Anti-HLA Donor-Specific IgG Subclasses and C1q-binding Evolution in Posttransplant Monitoring

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**Background.** The identification of low-level antibodies by single-antigen bead methodology has brought advancements to risk evaluation of kidney transplant recipients. However, the use of mean fluorescence intensity (MFI) to quantify antibodies and to guide therapy is not enough. Notably, immunoglobulin G (IgG) subclass switching is hypothesized to follow a programmed sequence after an emergency signal from the germinal center. In transplantation this process is not clear yet. In the present study, we sequentially evaluate anti-HLA donor specific antibody (DSA) subclasses, their profile changes, and C1q-binding ability and the influence of those characteristics on antibody mediated rejection (AMR) occurrence and allograft function. **Methods.** A total of 30 DSA-positive patients were tested for IgG subclass content and C1q-binding in sequential serum samples. **Results.** Twenty-one patients were DSA-positive before transplant; patients sensitized only by transfusion or pregnancies had IgG1 and/or IgG3, and patients sensitized by both transfusion and pregnancies or previous transplant showed a broader range of IgG subclasses. C1q binding was detected in high MFI made up of IgG1 or multiple IgG subclasses. Only 4 patients were positive for C1q posttransplantation and 3 of these showed an increase in MFI, changes in subclasses patterns, AMR, and allograft dysfunction. **Conclusions.** Posttransplant evaluation of DSA subclasses and the ability to bind C1q may be informative for both AMR occurrence and allograft dysfunction. Monitoring these events may help to better define risk and interventional time points.

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**Laboratory Method**

**OPEN**

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Lefaucheur et al showed in a pretransplant setting that patients with donor-specific antibody (DSA) higher than 6000 MFI presented a 100-fold increased risk for antibody-mediated rejection (AMR) (relative risk, 113.0; 95% confidence interval [CI], 30.8-414), however, the CI showed by the data suggests that some of the patients in this group presented lower risk for AMR than patients with MFI between 465 and 1500 (relative risk, 24.8; 95% CI, 4.6-134.8. When evaluating large cohorts, it is possible to find greater risk associated to higher MFI values, but the question remains, what differs in patients with high MFI values that develop AMR versus those that do not?

In the posttransplant setting, the appearance of anti-HLA DSAs, as determined by MFI and the subsequent rise and/or fall of the MFI value, although implying risk, does very little to define the function and activity of that antibody. When considering the functionality of DSA, it was recently demonstrated by Loupy et al that allograft survival in the presence of C1q-binding DSA was significantly lower than that in patients with non-C1q-binding DSA and no DSA. However, in their cross-sectional analysis of 1016 patients tested at 1 year or at the time of rejection with a 3-year follow-up, they found only 77 patients with C1q-positive DSA. Although providing a highly significant cohort of patients at higher risk for allograft loss, suggesting the functionality of DSA at a specific time point, these data do little to elucidate the true evolution of the immune response. One could argue that instead of being a marker for rejection and possibly an opportunity for intervention, it is nothing more than evidence of a predetermined fate.

It has recently been shown that the presence of complement-fixing IgG (IgG1 and/or IgG3) is abundant in kidney transplant patient serum but is not a determinant of the detection of C1q-binding. Moreover, subclass switching is hypothesized to follow a programmed sequence after an emergency signal from the germinal center leading to the production of IgM followed by IgG3, then IgG1, then IgG2, and finally IgG4. This process is influenced by the initial immune response, the microenvironment of cytokines, and the signaling produced. There are many reports suggesting that IgG3 and IgG1 appear relatively early in the immune response and are often the only subclasses detected, which could mean early antigen clearance. Arnold et al observed that AMR features were more common in patients

| TABLE 1. Patient demographics |
|-----------------------------|
| Characteristics             | All patients | DSA+C1q− | DSA+C1q+ | P   |
| N total                     | n (%)        | 30 (100) | 23 (76.7) | 7 (23.3) |
| Female                      | n (%)        | 17 (56.7) | 12 (62.2) | 5 (71.4) | 0.38 |
| Age, y                      | Mean ± SD    | 42.17 ± 13.70 | 41.09 ± 13.82 | 45.71 ± 13.68 | 0.44 |
| Transplant type             |              |          |          |      |
| Live unrelated              | n (%)        | 12 (40.0) | 8 (34.8) | 4 (57.1) | 0.30 |
| Live related                | n (%)        | 18 (60.0) | 15 (62.6) | 4 (23.9) | 0.32 |
| Transplants                 | Median (min-max) | 2.0 ± 2.9 | 2.1 ± 3.2 | 1.8 ± 2.2 | 0.85 |
| Pregnancies, n = 17         | Median (min-max) | 3.5 ± 0.8 | 2.5 ± 1.5 | 3.0 ± 2.2 | 0.38 |
| No. mismatches              | mean ± SD    | 4.23 ± 1.48 | 4.2 ± 1.6 | 4.4 ± 1.1 | 0.70 |
| PRA                         | mean ± SD    | 56.6 ± 39.8 | 54.9 ± 38.4 | 62.1 ± 46.8 | 0.68 |
| Clinical events             |              |          |          |      |
| Induction therapy           | n (%)        | 17 (56.7) | 15 (65.2) | 2 (28.6) | 0.09 |
| Dialysis time, d            | Median (min-max) | 730 (0-4380) | 700 (0-3650) | 1278 (153-4380) | 0.22 |
| Diabetes                    | n (%)        | 1 (3.3) | 0 (4.3) | 0 | 0.58 |
| Hypertension                | n (%)        | 20 (66.7) | 15 (65.2) | 5 (71.4) | 0.76 |
| Follow-up time, d           | Median (min-max) | 1801.5 (289-2176) | 1833 (851-2176) | 1770 (289-1914) | 0.33 |
| Infection                   | n (%)        | 20 (66.7) | 15 (65.2) | 5 (71.4) | 0.76 |
| Graft loss                  | n (%)        | 2 (6.7) | 0 | 2 (28.6) | 0.009 |
| Patient death               | n (%)        | 3 (10.0) | 2 (8.7) | 1 (14.3) | 0.67 |
| Histological factors        |              |          |          |      |
| AMR                         | n (%)        | 4 (13.3) | 0 | 4 (57.1) | 0.0001 |
| TCMR                        | n (%)        | 2 (6.7) | 2 (8.7) | 0 | 0.63 |
| CsA nephropathy             | n (%)        | 1 (3.3) | 0 | 1 (4.3) | 0.58 |
| BKV nephropathy             | n (%)        | 4 (13.3) | 4 (17.4) | 0 | 0.24 |

χ² tests were used for comparison of categorical variables and 1-way ANOVA was used for the comparison of parametric continuous variables. Comparison between groups of nonparametric variables was performed by the Kruskal-Wallis method. Nonparametric variables are presented as the median (range). BKV, BK virus; CsA, cyclosporine; min, minimum; max, maximum.
with an expansion to non-complement-fixing DSA. This suggests that the expansion of complement-fixing to non-complement-fixing DSA shows an evolution of the immune response. Little has been described in transplantation about this process, because most studies evaluate pretransplant sera alone or pretransplant sera with only 1 posttransplant time point.16-21

In the present study, we sequentially evaluated anti-HLA DSA subclasses, their profile changes, and C1q-binding ability while observing the influence of those characteristics on AMR and allograft function in live donor kidney transplant recipients.

**METHODOLOGY**

**Patients and Sera Selection**

From January of 2007, we prospectively monitored kidney transplant recipients for the presence of donor-specific anti-HLA antibodies (DSA) by SAB. Between 2007 and 2010, 158 patients received kidneys from live donors at the Hospital Universitário Evangélico de Curitiba. From those, 63 patients were excluded due to lack of complete follow-up or available serum for new tests, and 65 did not present DSA during the 5 years of posttransplant monitoring. Thirty patients were included in the study, 21 with preformed DSA and 9 with de novo DSA. All transplants required a negative complement-dependent cytotoxicity crossmatch for IgG T cell and B cell, and ABO blood group compatibility. Sera collection is shown in Figure 1. The study was approved by the Ethics Committee for Research from the Pontifícia Universidade Católica do Paraná.

**Clinical Data**

Clinical data on donors and recipients were obtained from the original medical records. Immunosuppression included prednisone, cyclosporine, and mycophenolate mofetil. Acute clinical rejection was characterized by deterioration of allograft function, proteinuria, and histopathological evidence. Allograft function is shown as estimated glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease (MDRD) formula in mL/min per 1.73 m². Proteinuria is presented in the following categories: P+, 150 to 500 mg; P++, 500 to 1000 mg; P+++, 1000 to 3500 mg; P++++, >3500 mg. Biopsies were reanalyzed by 2 different pathologists without any clinical information of the recipient and were classified according to the most recent Banff classification criteria. C4d was performed for all biopsies.

**Detection of IgG and DSA Characterization**

Pretransplant and posttransplant sera were tested for class I and class II anti-HLA antibodies (SAB-IgG_total) with commercially available, Luminex-based Single Antigen Bead assay kits (LABScreen Single Antigen LS1A04 and LS2A01; One Lambda, Inc.; Canoga Park, CA) per the manufacturer’s protocol and analyzed with HLA FUSION software (One Lambda, Inc.). A positive result was defined as a baseline normalized mean fluorescence intensity (MFI) greater than 500. Donor specificity for anti-HLA antibodies was determined by the comparison of the HLA antibody specificities with the HLA typing of the donor for HLA-A, -B, -DRB1, -DRB3, -4 and -5, -DQB1, and -DQA1 loci. HLA typing for both patient and donor was performed by LABType SSO (One Lambda, Inc.).

![Figure 2](image-url)  
**FIGURE 2.** Study population according to anti-HLA DSA and C1q-binding status. C1q+—C1q-binding positive; C1q−—C1q-binding negative.
Detection of IgG Subclasses

DSA-positive samples were tested with a modified SAB assay to determine the IgG subclass of the antibody. The generic secondary antibody IgGtotal (One Lambda, Inc.) was replaced by monoclonal secondary antibodies specific for IgG1-4 subclasses conjugated with phycoerythrin (PE) (IgG1 clone HP6001, IgG2 clone 31-7-4, IgG3 clone HP6050, IgG4 clone HP6025; Southern Biotech, Birmingham, AL). A positive result was defined as an MFI above the cutoff ratio that was generated for each individual bead of each subclass using 3 negative control sera (NC1-3) obtained from healthy, nonsensitized, anti-HLA antibody negative males, and 1 commercially available negative control serum (NC4). The cutoff ratio was calculated as follows: cutoff MFI = 2 × ((mean NC1-4) + 3 × (standard deviations NC1-4)); cutoff ratio = MFI/cutoff MFI. A ratio above 5 was considered positive.

Detection of Complement-fixing Antibodies

DSA-positive samples were tested for C1q-binding anti-HLA antibodies (SAB-C1q) using commercially available kits (C1qScreen; One Lambda). The serum samples were heat-treated (56°C for 30 minutes) to denature endogenous complement components and the test was performed per the manufacturer’s protocol. The analyses of C1q results were performed by HLA FUSION software (One Lambda, Inc.) following the interpretation method published by Tyan et al.22

Statistics

Comparison of patient and donor characteristics between groups according to sensitization status was performed with the χ² test for discrete variables and with analysis of variance (ANOVA) or the Kruskal-Wallis test, as applicable, for quantitative variables. Survival was analyzed from the time of transplantation to a maximum of 5 years using kidney allograft loss as the event of interest. Survival rates were compared according to the presence of anti-HLA DSA C1q-binding status using the log-rank test. Allograft function was evaluated by comparing the means of groups with DSA C1q-binding and DSA without C1q-binding by ANOVA 1-way on the following posttransplant days: 30, 180, 365, 730, 1095, 1460, and 1825. We further applied repeated-measures ANOVA to evaluate the variance in eGFR MDRD over time after transplant according to the presence of DSA C1q-binding. All statistical analyses were performed using MedCalc for Windows, version 13.0 (MedCalc Software, Ostend, Belgium). Statistical significance was set at P less than 0.05.

RESULTS

Study Population Demographics

Patient characteristics according to C1q DSA status are shown in Table 1. Patients with C1q DSA-positive at any time during the study were included in the group DSA+C1q+. We tested a mean of twelve serum samples per patient from pretransplant up to 5 years after transplantation for the presence of DSA. In the posttransplant evaluation, anti-HLA class II DSA was more frequent than class I alone and classes I and II together. IgG subclasses were primarily found in combinations, IgG1 being the most frequent. C1q-binding was detected in 3 pretransplant patients, 2 of whom

| Table 2. Mean fluorescence intensity in SAB IgG total and SAB C1q by groups of IgG subclasses |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Anti-HLA antibody IgG subclass composition | No subclass IgGt | IgG1 | IgG2 | IgG3 | IgG4 | IgG1 + IgG2 | IgG1 + IgG3 | IgG1 + IgG4 | IgG3 + IgG4 | IgG2 + IgG4 | IgG1 + IgG2 + IgG3 | IgG1 + IgG2 + IgG4 | IgG3 + IgG4 + IgG1+ IgG2 + IgG3 | IgG3 + IgG4 + IgG1+ IgG2 + IgG4 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| No. anti-HLA class I IgGt-positive beads | 630 | 441 | 17 | 78 | 96 | 121 | 37 | 96 | 37 | 192 | 1088 | 5922 | 4156 | 1041 |
| Mean MFI IgGt class I | 1780 | 4931 | 4512 | 7610 | 1664 | 6411 | 8892 | 1538 | 6438 | 89 | 234 | 48 | 9292 | 10310 |
| Mean MFI C1q class I | 57 | 790 | 1273 | 49 | 64 | 1538 | 80 | 64 | 64 | 64 | 68 | 3472 | 70 | 110 |
| No. anti-HLA class II IgGt-positive beads | 899 | 511 | 1 | 39 | 54 | 123 | 65 | 123 | 2 | 1 | 68 | 110 | 3 | 88 |
| Mean MFI IgGt class II | 2830 | 6414 | 2916 | 2136 | 2619 | 10112 | 6883 | 9843 | 2040 | 9364 | 9140 | 10547 | 9443 | 7107 |
| Mean MFI C1q class II | 31 | 1040 | 4881 | 682 | 5748 | 1480 | 50 | 40 | 22 | 10422 |

All beads with mean fluorescence intensity above 500 were considered.

IgGt, Immunoglobulin G total.
| Patient ID | Sensitization | Sensitization PRA (%) | HLA class | Pretransplant Antibody Profile | Anti-HLA Antibodies IgGt with no subclass (MFI) | C1q binding (MFI) |
|------------|---------------|-----------------------|-----------|-------------------------------|-----------------------------------------------|-------------------|
| 1          | Transfusion   | 76%                   | II        | DQ7(3)* (2934-IgG1)           |                                               | Negative          |
| 2          | Not Known     | 44%                   | I         | B12* (2121-IgG1) B8 (2297-IgG1) |                                               | B12* (3083) B37 (4001) B82 (6173) |
| 3          | Transfusion + Pregnancies | 84% | II | DR53* (4717-IgG3) | DR9* (4547) DR18 (738) DR17 (4471) | Negative          |
| 4          | Transfusion + Pregnancies | 97% | I and II | A9* (2238-IgG1) B27 (719-IgG1) B39 (743-IgG1) B45 (78-IgG1) B21 (677) B22 (1143) B6 (591-IgG1 + IgG4) DR3* (6255-IgG1 + IgG2) DR4 (1259-IgG4) DR7 (3327-IgG1 + IgG4) DR8 (2188-IgG1) DR9 (2905-IgG1) DR6 (5626-IgG1 + IgG2 + IgG4) DR6 (7787-IgG1 + IgG2) DR5 (5826-IgG1 + IgG2 + IgG4) DR8 (1038-IgG1) DR9 (5051-IgG1 + IgG4) DQA1*03:01 (6998-IgG1) DQA1*03:02 (6998-IgG1) DQA1*03:05 (6998-IgG1) DQA1*03:06 (6998-IgG1) | A10* (6999) B8* (1268) B14 (1165) B15 (677) B70 (661) B42 (964) B82 (801) | Negative          |
| 5          | Previous Transplantation | 98% | II | DR4* (7611-IgG1 + IgG2 + IgG4) DR53* (12593-IgG1 + IgG2 + IgG4) DR1 (5063-IgG1 + IgG2 + IgG4) DR7 (13794-IgG1) DR9 (11016-IgG1 + IgG4) DR10 (8489-IgG1 + IgG2 + IgG4) DR4 (6518-IgG1) DR15 (9556-IgG1 + IgG2 + IgG4) DR16 (6434-IgG1 + IgG4) DR5 (13501-IgG1 + IgG2 + IgG4) DR12 (3484-IgG1) DQA1*03:01 (6056-IgG1 + IgG2 + IgG4) DQA1*03:02 (6056-IgG1 + IgG2 + IgG4) DQBI*05:01 (6056-IgG1 + IgG2 + IgG4) | DR1 (5091) DR7 (1171) DR9 (1436) DR10 (1225) DR15 (726) DR16 (772) DR5 (1088) DQA1*05:01 (2209) | DR10 (1225) DR15 (726) DR16 (772) DR5 (1088) DQA1*05:01 (2209) |
| Patient ID | Sensitization | PRA (%) | HLA class | Anti-HLA Antibodies (IgG MFI-IgG subclass) | Anti-HLA Antibodies IgG+ with no subclass (MFI) | C1q binding (MFI) |
|------------|---------------|---------|-----------|------------------------------------------|-----------------------------------------------|------------------|
| 6          | Transfusion + Pregnancies | 99%     | I         | A1* (3502-IgG1) A2 (686-IgG1 IgG2 + IgG3) A3 (5545-IgG1) A11 (4895-IgG1) A9 (6621-IgG1 + IgG3) A10 (5367-IgG1 + IgG2) A*301 (5550-IgG1 + IgG2) A19 (2967-IgG1) A36 (2967-IgG1) A28 (12043-IgG1 + IgG2 + IgG3) A80 (1640-IgG1) B13 (3633-IgG1 + IgG2) B15 (4174-IgG1 + IgG2) B27 (4158-IgG1 + IgG2) B37 (2979-IgG1) B16 (3972-IgG1 + IgG2) B39 (1865-IgG1) B12 (3455-IgG1 + IgG2) B47 (2999-IgG1) B21 (6413-IgG1 + IgG2) B5 (3771-IgG1 + IgG2) B53 (4898-IgG1 + IgG2) B17 (7549-IgG1 + IgG2) B59 (3475-IgG1 + IgG2) | | |
| 7          | Transfusion   | 40%     | I         | B7* (3998-IgG1) B27 (2650-IgG1) B81 (3915-IgG1) | B13 (1008) B40 (693) B42 (2059) B48 (1122) B22 (2530) B67 (738) | Negative |
| 8          | Transfusion + Pregnancies | 80%     | I         | A28* (5207-IgG1 + IgG2 + IgG3) A2 (10752-IgG1 + IgG2 + IgG3 + IgG4) B15 (10609-IgG1 + IgG2 + IgG4) B17 (2533-IgG1) B82 (7116-IgG1 + IgG2) | | |
| 9          | Transfusion   | 86%     | I         | B7 (3951-IgG1 + IgG2) B27 (1745-IgG1 + IgG3) B40 (595-IgG3) B42 (1760-IgG3) B48 (1208-IgG3) B81 (3746-IgG1 + IgG2) | | |
| 10         | Transfusion + Pregnancies | 72%     | I         | A1* (3689-IgG1 + IgG3) A3 (1437-IgG1 + IgG3) A11 (154-IgG1 + IgG3) A9 (1195-IgG1 + IgG3) A36 (245-IgG1 + IgG2) | B15 (759) | Negative |
| 11         | Not Known     | 20%     | I         | | | |
| 12         | Transfusion   | 31%     | II        | | | |
| 13         | Transfusion   | 75%     | II        | DQA1*01:01 (1743-IgG1) DQA1*01:03 (2210-IgG1) | | |
| 14         | Pregnancy     | 80%     | II        | A10 (2477-IgG1 + IgG3) DQ7(3)* (4084-IgG1) DR18(3) (616-IgG3) | | |
| 15         | Transfusion + Pregnancies | 80%     | II        | | | |
| 16         | Transfusion   | 82%     | II        | | | |
### Transfusion + Pregnancies

| Transfusion + Pregnancies III | DR13* (5988-IgG1) | DQ6* (1) (12651-IgG1 + IgG2 + IgG3 + IgG4) |
|-----------------------------|------------------|------------------------------------------|
| DR17(3) (2211-IgG1) DQ4 (1612-IgG1) DR52 (3616-IgG1) DQ6* (1) (15245-IgG1 + IgG2 + IgG3) DQB1*03:03 (3251-IgG1) | DR9 (686) DR11(5) (2022) DR15(2) (867) | DQB1*05:01/DQA1*01:01 (5541) DQ6* (1) (6198) |
| DR11(5) (2149) | DR16(2) (2917) DQ5 (5111) | DR12(5) (2317) |
| DR51 (1265) | DQ5 (3) (1) (8610-IgG1) | DR2 (4033-IgG1) |

### Previous Transplantation

| Previous Transplantation I | DR9 (686) DR11(5) (2022) DR15(2) (867) |
|-----------------------------|------------------------------------------|
| DR51 (1265) | DQ5 (3) (1) (8610-IgG1) | DR2 (4033-IgG1) |

### Negative

**HLA antibodies are shown in serologic groups; *Anti-HLA donor specific antibodies are highlighted. HLA, human leucocyte antigen; MFI, mean fluoresce intensity; IgGt, immunoglobulin total.**
continued to present during the posttransplant period. Four patients presented C1q-binding during posttransplant monitoring, and 2 of these presented pretransplant DSA, whereas 2 formed posttransplant DSA (Figure 2). Four patients presented antibody-mediated rejection (AMR), 3 presensitized and 1 with de novo DSA. T cell–mediated rejection (TCMR) occurred in 2 patients, both with de novo DSA. Two patients lost their allograft, 1 with preformed DSA, and 1 with de novo DSA. Three patients died during the study, 2 with functioning allografts and 1 who developed DSA, C1q-binding, and AMR.

**MFI of IgG total and C1q-binding According to IgG Subclass Groups**

We analyzed 1598 beads with positive reactions in IgG total for anti-HLA class I, and 2087 positive reactions for anti-HLA class II antibodies. In 630 (39.4%) of class I beads, and 899 (43.1%) of class II beads with low MFI, it was not possible to define the IgG subclass. IgG1 was the predominant IgG subclass found either alone or in combinations with other subclasses; 811 (50.8%) for class I and 1091 (52.3%) for class II antibodies. C1q-binding reactions showed a higher MFI when IgG1 and IgG3 were positive (Table 2).

**Pretransplant IgG Subclass Pattern, C1q Reactivity and Previous Sensitization**

Twenty-one patients had preformed DSA. Pretransplant antibody profile and sensitization are shown in Table 3. Previous sensitization information was not available for 2 patients. Patients with only transfusions or only pregnancies presented IgG1 and/or IgG3 with lower MFI, whereas patients with both transfusion and pregnancy or previous transplant presented a broader antibody profile with a higher MFI (P < 0.00001) (Table 4). 90.7% of the positive beads in previously transplanted patients had IgG2 and IgG4 in its composition and 64.3% of the positive bead reactions in patients with pregnancy plus transfusion presented IgG1 and IgG3 (χ² = 97.504; P < 0.0001). C1q-binding was detected in 6 pretransplant patients, of these, 2 were DSA. Patients with C1q-binding antibodies were sensitized by transfusions and pregnancy or previous transplantation.

**Posttransplant Changes in IgG Subclass Patterns and C1q Reactivity**

**Presensitized Patients**

Table 5 shows posttransplant evolution of preformed DSA for each patient with the changes in DSA IgG subclasses, ability to bind C1q, allograft function, and clinical events over time during follow-up. Patients 2, 6 and 17 had preformed C1q-binding DSA; but only patients 2 and 17 remained positive after transplantation. Patient 17 presented AMR with minimal (10%) C4d deposition. Patients 4 and 20 were presensitized after multiple transfusions and pregnancies and developed posttransplant C1q-binding DSA showing an increase in MFI values and changes in IgG subclasses patterns, C4d deposition, and AMR. Patient 20 lost the allograft before completing 1 year of transplant.

**De Novo DSA Patients**

Table 6 shows the posttransplant follow-up for patients that developed DSA after transplantation, with the changes
| Patient ID | Induction | Therapy | Number days posttransplant | DSA profile | C1q-binding | eGFR MDRD | Proteinuria | C4d | Biopsies (BANFF 2017 score) |
|------------|-----------|---------|--------------------------|-------------|-------------|-----------|-------------|-----|----------------------------|
| 1          | T         | 0       | DQ7(3) (2934-IgG1)       | Negative    |             |           |             |     |                            |
| 5          |           |         | DQ7(3) (1148-IgG1)       | Negative    | 44          | P+        |             |     |                            |
| 16         |           |         | DQ7(3) (1290-IgG1)       | Negative    | 74          |           |             |     |                            |
| 30         |           |         | DQ7(3) (690-IgG1)        | Negative    | 82          |           |             |     |                            |
| 76         |           |         | DQ7(3) (2297-IgG1)       | Negative    | 69          |           |             |     |                            |
| 94         |           |         | DQ7(3) (1362-IgG1)       | Negative    | 68          |           |             |     |                            |
| 184        |           |         | DQ7(3) (878-IgG1)        | Negative    | 54          |           |             |     |                            |
| 549        |           |         | DQ7(3) (2297-IgG1)       | Negative    | 75          |           |             |     |                            |
| 757        |           |         | DQ7(3) (891-IgG1)        | Negative    | 73          |           |             |     |                            |
| 2          | N         | 0       | B*44:02 (2121-IgG1)      | B*44:02 (3083) | 4          | P++++    |             |     |                            |
| 3          |           |         | B*44:02 (3111-IgG1)      | B*44:02 (7116) | 33         |          |             |     |                            |
| 16         |           |         | B*44:02 (6960-IgG1)      | B*44:02 (6680) | 68         |          |             |     |                            |
| 27         |           |         | B*44:02 (3475-IgG1)      | B*44:02 (1063) | 70         | P++      |             |     |                            |
| 164        |           |         | B*44:02 (5035-IgG1)      | B*44:02 (1058) | 68         |          |             |     |                            |
| 369        |           |         | B*44:02 (8892-IgG1)      | Negative    | 60          |          |             |     |                            |
| 540        |           |         | B*44:02 (2102-IgG1)      | Negative    | 79          |          |             |     | Negative No rejection      |
| 842        |           |         | B*44:02 (1678-IgG1)      | Negative    | 44          |          |             |     |                            |
| 1266       |           |         | B*44:02 (3334-IgG1)      | B*44:02 (1084) | 47         |          |             |     |                            |
| 1432       |           |         | B*44:02 (4029-IgG1)      | Negative    | 66          |          |             |     |                            |
| 3          | P         | 0       | DR9 (4547) DR53 (4717-IgG3) | Negative    |             |          |             |     |                            |
| 3          |           |         | DR9 (1266)               | Negative    | 14          | P+       |             |     |                            |
| 8          |           |         | DR9 (1742-IgG1) DR53 (1138) | Negative    | 76          | P+       |             |     |                            |
| 13         |           |         | DR9 (1647-IgG1) DR53 (10231-IgG12) | Negative | 65          | P+       |             |     |                            |
| 38         |           |         | DR53 (9607-IgG12)        | Negative    | 74          | P+       |             |     |                            |
| 90         |           |         | DR53 (7348-IgG12)        | Negative    | 59          |          |             |     |                            |
| 742        |           |         | DR9 (756) DR53 (4174)    | Negative    | 56          |          |             |     |                            |
| Patient ID | Induction Therapy | Number days posttransplant | DSA profile | C1q-binding | eGFR MDRD | Proteinuria | C4d | Biopsies (BANFF 2017 score) |
|------------|------------------|---------------------------|-------------|-------------|-----------|-------------|-----|--------------------------|
| 4          | N                | 0                         | A*24:02 (1847-IgG1) A*26:01 (695) B*08:01 (1269) DRB1*03:01 (9329-IgG12) DRB1*13:01 (9111-IgG12) DR52 (3827-IgG124) | Negative | | | | |
| 3          |                  |                           | A*24:02 (8396-IgG123) A*26:01 (7633-IgG1) B*08:01 (5418-IgG123) B*38:01 (10487-IgG12) DRB1*03:01 (5101-IgG12) DRB1*13:01 (11961-IgG12) DR52 (10547-IgG124) D22 (8770-IgG1234) | Negative | | | | |
| 7          |                  |                           | A*24:02 (5726-IgG1234) A*26:01 (7749-IgG1234) B*08:01 (4799-IgG123) B*38:01 (12140-IgG123) DRB1*03:01 (6852-IgG123) DRB1*13:01 (14181-IgG123) DR52 (12924-IgG1234) D22 (13217-IgG1234) | A*24:02 (11306) A*26:01 (7882) B*08:01 (11467) B*38:01 (7437) DRB1*03:01 (16344) DRB1*13:01 (11553) DR52 (10752) D22 (7436) | 24 | P++ | |
| 11         |                  |                           | A*24:02 (7919-IgG1234) A*26:01 (2380-IgG13) B*08:01 (9730-IgG123) B*38:01 (2537) DRB1*03:01 (9382-IgG12) DRB1*13:01 (7822-IgG12) DR52 (7161-IgG14) D22 (11396-IgG1234) | A*24:02 (12165) DRB1*03:01 (6995) D22 (1178) | 4 | P++++ | |
| 30         |                  |                           | A*24:02 (1664) DRB1*03:01 (1269) DR52 (1263) D22 (1139) | Negative | | | | |
| 218        |                  |                           | A*24:02 (1664) DRB1*03:01 (1269) DR52 (1263) D22 (1139) | Negative | | | | |
| 390        |                  |                           | DRB1*03:01 (781) DR52 (1020) D22 (824) | Negative | | | | |
| 745        |                  |                           | Negative | | | | | |
| 5          | OKT3             | 0                         | DRB4*01:01 (12416-IgG14) DBR1*04:05 (9364-IgG24) | Negative | | | | |
| 15         |                  |                           | DBR4*01:01 (8996-IgG14) DBR1*04:05 (1798-IgG6) | Negative | | | | |
| 20         |                  |                           | DBR4*01:01 (8793-IgG14) DBR1*04:05 (1427-IgG4) | Negative | | | | |
| 28         |                  |                           | DBR4*01:01 (8427-IgG14) DBR1*04:05 (688-IgG4) | Negative | | | | |
| 52         |                  |                           | DBR4*01:01 (13247-IgG4) DBR1*04:05 (1614-IgG4) | Negative | | | | |
| 92         |                  |                           | DBR4*01:01 (6069-IgG4) DBR1*04:05 (1001-IgG4) | Negative | | | | |
| 210        |                  |                           | DBR4*01:01 (798-IgG4) DBR1*04:05 (650-IgG4) | Negative | | | | |
| 368        |                  |                           | DBR4*01:01 (7717-IgG4) DBR1*04:05 (727-IgG4) | Negative | | | | |
| 509        |                  |                           | DBR4*01:01 (9913-IgG4) DBR1*04:05 (650-IgG4) | Negative | | | | |
| 754        |                  |                           | DBR4*01:01 (8746-IgG4) DBR1*04:05 (545) | Negative | | | | |
|   |   |   |   |
|---|---|---|---|
| N | 0 | A*01:01 (3502-IgG1) B*39:01 (1866-IgG1) | A*01:01 (5011) |
| 3 | A*01:01 (5304-IgG1) B*39:01 (859) | Negative | 5 |
| 5 | A*01:01 (2061-IgG1) | Negative | 3 |
| 30 | A*01:01 (4736-IgG1) DQA1*01:02 (1973) | Negative | 19 |
| 61 | A*01:01 (7846-IgG1) B*39:01 (1591) DQA1*01:02 (1952) | Negative | 50 |
| 89 | A*01:01 (5304-IgG1) B*39:01 (859) DQA1*01:02 (1952) | Negative | 33 |
| 190 | A*01:01 (5022-IgG1) | Negative |   |
| 562 | A*01:01 (3016-IgG1) | Negative | 47 |
| 761 | A*01:01 (4178-IgG1) | Negative | 56 |
| 23 | B*07:02 (3998-IgG1) | Negative | 23 |
| 10 | B*07:02 (1499-IgG1) | Negative | 25 |
| 20 | B*07:02 (1324-IgG1) | Negative | 35 |
| 23 | B*07:02 (726-IgG1) | Negative | 36 |
| 1852 | Negative | Negative | 55 |
| 8 | T | A*68:02 (5080-IgG12) | Negative |
| 5 | Negative | Negative | 35 |
| 14 | Negative | Negative | 45 |
| 18 | Negative | Negative | 43 |
| 20 | Negative | Negative | 61 |
| 35 | Negative | Negative | 53 |
| 60 | Negative | Negative | 60 |
| 125 | Negative | Negative | 53 |
| 938 | Negative | Negative | 32 |
| 1048 | Negative | Negative | 22 |
| 1227 | Negative | Negative | 17 |
| 9 | N | B*13:02 (665) | Negative |
| 3 | Negative | Negative | 72 |
| 10 | B*13:02 (1022) | Negative | 102 |
| 23 | Negative | Negative | 109 |
| 79 | Negative | Negative | 85 |
| 362 | Negative | Negative | 58 |
| 708 | Negative | Negative | 76 |

Continued next page
| Patient ID | Induction | Therapy | Number days post transplant | DSA profile | C1q-binding | eGFR MDRD | Proteinuria | C4d | Biopsies (BANFF 2017 score) |
|-----------|-----------|---------|-----------------------------|-------------|-------------|-----------|-------------|-----|-----------------------------|
| 10        | T         | 0       |                             | A*01:01 (3689-IgG13) | Negative    |           |             |     |                             |
| 6         | A*01:01   | 11      |                             | 1681-IgG13  | Negative    | 53        |             |     |                             |
| 10        | A*01:01   | 5       |                             | 5370-IgG13  | Negative    | 66        |             |     |                             |
| 19        | A*01:01   | 5       |                             | 5481-IgG13  | Negative    | 66        |             |     |                             |
| 29        | A*01:01   | 2       |                             | 2710-IgG13  | Negative    | 51        |             |     |                             |
| 69        | A*01:01   | 3       |                             | 3668-IgG13  | Negative    | 64        | P+          |     |                             |
| 138       | A*01:01   | 28      |                             | 2829-IgG13  | Negative    | 41        |             |     |                             |
| 208       | A*01:01   | 86      |                             | 189-IgG3    | Negative    | 49        |             |     |                             |
| 11        | T         | 0       |                             | A*31:01 (2228) | Negative    |           |             |     |                             |
| 10        | A*31:01   | 56      |                             | 5571-IgG1   | Negative    | 27        |             |     |                             |
| 20        | A*31:01   | 27      |                             | 2739-IgG1   | Negative    | 109       |             |     |                             |
| 63        | A*31:01   | 98      |                             | 1630        | Negative    | 98        |             |     |                             |
| 90        | A*31:01   | 97      |                             | 1814        | Negative    | 97        |             |     |                             |
| 176       | A*31:01   | 51      |                             | 1503        | Negative    | 51        |             |     |                             |
| 358       | A*31:01   | 79      |                             | 1479        | Negative    | 79        |             |     |                             |
| 601       | A*31:01   | 93      |                             | 1500        | Negative    | 93        |             |     |                             |
| 772       | A*31:01   | 93      |                             | 1166        | Negative    | 93        |             |     |                             |
| 12        | N         | 0       |                             | DQB1*03:01  | 3278        | Negative   |             |     |                             |
| 5         | DQB1*03:01| 33      |                             | 1380        | Negative    | 33        |             |     |                             |
| 12        | DQB1*03:01| 45      |                             | 1058        | Negative    | 45        |             |     |                             |
| 29        | Negative  | 61      |                             |             | Negative    | 61        |             |     |                             |
| 56        | Negative  | 56      |                             |             | Negative    | 56        |             |     |                             |
| 92        | Negative  | 60      |                             |             | Negative    | 60        |             |     |                             |
| 196       | Negative  | 57      |                             |             | Negative    | 57        |             |     |                             |
| 390       | Negative  | 52      |                             |             | Negative    | 52        |             |     |                             |
| 548       | Negative  | 65      |                             |             | Negative    | 65        |             |     |                             |
| 13        | P         | 0       |                             | DQB1*06:09:0QA1*01:02 (2706-IgG1) | Negative    |           |             |     |                             |
| 01        | DQB1*06:09:0QA1*01:02 (1298) | Negative  | 4                          |             |             |     |                             |
| 15        | DQB1*06:09:0QA1*01:02 (3797) | Negative  | 12                         |             |             |     |                             |
| 20        | DQB1*06:09:0QA1*01:02 (6663-IgG13) | Negative  | 12                         |             |             |     |                             |
| 30        | DQB1*06:09:0QA1*01:02 (5989-IgG13) | Negative  | 12                         |             |             |     |                             |
| 14        | I         | 0       |                             | B*37:01 (1862) | DQB1*03:01 (4936-IgG1) | Negative |             |     |                             |
| 5         | B*37:01   | 54      | (1110) DQB1*03:01 (3724-IgG1) | Negative    | 54        |             |     |                             |
| 16        | DQB1*03:01(4120-IgG1) | Negative | 54                          |             |             |     |                             |
| 20        | DQB1*03:01 (5951-IgG1) | Negative | 62                          |             |             |     |                             |
| 35        | DQB1*03:01 (2189-IgG1) | Negative | 51                          |             |             |     |                             |
| 63        | DQB1*03:01 (912-IgG1) | Negative | 46                          |             |             |     |                             |
| 180       | DQB1*03:01 (4952-IgG1) | Negative | 51                          |             |             |     |                             |
| 365       | DQB1*03:01 (1666-IgG1) | Negative | 57                          |             |             |     |                             |
| P | DQB1*06:03/DQA1*01:03 (1002) | Negative |
|---|-----------------------------|----------|
| 10 | B*51:01 (1017) DQB1*06:03/ DQA1*01:03 (2390-IgG1) | Negative 74 |
| 15 | B*51:01 (1168) DQB1*06:03/ DQA1*01:03 (1838-IgG1) | Negative 74 |
| 64 | Negative | Negative 57 |
| 88 | Negative | Negative 60 |
| 730 | Negative | Negative 46 |

| N | DQB1*03:02 (1527) | Negative |
|---|------------------|----------|
| 5 | DQB1*03:02 (1713) | Negative 8 |
| 15 | DQB1*03:02 (1606) | Negative 43 |
| 30 | DQB1*03:02 (1645) | Negative 62 |
| 92 | DQB1*03:02 (3602) | Negative 86 |
| 142 | DQB1*03:02 (2086) | Negative 26 |

| T | DRB1*13:01 (5635-IgG1)/ DQA1*01:02 (13658-IgG1234) | Negative |
|---|--------------------------------------------------|----------|
| 17 | DRB1*13:01 (11917-IgG123) DQA1*01:02 (16358-IgG1234) | Negative |
| 18 | DRB1*13:01 (11436) DQA1*01:02 (16358-IgG1234) | Negative |
| 21 | DRB1*13:01 (11436) DQA1*01:02 (16464) | Negative |
| 626 | Negative | Negative 27 P+ C4d+ AMR (i1; t2; v0; g1; v0; c1; g0; mm0; a00; ph0) |

| T | DQB1*03:01 (9486-IgG1) | Negative |
|---|------------------|----------|
| 18 | DQB1*03:01 (9486-IgG1) | Negative |
| 3 | DQB1*03:01 (5427-IgG1) | Negative 69 P++ |
| 15 | DQB1*03:01 (3453-IgG1) | Negative 55 |
| 21 | DQB1*03:01 (1821-IgG1) | Negative 52 |
| 83 | DQB1*03:01 (2906-IgG1) | Negative 66 P+ |
| 169 | DQB1*03:01 (2417-IgG1) | Negative 79 P+ |
| 289 | DQB1*03:01 (1367-IgG1) | Negative 55 |
| 362 | DQB1*03:01 (1386-IgG1) | Negative 48 |
| 519 | DQB1*03:01 (1489-IgG1) | Negative 49 Negative No rejection |

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| Patient ID | Induction | Number days posttransplant | DSA profile | C1q-binding | eGFR MDRD | Proteinuria | C4d | Biopsies (BANFF 2017 score) |
|------------|-----------|---------------------------|-------------|-------------|-----------|-------------|-----|-----------------------------|
| 19         | T         | 0                         | A*30:02 (723) A*32:01 (827) B*44:02 (808) B*49:01 (1251) DQA1*02:01 (6773-IgG124) | Negative    |           |             |     |                             |
| 3          | B*49:01   | 3                         | A*32:01 (6060-IgG124) | Negative    | 37        |             |     |                             |
| 6          | DQA1*02:01| 45                        |             | Negative    | 45        |             |     |                             |
| 10         | DQA1*02:01| 46                        |             | Negative    | 46        |             |     |                             |
| 17         | DQA1*02:01| 48                        |             | Negative    | 48        |             |     |                             |
| 27         | DQA1*02:01| 48                        |             | Negative    | 48        |             |     |                             |
| 35         | DQA1*02:01| 40                        |             | Negative    | 40        |             |     |                             |
| 60         | DQA1*02:01| 29                        |             | Negative    | 29        |             |     |                             |
| 137        | DQA1*02:01| 45                        |             | Negative    | 45        |             |     |                             |
| 272        | DQA1*02:01| 57                        |             | Negative    | 57        | Negative    |     | No Rejection                |
| 553        | DQA1*02:01| 57                        |             | Negative    | 57        |             |     | P+                          |
| 20         | T         | 0                         | A*02:01 (6635-lgG123) B*57:01 (4483-lgG1) DRB1*16:01 (15645-lgG12) DRB1*11:04 (6595-lgG1) DQB1*03:01 (1372) DQA1*01:02 (2282-lgG1) DRB3*02:02 (7956-lgG1) DRB5*02:02 (17446-lgG12) | Negative    | 56        |             |     |                             |
| 5          | A*02:01   | 56                        |             | Negative    | 56        |             |     |                             |
| 20         | A*02:01   | 6                         | A*02:01 (7179) B*57:01 (1861) DRB1*16:01 (1534) DQA1*01:02 (754) DRB5*02:02 (12402) |             |           |             |     |                             |
|   | A*02:01 (10:933-IgG123) | B*57:01 (6616-IgG123) | DRB1*16:01 (11:727-IgG13) | DRB1*11:04 (5:157-IgG1) | DQB1*03:01 (1:8207-IgG123) | DQA1*01:02 (8:923-IgG12) | DRB3*02:02 (4:754-IgG1) | DRB5*02:02 (7:161-IgG123) |
|---|---------------------|----------------------|------------------------|-------------------|----------------------|-----------------|-----------------|----------------------|
| 30| A*02:01 (17:486)   | B*57:01 (14:806)     | DRB1*16:01 (3:005)    | DQA1*01:02 (2:303) | DRB5*02:02 (1:4071) |
|   |                     |                      |                       |                   |                     |                 |                 |                      |
| 75| A*02:01 (3:403-IgG123) | DRB1*16:01 (3:921-IgG123) | DRB1*11:04 (2:694-IgG1) | DQB1*03:01 (2:5596-IgG123) | DQA1*01:02 (3:433-IgG123) | DRB3*02:02 (3:342-IgG1) | DRB5*02:02 (1:926-IgG123) |
|   |                     |                      |                       |                   |                     |                 |                 |                      |
| 90| A*02:01 (7:201-IgG123) | B*57:01 (10:173)     | DRB1*16:01 (14:792-IgG123) | DRB1*11:04 (5:106-IgG1) | DQB1*03:01 (12:126-IgG123) | DQA1*01:02 (4:1341-IgG12) | DRB3*02:02 (6:660-IgG123) |
|   |                     |                      |                       |                   |                     |                 |                 |                      |
| 191| A*02:01 (1:958-IgG123) | DRB1*16:01 (12:159-IgG123) | DRB1*11:04 (10:1040) | DQB1*03:01 (4:805-IgG123) | DQA1*01:02 (6:732-IgG123) | DRB3*02:02 (2:457-IgG1) | DRB5*02:02 (6:732-IgG123) |
|   |                     |                      |                       |                   |                     |                 |                 |                      |
| 237| A*02:01 (3:845-IgG123) | DRB1*16:01 (13:954-IgG13) | DRB1*11:04 (1:968) | DQB1*03:01 (6:611-IgG123) | DQA1*01:02 (6:627-IgG123) | DRB3*02:02 (3:604) | DRB5*02:02 (6:630-IgG1) |
|   |                     |                      |                       |                   |                     |                 |                 |                      |

Continued next page
of IgG subclasses, ability to bind C1q, allograft function, and clinical events. Patients 22 and 25 formed C1q-binding DSA. The first anti-HLA DSA detected in patient number 22 was on day 90 after transplant with no C1q binding, followed by an increase in MFI and change in the subclass profile from IgG1 and/or IgG3 to all subclasses and C1q-binding. This patient developed AMR and lost the allograft before completing 2 years of transplant.

C1q Reactivity and Allograft Function and Survival

Allograft function over time was evaluated by comparing the mean eGFR MDRD by period (Figure 3A) and by ANOVA repeated measurements (Figure 3B). Patients with C1q binding anti-HLA DSA showed lower allograft function from the first year of transplant through the fifth year. Allograft loss was only observed in patients with C1q binding anti-HLA DSA (100% survival rate at 5 years for DSA+C1q− patients compared to 71.4% survival rate for patients DSA+C1q+).

DISCUSSION

In the present study, we sequentially followed up 30 patients with anti-HLA DSA (mean of 12 samples per patient) to evaluate characteristics such as HLA class, IgG subclass, C1q-binding ability, changes in reaction patterns over time after transplant, time of AMR, TCMR, and allograft dysfunction. All transplants required a negative T and B cell CDC-XM at the time of transplantation. The presence of DSA of any MFI value was not a contraindication for transplantation.

In pretransplant and posttransplant sera, IgG1 was the most common IgG subclass. Presensitized patients showed different compositions of IgG subclasses according to the cause of sensitization. Patients sensitized by only transfusion or pregnancies had anti-HLA antibodies of IgG1 and/or IgG3 subclasses, whereas patients with both transfusion and pregnancies and previous transplant showed a broader range of IgG subclasses. This is in accordance with data presented by Lowe et al.19

We found IgG3 more frequently than IgG2 in posttransplant sera rather than the expected order of the IgG subclass concentration IgG1, IgG2, IgG3, and IgG4.23 The evaluation of sequential sera allowed for detection of subclasses at different stages of the immune response. We hypothesize that like other diseases, such as membranous glomerulonephritis,24,25 development of AMR and its progression is related to subclass switching. There were also differences between patients with preformed DSA and de novo DSA, in which de novo DSA were primarily made up of IgG1 and IgG3 alone. Only 1 patient with de novo DSA presented AMR, and subsequently, allograft dysfunction. The first IgG detected was a class II DSA of IgG3 subclass with no C1q-binding ability in vitro. We subsequently detected all subclasses and C1q binding in the next serum, which presented AMR features in the tissue with minimal C4d deposition. It has been suggested that subclass switching occurs first from IgM to IgG3 and then to IgG1, IgG2, and IgG4. In many responses early antigen clearance would prevent the appearance of IgG2 and IgG4.12 IgG2 and IgG4 were detected, but only in combination with other subclasses, demonstrating an evolution of the immune response. The presence of IgG2 and IgG4 was shown in elutes of rejected renal allografts confirming sequential subclass switching.16 Moreover,

| Patient ID | Induction Therapy | Number days posttransplant | DSA profile | Biopsies (BANFF 2017 score) |
|------------|-------------------|-----------------------------|-------------|-----------------------------|
| 21         | T                 | 0                           | B*39:01 (IgG12) | Negative                   |
| 21         | T                 | 5                           | B*39:01 (1318-IgG1) | Negative                   |
| 21         | T                 | 10                          | B*39:01 (1318-IgG1) | Negative                   |
| 21         | T                 | 19                          | B*39:01 (1318-IgG1) | Negative                   |
| 21         | T                 | 390                         | B*39:01 (1318-IgG1) | Negative                   |
| 21         | T                 | 416                         | B*39:01 (1318-IgG1) | Negative                   |
| 21         | T                 | 751                         | B*39:01 (1318-IgG1) | Negative                   |

Induction therapy: N, none; T, thymoglobulin; I, anti-IL2mAb; P, prednisone; OKT3, MuronAb-CD3. Proteinuria: P+, 150-500 mg; P++, 1500-3000 mg; P+++ , >3000 mg. C4d: C4d+, minimal deposition (<10%); C4d++, focal deposition (10-50%); C4d+++ , diffuse (>50%).
| Patient ID | No. days posttransplant | DSA profile | C1q-binding | eGFR MDRD | Proteinuria | C4d | Biopsies (BANFF 2017 score) |
|------------|-------------------------|-------------|-------------|-----------|------------|-----|-----------------------------|
| 22         | 3                       | 0           | 0           | 0         | 45         |     |                             |
| 5          | 0                       | 0           | 0           | 0         | 66         |     |                             |
| 15         | 0                       | 0           | 0           | 0         | 57         |     |                             |
| 27         | 0                       | 0           | 0           | 0         | 76         |     |                             |
| 30         | 0                       | 0           | 0           | 0         | 87         |     |                             |
| 62         | DQB1*03:01 (IgM)        | 0           | 0           | 0         | 53         | P++ |                             |
| 90         | DR8 (1422-IgG3)         | 0           | 0           | 0         | 65         | P++ |                             |
| 182        | B35 (IgM) B53 (IgM)     | DQB1*03:01 (3020) | 0 | 0 | 59 | P++ | C4d+ | AMR (2; 12; v0; g1; c0; cg0; mm0; ah0; ptc1) |
| 432        | A1 (869-IgG13) A36 (339-IgG13) | A36 (827) DQB1*03:01 (11275) | 64 | P++ | | | |
| 687        | A1 (4164-IgG134) A36 (4408-IgG134) | DQB1*03:01 (2989-IgG134) | 37 | P++ | | | Allograft Loss |
| 23         | 10                      | 0           | 0           | 0         | 62         |     |                             |
| 13         | 0                       | 0           | 0           | 0         | 48         |     |                             |
| 20         | 0                       | 0           | 0           | 0         | 44         |     |                             |
| 27         | 0                       | 0           | 0           | 0         | 40         |     |                             |
| 1832       | DQB1*03:01 (2523)       | 0           | 0           | 0         | 70         |     |                             |
| 24         | 3                       | DG2/DQA1*02:01 (3130) | 0 | 0 | 51 | P++ | | |
| 5          | 0                       | 0           | 0           | 0         | 54         | P++ | | |
| 12         | 0                       | 0           | 0           | 0         | 71         |     |                             |
| 30         | 0                       | 0           | 0           | 0         | 65         |     |                             |
| 48         | DQ2/DQA1*02:01 (876)    | 0           | 0           | 0         | 31         |     |                             |
| 71         | 0                       | 0           | 0           | 0         | 46         |     |                             |
| 168        | DQ2/DQA1*02:01 (1398)   | 0           | 0           | 0         | 55         |     |                             |
| 365        | 0                       | 0           | 0           | 0         | 66         |     |                             |
| 580        | DQ2/DQA1*02:01 (1071)   | 0           | 0           | 0         | 61         |     |                             |
| 760        | DQ2/DQA1*02:01 (1190)   | 0           | 0           | 0         | 53         |     |                             |
| 1024       | 0                       | 0           | 0           | 0         | 41         |     |                             |
| 1189       | 0                       | 0           | 0           | 0         | 43         |     |                             |
| 1290       | 0                       | 0           | 0           | 0         | 31         |     |                             |
| 25         | 2                       | 0           | 0           | 0         | 37         | P+  |                             |
| 5          | 0                       | 0           | 0           | 0         | 42         | P+  |                             |
| 8          | 0                       | 0           | 0           | 0         | 59         | P+  |                             |
| 1717       | DQ5(1) (7601-IgG1)      | DQ5(1) (1276) | 71 |     | | | |
| 26         | 10                      | DQB1*02:01/DQA1*05:01 (2477-IgG2) | 0 | 0 | 31 | | | |
| 15         | DQB1*02:01/DQA1*05:01 (2916-IgG2) | 0 | 35 | P++ | | | |
| 30         | 0                       | 0           | 0           | 0         | 43         |     |                             |
| 34         | 0                       | 0           | 0           | 0         | 39         |     |                             |
| 63         | 0                       | 0           | 0           | 0         | 41         |     |                             |
| 92         | 0                       | 0           | 0           | 0         | 39         |     |                             |
| 187        | 0                       | 0           | 0           | 0         | 43         |     |                             |

Continued next page
there seemed to be a correlation between the occurrence of AMR and the expansion of complement-fixing to noncomplement fixing DSA.\textsuperscript{15}

Another important observation was that despite the high prevalence of IgG1 subclass, C1q-binding was found in less than 15% of the sera tested, and only in the presence of high MFI IgG1 or, most frequently, in the presence of a combination of all 4 subclasses. As recently shown by Schaub et al, C1q binding is related to anti-HLA antibody density, and furthermore, a great number of HLA antibodies found in sera that do not induce C1q-binding in vitro do, however, contain C-binding IgG subclasses (IgG1 and IgG3).\textsuperscript{11} The presence of all 4 subclasses can indicate a higher antibody concentration, thus providing higher density for C1q-binding. The presence of a high concentration of antibodies sequentially binding to antigens leads to hexamer formation that binds to C1q with higher avidity than monomeric IgG, inducing activation of the complement system.\textsuperscript{26}

Antibody-mediated rejection is one of the leading causes of allograft failure.\textsuperscript{27} Although the presence of DSA implies risk for AMR, long-term survival of patients with DSA has been reported.\textsuperscript{28,29} Despite our small population number, we observed that most patients diagnosed with AMR presented an increase in MFI, changes in IgG subclasses, and C1q-binding DSA. Loupy et al reported that patients with donor specific C1q-binding DSA present lower allograft survival
within 5-years of follow-up when compared with non-complement-binding DSA and non-DSA patients. Moreover, pediatric kidney recipients with de novo C1q DSA reactivity showed higher rates of rejection and increased risk of allograft loss. The presence of a combination of IgG subclasses with C1q-binding DSA could also be related to AMR occurrence, and diminished allograft function. It was previously shown that liver transplant patients with IgG subclass combinations containing IgG3 presented allograft survival that was significantly lower than patients who presented a single IgG subclass. Changes in the profile of antibodies during posttransplant follow-up demonstrate the importance of close anti-HLA DSA monitoring after transplantation. Moreover, the presence of different subclasses can indicate distinct phenotypes of AMR. IgG4-containing DSA was associated with features of subclinical AMR, whereas IgG3-containing DSA was associated with an acute form of AMR and represented a greater risk for allograft loss.

Our study presents certain limitations, including the small population and testing at different time points with different lots of SAB for DSA. In addition, we were unable to evaluate denatured antigens to confirm the IgG subclass negative reactions.

Recently, 3 different stages of AMR were described by molecular diagnosis of kidney biopsies; early-stage AMR, fully
formed AMR, and late-stage AMR. Understanding the natural evolution of anti-HLA antibodies during the process of AMR and correlating to its stages is essential to define treatment. Although our data are not conclusive, we demonstrate that there is a progression of the immune response and it can begin at the sensitization cause and may consequently lead to allograft loss. These insights should be considered if patients are not consistently monitored for anti-HLA antibodies after transplantation. Thus, a single time point evaluation after transplantation may not be sufficient to provide all the information needed to make clinical decisions.

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