

# COVID-19 Infection Does Not Alter HLA Antibody Reactivity



Aileen C. Johnson<sup>1</sup>, Christian P. Larsen<sup>1</sup>, Howard Gebel<sup>2</sup> and Robert Bray<sup>2</sup>

<sup>1</sup>Department of Surgery, School of Medicine, Emory University, Atlanta, Georgia, USA; and <sup>2</sup>Department of Pathology, School of Medicine, Emory University, Atlanta, Georgia, USA

**Correspondence:** Robert Bray, Department of Pathology, School of Medicine, Emory University Hospital, 1364 Clifton Road NE, Atlanta, Georgia, 30322 USA. E-mail: [rbray@emory.edu](mailto:rbray@emory.edu)

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## INTRODUCTION

The requirement for immunosuppression in organ transplant recipients, particularly suppression of the T cell response, has made this population especially vulnerable to prolonged and severe infections during the ongoing SARS-CoV-2 pandemic.<sup>1</sup> Acknowledgment of this increased risk led to the reduction of deceased-donor organ transplantation, and for many programs, complete cessation of living-donor operations during the early periods of the COVID-19 pandemic.<sup>2,3</sup> Nevertheless, for transplant recipients, the risk of contracting viral disease does not exist in a vacuum. Solid organ transplant candidates have a number of characteristics that increase their morbidity and mortality risk above that of the general population. Delineating the presentation and effects of SARS-CoV-2 in this cohort is critical to understanding the new landscape of organ transplantation in the context of an ongoing pandemic.<sup>4-6</sup>

Of particular concern in both transplant recipients and candidates is the possibility of heterologous immunity after infection, with cross-reactive T cell receptor specificity leading to unanticipated allosensitization. Virus-specific T cells that harbor cross-reactivity to human leukocyte antigen (HLA) antigens have been identified for Epstein-Barr virus, cytomegalovirus, varicella zoster virus, HIV, herpes simplex virus 2, and Influenza.<sup>55-511</sup> Whereas T cell cross-reactivity is not easily determined, HLA antibodies are routinely tested in this population and provide a useful surrogate endpoint. A recent report describes the presence of HLA antibodies in the convalescent serum of male patients without any known allosensitizing events.<sup>7</sup> In response to this, the HLA antibody profile among a small series of waitlist candidates who developed symptomatic COVID-19 was

assessed.<sup>8</sup> The study was encouraging for the following reasons: none of the patients developed *de novo* HLA antibodies, and pre-existing HLA antibodies in highly sensitized candidates did not increase. Nevertheless, larger numbers are needed to determine whether repeat HLA antibody testing prior to kidney transplantation is necessary after COVID-19 infection.

The kidney transplant waitlist at our institution, Emory University Hospital, consists of approximately 2000 patients across southeastern United States. Patients send in monthly serum samples for panel reactive antibody (PRA) testing to maintain updated data on sensitization status. We aimed to use historical serum samples from these patients to determine the prevalence of SARS-CoV-2 seropositivity among our kidney transplant waitlist population, across the state of Georgia, during the summer of 2020. In addition, we aimed to monitor the duration of humoral immunity in the waitlist population, and to evaluate SARS-CoV-2 seropositive patients for changes in HLA alloreactivity.

## RESULTS

### Patient Demographics

The Emory kidney transplant waitlist consisted of 2010 candidates overall with 1188 hemodialysis patients, 390 peritoneal dialysis patients, 234 candidates not on dialysis, and 198 on home hemodialysis (Supplementary Figure S1a). Four hundred dialysis-dependent candidates were selected from high-risk counties, defined as having a case rate above the average (2229/100,000 residents, August 2020). Of the 400 patients tested, 28 (7%) were positive (Supplementary Methods, Supplementary Figure S1b). Patients who tested positive had a lower mean age compared to those who tested negative (44 years vs. 55 years,  $P < 0.01$ ,

**Table 1.** Demographic analysis of the tested waitlist candidates

Patient demographics by SARS-CoV-2 serology			
Characteristic	Negative, <i>n</i> = 374 <sup>a</sup>	Positive, <i>n</i> = 28 <sup>a</sup>	<i>P</i> -value <sup>b</sup>
Age, mean (SD)	55 (13)	44 (10)	< 0.001
Sex, <i>n</i> (%)			0.085
Female	114 (36%)	4 (17%)	
Male	200 (64%)	20 (83%)	
Race, <i>n</i> (%)			0.8
African-American	192 (61%)	16 (67%)	
Caucasian	76 (24%)	4 (17%)	
Other	46 (15%)	4 (17%)	
Time since referral (yrs), mean (SD)	4.05 (2.23)	3.84 (2.27)	0.6
Dialysis method			0.8
Hemodialysis	270 (72%)	19 (68%)	
Peritoneal dialysis	104 (28%)	9 (32%)	

<sup>a</sup>Statistics presented: mean (SD), *n* (%).

<sup>b</sup>Statistical tests performed: Wilcoxon rank-sum test,  $\chi^2$  test of independence, Fisher's exact test.

Clinical and demographic variables were compared between waitlist candidates who tested positive and those who tested negative.

Table 1). This was the only significant difference between groups, who were majority male (65%), Black (62%), and approximately 4 years from their initial referral. There was a similar distribution of hemodialysis and peritoneal dialysis between groups. Spatial analysis of these cases did not demonstrate any evidence of geographic clustering (Supplementary Results).

Patients who tested positive were interviewed by phone, and 19 of 28 patients responded. Ten of the seropositive patients who responded were aware of prior COVID infection, and most of them endorsed a symptom history (9/10) along with a positive test (10/10). As of April 2021, 9 of 19 patients were vaccinated, and the rest expressed interest in future vaccination.

## Serologic Response

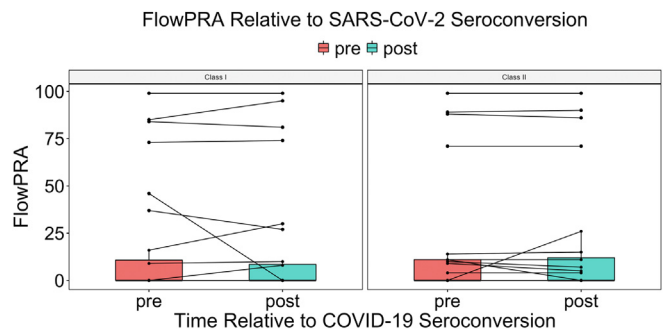
### COVID Serology

Antibodies to full-spike were nearly ubiquitous in patients who tested positive, with corresponding bimodal distribution of S1 and S2 antibodies. Trimmed mean fluorescent intensity was compared between positive patients who were symptomatic and asymptomatic (Supplementary Figure S2b). Across all SARS-CoV-2 proteins tested, symptomatic patients appeared to have a higher average trimmed mean fluorescent intensity than asymptomatic patients, however this difference was not statistically significant for any individual protein.

Follow-up testing was performed in April 2021. All patients maintained seropositivity over the course of available samples, which is a mean of 220 days follow-up (Supplementary Figure S2c). Only samples prior to patient vaccination were tested.

### Panel Reactive Antibodies

Transplant waitlist candidates who tested positive for SARS-CoV-2 antibodies were examined for changes in



**Figure 1.** PRA testing of SARS-CoV-2 seropositive patients. For SARS-CoV-2 seropositive patients, the closest FlowPRA testing prior to seroconversion was compared to the nearest FlowPRA testing subsequent to seroconversion. Paired *t*-test failed to detect any significant increase in FlowPRA chronologically related to SARS-CoV-2 seroconversion. This was true for nonsensitized, intermediate, and highly sensitized patients. FlowPRA, flow panel reactive antibody.

PRA at the time of seroconversion. Each SARS-CoV-2 positive sample was compared to the sample immediately prior to seroconversion. Details of prior sensitization exposures along with individual demographics and calculated PRA levels were compiled (Supplementary Table S1). Paired sample *t*-test demonstrated no significant increase in FlowPRA values before and after seroconversion (Figure 1). Across highly sensitized candidates, there was no consistent change in HLA antibody specificity as evaluated by single antigen bead testing. Patients were examined on an individual level, and there was no sustained change in antibody specificity associated with seroconversion.

## DISCUSSION

The COVID-19 pandemic has disproportionately impacted immunosuppressed or immunocompromised populations, including solid organ transplant recipients.<sup>1-6</sup> Though most transplant programs have instituted vaccination requirements for waitlist candidates, the sequelae of natural infection is still relevant. We aimed to add to the current literature surrounding the duration of the humoral response in the immunodysregulated chronic kidney disease population and to examine HLA antibody positive patients for evidence of heterologous immunity related to SARS-CoV-2 infection.

Of the 400 waitlist candidates tested, 28 tested positive for antibodies against SARS-CoV-2. Testing of serum samples immediately preceding and following SARS-CoV-2 seroconversion failed to detect a significant increase in PRA values subsequent to SARS-CoV-2 infection. In addition, individual antibody specificities did not demonstrate any consistent or sustained changes after infection. Seroconversion does not appear to be a significant risk factor for development of donor specific antibodies in this cohort of patients. Whereas

larger sample sizes are needed to fully understand the effects of SARS-CoV-2 on the alloimmune response, these results are reassuring and add to the prior literature that has, to date, only examined calculated PRA values in nonsensitized and a small number ( $n = 4$ ) of highly sensitized patients.<sup>7</sup>

This study has a number of limitations, because it is a single-institution, retrospective study with relatively small sample size. The study only included patients with natural immunity to SARS-CoV-2 acquired prior to vaccine release and did not examine the effects of subsequent vaccination on antibody profiles. The intermittent nature of PRA testing among waitlist candidates also allows the possibility that a transient increase in alloreactivity may occur without being captured by this dataset.<sup>9</sup> In addition, patients were not followed up post-transplant to monitor the effects of immunosuppression. Finally, the immune response to SARS-CoV-2 was quantified one-dimensionally, without consideration for cell-mediated immunity, and without assessment of the neutralizing capability of antibodies that were detected.

Although this was a small sample size and retrospective study, the results add to the developing body of knowledge surrounding transplant candidates and recipients during the ongoing COVID-19 pandemic. Hemodialysis patients were at no greater risk than peritoneal dialysis patients of seroconversion. Rather, younger age was the only significant difference between cohorts. Seroconversion was not associated with any increase in PRA. Waitlist candidates demonstrated a sustained natural humoral immune response over a mean of 220 days follow-up. These findings reinforce the importance of vaccination in the waitlist population, in whom a more robust immune response is possible. More research is needed to understand the effect of SARS-CoV-2 infection on the alloimmune response of transplant candidates in order to guide their calculated PRA and SARS-CoV-2 monitoring. Whereas testing for active SARS-CoV-2 infection by polymerase chain reaction test upon receipt of an organ offer remains necessary to avoid infection in the setting of compromised protective immunity, the current data does not suggest any increased risk of allo-sensitization in these individuals. Therefore, we recommend continuing with standard calculated PRA testing for candidates on the kidney transplant waitlist, without any increase in frequency in the setting of SARS-CoV-2 infection.

## DISCLOSURE

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All the other authors declared no competing interests.

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## Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Supplementary Results.

Supplementary References.

**Figure S1.** Geographic analysis of SARS-CoV-2 serology in Georgia kidney transplant waitlist candidates.

**Figure S2.** Analysis of SARS-CoV-2 seropositive patients.

**Table S1.** Details of seropositive patients and pre/post seroconversion FlowPRA testing

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