Ultra-sensitive Serial Profiling of SARS-CoV-2 Antigens and Antibodies in Plasma to Understand Disease Progression in COVID-19 Patients with Severe Disease

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Keywords: SARS-CoV-2, viral antigen, serological, longitudinal plasma samples, single molecule arrays

List of abbreviations: Coronavirus disease (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), reverse transcription polymerase chain reaction (RT-PCR), nasopharyngeal (NP), Single Molecule Array (Simoa), S1-S2 extracellular domain (spike), nucleocapsid (N), receptor binding domain (RBD)
Abstract:

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 21 million people worldwide since August 16, 2020. Compared to PCR and serology tests, SARS-CoV-2 antigen assays are underdeveloped, despite their potential to identify active infection and monitor disease progression.

Methods: We used Single Molecule Array (Simoa) assays to quantitatively detect SARS-CoV-2 spike, S1 subunit, and nucleocapsid antigens in the plasma of coronavirus disease (COVID-19) patients. We studied plasma from 64 COVID-19 positive patients, 17 COVID-19 negative patients, and 34 pre-pandemic patients. Combined with Simoa anti-SARS-CoV-2 serological assays, we quantified changes in 31 SARS-CoV-2 biomarkers in 272 longitudinal plasma samples obtained for 39 COVID-19 patients. Data were analyzed by hierarchical clustering and were compared to longitudinal RT-PCR test results and clinical outcomes.

Results: SARS-CoV-2 S1 and N antigens were detectable in 41 out of 64 COVID-19 positive patients. In these patients, full antigen clearance in plasma was observed a mean ± 95%CI of 5 ± 1 days after seroconversion and nasopharyngeal RT-PCR tests reported positive results for 15 ± 5 days after viral antigen clearance. Correlation between patients with high concentrations of S1 antigen and ICU admission (77%) and time to intubation (within one day) was statistically significant.

Conclusions: The reported SARS-CoV-2 Simoa antigen assay is the first to detect viral antigens in the plasma of COVID-19 positive patients to date. These data show that SARS-CoV-2 viral antigens in the blood are associated with disease progression, such as respiratory failure, in COVID-19 cases with severe disease.
Main Text:

Introduction

The current pandemic of coronavirus disease (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has resulted in over 21,000,000 confirmed cases globally and over 167,000 deaths in the United States alone, as of August 16, 2020. (1) While reverse transcription polymerase chain reaction (RT-PCR) tests remain the gold standard for diagnosing COVID-19, RT-PCR tests do not provide adequate information on progression of the disease. Furthermore, RT-PCR tests can give positive results for several weeks after a patient has seroconverted or recovered. (2) Serological tests can identify individuals who have mounted an immune response but cannot necessarily be used for monitoring disease in the early stages of infection. (3–5) SARS-CoV-2 antigen assays can complement PCR and serological tests by identifying active infection or monitoring disease progression by measuring viral antigens in biofluids. Currently there are two FDA approved SARS-CoV-2 antigen tests that detect nucleocapsid in nasopharyngeal (NP) swabs. (6,7) However, both tests provide qualitative results and may only detect viral antigen within the first five days of symptom onset. Quantitative and ultra-sensitive SARS-CoV-2 antigen assays could enable detection of viral antigens in blood, saliva, or NP swabs and, in combination with serological assays, could enable analysis of COVID-19 progression from early infection to seroconversion.

To address the need for a quantitative antigen assay, we developed ultra-sensitive Single Molecule Array (Simoa) SARS-CoV-2 antigen assays for S1, S1-S2 extracellular domain (spike), and nucleocapsid (N). The ultra-sensitivity of Simoa enables detection of SARS-CoV-2 antigens in the plasma of COVID-19 positive patients. Additionally, Simoa provides a dynamic range that allows quantification of antigens over a concentration range of four orders of
magnitude. This precise quantification is advantageous for capturing the wide range of antigen concentrations in COVID-19 patient plasma throughout the course of hospitalization. We combined our Simoa SARS-CoV-2 antigen assays with previously developed Simoa serological assays to monitor SARS-CoV-2 antigens and anti-SARS-CoV-2 immunoglobulins in longitudinal plasma samples of COVID-19 patients. These measurements provide direct evidence of the inverse correlation between anti-SARS-CoV-2 antibody production and viral antigen clearance from plasma, which provides a unique view of viral infection and immune response from the beginning of hospitalization through recovery or death.

**Methods:**

**Plasma Samples**

All plasma was collected in purple top K2-EDTA tubes and centrifuged at 2,000 × g at 4°C for 10 min prior to analysis. COVID-19 positive and negative samples were obtained from adult patients presenting to Brigham and Women’s Hospital or Massachusetts General Hospital. We received 17 samples from patients who tested negative for SARS-CoV-2 using NP RT-PCR. We received 64 samples from patients who tested positive for SARS-CoV-2 using NP RT-PCR. We received 34 pre-pandemic samples, defined by a collection date before October 1, 2019, from the Mass General Brigham Biobank. 20 pre-pandemic samples were from healthy patients and 14 pre-pandemic samples from sick patients with upper respiratory infections, bacterial pneumonia, viral pneumonia, or unspecified virus positive.

Serial timepoint clinical samples were obtained from patients admitted to Massachusetts General Hospital (n= 67 samples from 19 individual patients) and Brigham and Women’s Hospital (n= 205 samples from 24 patients) diagnosed as SARS-CoV-2 positive by a NP RT-PCR. On average, seven serial samples per patient were taken 0-30 days after the first SARS-CoV-2
positive NP RT-PCR. All plasma samples were collected under approval of the Mass General Brigham Institutional Review Board for Human Subjects Research.

**Simoa assays**

Antigen Assays

Preparation of viral antigen assay Simoa reagents is in the online *Supplemental Materials*. Simoa assays were performed on an HD-X Analyzer (Quanterix) in an automated three-step assay format according to the manufacturer’s instructions and as previously described. (8) Plasma samples were diluted 8-fold in Homebrew Detector/Sample Diluent (Quanterix) with Halt Protease Inhibitor Cocktail (ThermoFischer Scientific) and EDTA. Detector antibodies were diluted in Homebrew Detector/Sample Diluent to 0.3 µg/mL, and streptavidin-β-galactosidase (SβG) concentrate (Quanterix) was diluted to 150 pmol/L in SβG Diluent (Quanterix). Antibody-conjugated capture beads were diluted in Bead Diluent, with a total of 500,000 beads per reaction (125,000 S1 beads, 125,000 S2 beads, and 250,000 647 nm dye-encoded helper beads for the S1/S2 multiplex assay, and 125,000 nucleocapsid beads and 375,000 647 nm dye-encoded helper beads for the nucleocapsid assay). All reagents were diluted in plastic bottles that were loaded into the HD-X Analyzer, and all assay steps were performed in an automated manner on the instrument. In each assay, capture beads were incubated with the sample for 15 minutes, detector antibody for 5 minutes, and SβG for 5 minutes, with washing steps in between. The beads were then resuspended in 25 µL resorufin-β-galactopyranoside and loaded into the microwell array for imaging. Average enzyme per bead (AEB) and sample concentration values were calculated by the HD-X Analyzer software. All samples were measured in duplicates. Immunoglobulin assays were performed using a similar procedure (online *Supplemental Materials*).
Data Analysis

Seroconversion Thresholding

Seroconversion classification was determined based upon the early stage classification model (9) trained using an independent panel of 142 samples positive by RT-PCR SARS-Cov-2 and 200 negative pre-pandemic controls. The markers for this model were chosen using a five-fold cross-validation step as previously reported. (9) Two cross validations were run: 1) Early-stage cases and pre-pandemic controls; 2) late-stage cases and all controls. Each training set initially considered all 12 markers (IgG, IgM, and IgA each against S1, Spike, N, and RBD). This cross-validation yielded four markers (IgA S1, IgA Nucleocapsid, IgG Nucleocapsid, and IgG Spike) and exhibited the best performance in the training set. The threshold for a positive test result for the unknown samples was determined based on the cutoff that yielded 100% specificity in the training set.

Cluster Map Analysis

The cluster map analysis was performed on standardized biomarker data. An example (online Supplemental Fig. S1) and detailed description of the standardization is provided in the online Supplemental Materials. Samples were removed if they had any missing values (reduced the number of samples from 272 to 252). Specifically, the cluster map is a hierarchical clustering based on the Ward variance minimization algorithm. (10)
Results & Discussion:

For SARS-CoV-2 antigen Simoa assays, three types of dye-encoded paramagnetic beads were functionalized with antibodies against each viral antigen and incubated with plasma samples for multiplexed Simoa measurements (Fig. 1a, Methods) as described previously.(8) The SARS-CoV-2 Simoa antigen assays detect S1, spike, and N antigens with limits of detection (LOD) of 5 pg/mL (0.07 pmol/L), 70 pg/mL (0.39 pmol/L), and 0.02 pg/mL (0.4 fmol/L), respectively (online Supplemental Table S2). The range in LODs for each viral antigen assay is primarily due to differences in affinities of the capture and detection antibodies, as antibody dissociation rate constants are an important factor for Simoa assay sensitivity.(11) All SARS-CoV-2 antigen assays were validated in commercial human plasma by spike-and-recovery and dilution-linearity experiments prior to analysis of clinical plasma samples (online Supplemental Figure S2-S3, Table S3). We measured anti-SARS-CoV-2 immunoglobulins (total IgA, IgM, and IgG) using our recently established SARS-CoV-2 serological assays(8) to correlate viral antigens with immunoglobulin levels (Fig. 1a). In addition, we developed SARS-CoV-2 serological Simoa assays for IgG1, IgG2, IgG3, and IgG4 detection (online Supplemental Materials). The combined measurements of three SARS-CoV-2 antigens and seven anti-SARS-CoV-2 immunoglobulin isotypes against four SARS-CoV-2 antigens enables quantification of 31 biomarkers from 70 µL of a plasma sample.

To probe for the presence of viral antigens in plasma, we tested samples from COVID-19 positive patients using our SARS-CoV-2 Simoa antigen assays for S1, spike, and N. These patients were determined to be COVID-19 positive by NP RT-PCR and all plasma samples were obtained within the first ten days of the initial NP RT-PCR test. Corresponding immunoglobulin levels are presented in online Supplemental Figures S4 and S5. S1 and N were detected in 41
of 64 COVID-19 positive patients (Fig. 1b and 1c), who we identify as “viral antigen positive.”

Despite the presence of S1 and N in some samples, spike was only detectable in 5 of 64 COVID-19 positive patients (online Supplemental Figure S6). Spike may be undetectable in some samples since the LOD is one order of magnitude higher than the LOD of the S1 assay. Additionally, in the Simoa assay for spike, the formation of a full immunocomplex depends on spike binding to the S2 subunit capture beads and the S1 subunit detection antibody. Therefore, we hypothesize that free spike antigen in plasma is likely proteolytically cleaved, releasing the S1 subunit, and the remaining spike protein fragment is undetectable by our assay.

Cross-reactivity of the SARS-CoV-2 antigen assays was assessed using plasma samples from three control patient cohorts: (1) samples from individuals who tested negative for COVID-19 by NP RT-PCR, (2) pre-pandemic samples from healthy individuals, and (3) pre-pandemic samples collected from sick individuals (Methods). Viral antigen concentrations are plotted for each individual patient in online Supplemental Figure S7. We observed cross-reactivity that limited the SARS-CoV-2 antigen assays for diagnostic applications and highlighted their use as a complementary technique to RT-PCR diagnosis (Fig. 1b, c). However, based on our preliminary results, a multiplexed approach for the detection of S1 and N can improve analytical specificity of these assays. A more detailed discussion is provided in the online Supplemental Materials. We attribute detection of viral antigens in COVID-19 negative patients to either (1) assay cross-reactivity with other coronaviruses or (2) the patients with a negative NP RT-PCR test were COVID-19 positive but received a false negative result.(12) We focused on the utility of the SARS-CoV-2 Simoa antigen assays for monitoring disease progression in COVID-19 patients diagnosed by NP RT-PCR. When combined with the diagnostic accuracy of RT-PCR, the ultra-sensitivity of Simoa enabled high-resolution
quantification of changes in viral antigen concentrations over the course of disease in COVID-19 patients. Future studies aim to further optimize capture and detection antibodies, assay conditions, and add additional SARS-CoV-2 antigens to our marker panel to improve analytical specificity for diagnostic applications.

To understand how antigen and immunoglobulin levels change with time after infection, we performed longitudinal studies in COVID-19 positive patients to monitor viral antigen and immunoglobulin levels during the course of hospitalization. We measured viral antigen concentrations (S1, spike, and N) and immunoglobulin levels (total IgA, IgM, and IgG and IgG1-4) in serial plasma samples from 39 admitted patients at Brigham and Women’s Hospital and Massachusetts General Hospital (patient IDs 1-39, online Supplemental Fig. S8). All patients were diagnosed as COVID-19 positive by NP RT-PCR. Longitudinal antigen and immunoglobulin levels for four representative patients are plotted in Fig. 2; plots for the remaining 35 patients are shown in the online Supplemental Materials. In this patient cohort, 25 of 39 patients had detectable concentrations of both S1 and N antigen in their plasma, whereas spike was detectable in only 6 of 39 patients during their hospitalization.

The ultra-sensitivity of Simoa assays provides quantitative resolution of viral antigen concentrations and enables us to measure even the earliest stages of antibody production for a detailed view of patient responses over time. For example, in Fig. 2, Patient 21 originally had high concentrations of N (1,240 pg/mL) and S1 (75.3 pg/mL) and low concentrations of anti-SARS-CoV-2 immunoglobulins in their plasma on Day 0. As patient 21 mounted an antibody response against the virus, indicated by the increase in immunoglobulin levels over time, we observe a decrease in viral antigen concentrations in plasma. This trend is observed for a majority of viral-antigen positive patients (Patient 21, 22, and 26 in Fig. 2 and Patients in online
Supplemental Figures S10, S12-14, S19-20, S23, S26, S31, S34, S36). As shown in Fig. 2, among Patients 21, 22, and 26, once a patient has seroconverted and their plasma immunoglobulin levels reach a steady state, there are typically no detectable concentrations of viral antigen. All patients seroconverted a mean ± (95% CI) of 7 ± 1 days after the first NP RT-PCR positive test, in agreement with previous serological studies.(13–16) We defined viral antigen clearance as the first day that both S1 and N were undetectable in patient’s plasma. For viral antigen positive patients, full antigen clearance in plasma was observed 5 ± 1 days after seroconversion.

Furthermore, 13 of 15 patients with undetectable viral antigen concentrations in plasma were already seroconverted at their first NP RT-PCR test (Patient 39 in Fig. 2 and online Supplemental Figures S11, S15-17, S18, S24, S27-28, S30-33, S35, S7-44). We propose three possibilities for the lack of detectable antigen in some patient plasma: (1) patients are presenting to the hospital after seroconversion, and therefore most viral antigens have been cleared from the plasma, (2) we hypothesize that COVID-19 cases with very severe disease will have viral antigen leakage into the blood, and therefore patients that do not progress to more severe forms of the disease will not have viral antigen leakage into the blood, or (3) viral antigens are present but are bound to an immunoglobulin, which blocks a binding epitope of the capture or detection antibody, resulting in an antigen-immunoglobulin complex that is undetectable by our Simoa assays. However, even with no detectable concentrations of viral antigen, 12 of 16 patients were admitted or transferred to the ICU during hospitalization and intubated, indicating that these patients were severe cases. Therefore, we propose a third possibility where patients have seroconverted and despite reaching viral clearance, suffer from severe respiratory damage that inhibits recovery.
Next, we performed a cluster map analysis to determine if antigen and immunoglobulin levels in COVID-19 positive patients could differentiate different stages of infection across all patients. The cluster map shows three dominant branches (Fig. 3). The first branch included a majority of early time points where we observed high concentrations of viral antigens and low levels of antibodies. The second branch showed a transition in patient samples where antigen concentrations began to decrease as total IgM, IgA, and IgG (as well as IgG subclasses IgG1 and IgG3) began to increase. In the third branch, we observed an even further reduction in antigen concentrations with small increases in IgG subclasses IgG2 and IgG4. The third branch was observed for later time points where we propose that patients had cleared viral antigens from the plasma and antibodies had reached their steady-state levels. For all 39 patients in this cohort, we observed that IgG1 and IgG3 showed large increases in plasma concentrations over time. In contrast, IgG2 and IgG4 showed little to no changes above background levels over time. Both IgG1 and IgG3 are subclasses of IgG that predominantly respond to viral antigens and mediate neutralization.(17–21) Based on these measurements, the IgG response that was mounted by the immune system was primarily mediated by IgG1 and IgG3 for SARS-CoV-2, in agreement with previous serological studies.(4,5) The neutralization effects of IgG1 and IgG3 on viral antigens should be explored in future studies that include correlative neutralization titer assays.

We next explored how antigen clearance compared with longitudinal RT-PCR tests and clinical outcomes. The measured SARS-CoV-2 antigen and immunoglobulin levels in plasma, NP RT-PCR tests, and select clinical outcomes for each of these patients are displayed in Fig. 4 and patient features such as age and gender are summarized in online Table S4. In this patient cohort, NP RT-PCR tests reported positive results for a mean of 15 ± 5 days after viral antigen clearance and 18 ± 4 days after seroconversion. Furthermore, several patients never received a
negative RT-PCR result before being discharged from the hospital. These observations are in agreement with recent virological studies that confirmed persistence of viral RNA and showed how seroconversion was not immediately followed by a decrease in NP viral RNA in hospitalized patients. (22) Because RNA shedding can occur for several weeks after infection and recovery, measurements of viral antigens and immunoglobulins may provide more timely indicators of infectivity compared to viral RNA, but additional studies such as viral load measurements are necessary to corroborate these findings.

To understand the correlation between SARS-CoV-2 antigen concentrations and disease severity, clinical features for the 64 COVID-19 positive patients (Fig. 5, patient IDs 1-64) were compared with viral antigen concentrations in plasma. It is important to note that all samples were obtained from patients admitted to the hospital, resulting in a cohort of cases primarily with severe disease. S1 shows higher correlation with clinical severity than N in plasma (online Supplemental Table S5-8, Figure S45). Confounding factors such as age and sex were also assessed and showed no correlation with clinical outcomes (online Supplemental Table S9, S10 and Figure S46). Therefore, patients were grouped into three categories of S1 concentrations: (1) 23 patients with undetectable S1 concentrations (below the limit of detection), (2) 23 patients with low concentrations of S1 (6-50 pg/mL, 0.08–0.65 pmol/L), and (3) 18 patients with high concentrations of S1 (>50 pg/mL, >0.65 pmol/L). There is a significant difference in rates of ICU admission upon presentation for the three patient groups based on S1 concentrations in plasma (P-value: 0.0107). Patients with zero, low, and high concentrations of S1 were admitted to the ICU upon presentation to the hospital at rates of 30% (7 of 23 patients), 52% (12 of 23 patients), and 77% (14 of 18 patients), respectively (Fig. 5a). Among all COVID-19 positive patients, over 60% were intubated during hospitalization with no statistically significant
difference in intubation rates among groups (Fig. 5b). The difference in mean times to intubation between patients with high concentrations of S1 and patients with no detectable S1 is significant (P-value: 0.0050), where all patients with high concentrations of S1 were intubated within one day of hospitalization (Fig. 5c). These results suggest that high S1 concentrations in plasma upon presentation to the hospital correlate with severe cases of COVID-19 that can result in respiratory failure and require immediate intubation. Among all patients, a broad range of intubation periods, up to 32 days, was observed (Fig. 5d). Patients grouped by S1 concentrations, age, and sex showed no statistically significant difference in death rate (online Supplemental Table S9, S10 and Figure S46). Although six patients showed detectable concentrations of spike protein, there was no correlation between spike concentrations and ICU admission, intubation rate, or death rate. There are potentially more features in these data that will lead to further correlations, but a large cohort of patients that include asymptomatic and mild cases will need to be tested to elucidate other patterns.

Plasma is a readily available biofluid from hospitalized patients but is more difficult to obtain in non-clinical settings. In comparison, saliva is a non-invasive biofluid that is easier to use for wide-scale testing and may be more interesting for monitoring viral antigen concentrations to understand active viral infection from COVID-19, which is a respiratory disease. We tested 17 saliva samples from patients presenting to the Emergency Department at Brigham and Women’s Hospital who were tested by NP RT-PCR for COVID-19 (online Supplemental Figure S47, S48, S49). We found that S1 and N was detectable in 7 of 11 COVID-19 patients when compared to healthy saliva controls. Similar to our observation of antigen and immunoglobulins in plasma, there were 7 of 11 patients with low S1 concentrations and high levels of IgA-S1. One patient showed a background concentration of IgA-S1 and
notably high concentration of S1 (135 pg/mL). However, a deep understanding of the correlation between viral antigens and immunoglobulins in saliva will require a larger sample cohort. Nonetheless, these initial results indicate the presence of SARS-CoV-2 antigens and anti-SARS-CoV-2 immunoglobulins in saliva and highlight the potential for adapting our assays to a diagnostic test for COVID-19. Future studies on saliva will include longitudinal sample analysis for mild and severe cases of COVID-19 patients and will explore the potential for developing a saliva-based COVID-19 antigen screening tool.

**Conclusion:**

Using SARS-CoV-2 Simoa assays, we have demonstrated quantitative detection of SARS-CoV-2 antigens and anti-SARS-CoV-2 immunoglobulins in plasma of COVID-19 patients. While detection of N in NP swabs has been cited,(6) we present the first report of SARS-CoV-2 spike, S1, and N detection in plasma. The presence of S1 and N in plasma suggest that fragments of virus are entering the bloodstream, potentially due to tissue damage. Although spike is undetectable in most COVID-19 patients, possibly due to proteolytic cleavage, six patients showed high concentrations of spike in plasma. No evidence has been reported yet for full viral particles in blood, though we cannot rule out this possibility.(23) Nonetheless, severe COVID-19 cases with acute respiratory distress syndrome can result in damage to endothelial cells and vascular leakage(24–27) and we propose that this damage can lead to discharge of viral antigens into the blood. Patients with lung damage can suffer from respiratory failure and require intubation or mechanical ventilation. We also found significant correlation between high S1 concentrations in plasma and time between hospital admission and intubation. Although we hypothesize that asymptomatic or mild cases will likely not show viral antigen concentrations in
plasma, future studies that include mild cases will be used to probe SARS-CoV-2 antigen and antibody concentrations over time in comparison to the severe cases presented here.

Acknowledgments:

The authors acknowledge Dr. Sanjat Kanjilal for collecting saliva samples.

Author Declaration

A version of this paper was previously posted as a preprint on medRxiv as https://www.medrxiv.org/content/10.1101/2020.07.20.20156372v1.

The anti-SARS-CoV-2 Simoa assays in this publication have been licensed by Brigham and Women’s Hospital to Quanterix Corporation.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

A.F. Ogata, A.M. Maley, C. Wu, T. Gilboa, M. Norman, and D.R. Walt conceived the approach. A.F. Ogata, A.M. Maley, C. Wu, T. Gilboa, M. Norman, and G. Newton performed the experiments, A.F. Ogata, A.M. Maley, C. Wu, T. Gilboa, M. Norman, R. Lazarovits, C.-P. Mao, and T.E. Gibson analyzed the data, M. Chang, K. Nguyen, M. Kamkaew, Q. Zhu, and W.A. Marasco produced and purified the anti-S1 antibody, R.C. Charles and E.T. Ryan collected the samples and performed chart review for patients from Massachusetts General Hospital, C.-P. Mao performed chart review for patients from Brigham and Women’s Hospital. A.F. Ogata, A.M. Maley, and D.R. Walt co-wrote the paper. All authors were involved in designing experiments, reviewing and discussing data, and commented on the manuscript.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. Disclosures and/or potential conflicts of interest:

Employment or Leadership: D. Walt is an inventor of the Simoa technology, a founder of the company and also serves on its Board of Directors. Dr. Walt’s interests were reviewed and are managed by BWH and Partners HealthCare in accordance with their conflict of interest policies.

Consultant or Advisory Role: None declared.

Stock Ownership: D. Walt has a financial interest in Quanterix Corporation, a company that develops an ultra-sensitive digital immunoassay platform.

Honoraria: None declared.
Research Funding: Funding for this work came from a generous donation from Barbara and Amos Hostetter and the Chleck Foundation. This work was also funded through grants from the Massachusetts Consortium on Pathogen Readiness and the Centers for Disease Control and Prevention (U01CK000490). C. Wu, National Institutes of Health (1F32EB029777-01).

Expert Testimony: None declared.

Patents: A.M. Maley, patent application in progress; T. Gilboa, patent application in progress; M. Norman, patent application in progress; D.R. Walt, many patents.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.
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Figure Captions:

Figure 1. SARS-CoV-2 Antigen and anti-SARS-CoV-2 Immunoglobulin Detection in Plasma. a.) Schematic of Simoa detection of SARS-CoV-2 S1, spike, and N antigens and anti-SARS-CoV-2 immunoglobulins IgG, IgA, and IgM. Measurements for all antigens and immunoglobulins can be obtained from a single plasma sample (70 µL). b-c) Simoa SARS-CoV-2 antigen assay results for plasma samples collected from pre-pandemic healthy patients, pre-pandemic sick patients, COVID-19 negative patients, and COVID-19 positive patients. 41 of 64 COVID-19 positive patients show detectable b.) S1 and c.) N concentrations in plasma. Each data point represents the average of two replicate measurements.
Figure 2. Serial data for four individual COVID-19 positive patients from admission to clinical recovery or death. Simoa antigen and serological results for serial plasma samples. (1) the total IgA, IgM, and IgG levels against four viral antigens: nucleocapsid (N), receptor binding domain (RBD), S1, and spike, for a total of 12 interactions, (2) N antigen concentrations with IgA, IgM, and IgG against N, (3) S1 antigen concentrations with IgA, IgM, and IgG against S1, and (4) S1 antigen concentrations with IgG1-4 against S1. Data points represent the mean concentration or AEB from two replicate measurements and error bars represent the standard error of the mean from two replicate measurements.
Figure 3. Clustergram of Simoa antigen and immunoglobulin results from 252 samples from 39 patients. Cluster map produce by Ward Variance Minimization algorithm. 252 samples from 39 patients were analyzed as described in the Methods section. Data were standardized after a non-linear transformation and each row represents a single sample. Days since first RT-PCR test for each sample are presented at the far left of the clustergram. Longitudinal samples are clustered by dendrograms to the left of the clustergram. SARS-CoV-2 antigen and immunoglobulin markers are clustered by dendrograms at the top of the clustergram. The dotted line represents the cutoff that results in three branches: branch 1 (top), branch 2 (middle), and branch 3 (bottom). RBD: Receptor binding domain. N: Nucleocapsid.
Figure 4. Summary of clinical data and Simoa SARS-CoV-2 antigen and immunoglobulin assays for individual patients in the longitudinal analysis. A black X indicates a viral antigen positive test, whereas a blue X indicates a viral antigen negative test. A black circle indicates a positive NP RT-PCR test, whereas an orange circle indicates a negative NP RT-PCR test. For example, on May 3, 2020, Patient 34 received both a positive and negative NP RT-PCR result, indicated by the red circle. The intubation and extubation dates of each patient are indicated by green squares. The survival outcome for each patient is marked by the patient ID number color. Patient IDs are coded black for recovered patients who were discharged from the hospital, whereas patient IDs are coded red for deceased patients. Pink boxes represent the date of seroconversion for each patient.
Figure 5. Indicators of disease severity based on S1 concentrations in plasma for 64 COVID-19 positive patients. COVID-19 positive patients were separated into three groups based on S1 concentrations. The cutoff between groups 2 and 3 (50 pg/mL, 0.65 pmol/L) was chosen as five standard deviations above the LOD. The fraction of patients admitted to the ICU or who were intubated was calculated for each group independently. a.) Fraction of COVID-19 positive patients who were immediately admitted to the ICU upon presentation to the hospital. b.) Fraction of COVID-19 positive patients who were intubated during hospitalization. c.) Days between date of presentation to the hospital and intubation date for intubated COVID-19 positive patients. d.) The length of intubation for intubated COVID-19 positive patients. For all plots, significance indicated by the asterisks (P value <0.05).

a. Patients admitted to ICU

b. Patients intubated
c. Days between hospitalization and intubation

d. Length of intubation