Novel diagnostics and therapeutics to prevent injury in native and transplanted kidneys

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Chapter 5

Single antigen testing to reduce early antibody-mediated rejection risk in female recipients of a spousal donor kidney

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Abstract

Background

Female recipients of a spousal donor kidney transplant are at greater risk of donor-specific pre-immunization, which may increase the risk of acute antibody-mediated rejection (ABMR).

Methods

We assessed the incidence of early ABMR (within two weeks after transplantation), risk factors for ABMR and graft function in 352 complement-dependent cytotoxicity test-negative LURD transplant recipients, transplanted between 1997-2014 at the Leiden University Medical Center in The Netherlands. Risk factors for immunization were retrieved from the health records. As methods to screen for preformed donor-specific antibodies (pDSA) have developed through time, we retrospectively screened those with ABMR for pDSA using pooled-antigen bead (PAB) and single-antigen bead (SAB) assays.

Results

The cumulative incidence of rejection in the first six months after transplantation was 18% (TCMR 15%; early ABMR 3%). Early ABMR resulted in inferior graft survival and was more common in women who received a kidney from their spouse (10%) than in other women (2%) and men (<1%). The SAB assay retrospectively identified pDSA in seven of nine cases of early ABMR (78%), while the PAB detected pDSA in only three cases (33%).

Conclusions

Seeing that early ABMR occurred in 10% of women who received a kidney from their spouse, a SAB assay should be included in the pre-transplant assessment of this group of women, regardless of the result of the PAB assay.

Introduction

Kidney transplantation improves life expectancy and quality of life for patients with end-stage kidney disease, as compared with dialysis.[1-3] There is an ongoing shortage of deceased donor kidneys that are suitable for transplantation. This has contributed to an increase in living unrelated kidney donation (LURD).[4-6] In the Netherlands up to 60% of the annual transplants now stem from a living kidney donor. A sizeable proportion receives a kidney from a spouse.[7] This is encouraged by several studies that have documented excellent outcomes.[8,9] However, early antibody-mediated rejection (ABMR) can adversely affect outcome.[10-14] ABMR is a result of the formation of antibodies, directed against human leukocyte antigen (HLA) or non-HLA antigens of the donor. Development of ABMR during the first weeks after transplantation suggests the presence of preformed donor specific antibodies (pDSA) and/or dormant HLA specific B cell memory. It is known that women may have more pDSA from previous pregnancies[15] and that the presence of pDSA in the absence of a positive complement-dependent cytotoxicity (CDC) test results in a higher risk of acute rejection and subsequent graft loss.[16] On the other hand, not all low titer pDSA are harmful, but nevertheless may prohibit transplantation. There is no effective therapy to treat ABMR. Plasma exchange, and/or intravenous immune globulin, and glucocorticoids are considered as standard of care, though evidence for these treatment options is scarce and mainly based on small studies and expert consensus.[17]

It is paramount to optimize the pre-transplant assessment of the risk for acute ABMR in LURD. Therefore, the aim of this study was to assess the incidence of early ABMR in LURD and to identify risk factors for ABMR, in particular relevance of pDSA. Furthermore, we studied the effect of early ABMR on subsequent graft function and kidney graft loss (GL).

Materials and methods

Study design and population

This single center, observational, cohort study consisted of all LURD recipients of a blood type (ABO) compatible renal allograft at the Leiden University Medical Center (LUMC) transplanted between 1997 and 2014. The cohort consisted of 352 recipients, including 35 repeat transplants (10%). The majority (85%) had been transplanted after 2004 (*Supplementary Figure 1*). Based on the recipient-donor relationship, the population was divided into four groups: female recipients with either a spousal male donor (n=61), a non-spousal male donor (n=36) or a female donor (n=46) and male recipients (n=209) (*Figure 1*).

Clinical data was obtained from the departmental database containing information that is updated regularly and sent to the Dutch Organ Transplant Registry. This type of retrospective study with data from a registry was exempt from approval from an ethics board. The study was performed in accordance with the FEDERA Code of Conduct.[18]

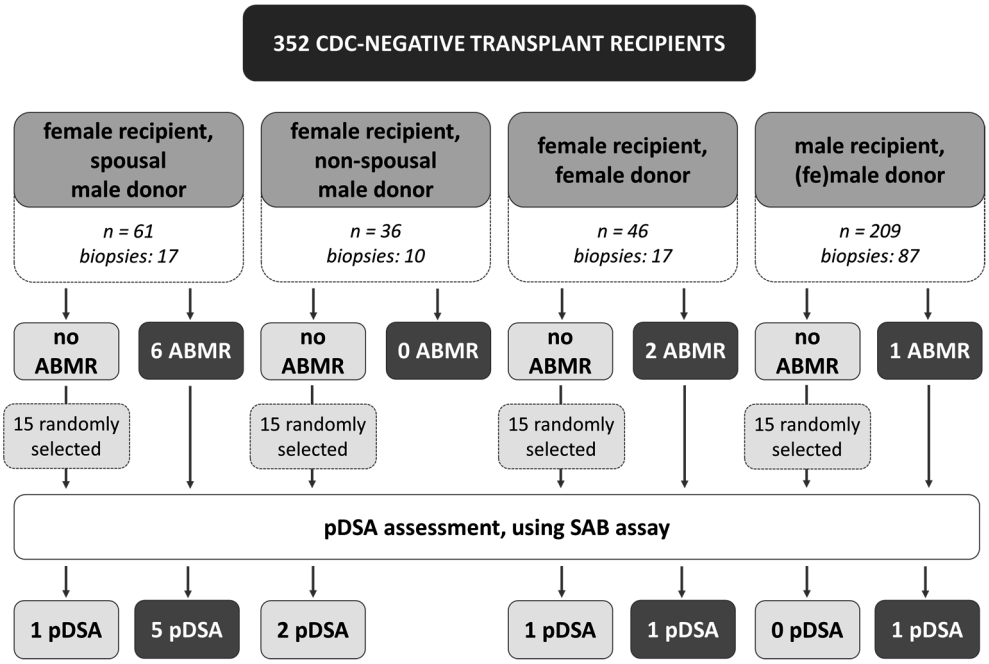

Figure 1. Schematic representation of the cohort and presence of preformed donor specific antibodies (pDSA). *The cohort, consisting of 352 renal recipients with a negative complement-dependent cytotoxicity (CDC) test, was divided into four groups; female recipient with either a spousal male donor (n=61), a non-spousal male donor (n=36) or a female donor (n=46), and male recipients (n=209). All patients with early antibody-mediated rejection (ABMR), supplemented with a randomly selected group (n=60) of patients without ABMR, were tested with a single antigen bead (SAB) assay. The randomly selected group consisted of 56 patients from the 'no rejection' group and 4 from the 'TCMR' group. All four patients with pDSA in the randomly selected group were patients from the 'no rejection' group.*

Biopsy assessment and classification of allograft rejection

All for cause biopsies taken in the first six months after transplantation were re-assessed and classified according to the BANFF 2017 classification.[19,20] Patients empirically treated for rejection without confirmation by a renal biopsy (not performed or no histopathologic changes in the biopsy) were not included in the study (n=14). All diagnoses of 'T cell-mediated rejection' (TCMR) or 'ABMR' were based on histopathologic assessment of a kidney transplant biopsy and serological assessment of DSA, in accordance with the BANFF 2017 classification. For ABMR this consists of histologic evidence of acute tissue injury and of evidence of current/recent antibody interaction with vascular endothelium and of serological evidence for DSA or C4d staining in the biopsy. Cases with ABMR were subsequently subdivided into early (≤14 days) and late (between 15 days and 6 months) rejection. In cases where the biopsy only showed borderline rejection, initiation of treatment for rejection was used to classify the patient as having either TCMR (n=4) or 'no

rejection' (n=4; details in *Supplementary Table 1*). Mixed rejection (ABMR and TCMR) was classified as ABMR (n=1). Some patients had a repeat biopsy. None of the repeat biopsies led to reclassification of the type of rejection.

Baseline characteristics and donation type

Patient, donor and transplantation characteristics and specific risk factors for the development of rejection (including previous transplantations, panel-reactive antibody percentage (PRA), HLA typing, mismatch (HLA-A, HLA-B and HLA-DR), immunosuppressive therapy) were extracted from the electronic health record.

Immunosuppressive regime and induction therapy

Patients received the immunosuppressive regimen according to the standard of care at the time of transplantation. All patients were treated with a combination of a calcineurin inhibitor, prednisolone and mycophenolate mofetil or mycophenolic acid. Before 2001 induction therapy was not part of the standard protocol. In 2001 induction with human interleukin-2 receptor monoclonal antibodies (basiliximab) was introduced, with reduced calcineurin inhibitor exposure. Lymphocyte depleting induction therapy with anti-CD52 monoclonal antibodies (alemtuzumab) was introduced in 2009. The preferred induction therapy depended upon the risk as assessed by the treating physician.

Renal function, patient- and graft survival

Serum creatinine levels at 6 and 12 months after transplantation and information on patient survival and graft survival were obtained from the electronic health record. The estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula.[21] Based on the eGFR, four groups were defined; >50, 30-50 and <30 mL/min/1.73m² and GL (defined as initiation of dialysis as renal replacement therapy).

Assessment of donor specific antibodies

The standard complement dependent cytotoxicity (CDC) test that employs lymphocyte targets to detect complement-fixing IgG and IgM antibodies before transplantation was negative in all patients. A positive CDC test was considered a contra-indication for transplantation. Currently, many transplant centers use a pooled antigen bead (PAB) Luminex assay for the standard work up for a kidney transplantation. In this PAB assay the complete phenotype of class I and II are present on beads and binding of IgG antibodies can be detected by a fluorescence signal. The PAB Luminex assay detects the presence of class I and/or II without specification of the exact antibody. In case of a negative result, absence of antibodies is assumed and further analysis is considered to be redundant. However, in case of a positive result, a single antigen bead (SAB) assay is performed, in order to identify the antibodies and specify donor specificity.

In this cohort both the PAB and SAB assays (regardless of the result of the PAB assay) were performed retrospectively on stored samples of all patients with early ABMR. The samples had been obtained and stored at two timepoints: before transplantation and at the time of rejection. In addition, stored pre-transplant samples of 60 randomly selected patients, who did not develop ABMR, were tested with the SAB assay (15 female recipients with a spousal donor, 15 female recipients with a non-spousal male donor, 15 female recipients with a female donor and 15 male recipients) (*Figure 1*). "The following assays were used. SAB: One Lambda (SA), class 1, catalogue number LSA04NC19_011_00, lot number 3007441, One Lambda (SA), class 2, catalogue number LSA01NC17_012_00, lot number 3007379, LifeCodes (Luminex), class 1, lot number 3008213 and LifeCodes (Luminex), class 2, lot number 3008357. The vendors' protocols and cutoff values were followed"

Statistical analysis

Patient characteristics and risk factors were described as mean ± standard deviation and categorical data as numbers and percentage of the total number. Analysis of differences was performed by Fisher's Exact Test, Independent Samples T-test, and Pearson Chi-square Test. Kidney function was analyzed as a categorical variable. A p-value of <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS version 23.0 (IBM SPSS, Inc., Chicago, IL) and graphs were created with Graphpad Prism version 8.0 (Graphpad Prism Software, Inc., San Diego, CA).

Results

Patient characteristics and the incidence of acute rejection

Baseline characteristics of patients with and without ABMR are summarized in *Table 1*. In the first six months after transplantation, 131 for-cause biopsies were performed in 107 patients. No rejection, TCMR and ABMR was observed in 83% (n=288), 14% (n=53) and 3% (n=11) of patients, respectively. Nine of eleven cases of ABMR were early ABMR and occurred at a median of eight days after transplantation (range 5-14 days). In five of these patients DSA were detected during the rejection episode and eight out of nine patients with early ABMR had C4d positivity in the peritubular capillaries in the biopsy. Two male recipients were diagnosed with late ABMR, 35 and 75 days after transplantation. These patients had de-novo DSA, but no pDSA. All patients with early ABMR were transplanted between 2005 and 2014, none had received a repeat transplant and seven out of nine were non-immunized (i.e. PRA ≤5%). The mean age of the recipient and donor, as well as the degree of HLA mismatch, were not different between patients with and without ABMR. The standard immunosuppressive regimen included steroids, mycophenolate mofetil and a calcineurin inhibitor (either tacrolimus or ciclosporin) and there were no differences between those with and without ABMR (*Table 1* and *Supplementary Table 2*). Patients with ABMR were more likely to have received alemtuzumab as induction therapy. This is most

likely explained by the fact that induction therapy with Alemtuzumab (introduced in 2009) was used more frequently in male to female spousal transplantation, in relation to the clinical perception of an increased risk of early acute rejection.

Table 1. *Patient characteristics stratified by the occurrence of early ABMR (i.e. within two weeks after transplantation) in 352 living unrelated donor kidney transplant recipients, transplanted between 1997-2014 (median 2009, IQR 2006-2012).*

Characteristics	All n = 352	Early ABMR n = 9	No early ABMR n = 343	p-value
Recipient sex - female (%)	143 (41%)	8 (89%)	135 (39%)	<0.01[1]
Recipient age - years (SD)	54 ± 11	55 ± 7	54 ± 11	0.82[2]
Previous transplantation(s) - n (%)	35 (10%)	0	35 (10%)	0.61[1]
Pre-emptive - n (%)	118 (34%)	4 (44%)	114 (33%)	0.49[1]
Donor sex - female (%)	217 (62%)	3 (33%)	214 (62%)	0.09[1]
Donor age - years (SD)	53 ± 11	56 ± 12	53 ± 11	0.45[2]
Mismatch				
HLA A - 0/1/2	31/165/156	0/6/3	31/159/153	0.40[3]
HLA B - 0/1/2	12/125/215	0/3/6	12/122/209	0.83[3]
HLA DR - 0/1/2	22/164/166	0/6/3	22/158/163	0.42[3]
Immunosuppression, induction				
No induction	16 (4%)	0	16 (4%)	
Alemtuzumab	29 (8%)	5 (56%)	24 (7%)	<0.01[3]
Basiliximab	307 (87%)	4 (44%)	303 (88%)	
Immunosuppression, CNI				
Tacrolimus	243 (69%)	5 (56%)	238 (69%)	0.47[1]
Ciclosporin	109 (31%)	4 (44%)	105 (31%)	

Early ABMR = antibody-mediated rejection ≤14 days after transplantation. Pre-emptive = no dialysis treatment before transplantation, CNI = calcineurin inhibiter. [1] Fisher's Exact Test, [2] Independent Samples T-test, [3] Pearson Chi-square Test.

Table 2. *Type of histologically confirmed rejection for the entire cohort in the first six months after transplantation.*

Characteristics	All n = 352	Female recipient male donor spousal n = 61	Female recipient male donor non-spousal n = 36	Female recipient Female donor n = 46	Male recipient n = 209
Early ABMR - *n* (%)	9 (3%)	6 (10%)	0	2 (4%)	1 (<1%)
Late ABMR - *n* (%)	2 (1%)	0	0	0	2 (1%)
TCMR - *n* (%)	53 (15%)	3 (5%)	6 (17%)	4 (9%)	40 (19%)
No rejection[+] - *n* (%)	288 (82%)	52 (85%)	30 (83%)	40 (87%)	166 (79%)

Early ABMR = antibody-mediated rejection ≤14 days after transplantation, late ABMR = antibody-mediated rejection between 15 days and 6 months after transplantation, TCMR = T cell-mediated rejection. [+]No rejection indicates that there was no rejection upon biopsy, or that no biopsy was performed (because there were no clinical signs for rejection).

High incidence of early ABMR in females who received a kidney transplant from their spouse

In order to identify recipients that are particularly at risk for ABMR, the cohort was divided into four groups, based on the recipient sex and donor-recipient relationship (*Table 2*). Overall, 41% of the recipients was female and 43% of these females received a kidney from their male spouse. The pretransplant test for panel reactive antibodies (PRA) was negative in 94% of male recipients and 89% of female recipients. Stratified by type of donor, PRA was negative in 98% of female recipients with a spousal male donor, 81% of female recipients with a non-spousal male donor and 83% of female recipients with a female donor. The fact that nearly all female recipients of a spousal male donor kidney had tested negative in the PRA test before transplantation, reflects clinical practice in which more stringent criteria are applied to these higher risk transplantations.

Table 2 shows that the overall incidence of rejection in the first six months after transplantation was 18% (TCMR 15.1%, early ABMR 2.6%, late ABMR 0.6%). The incidence of TCMR was 19% in male recipients, while only 1% of males developed ABMR. Female recipients, who received a kidney from a male spouse, had a significantly higher incidence of early ABMR compared with the rest of the cohort (10% vs 1%, relative risk 9.5, $p<0.001$), while the incidence of TCMR was 5% in this group. Of note, there were six cases of early ABMR in 61 women who received a kidney from a male spouse, two cases in the other 82 women (relative risk 4.0, $p=0.06$) and only one case in more than 209 men. The low TCMR incidence in female recipients of a spousal donor kidney is most likely due to the choice of induction therapy. After the introduction of alemtuzumab induction therapy in 2009, a higher proportion of female recipients of a spousal donor kidney was treated with alemtuzumab (20/35; 57%), compared with other recipients (9/133; 7%).

Early ABMR leads to a severe reduction in renal function and death censored graft survival

Patient survival was 100% and 99.7% at six and twelve months after transplantation. One patient with polycystic kidney disease, died due to a subarachnoid hemorrhage eight months after transplantation, while having a stable and good kidney function. For the entire cohort, death censored transplant survival in the first year after transplantation was 97%. In the group with early ABMR, this was only 56% (*Figure 2*). Mean eGFR in patients without rejection, TCMR and early ABMR was 57, 47 and 36 ml/min/1.73m^2 respectively, one year after transplantation (in case of a functioning allograft). In the first year GL due to ABMR occurred in four out of nine patients with early ABMR (44%). These patients required dialysis as renal replacement therapy at 9, 11, 96 and 283 days after transplantation. Only one of nine patients with early ABMR reached an eGFR above 50 ml/min/1.73m^2, whereas 69% of those without rejection did so.

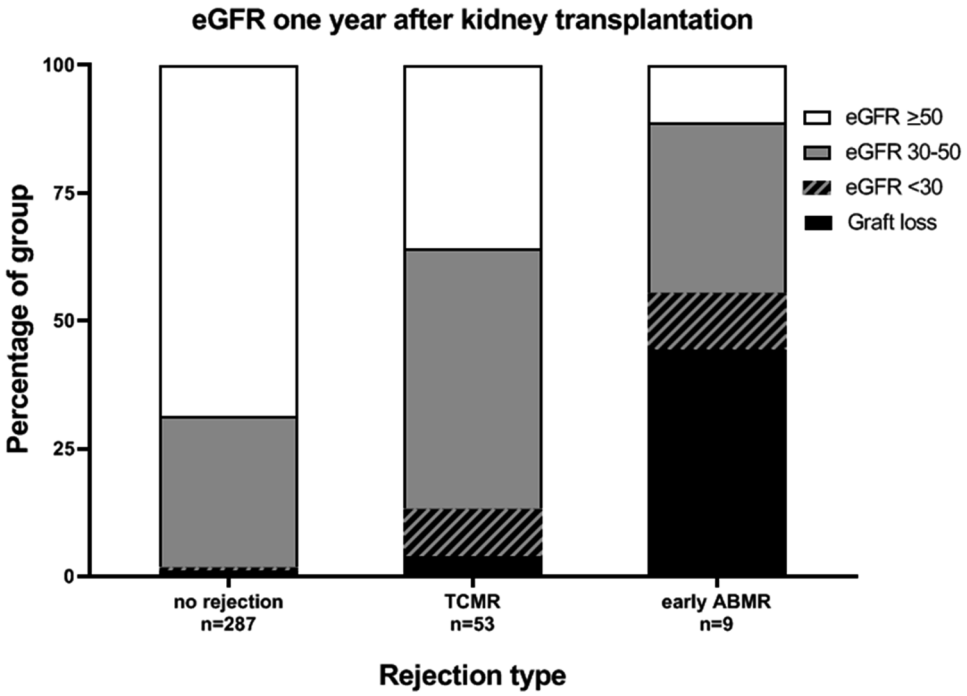

Figure 2. Kidney function one year after transplantation depends on the presence and type of rejection. *56% of patients with early antibody-mediated rejection (ABMR) had kidney graft loss (GL) or an eGFR <30 ml/min/1.73m^2 within 1 year after transplantation. One of the patients with early ABMR reached a kidney function >50 ml/min/1.73m^2, while 69% of patients without rejection reached this level. One patient without rejection died at eight months. Two patients with late ABMR (between 15 days and 6 months after transplantation) are not depicted (resp. eGFR 17 and 51 ml/min/1.73m^2).*

Table 3*. Association between early ABMR and history of pre-transplant blood transfusions and pregnancies in women who received a kidney from an unrelated donor.*

Characteristics	All n = 93	Early ABMR n = 7	No ABMR n = 86	Univariate OR (95% CI)
Blood transfusions				
None	40	4 (57%)	36 (42%)	
≥ 1	48	2 (29%)	46 (53%)	0.4 (0.1-2.3)
Unknown	5	1 (14%)	4 (5%)	
Pregnancies				
None	16	1 (14%)	15 (17%)	
≥ 1	76	6 (86%)	70 (81%)	1.4 (0.2-12.2)
Unknown	1	0	1 (1%)	

Missing data: one in the 'early ABMR' group, 49 in the 'No ABMR' group. Early ABMR = antibody-mediated rejection ≤14 days after transplantation.

Blood transfusions and pregnancies did not correlate with ABMR

In order to clarify the role of immunizing events among female recipients, we analyzed the association between pre-transplant blood transfusions and pregnancies and the development of ABMR. Data was complete for 65% (93/143) of female recipients, for 67% (41/61) of female recipients who received a transplant from their spouse and for seven out of eight female recipients with ABMR. The prevalence of blood transfusions before transplantation and pregnancies did not differ between women with or without ABMR, as shown in *Table 3*. Furthermore, in the subset of women who received a kidney from their spouse, the percentage of women who had been pregnant with the donor's child was no different in those with ABMR (67%, 4/6) than in those without ABMR (66%, 23/35).

Patients with early ABMR had preformed DSA in spite of having tested negative in pre-transplantation CDC test and pooled antigen bead assay

As described above, the median time to early ABMR after transplantation was eight days (range 5-14). Such an early onset of a humoral response is a strong indication that ABMR was caused by pDSA. We retrospectively performed testing for pDSA, using a SAB assay. Analysis of the cases in which ABMR occurred within two weeks after transplantation, showed that seven out of nine patients (78%) had pre-formed class I and/or II anti-HLA DSA (*Table 4*). In four of these patients, pDSA were only detected by using the SAB assay, but not in the PAB assay. pDSA were HLA class I in five patients, class II in one patient and both class I and II in one patient. The median MFI was 2200 (IQR 1400-2700, range 700-5500). pDSA were found in all three patients with GL within the first six months after transplantation. Only one of them had a positive PAB assay, while all three had pDSA in the SAB assay. At the time of rejection, five out of nine patients with early ABMR had DSA (class I in two patients, class II in one patient and class I and II in two patients).

In order to assess the prevalence and clinical significance of a positive single antigen test before transplantation, we performed a SAB assay in 60 randomly selected patients without signs of ABMR (*Figure 1*). The test yielded pDSA in four patients (7%). Of 15 female recipients without ABMR and with a spousal donor, one had pDSA. In comparison, pDSA were detected in five of six female recipients with ABMR and with a spousal donor, resulting in a specificity of 93% and sensitivity of 83% of pDSA for early ABMR within this group of recipients.

Table 4. *Patient characteristics, presence of (p)DSA and kidney function for nine renal recipients with early ABMR.*

Recipient sex	Donor type	Initial kidney disease	Risk factors before Tx	Induction therapy	ABMR days after Tx	DSA (MFI) before Tx	DSA (MFI) during ABMR	eGFR Tx + 1y
female	male spousal	MPGN	reTx: - preg: + PRA: - btf: -	Amab	7	A1 (900), DQ2 (1,900)	DQ2 (3,100)	GL
female	male spousal	PKD	reTx: - preg: + PRA: - btf: -	Amab	8	B51 (2,700) *	B51(2,200), B38(2,200), DR5 (3,400)	GL
female	male spousal	secondary hyperoxaluria	reTx: - preg: + PRA: + btf: +	Amab	14	A2 (2,300), B57 (2,800) *	A2 (7,000), B44 (8,300)	GL
female	male spousal	hypertension	reTx: - preg: + PRA: - btf: +	Amab	11	B58 (730) *	neg	26
female	male spousal	Secondary FSGS	reTx: - preg: - PRA: - btf: -	Bmab	5	DQ2 (2,200) *	neg	59
female	male spousal	diabetic nephropathy	reTx: - preg: + PRA: - btf: -	Amab	7	neg	neg	47
female	female	FSGS	reTx: - preg: + PRA: - btf: u	Bmab	7	A2 (5,500)	A2 (15,300), A3 (14,200)	45
female	female	PKD	reTx: - preg: + PRA: + btf: -	Bmab	12	neg	neg	GL
male	female	unknown cause	reTx: - preg: PRA: - btf: u	Bmab	8	B7 (1,900)	B7 (15,300), DR13 (20,400), DR52 (11,000)	40

*The pre-transplantation complement dependent cytotoxicity test was negative in all patients. The PRA was positive in two patients (26% and 36%). Early ABMR = antibody-mediated rejection ≤14 days after transplantation, Tx = transplantation, DSA = donor specific antibodies, MFI = Mean Fluorescence index, eGFR in ml/min/1.73m2, MPGN = membranoproliferative glomerulonephritis, PKD = polycystic kidney disease, FSGS = focal segmental glomerulosclerosis, SLE = systemic lupus erythematosus. reTx = repeat transplant, PRA = panel-reactive antibody (<5% considered negative), preg = pregnancy, btf = blood transfusion, u = unknown. Amab = Alemtuzumab. Bmab = Basiliximab. GL = graft loss and was due to ABMR. *solely detected by single antigen bead assay.*

Discussion

We found that early ABMR occurred in one in ten women who received a kidney transplant from a male spouse, with detrimental consequences to graft function. The incidence of early ABMR was 2% in other women and <1% in men. Furthermore, we show that in the pre-transplant assessment of women who receive a kidney transplant from their male spouse, even when the PAB is negative a SAB assay should be performed to lower the risk of early ABMR. The risk of developing early ABMR could not be predicted by assessing classical clinical patient characteristics, such as prior blood transfusion or pregnancy.

The median time from transplantation to ABMR was very short (eight days in early ABMR). Therefore, it is not surprising that in retrospect, pDSA were present in 78% of cases with early ABMR. In general, de-novo DSA would take more time to develop and are not likely to be formed so early, particularly given the current potent immunosuppressive drug regimen.

In our cohort, early ABMR only occurred in recipients of a living donor kidney transplanted after 2004. Most likely, this reflects a change in practice through time. Firstly, living kidney donation has become more common. Secondly, with the availability of more sensitive assays to screen for pDSA and the advent of stronger immunosuppressive drugs, male to female spousal transplantations, which traditionally have been viewed as carrying a higher immunological risk and were often avoided, were deemed safe. It is important to note that in spite of testing for pDSA with a combination of assays (CDC test and PAB assay) in an experienced, specialized laboratory, pDSA can go undetected. The SAB assay revealed pDSA in up to 57% of patients with early ABMR, despite a negative PAB assay.

In accordance with previous studies, patients with ABMR had an inferior outcome in terms of eGFR and/or graft loss in the first year after transplantation, compared with recipients without ABMR or pDSA.[22] Furthermore our study corroborates the fact that presence of pDSA, despite a negative CDC test, is a key parameter, indicating a strong increase in the risk of early ABMR.[23] Other studies show that especially pDSA that persist after transplantation cause ABMR and a worse outcome, while recipients with pDSA that disappear after transplantation tend to have the same outcome as recipients without pDSA.[24]

In our cohort, prior pregnancies were as common in women with, as in women without early ABMR. Nevertheless, in the literature there are several indications that a proportion of renal recipients develop DSA due to sensitization by a previous pregnancy. After a failed previous transplant, pregnancy is considered to be the second most prominent immunizing event.[25] Terasaki reported that in spousal donations, females who had been pregnant before transplantation tended to have a worse three-year graft survival than female recipients without pregnancies.[26] This type of immunization is a risk factor for the development of DSA[27] and early graft loss, in particular if the mismatch with the donor

kidney is repeated in the HLA profile of the father of the child.[28,29] In addition, a higher rate of hyperacute rejection and GL has been observed in spousal male to female donations in general, compared with living related donations.[30] With respect to the type of rejection, ABMR has been reported to be more frequent in spousal kidney transplantation than in living related kidney transplantation, in particular in patients with a low-risk pre-transplant risk profile for ABMR.[15,31] In the majority of these cases, changes in kidney function are not reported. Despite these risks, spousal LURD kidney donation is generally considered to be relatively safe, compared with other living kidney donations. Our study adds data showing that this is not the case and that additional care is needed to safely conduct male to female spousal LURD.

In contrast with other studies, we did not find a higher incidence of ABMR in patients who received blood transfusions before transplantation. It is reported that especially blood transfusions that share HLA antigens with the allograft are a risk factor for the development of transfusion specific antibodies that may harm the allograft.[32] This discrepancy between the literature and our results may be related to the fact that blood transfusion products are entirely leukocyte depleted since 2001 which significantly reduces the immunological risk.

This study has a number of strengths. First, the cohort was large and well defined and focused both on clinical and immunological risk factors for ABMR. Second, biopsies were assessed by an experienced nephropathologist and classified using the most recent guideline (BANFF 2017 classification[33]). Furthermore, extensive DSA testing was performed by a Eurotransplant reference laboratory. Last, we assessed the prevalence of pDSA in a random selection of recipients without ABMR, to gain insight into the occurrence and relevance of pDSA that are detected with the SAB assay, while not detected by the PAB assay.

Our study also has limitations. Firstly, despite it being a large cohort, the overall incidence of early ABMR was low; nine cases in total. Based on our results, we can confidently state that screening for pDSA with a PAB assay suffices for male transplant recipients and that screening with a SAB assay should be included in the pre-transplant assessment of women who are to receive a kidney from their spouse. Uncertainty remains regarding other female recipients. In our study, only women with a male spousal donor had pDSA that were not detected with a PAB assay. Therefore, we limit the recommendation to include a SAB assay in the pre-transplant work-up to women who are to receive a kidney from a spouse. Secondly, we did not test C1q binding by DSA. Since DSA that bind C1q are associated with an increased risk of ABMR and a higher risk of graft loss,[34-36] this test could be of value in discerning relevant from irrelevant pDSA. However, the increased risk of ABMR is described for de-novo DSA in particular and there is no consensus about the relevance of C1q binding for clinical outcomes.[37,38] The same applies to the relation between DSA and the role of T-cells and NK cells in ABMR.[39] Furthermore, information on previous blood transfusions and pregnancies was incomplete. The results of our analysis, however, do suggest that it is

unlikely that these clinical characteristics have a reliable predictive value for early ABMR. Lastly, female recipients who received a kidney from their spouse were more often treated with alemtuzumab as induction therapy and this may have lowered the incidence of ABMR in this specific group. In fact, 25% of female recipients with a spousal donor developed early ABMR, despite having received alemtuzumab induction therapy.

We conclude that risk assessment for ABMR benefits from the addition of the SAB assay in all female recipients of a spousal donor kidney transplant. We observed a high incidence of ABMR in this subgroup as well as a significantly inferior outcome in terms of eGFR and graft survival. A positive SAB in this group, should encourage the option of indirect (cross-over) donation.

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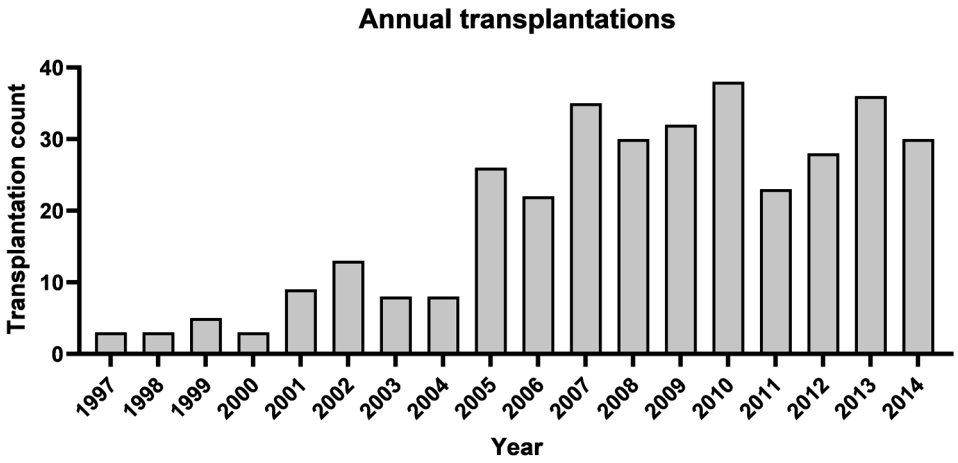
Supplementary information



Supplementary Figure 1. *The year of transplantation of the 352 individuals in the cohort.*

Supplementary Table 1. *Patients with borderline rejection that are classified as 'TCMR' or 'no rejection', based on clinical treatment.*

Donation type (age recipient)	Initial disease	Biopsy after Tx (days)	eGFR at 1Y after Tx (ml/min/1.73m^2)	Year of Tx
Borderline rejection classified as TCMR				
Female to male (60Y)	MPGN	8	42	1999
Female to male (65Y)	Unknown	8	35	2007
Female to male (60Y)	Unknown	10	36	2013
Male to male (39Y)	Unknown	3	47	2013
Borderline rejection classified as No rejection				
Female to female (68Y)	IgA	145	43	2005
Female to female (70Y)	Hypertension	164	39	2010
Female to female (67Y)	Hypertension	12	77	2008
Female to male (55Y)	Diabetes	70	30	2008

Tx = transplantation, MPGN = membranoproliferative glomerulonephritis.

Supplementary Table 2. *Induction therapy and calcineurin inhibitor in donation groups.*

	All	Female recipient male donor		Female recipient Female donor	Male recipient
		spousal	non-spousal		
Early ABMR	n = 9	n = 6	n = 0	n = 2	n = 1
Induction – *n* (%)					
No induction	-	-	-	-	-
Alemtuzumab	5 (56%)	5 (83%)	-	-	-
Basiliximab	4 (44%)	1 (17%)	-	2 (100%)	1 (%)
CNI – *n* (%)					
Tacrolimus	5 (56%)	5 (83%)	-	-	-
Ciclosporin	4 (44%)	1 (17%)	-	2 (100%)	1 (%)
No ABMR	n = 343	n = 55	n = 36	n = 44	n = 208
Induction – *n* (%)					
No induction	16 (5%)	4 (7%)	-	1 (2%)	11 (5%)
Alemtuzumab	24 (7%)	15 (27%)	4 (11%)	2 (5%)	3 (1%)
Basiliximab	303 (88%)	36 (65%)	32 (89%)	41 (93%)	194 (93%)
CNI – *n* (%)					
Tacrolimus	238 (69%)	39 (71%)	23 (64%)	32 (73%)	144 (69%)
Ciclosporin	105 (31%)	16 (29%)	13 (36%)	12 (27%)	64 (31%)

Next to a calcineurin inhibitor, all patients were treated with prednisolone and either mycophenolate mofetil or mycophenolic acid. Early ABMR = antibody-mediated rejection ≤14 days after transplantation, CNI = calcineurin inhibitor.