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ORIGINAL ARTICLE

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# Predictive value of molecular matching tools for the development of donor specific HLA-antibodies in patients undergoing lung transplantation

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Molecular matching is a new approach for virtual histocompatibility testing in organ transplantation. The aim of our study was to analyze whether the risk for de novo donor-specific HLA antibodies (dnDSA) after lung transplantation (LTX) can be predicted by molecular matching algorithms (MMA) and their combination. In this retrospective study we included 183 patients undergoing LTX at our center from 2012–2020. We monitored dnDSA development for 1 year. Eplet mismatches (epMM) using HLAMatchmaker were calculated and highly immunogenic eplets based on their ElliPro scores were identified. PIRCHE-II scores were calculated using PIRCHE-II algorithm (5- and 11-loci). We compared epMM and PIRCHE-II scores between patients with and without dnDSA using t-test and used ROC-curves to determine optimal cut-off values to categorize patients into four groups. We used logistic regression with AIC to compare the predictive value of PIRCHE-II, epMM, and their combination. In total 28.4% of patients developed dnDSA ($n = 52$), 12.5% class I dnDSA ($n = 23$), 24.6% class II dnDSA ($n = 45$), and 8.7% both class II and II dnDSA ($n = 16$). Mean epMMs ($p$-value $= 0.005$), mean highly immunogenic epMMs ($p$-value $= 0.003$), and PIRCHE-II (11-loci) ($p = 0.01$) were higher in patients with compared to without class II dnDSA. Patients with highly immunogenic epMMs above 30.5 and PIRCHE-II 11-loci above 560.0 were more likely to develop dnDSA (31.1% vs. 14.8%, $p$-value $= 0.03$). The logistic regression model including the grouping variable showed the best predictive value. MMA can support clinicians to identify patients at higher or lower risk for developing class II dnDSA and might be helpful tools for immunological risk assessment in LTX patients.

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**KEYWORDS**
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## 1 | INTRODUCTION

Unlike patients with severe kidney disease, for whom replacement therapy is available in addition to transplantation, transplantation is the last treatment option for many patients with end-stage lung disease. Because the urgency of transplantation is often very high, only minimal matching criteria such as donor-recipient size match and ABO blood group compatibility are considered in lung allocation in addition to the Lung Allocation Score (LAS). Currently, the median survival time after lung transplantation is approximately 6 years.[1] Humoral and/or cellular rejection reactions but also the side effects of immunosuppressive therapy are feared complications after transplantation and common causes of chronic lung allograft dysfunction (CLAD). In solid organ transplantation (SOT), human leukocyte antigens (HLA) play a crucial role in assessing histocompatibility between donor and recipient.[2] Unlike kidney transplantation, HLA matching is currently not performed in lung organ allocation, and the extent to which transplant centers consider the HLA immunization status of their patients when accepting a lung offer is at their decision. As a result, there may be a large number of HLA mismatches between donor and recipient and patients might be transplanted with preformed donor-directed HLA antibodies. However, the better the histocompatibility between recipient and donor, the lower the risk of acute cellular reaction (ACR) or antibody mediated reaction (AMR).

The basis for the direct pathway of antigen recognition is formed by so-called donor passenger leukocytes, antigen-presenting cells of the donor, which migrate out of the graft after transplantation and enter the lymph nodes of the recipient via the lymphatic pathway, where they are recognized by CD8+ and CD4+ cells of the recipient. This mechanism is thought to be responsible for acute cellular rejection in the early post-transplant period. The indirect pathway is based on the reactivity of CD4+ T cells against allopeptides bound to MHC class II molecules on recipient APCs leading to a chronic rejection of the allograft.[3]

In addition to T cells, B cells are another essential component of the acquired immune defense. They express B cell receptors (BCR) on their cell surface in the form of immunoglobulins (Ig) specific for a particular antigen. Cytokines cause the B cells to differentiate into antibody-secreting plasma cells. The antibodies secreted by the plasma cells have several effects that can lead to antibody-mediated rejection in a complement-dependent or complement-independent manner. With the availability of high-resolution HLA typing of up to 11 loci and the three-dimensional structures of HLA molecules, the potentially immunogenic parts of each HLA can be determined using current epitope prediction algorithms. An epitope consisting of a structural and a functional part is a specific area on the surface of an antigen that can be bound by the paratope of an antibody or the BCR leading to a specific immune response. The CDR H3 of the paratope is thought to bind to the functional part of the epitope (eplet) determining the specificity of the antibody.[4]

In context of transplantation HLAMatchmaker is a molecular mismatch algorithm for histocompatibility determination, describing the compatibility at the structural level of recognized B-cell epitopes while non-self eplets according to the structural similarity or dissimilarity of the patient and donor HLAs are taken into account.[5] According to Duquesnoy, eplets are amino acid residues at polymorphic positions of the HLA, arranged continuously or discontinuously within 3.0–3.5 Ångstroms radius. The HLAMatchmaker algorithm sums the amount (eplet load) and type of mismatched eplets and is available on R. Duqesnoy's website at http://www.epitopes.net. Meanwhile this algorithm is also included in actual antibody software programs such as HLA Fusion™ (One Lambda, Inc., Canoga Park, CA, USA). Currently known and defined HLA epitopes are listed with additional information such as polymorphic residues or antibody reactivity in the international HLA Epitope Registry (https://www.epregistry.com.br/). The epitope registry also provides ElliPro scores derived from the ElliPro antibody epitope prediction tool which are divided in categories for each listed epitope to determine the immunogenicity of the eplet. Based on the three-dimensional structure and amino acid properties of the eplet, the ElliPro score algorithm estimates the interactive potential to cause antibody binding.[6]

Regarding the above mentioned reactivity of CD4+ T cells against allopeptides bound to MHC class II molecules on recipient APCs this indirect pathway plays another main role in allorecognition. With the **P**redicted **I**ndirectly **R**ecognizable **H**LA **E**pitope (**PIRCHE**) algorithm T-cell epitopes can be forecasted. The PIRCHE-II algorithm predicts the amount of mismatched HLA-peptides that can bind to the HLA class II molecule of the recipient. HLA-class II presentation of mismatched donor HLA peptides to CD4+ T-cells of the recipient might lead to B-cell activation that triggers HLA- antibody production.[3,7] In theory, the higher the number of

mismatched HLA peptides presented to the host's CD4+ T-cells via self-HLA class II molecules, the higher the PIRCHE-II scores and the higher the likelihood for the patient to develop a rejection.[3]

Both molecular matching algorithms require the input of recipient and donor HLA typings at least at the intermediate resolution level. Calculations are possible with any number of HLA loci, depending on availability.

Several studies have already shown that a high number of eplet mismatches (MM) and a higher PIRCHE-II score are associated with the development of de novo donor-specific HLA antibodies (dnDSA) in SOT.[8–10] In case of lung transplantation dnDSA are associated with ACR, CLAD and worse graft survival.[11–13] At present, both epitope prediction algorithms are still fraught with uncertainties and neither of them has yet been adapted in routine clinical practice. Clinicians and laboratories are challenged with a series of problems using these algorithms and the immunogenic potential of the individual epitopes is still a matter of debate. Different sets of loci, different resolution levels of HLA typing, and different versions of the same algorithm program make it difficult to perform analysis and compare data consistently.

Our aim in this retrospective study was to describe the predictive value of the epitope matching algorithms HLA-Matchmaker and PIRCHE-II in relation to the development of dnDSA in lung transplant patients. To extend the HLAMatchmaker results, we aimed to determine the utility of information on the immunogenicity of eplets using their ElliPro scores. Therefore, in addition to the amount of eplets, their immunogenicity is also considered in our results. Regarding PIRCHE-II algorithm, we aimed to demonstrate the utility of information with the availability of full 11 loci typing of donors and recipients (HLA-A, B, C, DRB1, DRB345, DQA1, DQB1, DPA1 and DPB1) compared to the 5 loci typing (HLA- HLA-A, B, C, DRB1 and DQB1). We also aimed to determine optimal cut-off values for use in clinical practice for each method. Finally, we examined whether not each algorithm alone but the combination of both is helpful for HLA-antibody prediction. Because the focus of this study is on the informative value of the two molecular matching algorithms with respect to the development of dnDSA, associations with clinical outcome parameters are not the subject of this study.

## 2 | METHODS

### 2.1 | Study population

This retrospective study used data from lung transplant recipients and their donors who underwent organ transplantation between 2012 and 2020 in the Munich Lung Transplant Program at LMU University Hospital. The main inclusion criteria was complete HLA typing at 11 loci in both donor and recipient (HLA-A, B, C, DRB1, DRB345, DQA1, DQB1, DPA1 and DPB1). Patients with pre-transplant HLA antibodies were excluded from the study, as well as patients with neither class I nor class II HLA mismatches. Baseline patient and donor characteristics such as sex, age at transplantation, preoperative diagnosis, single or bilateral lung transplantation, HLA mismatch, BMI, CMV status and blood type were obtained from the hospitals electronic records and the Eurotransplant database. According to the transplant centre's standard practice, all patients received a triple immunosuppressive therapy regimen consisting of prednisolone, mycophenolate mofetil and tacrolimus or ciclosporin. This study was approved by the Ethics Committee of the Ludwig Maximilian University of Munich, Germany (reference number 22–0166). The study was conducted in accordance with the Declaration of Helsinki, guideline for good clinical practice, and local ethical and legal requirements.

### 2.2 | HLA-typing

Recipients were routinely HLA-11 loci typed (HLA-A, B, C, DRB1, DRB345, DQA1, DQB1, DPA1 and DPB1) using the Luminex sequence-specific oligonucleotide technique (LABType™ SSO Typing Kits, One Lambda, Inc., Canoga Park, CA, USA, Database: IMGT/HLA 3.45.1). Donors were also HLA-11 loci typed using either the sequence-specific oligonucleotide technique or a real-time PCR genotyping assay using sequence-specific primers (LinkSēq™ HLA-ABCDRDQB1 384 Kit, One Lambda, Inc., Canoga Park, CA, USA, Database: CWD_TDX_3.49.0). Intermediate resolution HLA typing (2-fields) were obtained with both techniques. The most common allele was used for molecular matching. Rare alleles could not be excluded.

### 2.3 | HLA-antibody detection

In line with the policies of the transplant program, patients` antibody status was regularly monitored for HLA-antibodies using Luminex-based screening and Single Antigen Bead Technology (LABScreen™ and LABScreen™ Single-antigen Bead assay Class I and Class II, One Lambda, Inc., Canoga Park, CA, USA). If data was available, antibody status was determined before transplantation as well as at one, three, six and 12 months after transplantation. HLA antibody specificities above a mean fluorescence intensity (MFI) of approximately 1.000 were considered positive. All reported donor specificities could be explained by one or more of the mismatched eplets.

In this study, we evaluated molecular matching algorithms with respect to the development of class I and

class II antibodies. However, the main analysis focuses on class II antibodies because the detected class II antibodies were predominantly directed against the donor. Immunization against HLA class I due to transfusion during surgery cannot be excluded and could influence the immunization outcome.

## 2.4 | Molecular matching algorithms

HLAMatchmaker algorithm (integrated in One Lambda Fusion software, One Lambda, Inc., Canoga Park, CA, USA) was used to calculate the number of eplet MM based on HLA 11-loci typing results. No distinction was made between "antibody verified" eplets which were experimentally verified by a research group, and "antibody unverified" eplets. Class II interloci eplets have been excluded from the analysis. Based on the description of the ElliPro scores in the Epitope Registry (HLA Epitope Registry, HLA Epitope Registry (https://www.epregistry.com.br/ version 3.0) the eplets were classified according to their immunogenicity into very low, low, intermediate and high immunogenic. Both the number of all eplet MMs as well as the number of high immunogenic eplet MMs (high ElliPro scores) were calculated. To determine the total amount of foreign peptides presented by self HLA class II molecules, both the 5 loci (HLA-ABCDRB1DQB1) as well as 11 (HLA-ABCDRB1DRB345DQA1DQB1DPA1DPB1) loci HLA typing of donor and recipient using the PIRCHE-II SOT module (available on the PIRCHE Website www.pirche.com) were entered and the corresponding PIRCHE-II scores for each donor-recipient combination could be calculated.

## 2.5 | Statistical analysis

We reported categorical variables as absolute and relative frequencies and numerical variables as means with standard deviation (sd). We compared differences in frequencies between patients with dnDSA and patients without dnDSA using $Chi^2$ or fisher exact-test (cell-numbers <6) (Tables 1 and 2). We tested numeric variables for normal distribution using graphical inspection of QQ-plots and histograms. For normally distributed variables, we used Students t-tests, otherwise we used Mann–Whitney *U* test for comparison. Receiver operating characteristic (ROC) curves based on sensitivity, specificity, and area under the curve (AUC) were used to determine optimal cut-off values for the number of MM eplets and PIRCHE-II scores (Table 3). For further analyses we categorized patients according to these cut-offs from ROC into both molecular mismatch algorithms values above cut-off, value of PIRCHE-II score above cut-off, value of eplet MM above cut-off, and both values lower than cut-off (Table 4). Finally, to determine whether the Eplet MM, the PIRCHE-II score, or a combination of both is most appropriate to identify patients at high risk for developing dnDSA, we used logistic regression models using logistic regression analysis with log link.

We compared the model fit of six different models including different combinations of the algorithms (Table 5) using Akaikes information criterion (AIC). Additionally, we included age, sex, blood group, and CMV risk combination status to all regression models to adjust for these confounding variables. We reported results from regression analysis as Odds ratios (OR) with p-values, and determined statistical significance in all analysis using two-sided p-values with alpha errors <0.05. R Version 4.0.0 and RStudio Version 1.4 were used to perform the data analysis and tables and figures were created in RStudio and Microsoft Excel.

# 3 | RESULTS

## 3.1 | Study population

In total, 608 patients underwent lung transplantation in the Munich Lung Transplant Program of the LMU University Hospital between 2012 and 2020. Complete HLA-11 loci typing results for donors and recipients was available for 220 patients. Of these, we excluded one patient due to missing HLA antibody follow-up information, 32 patients due to pre-transplant HLA antibodies, and four patients due to no HLA class I or II mismatches. As a result, we included 183 patients in our study.

In total 28.4% of patients developed dnDSA ($n = 52$), 12.5% class I dnDSA ($n = 23$), 24.6% class II dnDSA ($n = 45$), and 8.7% both class I and II dnDSA ($n = 16$). The distribution of dnDSA across all loci was as follows: HLA-A ($n = 10$), HLA-B ($n = 9$), HLA-C ($n = 7$), HLA-DRB1 ($n = 5$), HLA-DQ ($n = 42$), HLA-DP ($n = 2$).

Remarkably among class II dnDSA, 93.3% were directed against HLA-DQ. Table 1 describes the baseline characteristics of the study cohort stratified by development of dnDSA. With regard to age, BMI, sex, underlying diseases, type of transplantation, blood type, and CMV risk group there was no significant difference in the group of patients with dnDSA compared to those without dnDSA.

Regarding the HLA mismatch level of the different HLA loci, we also did not find a significant difference between patients with and without dnDSA, not even for the DQ locus, although most dnDSA were directed against HLA-DQ.

**TABLE 1** Patients characteristics stratified by development of DSA

| | All patients ($n = 183$) | | DSA ($n = 52$) | | no DSA ($n = 131$) | | | |
|---|---|---|---|---|---|---|---|---|
| | mean | sd | mean | sd | mean | sd | | *p*-value |
| **age in years** | 51.8 | 13.0 | 50.7 | 12.9 | 52.2 | 13.1 | | 0.48 |
| **BMI** | 23.1 | 4.5 | 23.5 | 4.8 | 22.9 | 4.4 | | 0.42 |
| | | | *n* | % | *n* | % | *n* | % | |
| **sex** | | | | | | | | |
| female | | | 69 | 37.7% | 22 | 42.3% | 47 | 35.9% | |
| male | | | 114 | 62.3% | 30 | 57.7% | 84 | 64.1% | 0.52 |
| **underlying condition** | | | | | | | | |
| COPD | | | 45 | 24.6% | 11 | 21.2% | 34 | 26.0% | |
| CF | | | 35 | 19.1% | 10 | 19.2% | 25 | 19.1% | |
| ILF | | | 26 | 14.2% | 4 | 7.7% | 22 | 16.8% | |
| other (e.g. PPH,EAA, bronchiectasis, sarcoidosis) | | | 77 | 42.1% | 27 | 51.9% | 50 | 38.2% | 0.24 |
| **type of surgery** | | | | | | | | |
| single lung | | | 28 | 15.3% | 5 | 9.6% | 23 | 17.6% | |
| double lung | | | 155 | 84.7% | 47 | 90.4% | 108 | 82.4% | 0.26 |
| **blood type** | | | | | | | | |
| O | | | 73 | 39.9% | 23 | 44.2% | 50 | 38.2% | |
| A | | | 77 | 42.1% | 19 | 36.5% | 58 | 44.3% | |
| B | | | 29 | 15.8% | 9 | 17.3% | 20 | 15.3% | |
| AB | | | 4 | 2.2% | 1 | 1.9% | 3 | 2.3% | 0.85 |
| **CMV** | | | | | | | | |
| R-D- | | | 42 | 22.5% | 8 | 15.4% | 33 | 25.2% | |
| R-D+ | | | 59 | 31.6% | 15 | 28.8% | 42 | 32.1% | |
| R + D- | | | 30 | 16.0% | 9 | 17.3% | 21 | 16.0% | |
| R + D+ | | | 44 | 23.5% | 11 | 21.2% | 32 | 24.4% | 0.77 |
| unknown | | | 12 | 6.4% | 9 | 17.3% | 3 | 2.3% | |
| **HLA mismatch** | | | | | | | | |
| A locus | | | | | | | | |
| 0 | | | 41 | 22.4% | 2 | 3.8% | 4 | 3.1% | |
| 1 | | | 57 | 31.1% | 11 | 21.2% | 51 | 38.9% | |
| 2 | | | 30 | 16.4% | 39 | 75.0% | 76 | 58.0% | 0.05 |
| B locus | | | 43 | 23.5% | | | | | |
| 0 | | | 12 | 6.6% | 0 | 0.0% | 0 | 0.0% | |
| 1 | | | | | 9 | 17.3% | 14 | 10.7% | |
| 2 | | | | | 43 | 82.7% | 117 | 89.3% | 0.33 |
| C locus | | | 6 | 3.3% | | | | | |
| 0 | | | 62 | 33.9% | 0 | 0.0% | 1 | 0.8% | |
| 1 | | | 115 | 62.8% | 9 | 17.3% | 21 | 16.0% | |
| 2 | | | | | 43 | 82.7% | 109 | 83.2% | 0.88 |
| DRB1 locus | | | 0 | 0.0% | | | | | |
| 0 | | | 23 | 12.6% | 0 | 0.0% | 0 | 0.0% | |
| 1 | | | 160 | 87.4% | 6 | 11.5% | 25 | 19.1% | |
| 2 | | | | | 46 | 88.5% | 106 | 80.9% | 0.28 |

(Continues)

**TABLE 1** (Continued)

| | *n* | % | *n* | % | *n* | % | |
|---|---|---|---|---|---|---|---|
| DQ locus | 1 | 0.5% | | | | | |
| 0 | 30 | 16.4% | 1 | 1.9% | 2 | 1.5% | |
| 1 | 152 | 83.1% | 8 | 15.4% | 29 | 22.1% | |
| 2 | | | 43 | 82.7% | 100 | 76.3% | 0.53 |

*Note*: Baseline characteristics of our study collective of lung transplanted patients, stratified by development of donor-specific antibodies (DSA) during the first year after transplantation. Further information such as CMV serostatus was determined by measuring anti-CMV IgG by PCR. HLA mismatch between donor and recipient was calculated by comparing the HLA-antigen typing. Categorical variables are reported as absolute and relative frequencies and numerical variables as means with standard deviation. P-values between frequencies and mean values between patients with DSA and patients without DSA are from $Chi^2$ and fisher exact-test (cell-numbers <6), and Students *t*-tests, respectively.
DSA = donor-specific antibody, sd = standard deviation, BMI = body mass index, COPD = chronic obstructive pulmonary disease CF = cystic fibrosis ILF = idiopathic lung fibrosis CMV = cytomegalovirus PPH = primary pulmonary hypertension EAA = exogenous allergic alveolitis HLA = human leukocyte antigen.

**TABLE 2** Eplet-MM load and PIRCHE-II scores

| | **DSA (*n* = 52)** | | **no DSA (*n* = 131)** | | |
|---|---|---|---|---|---|
| | **mean** | **sd** | **mean** | **sd** | ***p*-value** |
| number of epMM | 72.3 | 19.2 | 66.8 | 19.5 | 0.09 |
| number of highly immunogenic epMM | 46.6 | 12.6 | 41.8 | 14 | 0.03 |
| PIRCHE-II (5 loci) | 94.2 | 41.6 | 89 | 43.7 | 0.47 |
| PIRCHE-II (11 loci) | 595.0 | 198.0 | 529.7 | 197.5 | 0.053 |
| | **class I DSA (*n* = 23)** | | **no class I DSA (*n* = 160)** | | |
| | **mean** | **sd** | **mean** | **sd** | ***p*-value** |
| number of epMM | 36.0 | 11.9 | 31.0 | 10.4 | 0.04 |
| number of highly immunogenic epMM | 17.5 | 7.2 | 15.1 | 5.92 | 0.07 |
| PIRCHE-II (5 loci) | 97.7 | 36.8 | 89.5 | 43.9 | 0.39 |
| PIRCHE-II (11 loci) | 604.4 | 162.6 | 540.2 | 211.2 | 0.16 |
| | **class II DSA (*n* = 45)** | | **no class II DSA (*n* = 138)** | | |
| | **mean** | **sd** | **mean** | **sd** | ***p*-value** |
| number of epMM | 41.3 | 13.4 | 35.2 | 14.2 | 0.01 |
| number of highly immunogenic epMM | 31.8 | 9.7 | 26.6 | 11.1 | 0.01 |
| PIRCHE-II (5 loci) | 94.9 | 41.7 | 89 | 43.5 | 0.43 |
| PIRCHE-II (11 loci) | 608.9 | 205.0 | 528.5 | 203.8 | 0.02 |
| | **DQ DSA (*n* = 42)** | | **no DQ DSA (*n* = 141)** | | |
| | **mean** | **sd** | **mean** | **sd** | ***p*-value** |
| # of DQ epMM | 16,8 | 6,42 | 12,6 | 6,23 | 0.0002 |
| # of highly immunogenic DQ epMM | 14,1 | 4,67 | 10,2 | 5,4 | <0.0001 |

*Note*: The amount of eplet mismatches was quantified with HLA-Matchmaker algorithm using One-Lambda Fusion software. Only Eplets with high ElliPro scores according to the HLA Epitope Registry 3.0 were taken into account for the number of highly immunogenic eplet mismatches. The PIRCHE-II scores of each donor-recipient combination based on 5 or 11 loci HLA-typing was delivered by the SOT function of the PIRCHE-II website. The results were stratified by development of either DSA class I, class II or both and only DQ-DSA. Mean values were compared using two-sided *p*-values from Students' *t*-test.
epMM = eplet mismatches DSA = donor-specific antibody, PIRCHE-II = Predicted Indirectly ReCognizable HLA Epitope Algorithm.

**TABLE 3** Optimal cut-off values

| class II DSA | AUC | sensitivity | specificity | optimal cut-off |
|---|---|---|---|---|
| # of epMM | 0.63 | 0.53 | 0.73 | 42.50 |
| # of high immunogenic epMM | 0.65 | 0.53 | 0.70 | 30.50 |
| PIRCHE-II (5 loci) | 0.62 | 0.58 | 0.57 | 85.50 |
| PIRCHE-II (11-loci) | 0.62 | 0.44 | 0.61 | 560.00 |
| **DQ DSA** | **AUC** | **sensitivity** | **specificity** | **optimal cut-off** |
| # of epMM | 0.60 | 0.83 | 0.50 | 17.50 |
| # of high immunogenic epMM | 0.60 | 0.78 | 0.57 | 13.50 |

*Note*: Optimal cut-off values of eplet mismatches and PIRCHE-II scores between donor and recipients were calculated using ROC curves. The number of eplet mismatches in total and only high ElliPro Eplets (according to the HLA Epitope Registry 3.0) were compared as well as the PIRCHE-II scores based on 5 and 11 loci HLA typing.
epMM = eplet mismatches DSA = donor-specific antigen AUC = area under the curve, PIRCHE-II = Predicted Indirectly ReCognizable HLA Epitopes Algorithm.

**TABLE 4** Combination of both HLA-molecular mismatch algorithms

| | class II DSA ($n = 45$) | | no class II DSA ($n = 138$) | | |
|---|---|---|---|---|---|
| | ***n*** | **%** | ***n*** | **%** | ***p*-value** |
| **combination of PIRCHE-II and eplet MM** | | | | | |
| both above cut-off | 14 | 31.1% | 22 | 15.9% | 0.04 |
| PIRCHE-II above cut-off | 15 | 33.3% | 32 | 23.2% | 0.32 |
| epMM above cut-off | 10 | 22.2% | 20 | 14.5% | 0.25 |
| none | 6 | 13.3% | 64 | 46.4% | 0.0002 |

*Note*: Table 4 subdivides patients depending on their molecular mismatch results below or above the cut-offs. For highly immunogenic eplet the cut-offs of 30.5 was used. For the PIRCHE-II scores (11 loci) a cutoff of 560.0 was applied. Proportions between groups are compared using $Chi^2$-test.
epMM = eplet mismatches DSA = donor-specific antibody, PIRCHE-II = Predicted Indirectly ReCognizable HLA Epitope Algorithm.

## 3.2 | Univariate comparison of eplet MMs and PIRCHE-II scores stratified by development of dnDSA

Mean number of eplet MMs ($p$-value = 0.03) and highly immunogenic eplet MM ($p$-value = 0.02) differed significantly between patients with dnDSA and patients without dnDSA. In addition, the mean number of eplet MMs ($p$-value = 0.005) as well as the mean number of highly immunogenic eplet MMs ($p$-value = 0.003) in patients with class II dnDSA compared to patients without class II dnDSA were significantly higher. Regarding the development of DQ-DSA, mean number of eplet MM ($p$-value = 0.0002) and highly immunogenic eplet MM ($p$-value = <0.0001) were significantly higher in patients with DQ dnDSA than in patients without DQ dnDSA. With regard to class I DSA, we found significant difference regarding the number of eplet MM ($p$-value = 0.03), but not for highly immunogenic eplet MMs. The mean PIRCHE-II 5 loci score was not significantly different between patients who developed class I and/or class II dnDSA and patients without dnDSA. In contrast, mean PIRCHE-II 11 loci score was significantly higher in patients with dnDSA compared to patients without dnDSA ($p$-value 0.03), and in patients with class II dnDSA compared to patients without class II dnDSA ($p$-value = 0.01). Mean values of eplet numbers as well as PIRCHE-II 5 loci and 11 loci scores are displayed in Table 2.

## 3.3 | Cut-off values for PIRCHE-II and eplet MM stratified for the development of HLA-class II dnDSA

Regarding class II dnDSA, optimal cut-off values for eplet MMs from ROC were 43.5 for all eplet MMs and 30.5 for highly immunogenic eplet MMs, respectively. AUC for these cut-offs were 0.64 for all eplet MM and 0.66 for highly immunogenic eplet MM. AUC values were slightly lower for PIRCHE-II 5 loci and 11 loci scores (both 0.63) with optimal cut-offs of 85.5 and 560.0, respectively. We summarized sensitivity and specificity of all ROC as well as optimal cut-off values from ROC for class II dnDSA in Table 3.

Cut-off values of highly immunogenic eplet MM (30.5) and PIRCHE-II score of 11 loci (560.0) from ROC were used to classify patients into the following four groups: Patients

**TABLE 5** Results from logistic regression models with log link for DSA class II

| | OR | ß | se | *z*-value | *p*-value | AIC |
|---|---|---|---|---|---|---|
| **model 1** | | | | | | |
| number of epMM | 1.03 | 0.03 | 0.01 | 2.10 | 0.04 | 203.9 |
| **model 2** | | | | | | |
| number of highly immunogenic epMM | 1.04 | 0.04 | 0.02 | 2.27 | 0.02 | 203.1 |
| **model 3** | | | | | | |
| PIRCHE-II (5 loci) | 1.00 | 0.00 | 0.00 | 0.78 | 0.43 | 207.8 |
| **model 4** | | | | | | |
| PIRCHE-II (11 loci) | 1.003 | 0.003 | 0.001 | 2.70 | 0.007 | 200.8 |
| **model 5** | | | | | | |
| PIRCHE-II (11 loci) | 1.002 | 0.002 | 0.001 | 2.23 | 0.03 | 200.0 |
| Number of highly epMM | 1.03 | 0.03 | 0.02 | 1.68 | 0.09 | |
| **model 6 (combination variable)** | | | | | | |
| both values above cut-off vs. none | 7.93 | 2.07 | 0.60 | 3.46 | 0.001 | 194.2 |
| PIRCHE-II (11 loci) above cut-off vs. none | 6.51 | 1.87 | 0.57 | 3.27 | 0.001 | |
| highly immunogenic epMM above cut-off vs. none | 4.94 | 1.60 | 0.62 | 2.56 | 0.01 | |

*Note*: Results from multivariate logistic regression models with log link for DSA class II. All regression models are adjusted for age, sex, blood type, and CMV risk combination status. Comparison of model fit using Akaikes information criterion (AIC), Molecular mismatch algorithms were used in order to evaluate histocompatibility between donor and recipients. Comparisons with the effect of only taking highly immunogenic eplets into account and using the full 11 loci typing were made.

OR = Odds ratio, se = standard error, AIC = Akaike information criterion, epMM = eplet mismatches PIRCHE-II = Predicted Indirectly ReCognizable HLA Epitope Algorithm.

with eplet MM and PIRCHE-II 11 loci above cut-off ($n = 35$), patients with only PIRCHE-II 11 loci above cut-off ($n = 44$), patients with only eplet MM above cut-off ($n = 31$) and patients with eplet MM and PIRCHE-II 11 loci below the cut-off ($n = 77$), stratified for the development of HLA-class II dnDSA. Distribution of this combination variable overall was significantly different between patients with HLA-class II dnDSA and patients without dnDSA ($p$-value = 0.002). The proportion of patients with both PIRCHE-II and immunogenic eplet MMs above the cut-offs of 560.0 and 30.5 was significantly higher in patients who developed HLA-class II dnDSA (31.1% vs. 14.8%, $p$-value = 0.03). Accordingly, the proportion of patients with both values below the cut-offs was significantly lower in patients with HLA-class II dnDSA (15.6% vs. 49.3, $p$-value = 0.0001, Table 4).

### 3.4 | Multivariate analysis of eplet MMs and PIRCHE-II scores for HLA- class II dnDSA

We performed six logistic regression models for HLA-class II dnDSA. The first four each contained either the number of eplet MM, the number of highly immunogenic eplet MM, PIRCHE-II 5 loci score or PIRCHE- II 11 loci score. The fifth included the total number of highly immunogenic eplet MM and PIRCHE-II 11 loci score, and the last and sixth model included the combination variable of cut-offs values. Model number six had the lowest AIC and therefore the best model fit. Therefore, using cut-off values of 30.5 in eplet MM and 560.0 for PIRCHE-II score for risk assessment regarding the development of HLA-class II dnDSA seems to be a valuable tool. We displayed ORs and $p$-values for the variable of interest as well as AICs of all models in Table 5. Supplemental Table S1 displays results with ORs and p-values for all models including the adjusting variables.

## 4 | DISCUSSION

In our study of lung transplant patients, we have shown that molecular matching methods can aid in the assessment of histocompatibility between donor and recipient. Furthermore, we have shown that molecular matching methods bear a higher potential to identify patients at higher risk for developing dnDSA than classical antigen matching. Approximately one-third of patients (52/183 patients, 28.4%) in our cohort developed dnDSA in the first year after lung transplantation. Other studies even found higher numbers like

Bedford et al, 2022 (36%)[14] and Tikkanen et al., 2016 (47%).[15] The large number of patients with nonDSA, especially in class I, might be explained by blood transfusions during surgery. Most dnDSA were detected against HLA class II, especially HLA-DQ, which is comparable to other published data. Tikkanen et al. studied 340 lung transplant recipients, and the prevalence of dnDSA was 47%, of which 76% were against DQ.[15] Clustered immunization against HLA-DQ has also been observed in renal[16] and cardiac transplantation.[17] Increased immunogenicity of HLA class II molecules might be related to increased expression on lung tissue during inflammation and rejection episodes.[18–20]

Regarding the eplet MM analysis by HLAMatchmaker, our data demonstrate that the number of eplet MMs in patients developing dnDSA was significantly higher than in patients who did not develop dnDSA. Additionally, the difference in class II was more pronounced than in class I. Walton et al. also showed that the number of class II eplet MM was associated with the formation of dnDSA in the early post-transplant period.[21] Bedford et al., who also investigated the importance of different molecular matching algorithms in heart and/or lung transplant patients, also demonstrated that in the subgroup of lung transplant patients, patients with a higher HLA-DQ-Eplet-MM load had a higher risk of developing dnDSA.[14]

Regarding the calculation of the eplet MM load, the reader is referred to the work by Tassone et al. The authors illustrated that the use of different applications of the HLAMatchmaker algorithm (eg. using the Excel sheet provided from R.Duquesnoy website vs. the integrated algorithm in the HLA Fusion software) can lead to discordant results concerning the amount and type of eplet mismatches.[22]

In addition to the significance of the eplet MM load, we were particularly interested in determining the significance of the immunogenicity of the eplets in our patient group, which was classified using the ElliPro score. Renée Duquesnoy has previously demonstrated the utility of the ElliPro score in identifying eplets with higher immunogenic potential.[23] We found that the association between the number of highly immunogenic eplets and the development of class II dnDSA was more pronounced than the association between the number of highly immunogenic eplets and the development of dnDSA in general and class I dnDSA, respectively. Currently, a HLA workshop of international experts is trying to assess the immunogenicity of each individual eplet, which may lead to further clarification. Bezstarosti et al. recently published their in-depth analysis of HLA-DQ molecules, which provides further insight into the particular immunogenicity of these molecules.[24] In addition, Tambur et al. demonstrated that the 'epitope footprint' of DQA1 and DQB1 eplets may contribute more to immunogenicity than the mere presence of specific eplets.[25] Several research groups are making efforts to define high risk eplets.[26,27] Further research on HLA-DQ eplets and the inclusion of their immunogenicity into HLA Matchmaker may help to improve its accuracy.

With regard to the PIRCHE-II score as another molecular matching algorithm tool, there is little data on antibody formation in lung transplant patients. Bedford and colleagues investigated a possible association between the eplet loads and PIRCHE-II score and the development of dnDSA in a study population of lung and heart transplanted patients. Their calculation was based on HLA class I and/or class II and additionally locus-specific for DQ locus and DR + DQ loci. Using HLA-Matchmaker the authors found no significant association between the development of dnDSA and eplet load neither for the entire cohort nor for lung transplant patients only. But in their complete cardiothoracic transplant cohort class II PIRCHE-II scores reached statistical significance for the development of dnDSA, especially when calculation was based only on DQ locus.[14]

It is important to note that currently only data on HLA typing at 5 loci is available for both donor and recipient within Eurotransplant region. According to the local transplant protocol, all patients and donors included in our study were typed for DRB345, DQA1, DPB1 and DPA1 additionally, allowing the calculation of PIRCHE-II 5 loci as well as of PIRCHE-II 11 loci. Due to these additional HLA class II typings, the PIRCHE-II score values increased from a maximum of 230 at 5 loci to up to 1244 at 11 loci, since more peptides are included in the calculation. Our data show that when full HLA 11 loci typing is used, there is a significant association between elevated PIRCHE-II scores and the development of class II dnDSA. This shows that the typing of these additional class II loci is of great value and underlines the focus on HLA-class II. Up to now, the PIRCHE-II web service does not report the contribution of individual loci to the overall PIRCHE-II result because there are a lot of overlapping peptides.

Using optimal cut-off values of 560.0 for PIRCHE-II scores with 11 loci and 30.5 for highly immunogenic eplet MM from ROC curves, we were able to categorize patients according to their risk of developing dnDSA. Although values for AUC, sensitivity and specificity resulting from ROC curves were only moderate, they were comparable to the work of Senev et al. who performed ROC curves for the total "antibody-verified" eplet mismatch load and the development of dnDSA in kidney transplantation.[28] In our study, "antibody-verified" and

unverified eplets were considered equal because classification is a dynamic process. One eplet that has the status ab-unverified today might be experimentally validated by a research group the near future and the verification process itself is yet not clearly regulated and not always comparable.[29]

The results of the multivariate regression analyses showed that the aforementioned combination of PIRCHE-II 11 loci and highly immunogenic eplet MMs was best in identifying the patients at highest risk for developing class II dnDSA. Both the crude number of eplet MM load, the crude number of highly immunogenic eplet MM, the sole PIRCHE-II 5 loci or 11 loci score and the combination of the number of highly immunogenic eplets and the PIRCHE-II 11 loci score (without cut-off) showed a lower model fit compared to the combination variable. Therefore, model 6 of multivariate regression analysis demonstrates the potential benefits of using a cut-off (highly immunogenic eplet MM > 30.5; PIRCHE-II 11 loci >560.0), with the combination of both molecular matching algorithms being more informative than either algorithm alone.

This supports the idea that the alloimmune response is a complex mechanism involving multiple factors and both pathways of allorecognition, direct and indirect, as summarized by Geneugelijk and Spierings, contribute to the process of antibody formation.[3] These results agree with those reported recently by Mangiola et al. who investigated the combination of both algorithms in pediatric cardiac transplant patients.[9] The aim of their study was to identify patients at low risk of alloimmunization after transplantation. They were able to work out the benefits that result from the combination of both algorithms.

Overall, 35/183 (19.1%) of the patients in this study revealed results from both algorithms above the respective cut-off. Translated to daily routine, it has to be assumed that approximately every fifth lung transplanted patient is a high-risk patient with regard to dnDSA development.

One of the limitations of our study is the uncertainty about the immunogenicity of each eplet. Nevertheless, the ElliPro score seems to be a useful approach to categorize eplets according to their ability to lead to antibody formation. It is easily reproducible because it is freely accessible in the epitope registry. Still, further in depth-analysis of individual eplet mismatches will be helpful to identify eplets with higher immunogenic potential. Because there are different ways for performing eplet analysis, as mentioned earlier, the total numbers of eplet mismatches as well as our cut-off values must be treated with caution and determined for each centre itself. Furthermore, because of the moderate values for sensitivity and specificity, confirmation of the cut-off values in a larger cohort of patients might be beneficial. Since the HLA typing for patients and donors are not high-resolution but only intermediate resolution, rare alleles cannot be excluded in both patients and donors. Although this study did not examine associations with clinical parameters, we are aware that the development of HLA antibodies has consequences for allograft outcomes. Further data on this topic, particularly on the impact of HLA-DQ eplet mismatches on de novo HLA antibody formation and clinical outcome of patients, are currently being collected. Hopefully, this will help to improve risk assessment in lung transplant candidates.

A strength of this study is the large number of lung transplant patients ($n = 183$). We consider our results generalizable since our patient cohort is comparable to the general population of lung transplant patients in terms of age, sex, and underlying disease. Generally, about half of the patients on the waiting list are under the age of 55 and half of the patients are over the age of 55, which corresponds to the average age of 51.2 years in our cohort. In recent years, men and women have been almost equally represented on the waiting list. Since women have a higher rate of immunization compared to men due to pretransplant pregnancies, they may be slightly underrepresented since immunized patients were excluded from the study. As listed in the annual report of the German Organ Transplantation Foundation (DSO, Annual Report 2021), COPD, idiopathic pulmonary fibrosis (ILF), and cystic fibrosis (CF) are the most important underlying diseases. This is also the case in our study population. Another strength of our study is that we did not only focus on the eplet load but also tried to consider the immunogenicity of the eplets according to the current knowledge. In addition, we have demonstrated the benefit of performing PIRCHE-II analysis based on HLA typing at 11 loci rather than at 5 loci. The advantage of molecular epitope matching algorithms, especially synergistically and with defined cut-offs, is that the risk of immunization can be calculated immediately before or after transplantation without prolonging ischemia time or rejecting an organ offer. Thus, this model seems to be a helpful tool to identify patients at higher risk of developing class II dnDSA after lung transplantation. These patients could subsequently be monitored more closely for the development of HLA-antibodies or even benefit from individually adapted immunosuppressive therapy.

## 5 | CONCLUSION

Our results substantiate that molecular matching algorithms can be useful tools to identify patients at higher

risk for developing class II dnDSA. The immunogenicity of eplets as currently classified by ElliPro scores needs further development. Regarding indirect allorecognition, the input of full HLA 11-loci typing of donor and recipient was crucial for the predictive value of PIRCHE-II scores. Finally, the combination of molecular algorithms using the cut-offs developed in our study (highly immunogenic eplet MM > 30.5; PIRCHE-II 11 loci >560.0) may further specify the immunologic risk for the development of class II dnDSA in patients after lung transplantation. And conversely, patients whose scores are below the thresholds for both algorithms might have the best outcome prognosis.

## AUTHOR CONTRIBUTIONS

**Lisa Kleid** was responsible for conceptualization, data collection, data interpretation and writing of the original draft. **Julia Walter** was responsible for data analysis and interpretation, and writing the original draft. **Maximilian Vorstandlechner** was responsible for data collection and analysis. **Teresa Kauke** and **Andrea Dick** were responsible for conceptualization, funding, and participated in writing and editing of the original draft. **Christian Schneider**, **Sebastian Michel**, **Nikolaus Kneidinger**, **Michael Irlbeck**, **Patrick Möhnle**, **Christian Wichmann**, and **Andreas Humpe** were involved in writing and editing the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## REFERENCES

1. Khush KK, Cherikh WS, Chambers DC, et al. The international thoracic organ transplant registry of the International Society for Heart and Lung Transplantation: thirty-sixth adult heart transplantation report - 2019; focus theme: donor and recipient size match. *J Heart Lung Transplant*. 2019;38(10):1056-1066.
2. Opelz G, Wujciak T, Döhler B, Scherer S, Mytilineos J. HLA compatibility and organ transplant survival. Collaborative transplant study. *Rev Immun*. 1999;1(3):334-342.
3. Geneugelijk K, Thus KA, Spierings E. Predicting alloreactivity in transplantation. *J Immunol Res*. 2014;2014:159479.
4. Duquesnoy RJ. The antibody response to an HLA mismatch: a model for nonself–self discrimination in relation to HLA epitope immunogenicity. *International Journal of Immunogenetics*, 2012;39:1-9.
5. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. *Hum Immunol*. 2002;63(5):339-352.
6. Ponomarenko J, Bui HH, Li W, et al. ElliPro: a new structure-based tool for the prediction of antibody epitopes. *BMC Bioinform*. 2008;9:514.
7. Geneugelijk K, Spierings E. PIRCHE-II: an algorithm to predict indirectly recognizable HLA epitopes in solid organ transplantation. *Immunogenetics*. 2020;72(1):119-129.
8. Hamada S, Dumortier J, Thévenin C, et al. Predictive value of HLAMatchmaker and PIRCHE-II scores for de novo donor-specific antibody formation after adult and pediatric liver transplantation. *Transpl Immunol*. 2020;61:101306.
9. Mangiola M, Ellison MA, Marrari M, et al. Immunologic risk stratification of pediatric heart transplant patients by combining HLAMatchmaker and PIRCHE-II. *J Heart Lung Transplant*. 2022;41:952-960.
10. Sakamoto S, Iwasaki K, Tomosugi T, et al. Analysis of T and B cell epitopes to predict the risk of de novo donor-specific antibody (DSA) production after kidney transplantation: a two-center retrospective cohort study. *Front Immunol*. 2000;2020:11.
11. Lobo LJ, Aris RM, Schmitz J, Neuringer IP. Donor-specific antibodies are associated with antibody-mediated rejection, acute cellular rejection, bronchiolitis obliterans syndrome, and cystic fibrosis after lung transplantation. *J Heart Lung Transplant*. 2013;32(1):70-77.
12. Safavi S, Robinson DR, Soresi S, Carby M, Smith JD. De novo donor HLA-specific antibodies predict development of bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant*. 2014;33(12):1273-1281.
13. Girnita AL, Duquesnoy R, Yousem SA, et al. HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. *Am J Transplant*. 2005;5(1):131-138.
14. Bedford A, Jervis S, Worthington J, Lowe M, Poulton K. HLA epitope mismatch loads and the development of de novo donor-specific antibodies in cardiothoracic organ transplantation. *Int J Immunogenet*. 2022;49(1):30-38.
15. Tikkanen JM, Singer LG, Kim SJ, et al. De novo DQ donor-specific antibodies are associated with chronic lung allograft dysfunction after lung transplantation. *Am J Respir Crit Care Med*. 2016;194(5):596-606.
16. Willicombe M, Brookes P, Sergeant R, et al. De novo DQ donor-specific antibodies are associated with a significant risk of antibody-mediated rejection and transplant glomerulopathy. *Transplantation*. 2012;94(2):172-177.
17. Zhang X, Kransdorf E, Levine R, Patel JK, Kobashigawa JA. HLA-DQ mismatches stimulate de novo donor specific antibodies in heart transplant recipients. *Hum Immunol*. 2020;81(7):330-336.
18. Wosen JE, Mukhopadhyay D, Macaubas C, Mellins ED: Epithelial MHC class II expression and its role in antigen presentation in the gastrointestinal and respiratory tracts 2018, 9.

19. CHANG S-C, HSU H-K, PERNG R-P, SHIAO G-M, LIN C-Y: Increased expression of MHC class Ii antigens in rejecting canine lung allografts 1990, 49(6):1158–1163.
20. Cross AR, Lion J, Poussin K, Glotz D, Mooney N. Inflammation determines the capacity of allogenic endothelial cells to regulate human Treg expansion. *Front Immunol*. 2021;12: 666531.
21. Walton DC, Cantwell L, Hiho S, et al. HLA class II Eplet mismatch predicts De novo DSA formation post lung transplant. *Transpl Immunol*. 2018;51:73-75.
22. Tassone G, de Santis D, Vukovic I, Downing J, Martinez OP, D'Orsogna LJ. Different eplet software programs give discordant and incorrect results: an analysis of HLAMatchmaker vs fusion matchmaker Eplet calling software. *HLA*. 2020;96(1): 52-63.
23. Duquesnoy RJ, Marrari M. Usefulness of the ElliPro epitope predictor program in defining the repertoire of HLA-ABC eplets. *Hum Immunol*. 2017;78(7–8):481-488.
24. Bezstarosti S, Kramer CSM, Franke-van Dijk MEI, et al. HLA-DQ-specific recombinant human monoclonal antibodies allow for In-depth analysis of HLA-DQ epitopes. *Front Immunol*. 2021;12:761893.
25. Tambur AR, Rosati J, Roitberg S, Glotz D, Friedewald JJ, Leventhal JR. Epitope analysis of HLA-DQ antigens: what does the antibody see? *Transplantation*. 2014;98(2):157-166.
26. Schawalder L, Hönger G, Kleiser M, et al. Development of an immunogenicity score for HLA-DQ eplets: a conceptual study. *HLA* 2021;97:30-43.
27. McCaughan JA, Battle RK, Singh SKS, et al. Identification of risk epitope mismatches associated with de novo donor-specific HLA antibody development in cardiothoracic transplantation. *Am J Transplant*. 2018;18(12):2924-2933.
28. Senev A, Coemans M, Lerut E, et al. Eplet mismatch load and De novo occurrence of donor-specific anti-HLA antibodies, rejection, and graft failure after kidney transplantation: an observational cohort study. *J Am Soc Nephrol: JASN*. 2020; 31(9):2193-2204.
29. Bezstarosti S, Bakker KH, Kramer CSM, et al. A comprehensive evaluation of the antibody-verified status of Eplets listed in the HLA epitope registry. *Front Immunol*. 2021;12:800946.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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