Donor-specific B cell memory in alloimmunized kidney transplant recipients – first clinical application of a novel method

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C.W. designed and performed the research, analyzed the data and wrote the article. G.K. analyzed the data and wrote the article. J.K., Y.d.V. and J.L.-L. performed the research and analyzed the data. I.B. participated in study design. S.S. and F.C. participated in study design and wrote the paper. D.R. assisted in data acquisition and wrote the paper. S.H. designed the research, analyzed the data and wrote the article.

Disclosure

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Abbreviations

ABMR, antibody-mediated rejection; ATG, anti-thymocyte globulin; DSA, donor-specific HLA antibodies; DSA-M, donor-specific memory B cell-derived antibodies; EDTA, ethylene-diamine-tetra-acetate; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot assay; i.v., intravenous; HLA, human leucocyte antigen; IvIg, intravenous immunoglobulins; MFI, mean fluorescence intensity; PBMC, peripheral blood mononuclear cells; p.o., peroral; SAB, single antigen bead; TCMR, T cell-mediated rejection

Keywords

Kidney transplantation, memory B cells, donor-specific antibodies, allograft rejection, antibody-mediated rejection

Abstract

Background: HLA-specific memory B cells may contribute to the serum HLA antibody pool upon antigen re-exposure. The aim of this pilot study was to investigate the presence of concurrent donor-specific memory B cell-derived HLA antibodies (DSA-M) in renal allograft recipients with pre-transplant donor-specific HLA antibodies (DSA) and its association with occurrence of antibody-mediated rejection (ABMR) using a recently developed method.

Methods: Twenty patients with Luminex single antigen bead (SAB) assay-defined DSA but negative complement-dependent cytotoxicity crossmatches were enrolled. Plasma samples and peripheral blood mononuclear cells (PBMC) were collected at 3 timepoints (pre-transplant, month 6, month 12). We analyzed IgG-purified and concentrated culture supernatants from polyclonally activated PBMC using SAB assays and compared HLA antibody profiles with same day plasma results.

Results: Plasma SAB analysis revealed 35 DSA in 20 patients pre-transplant. DSA-M were detected in 9/20 (45%) patients and for 10/35 specificities (29%). While median mean fluorescence intensity (MFI) values of DSA with concurrent DSA-M (5877) were higher than those of DSA without DSA-M (1476), 3/6 patients with ABMR and low MFI DSA (<3000) had DSA-M. Overall, pre-transplant DSA/DSA-M_{pos} allograft recipients showed a higher incidence of biopsy-proven (sub)clinical ABMR (p=0.032) and a higher extent (g≥1+ptc≥1) of microvascular inflammation (67% versus 9%, p=0.02). In 17 patients (28 DSA) with post-transplant analyses, persisting DSA post-transplant had more often DSA-M (6/12; 50%) than non-persisting DSA (2/16; 13%).

Conclusion: Assessment of DSA-M might be a novel tool to supplement serum HLA antibody analysis for pre-transplant risk stratification in patients with DSA.

1. Introduction

In kidney transplantation, current immunological pre-transplant risk assessment is based on detection of donor-specific HLA antibodies (DSA) in the patients' serum. Their presence is a major risk factor for antibody-mediated rejection (ABMR) and inferior allograft outcomes.[1,2] In the era of highly sensitive Luminex single antigen bead (SAB) technology, transplantation in the absence of DSA is not always possible. For DSA-positive patients, options to transplant include desensitization as well as treatment with an intensified induction immunosuppression.[3-5] However, the post-transplant course of patients transplanted across the DSA barrier is highly variable and cannot be sufficiently predicted by antibody properties, such as antibody strength, composition of IgG subclasses and complement-binding capacities.[6,7]

Previous studies showed that persisting or increasing levels of HLA antibodies after transplantation are associated with a higher risk for ABMR.[8-10] Rebound and/or persistence of DSA post-transplant may be derived from dormant circulating memory B cells that differentiate into antibody-producing cells upon antigen re-encounter or bystander activation.[11-13]

So far, assessment of the peripheral HLA-specific memory B cell pool for risk stratification has been either labor-intensive or lacked sensitivity due to low IgG concentrations and possibly IgM interference in B cell supernatants following *in vitro* stimulation.[14-17] Our group recently presented an easy-to-perform technique for sensitive detection of HLA antibodies derived from circulating memory B cells.[18] For this, IgG of supernatants from polyclonally activated memory B cells is isolated,

concentrated, and analyzed using SAB technology, enabling direct comparison with serum HLA antibody profiles.

In this pilot study, the method was applied to a cohort of patients transplanted in the presence of DSA. The aim was to investigate the presence of concurrent donor-specific memory B cell-derived HLA antibodies (DSA-M) and its association with occurrence of ABMR.

2. Material and Methods

2.1 Study design

The study (project number FUP092) was conducted within the framework of the Swiss Transplant Cohort Study (STCS), supported by the Swiss National Science Foundation and the Swiss University Hospitals (G15) and transplant centers.[19] Signed written informed consent was obtained, as approved by the local institutional review board and the STCS committee. We investigated a cohort of DSA-positive patients transplanted at the University Hospital Basel and took advantage of the STCS bio-banking, collecting PBMC and plasma of participating patients at 3 timepoints within the first year: pre-transplant, at month 6 (m6) and at month 12 (m12) after transplantation. Pre-transplant samples were either collected on the day of transplantation or maximum 3 days in advance. Biological material was shipped and all analyses were performed at the Leiden University Medical Center (the Netherlands).

2.2 Patient population

We identified 64 DSA-positive patients transplanted between May 2008 and December 2015 and participating in the STCS. All patients had pre-transplant DSA defined by SAB testing using high resolution HLA typing results but negative T and B cell complement-dependent cytotoxicity crossmatches at the time of transplantation. No flow cytometric crossmatches were performed. In total, we excluded 23 patients for the following reasons: (1) Less than two allograft biopsies (indication or surveillance) within the first year post-transplant (n=9), (2) no induction therapy as intended by the protocol (n=5), (3) no complete follow-up of at least 12 months (n=6), (4) lacking or insufficient biological material at one or more of the investigated timepoints (n=3). For this pilot study, we selected 20 out of the remaining 41 patients with prioritizing those that had higher number of frozen PBMC and distinct histological phenotypes (e.g. an ABMR phenotype in ≥ two biopsies or no signs of ABMR in any biopsy).

2.3 Allograft biopsies and definition of rejection

Indication biopsies were performed at any time in case of inadequate or worsening graft function. According to the local protocol, surveillance biopsies were scheduled at month 3, 6 and 12 post-transplant. Rejection was defined according to the most recent Banff guidelines at the time of study performance.[20]

2.4 Immunosuppression

All patients received induction therapy with a polyclonal T cell-depleting agent (ATG-Fresenius total dose 21 mg/kg body weight or Thymoglobulin total dose 6 mg/kg body) plus intravenous immunoglobulins (Ivlg) (total dose 2 g/kg body weight) and

indefinitely continued triple maintenance immunosuppression with tacrolimus, mycophenolic acid and steroids, as described previously.[2,3]

2.5 HLA typing

For the purpose of this study, both recipients and donors were retrospectively HLA typed by next generation sequencing (NGS) for all loci on an Illumina platform (Illumina, California, USA) at the Leiden University Medical Center. NGS was performed as previously described.[18]

2.6 Polyclonal activation of B cells and supernatant preparation

PBMC were isolated using Ficoll-Hypaque density gradient centrifugation and kept frozen in liquid nitrogen until use. Upon thawing, PBMC were polyclonally stimulated for 6-10 days, culture supernatants harvested and IgG isolated and concentrated, as reported before.[18] Flow cytometry to obtain CD19+ percentages within PBMC was performed before and after stimulation. We determined total IgG concentrations of neat supernatants and eluates by ELISA, as described previously.[18]

2.7 HLA antibody detection and DSA assignment

Plasma samples and eluates were analyzed for the presence of HLA antibodies by using Lifecodes SAB kits (LSA, Immucor Transplant Diagnostics, Stamford, USA). Performance of the assay was based on a previously described protocol.[21] All plasma samples were treated with EDTA. Data analysis was performed using MATCHIT software version 1.3.0 (Immucor). Background corrected mean fluorescence intensity (MFI) values (BCM), BCR (BCM divided by the raw MFI of the lowest ranked bead for a locus) and AD-BCR (antigen density corrected BCR values) were calculated by the

software. Beads were assigned as positive if at least 2 of 3 of the following criteria were met: for HLA class I: BCM>1000, BCR>3, AD-BCR>4 and for HLA class II: BCM>1000, BCR>4, AD-BCR >5.

HLA mismatches were defined on the allelic level. DSA were only assigned if the bead of the mismatched allele was present.

2.8 Statistical analyses

Data were analyzed using JMP Version 13 software (SAS institute Inc., Cary, NC, USA). Categorical data are presented as counts and/or percentages and were analyzed by Fisher's exact test. Continuous data are shown as median and interquartile ranges (IQR) and compared by Wilcoxon rank sum tests. The Spearman correlation was used as a non-parametric measure of association. Time-to-rejection curves were generated by the Kaplan-Meier method, and the groups compared using the log-rank test. For all statistical tests, a two-tailed p-value <0.05 was considered statistically significant.

3. Results

3.1 Study population characteristics

In total, 20 kidney transplant recipients were investigated and their characteristics are summarized in ***Table S1***. Patients had a median age of 58 years and 45% were women. Eighty percent of patients received deceased donor transplants. All but one patient (95%) had a history of known sensitizing events and 13 patients (65%) were previously transplanted. Most patients had either 1 or 2 DSA (17/20; 85%) and 16/20 patients (80%) class II or class I+II DSA. HLA mismatches and the assigned DSA of all patients are detailed in ***Table S2***.

3.2 Polyclonal B cell stimulation

Polyclonal activation of PBMC was performed in all patients using samples obtained pre-transplant, in 17/20 (85%) patients at m6 and in 16/20 (80%) patients at m12. Excluded samples belonged to patients who received rituximab as rejection treatment resulting, as expected, in profound and persisting peripheral B cell depletion precluding successful polyclonal stimulation of B cells.

Per patient and timepoint, a median number of 16 million PBMC (minimum 8 million) were cultured. The median percentage of CD19+ B cells within the lymphocyte gate determined by flow cytometry on day 0 of the culture was 6.3% (4.1-13.5) pre-transplant, 9.9% (7.4-26.3) at m6 and 10.8% (5.7-20.0) at m12. To assure successful polyclonal stimulation, we measured total IgG concentrations by ELISA. Neat culture supernatants showed a median total IgG concentration of 8.8 µg/ml (4.6-19.8), 12.7 µg/ml (7.7-27.4) and 15.8 µg/ml (8.1-25.6) at the 3 timepoints, respectively, which was within the expected range considering the B cell percentages and our previous experience.[18] Following IgG isolation and concentration, the median total IgG concentration pre-transplant was 1189 µg/ml (547-4116) and 2240 µg/ml (1200-4132) and 2237 µg/ml (1574-4641) at m6 and m12, respectively.

3.3 Comparison of HLA antibody profiles in plasma and eluates

3.3.1 Pre-transplant

All patients had pre-transplant plasma HLA antibodies (DSA and non-DSA). Of those, 80% had also detectable HLA antibodies deriving from memory B cells (***Table S3***). We subsequently focused on donor-directed antibody specificities. In total, the cohort had 35 pre-transplant DSA (14 class I, 21 class II). Of those, we detected concurrent

DSA-M in 9 patients (45%) and for 10 specificities (29%) ***(Figure 1A)***. Of the latter, 4 were class I and 6 class II antibodies. MFI values of DSA with concurrent DSA-M were higher than those of DSA without DSA-M (median 5877 (2441-10003) and 1476 (943-3901)). Noteworthy, both groups contained DSA with a broad range of MFI values (DSA/DSA-M_{pos}: MFI 783-18919, DSA/DSA-M_{neg}: MFI 447-18808). This was also observed when we investigated class I and II separately. In only one patient (#9), a class II DSA-M without concurrent DSA was detected.

3.3.2 Post-transplant

At m6 and m12 post-transplant, only 19 (95%) and 17 (85%) patients still had plasma antibodies (DSA and non-DSA). However, the proportion of patients with detectable memory B cell-derived HLA antibodies remained stable (82% and 88%, respectively) (***Table S3***).

As illustrated in ***Figure 1A***, there were also less donor-directed antibody specificities detected post-transplant. At m6, we found 12 DSA in 9 patients and concurrent DSA-M in 2/9 (22%) patients and for 2/12 (17%) specificities. In one patient and for one specificity (A*32:01, #6), DSA-M was detected at m6 while the plasma antibody was not present anymore at this timepoint. At m12, we detected only 7 DSA in 6 patients and one patient (17%) had concurrent DSA-M.

Based on their persistence or disappearance post-transplant, we then grouped pre-transplant DSA of patients who had also post-transplant eluate analyses (n=17 patients with n=28 specificities) into persisting and non-persisting DSA (***Figure 1B***). Interestingly, persisting DSA had more frequently DSA-M (6/12; 50%) than non-persisting DSA (2/16; 13%).

3.3.3 De novo DSA and DSA-M development

We did not detect *de novo* DSA in any of the 20 study patients at m6 and m12 post-transplant. *De novo* DSA-M without DSA was found in only one patient (#13) and for one specificity (A*31:01, data not shown) at m6, but was not anymore detectable at m12.

3.4 Association of pre-transplant DSA-M status with transplant outcomes

Next, we correlated transplant outcomes with pre-transplant presence and absence of DSA-M (***Table 1***). All patients had a minimal follow-up of one year and a median follow-up of 5 years (3.1-7.9).

In total, 73 allograft biopsies were performed. A median number of 4 allograft biopsies was obtained in both DSA-M_{pos} and DSA-M_{neg} patients in the first year. Pre-transplant DSA-M_{pos} patients showed a significantly higher incidence of (sub)clinical ABMR (p=0.032, ***Figure 2***). In addition, DSA-M_{pos} patients had also a higher extent of microvascular inflammation as defined by glomerulitis and peritubular capillaritis scores ≥1 (67% versus 9%, p=0.02, ***Table 1***). C4d positive ABMR episodes as well as persistent ABMR (defined as an ABMR phenotype in at least 2 subsequent biopsies) were more frequently observed in pre-transplant DSA-M_{pos} patients, but this did not reach statistical significance.

Interestingly, 7/9 (78%) patients who had persisting DSA (***Figure 1A***) developed ABMR post-transplant. Of those, DSA-M pre-transplant was detected in 5 cases (5/7; 71%).

Three patients lost their graft during the follow-up period. In 2/3 (66%) graft failure was due to ongoing humoral rejection (one DSA-M_{pos} and one DSA-M_{neg}, respectively).

One patient lost the graft following an ischemic event (pneumonia with severe hypotension and stenosis of transplant artery).

Among all patients with a functioning graft at the time of last follow-up, there was a trend towards lower estimated glomerular filtration rate (eGFR) in pre-transplant DSA-M_{pos} compared with DSA-M_{neg} patients (***Table 1***).

4. Discussion

The main findings of this study are that 45% of pre-transplant DSA-positive patients had detectable concurrent DSA-M and that DSA/DSA-M_{pos} individuals more frequently developed (sub)clinical ABMR in the first year than DSA/DSA-M_{neg} patients. Furthermore, DSA/DSA-M_{pos} patients showed a higher extent of microvascular inflammation in their allograft biopsies, and had a tendency towards worse allograft function at last follow-up. These results are in line with the association between post-transplant detection of circulating HLA-specific donor-directed memory B cells and ABMR lesions found in a recently published study using HLA-specific B cell ELISPOT assays.[17] Importantly, our findings suggest that assessment of the peripheral donor-specific memory B cell pool may be used before transplantation, which is clinically desirable.

An interesting observation was that MFI values of DSA with concurrent DSA-M were higher than those of DSA without DSA-M, although MFI distribution showed a broad range in both groups. On the patient level, pre-transplant DSA with MFI >3000 were found in 50% of patients developing ABMR and all but one had detectable DSA-M (data not shown). Among 6 patients experiencing ABMR in the presence of low MFI DSA (MFI <3000, one of them MFI <1000), 3 otherwise unrecognized patients would

have been identified as being at higher risk by using the new method. Supported by these findings, use of an integrative approach with assessment of multiple factors being involved in the alloimmune response is advisable.[22-24]

Mechanistically, HLA-specific memory B cells can contribute to the antibody repertoire by differentiating into antibody producing cells following antigen re-challenge, thereby serving as a replenishing source of circulating DSA.[25] Not surprisingly, we observed that persisting post-transplant DSA were more often found to have DSA-M than those becoming undetectable. In the majority (7/9; 78%) of patients with persisting DSA ABMR was diagnosed within the first year. Future studies should also address the question whether detection of pre-transplant DSA-M predicts the persistence of DSA, since this may abolish the need to monitor the evolution of pre-existing antibodies after transplantation.

Several studies have shown that HLA antibody profiles from serum and culture supernatants overlap but are not mirror images of each other.[14,16,26,27] An important clinical question is whether DSA-M can be detected while plasma antibodies are absent. In this study, we detected only one DSA-M that was not present in the plasma, which might be due to the study design focusing on DSA-positive patients. Likely, the frequency of detecting DSA-M without DSA will be considerably higher in specific clinical situations such as husband-to-wife (with shared children) or child-to-mother transplantation.

Absence of *de novo* DSA and only transient *de novo* DSA-M formation for one specificity was observed in this study. Noteworthy, lacking detection of *de novo* DSA

up to one year post-transplant is not surprising as the frequency of *de novo* DSA in the first year has been found to be around 2%.[28,29] In addition, T cell depleting therapy with ATG has recently been shown to dampen both naïve and memory DSA responses by impaired CD4+ T cell help and can therefore be considered as an effective prevention of *de novo* DSA development until T cell recovery.[30]

This study has several strengths. Assignment of DSA was performed in the most accurate way, namely by combination of high resolution HLA typing results of all loci of donors and recipients and antibody detection using SAB technology. All patients received uniform induction therapy and maintenance immunosuppression. In addition, all transplant recipients underwent surveillance biopsies and had a median number of 4 allograft biopsies per patient, making it rather unlikely that ABMR episodes within the first year were missed.

The main limitation is the small number of patients, which is inherent to the pilot character of this study and only allows the detection of a possible association with the occurrence of ABMR. Since we focused on a specific subgroup of patients transplanted in the presence of DSA following T cell-depleting therapy and retrospectively selected patients based on availability of biological material as well as histological phenotypes, a selection bias cannot be ruled out. Methodologically, antibodies contained in culture supernatants are only qualitatively analyzed. Quantification of memory B cells is, however, currently only possible by performing labor-intensive and less sensitive ELISPOT assays.[15,16] In addition, due to circulation between the secondary lymphoid organs and the peripheral blood, memory B cells may appear in the latter in low frequencies, which potentially precludes their detection.

This limitation applies to both ELISPOT assays and culture supernatant analyses. For clinical purposes, it is therefore important to focus on their detectability instead of their absence, which is the approach we followed in this study.

In conclusion, the current data suggest that assessment of DSA-M provides a new tool that could supplement serum HLA antibody analysis for pre-transplant risk stratification in DSA-positive patients. Validation of our findings in a larger cohort and expansion to other patient groups, such as patients without pre-transplant DSA, is warranted.

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References

1. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *J Am Soc Nephrol.* 2010;21(8):1398-1406.
2. Wehmeier C, Hoenger G, Cun H, et al. Donor Specificity but Not Broadness of Sensitization Is Associated With Antibody-Mediated Rejection and Graft Loss in Renal Allograft Recipients. *Am J Transplant.* 2017;17(8):2092-2102.
3. Baechler K, Amico P, Hoenger G, et al. Efficacy of induction therapy with ATG and intravenous immunoglobulins in patients with low-level donor-specific HLA-antibodies. *Am J Transplant.* 2010;10(5):1254-1262.
4. Marfo K, Ajaimy M, Colovai A, et al. Pretransplant immunologic risk assessment of kidney transplant recipients with donor-specific anti-human leukocyte antigen antibodies. *Transplantation.* 2014;98(10):1082-1088.
5. Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-Incompatible Kidney Recipients and Survival. *New England Journal of Medicine.* 2011;365(4):318-326.
6. Courant M, Visentin J, Linares G, et al. The disappointing contribution of anti-human leukocyte antigen donor-specific antibodies characteristics for predicting allograft loss. *Nephrol Dial Transplant.* 2018.
7. Valenzuela NM, Schaub S. The Biology of IgG Subclasses and Their Clinical Relevance to Transplantation. *Transplantation.* 2018;102(1S Suppl 1):S7-S13.
8. Burns JM, Cornell LD, Perry DK, et al. Alloantibody levels and acute humoral rejection early after positive crossmatch kidney transplantation. *Am J Transplant.* 2008;8(12):2684-2694.
9. Kimball PM, Baker MA, Wagner MB, King A. Surveillance of alloantibodies after transplantation identifies the risk of chronic rejection. *Kidney Int.* 2011;79(10):1131-1137.
10. Redondo-Pachon D, Perez-Saez MJ, Mir M, et al. Impact of persistent and cleared preformed HLA DSA on kidney transplant outcomes. *Hum. Immunol.* 2018;79(6):424-431.
11. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. *Science.* 2002;298(5601):2199-2202.
12. Crotty S, Ahmed R. Immunological memory in humans. *Semin. Immunol.* 2004;16(3):197-203.
13. D'Orsogna L, van den Heuvel H, van Kooten C, Heidt S, Claas FHJ. Infectious pathogens may trigger specific allo-HLA reactivity via multiple mechanisms. *Immunogenetics.* 2017;69(8-9):631-641.
14. Han M, Rogers JA, Lavingia B, Stastny P. Peripheral blood B cells producing donor-specific HLA antibodies in vitro. *Hum. Immunol.* 2009;70(1):29-34.
15. Karahan GE, de Vaal YJH, Krop J, et al. A Memory B Cell Crossmatch Assay for Quantification of Donor-Specific Memory B Cells in the Peripheral Blood of HLA-Immunized Individuals. *Am J Transplant.* 2017;17(10):2617-2626.
16. Lucia M, Luque S, Crespo E, et al. Preformed circulating HLA-specific memory B cells predict high risk of humoral rejection in kidney transplantation. *Kidney Int.* 2015;88(4):874-887.

17. Luque S, Lucia M, Melilli E, et al. Value of Monitoring Circulating Donor-Reactive Memory B Cells to Characterize Antibody-Mediated Rejection after Kidney Transplantation. *Am J Transplant.* 2018.
18. Karahan GE, Krop J, Wehmeier C, et al. An Easy and Sensitive Method to Profile the Antibody Specificities of HLA-Specific Memory B Cells. *Transplantation.* 2018.
19. Yamakuchi M, Kirkiles-Smith NC, Ferlito M, et al. Antibody to human leukocyte antigen triggers endothelial exocytosis. *Proc Natl Acad Sci USA.* 2007;104(4):1301-1306.
20. Loupy A, Haas M, Solez K, et al. The Banff 2015 Kidney Meeting Report: Current Challenges in Rejection Classification and Prospects for Adopting Molecular Pathology. *Am J Transplant.* 2017;17(1):28-41.
21. Kamburova EG, Wisse BW, Joosten I, et al. How can we reduce costs of solid-phase multiplex-bead assays used to determine anti-HLA antibodies? *Hla.* 2016;88(3):110-119.
22. Lefaucheur C, Viglietti D, Mangiola M, Loupy A, Zeevi A. From Humoral Theory to Performant Risk Stratification in Kidney Transplantation. *J Immunol Res.* 2017;2017:5201098.
23. Tambur AR, Campbell P, Claas FH, et al. Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *Am J Transplant.* 2018;18(7):1604-1614.
24. Wiebe C, Ho J, Gibson IW, Rush DN, Nickerson PW. Carpe diem-Time to transition from empiric to precision medicine in kidney transplantation. *Am J Transplant.* 2018;18(7):1615-1625.
25. Chong AS, Ansari MJ. Heterogeneity of memory B cells. *Am J Transplant.* 2018;18(4):779-784.
26. Snanoudj R, Claas FH, Heidt S, Legendre C, Chatenoud L, Candon S. Restricted specificity of peripheral alloreactive memory B cells in HLA-sensitized patients awaiting a kidney transplant. *Kidney Int.* 2015;87(6):1230-1240.
27. Karahan GE, Claas FH, Heidt S. Detecting the humoral alloimmune response: we need more than serum antibody screening. *Transplantation.* 2015;99(5):908-915.
28. Wehmeier C, Amico P, Hirt-Minkowski P, et al. Acute Rejection Phenotypes in the Current Era of Immunosuppression: A Single-Center Analysis. *Transplant Direct.* 2017;3(3):e136.
29. Wiebe C, Gibson IW, Blydt-Hansen TD, et al. Rates and determinants of progression to graft failure in kidney allograft recipients with de novo donor-specific antibody. *Am J Transplant.* 2015;15(11):2921-2930.
30. Chen CC, Koenig A, Saison C, et al. CD4+ T Cell Help Is Mandatory for Naive and Memory Donor-Specific Antibody Responses: Impact of Therapeutic Immunosuppression. *Front. Immunol.* 2018;9:275.

Tables

Table 1. Association of pre-transplant DSA-M status with transplant outcomes

	DSA-M_{pos} pre-transplant (n=9)	**DSA-M_{neg} pre-transplant (n=11)**	**p-level**
Total number of biopsies within 1st year	35	38	n.a.
Median number of biopsies within 1st year	4 (3-5)	4 (3-4)	0.28
Frequency of rejection in the 1st year			
- Any rejection (subclinical or clinical)	8 (89%)	6 (55%)	0.16
- Any TCMR (subclinical or clinical)	7 (78%)	5 (45%)	0.2
- Any ABMR (subclinical or clinical)	8 (89%)	4 (36%)	0.03
ABMR details in the 1st year			
- Clinical ABMR	3 (33%)	1 (9%)	0.28
- g≥1 and ptc≥1 in ≥ one biopsy	6 (67%)	1 (9%)	0.02
- C4d positive ABMR in ≥ one biopsy	4 (44%)	3 (27%)	0.64
- Persistent ABMR	6 (67%)	4 (36%)	0.37
Graft failure	2 (22%)	1 (9%)	0.57
Death with functioning graft	1 (11%)	2 (18%)	1.0
Functioning graft at last follow-up			
- creatinine [µmol/l]	188 (135-237)	126 (105-175)	0.16
- eGFR MDRD [ml/min]	32 (25-41)	52 (28-63)	0.22
- protein/creatinine ratio [mg/mmol]	19 (9-109)	15 (7-37)	0.59

DSA-M=donor-specific memory B cell-derived HLA antibodies; TCMR=T cell-mediated rejection; ABMR=antibody-mediated rejection; g=glomerulitis; ptc=peritubular capillaritis; eGFR=estimated glomerular filtration rate; MDRD=Modification of Diet in Renal Disease

Figure Legends

Figure 1. (A) Comparison of donor-specific HLA antibodies (DSA) and memory B cell-derived donor-specific HLA antibodies (DSA-M) before transplantation (pre-tx), at month 6 (m6) and month 12 (m12) in the study population (n=20). (B) Comparison of donor-specific HLA antibodies (DSA) and memory B cell-derived donor-specific HLA antibodies (DSA-M) before transplantation (pre-tx), at month 6 (m6) and month 12 (m12) in patients with post-transplant eluate analyses (n=17 patients with n=28 specificities), grouped according to persistence and HLA class of DSA.
Grey-shaded boxes indicate assigned positivity. Boxes marked with X represent samples not tested due to low B cell counts. Patients marked by an asterisk developed antibody-mediated rejection (ABMR) post-transplant.

Figure 2. Incidence of (sub)clinical antibody-mediated rejection (ABMR) in patients with (DSA-M_{pos}) and without (DSA-M_{neg}) pre-transplant memory B cell-derived donor-specific HLA antibodies.