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Anti-perlecan antibodies and acute humoral rejection in hypersensitized patients without forbidden HLA specificities after kidney transplantation

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ABSTRACT

Background: The improvement in the definition of serum anti-HLA antibodies (HLA-Abs) profiles after Luminex-assay implementation in transplant patients follow-up is clear. This success has permitted the development of hypersensitized-recipient allocation and donor-paired exchange programs improving the access to transplantation. However, non-HLA Abs have been described in transplanted patients but their effect in hypersensitized transplanted recipients is unclear.

Methods: Twenty-seven HLA hypersensitized patients awaiting for kidney transplantation (KT) were studied and 11 of them were followed after KT. The HLA Abs profile was confirmed in serum by Single Antigen Luminex assay and panel reactive of antigens > 98% was achieved in all patients. Subsequently, the ability to fix complement by C1q test was also assessed. Serum non-HLA Abs before and 1 month after transplantation were measured in the 11 hypersensitized recipients.

Results: 95.2% of the hypersensitized on waiting list had concomitant serum anti-HLA and non-HLA Abs. The more frequent specificity in non-HLA Abs were found against Glutathione S-transferase theta-1 (GSST-1) (in 62%) and C-terminal fragment of perlecan (LG3) (in 52%). Four out of 11 transplanted patients presented early antibody-mediated rejection (ABMR) confirmed by biopsy and had serum anti-LG3 antibodies, two of them with concomitant anti-anti-angiotensin II type I receptor. Only one patient developed *de novo*-donor specific HLA antibodies.

Conclusions: The incidence of non-HLA antibodies in patients in the waiting list is largely underestimated. The concomitance anti-HLA and non-HLA Abs in hypersensitized patients is very common and the detection of non-HLA Abs in this population could allow to identify patients with an increased risk of humoral rejection.

1. Introduction

The anti-HLA antibodies (HLA-Abs) assays have been developed from cell-based assays to solid-phase based assays (SBA). Despite several pitfalls [1], SBA assay on Luminex technology has been implemented in histocompatibility laboratories to measure HLA Abs. The patients previously sensitized with HLA antigens by transfusions, pregnancy or previous transplants, may react against shared epitopes on HLA molecules [2,3] increasing forbidden HLA antigens and waiting time in the list for transplantation [4]. The level of sensitization is given by calculated panel reactive of antibodies (cPRA), and generally PRA above 80–90% identifies an hypersensitized patient [5].

Abbreviations: ABMR, antibody-mediated rejection; Abs, antibodies; AT1R, anti-angiotensin II type I receptor; CDC, complement-dependent-cytotoxic; cPRA, calculated panel reactive of antibodies; DSA, donor-specific anti-HLA antibodies; FC, flow cytometry; GSTT-1, Glutathione S-transferase theta-1; HLA-Abs, anti-HLA antibodies; KT, Kidney transplantation; LG3, C-terminal fragment of perlecan; PATHI, hypersensitized Spanish program; SA, Single Antigen; SBA, solid-phase based assays

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The SBA assays allow the definition of HLA Abs reactions and forbidden HLA antigens while the patient is in the waiting list. At the same time virtual crossmatch after donor typing is performed with these assays [6]. In fact, virtual crossmatch has been implemented in some countries to allocate organs for transplantation [7]. This technical improvement has permitted the development of hypersensitized patients allocation and donor-paired exchange programs improving their access to transplantation.

However, despite the fine tune anti-HLA specificities there is still a gap for patients in whom antibody-mediated rejection (ABMR) is developed and not well explained by the production of HLA Abs. An increasing incidence of non-HLA Abs with or without concomitant HLA Abs has been recently proposed [8]. The presence of non-HLA Abs was associated with worse graft function, but the role of non-HLA antibodies in rejection remains to be fully elucidated [9,10].

The incidence of non-HLA Abs in HLA hypersensitized patients is largely unknown as well as their involvement in graft outcome. In the present work, we assessed the effect of non-HLA Abs in a cohort of 11 hypersensitized renal transplant patients that could be transplanted at our center.

2. Materials and methods

A total of 27 hypersensitized patients waiting for kidney transplantation at our institution in December 2016 were recruited for the study. The study was conducted following the principles of Declaration of Helsinki and approved by the Ethics Committee from our institution (Comité Ético de Investigación Clínica de Cantabria). The patients signed the informed consent to be included in the waiting list and the sera collection is registered in ISCIII with number C.0003580. Eleven out of 27 patients were finally transplanted and the demographic, clinical and immunological variables are included in Table 1.

The HLA Abs profile and anti-MICA Abs were assessed by Luminex (LABScreenMix, One Lambda) and confirmed by Single Antigen (SA) (LABSCreenSingle Antigen, One Lambda) in ethylene-diamine-tetra-acetic acid treated sera with at least 2 assays. The cPRA was > 98% in all the patients, who were included in the hypersensitized Spanish program (PATHI) [11]. The ability to fix complement of anti-HLA Abs was assessed by C1q test (One Lambda). Besides, serum detection of Abs against non-HLA antigens was performed by Luminex in all the sera (LABScreen® Autoantibody, One Lambda). The criteria for a positive result in anti-HLA and C1q test were previously described [12]. For non-HLA Abs the cut off was set in > 10000 MFI, and negative and positive sera provided with the kit were assessed and the values obtained within ranges (the MFI values are detailed in Supplementary Table 1). Since they were not included in the Luminex assay, anti-angiotensin type 1 receptor (AT1R) antibodies were tested by ELISA, following manufacturer instructions (One Lambda). Eleven hypersensitized patients were transplanted without donor-specific anti-HLA antibodies (DSA) through PATHI program. Clinically indicated biopsy was performed and ABMR was diagnosed following Banff criteria [13,14].

Further follow-up of the transplanted hypersensitized patients included DSA monitoring by SA within the first month post-KT and non-HLA antibodies at first month post-KT.

3. Results

3.1. Non-HLA Abs in hypersensitized patients in the waiting list

The presence of non-HLA Abs was confirmed in 95.2% of hypersensitized patients on waiting list. The screening for anti-non-HLA antibodies includes a wide range of antigens that are summarized in Supplementary Table 2. Reaction against Glutathione S-transferase theta-1 (GSTT-1) was present in 61.9% of hypersensitized patients whereas 52.4% of them had anti-C-terminal fragment of perlecan (LG3) Abs. The frequency of other non-HLA Abs was lower than 50% (Supplementary Fig. 1), including anti-AT1R (19%).

3.2. HLA Abs in transplanted hypersensitized patients

The HLA Abs profiles in hypersensitized patients were confirmed while listed at least in two sera within one year before kidney transplantation. The graft was allocated following criteria of PATHI program (https://portal.ont.es) and after rigorous virtual, complement-dependent-cytotoxic (CDC) and flow cytometry (FC) crossmatches negative results. Three serum samples from each patient were studied and absence of serum donor-specific HLA Abs (DSA) was observed in all the cases within the first month after KT. However, one patient developed *de novo* DSA with biopsy-proven ABMR at day 135 after KT (Table 1), without evidence of anti-AT1R, anti-MICA or anti-LG3.

3.3. Non-HLA Abs in transplanted hypersensitized patients

A total of 2 out of 11 hypersensitized patients had anti-AT1R Abs whereas 5 out of 11 had anti-LG3 Abs before KT. The presence of non-HLA Abs was tested also at one month after transplantation, all non-HLA Abs remained at the same pretransplant MFI except those directed against LG3 (Fig. 1).

3.4. Non-HLA Abs and acute rejection in transplanted hypersensitized patients

The two patients with anti-AT1R antibodies prior KT had early graft lost, one of them with biopsy-proven ABMR and also with concomitant anti-LG3 (Table 1). The remaining two patients with early biopsy-proven ABMR, had no evidence of DSA nor anti-MICA Abs, however one presented anti-LG3 Abs before KT and the other one developed *de novo* anti-LG3 Abs at 1 month after KT. Finally, one patient was treated as ABMR based on clinical data without biopsy confirmation and with the demonstration of serum anti-MICA Abs before the transplant (Table 1).

4. Discussion

The appearance of non-HLA Abs in patients on the waiting list for KT has been identified in several unicenter studies and its incidence has been reported to be high [15]. Thus, the presence of anti- AT1R and anti-endothelin1 type A receptors (ETAR) Abs in end-stage renal disease has been reported to be 23% [16] and 47%, respectively [17], and, in both cases, their presence was associated with worse graft function one year after KT. Moreover, the AT1R Abs have been associated with rejection in KT [16–18]. Our selected cohort of hypersensitized patients with anti AT1R Abs that had early graft lost (one patient with early biopsy-proven ABMR) also showed anti-LG3 Abs. Recently, Philogene et al., described a cohort of pre-transplant hypersensitized patients with donor-specific anti-endothelial Abs based on XM-ONE [10]. To our knowledge, this is the first study assessing the variation in non-HLA Abs levels pre- and post-transplantation in hypersensitized patients.

More than 95% of hypersensitized patients had concomitantly both types HLA and non-HLA Abs. This observation claims for screening non-HLA Abs in hypersensitized patients in the waiting list for KT, as previously suggested [9,10], more specifically in those patients included in hypersentized allocation programs. The presence of non-HLA Abs would suggest the need for desensitization procedures in order to minimize a potential humoral damage in a similar way to HLA Abs, but guidelines in the treatment of patients with non-HLA Abs are still lacking.

In our cohort, the source of sensitization cannot be established because all hypersensitized transplanted patients were transfused while listed and 9 out of 11 were retransplanted, being difficult to discern the origin of non-HLA Abs.

Table 1
Demographic, clinical and immunological parameters of hypersensitized patients transplanted without forbidden HLA specificities.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11
Recipient Sex	F	M	F	M	M	F	F	F	M	M	M
Recipient Age (years)	52	63	49	51	45	50	45	61	48	30	58
Donor Sex	M	ND	M	M	M	M	M	M	ND	M	M
Donor Age (years)	49	55	53	74	69	23	62			64	24
N° of Transplant	1	3	3	2	3	3	2	3	3	2	3
N° of transfusions	2	> 2	4	3	6	> 5	0	0	> 5	0	0
N° of pregnancies/ miscarriages	3	–	0	–	–	ND	ND	ND	–	–	–
Renal disease	Diabetic nephropathy	Focal and segmentary hialinosis	Systemic Lupus erithematous	Chronic pielonephritis	Diabetic nephropathy	Intertitial nephritis /pielonephritis	Membranoprolferative GN	Polycystic disease	Vascular Renal Disease	Vascular Renal Disease	Focal and segmental hyalinesis
cPRA	> 98%	> 98%	98%	> 98%	> 98%	> 98	> 98	> 98%	> 98%	98%	99%
DSA (at 0/1/3 months)	N/N/N	N/N/N	N/N/N	N/N/N	N/N/N	N/N/P	N/N/N	N/N/N	N/N//N	N/N/N	N/N/N
Anti-MICA	N	N	N	N	**P**	N	N	N	N	N	N
Pre Tx Anti-LG3	**P**	P	N	**P**	N	N	N	**P**	N	N	N
Post Tx Anti-LG3	**P**	**P**	**P**	**P**	N	N	N	U	N	N	N
Pre Tx Anti-AT1R	N	N	**P**	**P**	N	N	N	N	N	N	N
Post Tx Anti-AT1R	N	N	N	N	N	N	N	N	N	N	N
Mismatches A	2	1	1	1	0	1	1	0	0	0	1
Mismatches B	1	2	1	0	2	0	0	1	0	1	0
Mismatches DR	1	2	2	2	1	0	0	0	1	1	1
Induction Therapy	Thymo	Thymo	Thymo	Thymo	Thymo	Thymo	Thymo	Thymo	Thymo	Thymo	Thymo
Biopsy proven ABMR	Y	Y	Y	N	NP(***)	Y	N	N	N	N	N
Time of Biopsy (days)	25	13	10	–	–	135	–	–	–	–	15
Histology*	C4d2 ptc2 g1i1mm1	C4d2 ptc2 ct2 g2ci2ct2cv1	C4d2 ptc2cv1	–	–	C4d3 ptc2 ti1 ci1 i1	–	–	–	–	i3t3
ABMR treatment	6 Pl + 6 IAs + IVIGs + RTX	Pl + IVIGs + RTX + Eculiz	Pl + IVIGs +2 RTX + 5 Eculiz	–	6 Pl + 5 IAs + IVIGs	6PL + IVIGs	–	–	–	–	–
sCr after 120 days post-transplant (mg/dL)	1.7	1.8	7.1	6.0	1.9	0.6	1.7	1.6	1.4	1.5	6.3
Proteinuria after 120 days post-transpl year (g/L)	0.14	NP	NP	0.8	0.19	NP	N	0.54	0.7	0.3	NP
Time of Follow-up (days)	240	660	0	0	240	240	240	> 240	> 240	150	240
Graft functioning	Y	Y	N	N(**)	Y	Y	Y	Y	Y	Y	N

(*) Analysed (t)(i)(v)(g)(ct)(ci)(cv)(cg)(mm)(ti)(ptcbm)(aah)(ptc)(C4d), 0 classifications are not shown.
(**) Never functioning.
(***) Patient with clinical humoral rejection but biopsy contraindicated by anticoagulant treatment.
N, Negative; ND, No data; NP, Not performed; U, undetermined; P, Positive; Pl, plasmaferesis; IA, immune absortion; IVIGs, intra venous immunoglobulins; RTX, rituximab; Eculiz, eculizumab; cPRA, calculated panel reactive of antibodies; LG3, C-terminal fragment of perlecan; MICA, major histocompatibility complex class I chain-related molecule A; AT1R, anti-angiotensin II type I receptor; DSA, donor-specific anti-HLA antibodies; ABMR, antibody-mediated rejection; BP, biopsy proven; SCr, serum creatinine; Tx, transplant.

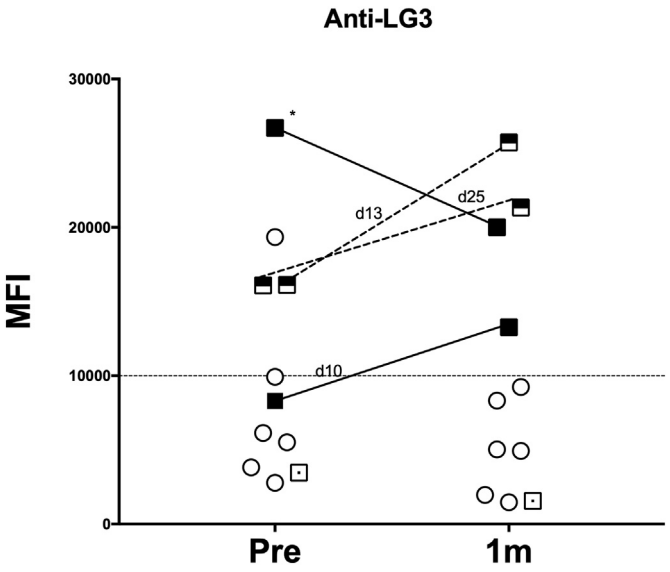

Fig. 1. Association of non-HLA antibodies with antibody-mediated acute rejection in hypersensitized patients after kidney transplantation. The levels of mean fluorescence intensity for anti-perlecan (LG3) antibodies were quantified before (pre) and after one month (1 m) of kidney transplantation. The patients with biopsy-proven antibody-mediated rejection (ABMR) (squares) and without ABMR (circles) are depicted. The patients with anti-LG3 (semi-black squares) and concomitant anti-LG3 and angiotensin II type 1 receptor antibodies (black squares) before the transplant are shown. One patient only with anti-MICA antibodies before kidney transplantation (dot in square) was treated with desensitization due to contraindication of biopsy.(*) Allograft never working. The day of biopsy is indicated in each case (d#).

The deleterious impact of preformed anti-MICA Abs has been previously reported [19]. In our cohort, the patient without biopsy-proven ABMR but with improvement of graft function after desensitization treatment, only presented anti-MICA Abs. Moreover, one patient presented anti-LG3 Abs prior KT without evidence of ABMR.

Here, we describe 5 out of 11 hypersensitized patients with negative virtual, CDC and FC crossmatches that suffered acute ABMR. They did not develop *de novo* DSA within first month of KT, but one had serum anti-MICA Abs and four had anti-LG3 Abs at first month of KT. Two of them demonstrated higher MFI levels with respect to pre-transplant serum sample and one produced *de novo* anti-LG3 Abs. These findings point to anti-LG3 Abs as inducer of vascular/ABMR rejection in these cases. LG3 Abs have been related to accelerated immune-mediated vascular injury in animal models [20] and their presence prior transplantation is associated with delayed graft function [21]. Moreover, anti-endothelial cell Abs (AECA) have been associated with poorer graft survival [22], and in a cross-sectional study the *de novo* development of AECAs after KT has been associated with allograft rejection [23]. In lung transplantation, preformed or *de novo* non-HLA Abs against K-alpha-1 tubulin and collagen V were associated with chronic rejection [24]. In liver transplantation, concomitant non-HLA and HLA Abs were associated with increased risk of decease [25].

Our results stress the importance of monitoring non-HLA Abs in hypersensitized patients while listed in order to identify potential hypersensitized patients with increased risk of early ABMR after transplantation.

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Author contribution

Participated in research design (LR, JI, ER, MLH, DSS), writing the paper (ER, JCR, MLH, DSS), performance of the research (LR, JI, JG, SG), new reagents o analytic tools (LR, JI, SG, JG), participated in data analysis (LR, JI, ER, SG, JCR, JG, ML, DSS).

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