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Significance of HLA-matching and anti-HLA antibodies in heart transplant patients receiving induction therapy?

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ABSTRACT

Objectives: Though Human Leukocyte Antigen (HLA) matching benefits are demonstrated in renal transplantation, evidence in heart transplantation is lacking, and its clinical feasibility is uncertain. Post-transplantation anti-HLA antibodies are being increasingly studied in organ transplantation, with diverging conclusions between transplantated organs.

Methods: We analyzed retrospectively the influence of HLA matching and anti-HLA antibodies on overall survival, acute rejection and chronic allograft vasculopathy in 309 patients receiving induction therapy and triple-drug immunosuppression.

Results: The average number of HLA-A/B/DR mismatches between donor and recipient was 4.9 ± 1. The majority of mismatches was for Class I HLA-A/B with an average of 3.3, then for Class I HLA-DR with an average of 1.6. Overall, the HLA-A/-B/-DR mismatches had no influence on the cardiac allograft survival ($p = 0.28$). However, HLA-DR mismatches were negatively correlated to severe cellular and/or humoral allograft rejection ($p = 0.04$). Our analysis found anti-HLA antibodies in 27% of recipients, de novo anti-HLA antibodies in 16% of recipients, and donor-specific anti-HLA (DSA) antibodies in 8% of recipients. Furthermore, de novo DSA had no influence on the 5-year survival (78% with DSA vs. 92% without DSA; $p = 0.49$), which may be masked by the limited number of recipients in analysis By univariable analysis, anti-HLA antibodies (preexisting or de novo) unrelated or related to the donor had no influence on severe cellular and/or humoral rejection or on chronic allograft vasculopathy.

Conclusions: HLA-DR mismatch was negatively correlated to severe cellular and/or humoral allograft rejection but had no influence on cardiac allograft survival. In this study, anti-HLA antibodies (preexisting or de novo) unrelated or related to the donor had no influence on cellular and/or humoral rejection or on chronic allograft vasculopathy. The results of this study add to the controversy on the impact of allo-antibodies in heart transplant recipients receiving induction therapy and contemporary immunosuppression.

1. Introduction

Despite the use of contemprorary immunosuppressive regimen, there remains a 5–10% annual attrition of functioning graft across all solid organs transplantation [1]. Causes of failure are multifactorial, namely ischemia-reperfusion injury [2], drug-induced vasculopathy [3] and chronic allograft rejection (CAV) [4].

While the benefits of HLA matching in renal transplantation are undisputed [5,6], hard evidence in heart transplantation is still lacking. Historical cardiac studies were performed in the 90's, and recent studies using contemporary immunosuppression did not demonstrate any correlation between HLA matching and the incidence of CAV, neither on graft rejection, nor on patient survival [7–10].

With the renewed interest in donor organ resuscitation as well as the

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availability of continuous perfusion devices [11], we could foresee the possibility to extend the delay between donor heart procurement and its transplantation, allowing improved HLA matching among donor-recipient pairs across Eurotranplant.

Besides HLA matching, there is a growing interest in post-transplantation surveillance of anti-HLA antibodies, with diverging conclusions upon organs and/or studies [12]. Unlike kidney transplantation, the impact of anti-HLA antibodies on heart transplantation outcome is still unclear. Some studies suggested that donor specific anti-HLA antibodies (dsAnti-HLA) may affect heart transplantation outcome in both short- and long-term analysis [13–15] whereas other contemporary studies demonstrated a clear association with antibody-mediated rejection (AMR) but no influence on long-term graft survival [16].

2. Objective

In this retrospective study, we analyzed whether a) some degree of HLA matching in heart transplant patients and b) the presence of anti-HLA antibodies would influence any of the following endpoints: overall survival, survival without severe acute rejection (cellular or humoral) and survival without chronic allograft vasculopathy (or CAV).

3. Materials and methods

3.1. Study population and sample collection

From 1985 to 2013, 453 cardiac transplantations were performed at the Cliniques Universitaires Saint-Luc Brussels (26 *Re*-Transplantations).

For this study, we included only patients with complete dataset of donor/recipient HLA typing (HLA –A, —B and –DR) ($n = 309$).

From this cohort, 219 patients (71%) had at least one pre-transplantation and one post-transplantation sera tested for anti-HLA antibodies. All patients with anti-HLA antibodies prior to transplantation (PRA >10%, $n = 39$) had a prospective negative T- and B-cell cross-match before transplantation.

Information was collected from the patient hospitalization charts and our prospective transplantation database.

Follow-up was available in all patients, with a median time of 101 months (IQR 42.4–164.5 months).

The Committee on Human Rights in Research (Institutional Review Board) of Cliniques Universitaires Saint-Luc approved this study performed in accordance with the 2000 Declaration of Helsinki as well as the Declaration of Istanbul 2008 (CEHF 2015/316). All patients consented to the study.

3.2. Immunosuppression

All patients received induction therapy with rabbit anti-thymocyte globulin (RATG, Fresenius AG, Bad Homburg, Germany) immediately before transplantation and repeated daily for up to 5 days depending upon post-operative renal function recovery. During the first decade, maintenance immunosuppression included cyclosporine (CsA), azathioprine and steroids. After 1995, azathioprine was progressively replaced by mycophenolate mofetil (MMF). Since 2000, a tacrolimus-based regimen was selected for young patients and/or female recipients and an early steroid.

withdrawal protocol was initiated (progressive tapering and wean off after 6 months in the absence of treated rejection). Most patients transplanted before 1995 were switched from azathioprine to MMF when it became available. Cyclosporin was switched to tacromimus in patients with either drug-specific side effects or recurrent episodes of cellular rejection.

3.3. HLA typing

Donors and recipients were typed for major histocompatibility class I antigens (HLA-A and -B) by conventional microlymphocytotoxicity. Major histocompatibility class II antigens (HLA-DR and -DQ) typing was performed by molecular techniques using polymerase chain reaction and amplification with sequence-specific oligonucleotide primers.

3.4. Detection of anti-HLA antibodies

During the first decade of our transplantation program (1985–1995), pre-Tx anti-HLA antibodies were rarely assessed. Patients in whom allosensitization was suspected underwent prospective cross-matching with donor lymphocytes and/or splenocytes prior to organ acceptance.

After 1995, panel-reactive antibodies against B and T lymphocytes were systematically assessed in listed patients supported by left ventricular assist devices. This screening was progressively extended to all patients [17].

After transplantation, the presence of anti-HLA antibodies were tested yearly during follow-up, with additional testing depending on the pre-transplantation anti-HLA status and/or if rejection was suspected.

Sera were first heat-treated to remove complement lytic activity and tested by complement-dependent cytotoxicity against a comprehensive cell panel of HLA-typed donors representing most of the defined HLA specificities. Positive reactions were expressed as a percentage of total T-cell panel using cytotoxicity by standard dye-exclusion assay (PRAs).

Solid phase immunoassays were used to determine the presence of Class I and Class II antibodies, including the LAT mixed antigen tray (LATM) ELISA (One Lambda, USA) and the LAT 1240/240 to determine anti-HLA specificities. In all instances, kit protocols were followed as per manufacturers' instructions.

Since 2008, HLA antibody detection was performed using single beads on Luminex platform, with a mean fluorescence index (MFI) cut-off value of 1500 [18].

Donor-specific anti-HLA (dsAnti-HLA) detection was conducted for detectable HLA antibodies.

In this study, patients with anti-HLA titers ≤10% without donor specific antibody (or any anti-HLA with MFI ≤ 1500) were considered not sensitized.

3.5. Endomyocardial biopsies

Endomyocardial biopsies were performed weekly during the first month, every second week for the next 2 months, monthly until month 6, and then every 6 weeks up to 1-year post-transplantation. Scoring was done according to the International Heart and Lung Transplantation criteria. High-grade cellular rejection was defined pathologically as grade 3A or 3B (greater or equal to Grade 2R) [19,20].

The combination of clinical, histologic and immunopathologic findings, as well as demonstration of circulating donor specific antibodies were requested to diagnose acute antibody-mediated rejection. Those included a) the presence of acute cardiac graft dysfunction, b) histologic evidence of acute capillary injury (capillary endothelial changes and macrophages in capillaries), c) Immunopathologic evidence for antibody mediated injury (C3d and/or C4d or C1q or CD68 positivity for macrophages in capillaries), d) Serologic evidence of anti-HLA class I and/or class II antibodies at time of biopsy [21].

3.6. Detection of transplant-associated coronary allograft vasculopathy (CAV)

All patients had a protocol coronary angiography within 3 months of transplantation, and yearly thereafter. CAV was classified as absent, mild, moderate, or severe according to the amount of stenosis in the most severely affected vessel. Our definition of significant CAV was a lesion >50% of a proximal or mid-portion of one major coronary graft vessel [22,23].

All angiographies were serially reviewed and compared with each patient's prior exam to determine the onset of graft vasculopathy.

Coronary angiography was available in 243 patients (79%) among those with complete HLA dataset ($n = 309$).

3.7. Statistical analysis

Survival was calculated from the date of transplantation to the date of follow-up (or death/graft loss). Patients were stratified by either the total number of HLA mismatches or the numbers of mismatches at each HLA locus. For each patient, the date of the first acute cellular (or humoral) rejection grade 3A or greater was recorded as well as the number of rejection episodes. For patients presenting with CAV, the number of days was calculated from the date of transplantation to the first abnormal coronary angiography.

For analysis of descriptive statistics and categorical variables, Chi-square or Fisher's exact test were used as appropriate whereas for analysis of continuous variables, student's *t*-test was used. Survival analysis was performed according to Kaplan-Meier method. Univariate analysis by the Log-rank test and Cox regression model were used to compare survival, and identify predictors of death. To determine the independent predictors of each outcome of interest, variables with a *p*-value <0.20 in the univariate analysis were entered into a multivariate conditional forward stepwise selection procedure. Results are displayed as hazard ratio or adjusted hazard ratio with 95% confidence interval.

The level of statistical significance was set at a p value <0.05. All statistical analyses were performed with SPSS version 25.0 software (SPSS, Inc., Chicago, IL).

4. Results

4.1. Overall survival

4.1.1. HLA matching and overall survival

All demographics and clinical data of the study cohort are provided in Table 1.

For the entire cohort ($n = 453$), 30-days and 90-days mortality were 8.9% and 13.9%, respectively.

Overall survival at 1-, 5- and 10-years in this cohort was 81.9%, 73.3% and 58.2%, respectively.

As the study period extended over nearly 30 years, which could potentially represent a confounding factor for our survival analysis, we first tested the "era effect" by dividing the study cohort in terciles.

The one-year and five-year survival rates were 85% and 79%, 85% and 72%, and 83% and 78% for each tertile, respectively ($p = 0.59$) (Fig. 1).

4.1.2. Demographic and clinical data of the study cohort

Fig. 2 summarizes the distribution of HLA-A, -B or –DR mismatches (MM) in the entire cohort (n = 309). The average number of HLA-A/B/DR MM between donor and recipient was 4.9 ± 1. One recipient (0.3%) received an HLA-identical allograft. There were 2 MM in 4 patients (1.3%); 3 MM in 25 (8.1%); 4 MM in 60 (19.4%); 5 MM in 122 (39.5%); and 6 MM in 97 patients (31.4%). The relationship between the total number of HLA-A, -B and -DR mismatches, calculated from 0 to 6 MM, and long-term survival revealed no statistically significant difference ($p = 0.28$, not shown).

Analysis of the impact of mismatches on survival with respect to the level of HLA class I loci, comparing patients with 1 MM (or less) at each locus A/B ($n = 34$) versus all others (>2 HLA A/B MM $n = 275$), revealed no statistical significant differences for HLA class I loci (Table 2).

Similarly, the number of HLA-DR mismatches had no significant influence on long-term outcome: among recipients of grafts without DR mismatch ($n = 8$), with 1DR MM ($n = 97$) or 2 DR MM ($n = 204$), cumulative 5-year survival was 50%, 78.3 and 76.1%, respectively ($p = 0.35$). Of note, three early deaths were encountered in the 0 MM group (Two septic shock, one late tamponade, all within the first 3 months).

Table 1
Characteristics.

Total (n)	**309**
Gender	
Male/Female (%)	237/72(77/23)
Mean age at transplantation (years)	**49.7 ± 13**
Mean waiting time (days) [range]	**167 [1−1100]**
Recipients blood group	
A	*137 (44.3%)*
B	*11 (3.6%)*
AB	*39 (12.6%)*
O	*122 (39.5%)*
Diagnosis	
DCM	*109 (35.3%)*
ICM	*124 (40.1%)*
Congenital	*16 (5.2%)*
Retransplantation	*7 (2.3%)*
Other	*53 (17.2%)*
Donor Gender	
Male/Female (%)	*188/121 (61/39)*
Donor mean age (years)	**35.0 ± 12.6**
Donor blood group	
A	*118 (40.8%)*
B	*5 (1.7%)*
AB	*26 (9.0%)*
O	*136 (47.1%)*
ABO match	
Identical	*276 (89.3%)*
Compatible	*33 (10.7%)*
Ischemic Time (min)	**147 +/− 43**
Year of transplantation	
1985–1993	*69 (22.3%)*
1993–2002	*108 (35.0%)*
2002–2013	*132 (42.7%)*
LVAD prior to transplantation	**49 (16%)**
Mean Follow-up (years)	**9.2+/− 4.4**
Death	**158 (51.1%)**
Cause of death	
Sudden death	*27 (17.8%)*
Cardiac related	*18 (11.9%)*
Infection	*22 (14.5%)*
Cancer	*32 (21.1%)*
Other	*59 (39.0%)*

4.1.3. Anti-HLA antibodies and overall survival

As described earlier, out of 309 patients, only 219 could be analyzed (availability of both « anti-HLA screening » pre-transplantation and « at least one post-transplantation sera tested for anti-HLA » during follow-up. One hundred and one patients (46%) were tested within 5 years after transplantation, whereas 118 patients (54%) were tested beyond 5 years of transplantation.

Anti-HLA antibodies were detected in 60 patients (anti-HLA class I $n = 25$, anti-HLA class II $n = 35$). « De novo » anti HLA antibodies were found in 36 patients (anti-HLA class I $n = 14$, anti-HLA class II $n = 19$, both anti-HLA classes I and II n = 3). Analysis of HLA donor-recipient pairs revealed that only 18 patients (8.2%) developed donor-specific anti-HLA antibodies (ds anti-HLA class I $n = 8$, ds anti-HLA class II $n = 9$, ds anti-HLA classes I and II n = 1).

Overall, patients without or with « de novo » anti-HLA antibodies had a cumulative 5-year survival of 77.1% and 90.6%, respectively ($p = 0.34$). In addition, patients without or with « de novo » donor specific anti-HLA antibodies had a cumulative 5-year survival of 78.3 and 92.3%, respectively ($p = 0.49$).

By univariable analysis, none of the six categories studied influenced survival (See Table 2).

4.1.4. Other predictors of overall survival after transplantation

Among predictors of long-term survival, by univariable analysis, we found that recipient older age (HR 1.5 95% CI 1.05–2.1), prior immunization against CMV (HR 0.7 95% CI 0.5–0.9), or the development of CAV at follow-up (HR 1.5 95%CI 1.1–2.2) had a significant impact on patients survival. Variables such as acute cellular rejection (3A or more)

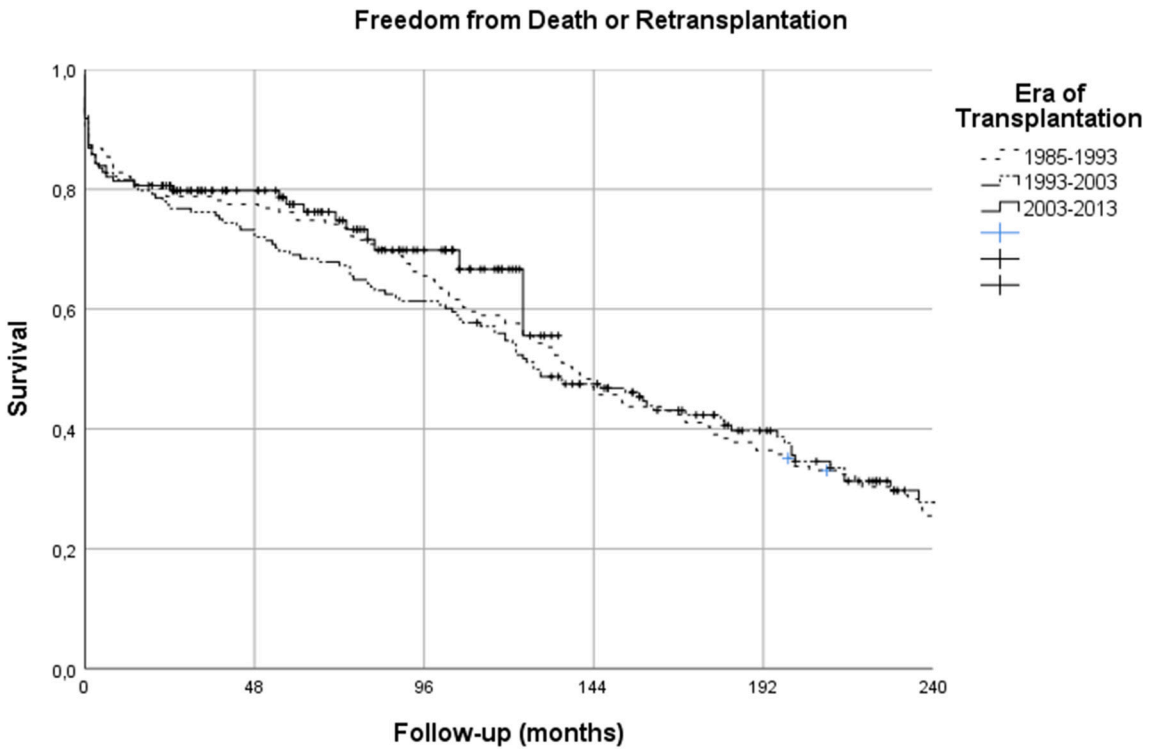

Fig. 1. Distribution of Donor-Recipient HLA mismatches in the cohort of isolated heart transplantation patients ($n = 309$).

within the first year (HR 1.4 95%CI 0.9–2.3), evidence of humoral rejection (HR 1.7 95%CI 0.8–3.5) and IS scheme ($p = 0.15$) were of borderline significance (Table 3).

By multivariable analysis, older recipient age (HR 2.2 95% CI 1.4–3.6), acute cellular rejection within the first year (3A or more) (HR 1.7 95%CI 1.04–3), any episode of humoral rejection after transplantation (HR 1.2 95%CI 1.03–1.4) or the development of CAV (HR 1.1 95%CI 1.04–1.3) were all significantly associated with patients survival (Table 4).

4.2. Cellular and humoral rejection

4.2.1. HLA matching and freedom from rejection grade ≥ 3A/humoral rejection

The relationship between the total number of HLA-A, -B and -DR mismatches, calculated from 0 to 6 MM, and freedom from cellular rejection ≥3A and/or humoral rejection revealed no significant difference ($p = 0.27$, not shown).

Analyzing differences in HLA class I, patients with 1 MM (or less) at each locus A, B (n = 34) versus all others ($n = 278$), revealed no statistical significant differences in freedom from cellular or humoral rejection at (93.4% vs 85.4% at 5-yr, $p = 0.22$) (Table 5).

However, the presence of HLA-DR mismatches had a significant influence on freedom from cellular rejection ≥3A and/or humoral rejection. Among recipients of grafts without DR mismatch (n = 8), with 1DR MM (n = 97) or 2 DR MM (n = 204), freedom from cellular rejection (3A or more) and/or humoral rejection was 100%, 94% and 82% at 5-yr, respectively (p = 0.04) (Fig. 3 and Table 5).

4.2.2. Anti-HLA antibodies and freedom from rejection grade ≥ 3A/humoral rejection at follow-up

Patients without or with « de novo » anti-HLA antibodies had a cumulative 5-year freedom from severe cellular or humoral rejection of 87.3% and 74.2%, respectively ($p = 0.15$). Those without or with « de novo » donor-specific anti-HLA antibodies had 5-year rejection-free survival of 85.6% and 73.4%, respectively ($p = 0.7$).

By univariable analysis, neither the class of anti-HLA antibodies (preexisting or de novo) nor the presence of donor-specific anti-HLA influenced the occurrence of severe cellular or humoral rejection (See Table 5).

However, looking more specifically at humoral rejection, 11 patients presented at least one episode of acute humoral rejection, 3/18 patients (16.7%) in the group of patients with donor-specific de novo anti-HLA antibodies, and 8/201 (3.9%) patients without de novo DSA ($p = 0.004$).

Among the group with humoral rejection ($n = 11$), those rejections were not concomitant with ACR, and the episode of ACR always preceded AHR.

Among the 262 patients surviving at least one year, severe ACR during the first year was present in 27 patients (10.3%). There was no correlation between acute cellular and humoral rejection ($p = 0.97$). However, over the entire follow-up, severe ACR was found in 45 patients (17.2%) and was associated with acute humoral rejection (4/10 with AHR vs 41/252 without AMR) ($p = 0.05$).

4.3. Chronic allograft vasculopathy

4.3.1. Influence of HLA matching on incidence of chronic allograft vasculopathy

There was no correlation between the number of HLA-A, -B and -DR mismatches and the presence of chronic allograft vasculopathy ($p = 0.59$).

Analysis of the impact of mismatches with respect to the level of HLA class I loci, comparing patients with 1 MM (or less) at each locus A, B ($n = 28$) versus all others ($n = 215$), revealed no statistical significant differences for HLA class I loci (Table 6).

Similarly, analysis with respect to the number of HLA-DR mismatches had no significant influence on the incidence of CAV. Freedom from CAV at 5- and 10-years were 100% and 50% in the group with 0MM (n = 4), 91.1% and 57.9% in the group with 1MM ($n = 75$) and 94.5% and 79% in the group with 2MM ($n = 164$) ($p = 0.13$) (Table 6).

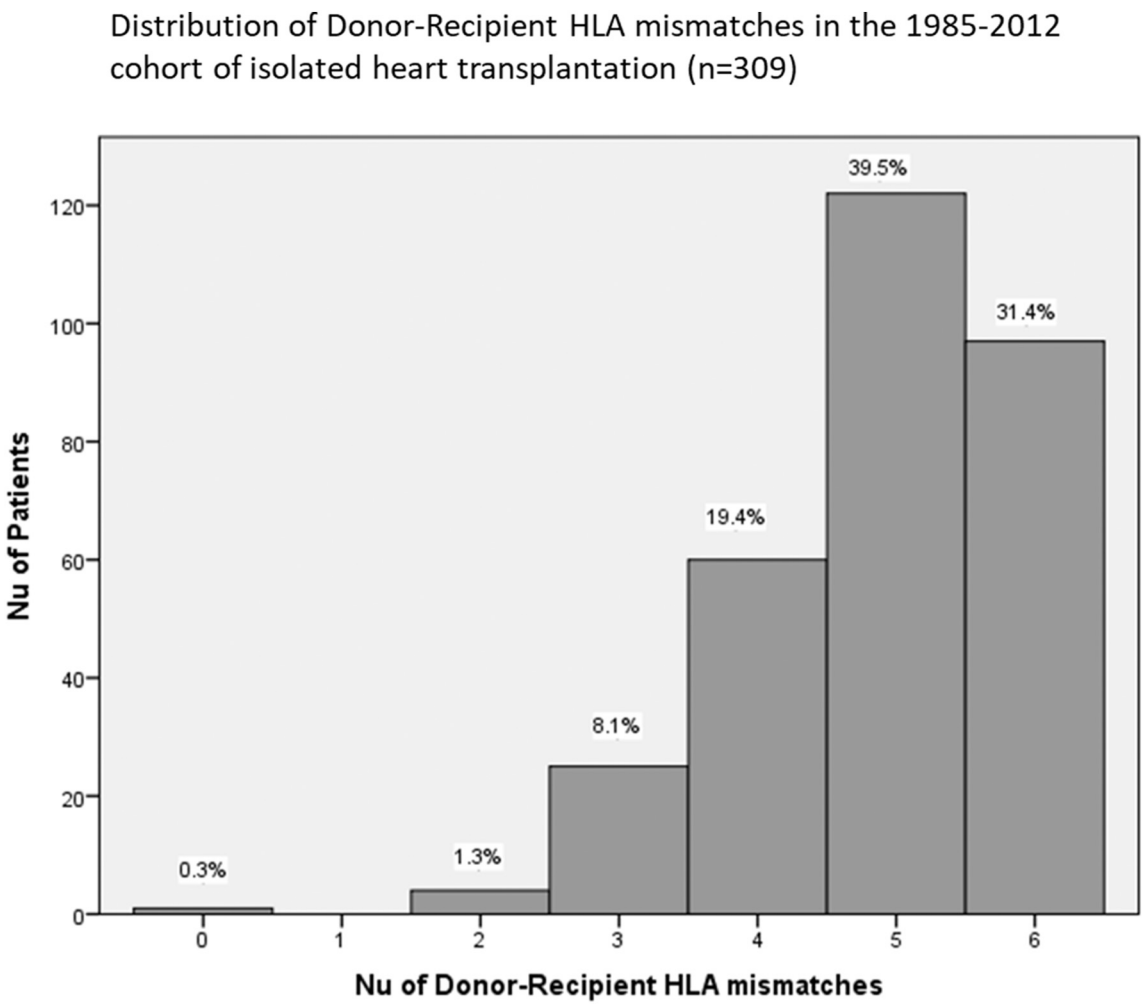

Fig. 2. Freedom from ACR 3A or more and/or AHR (months).
Freedom from acute cellular rejection ≥3A and/or acute humoral rejection in the entire cohort of patients (n = 309) according to the number of HLA-DR mismatch (es). (0 DR MM: solid line; 1 DR MM: dotted line; 2 DR MM: dashed line).

Table 2
Influence of HLA matching and anti-HLA antibodies on long-term survival by univariable analysis.

	H. R.	95 CI		ρ value
		Low	Upp	
Effect of HLA Matching				
Class I (Others vs ≤ 1 MM at locus A and B each)	1.2	0.7	2.0	0.51
Class II (0–1 MM vs 2 MM)	1.0	0.7	1.4	0.96
Effect of allo-immunization				
Anti-HLA Class I	1.3	0.6	2.8	0.49
Anti-HLA Class II	1.2	0.7	2.1	0.59
De novo Anti-HLA Class I	0.5	0.1	2.0	0.30
De novo Anti-HLA Class II	0.7	0.3	2.1	0.53
De novo Donor Specific Anti-HLA Class I	0.4	0.1	3.0	0.38
De novo Donor Specific Anti-HLA Class II	0.5	0.1	2.0	0.31

4.3.2. Influence of anti-HLA antibodies on incidence of chronic allograft vasculopathy

Overall, 5-year and 10-yr freedom CAV were 92% and 72% in patients without « de novo » anti-HLA antibodies and 96% and 67.5% in those who developed « de novo » anti-HLA antibodies after transplantation ($p = 0.71$).

Similarly, those patients without or with « de novo » donor specific

Table 3
Other predictors of Long-term mortality by Cox Univariable Analysis ($n = 309$).

	HR	95% CI		ρ value
		Low-Up		
Age at transplantation > 50yo	1.5	1.05	2.1	0.02
Indication for Tx (vs idiopathic)				0.35
Ischemic	1.1	0.7	1.6	0.69
Congenital	0.4	0.2	1.2	0.12
Re-transplantation	0.4	0.1	1.8	0.25
Others	1.1	0.7	1.8	0.72
Era of Tx (vs 2003–2013)				0.59
1985–1993	1.3	0.8	2.1	0.35
1993–2003	1.2	0.8	1.9	0.35
Diabetes pre-Tx	1.6	0.8	3.1	0.19
CMV (+) status pre-Tx (vs CMV naive)	0.7	0.5	0.9	0.04
VAD at Tx	0.9	0.7	1.4	0.58
Female-Male (D-R)	1.1	0.9	1.6	0.60
Graft Ischemic time > 180 min	1.1	0.7	1.7	0.63
Immunosuppression Scheme (vs CNI-AZA-Ster)				0.15
CNI-MMF-Ster	0.8	0.5	1.2	0.23
EVE-MMF-Ster	1.8	0.7	4.8	0.19
Rejection ≥3A during 1st Yr	1.4	0.9	2.3	0.14
Any humoral rejection at f-up	1.7	0.8	3.5	0.17
Diagnosis of CAV at f-up	1.5	1.1	2.2	0.02

Table 4
Other predictors of Long-term mortality by Cox Multivariable Analysis (n = 309).

	HR	95% CI		ρ value
		Low-Up		
Age at transplantation >50yo	2.2	1.4	3.6	0.001
CMV (+) status pre-Tx (vs CMV naive)	0.7	0.5	1.2	0.23
Immunosuppression Scheme	1.4	0.8	2.2	0.20
Rejection ≥3A during 1st Yr	1.7	1.04	3	0.03
Any humoral rejection at f-up	1.2	1.03	1.4	0.02
Diagnosis of CAV at f-up	1.1	1.04	1.3	0.003

Table 5
Influence of HLA matching and anti-HLA antibodies on Acute Cellular/Humoral rejection at follow-up.

	HR	95 CI		ρ value
		Low-Up		
Effect of HLA Matching				
Class I (Others vs ≤ 1 MM at locus A and B each)	2.42	0.58	10.06	0.22
Class II (≤ 1MM vs 2 MM)	0.28	0.11	0.70	0.007
Effect of allo-immunization				
Anti-HLA Class I	1.93	0.72	5.16	0.19
Anti-HLA Class II	1.59	0.73	3.43	0.24
De novo Anti-HLA Class I	1.44	0.51	4.11	0.49
De novo Anti-HLA Class II	1.25	0.44	3.60	0.67
De novo Donor Specific Anti-HLA Class I	1.59	0.37	6.80	0.52
De novo Donor Specific Anti-HLA Class II	0.88	0.12	6.51	0.90

anti-HLA antibodies had a cumulative 5-year and 10-yr freedom from CAV of 93% and 71%, and 90% and 67.5% respectively ($p = 0.77$).

By univariate analysis, neither the class of anti-HLA antibodies (preexisting or de novo) nor the presence of donor-specific anti-HLA had any significant influence on the occurrence of CAV (See Table 6).

4.3.3. *Other predictors of chronic allograft vasculopathy*

Among risk factors of CAV, by univariable analysis, we found that a younger donor age (HR 0.6 95%CI 0.3–1.0), indication for transplantation ($p = 0.02$) and a female donor-to-male recipient (HR 1.8 95% CI 1.1–3.1) had a significant impact on the development of CAV (Table 7).

5. Discussion

Our study showed that in our cohort of heart transplant patients, HLA matching did not influence overall survival, whether studied by the number of MM or by the MM at each loci.

Those results contradict one of the first seminal report by Hosenpund et al. [24] which demonstated in a very large set of patients a stepwise reduction in survival with decreasing levels of total HLA matching, with

Table 6
Influence on HLA matching and anti-HLA antibodies on incidence of Chronic Allograft Vasculopathy.

	HR	95 CI		ρ value
		Low-Up		
Effect of HLA Matching				
Class I (Others vs ≤ 1 MM at locus A & B each)	1.1	0.5	2.3	0.85
Class II (≤1 MM vs 2 MM)	1.5	0.9	2.5	0.10
Effect of allo-immunization				
Anti-HLA Class I	0.7	0.3	1.8	0.48
Anti-HLA Class II	1.1	0.6	2.0	0.70
De novo Anti-HLA Class I	0.9	0.2	3.7	0.83
De novo Anti-HLA Class II	1.1	0.4	2.9	0.90
De novo Donor Specific Anti-HLA Class I	1.2	0.3	5.2	0.81
De novo Donor Specific Anti-HLA Class II	1.5	0.5	4.4	0.46

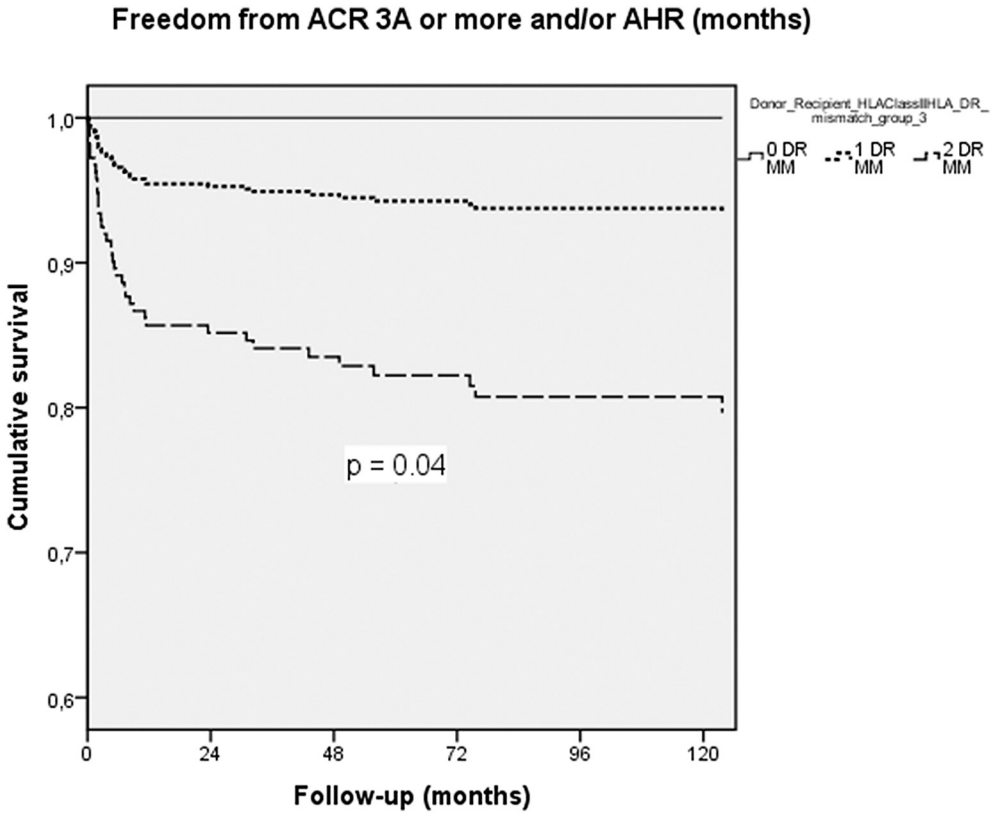

Fig. 3. D/R HLA class II mismatch and Freedom from Rejection.

Table 7
Other predictors of Chronic Allograft Vasculopathy by Univariable analysis.

	HR	95% CI		ρ value
		Low	Upp	
Age at transplantation <50yo	0.8	0.6	1.2	0.35
Indication for Tx (vs Others)				0.02
Ischemic	1.2	0.7	2	0.50
Congenital	0.2	0.1	0.8	0.02
Re-transplantation	0.7	0.2	3.2	0.69
Idiopathic	0.7	0.4	1.2	0.21
Era of Tx (vs 2003–2013)				0.83
1985–1993	0.8	0.4	1.7	0.55
1993–2003	0.8	0.4	1.8	0.58
Diabetes pre-Tx	1.2	0.4	3.8	0.75
CMV (+) status pre-Tx (vs CMV naive)	1.2	0.8	1.9	0.35
Sex (Male vs Female)	1.6	0.9	2.8	0.06
Graft Ischemic time > 180 min	0.9	0.4	1.8	0.67
Donor CMV seropositive status	1.5	0.9	2.6	0.09
Donor sex (Male vs Female)	1.5	0.4	6.2	0.19
Donor age (younger than 35 yo)	0.6	0.4	1	0.04
Immunosuppression Scheme (vs CNI-AZA-Ster)				0.28
CNI-MMF-Ster	1.5	0.8	2.7	0.15
EVE-MMF-Ster	2.6	0.3	19	0.36
Rejection ≥3A during 1st Yr	1.1	0.7	1.9	0.69
Acute Humoral Rejection	1.2	0.4	4.7	0.83
Post-Tx Diabetes	1.4	0.9	2.3	0.27

HLA class II (DR) and Class I (Locus A) being most influential. Unfortunately, those registry data did not include a wide range of covariates which might have influenced long-term survival and cause of death was not reported. Secondly, the immunosuppressive regimen available at the time of that study was different from our current standards of immunosuppression.

In a recent meta-analysis by Ansari and al. [25], only 4 studies reported on this subject since 2000. Our univariable analysis of the impact of HLA matching on survival is in line with those more recent monocentric studies [8,9]. Excluding early deaths and retransplantation from their analysis, Almenar et al. [9] reported a positive correlation between the degree of matching and a worsened overall survival, while Tenderich et al. [8] in a large dataset of >900 patients showed no effect of HLA matching on survival.

However, as already underscored by several studies [26–28], we found a protective effect of HLA-DR matching (0 or 1 MM) against the occurrence of severe acute cellular rejection and/or acute humoral rejection. During the entire follow-up, patients with 2MM in Class II-DR had a four-fold increased risk of developing rejection despite contemporary immunosuppression.

In our survival analysis of « non-HLA related » covariables, we found that, together with recipient's age and prior immunization against CMV, cellular rejection within the first year (≥3A) increased the risk of death by 70%. Intuitively, if DR-matching influences the occurrence of severe ACR, and the latter impact on survival, we would expect the former to also impact on overall survival.

One could hypothesized that failure to demonstrate a survival effect in our study is due to the combined low prevalence of HLA-DR matching (30% had 0MM or 1MM in our series) and the low prevalence of severe ACR during the first year (10.3%) in our cohort of patients receiving induction therapy and potent immunosuppressors.

Very few studies have tried to correlate the degree of HLA matching and chronic allograft vasculopathy [7,26,29].

Here, we were not able to detect any statistically significant impact of HLA-matching on the occurrence of CAV, with freedom from CAV at 5-year and 10-year of 90% and 61% in Class II 0–1 MM group and 94% and 80% in the Class II 2 MM group ($p = 0.10$). Methodological differences could explain those discordant results since we defined CAV as a narrowing of 50% or greater, in alignment with other studies [22] whereas Kaczmarek et al. [7] used a cut-off of 30% in any coronary artery.

Currently, HLA matching is not among the criteria for heart allocation, due to the short ischemic time, the lack of conservation techniques for thoracic organs, and the limited donor availability. However, based on recent successful experience of ex vivo continuous heart perfusion extending beyond 6 h [11] and on future refinements of this fast-developing area, it may not be long before we could be selecting donor-recipient pairs throughout the Eurotransplant zone based on well thought HLA criteria. Such innovative comparative and controlled studies are truely needed, acknowledging the lack of undisputable data on this subject.

In this study, we demonstrated that 26% (60/219) of patients presented with anti-HLA antibodies during follow-up. The prevalence of « de novo » anti-HLA antibodies was 16% (36/219) and only half of those were donor-specific anti-HLA antibodies (18/219 or 8.2%).

Though the latter figure is comparable to most studies [7,16], the prevalence of anti-HLA as a whole was much lower than previously reported. One hypothesis is that the MFI cut-off levels for the definition of HLA antibodies positivity varied widely among studies: cut-off MFI >1000 for Smith et al. [13], MFI >500 for Raess et al. [16] while we defined positivity at MFI >1500 [18].

Analyzing the effect of HLA antibodies on overall survival, on acute cellular and/or humoral rejection and chronic allograft vasculopathy, we could not demonstrate any detrimental effect of anti-HLA antibodies, whether those were « de novo » and/or « donor specific ».

As Smith et al. [13], we found that the most common donor-specific allosensitization occurred against Class II –DQ alleles (8/10 patients with Class II ds anti-HLA in our series).

Raess et al., [16] in a similar study design, reported a detrimental effect of DSA anti-HLA class I on overall survival in univariable analysis, but in the multivariable analysis, this effect was shadowed by the strongest impact of PRA positivity and (+) CDC screening test prior to transplant (despite pre-transplant T-cell crossmatch). Of note, in their study, the optimal MFI cut-off for predicting survival was ≥2000 MFI (specificity 73% and sensitivity 76%).

As in Smith et al. study [13], the proportion of cardio-vascular death was increased in patients with DSA, but in our study, it did not reach statistical significance. It also might be possible that the low prevalence of ACR/AHR in our cohort resulted in a failure to detect the effect of DSA on outcome measures. Indeed, for the entire cohort our 1-yr and 5-yr freedom from severe ACR/AHR of 90% and 85.6% respectively are much higher than other studies. (Raess et al. [16] reported a 62% rejection rate within the first year in their study).

A recent analysis of pediatric heart transplant recipients reported a high prevalence of de novo DSA associated with an increased risk of developing CAV [30]. Using established criteria for CAV diagnosis by angiography [22,23], our adult cohort had a 98% freedom from CAV at 1 year, and 93% at 5 year. Again, as Smith et al. [13], we found no correlation between the detection of DSA and chronic allograft vasculopathy. Again, it is possible that both adult studies were underpowered to detect significant differences.

In conclusion, the results of this study add to the controversy on the overall impact of allo-antibodies in adult heart transplant recipients receiving induction therapy and contemporary immunosuppression.

6. Limitations of this study

This study suffers several limitations as it is retrospective in design, with uncomplete dataset for allo-antibodies detection with regard to the HLA-matching population studied. Diagnostic tools were modernized over a period of 3 decades and multiple methods of detection were used. As the HLA typing techniques improved over the years, the number of HLA MM for patients in the early inclusion period could have been underestimated. The absence of longitudinal follow-up of sera for all patients (only 46% had sera tested starting immediately after transplantation) and the analysis of all patients (including early deaths) might have biased our results, translating in a positive selection of

survivors.

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Declaration of interest

None.

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