Pretransplant Single Antigen Bead–Detected HLA Antibodies in Kidney Transplant Long-term Outcome: A Single-Center Cohort Experience

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ABSTRACT

Single-antigen bead (SAB) platform permits the identification of antibodies not detectable by complement-dependent lymphocytotoxicity test, but their clinical significance is not completely understood. The aim of this study was to evaluate whether the presence of pretransplant SAB-detected antibodies is associated with the development of allograft failure.

This is a single-center cohort study with 10-year follow-up in which 573 kidney recipients with negative pretransplant complement-dependent lymphocytotoxicity crossmatch who received transplants at the Kidney Transplant Center of Policlinico, Milan, from deceased donors between 1996 and 2005 were evaluated. Pretransplant plasma samples were retrospectively analyzed by SAB assay. Survival analyses were performed to assess the risk of allograft failures by SAB-detected antibodies.

Pretransplant antibodies were found in 160 (28.0%) recipients, of whom 42 subsequently developed an allograft failure for a survival rate of 70.9% (95% confidence interval [CI], 63.5–78.4). Among those without antibodies, 58 (14.0%) returned to dialysis with a survival rate of 84.7% (95% CI, 81.0–88.4). In Cox regression analyses, patients with SAB-positivity had 2-fold higher risk of allograft failure than those who were SAB-negative (hazard ratio, 2.07; 95% CI, 1.39–2.79). Results did not change after adjustment for putative confounders. In conclusion, in this single-center cohort, 10-year allograft survival rate was significantly influenced by the presence of SAB-detected antibodies.

KIDNEY transplant is the treatment of choice for end-stage renal failure. Improvement in histocompatibility assays, surgical techniques, and immunosuppressive regimens have significantly improved the survival rates and the quality of life of adult and pediatric patients [1–3]. The availability of solid-phase immunoassays besides the complement-dependent lymphocytotoxicity (CDC) has radically changed the practice of antibody testing against HLAs in the context of organ transplantation. Although the solid-phase immunoassays performed on the single-antigen bead (SAB) platform permit the identification of antibodies not detectable by CDC, with the exception of donor-specific antibodies (DSA) that are known to be one of the most important cause of renal allograft loss [4], the clinical meaning of these non-DSA antibodies is not completely understood. The detection of these antibodies has changed the clinical management of sensitized patients [5–7], and, therefore, there is a persisting debate on which is the most appropriate policy for defining antibodies that better correlate with kidney transplant outcome. Several articles have been published in the last few years showing conflicting results [4,8–29], and laboratory and clinical experts have developed consensus guidelines regarding antibody testing

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in clinical transplantation [23]. In a previous article we presented the North Italy Transplant program policy for screening and identification of anti-HLA antibodies [15]. With this background, we followed a single-center cohort of patients who underwent kidney transplant, and we retrospectively investigated for the presence of pretransplant SAB positivity with the aim of evaluating the association of SAB-detected antibodies with allograft outcomes.

MATERIALS AND METHODS

Patient Cohort and Study Design

A total of 688 kidney recipients who received transplants at the Kidney Transplant Center at Ospedale Maggiore Policlinico, Milan, from deceased donors between January 1, 1996, and December 31, 2005, were eligible for this study. All patients, according to the North Italy Transplant program protocol, had a negative pretransplant CDC crossmatch. A total of 115 patients were excluded because pretransplant plasma samples for SAB testing were not available; therefore, the remaining 573 formed the final cohort. The SAB analyses were performed retrospectively.

Ten-year follow-up started immediately after the introduction of the immunosuppressive regimen and ended at the time of clinically documented allograft failure, after 10 years (administrative censoring), or after death, whichever came first. Allograft failure was defined as graft loss for any reason, with return to dialysis. Early allograft failure was defined as graft loss within the first year after transplant. Functional laboratory data (plasma creatinine levels and proteinuria) were collected, when available, in patients with a functioning graft at the end of follow-up.

Demographic data, medical history (including previous pregnancy and/or miscarriage, transfusions, waiting time list, previous transplant, and immunosuppressive therapies), and HLA mismatches were recorded. Immunosuppressive regimen included the induction therapy with basiliximab or antithymocyte globulin plus steroids, and the maintenance therapy with steroids, cyclosporine or tacrolimus, and mycophenolate mofetil/mycophenolic acid or azathioprine, according to local regimen. Clinically suspected and biopsy-proven rejection episodes were treated with steroid pulses and, in cases of steroid resistance and/or antibody-mediated rejection, with antithymocyte globulin and/or plasmapheresis and/or intravenous immunoglobulin and/or rituximab.

The SAB positivity was defined according to the median fluorescence intensity (MFI) level. The SAB assay was considered negative if MFI < 1000. Among the SAB-positive, intermediate (MFI between 1001–2999) and high (MFI > 3000) positivity were defined. The used MFI was the highest value of any MFI of any HLA antibodies for each patient. According to HLA class antibodies, 3 categories were also defined: class I–positive, class II–positive, and both class I– and class II–positive patients. The study was approved by the Hospital Institutional Review Board. All patients gave a written informed consent to participate the study.

Outcomes

Primary outcome was allograft survival rate. Moreover, histologically documented graft rejection and graft function parameters were evaluated, when available.

Kidney Allocation Criteria

Kidney allocation followed the NITK3 algorithm [24], which includes the following main immunologic criteria:

- ABO compatibility with the donor;
- HLA-A, -B, -DRB1 matching;
- absence of anti-HLA donor-specific cytotoxic antibodies;
- negative CDC crossmatch using the 3 most recent serum samples plus peak sera for sensitized patients;
- absence of previous donor-recipient HLA incompatibilities in case of retransplant.

The kidney allocation algorithm included the maximum historical percent panel-reactive antibodies level defined by CDC and not by SAB, as this methodology was not in use.

Laboratory Tests

Each recipient serum collected before transplant was retrospectively analyzed by SAB assay. All samples were treated as previously described [25]. The LABScreen Mixed kit (One Lambda, Canoga Park, Calif, United States), which simultaneously detects class I and class II antibodies with microbeads coated with purified class I and class II HLA antigens, was used. Results above a cutoff value of 3.0 ratio between sample and negative control were considered positive. Antibody specificity was determined with the LabScreen SAB (One Lambda). Patients were classified as positive when 1 or more antibody specificity was detected with a normalized MFI >1000.

When needed, the presence of "natural" anti-HLA antibodies (defined as antibodies reactive only with denatured HLA molecules) was excluded, as previously described [19]. No measurement of complement deposition on the beads (either C3d or C1q) was performed.

Anti-HLA antibodies were assigned by comparing antibody specificity against the mismatched donor HLA-A, -B, -C, -DRB, -DQB, and -DPB split antigens.

Histologic Evaluation

Renal biopsies were performed under ultrasound control with a 16 gauge needle in cases of (1) presence or increased proteinuria, (2) decreasing renal function defined as $> 25\%$ increased serum creatinine according to patient's basal level, and (3) clinical sign of graft dysfunction.

Tissue samples were processed following the International guidelines and examined by light microscopy and immunostaining. Immunoglobulins, complement components, light chains, and fibrinogen were assessed according to an established methodology. C4d was assessed by indirect immunohistochemistry. Histologic diagnoses were based on updated Banff '15 and grouped as normal or not according to international guidelines [26]. Biopsies were considered non-normal or normal according to the presence or absence of rejection (either antibody- or T-cell–mediated).

Statistical Analyses

Continuous variables were expressed as mean (SD) values and compared by independent t test. Categorical variables were expressed as frequencies and percentage values and compared by χ^2 test.

Kaplan-Meier survival analyses were performed to assess allograft survival rates by SAB serotype. The survival rates were compared using Cox regression survival analyses, taking into account, as covariates, CDC results; the presence and class of any DSA; the number of mismatches in HLA-A, -B, and -DR loci; recipient and donor sex; and recipient age. Donor and recipient age were considered as continuous and categorical variables. Adjustments in multivariable Cox models were made each individually, with derived confidence intervals (CIs).

Because of the occurrence of 55 deaths during the study, 2 sensitivity analyses were performed assuming that all patients who died had developed allograft failure at the truncated follow-up or had continued the study until 10 years of follow-up.

When kidney biopsies were performed, odds ratio (OR) and 95% CIs were used to assess the effect of SAB on histologic results.

Statistical analyses were performed within SPSS, version 23.0 (IBM, Armonk, NY, United States).

RESULTS

Single-Antigen Bead Results

A total of 573 patients were included in this cohort and followed for a mean period of 8.1 years (SD, 3.0; median, 10 y). According to SAB test, 413 patients were anti-HLA-negative and 160 (28.0%) were anti-HLA-positive. Out of these, 57 (35.6%) had class I, 18 (11.3%) had class II, and 85 (53.1%) had both classes of antibodies. Among SAB-positive patients, 83 (51.9%) had DSA and 77 (48.1%) had no DSA.

Table 1 shows the main characteristics of the study population according to SAB results. The SAB-positive group had a higher prevalence of female patients, a greater number of transfusions and retransplants, a longer time on dialysis, a lower degree of HLA mismatch, and a higher percentage of CDC–panel-reactive antibody–positive patients. Previous pregnancy or miscarriages were not different in the 2 groups. No significant difference was found between the 2 groups regarding the immunosuppressive protocols.

Survival Analyses

Of 573 patients, 100 (17.5%) returned to dialysis and 55 (9.6%) died during the median follow-up of 10 years. The overall 10-year survival rate was 80.9% (95% CI, 77.6–84.2).

Of the 160 who were SAB-positive, 42 patients returned to dialysis with a 10-year graft survival rate of 70.9% (95% CI, 63.5–78.4) compared with 58 of 413 SAB-negative patients with a survival rate of 84.7% (95% CI, 81.0–88.4) ($P < .001$; Fig 1A). Among SAB-positive subjects, 15 of 42 (36%) returned to dialysis within the first years for an early allograft failure of 93.1% (95% CI, 94.6–98.2), whereas 15 of 58 (26%) SAB-negative patients experienced an early allograft failure and had a survival rate of 96.4% (95% CI, 89.2–97.0) ($P < .005$). The SAB-positive patients had a 5-year survival rate of 81.1% (95% CI, 74.8–87.4) compared with SAB-negative patients with a rate of 93.1% (95% CI, 90.6–95.6) ($P < .001$). Forty-seven SAB-positive recipients had intermediate MFI antibody level and 8 developed an allograft failure with an allograft survival of 81.4% (95% CI, 69.6–93.2), whereas 34 of the 113 with a high MFI developed an allograft failure with a survival rate of 66.3% (95% CI, 56.9–75.7) (Fig 1B). Ten patients with SAB-detected class I,

Table 1. General Characteristics of the Study Population

	All Patients, No. (%) (N = 573)	SAB+, No. (%) (n = 160)	SAB− No. (%) (n = 418)	*P* Value
Transplant period				
1996–2000	282 (49.2)	87 (54.4)	195 (47.2)	
2001–2005	291 (50.8)	73 (45.6)	218 (52.8)	.12
Recipient sex, No. (%)				
Male	352 (61.4)	69 (43.1)	283 (68.5)	
Female	221 (38.6)	91 (56.9)	130 (31.5)	<.001
Recipient age, mean (SD), y	41.1 (15.9)	41.9 (14.5)	40.8 (16.4)	.42
Pregnancies/miscarriages				
Yes	128 (57.9)	57 (62.6)	71 (54.6)	.24
No	93 (42.1)	34 (37.4)	59 (45.4)	
Transfusions				
Yes	218 (38.0)	87 (54.4)	131 (31.7)	
No	355 (62.0)	73 (37.4)	282 (68.3)	<.001
Transplant, No. (%)				
1	514 (89.7)	105 (65.6)	409 (99.0)	
>1	59 (10.3)	55 (34.4)	4 (1.0)	<.001
Waiting time, mean (SD), y	3.3 (3.4)	4.3 (4.3)	2.9 (2.9)	.002
Immunosuppressive therapy				
CyA + AZA + steroid	141 (24.6)	46 (28.8)	95 (23.0)	
MMF + steroid	143 (25.0)	44 (27.5)	99 (24.0)	
TAC + MMF + steroid	138 (24.1)	30 (18.8)	108 (26.2)	
Basiliximab + any other association	151 (26.4)	40 (25.0)	111 (26.9)	.18
Donor age, mean (SD), y	41.7 (18.7)	43.6 (18.8)	40.9 (18.6)	.12
Cold ischemia time, mean (SD), h	15.7 (4.8)	15.5 (4.7)	15.7 (4.9)	.67
HLA-A, -B, -DR mismatch, mean (SD)	2.9 (1.1)	2.6 (1.1)	3.0 (1.1)	<.001

Results were reported for the overall population and according to SAB results.
Abbreviations: AZA, azathioprine; CyA, cyclosporine; MMF, mycophenolate mofetil; SAB, single-antigen bead–based screening assay; TAC, tacrolimus.

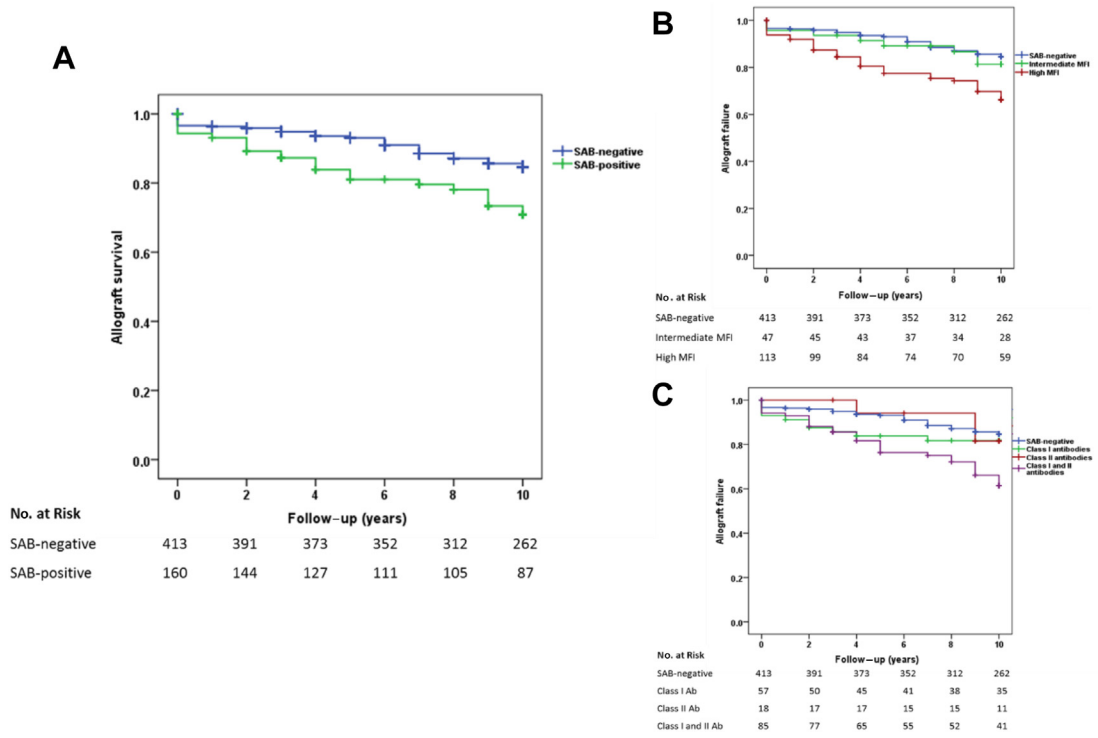

Fig 1. Kaplan-Meier survival curves for allograft survival by SAB serotype. **(A)** Allograft survival in SAB-positive and SAB-negative subjects. **(B)** Allograft survival in subjects without antibodies, with MFI 1000–2999, and MFI ≥ 3000; **(C)** Allograft survival in subjects without SAB-detected antibodies, with class I antibodies, with class II antibodies, and with both class I and class II antibodies. MFI, median fluorescence intensity; SAB, single-antigen bead.

3 with class II, and 29 with both class I and II HLA antibodies developed an allograft failure with a 10-year survival rate of 81.7% (95% CI, 71.5–91.9), 81.6% (95% CI, 53.0–94.0), and 61.4% (95% CI, 50.2–72.6) ($P < .001$), respectively (Fig 1C). Furthermore, 10-year survival rates were analyzed by locus-specific anti-HLA antibodies, and no differences were found in absolute and relative risk when compared with the absence of the locus-specific antibodies (data not shown).

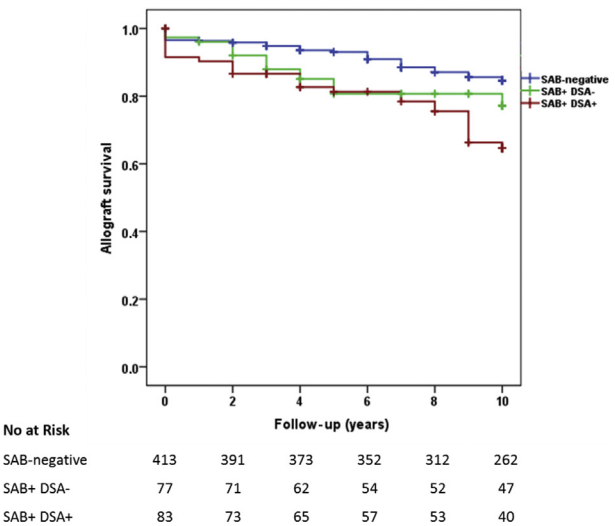

Fig 2. Kaplan-Meier survival curves for allograft survival by SAB and DSA serotype; subjects were categorized in SAB-negative, SAB-positive DSA-negative, and SAB-positive DSA-positive. DSA, donor-specific antibody; SAB, single-antigen bead.

To assess if the effect of the SAB results was influenced by the presence of DSA, the 160 SAB-positive patients were divided in 2 groups according to the presence of DSA. Of the 77 DSA-negative patients, 16 developed an allograft failure with a survival rate of 77.3% (95% CI, 67.5–87.1), whereas of 83 DSA-positive patients, 26 returned to dialysis with a survival rate of 64.7% (95% CI, 53.5–74.9) (Fig 2) ($P < .001$).

The risks of graft failure are shown in Table 2. Overall, SAB positivity was associated with a 2-fold higher risk of graft failure (hazard ratio [HR], 2.07; 95% CI, 1.39–3.08). The presence of any antibody with the highest MFI <3000 had no effect on allograft failure compared with those without antibodies (HR, 1.20; 95% CI, 0.57–2.50), whereas patients with any antibodies with MFI ≥ 3000 had 2-fold higher risk (HR, 2.46; 95% CI, 1.61–3.76) of allograft failure. Furthermore, subjects with both class I and II antibodies had an almost 3-fold higher risk compared with those without any antibodies (HR, 2.80; 95% CI, 1.79–4.37). The SAB-positive DSA-negative patients had a 60% increased risk of allograft failure (HR, 1.60; 95% CI, 0.92–2.79)

Table 2. Cox Regression for Allograft Survival by SAB Serotype and Other Potential Determinants Category

	Hazard Ratio	95% CI
Luminex-detected antibodies		
No	1.0	
Yes	2.07*	1.39–3.08*
MFI		
No antibodies	1.0	
MFI 1000–3000	1.23	0.59–2.57
MFI $\geq$ 3000	2.46*	1.61–3.76*
Luminex-detected antibodies class specificity		
No	1.0	
Class I HLA	1.37	0.70–2.68
Class II HLA	1.14	0.36–3.62
Class I and II HLA	2.80*	1.79–4.37*
DSA		
No	1.0	
Yes	2.31*	1.48–3.61*
Luminex vs DSA		
Luminex-negative	1.0	
Luminex-positive DSA-negative	1.60*	0.92–2.79*
Luminex-positive DSA-positive	2.52*	1.58–4.00*
CDC		
Negative	1.0	
Positive	2.16*	1.44–1.24*
Donor-specific antibodies		
No	1.0	
Yes	2.31*	1.48–3.61*
Class I DSA		
No	1.0	
Yes	2.18*	1.28–3.72*
Class II DSA		
No	1.0	
Yes	2.65*	1.48–4.75*
Class I and II DSA		
No	1.0	
Yes	3.62*	1.59–8.27*
HLA-A mismatch		
0	1.0	
1	0.97	0.62–1.52
2	1.20	0.67–2.15
HLA-B mismatch		
0	1.0	
1	0.96	0.53–1.74
2	1.0	0.54–1.86
HLA-DR mismatch		
0	1.0	
1	0.85	0.55–1.32
2	0.67	0.35–1.33
Donor age		
Continuous variable	1.02	1.01–1.03
Donor age categories, y		
15–59	1.0	
<15	1.08	0.54–2.17
>59	1.75*	1.13–2.73*
Recipient age		
Continuous variable	1.01	1.01–1.02
Recipient age categories, y		
18–59	1.0	
<18	0.73	0.37–1.46
>59	1.08	0.54–2.16
Sex		
Female	1.0	
Male	0.84	0.56–1.24
Immunosuppression therapy		
CyA + AZA + steroid	1.0	
MMF + steroid	1.18	0.71–1.97
TAC + MMF + steroid	0.64	0.35–1.17
Basiliximab + any other association	0.77	0.44–1.35

Hazard ratio compares the risk of allograft failure among those with SAB positivity before transplant vs those without. Furthermore, the effect of presence of other potential determinants was investigated vs the absence.

Abbreviations: AZA, azathioprine; CI, confidence interval; CyA, cyclosporine; DSA, donor-specific antibody; MFI, median fluorescence intensity; MMF, mycophenolate mofetil; SAB, single-antigen bead; TAC, tacrolimus.

*Statistically significant results.

compared with SAB-negative patients, whereas DSA-positive patients had an almost 3-fold higher risk (HR, 2.52; 95% CI, 1.58–4.00) compared with SAB-negative patients. Moreover, allograft failure was significantly influenced by a donor age older than 59 years, the presence of CDC-positive antibodies, and SAB-DSA positivity mostly when patients had both class I and II SAB-DSA. Mismatches in any HLA class, age of recipient, sex, and immunosuppression therapy had no effect on allograft failure.

After adjustment for the presence of DSA, the effect of SAB was not reversed with a favorable trend. This effect did not change after adjustment of any other putative confounders (Table 3).

Furthermore, early allograft failure was investigated by SAB positivity (HR, 1.90; 95% CI, 0.87–4.14). This effect disappeared after adjustment for DSA (HR, 1.07; 95% CI, 0.31–3.71).

Finally, 55 subjects died before completing the study; therefore, 2 sensitivity analyses were performed to exclude any potential effect on the main result. The first analysis assumed that all patients who died had developed an allograft failure at the truncated follow-up instead of dying. The second one assumed that all patients who died had been followed for 10 years. The effect of SAB on allograft survival did not change and was 1.86 (95% CI, 1.34–2.57) and 1.65 (95% CI, 1.20–2.28), respectively.

Histologic Results

During post-transplant follow-up, 253 patients underwent histologic evaluation on clinical indication. Among all the biopsied patients, 171 (67.6%) were SAB-negative and 82 (32.4%) were SAB-positive. Histologic evidence of rejection was found in 96 (38%) biopsy results, of which humoral and cellular rejection was present in 25 (9.9%) and 71 (28%), respectively.

The distribution of total, humoral, and cellular rejections by SAB is reported in Table 4. Compared with SAB-negative patients, SAB-positive patients had an increased risk of 68%

Table 3. Adjusted Analyses for Allograft Survival

Adjustment Variable	Hazard Ratio	95% CI
Crude*	2.07	1.39–3.08
DSA	1.60	0.92–2.79
HLA-A mm	2.12	1.42–3.17
HLA-B mm	2.08	1.40–3.11
HLA-DR mm	2.03	1.36–3.03
Age donor class	2.00	1.34–2.97
Age recipient class	2.05	1.38–3.05
Sex	2.07	1.37–3.12

Adjustments in multivariate Cox models were each performed individually.
Abbreviations: CI, confidence interval; DSA, donor-specific antibody; mm, mismatch.
*Unadjusted model.

(OR, 1.68; 95% CI, 0.98–2.88) of developing a histologic rejection. The OR increased to 5.33 (95% CI, 2.19–13.9) when the specific risk of humoral rejection was investigated by the presence of SAB. No difference was found in the risk of cellular rejection (OR, 0.55; 95% CI, 0.46–1.51).

Graft Function Outcomes

The creatinine levels at the end of follow-up were available in 358 patients. The mean creatinine level was 1.76 (SD, 1.04) mg/dL. The SAB-positive patients had a mean creatinine level of 1.86 (SD, 1.03) mg/dL, whereas those who were SAB-negative had a mean creatinine level of 1.72 (SD, 1.03) mg/dL ($P = .29$). Urinary protein levels were available in 269 patients with a mean proteinuria level of 0.30 (SD, 0.08) mg/dL. The SAB-positive patients had a mean proteinuria level of 0.33 (SD, 0.40) mg/dL, whereas the SAB-negative patients had a mean of 0.29 (SD, 0.23) mg/dL ($P = .32$).

DISCUSSION

In this single-center cohort of patients who underwent kidney transplant with negative crossmatch results, the prevalence of pretransplant antibodies detected by SAB was 28%, which was mainly associated with female sex and number of previous transfusions and transplants. Ten-year allograft survival rate was significantly influenced by the presence of pretransplant SAB-detected anti-HLA antibodies with a 2-fold higher risk of allograft failure. The survival rate was significantly lower when antibodies with the MFI value > 3000 were present and when both class I and II HLA antibodies were detected. The finding regarding differences in allograft outcomes according to anti-HLA classes and MFI results should be interpreted with caution because of the small size of some subgroups. These results were supported by the humoral histologic documented rejection when kidney biopsies were available. Finally, early allograft failure was influenced by SAB-detected antibodies, but the effect is mostly explained by the concomitant presence of DSA. Thus, our study confirmed the detrimental effect of preformed DSA in the early phase after transplant, as reported in previous studies [8–14].

Table 4. Histologic Results by SAB Serotype

	SAB-Negative	SAB-Positive	*P* Value*
Rejection, No. (%)			
No	113 (66.0)	44 (53.7)	
Yes	58 (34.0)	38 (46.3)	.06
Humoral, No. (%)			
No	163 (95.3)	65 (79.3)	
Yes	8 (4.7)	17 (20.7)	<.001
Cellular, No. (%)			
No	121 (70.8)	61 (74.4)	
Yes	50 (29.2)	21 (25.6)	.55

Data on rejection and humoral and cellular rejections were reported according to SAB results.
Abbreviation: SAB, single-antigen bead.
**P* value is the comparison of SAB-positive patients with SAB-negative patients.

In the last decade, several studies showed a relationship between SAB-detected DSA and impaired graft outcome [8–14], but this observation was not confirmed by others [16–22]. Those contradictory results may be explained by different sample sizes, follow-up duration, study design (multicenter vs single-center cohorts, cross-sectional vs cohort study), and by technical issues related to solid-phase antibody detection assays [23,25–29]. Furthermore, only the relationship between DSA and graft outcome was investigated, without taking into account the non-DSA antibodies. Quite recently, a large single-center cohort study of 837 patients with a long but inhomogeneous follow-up confirmed an increased risk of allograft failure in recipients with pretransplant DSA, without finding an effect of pretransplant non-DSA [12]. Interestingly, looking at the Kaplan-Meier curves reported in the previously mentioned article, subjects with non-DSA had a similar graft survival compared with patients with DSA, censoring the follow-up at 10 years. Secondly, kidney biopsy results were not evaluated.

To our knowledge, this is one of the largest single center cohort study with a homogeneous long-term follow-up in which all consecutive patients were included. This may avoid any selection bias regarding surgical procedure, organ allocation, and immunosuppressive regimen. Moreover, the centralization of SAB testing should have avoided a misclassification in antibody status. The retrospective design offers the unique advantage of collection data from a large cohort of patients with a long follow-up, which is a goal hardly achievable with a prospective design; on the other hand, missing data or errors are obviously more probable in retrospective studies than in prospective studies. However, these limitations are minimized in this study because most of the data were accurately collected, and all patients were strictly followed-up throughout their lifetime according to the protocol carried out in most transplant centers worldwide.

Some limitations need to be addressed. First, of 688 eligible patients, 115 were excluded. Although it was possible that this created a spurious effect, the only reason for exclusion was the absence of pretransplant blood samples, and that is not related to the outcome. Second,

because 55 patients died before completing 10 years of follow-up, 2 sensitivity analyses were performed, and no differences from the overall findings were found. Another limitation was considering the serum most approximate to kidney transplant, which caught only a limited picture of the alloimmunization at a single time point. No scheduled information regarding the alloimmunization status after transplant was available, and this made it difficult to draw any strong causative conclusion on SAB role.

Finally, the results regarding histologic findings and functional tests should be interpreted with caution because selection bias were likely and cannot be excluded.

In conclusion, this study supports the evidence that preformed SAB-detected antibodies were a risk factor for kidney loss both in the immediate post-transplant period and in the long term, mainly when both class I and II were detected, when MFI >3000, and when associated with DSA.

The identification of SAB-detected antibodies may be an additional marker for allograft failure, identifying those patients with a high immunologic risk. This finding needs to be validated in further studies and, once confirmed, may contribute to improved prediction scores, which in turn may lead to individualized interventions tailored to reduce the risk of allograft failure.

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