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ORIGINAL ARTICLE

Donor-specific anti-HLA antibodies detected by Luminex: predictive for short-term but not long-term survival after heart transplantation

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anti-HLA antibodies, heart transplantation, rejection, survival analysis, vasculopathy.

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Summary

In heart transplantation, the clinical significance of pretransplant donor-specific antibodies (DSA) detected by solid phase assay (SPA), which is more sensitive than the conventional complement-dependent cytotoxicity (CDC) assays, is unclear. The aim was to evaluate SPA performed on pretransplant sera for survival after heart transplantation. Pretransplant sera of 272 heart transplant recipients were screened for anti-HLA antibodies using CDC and SPA. For determination of pretransplant DSA, a single-antigen bead assay was performed. The presence of anti-HLA antibodies was correlated with survival. Secondary outcome parameters were acute cellular rejection, graft coronary vasculopathy and ejection fraction. In Kaplan–Meier analysis, SPA-screening did not predict survival ($P = 0.494$), this in contrast to CDC screening ($P = 0.002$). However, the presence of pretransplant DSA against HLA class I was associated with decreased short-term survival compared to non-DSA ($P = 0.038$). ROC curve analysis showed a sensitivity of 76% and specificity of 73% at a cutoff of 2000 MFI. In contrast, the presence of anti-HLA antibodies had no influence on long-term survival, rejection incidence, and graft function. Thus, detection of DSA class I in pretransplant serum is a strong predictor of short-term, but not long-term survival and may help in the early management of heart transplant patients.

Introduction

Heart transplantation in patients with preformed antibodies (Abs) against HLA bears an increased risk for acute rejection [1–4]. These antibodies are induced by alloimmunization resulting from pregnancy, blood transfusions, previous transplants or sensitization to ventricular assist devices (VAD), and cross-reactive microbial epitopes [5,6]. Acute rejection after HTx is associated with acute hemodynamic compromise, accelerated graft coronary artery vasculopathy (CAV), and death [7]. Therefore, the search for preformed anti-HLA antibodies before transplantation is of substantial clinical interest.

Since the introduction of the complement-dependent cytotoxicity (CDC) assay in the 1960s, it has remained the gold standard method for determination of preformed

antibodies because of its high positive predictive value for rapid humoral rejection [8,9]. However, there are patients with a negative CDC assay experiencing humoral rejection, which suggests that CDC fails to detect some clinically significant antibodies [10].

Recently, solid phase technology was introduced and improved sensitivity, specificity, and accuracy for detection of anti-HLA Abs [11]. Because the solid phase assay (SPA) cannot differentiate between complement-binding and noncomplement-fixing antibodies, it is not surprising that approximately five times as many patients have anti-HLA Abs compared with the CDC assay [12]. This raises the question about the clinical relevance of antibodies detected by solid phase technology and whether it is safe to transplant patients with a negative CDC assay, but a positive SPA.

The aim of this retrospective study was to test whether the presence of pretransplant DSA detected by SPA and their strength are correlated with survival, acute or chronic rejection, and graft function after HTx and to compare these data with results achieved with the standard CDC technology.

Materials and methods

Patients and serum samples

Between 1989 and 2010, 308 consecutive patients receiving a heart transplant at the University Hospital Zurich were evaluated for this retrospective study. Patients were excluded if pretransplant serum within 1 year before transplantation was missing ($n = 33$), if HLA typing of donor or recipient was unavailable ($n = 3$) or because of AB0-incompatibility ($n = 1$). One patient was retransplanted and counted as two transplantations for further analyses.

The 272 recipients included in this study underwent CDC assays, which involved CDC screening (Lymphoscreen) and crossmatch against donor T and B cells at the time of transplant. Recipient and donor typing for HLA-A, -B, -DR, and – DQ (the latter only in recent years) was done by serology and polymerase chain reaction with sequence-specific primers. Decision for transplantation was generally based on the presence of a negative T-cell crossmatch (with one exception), whereas results of PRA testing and virtual crossmatching had no influence on this decision.

Standard immunosuppression before 1997 consisted of cyclosporine, azathioprine, and prednisone. After 1997, azathioprine was replaced by mycophenolate mofetil and cyclosporine by tacrolimus in patients, who either experienced side effects or recurrent rejection. In addition, all patients received induction therapy with rabbit anti-thymocyte globulin (Thymoglobulin®). In general, dosing regimens of immunosuppressants were highly standardized within the first 6 months post-transplant, and no patient underwent desensitization using pre-emptive plasmapheresis or immunoadsorption treatment.

CDC assay

All serum samples were screened prospectively in a microlymphocytotoxicity assay against a lymphocyte panel of 56 donors using Lymphoscreen®ABC 60 (Bio-Rad Medical Diagnostics GmbH, Dreieich, Germany). Cells were incubated with serum for 30 min and then with complement for 60 min at room temperature. When panel reactivity (PRA) was >0% and the strength of reaction was >10% above background level, the patient was deemed to be antibody-positive. Furthermore, the serum samples underwent a crossmatch test prior to transplantation performed by the classical CDC method.

Anti-HLA antibody screening by Solid phase assay

Patients were retrospectively screened for anti-HLA Abs with Luminex LABScreen Mixed (One Lambda Inc., Canoga Park, CA, USA), which is from now on called SPA-Screen (Solid phase screen). This kit contains a panel of fluorescence-labeled microbeads coated with purified HLA antigens to identify anti-HLA class I or II IgG [13]. HLA FUSION 2.0 software (One Lambda) on the LABScan100 flow cytometer (Luminex Inc., Austin, TX, USA) was used for test interpretation. The positive cutoff, which was recorded according to the relative ratio between the patient sample and the negative control, was at 4.0 for HLA class I Abs and at 3.0 for HLA class II Abs.

Single-Antigen Bead Assay (SAB)

Sera positive in SPA-Screen were further tested to identify antibody specificity (Fig. 1). Therefore, a high-definition LABScreen Single Antigen (One Lambda) class I assay in SPA-Screen class I positive individuals and a class II assay in SPA-Screen class II positive patients was retrospectively performed according to the manufacturer's instructions [14]. For result interpretation, LABSCAN 100 software (One Lambda) was used. The cutoff for a positive result was set at 500 MFI according to the manufacturer's instruction. SP analysis has been highly standardized among all HLA laboratories across Switzerland and is regularly checked by quality controls twice yearly, resulting in concordance of >95% of the results for antibody determination with MFI >1000.

C1q assay

Sera positive in SAB were further tested to identify complement-fixing antibodies (Fig. 1). Therefore, a C1qScreen

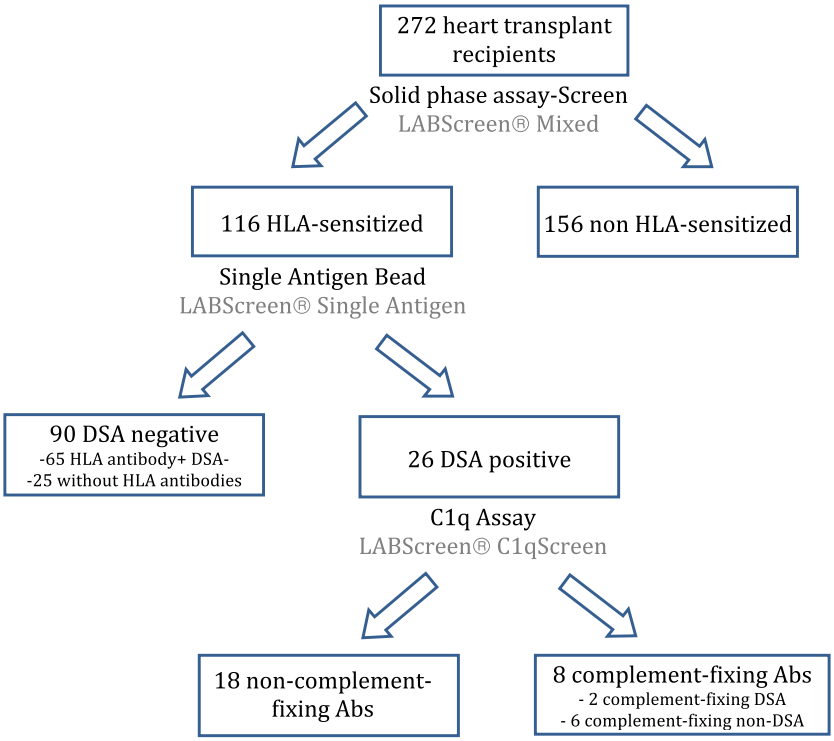

Figure 1 Study design. Screening of 272 heart transplant recipients by LABScreen Mixed assay for the presence of pretransplant anti-HLA antibodies. The 116 recipients positive in screening were further evaluated with the single-antigen bead assay.

(One Lambda) class I assay in DSA class I positive patients and a class II assay in DSA class II positive patients were retrospectively performed according to the manufacturer's instructions. HLA FUSION 2.0 software (One Lambda) on the LABScan100 flow cytometer (Luminex Inc.) was used for test interpretation. The cutoff for a positive result was set at 500 MFI according to the manufacturer's instruction.

Outcome parameters

Outcome parameters included patient survival, acute rejection, CAV (chronic rejection), and ejection fraction (EF). Acute cellular rejection, determined by endomyocardial biopsy and autopsy report, was graded according to the International Society for Heart and Lung Transplantation (ISHLT) system. ISHLT grade 2R or higher was considered to represent allograft rejection. Rejection treatment consisted of 3 × 1g prednisolone and adjusted immunosuppression. C4d staining for detection of acute humoral rejection (AMR) in endomyocardial biopsies is not routinely performed in the University Hospital Zurich, therefore only autopsy reports could be used as source. Results of EF after 1 year of transplantation were gained from echocardiography or coronary angiography, and information on CAV was gained from regularly performed coronary angiography (first coronary angiography 3 months after HTx, then every 2 years till occurrence of CAV, then at least once per year) or autopsy reports. If the obstruction was >50% or an intervention including percutaneous transluminal coronary angioplasty or aorto-coronary bypass was necessary, the patient was considered to be CAV positive.

Statistics

For comparison of two discrete variables, the chi-square test; for 2 × 2 tables, the Fisher exact test; for continuous variables between two groups, the Student's two-sample *t*-test and for more than two groups, the one-way ANOVA with Schéffe *post hoc* test were used. Analysis for survival, rejection-free survival and vasculopathy-free survival was conducted using actuarial Kaplan–Meier analysis. Observation was censored if the patient was alive at the end of the observation period and did not experience any event of interest. Univariable Cox regressions were computed and hazards ratios (HR) together with the corresponding 95% CI were provided. Backward elimination method assisted multiple Cox regression model choice.

Receiver operator characteristic (ROC) analysis, along with the area under the curve (AUC) together with the

corresponding 95% CI, was conducted in order to assess the ability of MFI to predict rejection and 1-year survival.

Statistical analysis was performed with SSPS version 18. *P*-values < 0.05 were considered statistically significant.

Results

Patient characteristics

Baseline characteristics are given in Table 1. Of the 272 enrolled heart transplant recipients, 116 (43%) were screened positive for anti-HLA Abs with SPA-Screen, whereas only 9 (3%) had a positive pretransplant PRA by CDC screening. Of the 116 sensitized patients, 26 had DSA (14 against class I, five against class II, seven against class I+II). Sixty-five patients had anti-HLA Abs, but no DSA, and 25 had no anti-HLA Abs. For further analysis, these were analyzed within the non donor-specific HLA-sensitized group (Fig. 1).

The DSA+ group (19%) was more often tested positive in CDC screening compared to the DSA- group (3%; $P = 0.014$). CDC crossmatch tests against T and B cells were performed in 267 (98%) and 40 (15%) patients, respectively. One patient had a positive CDC T-cell crossmatch but was negative by SPA-Screen, and three patients had a positive CDC B-cell crossmatch, but only two were positive by SPA-Screen.

To further analyze the complement-fixing capacity of DSA, a C1q assay was performed in all DSA-positive patients: eight patients were tested positive for complement-fixing Abs, but only two patients had donor-specific complement-fixing Abs (Fig. 1). Four positive tested patients also had a positive CDC Screen. Out of the 18 negative tested patients, all had a negative CDC Screen (Table S1).

Sensitizing events and histocompatibility

More HLA-sensitized patients (54%) received transfusions compared to the non-HLA-sensitized patients (39%; $P = 0.010$). VAD were more frequent in HLA-sensitized patients (trend, $P = 0.098$).

The number of HLA mismatches in the sensitized and nonsensitized group was identical. In the DSA+ group, the sum of HLA mismatches ($P = 0.032$) was higher, and the difference occurred mainly because of HLA-B mismatches ($P = 0.004$, Table 1).

Survival

The overall survival of the 272 heart transplant recipients was 80% at 1 year and 68% at 5 years and was equal between sensitized and nonsensitized patients as defined by

Table 1. Patient demographics including comparative statistics between patients with and without anti-HLA antibodies and patients with and without DSA.

		Solid phase assay screen*			Single antigen bead assay†		
Variable	All ($n = 272$)	HLA Abs− ($n = 156$)	HLA Abs+ ($n = 116$)	*P*-Value	DSA− ($n = 90$)	DSA+ ($n = 26$)	*P*-Value
Recipient age (year)	48 (±13)	48 (±12)	47 (±14)	0.530	48 (±14)	44 (±17)	0.254
Female sex	50 (18)	20 (13)	30 (26)	0.007	18 (20)	12 (46)	0.011
Cardiac diagnosis							
Ischemic	108 (40)	60 (39)	48 (41)	0.707	44 (49)	4 (15)	0.003
Nonischemic	164 (60)	96 (61)	68 (59)	0.707	46 (51)	22 (82)	0.003
Ischemia time (min)	107 (±52)	102 (±49)	114 (±54)	0.067	113 (±51)	116 (±65)	0.791
Sensitization events							
Transfusions	123 (45)	60 (39)	63 (54)	0.010	47 (52)	16 (62)	0.504
Pregnancy $n = 46$	30 (65)	15/19 (79)	15/27 (56)	0.126	6/15 (40)	9/12 (75)	0.121
VAD	45 (17)	24 (15)	21 (18)	0.098	15 (17)	6 (23)	0.048
All Devices‡	103 (38)	51 (33)	52 (45)	0.044	35 (39)	17 (65)	0.024
HLA mismatch							
HLA-A	1.3 (±0.6)	1.3 (±0.7)	1.3 (±0.6)	0.831	1.3 (±0.6)	1.4 (±0.6)	0.555
HLA-B	1.7 (±0.5)	1.6 (±0.5)	1.7 (±0.5)	0.419	1.6 (±0.5)	1.9 (±0.3)	0.004
HLA-DR	1.4 (±0.6)	1.4 (±0.6)	1.4 (±0.6)	0.488	1.4 (±0.6)	1.6 (±0.6)	0.206
Sum	4.4 (±1.1)	4.3 (±1.1)	4.4 (±1.1)	0.570	4.3 (±1.1)	4.8 (±1.0)	0.032
CDC Lymphoscreen							
Last PRA pos	9 (3)	1 (0.6)	8 (7)	0.005	3 (3)	5 (19)	0.014
Peak PRA pos	18 (7)	6 (4)	12 (10)	0.047	6 (7)	6 (23)	0.026

Abs, Antibodies; DSA, donor-specific antibody; CDC, complement-dependent cytotoxicity; PRA, panel reactive antibody.

*Screened with Luminex LABScreen®Mixed.

†Screened with Luminex LABScreen®Single Antigen.

‡All devices include ventricular assist device, pacemaker, intracardiac defibrillator, and intra-aortic balloon pump.

Data are presented as *n* (%) or mean (±standard deviation), where appropriate.

SPA-Screen assay (Fig. 2b). In contrast, only 44% of CDC screening positive patients were still alive at 1 year and 30% after 5 years ($P = 0.002$; Fig. 2a). However, caution is warranted for the interpretation of Fig. 2a because of the low patient number in the PRA+ group.

When focusing on patients with DSA determined by SAB, survival of patients with DSA class I was lower (62% at 1 year and 50% at 5 years) compared to patients without DSA class I (87% at 1 year and 73% at 5 years) ($P = 0.038$; Fig. 3a). In contrast, DSA class II had no predictive value ($P = 0.330$; Fig. 3b).

A ROC curve using MFI for the strength of anti-HLA antibodies was computed using either the maximal MFI among all DSA or the total MFI sum of all DSA. Only the maximal, but not the cumulative MFI was predictive for survival. Sensitivity and specificity were equally weighted at MFI 2000 corresponding to a sensitivity of 76% and a specificity of 73% [AUC 0.743, 95% CI (0.525; 0.961); $P = 0.052$; Fig. 3c].

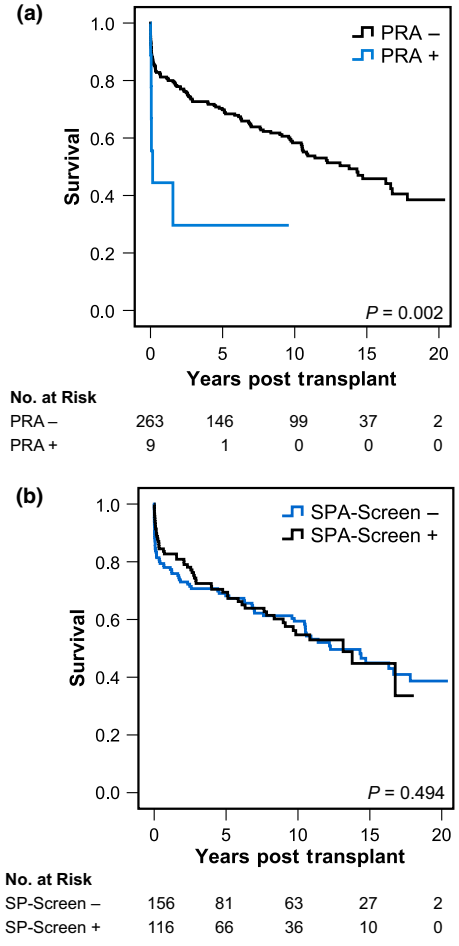

Figure 2 Survival after heart transplantation according to type of anti-HLA antibody screening. Comparison of two screening methods (CDC screening expressed as PRA (a) and SPA-Screen (b).

Since the survival curves diverge in the first 2 years post-transplant (Fig. 4a and b), we evaluated the utility of different HLA Abs assays to predict survival in this period (Fig. 4c). CDC screening had a sensitivity of only 9.2% [95% CI (4; 19)], but a specificity of 98.8% [95% CI (96; 100)] and an overall accuracy of 75.8% [95% CI (70; 81)]. In contrast, the sensitivity of SAB DSA class I to predict the death of a patient was with 39.1% [95% CI (22; 59)] more than four times higher compared with CDC, with only a slight loss in specificity to 88.5% [95% CI (80; 94)] and a similar overall accuracy of 78.2% [95% CI (70; 85)].

Univariable and multiple Cox analyses were computed in order to check for predictors influencing survival (Table 2). Univariable analysis confirmed positive PRA and presence of DSA class I as significant predictors of survival. In addition, HLA-DR mismatch ($P = 0.023$) and positive PRA in history ($P = 0.028$) were identified. There was no impact on survival by donor's age, VAD or other sensitizing events. In multiple Cox regression analysis performed on significant predictors of univariable analysis (Table 2), a positive pretransplant PRA detected by CDC screening (multiple analysis 1, $P = 0.056$, HR = 2.69) was found to be a stronger independent risk factor for survival than DSA class I detected by SAB ($P = 0.176$, HR = 1.66). However, the presence of DSA class I (multiple analysis 2, $P = 0.055$, HR = 1.95) was a better predictor for survival than HLA-DR compatibility ($P = 0.526$, HR = 1.16). In a multiple analysis including all three factors, positive CDC screening is the strongest independent risk factor (multiple analysis 3, $P = 0.036$).

When analyzing the complement-fixing capacity of anti-HLA antibodies in DSA-positive patients, a trend for lower survival in C1q positive patients was seen (Table S1). However, only two C1q-DSA-positive patients were found: one with a DSA class II (MFI 4611) showed long-term survival, whereas the other with DSA class I (MFI 19428) died after 3 months. When comparing the specific causes of death in a Fisher's exact test, no statistically significant differences between DSA+ and DSA- patients were observed, but a higher percentage of deaths because of acute rejections and infections were seen in DSA+ patients (Table S2).

Acute Rejection

The rejection-free survival rate was 38% at 1 year and 30% at 5 years. Interestingly, neither PRA positive (Fig. 5a) nor SPA-Screen positive (Fig. 5b) nor DSA class I-positive (Fig. 5c) patients showed a decreased rejection-free survival compared to negative patients. In our study, histocompatibility had the greatest impact ($P = 0.011$): 1-year rejection-free survival for transplants with two mismatches was 44%

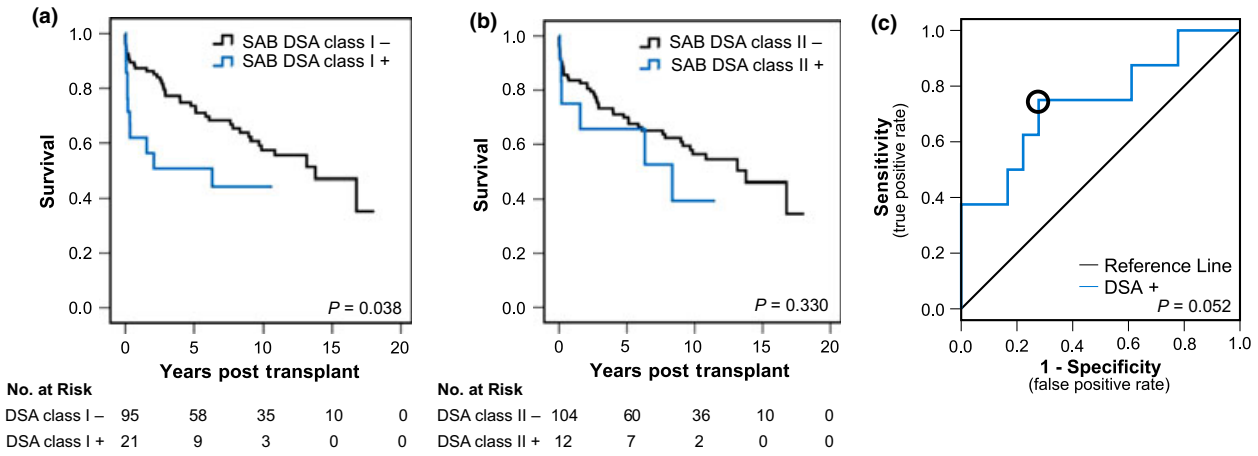

Figure 3 Survival after heart transplantation according to DSA status of pretransplant sera detected by single antigen bead. (a) Survival for patients with and without DSA class I. (b) Survival for patients with and without DSA class II. (c) ROC curve analysis for prediction of survival in relation to MFI for DSA by SAB (AUC 0.743, 95%CI (0.525; 0.961), $P = 0.052$). The MFI of the major DSA was significant, but not the cumulative MFI value (data not shown). The circle on the curve marks the point, where sensitivity and specificity are equally weighted (sensitivity = 0.76, specificity = 0.73) and corresponds to a DSA value of 2000 MFI.

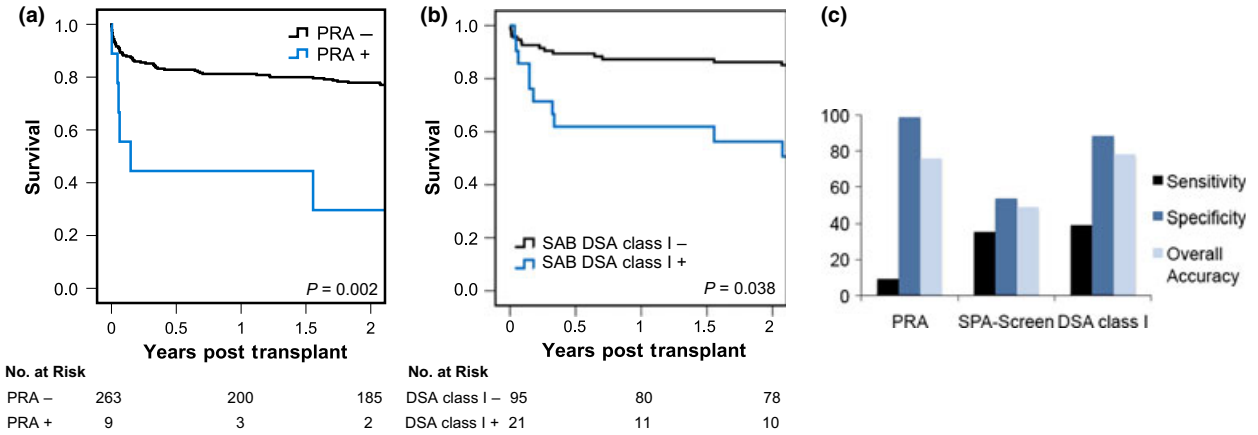

Figure 4 Two-year survival after heart transplantation. (a) Survival for patients with a PRA+ or PRA- in CDC screening. (b) Survival for patients with or without DSA class I. (c) Sensitivity, specificity, and overall accuracy of CDC screening (expressed as PRA), SPA-Screen and DSA class I for predicting of 2-year survival.

compared with 30% for six mismatches. The more HLA mismatches were present, the shorter was rejection-free survival (Fig. 5d).

The number of rejections of ISHLT 2R or greater was 1.4 (±1.6) per patient in the first year and 2.2 (±2.2) per patient in the whole follow-up (Table 3). Neither CDC screening nor SPA-Screen assay, nor C1q-binding (Table S1) was able to predict the incidence of acute cellular rejection in those patients who survived the first year.

Pathologically well-defined (characterized by positive C4d staining) *fatal* AMR happened to six patients (2%), all of them in the first month after transplantation. Three of them were in the DSA+ group and showed very high MFI rates of 6782, 12 259, and 15 349 (major DSA). All six patients had a negative CDC crossmatch, four had a negative pretransplant PRA. Only one had a negative SPA-Screen assay. Out of nine patients with MFI values over 4000, seven died within 1 year (four with a negative PRA).

Chronic Rejection

To screen for chronic rejection, 245 patients (90%) had coronary angiograms. The CAV-free survival rate in

Table 2. Univariate and multiple Cox regression analyses of survival.

	Survival		
Risk factor	*P*-value	Hazard ratio	95% CI
Univariate analysis			
Donor age	0.332	1.00	0.99; 1.02
Female recipient	0.069	1.50	0.97; 2.31
Ischemic diagnosis	0.945	1.01	0.70; 1.46
Ischemia time	0.204	1.00	0.99; 1.00
Transfusion	0.680	1.08	0.75; 1.55
Pregnancy	0.908	1.05	0.43; 2.59
VAD	0.772	1.04	0.82; 1.30
All devices	0.893	1.03	0.69; 1.53
HLA-A mismatch	0.549	0.92	0.71; 1.20
HLA-B mismatch	0.915	1.02	0.70; 1.48
HLA-DR mismatch	**0.023**	1.41	1.05; 1.91
Total mismatch	0.270	1.10	0.93; 1.30
PRA last	**0.004**	3.43	1.50; 7.88
PRA peak	**0.028**	2.18	1.09; 4.35
Anti-HLA Abs*	0.821	0.96	0.67; 1.38
DSA class I†	**0.042**	2.03	1.03; 4.00
DSA class II†	0.334	1.53	0.65; 3.61
Multiple analysis 1			
PRA last	**0.056**	2.69	0.97; 7.44
DSA class I†	0.176	1.66	0.79; 3.48
Multiple analysis 2			
DSA class I†	**0.055**	1.95	0.98; 3.88
HLA-DR Mismatch	0.526	1.16	0.73; 1.84
Multiple analysis 3			
PRA last	1.12	3.04	0.32; 29.26
PRA peak	**0.036**	1.04	0.14; 7.71
DSA class I†	0.431	1.54	0.72; 3.28
HLA-DR mismatch	0.256	1.29	0.79; 2.10

VAD, ventricular assist device; All devices include ventricular assist device, pacemaker, intra-aortic balloon pump; HLA, human leukocyte antigen; Total Mismatch includes HLA-A/-B/-DR; PRA, panel reactive antibody; DSA, donor-specific antibody.
*Screened with Luminex LABScreen®Mixed.
†Screened with Luminex LABScreen®Single Antigen.
Significant *P*-values are indicated in bold.

Kaplan–Meier analysis was 96% at 1 year and 86% at 5 years. No pretransplant anti-HLA Ab assay was able to predict CAV. No risk factor correlated with CAV aside from the primary heart disease: ischemic disease increased the risk by 1.8 times compared with nonischemic cardiopathy ($P = 0.025$).

Graft function

The mean EF 1 year after transplantation was 65% (±7). There was no difference between the sensitized and nonsensitized group (0.238) or between the DSA+ and DSA− group ($P = 0.423$). Univariable Cox regression demonstrated that a lower EF was associated with decreased survival ($P = 0.023$).

Discussion

In recent years, highly sensitive detection methods for anti-HLA Abs have been introduced to clinical routine, but their significance in heart transplantation is unclear. This study analyzed the predictive value of SPA performed on pretransplant sera for survival in 272 heart transplant recipients. The main findings were: (i) screening with SPA-Screen does not predict survival, this in contrast to the conventional CDC screening assay; (ii) pretransplant DSA class I predicted a decreased short-term survival with a four times higher sensitivity than CDC PRA; (iii) for patients surviving the first 2 years, the presence of pretransplant anti-HLA Abs had no influence on acute or chronic rejection and survival long-term survival.

CDC screening and crossmatch represent the current practice for pretransplant screening of heart transplant recipients. A positive PRA determined by CDC Lymphoscreeen was highly specific (98.8%) for early death, as previously described [15]. However, with a sensitivity of only 9%, it has a critical limitation by missing over 90% of the patients at high risk for early death. On the other hand, SPA-Screen, which detected 10 times more sensitized patients, seemed to be too sensitive leading to a large loss in specificity and no more predictive value. In contrast, DSA class I had a four times higher sensitivity compared with CDC screening with only a minor drop in specificity from 99% to 89%. Therefore, our study suggests that combining both screening tests – CDC screening with its high specificity and SPA-Screen with its higher sensitivity – allows more accurate pretransplant risk assessment of sensitized heart transplant recipients. Based on these tests, four groups of patients can be distinguished: (i) highest caution is warranted for patients with a positive PRA *and* DSA class I, as most of them (4/5) died in the first days after heart transplantation; (ii) caution is also warranted for patients with a negative PRA but positive for DSA class I. Special caution is necessary for MFI values over 4000: 7/9 patients (4 with a negative PRA) in our study died early; (iii) no special caution for patients with a positive PRA but negative in DSA class I: This combination may be a consequence of antibodies directed against non donor-type HLA molecules, against non-HLA determinants or of IgM autoreactive antibodies. SAB can determine the exact specificity of anti-HLA antibodies and neither detects non-HLA nor IgM antibodies, which are not deleterious to either graft survival or function [16]. In our study, those patients had a similar outcome as those with a negative CDC and SPA result; (iv) no special caution for patients with a negative PRA and no DSA class I. However, we would like to emphasize that these conclusions are only valid for patients transplanted with a negative CDC crossmatch, which has been a strict rule in our center for the last twenty years.

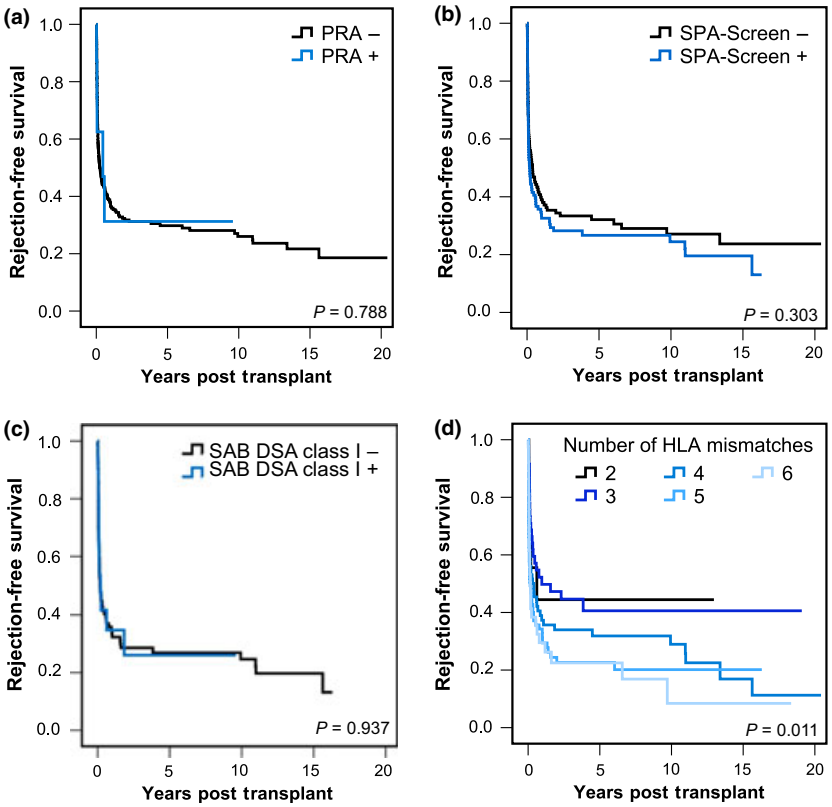

Figure 5 Freedom from acute cellular rejection after heart transplantation. Comparison of CDC screening (a), SPA-Screen (b), DSA class I (c) and number of HLA mismatches (d).

Few previous studies have investigated the significance of pretransplant DSA detected by SPA on short-term outcome: Smith *et al.* [12] detected a decreased survival in the first 500 days with 19 DSA-positive patients out of 565 heart transplant recipients. In a study of 63 patients, a correlation between DSA status and AMR was shown [17]. Another study of 144 heart transplant recipients observed decreased survival, more frequent acute rejection and CAV [18]. Irving *et al.* [19] showed an increased graft loss in the DSA+ group ($n = 4$) out of 59 pediatric patients. In contrast to our study, Gandhi *et al.* [20] found higher incidences of AMR and cellular rejection in 11 patients with DSA. However, none of these studies distinguished between DSA directed against class I or class II molecules. The finding that in particular DSA class I are relevant for early survival has only been demonstrated for kidney transplantation [21]. Two studies found a correlation between C1q-binding and the risk of AMR. We were not able to analyze this aspect in more detail because of the lack of C4d staining in biopsies and because of the low number of patients with C1q-binding Abs [22,23].

In heart transplantation, no previous study has analyzed the impact of pretransplant DSA on long-term outcome. Our finding that patients with DSA do well on the long-term is reassuring. It means that these patients need to be watched more closely in the early period post-transplant and surveillance biopsies should be screened for AMR (C3d/C4d staining [24]) in order to intervene rapidly. However, after this first critical phase, these patients seem not to be confronted more often with acute cellular and chronic rejection under stable immunosuppression. Previous studies reported a correlation between CAV and antibody status [25,26], but we cannot support this finding for the presence of pretransplant DSA. However, we still suggest close clinical and Ab monitoring of such patients at risk and, in case of graft dysfunction, evaluation for chronic AMR by biopsy.

Limitations

Our study has several inherent limitations. A first limitation is the absence of a systematic immunohistochemical analysis of surveillance biopsies for AMR, because of missing routine pathologic C4d examination. However, an association of DSA with early AMR is likely and may be the reason for the limited survival of DSA-positive patients [17].

A second limitation is the occurrence of false positive tests with the SPA-Screen because of the low cutoff level.

Table 3. Comparison of the incidence of acute cellular rejection episodes (ISHLT classification).

		CDC assay – Lymphoscreen®			Solid phase assay – Luminex®		
Variable	All* (n = 213)	PRA– (n = 209)	PRA+ (n = 4)	P value	HLA Abs– (n = 118)	HLA Abs+ (n = 95)	P value
Max. severity†, %							
First year	39/59/2	39/59/2	50/50/0	0.885	42/56/2	36/62/2	0.617
Follow-up	31/67/2	31/68/2	50/50/0	0.694	34/64/2	27/71/2	0.587
No ≥2R, mean (SD)							
First year	1.4 (±1.6)	1.4 (±1.6)	0.5 (±0.6)	0.235	1.4 (±1.6)	1.4 (±1.5)	0.357
Follow-up	2.2 (±2.2)	2.2 (±2.2)	1.3 (±1.9)	0.398	2.2 (±2.4)	2.2 (±2.1)	0.680
		Single antigen bead - Luminex®					
Variable	All* (n = 213)	DSA– (n = 77)	DSA+ (n = 18)	P value	DSA class I– (n = 82)	DSA class I+ (n = 13)	P value
Max. severity†, %							
First year	39/59/2	36/61/3	33/67/0	0.749	25/63/2	39/61/0	0.840
Follow-up	31/67/2	29/68/3	22/78/0	0.654	27/71/2	31/69/0	0.825
No ≥2R, mean (SD)							
First year	1.4 (±1.6)	1.4 (±1.5)	1.3 (±1.4)	0.781	1.5 (±1.5)	1.2 (±1.4)	0.620
Follow-up	2.2 (±2.2)	2.1 (±2.0)	2.2 (±2.3)	0.865	2.2 (±2.1)	1.8 (±1.8)	0.478

ISHLT, International Society for Heart and Lung Transplantation; CDC, complement-dependent cytotoxicity; PRA, panel reactive antibody; HLA, human leukocyte antigen; Abs, antibodies; DSA, donor-specific antibody.
*Only patients considered with a positive 1-year survival.
†ISHLT 1R/2R/3R.

However, from a clinical view, these false positive results were accurately indentified by SAB and would therefore not have influenced clinical decision-making. More worrying is the hidden false negative group, which would probably be treated differently. For detection of the false negative rate, it would be necessary to test all serum samples primarily with SAB instead of SPA-Screen, which in our study was not possible because of the high cost of the SAB.

A third limitation is a limited statement of the correlation of CDC crossmatch with DSA and its strength, because only very few patients with a positive CDC crossmatch were included. In a previous study in kidney transplantation, the highest DSA strength was measured in patients with a positive B-cell crossmatch [27]. Another study demonstrated that in the presence of DSA, a positive B-cell crossmatch was associated with a poorer graft outcome in renal transplantation [28]. Thus, we suggest using SAB for enhancing CDC crossmatch interpretation instead of replacing it. In general, we would like to underline the fact that our conclusions are only valid for heart transplant recipients receiving Thymoglobulin® and may differ in patients not receiving this type of induction therapy.

A fourth limitation is the lack of post-transplant anti-HLA Ab monitoring in our patients, which would allow to identify the role of persistent versus transient DSA positivity for long-term survival and occurrence of CAV. The relevance of post-transplant monitoring has clearly been demonstrated in a recent study by Ho *et al.* [29]. However, post-transplant DSA monitoring in our center was started only recently, and therefore no data are available in this respect, yet.

Conclusion

Detection of SAB DSA class I in pretransplant serum is a strong predictor of short-term, but not long-term survival and may help in the early management of heart transplant patients, particularly for deciding on type of immunosuppression and on the use of pre-emptive plasmapheresis.

Authorship

MR: performed study and wrote the paper. GF: collected data and designed study. MR: statistical analysis. BR: performed study and analyzed data. MJW, GN and FR: analyzed data and critical reading. TF: designed study and wrote the paper. FE: designed study and analyzed data.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. C1q Assay.

Table S2. Causes of death.

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