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Predictive value of HLAMatchmaker and PIRCHE-II scores for de novo donor-specific antibody formation after adult and pediatric liver transplantation

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ABSTRACT

Production of de novo DSA (dnDSA) is associated with an increased risk of antibody mediated rejection after liver transplantation. Antibodies not only recognize the entire antigen but are able to bind specific functional epitopes present on the HLA molecule surface. The HLAMatchmaker and the PIRCHE-II (predicted indirectly recognizable HLA epitopes) algorithms are able to determine predictive epitope mismatches scores and de novo DSA (dnDSA) synthesis based on alloreactive eplets' identification. The aim of the present study was to assess, for the first time in liver transplantation, the complementarity between these two algorithms. We retrospectively analyzed a cohort of 407 adult and 133 pediatric liver transplant patients without preformed DSA, transplanted between 1991 and 2019 in Lyon and Montpellier. HLA antibodies were detected by single antigen bead assay. HLA typing of the donor-recipient pair was achieved by serological and/or DNA-based techniques. PIRCHE-II and HLAMatchmaker algorithms were then applied on both groups. During follow-up, 27.3% of adults and 38.3% of children developed dnDSA. HLA-DRB1 and DQB1-PIRCHE-II and HLAMatchmaker scores were significantly higher in dnDSA group compared to no DSA group for both pediatric and adult patients (except for PIRCHE-II HLA-DRB1 locus score in pediatrics). ROC curves allowed determining score thresholds classifying patients in low- and high-risk of dnDSA synthesis. The two algorithms' Kaplan-Meier curves showed a predicted incidence of dnDSA 20 years after transplantation significantly lower in the low-risk group compare with the high-risk group (log rank < 0.05), in both cohorts, with a good negative predictive value. In conclusion, HLAMatchmaker and PIRCHE-II algorithms both are effective tools to identify anti-HLA immunization risk and to predict dnDSA formation after liver transplantation.

1. Introduction

The deleterious impact of donor-specific anti-HLA antibodies (DSA)-mediated rejection is well established in organ transplantation, including liver transplantation (LT) [1,2]. Both preformed and de novo DSA (dnDSA) are correlated with an increased risk of acute rejection and allograft injury after transplantation [3–6]. Donor-mismatched HLA antigens can lead to recipient DSA synthesis at any time after transplant via the indirect allorecognition pathway, which may adversely impact the liver graft. Thus, identifying dnDSA-generating risk factors is an important strategy to personalize the management and improve outcomes for LT patients.

Anti-HLA antibodies not only recognize the entire antigen but also bind to specific functional epitopes present at the HLA molecule surface [7]. Therefore, epitope-level matching between the donor and recipient represents an alternative to classic HLA matching that has already

Abbreviations: LT, liver transplantation; DSA, donor-specific alloantibodies; HLA, Human leukocyte antigen; PIRCHE-II, predicted indirectly recognizable HLA epitopes; MFI, mean fluorescence intensity;; dnDSA, de novo donor-specific HLA antibodies

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shown value in other organ transplants, especially kidney transplantation [8–10]. Some mismatched HLA epitopes generate antibody-mediated alloreactivity, while others are tolerated. Potentially immunogenic epitopes can be predicted using different algorithms that consider HLA molecules as polymorphic amino acid sequences and identify short amino acid configurations called eplets. The HLAMatchmaker algorithm determines immunogenic donor eplets based on high-resolution pair typing and calculates a predictive risk score represented by the number of eplet mismatches. Many studies, notably Kubal et al. [11], have already shown that the HLAMatchmaker score is predictive of dnDSA formation after LT, especially anti-class II HLA dnDSA. Similarly, the predicted indirectly recognizable human leucocyte antigen epitopes (PIRCHE-II) algorithm calculates a score taking into account the donor-derived HLA allopeptides that can be processed by recipient antigen-presenting cells through HLA-DRB1 molecules and presented to $CD4^+$ T helper (Th) cells [12,13]. The PIRCHE-II score thus reflects the level of indirect alloreactivity leading to dnDSA formation after transplantation. We previously demonstrated that LT recipients who develop class II dnDSA have significantly higher PIRCHE-II scores compared to patients without DSA [14].

Lachmann et al. [15] recently exposed that there is a moderate correlation between HLAMatchmaker and PIRCHE-II scores and that both are complementary but independent predictors of dnDSA formation and allograft survival following kidney transplantation. These authors established cut-offs to stratify kidney transplant recipients according to their dnDSA formation risk scores. Although there is less data on the pediatric population, several reports have already shown that HLAMatchmaker is useful in heart transplantation for identifying recipients at increased risk of graft loss [16] and in selecting donors for kidney transplantation based on DR and DQ eplet mismatch [17]. In pediatric LT, Ekong et al. established DQ locus epitope mismatch thresholds above which patients were more at risk to develop anti-DQ DSA [18].

The purpose of the present study was to compare, for the first time in LT, the HLAmatchmaker and PIRCHE-II scores in pediatric and adult patients. We hypothesized that these algorithms would be better predictors of dnDSA synthesis than classical HLA matching and that they would be correlated. We also aimed to determine thresholds to stratify patients according to the risk of developing dnDSA.

2. Materials and methods

2.1. Study population

This was a retrospective review of 407 adult patients who received a first LT between 1993 and 2019 (333 at Hospices Civils de Lyon, Lyon, France and 87 at Saint Eloi University Hospital, Montpellier, France) and 133 pediatric patients transplanted between 1991 and 2017 (Hospices Civils de Lyon).

All patients with at least one antibody monitoring test before LT and at least one during follow-up were included. Patients with preformed DSAs at the time of LT were excluded; all DSAs were therefore considered as dnDSA. The time between LT and DSA screening was calculated as the interval between transplantation and either the first positive DSA screening or the last negative DSA screening. Because of the heterogeneity of immunosuppressive therapies and incomplete data, immunosuppression and treatment adherence were not considered as variables.

2.2. HLA typing and antibody testing

Serum samples were analyzed using the Luminex Single-antigen Bead Assay (Immucor, Norcross, GA or One Lambda, Canoga Park, CA). DSAs were defined as positive by a mean fluorescence intensity (MFI) > 1000 for One Lambda tests and MFI > 1500 for Immucor tests.

Low-resolution HLA typing was performed using Luminex reverse polymerase chain reaction (PCR) sequence-specific oligonucleotides (SSO; One Lambda, Canoga Park, CA) for recipients and living donors. Deceased donors were typed by reverse PCR-SSO or by PCR-SSP using Linkage Biosciences (San Francisco, CA) kits. All tests were performed according to the manufacturers' instructions.

First field typing was available in 90% of donor-recipient pairs in adult and pediatric population and second field typing was available in 10% of the donor-recipient pairs. Retyping was not performed in this study. Missing typing and high-resolution typing were extrapolated from HaploStats based on the National Marrow Donor Program database 2007, selecting the most frequent typing in the population of interest and low-resolution typing data of patients and donors was extrapolated using a multiple imputation approach that was already described as very reliable by Geneugelijk et al. [19]. The number of mismatched HLA antigens between donors and recipients was determined in total, at the first and second field for each pair.

2.3. HLAMatchmaker analysis

HLAMatchmaker is a computer algorithm (http://www.epitopes.net) based on the analysis of HLA amino acid sequence polymorphisms and the identification of potentially immunogenic mismatched epitopes between donors and recipients (eplets). Eplets are small configurations of amino acids exposed on the donor HLA protein, not shared with the recipient, and accessible to recognition and alloantibody production.

Eplets for HLA-A, -B, -C, -DRB1, -DQB1 were assigned based on HLAMatchmaker version 2.1 and a score was calculated based on the number of mismatched eplets for each donor/recipient pair as determined by high-resolution HLA typing. HLAMatchmaker scores were calculated individually for HLA-A, -B, -C, -DRB1, -DQB1 by locus, for class II (as the sum of HLA-DRB1 and HLA-DQB1 loci) and for total loci for each donor/recipient pair. HLAMatchmaker was used considering all eplets.

2.4. PIRCHE-II analysis

The PIRCHE-II algorithm (https://www.pirche.com) is a refined HLA matching method designed to determine donor-recipient compatibility. The algorithm can predict donor HLA-derived peptides that will be presented by the recipient's HLA class II molecules and will activate $CD4^+$ T cells. The sum of all peptides results in the PIRCHE-II score, which reflects the level of indirect alloreactivity post transplantation [12,13]. The PIRCHE-II algorithm requires the second field HLA typing of the donor-recipient pair [20–22]. PIRCHE-II scores were calculated separately for HLA-A, -B, -C, -DRB1, -DQB1 by locus, for class II (as the sum of HLA-DRB1 and HLA-DQB1 loci) and for total loci for each donor/recipient pair.

2.5. Statistical analysis

Statistical analyses were performed using XLSTAT 2019.1.2. Continuous variables were expressed as means and standard deviations (SD) and categorical variables were expressed as percentages and analyzed using Chi-square test. Analysis for significance was performed using Student *t*-test in case of normal distributions. The incidence of dnDSA was analyzed with conventional Kaplan-Meier plots and log-rank tests, taking time-to-event data into account. The best predictive scores for PIRCHE II and HLAMatchmaker were obtained from the receiver operating characteristics (ROC) curves. As it was previously shown that PIRCHE-II is logarithmically correlated with HLA-antibody formation [15], the PIRCHE-II score was log-transformed for further univariate and multivariate analyses as ln(PIRCHE-II). Cox proportional hazards models were used to find variables that predicted dnDSA formation. A p-value < .05 was considered significant.

Table 1
Demographic and immunologic characteristics of patients for adult and pediatric cohorts.

	Adults $N = 407$			Children $N = 133$		
	No DSA $n = 296$ (72.7)	DSA $n = 111$ (27.3)	p-value	No DSA $n = 82$ (61.7)	DSA $n = 51$ (38.3)	p-value
Age						
Recipients	52 ± 11.1	52 ± 10.6	0.830	5 ± 4.6	5 ± 4.8	0.667
Donors	46 ± 18.7	46 ± 18.5	0.805	20 ± 13.8	20 ± 13.7	0.996
Sex						
F	87 (29.4)	32 (28.8)		44 (53.7)	23 (45.1)	
M	209 (70.6)	79 (71.2)		38 (46.3)	28 (54.9)	
Primary diagnostic						
OH (adults)/Biliary atresia (pediatrics)	144 (49)	51 (46)		36 (44)	25 (49)	
Others	152 (51)	60 (54)		46 (56)	26 (51)	
Living donors						
All living donors	18 (6.1)	5 (4.5)		15 (18.3)	9 (17.6)	
Haploidentical or identical donors	16 (5.4)	3 (2.7)		14 (17.0)	9 (17.6)	
Number of HLA mismatch						
Class I (A B C) 1st field	4.2	4.6	0.014	4.3	4.3	0.882
Class I (A B C) 2nd field	4.5	5	< 0.001	4.6	4.5	0.742
Class II (DRB1, DQB1) 1st field	2.2	2.9	< 0.001	2.1	2.6	0.009
Class II (DRB1, DQB1) 2nd field	2.8	3.3	< 0.001	2.9	3.1	0.186
Total 1st field	6.4	7.4	< 0.001	6.4	6.9	0.139
Total 2nd field	7.3	8.2	< 0.001	7.5	7.6	0.672
HLAMatchmaker score						
Class I	16.9	18.2	0.099	16.9	17.1	0.903
DRB1	10.5	13.6	< 0.001	10.1	12.6	0.022
DQB1	9.2	10.9	0.015	8.8	12.1	0.003
Class II	19.7	24.5	< 0.001	19.0	24.7	< 0.001
Total	36.6	42.7	< 0.001	35.9	41.8	0.013
PIRCHE-II score						
A	18.9	20.7	0.374	19.6	16.9	0.319
B	17.4	18.1	0.600	17.8	16.8	0.653
C	16.8	16.2	0.609	17.9	14.1	0.106
DRB1	15.4	18.3	0.016	16.9	16.5	0.863
DQB1	21.9	28.1	< 0.001	20.1	26.8	0.017
Class II	37.3	46.4	< 0.001	3.7	43.3	0.125
Total	88.8	97.3	0.082	91.1	89.5	0.849
DSA specificities						
A		3 (2.7)			1 (1.7)	
B		1 (0.9)			0	
C		7 (6.3)			0	
DR		41 (36.9)			14 (27.5)	
DQ		85 (76.6)			40 (78.4)	
DP		4 (3.6)			3 (5.9)	
dnDSA occurrence in years		6.5 ± 6.5			8.2 ± 6.5	

Data given as mean (%) and +/− SD.

3. Results

3.1. Study population, HLA antigen mismatch, PIRCHE-II and HLAMatchmaker scores

The adult cohort included 407 patients. Their demographic and immunologic characteristics are shown in Table 1. During follow-up, 111 patients (27.3%) developed dnDSA at a mean of 6.5 ± 6.5 years post transplantation. These 111 patients developed 141 dnDSA. Eleven (7.8%) were class I dnDSA and 130 (92.2%) were class II dnDSA. Three patients (2.7%) developed both class I and class II dnDSA. Patient and donor age at transplantation, sex, or primary liver disease were comparable between the two groups, with and without dnDSA.

Of 133 LT patients of the pediatric cohort, 51 patients (38.3%) developed dnDSA at a mean time of 8.2 ± 6.5 years post transplantation (Table 1). A total of 58 dnDSA were detected: one (1.7%) class I dnDSA and 57 (98.3%) class II dnDSA. Any of the pediatric patients developed both class I and class II dnDSA.

The distributions of the classical HLA mismatches, HLAMatchmaker scores, and PIRCHE-II scores in the adult cohort are shown in Fig. 1.

In the adult cohort, the number of first and second field class I, class II, and total classical HLA mismatches was significantly different between the no DSA and dnDSA groups ($p < .001$). Conversely, in children, only first field class II classical HLA mismatches were statistically different between the two groups ($p = .009$).

Total class I and II HLAMatchmaker scores ranged from 0 to 88 in adults (mean 38.3 ± 13.6, Fig. 1A) and were normally distributed. Total class I and II scores ranged from 0 to 94 in children (mean of 42.3 ± 20.7) and were also normally distributed. Total ($p < .001$ and $p = .013$), DRB1 locus ($p < .001$ and $p = .022$), DQB1 locus ($p = .015$ and $p = .003$), and class II DRB1 + DQB1 ($p < .001$ and $p < .001$) HLAMatchmaker scores were statistically different between no DSA and dnDSA groups, respectively, in both adult and pediatric cohorts (Table 1). Conversely, class I scores were comparable between groups in both cohorts ($p = .099$ and $p = .903$, respectively). High number of HLA mismatches were closely correlated with higher HLAMatchmaker scores ($R^2 = 0.9946$; Fig. 1B).

The total PIRCHE-II scores distribution for adults was much wider, with values ranging from 0 to 273.5 (mean 91.1 ± 43.5, median 83; Fig. 1C) and a left-skewed distribution. In adult patients, HLA-DRB1 + DQB1 ($p < .001$), -DRB1 ($p = .016$), and -DQB1 ($p < .001$) PIRCHE-II scores but not class I and total PIRCHE-II scores were significantly different between the no DSA and the dnDSA groups (Table 1).

In the pediatric cohort the total PIRCHE-II score distribution ranged from 0 to 244 (mean 90.5; median 85). Pediatric PIRCHE-II scores were

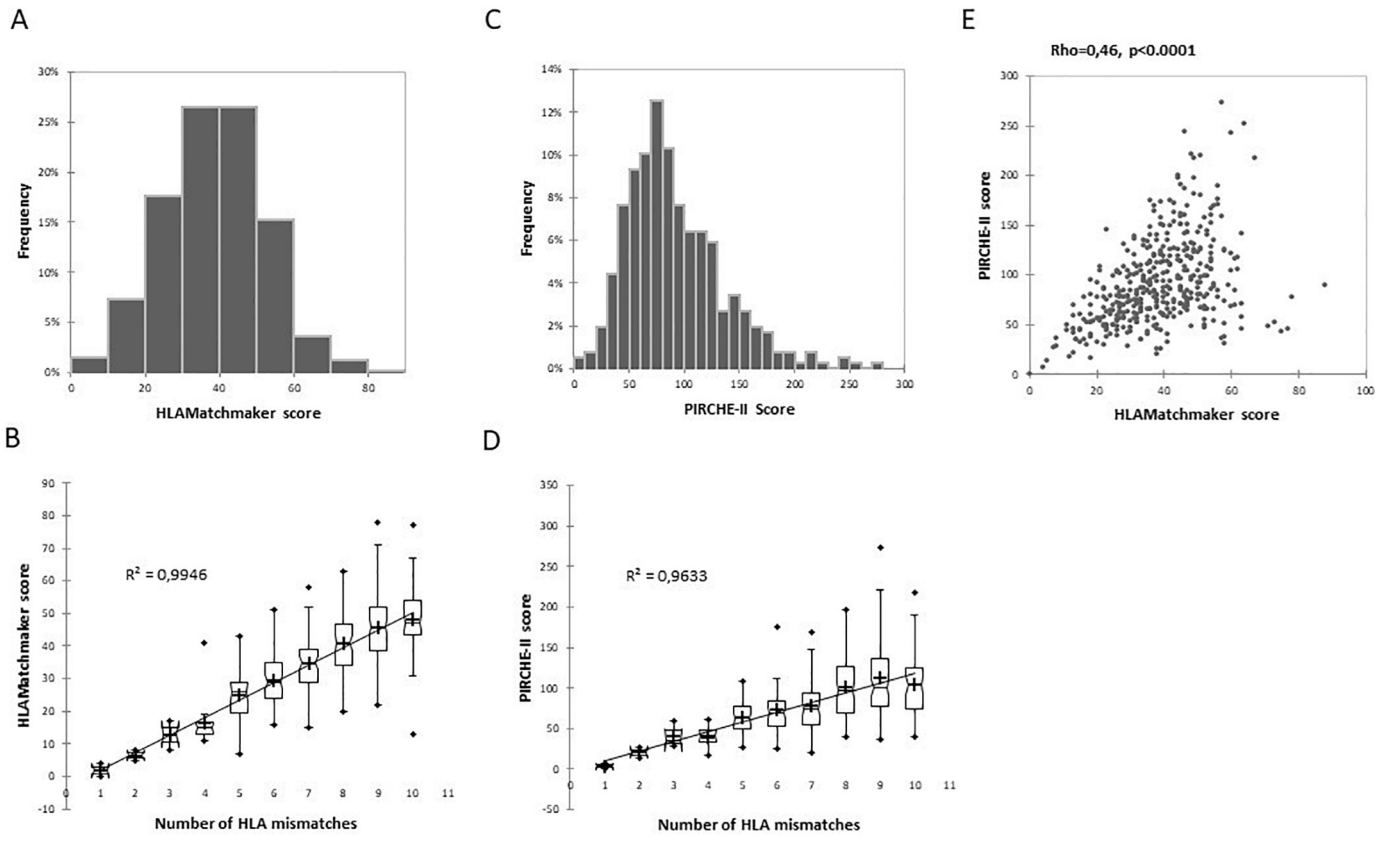

Fig. 1. Descriptive analysis of HLAMatchmaker and PIRCHE-II scores in the liver transplanted adults' cohort.
(A) Frequency distribution in percentage of HLAMatchmaker scores, (B) Association between HLAMatchmaker scores and the number of classical HLA ABCDRDQ mismatches at the one-field level (coefficient of determination R^2 = 0,9946), (C) Frequency distribution in percentage of PIRCHE-II scores, (D) Association between PIRCHE-II scores and the number of classical HLA ABCDRDQ mismatches at the one-field level (coefficient of determination R^2 = 0,9633), (E) Association of the PIRCHE scores with the HLAMatchmaker scores (Spearman rank-order correlation coefficient Rho of 0.46, p < .0001). The box plots of panels (B) and (D) represent the mean, the median and first to third quartile, the highest and lowest value.

not significantly different between groups except for the HLA-DQB1 PIRCHE-II score (p = .017) (Table 1).

In the adult cohort the number of HLA mismatches and PIRCHE-II scores were again highly correlated (R^2 = 0.9633), with a wider distribution of PIRCHE-II scores with greater numbers of HLA mismatches (Fig. 1D). HLAMatchmaker and PIRCHE-II scores were moderately correlated (Rho = 0.46, p < .0001) (Fig. 1E).

3.2. PIRCHE-II and HLAMatchmaker scores predict dnDSA synthesis

To identify high and low risk patients, thresholds were determined using ROC analysis (data not shown) for PIRCHE-II and HLAMatchmaker scores, with low-risk patients defined as having scores below the best predictive cutoff and high-risk patients with scores above the best predictive cutoff. Prediction of dnDSA synthesis during follow up was assessed by Kaplan-Meier analysis and log-rank testing (Figs. 2 and 3). Since class I scores were comparable between no DSA and dnDSA groups in both cohorts, Kaplan-Meier analysis was based on class II loci.

In adult patients the cut-off values were 11 for HLA-DRB1 PIRCHE-II and HLAMatchmaker scores, 12 for the -DQB1 HLAMatchmaker score, 27.6 for the -DQB1 PIRCHE-II score, 20 for the sum of -DRB1 + DQB1 HLAMatchmaker score, and 40 for the sum of - DRB1 + DQB1 PIRCHE-II score. After 20 years of follow-up post transplantation and using identified cut-offs, the HLAMatchmaker and PIRCHE-II algorithm predicted an incidence of dnDSA significantly lower in the low-risk group compare with the high-risk group (Fig. 2A–F).

In pediatric patients, thresholds were found as 12 for HLA-DRB1, 8 for HLA-DQB1 and 13 for the sum of HLA- DRB1 + DQB1 for HLAMatchmaker score and 5 for HLA-DRB1, 13 for HLA-DQB1 and 20 for the sum of HLA- DRB1 + DQB1 for PIRCHE-II scores.

Twenty years after transplantation, the predicted incidence of dnDSA was higher at all loci the high-risk group compare with the low-risk group (Fig. 3A–F).

Table 2 summarizes the uni- and multivariate Cox regression models comparing the predictive capacity for dnDSA formation of ln(PIRCHE-II) score, HLAMatchmaker score and the number of HLA antigen mismatches, adjusted for donor and recipient age. These three variables significantly contributed to univariate analysis but only ln(PIRCHE-II) and the number of HLA antigen mismatches contributed to predict independently dnDSA formation in a multivariate COX model.

The sensitivity, specificity, positive predictive value (PPV), as well as negative predictive value (NPV) for both algorithms depending on the determined thresholds were calculated. As shown in Table 3, NPV values for both HLAMatchmaker and PIRCHE-II algorithms ranged from 0.77 to 0.82 for adults and from 0.71 to 0.89 for children, while PPV values ranged from 0.32 to 0.39 for adults and from 0.45 to 0.46 for children. Misclassified patients were defined as patients without any DSA and classified in high-risk group with both algorithms, or patients with dnDSA and classified in low-risk group.

4. Discussion

There is increasing evidence that epitope-level matching between donors and recipients has additional benefits over classic HLA antigen matching in organ transplantation. To the best of our knowledge, our

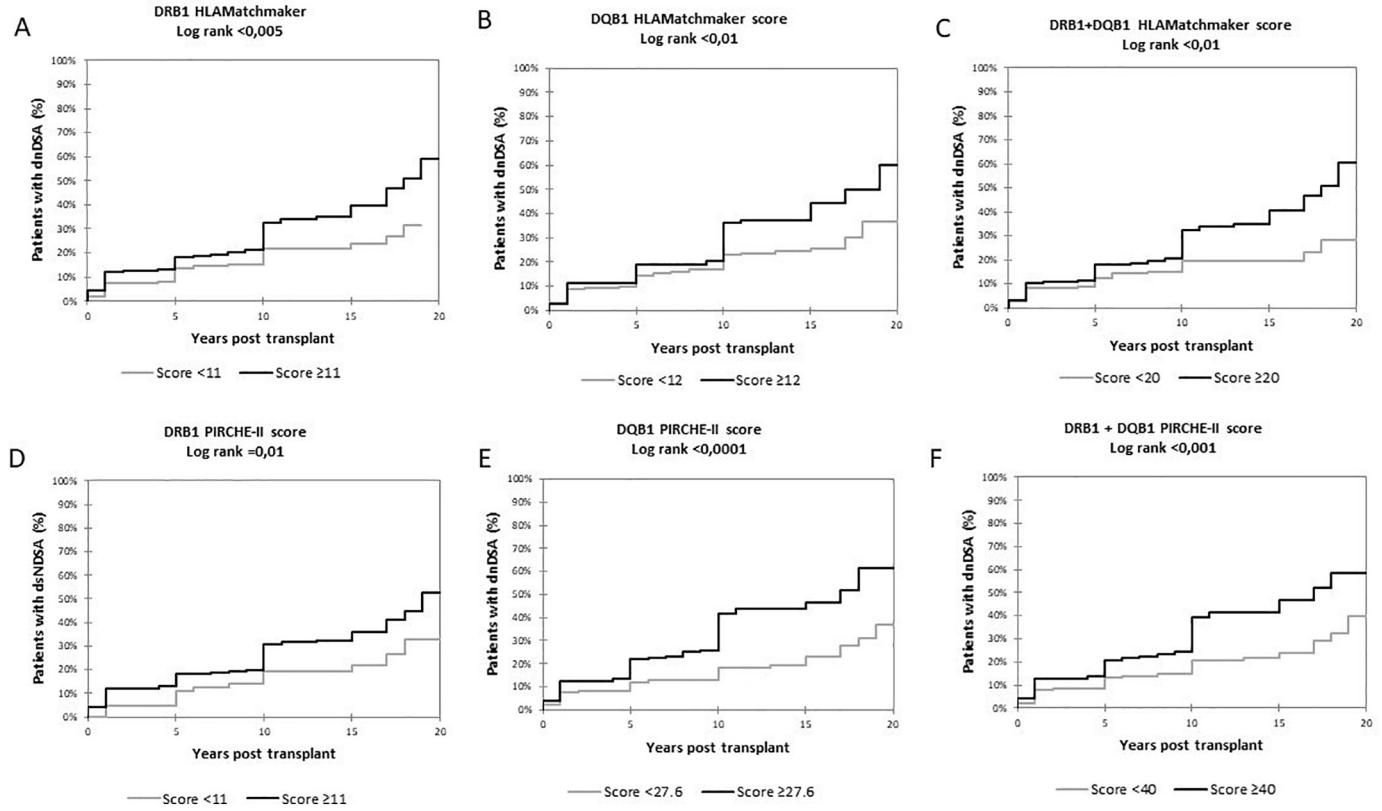

Fig. 2. Kaplan-Meier plots illustrating the predicted incidence (PI) of dnDSA after 20 years post liver transplantation in adults' cohort, stratifying patients in low-risk and high-risk groups according to their HLAMatchmaker score and their PIRCHE-II score (low-risk in grey line and high-risk in black line), for DRB1, DQB1 and DRB1 + DQB1 loci.
(A) DRB1 HLAMatchmaker locus (score < 11, PI = 31,6%; score ≥ 11, PI = 59%), (B) DQB1 HLAMatchmaker locus (score < 12, PI = 36,6%; score ≥ 12, PI = 60%), (C) DRB1 + DQB1 HLAMatchmaker locus (score < 20, PI = 28,4%; score ≥ 20, PI = 60,7%), (D) DRB1 PIRCHE-II locus (score < 11, PI = 32,6%; score ≥ 11, PI = 52,8%), (E) DQB1 PIRCHE-II locus (score < 27,6, PI = 37,1%; score ≥ 27,6, PI = 61,5%), (F) DRB1 + DQB1 PIRCHE-II locus (score < 40, PI = 40%; score ≥ 40, PI = 58,7%)

study is the first one comparing the predictive performance of HLAMatchmaker and PIRCHE-II algorithms for dnDSA occurrence in adult and pediatric LT recipients. Lachmann et al. recently reported a moderate correlation between the two algorithms and that PIRCHE-II predicted dnDSA formation and graft survival following kidney transplantation in adult patients [15].

We found that both HLAMatchmaker and PIRCHE-II algorithms were good predictors for dnDSA formation after LT for HLA class II loci in both adults and children. PIRCHE-II HLA-DRB1, -DQB1, and -DRB1 + DQB1 scores were significantly higher in adult patients who did develop dnDSA than those who did not, while in children only HLA-DQB1 PIRCHE-II score significantly differed between patients in the two groups. This may be explained by the fact that the pediatric population was smaller compared to the adult population and moreover recipients and donors are better matched in the pediatric population, with 17.6% and 17.0% of children receiving related living donor organs (with haploidentical or identical HLA typing) in each group, respectively, versus only 2.7% and 5.4% adult patients (Table 1). The moderate correlation between the two algorithms suggests that the two approaches may in some extent represent different aspects of epitope matching and therefore be complementary.

We defined HLAMatchmaker and PIRCHE-II scores thresholds for HLA-DRB1, HLA-DQB1, and HLA-DRB1 + DQB1 scores to stratify patients into low- and high-risk group of class II immunization post LT. The predicted incidence of dnDSA 20 years after LT in our adult population was significantly higher in high-risk group compared to patient in low-risk group with both algorithms. In their study, Lachmann et al. [15] evaluated the individual contributions of low or high PIRCHE-II scores using the first quartile versus the fourth quartile and focused on the development of locus-specific dnDSA for patients with one HLA mismatch at the corresponding locus. They showed that the probability of dnDSA for HLA-A, -B, -DR, and DQB loci was significantly higher in patients with correspondingly high PIRCHE-II scores (HLA-Matchmaker assessment was not performed for each locus). In a study of 286 kidney transplant recipients, Wiebe et al. reported that HLA-Matchmaker eplet mismatch scores < 10 for HLA-DR and < 17 for HLA-DQ were associated with minimal dnDSA synthesis [8]. Another small kidney transplant study reported that no patient with an HLA-Matchmaker score < 6 developed HLA-DQ dnDSA, while 84% of patients with a score ≥ 6 developed dnDSA [23]. PIRCHE-II stratification was based on quartiles, with no dnDSA patients in the first quartile and 100% in the third. In LT, Kubal et al. [11] calculated that an HLA-DR/DQ Abver eplet HLAMatchmaker threshold of 12 was associated with dsDSA development and found an association between class I eplet mismatch score and acute cellular rejection.

In our pediatric population, HLAMatchmaker and PIRCHE-II scores predicted higher proportions of dnDSA synthesis 20 years after transplantation than in adults, in both low- and high-risk groups. As for adults, there was greater immunoreactivity against the HLA-DQ locus. Although HLAMatchmaker has already been shown to be useful in pediatric transplantation populations [16–18], very few patients are well eplet matched. In a small cohort of pediatric kidney transplant recipients, 40% of one HLA-DR antigen-mismatched donors and 64% of two HLA-DR antigen-mismatched donors were in the high-risk group for both DR and DQ dnDSA synthesis (based on pre-established thresholds in a kidney transplantation study) [17]. In LT, only the

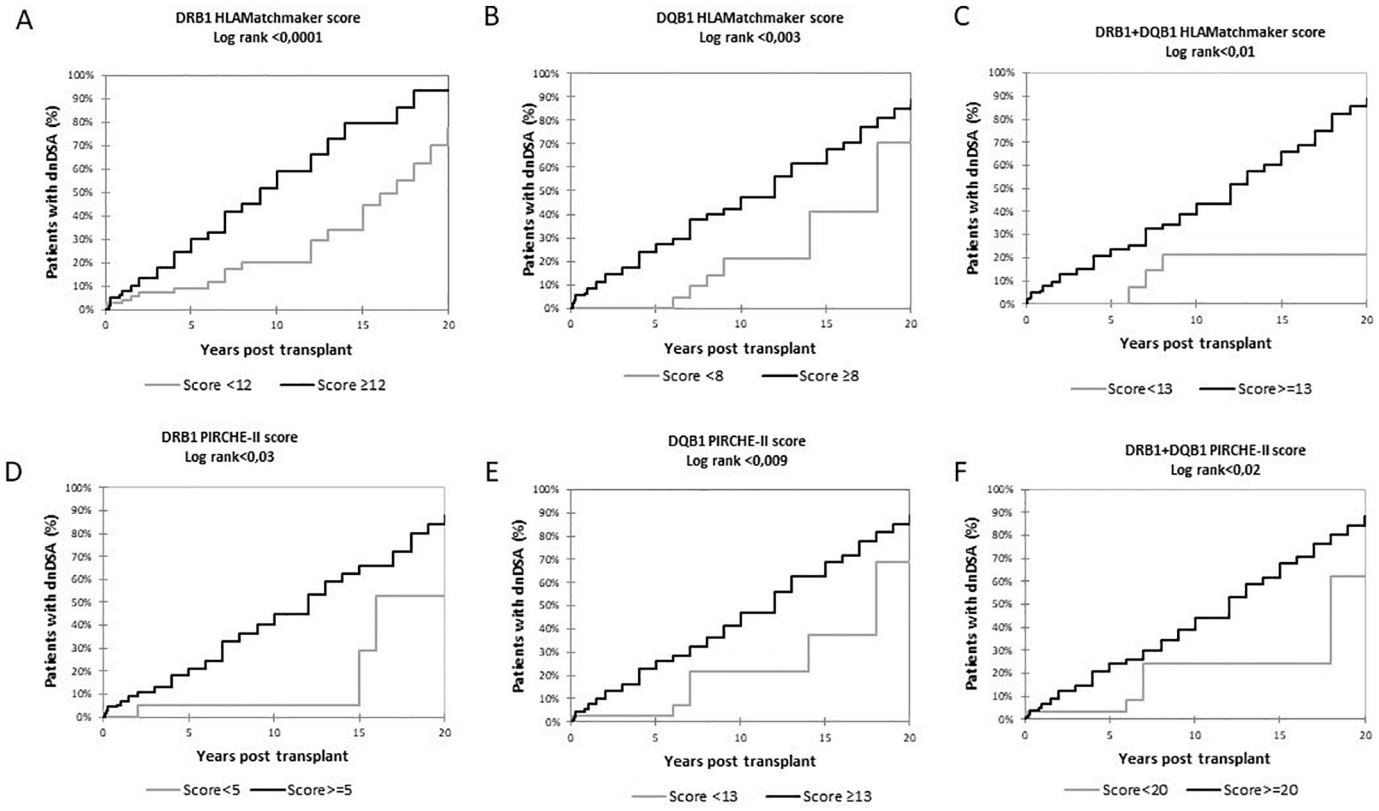

Fig. 3. Kaplan-Meier plots illustrating the predicted incidence (PI) of dnDSA after 20 years post liver transplantation in pediatric cohort, stratifying patients in low-risk and high-risk groups according to their HLAMatchmaker score and their PIRCHE-II score (low-risk in grey line and high-risk in black line), for DRB1, DQB1 and DRB1 + DQB1 loci.
(A) DRB1 HLAMatchmaker locus (score < 12, PI = 77,5%; score ≥ 12, PI = 93,2%), (B) DQB1 HLAMatchmaker locus (score < 8, PI = 70,5%; score ≥ 8, PI = 88,6%,(C) DRB1 + DQB1 HLAMatchmaker locus (score < 13, PI = 21,4%; score ≥ 13, PI = 89,3%), (D) DRB1 PIRCHE-II locus (score < 5, PI = 52,6%; score ≥ 5, PI = 87,9%), (E) DQB1 PIRCHE-II locus (score < 13, PI = 68,8%; score ≥ 13, PI = 89%), (F) DRB1 + DQB1 PIRCHE-II locus (score < 20, PI = 62,2%; score ≥ 20, PI = 92,1.

Table 2
Univariate and multivariable hazard ratios (HRs) of ln(PIRCHE-II score), number of HLA-A, -B, -DR, -DQ mismatches, age of donor,age of recipient, and HLAMatckmaker class II to predict de novo DSA in adult patients.

	Univariate Analysis			Multivariate analysis*		
	HR	P-value	CI 95%	HR	P-value	CI 95%
Recipient age	1	ns	0.9–1		ns	
Donor age	1	ns	0.9–1		ns	
ln(PIRCHE-II score)	**1.7**	**0.001**	**1.3–2.3**	**1.47**	**0.03**	**1.03–2.1**
ABCDRDQ total count of mismatch	**1.5**	**0.001**	**1.2–1.7**	**1.17**	**0.03**	**1.0–1.3**
HLA Matchmaker class II score	1.03	0.001	1.01–1.05		ns	

Bolds represent significative results in univariate and multivariate analysis.

HLAMatchmaker algorithm has been demonstrated to be predictive of anti-DQ dnDSA development, with cutoffs > 5 or 6 epitopes proposed [18]. The only available study on PIRCHE-II performance in pediatric LT recipients was performed in the context of intestinal and multivisceral transplantation (including liver), which did not reveal any association between PIRCHE-II and HLAMatchmaker epitope matching and dnDSA formation [24]. Interestingly, in our pediatric study even if mean PIRCHE-II HLA-DRB1 and PIRCHE-II HLA-DRB1 + DQB1 scores were comparable between dnDSA group and no DSA group 20 years post transplantation the predicted incidence of dnDSA based on our cut-offs was significantly higher in high-risk groups compared to low-risk groups.

Based on our data, HLAMatchmaker and PIRCHE-II have been demonstrated as good predictors for dnDSA formation, illustrated by the univariate cox regression. Contrariwise, HLAMatchmaker score did not seem to contribute to multivariate analysis, in contrast to Lachmann et al. [15] where both algorithms were found to be independent predictors of dnDSA synthesis. This could be explained by the lower number of transplant patients in our study compared to Lachmann et al. [15].

For both algorithms, our cut-offs had good negative predictive value in both populations. The few patients with dnDSA and misclassified in low-risk group with HLAMatchmaker and PIRCHE-II algorithms could be partially justified by an immunization from another source than the graft (such as transfusion, pregnancy or modification of the immunosuppressive treatment). A recipient with a predicted score below the threshold could be regarded as having only a low risk of developing dnDSA against the donor epitopes. This suggests that patients having a low risk score could be consider at low risk to develop dnDSA and DSA surveillance could be delayed until 6.5 years post transplantation except in case of clinical suspicion. Otherwise, PIRCHE-II and HLAMatchmaker scores might be useful in selecting the optimal graft with the lowest risk of future complications for the patient, limiting antibody-mediated rejection and increasing the possibility for future transplantations with low immunologic risk.

Patients without dnDSA and misclassified in high-risk groups in both algorithms could be explained by the time limit to dnDSA detection considering that mean occurrence of dnDSA is 6.5 years post LT for

Table 3
HLAMatchmaker and PIRCHE-II tests' positive predictive value and negative predictive value histogram for DRB1, DQB1and DRB1 + DQB1 loci determined according to the cutoffs established in adult and pediatric cohorts.

		HLAMatchmaker			PIRCHE-II		
		DRB1	DQB1	DRB1 + DQB1	DRB1	DQB1	DRB1 + DQB1
Positive predictive value	Adults	0.36	0.35	0.34	0.32	0.39	0.36
	Children	0.49	0.48	0.45	0.42	0.46	0.44
Negative predictive value	Adults	0.82	0.77	0.81	0.81	0.80	0.80
	Children	0.71	0.85	0.89	0.84	0.82	0.85

adults. Our study has some limitations. The retrospective nature of DSA monitoring after LT and the lack of monitoring homogeneity in patients might have underestimated the number of patients developing dnDSA. Some high-resolution HLA typing were not available, with two-fields level typing extracted by inference to select the most frequent typing in the population, which may have introduced bias. Our study was not focused on clinical outcomes and it might be interesting to integrate patients' survival, graft survival and immunosuppressive treatment in a future study to confirm our results. Moreover, we did not assess the dnDSA incidence correlated with the two algorithms' scores for each corresponding locus. Nevertheless, this is the first reported large cohort study on adult and pediatric patients comparing PIRCHE-II and HLA Matchmaker algorithms in predicting dnDSA after LT.

In conclusion, in the field of LT, the impact of histocompatibility, antigen levels, and epitope matching have probably been undervalued. HLAMatchmaker and PIRCHE-II algorithms could both help with graft allocation to select epitope mismatches between patient and donor with the lowest risk of dnDSA formation. In the future it will be interesting to explore the value of both algorithms on predicting, graft and patient survival after LT.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.trim.2020.101306.

References

[1] P.I. Terasaki, A personal perspective: 100-year history of the humoral theory of transplantation, Transplantation 93 (8) (2012) 751–756.
[2] R.N. Wesson, E.W. Etchill, J. Garonzik-Wang, Application and interpretation of histocompatibility data in liver transplantation, Curr. Opin. Organ. Transplant. 22 (2017) 499–504.
[3] P.I. Terasaki, M. Ozawa, R. Castro, Four-year follow-up of a prospective trial of HLA and MICA antibodies on kidney graft survival, Am. J. Transplant. 7 (2) (2007) 408–415.
[4] L.E. Morales-Buenrostro, R. Castro, P.I. Terasaki, A single human leukocyte antigen antibody test after heart or lung transplantation is predictive of survival, Transplantation 85 (3) (2008) 478–481.
[5] D. Cantarovich, S. De Amicis, A. Akl, et al., Posttransplant donor-specific anti-HLA antibodies negatively impact pancreas transplantation outcome, Am. J. Transplant. 11 (12) (2011) 2737–2746.
[6] K.M. Abu-Elmagd, G. Wu, G. Costa, et al., Preformed and de novo donor specific antibodies in visceral transplantation: long-term outcome with special reference to the liver, Am. J. Transplant. 12 (11) (2012) 3047–3060.
[7] A.R. Tambur, HLA epitopes as viewed by antibodies: what is it all about? Am. J. Transplant. 15 (5) (2015) 1148–1154.
[8] C. Wiebe, D. Pochinco, T.D. Blydt-Hansen, et al., Class II HLA epitope matching-a strategy to minimize de novo donor-specific antibody development and improve outcomes, Am. J. Transplant. 13 (12) (2013) 3114–3122.
[9] M.K.A. Dankers, M.D. Witvliet, D.L. Roelen, et al., The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched human leukocyte antigens, Transplantation 77 (2004) 1236–1239.
[10] G. Laux, U. Mansmann, A. Deufel, et al., A new epitope-based HLA-DPB matching approach for cadaver kidney retransplants, Transplantation 75 (2003) 1527–1532.
[11] C.A. Kubal, R. Mangus, B. Ekser, et al., Class II human leucocyte antigen epitope mismatch predicts de novo donor-specific antibody formation after liver transplantation, Liver Transpl. 24 (8) (2018) 1101–1108.
[12] K. Geneugelijk, K.A. Thus, E. Spierings, Predicting alloreactivity in transplantation, J Immunol Res 159479 (2014).
[13] K. Geneugelijk, E. Spierings, Matching donor and recipient based on predicted indirectly recognizable human leucocyte antigen epitopes, Int. J. Immunogenet. 45 (2018) 41–53.
[14] M. Meszaros, V. Dubois, M. Niemann, et al., Class II human leukocyte antigen epitope mismatch predicts de novo donor-specific antibody formation after liver transplantation, Liver Transpl. 25 (1) (2019) 184–185.
[15] N. Lachmann, M. Niemann, P. Reinke, et al., Donor recipient matching based on predicted recognizable HLA epitopes predicts the incidence of de novo donor-specific HLA antibodies following renal transplantation, Am. J. Transplant. 17 (12) (2017) 3076–3086.
[16] P.M. Sullivan, P. Warner, M.S. Kemna, et al., HLA molecular epitope mismatching and long-term graft loss in pediatric heart transplant recipients, J. Heart Lung Transplant. 34 (7) (2015) 950–957.
[17] C.F. Bryan, V. Chadha, B.A. Warady, Donor selection in pediatric kidney transplantation using DR and DQ eplet mismatching: a new histocompatibility paradigm, Pediatr. Transplant. 20 (7) (2016) 926–930.
[18] U.D. Ekong, S. Antala, L. Bow, et al., HLA, non-HLA antibodies, and eplet mismatches in pediatric liver transplantation: observations from a small, single-center cohort, Exp. Clin. Transplant. 17 (Suppl. 1) (2019) 6–17.
[19] K. Geneugelijk, J. Wissing, D. Koppenaal, M. Niemann, E. Spierings, Computational approaches to facilitate epitope-based HLA matching in solid organ transplantation, J Immunol Res 2017 (2017) 1–9, https://doi.org/10.1155/2017/9130879.
[20] K. Geneugelijk, M. Niemann, J. Drylewicz, et al., PIRCHE-II is related to graft failure after kidney transplantation, Front. Immunol. 9 (2018) 321.
[21] K. Geneugelijk, M. Niemann, T. de Hoop, et al., Completion of HLA protein sequences by automated homology-based nearest-neighbor extrapolation of HLA database sequences, Hum. Immunol. 77 (11) (2016) 1030–1036.
[22] M. Maiers, L. Gragert, W. Klitz, High-resolution HLA alleles and haplotypes in the United States population, Hum. Immunol. 68 (2007) 779–788.
[23] L. Daniëls, M. Naesens, J.L. Bosmans, et al., The clinical significance of epitope mismatch load in kidney transplantation: a multicentre study, Transpl. Immunol. 50 (2018 Oct) 55–59.
[24] P. Talayero, E. Ramos Boluda, E. Gómez Massa, et al., Donor-specific antibodies in pediatric intestinal and multivisceral transplantation: the role of liver and human leukocyte antigen mismatching, Liver Transpl. 24 (12) (2018) 1726–1735.