ORIGINAL RESEARCH

Human Leukocyte Antigen-Based Risk Stratification in Heart Transplant Recipients—Implications for Targeted Surveillance

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***Background*—**Human leukocyte antigen (HLA) matching isn't routinely performed in heart transplantation. Novel allograft perfusion methods may make HLA matching feasible. The purpose of this study is to reexamine whether HLA mismatch may be used in risk stratification to improve outcomes in heart transplantation.

***Methods and Results*—**We analyzed 34 681 recipients undergoing heart transplantation between 1987 and 2013. We used HLAMatchmaker to quantify HLA eplet mismatches and Cox regression for analysis of time to graft loss. Recipients with 4 mismatched HLA-DR/DQ alleles and >40 eplets reached an adjusted hazard ratio (HR) for graft loss of 1.17 (95% CI 1.07–1.28) and 1.11 (95% CI 1.03–1.21), respectively. We found significant interaction between recipient age and numbers of HLA-DR/DQ allele and eplet mismatches resulting in an adjusted HR of 1.78 (95% 1.13–2.80) and 1.82 (95% CI, 1.23–2.70), respectively. HR for both interaction terms was 0.99 (95% CI, 0.98–1.00). Risk of graft loss was more pronounced after 1 year, where recipient <40 years with 4 mismatched HLA-DR/DQ alleles and >40 eplets had an adjusted HR of 1.51 (95% CI 1.12–2.03) and 1.32 (95% CI 1.02–1.70), respectively. Pre-sensitized recipients with panel reactive antibodies >10% had an adjusted HR=1.27 (95% CI 1.16–1.40) for graft loss within 1 year but not thereafter. HLA eplet mismatch was independent of panel reactive antibodies on reduction of graft loss within and after 1 year, *P* (interaction)=0.888 and 0.389.

***Conclusions*—**HLA mismatch may be used in risk stratification for intensified post-transplant surveillance and therapy. **(*J Am Heart Assoc*. 2019;8:e011124. DOI: 10.1161/JAHA.118.011124.)**

Key Words: HLAMatchmaker • human leukocyte antigen • rejection • risk stratification • survival • transplantation

Survival after heart transplantation has improved markedly over the past 2 decades, particularly in the short-term, but graft dysfunction remains the leading cause of mortality.[1] Individual differences in the genetic constitution are likely to be important determinants of long-term graft loss, such as human leukocyte antigen (HLA) and potentially other polymorphic sites.[2,3] Age-related alterations in the immune system, HLA sensitization events, and differences in susceptibility to immunosuppressive regimens may also influence the outcomes.[4,5] By identifying patients at increased risk, a targeted strategy could be developed to individualize and intensify both monitoring and treatment post-transplant, thereby reducing mortality and morbidity.[1,6,7]

Several studies have shown a positive effect of HLA matching on survival. HLA matching avoids the production of donor specific antibodies (DSA) that are detrimental to the transplant. Matching of ≥3 HLA loci (Figure 1) improves survival in heart transplanted patient and decreases the risk of rejection during the first-year post-transplant.[8,9] However, studies differ on the HLA locus or loci identification of that influence the outcome. There is evidence from other solid organ transplants than heart that certain HLA allele mismatches may be more antigenic than others and that some allele mismatches may be inconsequential.[10] Anti-HLA antibodies recognize distinct exposed regions of the HLA antigen that consist of amino acid sequences located within the HLA

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Clinical Perspective

What Is New?

- Studies on how the structure of the human leukocyte antigen molecules influence on long-term graft loss in heart transplanted recipients are limited.
- This is the first study evaluating the human leukocyte antigen Matchmaker algorithm as a risk stratification tool in an adult heart transplantation population.
- Human leukocyte antigen-DR/DQ allele/eplet mismatch results in an increased risk of late graft loss.

What Are the Clinical Implications?

- The results of this study identify recipients with an increased risk of future rejection and graft loss based on their human leukocyte antigen-DR/DQ allele/eplet mismatch load.
- By identifying patients at increased risk, a targeted strategy could be developed to individualize and intensify both monitoring and treatment post-transplant, thereby reducing mortality and morbidity.

molecule. These so-called epitopes (Figure 1) are shared among HLA alleles and between HLA loci where the term eplet often is defined as clusters/patches of polymorphic residues ≈3 to 5 ångströms apart.[11] This observation may explain why sensitizing events such as a previous allograft, pregnancy, and blood transfusions can induce anti-HLA antibodies toward more than the specific HLA-antigens involved in the sensitizing event and as a consequence result in high panel reactive antibody status.[12] Furthermore, the epitope load of HLA mismatch correlates with the development of DSA which can result in rejection and graft loss.[12–14] The amount of mismatched epitope load could therefore be regarded as a risk factor and be used to adjust both monitoring and treatment post-transplant.

Prior studies evaluating the effect of HLA matching on post-transplant outcomes have focused on the measure of the level of HLA allele mismatch in adult heart transplanted patients. Studies on how the structure of the HLA molecules influences long-term graft loss in heart transplanted recipients are limited. Furthermore, donor-recipient HLA matching in heart transplantation is occasionally infeasible because of the time needed for advanced immunological analysis and evaluation. However, novel approaches to allograft perfusion may allow for longer times between allograft procurement and transplant, possibly making HLA matching feasible.[15] Whether HLA matching would improve long-term outcomes in heart transplantation should therefore be reexamined. In this study, we aimed to investigate the influence of HLA allele and HLA eplet mismatch on graft survival using a comprehensive approach in a large, contemporary cohort of heart transplant recipients.

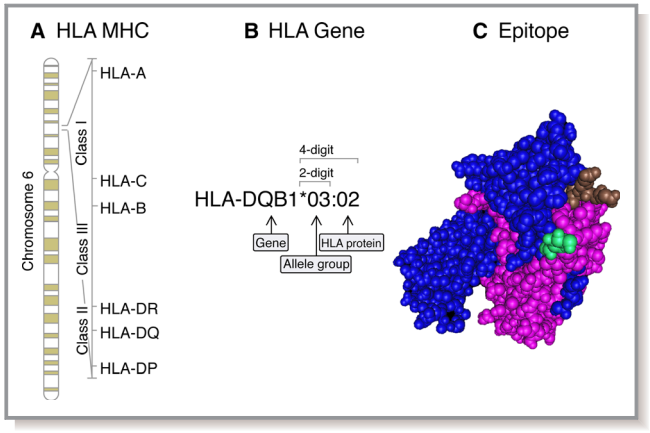

Figure 1. **A**, The human leukocyte antigen (HLA) gene complex encoding the major histocompatibility complex (MHC), class I (A, B, and C) and class II (DP, DQ, and DR). **B**, The HLA nomenclature (http://hla.alleles.org/nomenclature/naming.html). Each allele has a unique number, which consists of a sequence of 2 to 4 sets of digits separated by colons. All alleles receive at least a 4-digit name. The letters/number defines the locus/gene, the first field the allele group, and the second field the specific HLA protein. **C**, A 3-dimensional structure of an HLA-DQ molecule in which the pink amino acids compose the DQ α-chain, the blue amino acids compose the DQ β-chain, and the brown amino acids represent the peptide stuck within the peptide binding groove. The specific eplet identified is highlighted in blue. The crystalline model was downloaded from the National Center for Biotechnology Information website http://www.ncbi.nlm.nih.gov/Structure using Cn3D software. HLA indicates human leukocyte antigen; MHC, major histocompatibility complex.

Materials and Methods

Data Availability

The data that support the findings of this study are available from the SRTR (Scientific Registry of Transplant Recipients), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available.

Study Population

We extracted subjects from the SRTR (Scientific Registry of Transplant Recipients) undergoing primary heart transplantation in the United States between October 1, 1987 and September 30, 2013 (n=56 429). We excluded patients with incomplete follow-up or follow-up time <1 day, history of previous transplantation, pediatric cases (aged <18 years), or unknown age, incomplete typing of HLA-A and HLA-B and HLA-DRB1 (HLA-DR), n=21 748 (Figure 1). The final study

population was composed of 34 681 patients, with at least 1 day of follow-up duration.

Study Design and Definitions

The primary objective for the study was to evaluate the influence of the number of mismatched HLA alleles and eplets on graft loss (GL) after transplantation. We defined the primary end point for the study, GL, as patient death or re-transplantation within 20 years after transplantation. A secondary objective for the study was to evaluate the influence of the number of mismatched HLA alleles and HLA eplets on GL within 1 year and beyond 1 year after heart transplantation. We used HLA-typing data of class I (HLA-A, HLA-B, HLA-C) as well as class II (HLA-DRB1 [HLA-DR], HLA-DQB1 [HLA-DQ], HLA-DPB1 [HLA-DP], DQA1, DRB3, DRB4 and DRB5). We considered all the transplanted patients with complete HLA-A/B/DR data and we compared the outcomes among groups defined by the number of mismatched HLA-A/B/DR alleles. The study population was further sub-analyzed, including patients with complete HLA-A/B/C and HLA-DR/DQ, respectively. In this subgroup, we compared the outcomes among groups defined by the number of mismatched alleles and eplets, respectively, for HLA-A/B/C and HLA-DR/DQ. We stratified the HLA mismatch by number of allele mismatch and quartiles of the total number of the eplet mismatch distribution, for class I and class II. The mismatch configurations we evaluated are presented in Table 1.

The ascertainment of deaths by the SRTR is based on Organ Procurement and Transplantation Network reports from every US transplant program and monthly updates from the Social Security Administration Death Master File. The latest annual follow-up was on December 5, 2013. Demographic and clinical variables were defined at the time of transplantation.

The Ethics Committee for Clinical Research at Lund University, Sweden approved the study protocol. The data were anonymized and de-identified before analysis and the institutional review board waived the need for written informed consent from the participants.

HLA Typing and Epitope Mismatch Identification

The HLAMatchmaker 1000 pair (ABC epitope mismatch v02.0, June 2016 and DRDQDP epitope mismatch v02.1, January 2017, http://www.hlamatchmaker.net/) program was used to assess epitope mismatch for all transplants. HLAMatchmaker compares amino acid sequences between donor and recipient alleles to identify and quantify differences.[12] All donor and recipient HLA typing were entered, and the eplet mismatch load of each pairing was assigned by the program. The HLAMatchmaker requires high-resolution 4-digit HLA allele information (allele groups including the specific HLA protein) to calculate the eplet mismatch load (Figure 1). However, as only low-resolution 2-digit HLA allele information is available in the SRTR database, we had to generate the most likely high-resolution 4-digit alleles from the low-resolution 2-digit alleles. We performed this conversion with the 4-digit allele converter program v01, http://www.hlamatchmaker.net/. The low- to high-resolution conversion is based on the frequency of the most common 4-digit alleles in 4 major population groups (European whites, blacks, Hispanic, and Asian), which have been reported on the National Marrow Donor Program website.[16]

Statistical Analysis

Data are presented as mean±SD or as n (%) of patients. Baseline characteristics were compared between groups using the Chi-square test for categorical variables and the *t* test for continuous variables. Unadjusted survival rates were computed using Kaplan–Meier method and compared between treatment groups using the log-rank test for trend statistic. We used 1-year post-transplant as a landmark timepoint, and the maximum follow-up time was 20 years. We estimated the hazard ratios (HRs) and 95% CIs for the associations between HLA allele and HLA eplet-based matching and GL independent of other risk factors by fitting a multivariable Cox proportional hazards regression model. The following variables were considered to be potential confounders in examining the association between the number of HLA allele/eplet mismatches and graft failure: recipient age, recipient sex, pre-transplant diagnosis, pre-transplant diabetes mellitus, pre-transplant dialysis, history of previous blood transfusion, pre-sensitized (panel reactive antibodies [PRA] >10%), pre-transplant extra-corporeal membrane oxygenation, pre-transplant MCS, era of transplant, donor age, duration of ischemia, donor-recipient weight ratio, donor-recipient ethnicity match, donor-recipient sex match, donor-recipient blood group match, induction therapy, maintenance immunosuppression, and level of mismatching within

Table 1. The Human Leukocyte Antigen Mismatch Configurations Evaluated

	HLA-A/B/C								HLA-DR/DQ							
Type	Allele				Eplet				Allele				Eplet			
Mismatches	0 to 2	3	4	5 to 6	<10	10 to 12	13 to 16	>16	0 to 1	2	3	4	<18	18 to 28	29 to 40	>40

the other HLA class. A restricted cubic spline function was used on donor age, duration of ischemia, and donor-recipient weight ratio. In a secondary analysis, the Cox proportional hazard method was used to calculate the adjusted hazard ratios (HRs) for the associations between HLA allele and HLA eplet-based matching and GL in selected subgroups (recipient age <40, 40–60, and >60 years), and to test for interactions. For the youngest age group, we performed an additional subgroup analysis including recipient sex, pre-transplant PRA, pre-transplant transfusion and MCS. For each subgroup analysis, the HR for the HLA allele and HLA eplet-based matching were calculated by recalibrating a separate model including the interaction term and the same covariates as in the main effect model.

Table 2. Recipient Characteristics for Adult Heart Transplant Recipients (n=34 861)

Variables	Age <40 y (n=5259)	Age 40 to 60 y (n=20 963)	Age >60 y (n=8639)
Years of observation	6.2 ± 5.8†,‡	6.8 ± 5.7*,‡	5.3 ± 4.8*,†
Age at transplant, y	30.2 ± 6.6†,‡	52.1 ± 5.6*,‡	64.5 ± 2.9*,†
Recipient female sex	1852 (35.2%)†,‡	4697 (22.4%)*,‡	1463 (16.9%)*,†
Recipient ethnicity	†,‡	*,‡	*,†
White	3297 (62.7%)	16 117 (76.9%)	7254 (84.0%)
Asian	157 (3.0%)	372 (1.8%)	167 (1.9%)
Black or AA	1310 (24.9%)	2999 (14.3%)	768 (8.9%)
Hispanic/Latino	423 (8.0%)	1255 (6.0%)	402 (4.7%)
Miscellaneous	72 (1.4%)	220 (1.0%)	48 (0.6%)
Era of transplant	†,‡	*,‡	*,†
1987 to 1995	1533 (29.2%)	6944 (33.1%)	1617 (18.7%)
1996 to 2005	1794 (34.1%)	7642 (36.5%)	3085 (35.7%)
2006 to 2013	1932 (36.7%)	6377 (30.4%)	3937 (45.6%)
Pre-transplant diagnosis	†,‡	*,‡	*,†
Coronary artery disease	529 (10.1%)	10 362 (49.4%)	5452 (63.1%)
Cardiomyopathy	3968 (75.5%)	9379 (44.7%)	2843 (32.9%)
Congenital	514 (9.8%)	234 (1.1%)	33 (0.4%)
Heart valve disease	97 (1.8%)	608 (2.9%)	198 (2.3%)
Miscellaneous	151 (2.9%)	380 (1.8%)	113 (1.3%)
Diabetes mellitus	251 (6.4%)†,‡	3575 (23.8%)*,‡	2070 (28.3%)*,†
Last listing status	†,‡	*,‡	*,†
1A	1615 (30.7%)	5039 (24.0%)	2598 (30.1%)
1B	1191 (22.6%)	4369 (20.8%)	2399 (27.8%)
2	985 (18.7%)	5436 (25.9%)	2026 (23.5%)
Old status 1	1183 (22.5%)	4957 (23.6%)	1450 (16.8%)
Days listed	164.5 ± 284.3†,‡	199.0 ± 306.9*	213.4 ± 351.0*
Pre-transplant transfusion	911 (21.6%)†,‡	3348 (19.9%)*,‡	1357 (18.2%)*,†
PRA >10%	764 (14.5%)†,‡	2258 (10.8%)*	953 (11.0%)*
Positive crossmatch result	397 (7.5%)†,‡	1344 (6.4%)*	528 (6.1%)*
Pre-transplant dialysis	132 (2.5%)‡	480 (2.3%)‡	172 (2.0%)*,†
Pre-transplant MCS	1189 (28.2%)†,‡	3864 (23.6%)*	1680 (24.0%)*
Pre-transplant ECMO	40 (0.8%)†,‡	51 (0.2%)*	22 (0.3%)*
Pre-transplant ventilator	143 (2.7%)	479 (2.3%)	221 (2.6%)

Qualitative data are expressed as n (%) and quantitative data as mean±SD, as appropriate. Numbers for each categorical variable may not add up to total because of missing data. Symbols indicate groups when a significant difference was achieved; *age <40 years; †age 40 to 60 years; ‡age >60 years. AA indicates African American; ECMO, extracorporeal membrane oxygenation; MCS, mechanical circulatory support; PRA, panel reactive antibodies.

All tests were 2-sided, and *P*-values of <0.05 were deemed significant. Missing values (except HLA data) were imputed using the chained-equations multiple imputation techniques as described by White et al.[17] The imputation was performed by the Stata MP statistical package version 15.1 (2017) (StataCorp LP, College Station, TX).

Results

Study Population

The study cohort comprised 221 131 person-years, median survival time 10.7 (95% CI 10.5–10.8) years, with a median duration of follow-up 6.3±5.5 (range 0–26) years. The baseline characteristics for the recipients and their donors are shown in Tables 2 and 3, and cause of death 1 year post-transplant for recipients in Table 4. The mean recipient and donor age were 51.9±11.7 and 30.8±12.1 years, respectively, and 23% of the recipients and 29% of the donors were women. The cohort was 77% white followed by 15% black or African American; 2% underwent a dual organ transplantation. The most common diagnoses were ischemic cardiomyopathy (47%) and non-ischemic cardiomyopathy (46%). The Kaplan–Meier survival estimate was 53% after 10 years and 19% after 20 years. A total of 5959 patients (34%) achieved treatment for acute cellular rejection during the first-year post transplantation and 16 069 patients (46%) died during follow-up. The main causes of death were major adverse cardiovascular event (n=2660), graft failure (n=2068), infection (n=2043), and malignancy (n=1873). One year post transplantation, the 2 most common cause of death for the younger patients (aged <40 years) were graft failure and cardiovascular events, and for the older recipients (aged >60 years) malignancy and miscellaneous, Table 4.

For patients with PRA >10%, history of positive crossmatch results, ethnicity and sex mismatch were more common in the

Table 3. Donor Characteristics for Adult Heart Transplant Recipients (n=34 861)

Variables	Age <40 y (n=5259)	Age 40 to 60 y (n=20 963)	Age >60 y (n=8639)
Age, y	28.0 ± 10.9†,‡	30.7 ± 11.9*,‡	32.6 ± 13.0*,†
Female sex	1663 (31.6%)†,‡	5836 (27.8%)*,‡	2587 (29.9%)
Duration of ischemia, min	180.6 ± 66.0†,‡	177.5 ± 61.4*,‡	184.1 ± 62.9
Ethnicity	†,‡	*,‡	*,†
White	3715 (70.6%)	15 456 (73.7%)	6162 (71.3%)
Asian	66 (1.3%)	243 (1.2%)	119 (1.4%)
Black or AA	754 (14.3%)	2591 (12.4%)	1079 (12.5%)
Hispanic/Latino	658 (12.5%)	2447 (11.7%)	1194 (13.8%)
Miscellaneous	66 (1.3%)	226 (1.1%)	85 (1.0%)
Donor cause of death	†,‡	*,‡	*,†
Anoxia	549 (10.4%)	1913 (9.1%)	995 (11.5%)
CNS tumor	44 (0.8%)	150 (0.7%)	82 (0.9%)
CVA/stroke	1111 (21.1%)	5232 (25.0%)	2348 (27.2%)
Head trauma	3018 (57.4%)	11 453 (54.6%)	4665 (54.0%)
Miscellaneous	531 (10.1%)	2186 (10.4%)	539 (6.2%)
Donor/recipient weight ratio	1.0 ± 0.2†,‡	1.0 ± 0.2*,‡	1.0 ± 0.2*,†
Ethnicity match	2875 (54.7%)†,‡	13 265 (63.3%)*	5565 (64.4%)*
Sex match	3506 (66.7%)†,‡	15 168 (72.4%)*,‡	6243 (72.3%)*,†
Blood group match	†,‡	*	*
Compatible	861 (16.4%)	2980 (14.2%)	1228 (14.2%)
Identical	4395 (83.6%)	17 973 (85.7%)	7409 (85.8%)
Incompatible	3 (0.1%)	10 (0.0%)	2 (0.0%)

Qualitative data are expressed as n (%) and quantitative data as mean±SD, as appropriate. Numbers for each categorical variable may not add up to total because of missing data. Symbols indicate groups when a significant difference was achieved; *age <40 years; †age 40 to 60 years; ‡age >60 years. AA indicates African American; CNS, central nervous system; CVA, cerebrovascular accident.

Table 4. Recipient Cause of Deat h 1 Year Post Transplant, Stratified by Recipient Age

	Age <40 y (n=1187)	Age 40 to 60 y (n=5516)	Age >60 y (n=1934)
Cause of death			
Graft failure	307 (26%)†,‡	644 (12%)*,‡	145 (7.5%)*,†
Cardiovascular	470 (40%)†,‡	1350 (24%)*,‡	327 (17%)*,†
Infection	88 (7.4%)†,‡	695 (13%)*	271 (14%)*
Malignancy	80 (7.0%)†,‡	1159 (21%)*,‡	539 (28%)*,†
Cerebrovascular	30 (2.5%)†,‡	220 (4.0%)*,‡	97 (5.6%)*,†
Diabetes mellitus	0	12 (0.2%)	4 (0.2%)
Pulmonary	44 (3.7%)†,‡	349 (6.3%)*,‡	159 (8.2%)*,†
Trauma	55 (4.6%)†,‡	130 (2.4%)*,‡	28 (1.5%)*,†
Miscellaneous	113 (9.5%)†,‡	957 (17%)*	364 (19%)*

Qualitative data are expressed as n (%). Symbols indicate groups when a significant difference was achieved; *age <40 years; †age 40 to 60 years; ‡age >60 years.

younger patient cohort. Figure 2A and 2B shows the HLA-A/B/C and HLA-DR/DQ donor-recipient eplet mismatch distribution with averages of 13.4±5.3 and 29.6±17.2, respectively. The HLA-A/B/C and HLA-DR/DQ eplet mismatch did not vary by recipient age groups, P=0.585 and P=0.320 (one-way ANOVA).

The Degree of HLA-Mismatch and Graft Loss

Figure 3 shows Kaplan–Meier estimates of graft failure stratified by number of HLA allele mismatch for the whole study cohort (n=34 861). An increased number of HLA-A/B/DR allele mismatch decreased graft survival (P<0.001, log rank trend test). In the sub-analysis, patients with complete collection of HLA-A/B/C and HLA-DR/DQ, respectively, showed a similar trend; an increased number of allele mismatch results in impaired survival, P=0.073 and P<0.001, respectively. However, the number of mismatched HLA-A/B/C eplets was not associated with graft loss, P=0.584, as opposed to the HLA-DR/DQ eplet, P=0.025. These results could be confirmed in the unadjusted and adjusted Cox proportional hazard regression analysis. Recipient with >4 mismatched HLA-A/B/C alleles and 4 mismatched HLA-DR/DQ alleles reached an unadjusted HR for graft loss of 1.08 (95% CI 0.99–1.19; P=0.099) and 1.13 (95% CI 1.03–1.23; P=0.009), respectively, and an adjusted HR for graft loss of 1.07 (95% CI 0.97–1.18; P=0.165) and 1.17 (95% CI 1.07–1.28; P=0.001), respectively.

The number of HLA-A/B/C eplet mismatch was not associated with impaired graft survival. Recipients with >40 mismatched HLA-DR/DQ eplets achieved an unadjusted HR for graft loss of 1.10 (95% CI 1.02–1.19) and an adjusted HR for graft loss of 1.11 (95% CI 1.03–1.21). Recipient with PRA >10% achieved an unadjusted HR of 1.09 (95% CI, 1.04–1.15) and an adjusted HR of 1.13 (95% CI 1.07–1.20) for graft loss. The adjusted HR for HLA-A/B/C and HLA-DR/DQ, allele mismatch and eplet mismatch, were independent of the PRA level, P (interaction)=0.595 and 0.258; 0.555 and 0.538, respectively.

Influence of Recipient Age and Degree of HLA Mismatch on Graft Loss

We further analyzed the effect of an interaction between recipient age and HLA mismatch. Figure 4 shows a

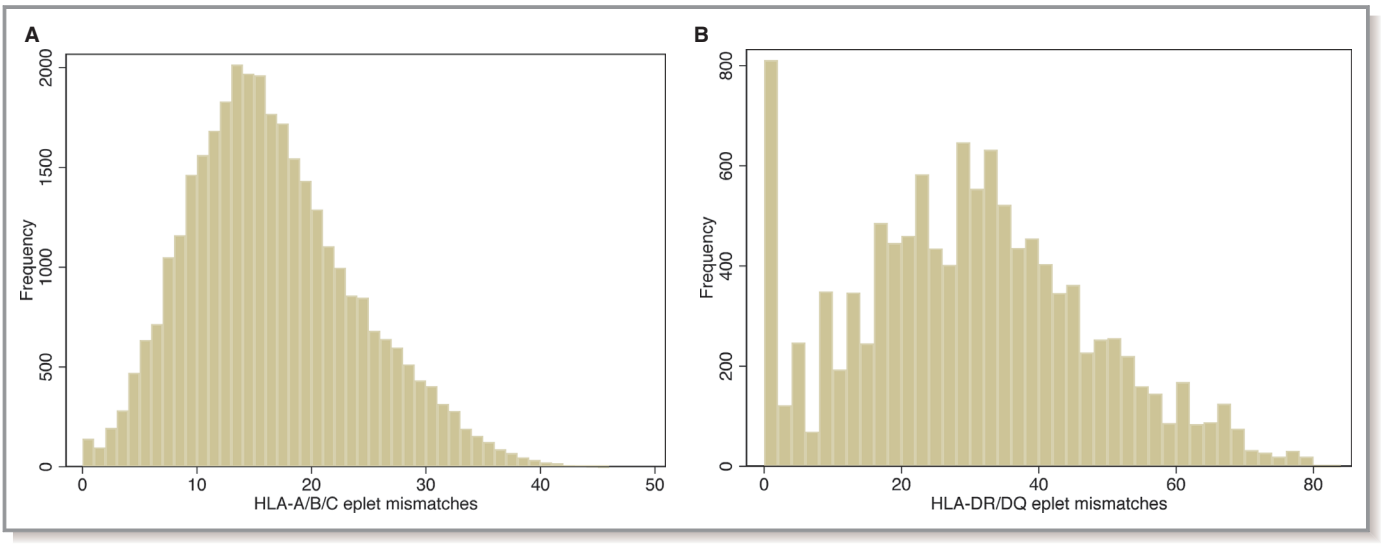

Figure 2. Histograms of the distribution of human leukocyte antigen epitope mismatch (mm). **A**, HLA-A/B/C eplet mismatch. **B**, HLA-DR/DQ eplet mismatch. The frequencies of total eplet mismatch are normally distributed with an average of 16.1±87.3 and 29.6±17.2, respectively. HLA indicates human leukocyte antigen.

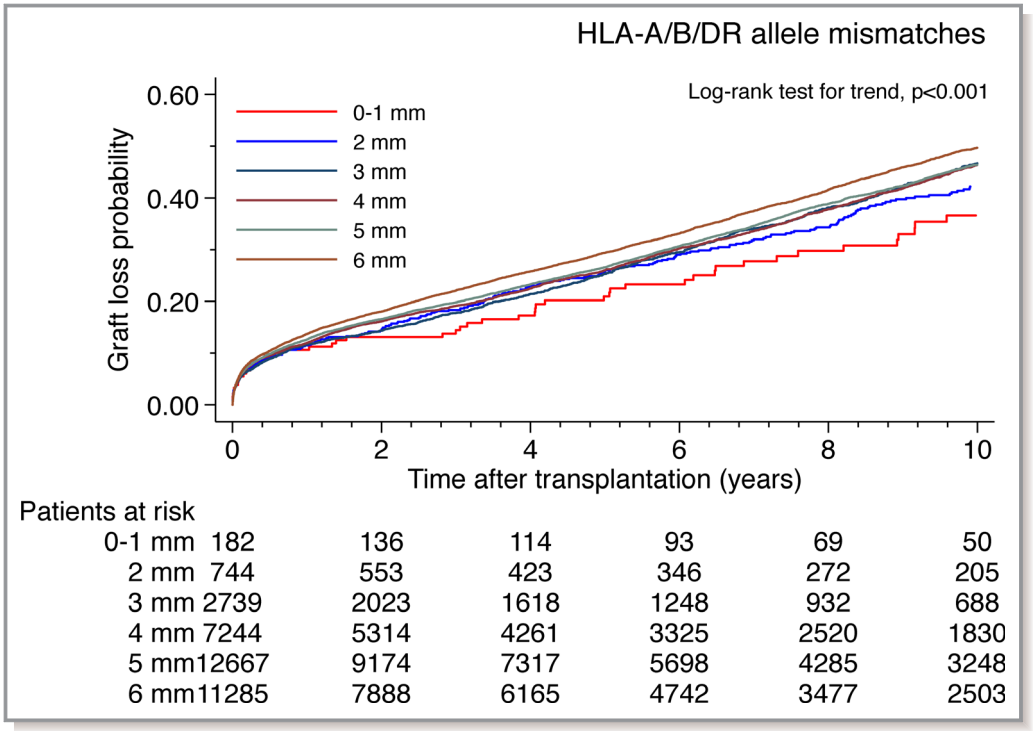

Figure 3. Kaplan–Meier graft loss curves stratified by the number of HLA-A/B/DR allele mismatch (mm). HLA indicates human leukocyte antigen.

Kaplan–Meier estimate of graft failure stratified by number of HLA alleles and HLA eplet mismatch for recipients aged <60 years. No significant interaction between age and HLA-A/B/C mismatch could be detected (Figure 4A and 4B), while an increased number of HLA-DR/DQ allele or eplet mismatches decreased graft survival and interact with age (Figure 4C and 4D). The adjusted HR was 1.78 (95% 1.13–2.80) for 4 mismatched HLA-DR/DQ alleles compared with 0 to 1 mismatches, and 1.82 (95% CI, 1.23–2.70) for >40 mismatched HLA-DR/DQ eplet compared with <18 mismatches. The HR for the interaction term was 0.99 (95% CI, 0.98–1.00) and 0.99 (95% CI, 0.98–1.00), respectively.

As shown in Tables 5 and 6, recipients in the 2 younger age groups (<40 years and 40–60 years old) with >40 mismatched HLA-DR/DQ eplets had an adjusted HR of 1.36 and 1.27, respectively. The older recipients (aged >60 years) had no association between HLA mismatch and graft loss. The HR for the interaction term was 0.70 (95% CI, 0.54–0.92). For HLA-DR/DQ allele mismatches, an even larger HR for the 2 youngest age groups was found. The HR for the interaction term was 0.73 (95% CI, 0.54–0.98).

We additionally evaluated the difference between early (within 1 year post-transplant) and late (after 1 year post-transplant) graft loss. The number of mismatched HLA-A/B/C alleles or eplets did not influence graft survival when we evaluated the difference between early and late graft loss. The impact of the number of HLA-DR/DQ allele and eplet mismatches on graft survival was more prominent after 1 year, where recipients aged <40 years with 4 HLA-DR/DQ allele mismatches had an HR of 1.51 (95% CI, 1.12–2.03) and recipients with >40 eplet mismatches had an adjusted HR of 1.32 (95% CI, 1.02–1.70), while there was no significant correlation to graft loss within 1 year after transplantation, adjusted HR of 1.22 (95% CI, 0.77–1.93) and 1.35 (95% CI, 0.90–2.01), respectively.

Recipients with PRA >10%, on the other hand, had an adjusted HR of 1.27 (95% CI, 1.16–1.40) for graft loss within 1 year. The PRA level did not influence outcome after 1 year, adjusted HR of 1.06 (95% CI, 0.99–1.13). The degree of HLA eplet match was independent of PRA on the prediction of graft loss within and after 1 year, *P* (interaction)=0.888 and 0.389.

Finally, we evaluated the influence on graft loss for patients aged <40 years of the interaction between known risk factors influencing immune function and >40 HLA-DR/DQ eplet mismatches. Here, we could not identify any significant interaction except for a trend in the recipient sex and mechanical circulatory support (MCS). Recipients (aged <40 years) without MCS pre-transplant and male recipients (aged <40 years), respectively, with >40 HLA-DR/DQ eplet mismatches had a 50% increased risk of graft loss compared with recipient with MCS and female recipients, respectively, Table 7.

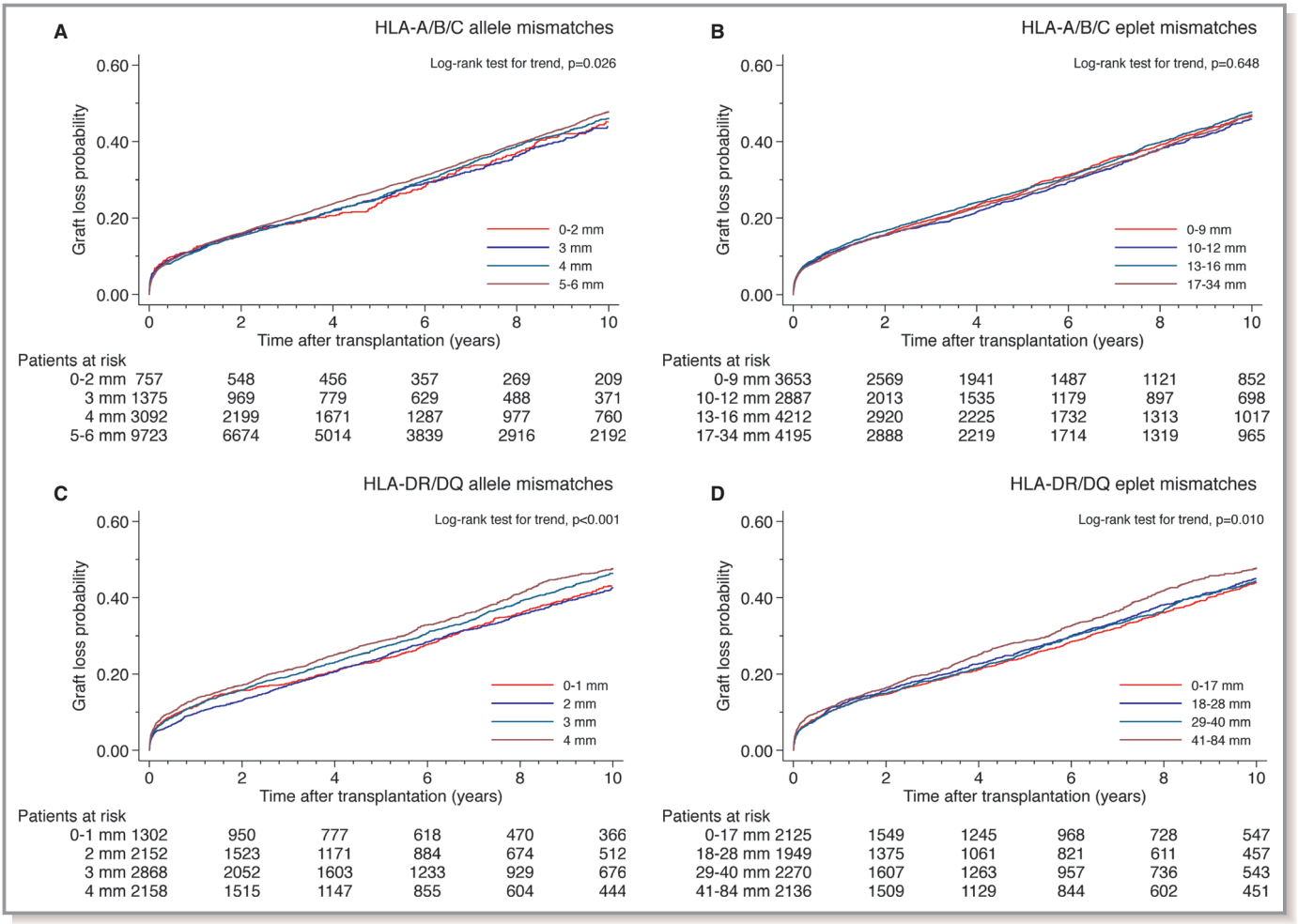

Figure 4. Kaplan–Meier graft loss curves by the number of HLA allele and HLA eplet mismatch (mm) for recipients aged <60 years. **A**, Stratified by the number of HLA-A/B/C allele mismatches. **B**, Stratified by the number of HLA-A/B/C eplet mismatches. **C**, Stratified by the number of HLA-DR/DQ allele mismatches. **D**, Stratified by the number of HLA-DR/DQ eplet mismatches. HLA indicates human leukocyte antigen; mm, mismatch.

Discussion

In this study, our main finding is that the HLA-DR/DQ mismatch results in an increased risk of late graft loss. Our results further indicate that eplet mismatch at the HLA-DRB1 and HLA-DQB1 loci did not influence graft survival more than the allele mismatch at the same loci. More importantly, we could show that there was a significant interaction between the number of HLA allele/eplet mismatches and recipient age.

The most common cause of death 1 year after a heart transplant is chronic rejection, leading to graft loss.[1] The factors that determine the development of chronic rejection are still not fully understood.[2,3] Preformed DSA is a known risk factor for hyper acute rejection, and regular pre-screening for HLA antibodies is therefore standard in most cardiac transplant programs.[18–20] DSA developed after heart transplantation, de novo DSA (dnDSA), and their impact on graft survival, on the other hand, are debated.[20–22] In recent years, studies have shown that patients with dnDSA and especially DSA against HLA class II antigen have a worse survival.[13,14,23,24]

In this study, we have chosen to focus on analysis of HLA-DR/DQ matches because of its strong linkage to post-transplantation outcome. We found an improved survival, which started 2 years after transplantation, in patients with less HLA class II eplet mismatch. The HLA-DR/DQ allele mismatch influenced survival much earlier. Fewer HLA DR/DQ eplet mismatches can thus be interpreted as a reduced risk of dnDSA development and less chronic rejection for these patients. This was further enhanced by the findings in the subgroup analysis, where the major influence of HLA-DR/DQ eplet mismatch differences on graft loss was observed in patients who had no risk factors for preformed DSA development, such as MCS. Furthermore, the effect of the number of HLA-DR/DQ allele mismatches and its influence on graft survival was more pronounced 1 year

Table 5. Crude and Adjusted Hazard Ratios for Graft Loss by Number of HLA-A/B/C Allele and Eplet Mismatches Stratified by Recipient Age (n=20 229)

	n	f	Crude			Adjusted		
			HR	95% CI	*P* Value	HR	95% CI	*P* Value
HLA-A/B/C allele								
Age 18 to 39 y								
0 to 2 mismatch	130	59	1	Ref		1	Ref	
3 mismatch	250	106	1.18	0.86 to 1.63	0.312	1.13	0.83 to 1.56	0.451
4 mismatch	639	258	1.17	0.88 to 1.55	0.287	1.13	0.85 to 1.51	0.393
5 to 6 mismatch	2011	840	1.30	0.99 to 1.69	0.056	1.26	0.97 to 1.65	0.088
Age 40 to 60 y								
0 to 2 mismatch	627	285	1	Ref		1	Ref	
3 mismatch	1125	503	0.97	0.84 to 1.13	0.729	0.97	0.83 to 1.12	0.643
4 mismatch	2453	1105	1.05	0.92 to 1.19	0.495	1.01	0.89 to 1.15	0.863
5 to 6 mismatch	7712	3375	1.05	0.93 to 1.19	0.429	1.03	0.91 to 1.16	0.649
Age 61 to 79 y								
0 to 2 mismatch	273	117	1	Ref		1	Ref	
3 mismatch	209	501	1.16	0.93 to 1.46	0.192	1.19	0.95 to 1.49	0.136
4 mismatch	441	1085	1.13	0.92 to 1.38	0.247	1.13	0.92 to 1.39	0.245
5 to 6 mismatch	1321	3423	1.07	0.89 to 1.29	0.475	1.08	0.89 to 1.30	0.452
HLA-A/B/C eplet								
Age 18 to 39 y								
0 to 9 mismatch	71	32	1	Ref		1	Ref	
10 to 12 mismatch	405	174	0.85	0.72 to 1.01	0.072	0.84	0.70 to 1.00	0.045
13 to 16 mismatch	1055	427	0.98	0.84 to 1.14	0.802	0.96	0.82 to 1.11	0.569
17 to 34 mismatch	1499	630	0.93	0.80 to 1.08	0.335	0.92	0.78 to 1.07	0.259
Age 40 to 60 y								
0 to 9 mismatch	406	177	1	Ref		1	Ref	
10 to 12 mismatch	1604	713	0.96	0.88 to 1.04	0.294	0.95	0.88 to 1.03	0.242
13 to 16 mismatch	4286	1904	1.00	0.93 to 1.08	0.979	1.00	0.93 to 1.08	0.925
17 to 34 mismatch	5621	2474	1.02	0.94 to 1.09	0.687	1.00	0.94 to 1.09	0.801
Age 61 to 79 y								
0 to 9 mismatch	168	71	1	Ref		1	Ref	
10 to 12 mismatch	767	304	0.98	0.86 to 1.12	0.781	0.97	0.85 to 1.11	0.691
13 to 16 mismatch	1869	753	1.02	0.91 to 1.15	0.738	1.00	0.89 to 1.13	0.985
17 to 34 mismatch	2478	960	1.01	0.90 to 1.14	0.844	1.00	0.89 to 1.13	0.999

HRs for graft loss were adjusted for recipient sex, sex match, ethnicity match, era of transplant, pre-transplant diagnosis, diabetes mellitus, panel reactive antibodies >10%, pre-transplant dialysis, pre-transplant extracorporeal membrane oxygenation, donor age, duration of ischemia, donor-recipient weight ratio, pre-transplant mechanical circulation support, previous blood transfusion, blood group match, induction therapy, maintenance immunosuppression, and level of mismatching within HLA-DR. f indicates number of graft loss; HLA, human leukocyte antigen; HR, hazard ratio; n, number of transplants.

and later after heart transplantation, which also supports our conclusion.

In kidney transplantation, it has been shown that the calculated HLA eplet mismatch load correlates well to both survival and dnDSA formation. Wiebe et al demonstrate that the risk of chronic rejection in renal transplanted patients was almost doubled in patients with ≥43 HLA-DR/DQ eplet mismatch compared with <43 mismatches. The conclusion was that HLA-DR and HLA-DQ eplet matching outperforms traditional low-resolution antigen-based matching.[14] Walton

Table 6. Crude and Adjusted Hazard Ratios for Graft Loss by Number of HLA-DR/DQ Allele and Eplet Mismatches Stratified by Recipient Age (n=11 570)

			Crude			Adjusted		
	n	f	HR	95% CI	*P* Value	HR	95% CI	*P* Value
HLA-DR/DQ allele								
Age 18 to 39 y								
0 to 1 mismatch	264	104	1	Ref		1	Ref	
2 mismatch	424	163	1.17	0.91 to 1.49	0.224	1.26	0.98 to 1.62	0.070
3 mismatch	563	243	1.32	1.05 to 1.66	0.018	1.39	1.10 to 1.76	0.005
4 mismatch	418	171	1.39	1.09 to 1.78	0.008	1.47	1.14 to 1.88	0.002
Age 40 to 60 y								
0 to 1 mismatch	1038	467	1	Ref		1	Ref	
2 mismatch	1728	685	0.94	0.83 to 1.06	0.296	0.95	0.85 to 1.07	0.426
3 mismatch	2305	1019	1.07	0.96 to 1.19	0.249	1.08	0.97 to 1.21	0.181
4 mismatch	1740	733	1.12	0.99 to 1.25	0.063	1.16	1.03 to 1.30	0.015
Age 61 to 77 y								
0 to 1 mismatch	468	197	1	Ref		1	Ref	
2 mismatch	763	304	1.01	0.84 to 1.21	0.920	1.06	0.89 to 1.27	0.505
3 mismatch	1015	436	1.06	0.89 to 1.25	0.505	1.08	0.91 to 1.28	0.383
4 mismatch	844	332	1.01	0.85 to 1.20	0.926	1.06	0.89 to 1.27	0.503
HLA-DR/DQ eplet								
Age 18 to 39 y								
0 to 17 mismatch	425	175	1	Ref		1	Ref	
18 to 28 mismatch	384	149	1.15	0.92 to 1.43	0.207	1.19	0.95 to 1.49	0.128
29 to 40 mismatch	459	459	1.23	1.00 to 1.52	0.049	1.27	1.03 to 1.57	0.025
41 to 84 mismatch	401	401	1.32	1.06 to 1.63	0.011	1.36	1.10 to 1.69	0.005
Age 40 to 60 y								
0 to 17 mismatch	1700	750	1	Ref		1	Ref	
18 to 28 mismatch	1565	662	1.00	0.90 to 1.11	0.991	1.02	0.92 to 1.13	0.749
29 to 40 mismatch	1811	1811	0.96	0.87 to 1.07	0.486	0.98	0.88 to 1.08	0.664
41 to 84 mismatch	1735	1735	1.11	1.01 to 1.11	0.036	1.14	1.02 to 1.26	0.015
Age 61 to 77 y								
0 to 17 mismatch	744	327	1	Ref		1	Ref	
18 to 28 mismatch	719	282	0.92	0.78 to 1.07	0.279	0.95	0.81 to 1.11	0.526
29 to 40 mismatch	826	334	0.97	0.84 to 1.13	0.729	0.99	0.86 to 1.16	0.930
41 to 84 mismatch	801	326	0.95	0.82 to 1.11	0.528	0.96	0.82 to 1.12	0.594

HRs for graft loss were adjusted for recipient sex, sex match, ethnicity match, era of transplant, pre-transplant diagnosis, diabetes mellitus, panel reactive antibody >10%, pre-transplant dialysis, pre-transplant extracorporeal membrane oxygenation, donor age, duration of ischemia, donor-recipient weight ratio, pre-transplant mechanical circulation support, previous blood transfusion, blood group match, induction therapy, maintenance immunosuppression, and level of mismatching within HLA-A/B. f indicates number of graft loss; HLA, human leukocyte antigen; HR, hazard ratio; n, number of transplants.

et al show that determination of donor/recipient HLA-DR/DQ incompatibility at the structural level could be used as a predictor for chronic lung allograft dysfunction.[25] Sullivan et al conclude that HLA eplet mismatch may aid in identifying heart transplanted patients at increased risk of long-term graft loss.[26] However, they could not demonstrate that an HLA class II epitope mismatch influenced the graft survival. Their different findings compared with the results from the present study may be partly explained by the fact that data from the HLA-DRB1 loci and not HLA-DQB1 loci were used, and that only pediatric recipients were included. Cardiac allograft vasculopathy seems to be less frequent and less

Table 7. Adjusted Hazard Ratios for Graft Failure by >40 HLA-DR/DQ Compared With <18 Eplet Mismatches for Recipient Aged <40 Years, for Different Subgroups

	n/f 0 to 17 EMM	n/f 41 to 84 EMM	Adjusted		
			HR	95% CI	*P* Value
Recipient sex					
Men	272/114	251/107	1.57	1.20 to 2.05	0.091
Women	153/61	150/62	1.07	0.75 to 1.52	
Pre-transplant PRA >10%					
No	335/137	303/135	1.42	1.12 to 1.80	0.359
Yes	29/69	22/65	1.07	0.62 to 1.86	
Pre-transplant transfusion					
No	258/101	285/124	1.36	1.07 to 1.74	0.766
Yes	96/33	69/17	1.25	0.73 to 2.15	
MCS					
No	233/88	226/89	1.53	1.19 to 1.96	0.083
Yes	107/38	96/26	0.96	0.61 to 1.51	

The *P* value for interaction represents the likelihood of an interaction between the subgroup variable and the treatment effect. The HRs for graft loss were adjusted for recipient sex, sex match, ethnicity match, era of transplant, pre-transplant diagnosis, diabetes mellitus, PRA >10%, pre-transplant dialysis, pre-transplant extracorporeal membrane oxygenation, donor age, duration of ischemia, donor-recipient weight ratio, pre-transplant MCS, previous blood transfusion, blood group match, induction therapy, maintenance immunosuppression, and level of mismatching within HLA-A/B. EMM indicates eplet mismatch; f, number of graft loss; HR, hazard ratio; MCS, pre-transplant mechanical circulation support; n, number of transplants; PRA, panel reactive antibody.

aggressive in pediatric recipients and previous studies have shown that HLA-DQ dnDSA occurs more commonly than HLA-DR dnDSA.[27] As previously reported, pre-sensitized patients have an increased risk of graft loss, which was confirmed in the present study.

In this patient cohort, malignancy and infection were the most common causes of death for patients aged >60 years a year after transplantation, which Wever-Pinzon et al also showed in a recently published study in an international patient cohort.[1] A novel finding in this study was that the effect of the HLA mismatch decreases with an increased age of the recipient. At the age of ≥60 years, the number of mismatched HLA did not affect the graft loss rate, while the younger patient has almost a doubled the risk of graft loss. Previous studies have suggested that age-related changes in the immune system can positively affect the transplantation results such as less chronic rejection.[1,4,5] The increased risk for infectious complications, renal failure, and neoplasms emphasized the need for individualized adjusted immunosuppression in older recipients.[28]

Although this study is based on the data prospectively collected, the retrospective design limits us from completely adjusting for differences between the comparison groups. However, most of the recipients are probably matched randomly to a donor without the knowledge of its HLA type, as HLA matching at transplantation is usually not performed routinely for a non-sensitized patient. We also adjust for clinical variables known to have an immunological effect, eg, female sex, previous blood transfusion, mechanical circulation support, and also time-era. The results of the subgroup analysis (Table 5) should be interpreted with caution. Because of the relatively small effect size only large interactions may be detected. HLA-A, HLA-B, and HLA-DRB1 loci have historically been considered to be the crucial sites for affecting clinical outcomes and have previously been the only site for which donor HLA typing is mandatory, limiting the collection of data at other loci in SRTR. The eplet assignments in the HLAMatchmaker software are based on the "most likely" high-resolution 4-digit alleles, converted from HLA typing information obtained from SRTR. This has shown to be sufficient in determining eplet mismatch load in kidney transplantation.[29] The assumptions of DRB3/4/5 linkages and assigning common alleles for low-resolution may have influenced the result. Furthermore, we had to assume that subjects with only one identified allele at a given locus were homozygous for this specific allele, which is the standard practice in the most laboratories. Despite these limitations, epitope mismatch using the HLAMatchmaker algorithm has been used in several studies and is clinically established, in kidney and lung transplantation.[25,26,30] This is the first time, to our knowledge, the HLAMatchmaker algorithm is used in an adult heart transplantation study.

Conclusions

Allograft rejection remains a major problem in heart transplantation, leading to increased mortality, morbidity, and costs. In this study, we have re-examined the influence of HLA mismatch and graft loss in a heart transplanted cohort. The results show that it is possible to identify recipients with an increased risk of future rejection and graft loss based on their HLA-DR/DQ allele/eplet mismatch load. By identifying patients at increased risk, a targeted strategy could be developed to individualize and intensify both monitoring and treatment post-transplant, thereby reducing mortality and morbidity. This would be even more clinically relevant and practicable when novel approaches to ex-vivo allograft perfusion may allow for longer times between allograft procurement and transplant.

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Disclosures

None.

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