LETTER TO THE EDITORS

Anti-HLA and anti-SARS-CoV-2 antibodies in kidney transplant recipients with COVID-19

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To the Editors,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and its associated illness coronavirus disease 2019 (COVID-19) have severely affected organ transplant recipients, with all-cause mortality rates exceeding 20% [1,2]. Although no clear guidelines exist on how to adjust immunosuppression, most centers reduce anti-rejection drugs to facilitate the T- and B-cell response against the virus. However, this maneuver may unleash the anti-donor immune response as well. Even if immunosuppression is not modified, infection episodes per se promote a proinflammatory state that may lead to an increased risk of rejection or of anti-HLA antibody development [3]. In most of the reports published so far, acute rejection episodes were uncommon during COVID-19 infection despite immunosuppression reduction [4]. However, no study has formally addressed the relationship between COVID-19 and the development of anti-HLA antibodies.

We recently showed that kidney transplant recipients with COVID-19 display a broad activation of B-cell subsets, together with detectable serum anti-SARS-CoV-2 IgM and IgG as early as 10 days after the onset of clinical symptoms [5], suggestive of a broad activation of the humoral response. Herein, we tested the hypothesis that COVID-19 associates with the development of anti-HLA antibodies in kidney transplant recipients.

The transplant center of the Parma University Hospital, Parma, Italy, actively follows up approximately 800 kidney transplant recipients. From March 1 to December 2, 2020, 17 of these patients were diagnosed with symptomatic SARS-CoV-2 infection (confirmed by RT-PCR testing). Thirteen patients were admitted at Parma University Hospital, Parma, Italy, and four were followed up as outpatients. Fourteen patients survived for more than 30 days after admission, and 7 of them had available serum that was collected at 3 months postinfection. In our center, kidney transplant recipients undergo regular measurements of donor-specific antibodies (DSA) and panel-reactive antibodies (PRA) and all the seven included patients had their measurements done no more than three months prior to infection. We used the sera collected at 3 months postinfection to measure anti-HLA (by Luminex Technology, One Lambda) and anti-SARS-CoV-2 IgM and IgG antibodies (JusChek; Acro Biotech, Rancho Cucamonga, CA, USA).

At diagnosis, all patients showed pneumonia and signs of systemic inflammations, such as elevated C-reactive protein (CRP), IL-6, and D-Dimer (Table 1). During COVID-19, six patients underwent reduction in immunosuppression that was resumed at discharge (Table 1). Two patients developed acute kidney injury, but serum creatinine fully recovered at 3 months after COVID-19 infection (Table 1). Contrary to our hypothesis, none of the patients developed anti-HLA antibodies, but all of them had detectable anti-SARS-CoV-2 IgM and IgG (Table 1). One patient (patient 2) had anti-donor antibodies before COVID-19, but neither their MFI nor their HLA specificities increased after COVID-19.

To the best of our knowledge, this is the first series of patients with serial anti-HLA antibody measurements. Despite significant reduction in immunosuppression in most of our patients and a systemic proinflammatory state associated with COVID-19, no patients developed signs of increased alloimmune response. This finding is in line with prior evidence that COVID-19 does not associate with disease relapses even in patients with autoimmune disease.
|                | Pt. 1 | Pt. 2 | Pt. 3 | Pt. 4 | Pt. 5 | Pt. 6 | Pt. 7 |
|----------------|-------|-------|-------|-------|-------|-------|-------|
| Age (years)    | 41    | 48    | 44    | 73    | 41    | 60    | 40    |
| Sex            | Male  | Male  | Female| Male  | Male  | Male  | Male  |
| Ethnicity      | African| Caucasian| Caucasian| Caucasian| Caucasian| Caucasian| Caucasian|
| Native nephropathy | Unknown| CAKUT| IgAN| ADPKD| IgAN| ADPKD| Chronic GN|
| Time after transplant (months) | 21    | 300   | 93    | 300   | 108   | 15    | 48    |
| Transplant type | DD    | DD    | DD    | DD    | DD    | LD, ABO-i| LD, ABO-i|
| HLA mismatches | A, B, DR, DQ | 2,1,1,0| 2,2,0,0| 0,1,0,0| 1,1,0,0| 0,2,2,2| 1,1,0,0|
| Prior pregnancies | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| Transplant number | 1     | 1     | 1     | 1     | 1     | 1     | 1     |
| Pretransplant class I PRA (%) | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| Pretransplant class II PRA (%) | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| Induction therapy | Basiliximab| None| Basiliximab| Rituximab| Basiliximab| Basiliximab| Basiliximab|
| Maintenance therapy | Tac, MMF, steroids | Tac, steroids| CsA, EVR, steroids| Tac, MMF, steroids| Tac, MMF, steroids| Tac, MMF, steroids| Tac, steroids|
| Baseline s-creatinine (mg/dl) | 1.1 | 2.1 | 0.8 | 1.1 | 1.6 | 1.0 | 2.3 |
| Prior rejection episodes | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Flu vaccination 2019–2020 | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| COVID-19 pneumonia (CT lung involvement) | Yes | Yes | Yes | Yes | Yes | Yes | Yes |
| Max CRP (mg/l) | 212 | 88  | 146  | NA    | NA    | NA    | 171   |
| Max IL-6 (ng/ml) | 223 | 37.04 | 1400  | NA    | NA    | NA    | 183   |
| Max D-dimer (ng/ml) | NA | 847 | NA  | 847 | NA  | 1079 | NA    |
| AKI during COVID-19 (peak s-creatinine mg/dl) | Yes | No | No | No | No | No | Yes |
| Immunosuppression changes during COVID-19 (days) | CNI Withdrawn (13) | Unchanged | Reduced (21) | Reduced (28) | Withdrawn (28) | Unchanged | Withdrawn (10) |
| MMF/mTORI | Withdrawn (25) | Unchanged | Withdrawn (67) | Withdrawn (41) | Withdrawn (40) | Unchanged | Unchanged |
| Steroids | Increased (28) | Increased (28) | Unchanged | Unchanged | Unchanged | Increased (28) | Increased (15) |
Table 1. Continued.

|                          | Pt. 1 | Pt. 2 | Pt. 3 | Pt. 4 | Pt. 5 | Pt. 6 | Pt. 7 |
|--------------------------|-------|-------|-------|-------|-------|-------|-------|
| Maximal steroid daily dose | MP 40 mg | MP 16 mg | – | – | MP 16 mg | – | MP 40 mg |
| Anti-viral therapy       | No    | Darunavir-cobicistat | No | No | No | No |
|                          |       | Anti-A2, MFI: 4971 |       |       |       |       |
| Pre-COVID DSA            | 0     | 0     | 0     | 0     | 0     | 0     |
| Pre-COVID class I PRA (%)| 0     | 41    | 0     | 0     | 0     | 0     |
| Pre-COVID class II PRA (%)| 0   | 0     | 0     | 0     | 0     | 0     |
| Post-COVID s-creatinine (mg/dl) | 0.9 | 1.9   | 0.8   | 1.1   | 1.7   | 1.0   |
| Post-COVID DSA           | No    | Anti-A2, MFI: 3987 | No | No | No | No |
| Pre-COVID class I PRA (%)| 0     | 41    | 0     | 2     | 0     | 0     |
| Pre-COVID class II PRA (%)| 0   | 5     | 0     | 0     | 80    | 0     |
| Anti-SARS-CoV-2 IgG      | +     | +     | +     | +     | +     | +     |
| Anti-SARS-CoV-2 IgM      | +     | +     | +     | +     | +     | +     |

ABO-I, ABO-incompatible; ADPKD, autosomal dominant polycystic kidney disease; AKI, acute kidney injury; ARPKD autosomal recessive polycystic kidney disease; CAKUT, Congenital Anomalies of the Kidneys and of the Urinary Tract; Chronic GN, chronic glomerulonephritis; CNI, calcineurin inhibitor; CsA, cyclosporine; CT, computerized tomography; DD, deceased donor; DSA, donor-specific antibody; EVR, everolimus; IgAN, IgA nephropathy; LD, living donor; MFI, mean fluorescent intensity; MMF, mycophenolate mofetil; MP, methylprednisolone; MPGN, membranoproliferative glomerulonephritis; NA, not available; PRA, panel-reactive antibody (cutoff MFI 3000); s-creatinine, serum creatinine; TAC, tacrolimus.

Pre-COVID DSA and PRA were measured no more than 3 months prior to infection. Post-COVID antibodies were measured at 3 months after infection.
conditions [6]. The mechanisms behind this unexpected phenomenon are unknown and worth investigating.

Our analysis is still preliminary, and we cannot exclude that COVID-19 does in fact increase the risk of DSA development. Although multiple series have reported a relatively low risk of acute rejection in kidney transplant recipients with COVID-19, careful monitoring of alloimmune response during COVID-19 should still be advised, especially in transplant recipients undergoing significant reduction in immunosuppressive therapy.

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**Conflict of interest**

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