6 ± 2.1</u>	<u>2.0 (1.1 - 3.3)</u>	<u>16</u>	<u>2.6 ± 2.7</u>	<u>1.9 (1.6 - 2.6)</u>	<u>0.80</u>

	<u>No DSA (n=132)</u>			<u>De novo DSA (n=18)</u>			<u>P-value</u>
	<u>N</u>	<u>Mean ± SD / n (%)</u>	<u>Median (Q1 - Q3)</u>	<u>N</u>	<u>Mean ± SD / n (%)</u>	<u>Median (Q1 - Q3)</u>	
<b><u>Transplantation data</u></b>							
<u>Viral status CMV donor/recipient</u>	<u>132</u>			<u>18</u>			<u>0.15</u>
<u>D-/R-</u>		<u>22 (16.7 %)</u>			<u>5 (27.8 %)</u>		
<u>D-/R+</u>		<u>32 (24.2 %)</u>			<u>2 (11.1 %)</u>		
<u>D+/R-</u>		<u>35 (26.5 %)</u>			<u>8 (44.4 %)</u>		
<u>D+/R+</u>		<u>43 (32.6 %)</u>			<u>3 (16.7 %)</u>		
<u>Type of donor</u>	<u>132</u>			<u>18</u>			<u>0.22</u>
<u>Living donor</u>		<u>50 (37.9 %)</u>			<u>6 (33.3 %)</u>		
<u>Standard criteria</u>		<u>48 (36.4 %)</u>			<u>10 (55.6 %)</u>		
<u>Expanded criteria</u>		<u>34 (25.8 %)</u>			<u>2 (11.1 %)</u>		
<u>Cold ischemia time (hours)</u>	<u>132</u>	<u>11.4 ± 8.0</u>	<u>11.0 (3.8 - 15.3)</u>	<u>18</u>	<u>11.2 ± 7.6</u>	<u>11.3 (4.2 - 15.0)</u>	<u>0.98</u>
<u>Induction treatment</u>	<u>132</u>	<u>123 (93.2 %)</u>		<u>18</u>	<u>17 (94.4 %)</u>		<u>1.00</u>
<u>Induction treatment</u>	<u>132</u>			<u>18</u>			<u>0.27</u>
<u>Thymoglobulin</u>		<u>69 (52.3 %)</u>			<u>13 (72.2 %)</u>		
<u>Basiliximab</u>		<u>54 (40.9 %)</u>			<u>4 (22.2 %)</u>		
<u>No induction</u>		<u>9 (6.8 %)</u>			<u>1 (5.6 %)</u>		
<b><u>Immunosuppressive regimen</u></b>							
<u>Cyclosporine</u>	<u>132</u>	<u>45 (34.1 %)</u>		<u>18</u>	<u>12 (66.7 %)</u>		<b><u>0.010</u></b>
<u>Tacrolimus</u>	<u>132</u>	<u>84 (63.6 %)</u>		<u>18</u>	<u>5 (27.8 %)</u>		<b><u>0.005</u></b>
<u>Mycophenolate mofetil</u>	<u>132</u>	<u>113 (85.6 %)</u>		<u>18</u>	<u>18 (100.0 %)</u>		<u>0.13</u>
<u>Azathioprin</u>	<u>132</u>	<u>3 (2.3 %)</u>		<u>18</u>	<u>0 (0.0 %)</u>		<u>1.00</u>
<u>Corticosteroids</u>	<u>132</u>	<u>130 (98.5 %)</u>		<u>18</u>	<u>18 (100.0 %)</u>		<u>1.00</u>
<b><u>Post-transplantation events</u></b>							
<u>Delayed graft function</u>	<u>132</u>	<u>41 (31.1 %)</u>		<u>18</u>	<u>4 (22.2 %)</u>		<u>0.59</u>
<u>Rejection</u>	<u>132</u>	<u>32 (24.2 %)</u>		<u>18</u>	<u>10 (55.6 %)</u>		<b><u>0.010</u></b>
<u>Type of rejection</u>	<u>32</u>			<u>10</u>			<u>0.42</u>
<u>Borderline</u>		<u>18 (56.2 %)</u>			<u>7 (70.0 %)</u>		
<u>Cellular</u>		<u>5 (15.6 %)</u>			<u>1 (10.0 %)</u>		
<u>Vascular cellular</u>		<u>8 (25.0 %)</u>			<u>1 (10.0 %)</u>		
<u>Acute humoral</u>		<u>0 (0.0 %)</u>			<u>1 (10.0 %)</u>		
<u>Chronic humoral</u>		<u>1 (3.1 %)</u>			<u>0 (0.0 %)</u>		
<u>Return to dialysis</u>	<u>132</u>	<u>5 (3.8 %)</u>		<u>18</u>	<u>4 (22.2 %)</u>		<b><u>0.013</u></b>
<u>Death with a functioning graft</u>	<u>132</u>	<u>7 (5.3 %)</u>		<u>18</u>	<u>0 (0.0 %)</u>		<u>1.00</u>
<u>Graft failure from any cause including death</u>	<u>132</u>	<u>12 (9.1 %)</u>		<u>18</u>	<u>4 (22.2 %)</u>		<u>0.10</u>
<u>Time to follow-up (years)</u>	<u>132</u>	<u>4.0 ± 1.5</u>	<u>3.9 (2.9 - 5.2)</u>	<u>18</u>	<u>4.6 ± 1.5</u>	<u>4.8 (3.1 - 5.9)</u>	<u>0.17</u>
<u>CNI Mean Absolute deviation</u>	<u>110</u>	<u>17.5 ± 8.8</u>	<u>15.7 (11.4 - 23.7)</u>	<u>15</u>	<u>17.9 ± 8.5</u>	<u>20.0 (8.6 - 23.3)</u>	<u>0.71</u>
<u>Last eGFR - CKD-EPI (mL/min/1.73m<sup>2</sup>)</u>	<u>123</u>	<u>57.5 ± 21.9</u>	<u>56.3 (41.3 - 68.9)</u>	<u>14</u>	<u>66.1 ± 27.0</u>	<u>58.6 (44.3 - 85.8)</u>	<u>0.28</u>

*BMI: body mass index; CMV: cytomegalovirus; D: donor's serology; DSA: donor specific antibody; dnDSA: donor specific antibody; eGFR: estimated Glomerular Filtration Rate; CNI: calcineurin inhibitor; Q1: first quartile; Q3: third quartile; R: recipient's serology; SD: standard deviation.*  
*MM: mismatch(es).*

**Table 2: Number of mismatches, amino-acid and physicochemical disparities according to the development or not of de novo DSA during follow-up**

	No DSA (n=132)			<i>De novo</i> DSA (n=18)			p-value
	N	Mean $\pm$ SD / n (%)	Median (Q1 - Q3)	N	Mean $\pm$ SD / n (%)	Median (Q1 - Q3)	
<b>Antigenic MM</b>	132	4.6 $\pm$ 2.0	5 (3 - 6)	18	5.9 $\pm$ 1.1	6 (5 - 7)	<b>0.004</b>
<b>Allelic MM</b>	132	5.0 $\pm$ 2.1	5 (4 - 7)	18	6.4 $\pm$ 1.1	7 (6 - 7)	<b>0.005</b>
<b>Epitope MM</b>	132	29.5 $\pm$ 15.5	29 (18 - 41.5)	18	39.9 $\pm$ 12.4	37.5 (30 - 46)	<b>0.008</b>
<b>AAMS</b>	114	36.7 $\pm$ 20.2	36.5 (22 - 51)	16	54.1 $\pm$ 19.8	47 (44.5 - 76)	<b>0.006</b>
<b>EMS</b>	114	45.6 $\pm$ 28.3	43.5 (25 - 63)	16	67.6 $\pm$ 30.9	64.5 (48 - 80.5)	<b>0.009</b>
<b>HMS</b>	114	35.9 $\pm$ 22.8	33.5 (20 - 50)	16	52.0 $\pm$ 23.9	49 (32 - 70)	<b>0.016</b>

*AAMS: amino-acid mismatch score; dnDSA: de novo donor specific antibody; EMS: electrostatic electrostatic mismatch score; HMS: hydrophobic mismatch score; MM: mismatch. Q1: first quartile; Q3: third quartile; SD: standard deviation.*



**Table 3: Association between *de novo* DSA development and antigenic, allelic, epitope, amino-acid and physicochemical mismatch variables (continuous variables and dichotomized according an optimal cutoff): univariable and adjusted Cox models**

	$N_{dnDSA}/N$	Univariable Cox model		Adjusted Cox model*	
		HR (CI 95 %)	p-value	HR (CI 95 %)	p-value
<b>Antigenic MM</b>					
Continuous	18 / 150 (12.0 %)	1.51 (1.10-2.07)	<b>0.011</b>	1.66 (1.17-2.35)	<b>0.004</b>
0-5 MM	5 / 88 (5.7 %)	1.00	-	1.00	-
> 5 MM	13 / 62 (21.0 %)	3.85 (1.37-10.82)	<b>0.010</b>	4.63 (1.53-14.02)	<b>0.007</b>
<b>Allelic MM</b>					
Continuous	18 / 150 (12.0 %)	1.52 (1.10-2.09)	<b>0.011</b>	1.62 (1.16-2.26)	<b>0.005</b>
0-6 MM	7 / 100 (7.0 %)	1.00	-	1.00	-
> 6 MM	11 / 50 (22.0 %)	3.31 (1.28-8.56)	<b>0.013</b>	3.76 (1.40-10.14)	<b>0.009</b>
<b>Epitope MM</b>					
Continuous	18 / 150 (12.0 %)	1.04 (1.01-1.07)	<b>0.016</b>	1.04 (1.01-1.08)	<b>0.010</b>
0-22 MM	0 / 46 (0.0 %)	-	-	-	-
> 22 MM	18 / 104 (17.3 %)	-	-	-	-
<b>AAMS</b>					
Continuous	16 / 130 (12.3 %)	1.04 (1.01-1.06)	<b>0.003</b>	1.04 (1.01-1.06)	<b>0.002</b>
0-42 MM	3 / 75 (4.0 %)	1.00	-	1.00	-
> 42 MM	13 / 55 (23.6 %)	6.60 (1.88-23.17)	<b>0.003</b>	6.85 (1.91-24.62)	<b>0.003</b>
<b>EMS</b>					
Continuous	16 / 130 (12.3 %)	1.02 (1.01-1.04)	<b>0.006</b>	1.02 (1.01-1.04)	<b>0.004</b>
0-45 MM	3 / 64 (4.7 %)	1.00	-	1.00	-
> 45 MM	13 / 66 (19.7 %)	4.63 (1.32-16.25)	<b>0.017</b>	5.13 (1.37-19.13)	<b>0.015</b>
<b>HMS</b>					
Continuous	16 / 130 (12.3 %)	1.03 (1.01-1.05)	<b>0.009</b>	1.03 (1.01-1.05)	<b>0.006</b>
0-44 MM	6 / 80 (7.5 %)	1.00	-	1.00	-
> 44 MM	10 / 50 (20.0 %)	2.99 (1.09-8.25)	<b>0.034</b>	3.22 (1.15-8.96)	<b>0.026</b>

*AAMS: amino-acid mismatch score; dnDSA: de novo donor specific antibody; EMS: electrostatic mismatch score; HMS: hydrophobic mismatch score; HR: hazard ratio; MM: mismatch(es).  $N_{dnDSA}$ : number of patients with dnDSA; N: number of non-missing patients.*

† adjusted for age, induction, living donor

**Table 4: C-index of antigenic, allelic, epitope, and physicochemical mismatch variables (continuous, dichotomized according an optimal cutoff) for the risk of *de novo* DSA development**

	C-index (CI 95 %)	p-value
<b>Antigenic MM</b>		
Continuous	69.3 (59.7 - 79.0)	<0.0001
> 5 MM	68.0 (57.8 - 78.1)	<b>0.0005</b>
<b>Allelic MM</b>		
Continuous	70.6 (60.7 - 80.6)	<0.0001
> 6 MM	67.7 (56.7 - 78.8)	<b>0.002</b>
<b>Epitope MM</b>		
Continuous	66.5 (55.8 - 77.2)	<b>0.003</b>
> 22 MM	65.7 (61.9 - 69.4)	<0.0001
<b>AAMS</b>		
Continuous	67.8 (56.6 - 79.1)	<b>0.002</b>
> 42 MM	70.4 (60.1 - 80.7)	<b>0.0001</b>
<b>EMS</b>		
Continuous	66.7 (54.9 - 78.4)	<b>0.005</b>
> 45 MM	65.9 (55.5 - 76.3)	<b>0.003</b>
<b>HMS</b>		
Continuous	66.9 (54.4 - 79.3)	<b>0.008</b>
> 44 MM	64.1 (51.9 - 76.3)	<b>0.024</b>

AAMS: amino-acid mismatch score; EMS: electrostatic mismatch score; HMS: hydrophobic mismatch score.

MM: mismatch(es).