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Donor-specific antibodies development in renal living-donor receptors: Effect of a single cohort

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Abstract

Minimization in immunosuppression could contribute to the appearance the donor-specific HLA antibodies (DSA) and graft failure. The objective was to compare the incidence of DSA in renal transplantation (RT) in recipients with immunosuppression with and without steroids. A prospective cohort from March 1st, 2013 to March 1st, 2014 and follow-up (1 year), ended in March 2015, was performed in living donor renal transplant (LDRT) recipients with immunosuppression and early steroid withdrawal (ESW) and compared with a control cohort (CC) of patients with steroid-sustained immunosuppression. All patients were negative cross-matched and for DSA pre-transplant. The regression model was used to associate the development of DSA antibodies and acute rejection (AR) in subjects with immunosuppressive regimens with and without steroids. Seventy-seven patients were included (30 ESW and 47 CC). The positivity of DSA class I (13% vs 2%; $P < 0.05$) and class II (17% vs 4%, $P = 0.06$) antibodies were higher in ESW versus CC. The ESW tended to predict DSA class II (RR 5.7; CI (0.93–34.5, $P = 0.06$). T-cell mediated rejection presented in 80% of patients with DSA class I ($P = 0.07$), and 86% with DSA II ($P = 0.03$), and was associated with DSA class II, (RR 7.23; CI (1.2–44), $P = 0.03$). ESW could favor the positivity of DSA. A most strictly monitoring the DSA is necessary for the early stages of the transplant to clarify the relationship between T-cell mediated rejection and DSA.

Keywords

acute rejection, donor-specific antibodies, immunosuppression, renal transplantation

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Introduction

The leading causes of graft loss in renal transplantation (RT) are due to recipient death (with functional allograft), and chronic graft dysfunction. Chronic graft dysfunction is multifactorial and it's associated with histopathological changes, as interstitial fibrosis and tubular atrophy (IFTA), these changes are attributed to acute rejection (AR) or adverse effects of immunosuppressors, among others factors.[1–4] In the past two decades, steroids withdrawal/avoidance has been used in the immediate or late post-transplant period to reduce complications associated to their use.[5–16] Despite some meta-analyses demonstrated a greater risk of AR with this intervention, nevertheless the majority shown mild or no-impact on renal function/allograft survival.[17–20] One of the clinical concern related to minimization/avoidance of immunosuppression is the development of donor-specific antibodies (DSA), during the post-transplant evolution. The DSA is associated with antibody-mediated rejection, and worsening in allograft function and survival.[4,21–29]

It is postulated that avoiding or withdrawing steroids in the post-transplant period, can promote the appearance of antibodies against HLA and/or other antigens from the donated kidney given the mechanism of suppression of antibodies by the B lymphocyte with the use of steroids, however, information regarding this issue is scarce and not conclusive.[30–32] Therefore, the aim of this study was to evaluate the development of DSA in RT recipients with early steroid withdrawal (ESW).

Patients and methods

A prospective cohort was performed (División de Trasplantes del Hospital de Especialidades, Centro Médico Nacional de Occidente; Instituto Mexicano del Seguro Social) patients were included from March 1st, 2013 to March 1st, 2014 and follow-up (1 year), ended in March 2015.

All subjects were >16 years old, recipients of a first graft from a living-donor. The exposed cohort was those with ESW patients, who received steroids only in the first five post-transplant days and later withdrawn, this protocol has been used in our center as a clinical practice since one decade.[15,16] ESW decision were responsible by Nephrologist according to clinical criteria. The control cohort (CC), included those patients with immunosuppression based on steroids throughout the post-transplant period (without suspension at any time). All received immunosuppressive scheme based on tacrolimus (TAC), mycophenolate mofetil (MMF). Nephrologists also decided the type of induction (thymoglobulin or basiliximab) and maintenance immunosuppression with or without steroids. Around three-quarters of patients had transfusion history, but all had negative results from cross-matching (flow cytometry) in the pre-transplant to determine sensibilization absence.

Sample size was calculated using a formula to determine risk factors[33] and were necessary 30 patients per group; in ESW group were included 30 patients and in control cohort 47 were included.

During follow-up, renal allograft biopsies reports were collected from medical chart. All biopsies were performed for medical indication and evaluated by the same pathologist using the Banff's 2017 histopathology classification[34] and were done among the third month to the end of the follow-up. Events that could confuse DSA development as pregnancy was recorded (none female case, get pregnant during the follow-up). Graft function was estimated with the Modification of Diet in Renal Disease (MDRD) formula.

Immunosuppression characteristics

Induction was based on thymoglobulin at a dose of 0.5 to 1 mg/kg/day (accumulated dosage 3–4 mg/kg) or basiliximab 20 mg at 0 and 4 days post-transplant. Maintenance immunosuppression was based on, MMF 2 g/day, TAC 0.1–0.2 mg/kg to achieve blood levels in days 1–30 post-transplant among 9–15 ng/mL, and since days 31–365 post-transplant to achieve 8–10 ng/mL. Prednisone (PDN) dose was 1 mg/kg/day starting from transplantation, and was adjusted to 50% in the first month and 75% reduction in the second month and finally to achieve 5 mg per day in the third month for the control cohort.[35] The withdrawal scheme of prednisone was as follows; day 0, methylprednisolone (MPD) 500 mg, day 1, MPD 250 mg, day 2, MPD 125 mg, day 3, MPD 60 mg, day 4, MPD 30 mg, and day 5, steroids were suspended.

Determination donor-specific anti-HLA antibodies

In all patients, donor-specific HLA antibodies were determined pre-transplant and at the end of follow-up (12 months), using the Luminex methodology

(LABScreen® single antigen HLA class I-combi y LABScreen® single antigen HLA class II, genprobe transplant diagnosis inc.). Biopsies and antibodies were not determined at the same time. Antibodies were considered positive when mean fluorescence intensity (MFI) >500 units (arbitrary cut-off point).

Statistical analysis

Data are presented as mean ± standard deviation or median (percentiles 25–75%), numbers, and percentages, as appropriate. Student *t* and Chi^2 test were used to compare groups. The regression model was used to associate the development of DSA antibodies and AR in subjects with immunosuppressive regimens with and without steroids. Statistical analysis was performed with SPSS™ software, version 17 (SPSS, Inc., Chicago, IL).

The results were considered significant with a value of $P \leqslant 0.05$.

Ethical considerations. All patients signed the informed consent previous to renal transplant and the study was evaluated and approved by the local Ethics and Research Committee with registration number: (R-2013-1301-91).

The study did not receive private and/or government funding.

Results

Demographic and transplant data are shown in Table 1. There were no differences between groups in age, donor gender, type and dialysis vintage, and HLA compatibility *(none has identical HLA)*. Patients of the ESW cohort were a majority male, and all received a graft from living related donors, compared with the control cohort (CC) ($P=0.017$). Pre-transplant blood transfusions were considerably high. However, the number of transfusions was not different between groups, and sensibilization was absent in both cohorts. Induction therapy was significantly different among the ESW. The ESW had most commonly basiliximab use (97%), whereas in the CC was close to half to half.

The majority of patients had at least one graft biopsy during the follow-up; 54 patients had one, 19 patients, two, and in one patient had three graft biopsies. Acute rejection was not different among the cohorts, and renal function was similar at baseline and at the end of the follow-up. The number of subjects with eGFR below 60 mL/min was not different between cohorts (Table 1).

Table 1. Comparison of socio-demographic and transplant data.

	ESW	CC
	n – 30	*n* – 47
Recipient age (years)	26.2 ± 8.4	27.7 ± 10.1
Recipient gender—male, *n* (%)	26 (87)*	31 (66)*
Donor age (years)	36 ± 10.5	34 ± 11.5
Donor gender—male, *n* (%)	11 (37)**	24 (51)
Type of donor (%)		
Living related donor	30 (100)	39 (83)
Living unrelated donor	0 (0)*	8 (17)*
History of transfusions (%)	22 (73)	28 (60)
Number of transfusions (*n*)	1 (0–2.3)	1 (0–3)
Dialysis vintage (months)	25 (18–31)	24 (16–38)
Type of dialysis (%)	47/50/3	53/43/4
HD/PD/pre-dialysis		
Cold ischemia (min)	52 ± 27	53 ± 27
Warm ischemia (min)	1.8 ± 1.3	2 ± 3.3
Compatibility of HLA antigens	4.3 ± 1.8	3.5 ± 1.8
Class I	2.2 ± 1.3*	1.6 ± 1.0
Class II	2.2 ± 1.0	2.0 ± 1.0
Induction immunosuppression, *n* (%)		
Thymoglobulin	1 (3)*	24 (51)
Basiliximab	29 (97)*	23 (49)
Graft biopsies during follow-up	30 (100)	45 (96)
Acute rejection	9 (31)	16 (36)
Graft function		
eGFR mL/min/1.73 m^2 (baseline)	5.7 ± 3.6	5.4 ± 2.6
eGFR mL/min/1.73 m^2 (follow-up)	77.6 ± 18.1	70.5 ± 20.9
eGFR below 60/mL/min	6 (20)	15 (33)

ESW: early steroid withdrawal; CC: control cohort; HD: hemodialysis; PD: peritoneal dialysis; LRD: living related donor; LURD: living unrelated donor; HLA: human leukocyte antigens; GFR: glomerular filtration rate.
*$P<0.05$. **$P=0.22$.

Post-transplant development of antibodies

DSA class I development was significantly higher and trend to be in class II, in the ESW cohort compared to the CC. The regression model showed that the AR could be associated with the positivity of de novo class II DSA (Table 2).

Only one patient of the ESW had DSA positivity concurrently for both classes. The DSA were directed mainly to antigen B of class I and DQ of class II in the ESW.

Association of DSA with the presence and severity of AR

The AR mainly T-cell mediated rejection was not different between groups; ESW 9/30 (30%) front

Table 2. Frequency of donor-specific antibodies (DSA) post-transplant.

Group	ESW (%)	CC (%)	*P*
DSA I	4/30 (13)*	1/47 (2)	<0.05
DSA II	5/30 (17)**	2/47 (4)	0.065

Comparison between groups.
*$P < 0.05$. **$P = 0.065$.

Table 3. Histopathological findings associated with donor specific antibodies.

BANFF classification	DSA antibodies positive ($n-12$)	
	Class I ($n-5$)	Class II ($n-7$)
Borderline	3	4
IA		1
IIA		
IB		
Toxicity + AR	1	1
Mixed AR		
Cumulative incidence of AR, *n* (%)	4/5 (80%)	6/7 (86%)*

Comparison between groups.
*$P < 0.05$.

Table 4. Acute rejection associated with donor specific antibodies and immunosuppressive scheme.

BANFF classification	DSA antibodies with AR positive $n=9$					
	ESW			CC		
	6			3		
	Class I	Class II	Class I/II	Class I	Class II	Class I/II
Borderline	2	3	1		2	
IA				1		

Comparison between groups.
$P <$ ns.

CC 16/47 (36%) ($P=0.70$). The histopathological characteristics, according to the presence of DSA they are shown in Table 3. The most common finding was borderline changes for AR in both groups.

According to the immunosuppressive regimen, five patients who were DSA antibody positive (two class I, three class II, and one for both classes) corresponded to the ESW, with borderline changes for AR, whereas three positives for DSA corresponded to de CC (two had borderline changes for AR and one T-cell mediated rejection type IA) (Table 4).

Non-difference was found between the different classes of antibodies and the different types of AR. All of the immunohistochemical stains performed in biopsies with cellular AR were negative for C4d.

Discussion

Minimization in immunosuppressive regimens have been practiced by several transplant centers to prevent undesirable effects, but there is not clarity regarding the possible repercussions on graft survival. Our group recently reported short-term results with the use of ESW in RT,[15,16] with no difference in acute rejection and graft function. However, there is still a concern if any state of sub-immunosuppression could be related to a subclinical immune response later on.

The present study shows that ESW has a tendency for the formation of DSA class II antibodies, and interestingly, the patients with T-cell mediated rejection presented the formation of such antibodies at the end of follow-up.[31]

The above is contrary to the results from Monfa et al.[36] even showed that late steroid withdrawal does not increase the risk of development antibodies with TAC and MMF.[37] Alonso-Titos et al.[38] in a study with recipients with low immunological risk in immunosuppressive regimens similar to ours (TAC + MMF) and ESW (<3 months post-transplant), did not find significant differences in the formation of DSA nor there was AR.

Similar results from Wiebe et al.[23] were reported, the authors demonstrated the presence of significantly more clinical episodes of T-cell mediated rejection (borderline, Banff IA/IB) in patients who later developed de novo DSA, compared to those who did not develop it. Logistic regression shows that the episodes of subclinical and clinical AR as associates for the positivity of de novo DSA. The majority of biopsies analyzed were recorded with borderline changes and C4d negative without the presence of clinical dysfunction at the time of the biopsy; *despite the significant difference in histopathological findings (most borderline changes), the negative result in C4d shows no immunological participation in those possible rejections.* Only one patient had histopathological characteristics of antibody-mediated rejection (Mixed AR), C4d positive in the absence of DSA. The explanation of this result is the potential participation of undetected Non-HLA antibodies[39,40] or the capacity of the allograft to absorb specific antibodies from donors, with difficulties in their detection and/or insufficient expression of antigens from the donor to

which the antibodies are directed, preventing their union and the complement activation.[22,41] Wiebe et al.[4] documented tubulitis (a marker of T-cell mediated rejection) as a strong predictor of progression of damage when the DSA were documented. Therefore the presence of antibodies may lead to mixed alloimmune injuries and require attention directed as much at T and B cells. T-cell mediated, and antibody-mediated rejection can occur concurrently in 50 to 60% of cases.[42] Zhang et al.[22] demonstrated the production of DSA in RT recipients, where 2% presented with antibody-mediated rejection, 8% with T-cell mediated rejection, and 14% with both types, with a significant correlation between the positivity of DSA and the presence of T-cell mediated and/or antibody-mediated rejection. Similar results were reported by Dieplinger et al.[21] with a greater deterioration of renal function, especially in those who were positive for both classes. DSA generated after the transplantation, (de novo antibodies), are more associated with antibody-mediated rejection. Lefaucheur et al.[43] demonstrated the incidence of antibody-mediated rejection in patients with DSA as nine times higher compared to patients without antibodies. Sub-immunosuppression (lack of adherence or minimization of immunosuppression) is documented as a possible risk factor for the formation of DSA. In relation to ESW yet, information is scarce.[31,32,36] Delgado et al.[31] in a retrospective study, showed that patients with ESW did not develop de novo anti-HLA antibodies compared to those with sustained steroids.

On the other hand, De Kort et al.[44] in a population with the withdrawal of steroids using Alemtuzumab and monotherapy with TAC, showed an increase in risk for development of DSA in the early post-transplant stage. Our study using a double immunosuppression scheme (TAC/MMF) and basiliximab showed a higher incidence of DSA (62.5%) in patients without steroids. DSA class I development was significantly higher and a non-significant trend in class II, in the ESW cohort. The clinical relevance of this finding could be the early DSA class I appearance and the possible association with antibody-mediated rejection, although other conditions can have an influence also (as their sub-class IgG1/IgG3 and complement-fixing capacity (C1q). However the trend in DSA class II, lead us to strictly monitorizing since they appear and evaluation of chronic antibody-mediated rejection.[29] Our study show that both antibodies can occur during the first year after kidney transplant and might be interesting to evaluate the effect in time.

The latter obligates to improve the evaluation of the use of immunosuppression in all patients subjected to steroid withdrawal, independently of their immunological risk.

The damaging effect of antibodies depends on their sub-class (IgG1/IgG3) and complement-fixing capacity (C1q), as well as the level of MFI. Current Guidelines[45] suggest that levels of DSA can be used to predict the risk for antibody-mediated AR. Some studies show a strong correlation between DSA positivity (levels <300) and results of graft damage.[23] Low levels of DSA can activate the memory B cells and favor the development of acute and chronic rejection. Dieplinger et al.[21] showed that an MFI >100 predicts a decrease of up to 25% of the glomerular filtration rate. We not measure sub-classes of antibodies and considered levels of MFI $\geqslant 500$ and did not find any association with the levels and damaging effects on the allograft.

Limitations of the study: The sample size could be considered as a limitation, however is a result of a mathematical calculation to discover a 30% incidence in antibodies DSA formation and the other hand, the follow-up could be considered a short-term, but the results could represent the DSA development in the first year after renal trasplant. Measurement of DSA was only baseline and at the end of the follow-up, the aim of the study was to evaluate the DSA development after 1 year of RT, and could be very interesting whether the antibodies were present/abscent before or during the AR.

In conclusion

The ESW is associated with the positivity of DSA class II in the living donor. It is necessary the most frequent and strictly monitoring of the antibodies in the early stages of the transplant to identify if the AR is associated with the DSA formation.

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Declaration of conflicting interests

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Ethics approval

Ethical approval for this study was obtained by the local Ethics and Research Committee (R-2013-1301-91). Specialties Hospital, National Western Medical Centre, Mexican Institute of Social Security, Guadalajara, Jalisco, México.

Informed consent

All patients signed the informed consent previous to renal transplant.

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