Early Antibody Responses Associated with Survival in Hospitalized COVID-19 Patients

Zhou, Zhao-Hua*, CDER/OPQ; Dharmarajan, Sai, CDER/OTS; Lehtimaki, Mari, CDER/OPQ; Kirshner, Susan L., CDER/OPQ; Kozlowski, Steven*, CDER/OPQ **Members of Office of Pharmaceutical Quality Center of Excellence for Infectious Diseases and Inflammation at CDER, FDA. Zhaohua.zhou@fda.hhs.gov; Steven.Kozlowski@fda.hhs.gov*

Abstract

Neutralizing antibodies to the SARS CoV-2 spike proteins have been issued Emergency Use Authorizations and are a likely mechanism of vaccines to prevent COVID-19. However, benefit of treatment with monoclonal antibodies has only been observed in clinical trials in outpatients with mild to moderate COVID-19 but not in patients who are hospitalized and/or have advanced disease. To address this observation, we evaluated the timing of anti SARS-CoV-2 antibody production in hospitalized patients with the use of a highly sensitive multiplexed bead-based immunoassay allowing for early detection of antibodies to SARS-CoV-2. We found that significantly lower levels of antibodies to the SARS-CoV-2 spike protein in the first week after symptom onset were associated with patients who expired as compared to patients who were discharged. We also developed a model, based on antibody level trajectory, to predict COVID 19 outcome that is compatible with greater antibody benefit earlier in COVID 19 disease.

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Introduction

SARS-COV-2 has led to more than 100 million cases of COVID-19 globally with high morbidity and mortality. There were over 485,000 deaths in the United States as of February 2021. Neutralizing antibodies to the SARS COV-2 spike protein are candidates for therapeutics. There have been three Emergency Use Authorizations issued for such antibodies and combinations, with many others in the development pipeline. Induction of neutralizing antibodies in immunized people is also likely to be a mechanism of vaccines to prevent COVID-19.

COVID-19 has an inflammatory phase associated with Acute Respiratory Distress Syndrome and severe disease. This phase has macrophage and monocyte activation, cytokine release, may involve viral transmission that is not dependent on the ACE2 viral receptor and may be enhanced by binding of SARS-CoV-2 specific antibodies to cells via Fc receptors. A host with activated immune and endothelial cells may also be more sensitive to antibody-virus immune complex- associated inflammation.

Some studies evaluating neutralizing antibody treatment in patients who are hospitalized and/or have advanced disease have been stopped due to lack of benefit. Current EUAs for neutralizing antibody are limited to outpatients with mild to moderate disease who are at high risk for progressing to severe disease. In contrast to findings of clinical benefit in outpatients early in disease progression with mild to moderate COVID-19, some studies in hospitalized patients have correlated high levels of neutralizing antibody and “early” seroconversion (8-16 days after onset as defined by these studies) with severity of disease for both SARS-Cov-1 and SARS-CoV-2.

To address this apparent gap, we evaluated the timing of anti SARS-CoV-2 antibody generation in patients who survived and in patients who expired with the use of a highly sensitive multiplexed bead-based immunoassay method allowing for earlier detection (within days of symptom onset) of antibody to SARS-CoV-2. We also developed a model that was predictive of outcome based on the trajectory of antibody levels over time.

Materials and Methods

Patient samples
Deidentified samples were obtained from Shady Grove Washington Adventist Hospital. Patients were hospitalized and diagnosed with COVID 19 by a PCR method. Onset of patient symptoms was in April through May 2020. Serial samples from 33 patients were evaluated; 11 of the patients expired and 22 were discharged. The patient manifestations, demographics and outcomes were blinded before evaluating sample antibody profiles detection and unblinded for correlation with outcomes after antibody levels were determined.

Multiplexed SARS CoV2 Antigen Beads Array
Multiplexed SARS CoV2 recombinant antigen-coupled target beads and BSA control beads (Figure 1) were prepared using sulfo-SMCC chemistry with Functional Bead Conjugation Buffer Set (BD Biosciences) according to manufacturer's instructions. Binding specificity was confirmed by free antigen inhibition of detection signal. The multiplexed beads array method was demonstrated to sensitively and specifically detect antibody signals in rabbit anti-SARS-CoV-2 immune serum, convalescent human COVID-19 serum, and serum samples from patients with PCR-confirmed COVID19.

Antibody Level Analysis and Predictive Modeling for Outcome
Log2 transformed antibody levels over time, starting from the earliest sample collection date after onset, in the expired and discharged patients were plotted. The trajectories of antibody levels are displayed for hospitalized patients who expired and who were discharged alive.

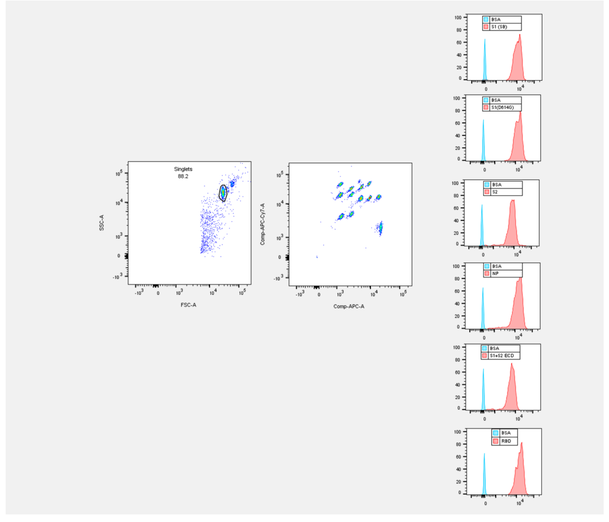

Figure 1. Multiplexed beads array for COV2 antibody and flow cytometry analysis. The singlet beads population were gated based on FSC-SSC display, followed by compensated APC-APC-Cy7 display to show different SARS-CoV2 antigen component-coated target and BSA-control beads. Histograms display overlays of antibody binding signals to antigen-target beads vs BSA beads as indicated.

Results

There were 4 to 16 collections per patient over a range of 0 to 42 days post-symptom onset. Antibody levels were plotted by time based on days post-symptom onset in the expired and discharged patients for the SARS-CoV-2 antigens. The trajectories of antibody levels appear to be different between hospitalized patients who expired and those who were discharged. Specifically, patients in the expired group seem to have lower antibody levels in the first week to 12 days after onset, after which values in the groups appear to converge (Figure 2 & 3).

We also fit a linear mixed model, accounting for patient-level correlation, to the data to model the antibody response using time, expired/discharged status, and the interaction between time and expired/discharged status as predictors. The mean antibody levels, at week one, were estimated to be significantly lower in the expired group than in the discharged group (Table 1). The mean increase from week one was also found to be significantly higher in the expired group at week two for both S1 and S2 spike proteins and at after week 2 for S1 spike protein (Table 1). These findings suggest that later increases in antibody response are unlikely to mitigate the negative effects of an initial slow response.

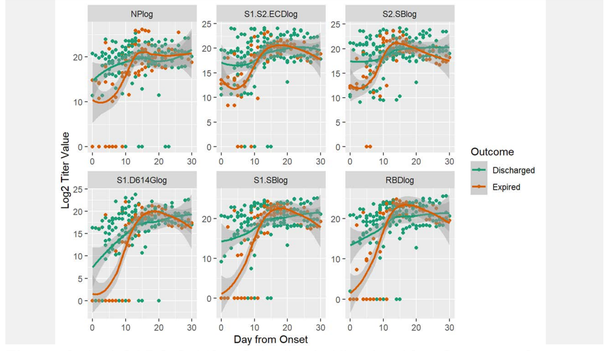

Figure 2. Anti-SARS CoV2 IgG Antibody Kinetics After Symptom Onset

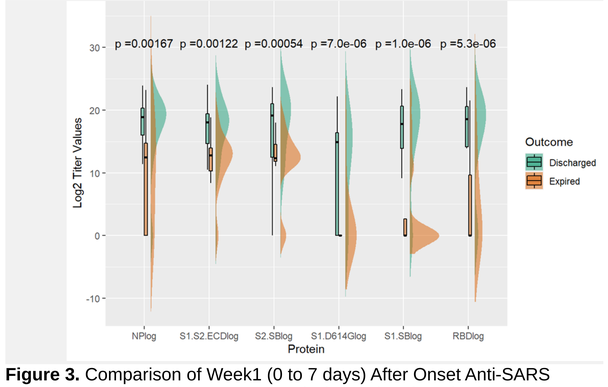

Figure 3. Comparison of Week1 (0 to 7 days) After Onset Anti-SARS COV2 IgG Antibody Titers Between Discharged and Expired Groups.

Table 1. Estimates from Linear Mixed Model (Model 1) of Anti-S1 and Anti-S2 IgG Titer Values (log) by Time and Outcome

	Antigen Targets					
	S1			S2		
	Expired	Discharged	Difference	Expired	Discharged	Difference
Wk1	6.65	13.5	6.86*	12.8	16.2	3.41*
Wk2	16.7	18.5	1.72	19.1	19.2	0.09
Beyond Wk2	17.9	20.6	2.66	18.5	20.4	1.91
Change from Wk1 to Wk2	10.1	4.95	-5.14*	6.32	2.99	3.33*
Change from Wk1 to beyond Wk2	11.2	7.04	-4.20*	5.73	4.23	1.50

*Statistically Significant Differences in IgG Titer Values between Expired and Discharged groups at α=0.05.

Discussion and Conclusion

We observed that lower levels of early antibody responses to several spike protein antigens correlated with poor outcomes. In addition, a joint model (Model 2) was developed, based on antibody trajectory, that was predictive of poor outcomes. Although early antibody detection is seen in other studies many assay formats have limited sensitivity and may not detect early antibody, at low levels. Bead-based immunoassays can be highly sensitive allowing for early antibody detection. In addition, the bead-based assay described here had a similar binding pattern to other assays using ten panel samples in the First WHO SARS-COV-2 International Standard harmonization exercise.

Although the mechanisms for these findings need further elucidation, these results align well with clinical studies on therapeutic antibodies. These antibodies show benefit in outpatients with mild to moderate COVID-19, before further disease progression whereas benefit of antibody treatment has not been observed in clinical trials in patients hospitalized due to COVID-19, and may be associated with worse clinical outcomes when administered to hospitalized patients with COVID-19 requiring high flow oxygen or mechanical ventilation. The importance of antibody early in disease also fits with data on early treatment using convalescent plasma but not later treatment and also with the initial evaluations of vaccines that generate neutralizing antibodies. The exact stage of disease progression that may have clinical benefit from antibody therapy needs further study and may be dependent on the nature of the antibodies, additional interventions and other factors.

In developing an early antibody response as a predictor of outcomes, it is important to have sensitive method to reliably detect antibody early in the course of disease, such as the flow cytometry bead-based method we describe. Such an assay with appropriate modeling could be studied as an approach to predict the clinical course of patients and aid in selection of the appropriate therapeutic interventions.