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ORIGINAL ARTICLE

Retrospective Cohort Study

Impact of preformed donor-specific antibodies against HLA class Ⅰ on kidney graft outcomes: Comparative analysis of exclusively anti-Cw *vs* anti-A and/or -B antibodies

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Abstract

AIM

To analyze the clinical impact of preformed antiHLA-Cw *vs* antiHLA-A and/or -B donor-specific antibodies (DSA) in kidney transplantation.

METHODS

Retrospective study, comparing 12 patients transplanted with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B.

RESULTS

One year after transplantation there were no differences

in terms of acute rejection between the two groups (3 and 6 cases, respectively in the DSA-Cw and the DSA-A-B groups; *P* = 1). At one year, eGFR was not significantly different between groups (median 59 mL/min in DSA-Cw group, compared to median 51 mL/min in DSA-A-B group, *P* = 0.192). Moreover, kidney graft survival was similar between groups at 5-years (100% in DSA-Cw group *vs* 91% in DSA-A-B group, *P* = 0.528). The sole independent predictor of antibody mediated rejection (AMR) incidence was DSA strength (HR = 1.07 per 1000 increase in MFI, *P* = 0.034). AMR was associated with shortened graft survival at 5-years, with 75% and 100% grafts surviving in patients with or without AMR, respectively (Log-rank *P* = 0.005).

CONCLUSION

Our data indicate that DSA-Cw are associated with an identical risk of AMR and impact on graft function in comparison with "classical" class I DSA.

Key words: Donor-specific antibodies; Antibody-mediated rejection; Anti human leukocyte antigen class Ⅰ; AntiHLA-Cw antibodies; Graft survival; Solid-phase immunoassays

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Core tip: The clinical importance of preformed antiHLA-Cw donor-specific antibodies (DSA) in kidney transplant patients remains controversial, so we performed a retrospective study comparing 12 patients with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B. Antibody-mediated rejection occurrence and graft survival frequency, respectively, at one and at five years of follow-up, were comparable between groups. Our data support a similar deleterious impact considering DSA-Cw or DSA-A/-B in terms of risk of AMR and impact on graft function.

Santos S, Malheiro J, Tafulo S, Dias L, Carmo R, Sampaio S, Costa M, Campos A, Pedroso S, Almeida M, Martins LS, Henriques C, Cabrita A. Impact of preformed donor-specific antibodies against HLA class Ⅰ on kidney graft outcomes: Comparative analysis of exclusively anti-Cw *vs* anti-A and/or -B antibodies. *World J Transplant* 2016; 6(4): 689-696 Available from: URL: http://www.wjgnet.com/2220-3230/full/v6/i4/689.htm DOI: http://dx.doi.org/10.5500/wjt.v6.i4.689

INTRODUCTION

In kidney transplantation the presence of preexisting anti human leukocyte antigen (HLA) donor-specific antibodies (DSA) has impact on graft outcomes. Their presence is associated with an augmented risk of antibody-mediated rejection (AMR)[1] and worst graft survival[2].

Classically, antibodies against major HLA Class Ⅰ (A and B) and Class Ⅱ (DR and DQ) antigens are considered to be responsible for most cases of AMR. AntiHLA-Cw are considered less immunogenic when are paralleled to other class I antiHLA antibodies, mainly due to minor HLA-Cw antigen expression on cell surface[3]. Indeed, some studies found that the incidence of antiHLA-Cw antibodies in sensitized patients was lesser than that for HLA-A or HLA-B antibodies[4-6].

However, the progress of additional sensitive assays that identify HLA antibodies, namely solid-phase immunoassays, demonstrated that HLA-C locus may induce an antibody reaction comparable to the other usually tested *loci*[4,5,7,8]. In 2012, Ling *et al*[5] showed that kidney transplantation in patients with isolated antiHLA-Cw antibodies was effective (no rejections occurred) when using induction treatment with anti-thymocyte globulin (ATG) and IVIG. Another study evaluated 22 patients with pretransplant DSA antiHLA-Cw in comparison with 88 patients allosensitized but with no detectable preformed DSA and concluded that they seem to be at superior risk for AMR occurrence[9]. Recently, Bachelet *et al*[10] in their retrospective and multicenter study showed that antiHLA-Cw DSA have the same effect on graft outcome as DSA against "classical" HLA *loci* (A, B, DR, DQ), suggesting that antiHLA-Cw should also be considered in transplant allocation procedures and in immunologic risk stratification of patients.

As this subject remains controversial, we decided to conduct a retrospective study in kidney transplant patients to investigate the clinical impact of preformed antiHLA-Cw DSA comparing them to DSA against the other HLA class I loci, namely antiHLA-A and/or B.

MATERIALS AND METHODS

Patients

From the database of our Histocompatibility Center 35 adults who received a kidney transplant since 2007 were identified as having pretransplant donor specific antibodies (DSA) exclusively antiHLA-A and/or -B or exclusively antiHLA-Cw. Twenty-three patients had DSA antiHLA-A and/or antiHLA-B: 6 with DSA antiHLA-A only; 11 with DSA antiHLA-B only and 6 with DSA antiHLA-A and -B. This group was designated DSA-A-B. Twelve patients had DSA exclusively antiHLA-Cw, and this group was designated DSA-Cw. The patients were all transplanted with a negative T- and B-cell cytotoxic crossmatch (standard NIH technique). The Institutional Review Board at Hospital Santo António, CHP approved this study.

AntiHLA antibody testing

Patients in the waiting list were examined for antiHLA IgG by multiplex microsphere based on Luminex X-map® Technology (LABScreen® Mixed kit, OneLambda, Canoga Park, CA, United States). The cut-off for positive samples was the Normalized Background (NBG) ratio advocated by the manufacturer and executed by the HLA fusion® software (One Lambda Inc.). To determinate the specificity of the HLA antibodies, single-antigen bead (SAB) assays (LabScreen Single Antigen Beads®, OneLambda, Canoga Park, CA) were executed in patients with a positive

screening, using the same pretransplant sera. The mean fluorescence intensity (MFI) was measured using LABScan ™ 100 flow analyzer (Luminex®, Austin, TX, United States). The analysis was performed using HLA fusion® software (One Lambda Inc.) and a cut-off for a positive reaction were set in MFI value of ⩾ 1000.

Donor typing and crossmatch

Samples of all deceased donors were routinely typed before recipient selection in loci HLA-A*, B*, Cw* and DRB1* using polymerase chain reaction (PCR) amplification with specific sequence primers (SSP; Olerup SSP® low resolution HLA typing kits, Stockholm, Sweden). After donor HLA typing, using that information, a virtual crossmatch (virtual XM) was executed. The strength of each single DSA was based on the MFI of one SAB. In the case of several DSA against different HLA-antigens, we considered the cumulative strength of all DSA by adding the individual MFI values.

Immunosuppression

Thirty-three of the total of 35 patients (94.3%) received induction therapy: Ten patients with a monoclonal antibody anti-IL-2 receptor (Basiliximab Novartis®, 20 mg twice at day 0 and 4), and 23 patients with polyclonal ATG Fresenius® (3 mg/kg for 5-7 d). All patients had an equivalent maintenance immunosuppression using three oral drugs: A calcineurin inhibitor [tacrolimus (FK-506) in the majority of patients (32/35 patients) or cyclosporine (CsA) in 3 patients], mycophenolate mofetil (MMF) and a corticosteroid. FK-506 was started at a dose of 0.1-0.15 mg/kg per day, and was adjusted to maintain levels between 8 and 12 ng/mL during the first month post-transplant, between 7 and 10 ng/mL the next 2-3 mo and between 5 and 8 ng/mL thereafter. MMF was started at a dose of 2000 mg/d, and decreased based on white blood cells count. Methylprednisolone was administered intravenously at doses of 500, 250 and 125 mg/d on the day of transplantation, days 1-2 and days 3-4 after the operation, respectively. Oral prednisolone was started on day 5 after the operation at the dose of 20 mg, being then tapered to 5-10 mg/d within 2-3 mo after transplant. Living donor recipients (n = 3) were prescribed FK-506 and MMF 7 d before transplant.

Eight patients underwent a desensitization protocol. Five patients received intravenous immunoglobulin (IvIg) 2 g/kg at transplant (0.5 g/kg immediately before transplant, and at day 1, 2 and 3) and 1-mo after transplant (1 g/kg in 2 consecutive days). One patient received a similar dose of IvIg and underwent plasmapheresis every other day (first session immediately before transplant, for a total of 6-9 sessions) and two other patients received additionally a dose of Rituximab (375 mg/m^2) on day 3 post-transplant.

Patients' data and outcomes

The data concerning patients' characteristics and transplantation variables was collected retrospectively. Estimated glomerular filtration rate (eGFR) was assessed using the 2006 Modification of Diet in Renal Disease (MDRD) equation and dialysis requirement in the first week post-transplant was defined as delayed graft function. Patients were followed until graft failure, death or end of follow-up (five years after transplant or December 31, 2015, which came first). Graft survival was evaluated considering graft failure censored for death with a functioning graft.

Follow-up

Graft biopsies were performed "for cause" only. Allograft rejection was classified according Banff classification (updated in 2013) and defined by biopsy where specimens were evaluated by light microscopy and immunofluorescence (with C4d staining). Mild acute cellular rejection (ACR Banff grade Ⅰ) was treated with 500 mg methylprednisolone for 3 d and increased maintenance immunosuppression. All other ACR were treated with ATG. AMR patients were treated with plasmapheresis every other day (the number of plasmapheresis sessions was 4 per protocol) and IvIg 100 mg/kg after each session. After the last plasmapheresis session, they received a high-dose IvIg (2 g/kg) divided in four daily doses and the same dose was repeated 1 mo later. If not used at transplant, patients received, additionally, one dose of rituximab (375 mg/m^2).

Statistical analysis

Categorical data were expressed as numbers (frequencies) and continuous data were described using median (interquartile range). Categorical data (demographic and medical characteristics) were compared using Pearson χ^2 test or Fisher's exact test, as appropriate. Continuous variables were compared with Mann-Whitney U test. Predictors of AMR were explored by univariate and multivariable (using a backward elimination method, with a P-value < 0.05 necessary for retention in the model) Cox regression. For graft survival curves was used the Kaplan-Meier method, and the comparison between groups was done by log-rank test.

RESULTS

Baseline characteristics

Baseline characteristics of DSA-Cw and DSA-A-B groups are given in Table 1. DSA-Cw patients tended to be younger compared to patients in DSA-A-B group (respectively, 39 years *vs* 48 years), (P = 0.061). There was no significant difference between groups concerning gender, history of previous transplant or previous pregnancies. However DSA-Cw patients had significantly higher prevalence of previous blood transfusions (75% *vs* 39%, P = 0.044).

Concerning donor characteristics and pretransplant immunological data, namely donor age, donor gender, type of donor transplant (living *vs* decease), peak PRA, and DSA number, none of these characteristics significantly differed between groups. Although DSA strength median was higher in DSA-A-B (MFI 7583) in comparison with DSA-Cw group (MFI 2939), this difference was not

Table 1 Baseline characteristics of donor-specific antibodies-Cw and donor-specific antibodies-A-B groups

	DSA-A-B *n* = 23	DSA-Cw *n* = 12	*P*
Recipient			
Age (yr), median (IQR)	48 (39-55)	39 (33-49)	0.061
Female gender, *n* (%)	13 (57)	6 (50)	0.713
Retransplant, *n* (%)	11 (48)	5 (42)	0.728
Previous blood transfusions, *n* (%)	9 (39)	9 (75)	0.044
Previous pregnancies, *n* (%)	8 (35)	8 (33)	1
Kidney-pancreas transplantation, *n* (%)	1 (4)	1 (8)	1
Donor			
Age (yr), median (IQR)	45 (36-56)	45 (32-54)	0.542
Female gender, *n* (%)	8 (35)	8 (33)	1
Living donor, *n* (%)	1 (4)	2 (17)	0.266
Pretransplant immunological data			
Peak PRA, median (IQR)	4 (0-80)	8 (0-52)	0.472
DSA number, median (range)	1 (1-3)	1 (1-2)	0.056
DSAsum MFI, median (IQR)	7583 (2320-12395)	2939 (2529-3650)	0.11
Transplant			
ABDR HLA mismatches, mean ± SD	3.22 ± 1.28	4.08 ± 1.16	0.056
FCXM-T + (*n* = 29), *n* (%)	1 (6)	3 (27)	0.139
FCXM-B + (*n* = 29), *n* (%)	2 (11)	0	0.512
ATG induction, *n* (%)	14 (61)	9 (75)	0.476
Tacrolimus (*vs* CsA), *n* (%)	20 (87)	12 (100)	0.536
Desensitized, *n* (%)	5 (22)	3 (25)	1
IvIg only, *n*	2	3	
IvIg + PP, *n*	1	0	
IvIg + Rtx + PP, *n*	2	0	

DSA: Donor-specific antibodies; MFI: Mean fluorescence intensity; IQR: Interquartile range; SD: Standard deviation; CKD: Chronic kidney disease; PRA: Panel reactive antibodies; HCV: Hepatitis C virus; CMV: Cytomegalovirus; HLA: Human leukocyte antigen; ATG: Anti-thymocyte globulin; CsA: Cyclosporin; IvIg: Intravenous immunoglobulin; PP: Plasmapheresis; Rtx: Rituximab.

Table 2 Clinical outcomes and follow-up

	DSA-A-B *n* = 23	DSA-Cw *n* = 12	*P*
Delayed graft function, *n* (%)	7 (30)	1 (8)	0.216
Acute rejection at 1-yr, *n* (%)	6 (26)	3 (25)	1
AMR at 1-yr, *n* (%)	6 (26)	2 (17)	0.685
ACR-only at 1-yr, *n* (%)	0	1 (8)	0.343
1 yr-eGFR (mL/min), median (IQR)	51 (46-60)	59 (47-64)	0.192
1 yr-ProtU, median (IQR)	0 (0-0.1)	0.1 (0-0.2)	0.163
Censored graft failure, *n* (%)	2 (9)	0	0.536
Follow-up time (mo), median (IQR) [range]	60 (45-60) [28-60]	18 (11-50) [3-60]	0.001

DSA: Donor-specific antibodies; AMR: Acute antibody-mediated rejection; ACR: Acute cellular rejection; eGFR: Estimated glomerular filtration rate; IQR: Interquartile range; ProtU: Proteinuria.

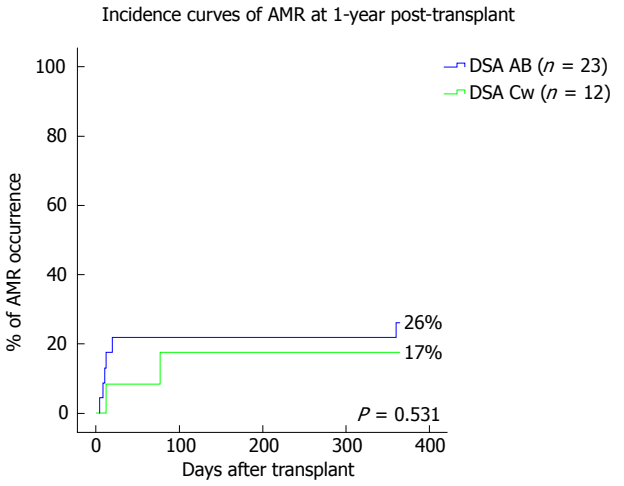

Figure 1 Incidence curves of antibody-mediated rejection at 1-year post-transplant. AMR: Antibody-mediated rejection; DSA: Donor-specific antibodies.

significant (P = 0.110).

Flow cytometry crossmatch (FCXM) was performed for 29 of 35 patients. Positive T- and/or B- cell FCXM was similarly uncommon between groups. Three (27%) patients had a positive T-cell FCXM in the DSA-Cw group and only one (6%) in the DSA-A-B group (P = 0.139). Only two patients had a positive B-cell FCXM and both belonged to the DSA-A-B group.

Immunosuppression and induction treatment were similar between groups. ATG induction was used in 14 (61%) and 9 (75%) patients from the DSA-A-B and DSA-Cw groups, respectively (P = 0.476). Additionally, 5 patients in the DSA-A-B group were desensitized: 2 of them using only IVIG, 1 with IVIG and plasmapheresis and another 2 combining IVIG, plasmapheresis and rituximab. In DSA-Cw group 3 patients were treated with IVIG.

Clinical outcomes

Transplant outcomes are detailed in Table 2. There was no difference in terms of acute rejection at one year between the two groups (6 and 3 cases, respectively in the DSA-A-B and the DSA-Cw groups; P = 1). All cases of acute rejection were diagnosed as AMR in the DSA-A-B group, while in the DSA-Cw group there were 2 cases of AMR and 1 of ACR. Figure 1 shows the incidence of AMR at one-year post-transplant, between DSA-A-B and DSA-Cw patients groups, (respectively, 26% and 17%, Log-rank P = 0.531) with no significant difference being detected. At one year, eGFR tended to be higher in DSA-Cw group (median 59 mL/min) compared to DSA-A-B group (median 51 mL/min), (P = 0.192) (Figure 2). Importantly, follow-up was significantly longer for the DSA-A-B group (median 60 mo) than in the DSA-Cw group (median 18 mo) (P < 0.001). Kidney graft survival at 5-years was also similar between groups (Figure 3, 91% for the DSA-A-B group *vs* 100% for the DSA-Cw group, P = 0.528).

Antibody-mediated rejection: Incidence, predictors and clinical impact

AMR occurred in 8 patients (23%) of the overall cohort. Possible associations between clinical and immunological data and AMR incidence through a Cox regression analysis is shown in Table 3. The sole independent predictor of AMR incidence was the DSA strength, both in uni- and multi-

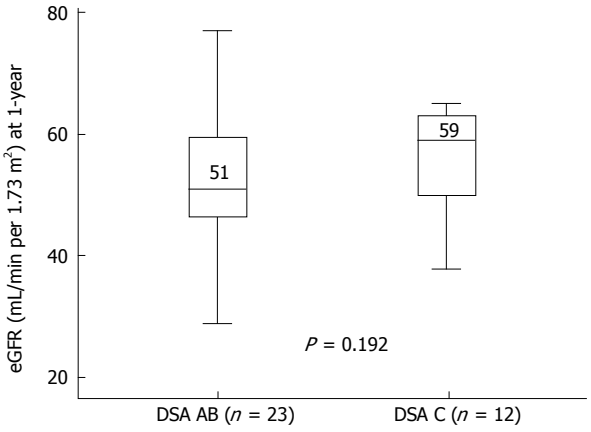

Figure 2 Graft function (estimated glomerular filtration rate at 1-year) post-transplantation according to donor-specific antibodies human leukocyte antigen loci. Boxes show the interquartile range of the values (median and percentile 25-75); whiskers show the lowest and the highest value within 1.5 times below or above the interquartile range, respectively. DSA: Donor-specific antibodies; eGFR: Estimated glomerular filtration rate.

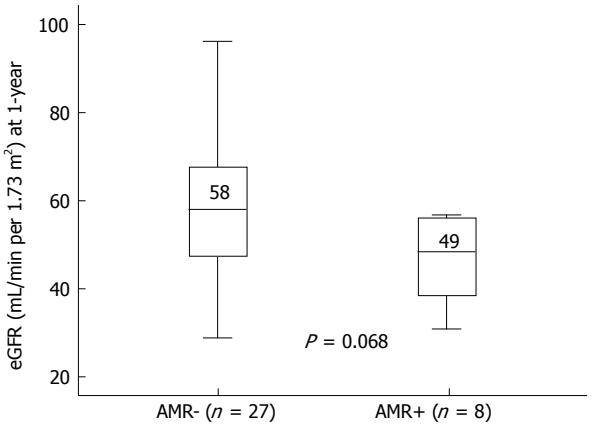

Figure 4 Graft function (estimated glomerular filtration rate at 1-year) post-transplantation according to antibody-mediated rejection occurrence. Boxes show the interquartile range of the values (median and percentile 25-75); whiskers show the lowest and the highest value within 1.5 times below or above the interquartile range, respectively. AMR: Antibody-mediated rejection; eGFR: Estimated glomerular filtration rate.

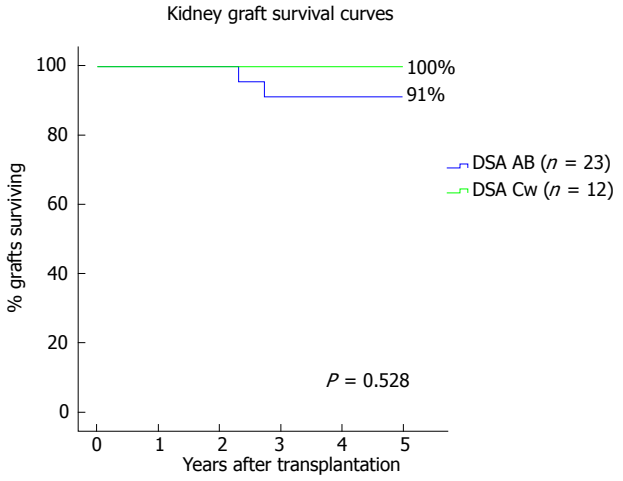

Figure 3 Kidney graft survival curves according with donor-specific antibodies human leukocyte antigen *loci*. DSA: Donor-specific antibodies.

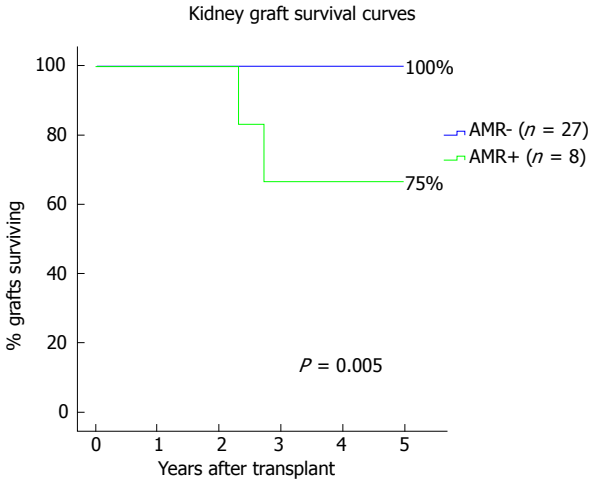

Figure 5 Kidney graft survival curves according with antibody-mediated rejection occurrence. AMR: Antibody-mediated rejection.

variable analysis (HR = 1.07 per 1000 increase in MFI, P = 0.034). At 1-year, eGFR was lower in AMR$^+$ (median 49 mL/min) in comparison with AMR$^-$ patients (median 58 mL/min) (P = 0.068), as shown in Figure 4. At the end of follow-up, kidney graft survival (Figure 5) was 75% in patients that experienced AMR and 100% in those who did not (Log-rank P = 0.005).

DISCUSSION

This retrospective study demonstrates that patients with preformed DSA solely antiHLA-Cw had a similar impact on post-transplant outcomes comparing to those patients with preformed antiHLA-A/-B DSA. Both groups had a relative high incidence of AMR at one year, 26% in the DSA-A-B group and 25% in DSA-Cw group. Also, the impact on graft outcomes measured by eGFR at one-year and graft survival at the end of follow-up was comparable between groups.

HLA-Cw molecules are scantily expressed at the cell surface compared with HLA-A and HLA-B *locus* products, but intracellular HLA-A, HLA-B and HLA-Cw alleles are expressed in similar quantities[3,11]. One reason pointed for this low amount at the cell surface is the fact that HLA-Cw alleles interact in a very stable way with the transporter associated with antigen processing (TAP) and they are kept in the endoplasmic reticulum, where they are degraded[11]. Another justification for finding low HLA-Cw at cell proposed by McCutcheon *et al*[3] is that HLA-Cw heavy chain mRNA is instable and rapidly degraded, resulting in a lower rate of protein. This fact, associated with the modest sensitivity of the lymphocytotocicity-based assays used in the past for identification of HLA-Cw antigens, probably explains why for many years they were considered less immunogenic and neglected in the matching systems of most kidney allocation procedures.

Table 3 Analysis of possible predictors of acute antibody-mediated rejection occurrence by univariable Cox regression

	HR for AMR	95%CI	*P*
Recipient			
Age (yr), per year	0.96	0.89-1.03	0.269
Female (*vs* male) gender	0.26	0.05-1.26	0.094
Retransplant	2.18	0.52-9.13	0.287
Previous blood transfusions	0.5	0.12-2.10	0.345
Previous pregnancies	0.24	0.03-1.99	0.187
Donor			
Age (yr), per year	1.01	0.96-1.06	0.684
Living donor	1.79	0.22-14.76	0.588
Pretransplant immunological data			
Peak PRA, per unit	1.01	1.00-1.03	0.149
DSA Cw (*vs* AB)	0.6	0.12-2.99	0.537
DSAsum MFI, per 1000[1]	1.07	1.01-1.15	0.034
Transplant			
ABDR HLA mismatches, per unit	0.84	0.50-1.41	0.512
ATG (*vs* basiliximab) induction	1.68	0.34-8.34	0.527
FCXM + (n = 29)	0.75	0.09-6.21	0.787
Desensitized	1.2	0.24-5.97	0.825
Delayed graft function	2.55	0.61-10.68	0.201

[1]Only independent predictor identified by multivariable Cox regression model (all variables included) using backward elimination (P-value < 0.050 needed for retention in the model). DSA: Donor-specific antibodies; AMR: Acute antibody-mediated rejection; MFI: Mean fluorescence intensity; ATG: Anti-thymocyte globulin; FCXM: Flow cytometry crossmatch.

Recent studies confirm their lower frequency. Bryan *et al*[6] in 2010 described in their sensitized transplant patients a 42% positivity to HLA-Cw, which was significantly lesser than sensitization to HLA-A (80%) and HLA-B (83%). In 2012, Ling *et al*[5], obtained similar results and showed that the frequency of antiHLA-Cw antibodies in sensitized patients was about 56%, lower than HLA-A (79%) and B (86%) antibodies. Our group evaluated 453 sensitized kidney transplantation candidates to determine the presence of antiHLA class Ⅰ and class Ⅱ antibodies, comparing how different sensitization events, such as pregnancy, transfusion or previous organ transplantation, affected the degree of HLA alloimmunization[12]. For antiHLA antibodies against class Ⅰ, if the sensitization event was previous transplant only, the antiHLA antibodies prevalence was 21.2% for -A, 28.8% for -B and 21.1% for -Cw; if the single sensitization event was previous transfusion, the antiHLA antibodies prevalence was 3.9% for -A, 5.5% for -B and 1.6% for -Cw. At last, if the sensitization event was pregnancy only, the antiHLA antibodies prevalence was 13.6% for -A, 11.1% for -B and 6.2% for -Cw.

In spite of their lower frequency, some reports have been published concerning their association with AMR and impact on graft function and survival[8,13,14]. Besides, the recent development of the solid-phase immunoassays, in particular the single-antigen flow bead (SAFB) assays, allowed us to detect and properly identify anti-HLA-Cw antibodies. Tambur *et al*[15] compared virtual flow-cytometry cross-match to actual cross-match and described that 40% of the cases with a positive actual flow-cytometry cross-match and negative virtual cross-match were explained by the presence of antiHLA-Cw antibodies. Gilbert *et al*[7] compared two groups of sensitized recipients, one group with only classical HLA-A, -B, -DR, -DQ antibodies (n = 176) and the other group with classical plus HLA-C and/or -DP antibodies (n = 27). They concluded that there was a significant increase in the number of AMR among the group with pre-transplant anti-Cw and -DP antibodies. However, they did not distinguish between pre-transplant anti-DP or anti-Cw antibodies, and they speculated that anti-DP antibodies seemed to be involved more often in poorer graft outcomes. Ling *et al*[5] investigated the clinical outcomes in kidney transplant patients with isolated Cw-DSA. They identified eight patients with pre-transplant DSA antiHLA-Cw, exclusively. During a median 6 mo of follow-up (range 3-24 mo), patient and graft survival was 100% without any acute rejection occurring. In this group, all the patients had induction therapy with thymoglobulin or basiliximab and additionally all patients received intravenous immunoglobulin, similar to patients with positive FCXM and/or cPRA > 50%. Even so, the median time of follow up was relatively short and may have underestimated the incidence of rejection. Aubert *et al*[9] evaluated retrospectively 22 renal transplant recipients with isolated antiHLA-Cw DSA at day 0 of renal transplant, comparing them with 88 allosensitized patients with no preformed DSA (control group), and followed for a period of 1 year. Acute AMR was diagnosed in six patients (27.3%) in patients with DSA-Cw *vs* 9% in those without DSA. In this study, the patients with DSA antiHLA-Cw received less-intensive immunosuppression than the control group of sensitized patients, including ATG induction (only 59.1%), and this may probably be a plausible explanation for this high rate of AMR. However they alert for the necessity of screening pre-transplant DSA HLA-Cw and subsequent modulation of immunosuppression in cases of positivity. More recently, Bachelet *el al*[10] investigated the clinical effect of DSA antiHLA-Cw and/or -DP, comparing 48 patients transplanted with isolated preformed DSA antiHLA-Cw and/or -DP with a group of HLA-sensitized recipients with no DSA (104 patients) and 47 kidney transplant recipients with preformed DSA antiHLA-A, -B, -DR, and/or -DQ. Two years after transplantation, the groups with DSA (both -Cw/-DP or -A/-B/-DR/-DQ) had similar incidence of AMR and graft survival (and worse than the group with no DSA), showing that preformed DSA anti-HLA-Cw and/or -DP were as deleterious as DSA anti-HLA -A/-B/-DR/-DQ.

Our data reached similar results of these previous studies, confirming that DSA-Cw is associated with a similar incidence of AMR and impact on graft survival in comparison with "classical" DSA against class Ⅰ [9,10].

We have also shown that patients that experienced AMR had a significant lower kidney graft survival in comparison to patients who did not (respectively, 75% *vs* 100%, Log-rank P = 0.005), with the sole independent predictor of AMR incidence being DSA strength. The negative impact of DSA for AMR occurrence and adverse results on kidney graft

survival has been previously established[2]. Lefaucheur *et al*[16] stated that it is the occurrence of AMR associated with DSA that has impact on graft survival, since graft survival of DSA-positive patients, in the absence of AMR, is the same as DSA-negative patients. Furthermore, DSA characteristics as number, class or strength may have a negative impact on graft outcomes[1,17-19]. Malheiro *et al*[20] showed that DSA strength (MFI) had a reasonable ability to predict AMR occurrence, with no cases of AMR occurring below a MFI < 3000. However when the MFI values increased from this value, also did the risk of AMR. Again, Aubert *et al*[9] in their retrospective study with 22 renal transplant recipients with preformed isolated antiHLA-Cw DSA, showed that the level of DSA at day 0 was predictive for AMR: Measurement of MFI was 4966 (978-17941) in the AMR group and 981 (530-8012) in the group of patients without AMR (P = 0.017).

This study has limitations. First, the small number of patients in the cohort limits our ability to generalize the results. Second, follow-up time difference may have limited the comparative analysis of graft survival according with DSA HLA loci. Contrarily, AMR incidence was not influenced by it, since it was analyzed at 1-year post-transplant. Third, there was no protocol biopsies performed in our patients and it is an important tool for HLA incompatible kidney transplantation[21,22]. Lastly, the limitations of SAB assay are well established and their reported MFI values should be considered for analyzing our results[23].

In summary, our data show that preformed DSA antiHLA-Cw exerts a deleterious effect in presensitized kidney transplant recipients that is similar when compared to antiHLA antibodies against other class I locus (antiHLA-A or -B). Also, the association between AMR occurrence and reduced graft survival is clear, with DSA strength being predictive of rejection. Therefore, HLA-C typing and respective antibody identification will benefit sensitized patients during organ allocation.

COMMENTS

Background

Classically, antibodies to major human leukocyte antigen (HLA) Class I (A and B) and Class Ⅱ (DR and DQ) antigens are considered to be responsible for most cases of AMR. Compared to other class Ⅰ antiHLA antibodies, antiHLA-Cw are considered less immunogenic.

Research frontiers

Preformed antiHLA-Cw donor-specific antibodies (DSA) seem to have the same impact on graft outcome as DSA against "classical" HLA *loci* (-A, -B, -DR and -DQ), suggesting that it should also be considered in transplant allocation systems and in immunologic risk stratification algorithms.

Innovations and breakthroughs

The clinical relevance of preformed antiHLA-Cw DSA in kidney transplant patients remains controversial, so the authors performed a retrospective study comparing 12 patients with DSA exclusively antiHLA-Cw with 23 patients with preformed DSA antiHLA-A and/or B. Antibody-mediated rejection occurrence and graft survival rates, respectively, at 1 and at 5-years of follow-up, were comparable between groups.

Applications

The data show that preformed DSA antiHLA-Cw exerts a deleterious effect in presensitized kidney transplant recipients that is similar when compared to antiHLA antibodies against other class Ⅰ locus (antiHLA-A or -B). Also, the association between AMR occurrence and reduced graft survival is clear, with DSA strength being predictive of rejection.

Terminology

HLA: Human leukocyte antigen; DSA: Donor-specific antibodies; AMR: Antibody-mediated rejection.

Peer-review

The topic is very interesting. The authors investigated the possible role of preformed donor-specific antibodies against HLA antigens, specially anti-Cw antibodies compared to standard anti A/B antibodies. The importance of Cw antibodies is still under investigation and this study is valuable about this topic. This article is worthwhile for publication.

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