Title

Detection of Nucleocapsid Antibody to SARS-CoV-2 is More Sensitive than Antibody to Spike Protein in COVID-19 Patients

Journal

The Journal of Infectious Diseases, 222(2)

ISSN

0022-1899

Authors

Burbelo, Peter D
Riedo, Francis X
Morishima, Chihiro
et al.

Publication Date

2020-06-29

DOI

10.1093/infdis/jiaa273

Peer reviewed

The Journal of Infectious Diseases

MAJOR ARTICLE

Sensitivity in Detection of Antibodies to Nucleocapsid and Spike Proteins of Severe Acute Respiratory Syndrome Coronavirus 2 in Patients With Coronavirus Disease 2019

Peter D. Burbelo,[1] Francis X. Riedo,[2] Chihiro Morishima,[3] Stephen Rawlings,[4] Davey Smith,[4] Sanchita Das,[5] Jeffrey R. Strich,[6] Daniel S. Chertow,[6] Richard T. Davey Jr,[7] and Jeffrey I. Cohen[8]

[1]National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA, [2]Division of Infectious Diseases and Travel Medicine, EvergreenHealth, Kirkland, Washington, USA, [3]Department of Laboratory Medicine, University of Washington, Seattle, Washington, USA, [4]Division of Infectious Diseases and Global Public Health, San Diego Center for AIDS Research, University of California San Diego, San Diego, California, USA, [5]Department of Laboratory Medicine, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA, [6]Critical Care Medicine Department, Clinical Center, National Institutes of Health, Bethesda, Maryland, USA, [7]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA, and [8]Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease 2019 (COVID-19), is associated with respiratory-related disease and death. Assays to detect virus-specific antibodies are important to understand the prevalence of infection and the course of the immune response.

Methods: Quantitative measurements of plasma or serum antibodies to the nucleocapsid and spike proteins were analyzed using luciferase immunoprecipitation system assays in 100 cross-sectional or longitudinal samples from patients with SARS-CoV-2 infection. A subset of samples was tested both with and without heat inactivation.

Results: At >14 days after symptom onset, antibodies against SARS-CoV-2 nucleocapsid protein showed 100% sensitivity and 100% specificity, whereas antibodies to spike protein were detected with 91% sensitivity and 100% specificity. Neither antibody levels nor the rate of seropositivity were significantly reduced by heat inactivation of samples. Analysis of daily samples from 6 patients with COVID-19 showed anti-nucleocapsid and spike protein antibodies appearing between days 8 and 14 after initial symptoms. Immunocompromised patients generally had a delayed antibody response to SARS-CoV-2, compared with immunocompetent patients.

Conclusions: Antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than spike protein antibody for detecting early infection. Analyzing heat-inactivated samples with a luciferase immunoprecipitation system assay is a safe and sensitive method for detecting SARS-CoV-2 antibodies.

Keywords: COVID-19; coronavirus; SARS-CoV-2; serology

Infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing coronavirus disease 2019 (COVID-19), were first reported in China [1–4]. The major clinical feature of SARS-CoV-2 infection is virus-associated pneumonitis [5–7]. In comparison to highly pathogenic coronaviruses such as SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV) [8], SARS-CoV-2 spreads more rapidly and reached 6 of the 7 continents, including North America [9], within 3 months of the initial outbreak. Nucleic acid-based testing of oropharyngeal or nasopharyngeal swab samples and saliva is useful for diagnosing acute infection. SARS-CoV-2 virus RNA can often be detected in upper respiratory secretions shortly before symptoms first appear, and these levels peak during the first week of symptoms and decline with time [10, 11]. RNA from SARS-CoV-2, like the related SARS-CoV-1 [12], can be detected in blood [11, 13], and high levels of circulating viral RNA are associated with more severe disease [13].

Assessment of the antibody response to SARS-CoV-2 should complement the RNA-based tests and improve our understanding of the pathogenesis and course of COVID-19, contribute to epidemiological studies, and inform vaccine development. Antibodies to the nucleocapsid protein are the most sensitive target for serological diagnosis of infection with SARS-CoV-1 [14, 15]. Antibodies against the spike protein of SARS-CoV-1, the target of neutralizing antibody and vaccine development, emerge later than those against the nucleocapsid protein.

Recently, several groups have reported serological tests using the nucleocapsid and/or spike protein from SARS-CoV-2 by enzyme-linked immunosorbent assay (ELISA) [11, 16, 17],

Received 14 April 2020; editorial decision 12 May 2020; accepted 16 May 2020; published online May 19, 2020.

Correspondence: Jeffrey I. Cohen, Bldg 50, Room 6134, 50 South Dr, MSC8007, National Institutes of Health, Bethesda, MD 20892 (jcohen@niaid.nih.gov).

The Journal of Infectious Diseases® 2020;XX:1–8

DOI: 10.1093/infdis/jiaa273

immunofluorescence [18] and even a lateral flow test [19]. One study using ELISA to measure only antibodies to the nucleocapsid protein found that patients become seropositive 10–18 days after the onset of symptoms [16]. A commercial ELISA using the spike protein demonstrated that immunoglobulin (Ig) G antibodies were detectable at a median of 14 days after onset of symptoms [17]. To et al [11] examined antibodies against both the spike and nucleocapsid by ELISA in a small number of samples and found that IgG antibodies against the nucleocapsid protein were generally detectable at about the same time as antibodies to the spike protein.

Despite these findings, further studies are needed to understand antibody dynamics in persons infected with SARS-CoV-2 to determine the most sensitive and specific antibody assays and to use these antibody-based tests to determine seroprevalence in different populations. In addition, it is currently unknown whether the viral RNA that has been detected in the blood [11, 13] indicates the presence of infectious virus, which has the potential to be a safety hazard for clinical laboratory technicians and researchers analyzing serological findings in persons infected with SARS-CoV-2. Thus, a sensitive and specific antibody assay using heat-treated plasma/serum may enhance safety for those working with these fluids.

We and others have used a liquid-phase immunoassay technology, a luciferase immunoprecipitation system (LIPS) assay, to measure antibodies against many viruses, to stratify infected patients based on antibody levels, and for virus discovery [20]. LIPS assays have shown promise for detecting antibodies against coronaviruses, including the nucleocapsid of MERS-CoV [21] and the spike protein of swine acute diarrhea syndrome coronavirus [22]. Unlike ELISA, which is solid phase, LIPS assays are performed in solution, thus maintaining the native antigen conformation. The antigen is produced in mammalian cells and often retains posttranslational modifications of the antigen, unlike bacterial recombinant proteins or peptide-based ELISAs.

LIPS assays typically have a dynamic range up to 6 $\log_{10}$ for some antigens and require <5 μ L of plasma or serum for testing. In the current study, recombinant nucleocapsid and spike protein from SARS-CoV-2 as antigens in LIPS assays were used to measure antibodies in patients with COVID-19 from 4 US locations. The assays showed high sensitivity and specificity for detecting SARS-CoV-2 antibodies and demonstrated that nucleocapsid antibodies emerge before spike antibodies. Moreover, because there are potential safety issues related to the presence of SARS-CoV-2 RNA in blood, we show that heat inactivation of plasma at 56˚C for 30 minutes does not significantly reduce the sensitivity of the LIPS assay and allows testing to be performed more safely.

METHODS

Characteristics of Patients With COVID-19

This retrospective study analyzed both cross-sectional and longitudinal blood samples collected from patients with COVID-19 or controls from 4 sites. Anonymized plasma or serum from patients from the University of California, San Diego (UCSD; n = 3), the University of Washington, Seattle (UW; n = 17), and EvergreenHealth, Kirkland, Washington (EH; n = 23) (Table 1) were obtained under an institutional review board exemption. Plasma from patients at the National Institutes of Health (NIH) Clinical Center (n = 6) were obtained under a protocol approved by the institutional review board of the NIH Intramural Research Program; all patients signed consent forms. Blood from anonymized blood bank donor controls (n = 32) collected at the NIH Clinical Center before 2018 were used as uninfected controls.

The time between the initial symptoms and the collection of plasma or serum samples from the 35 PCR-confirmed cases was variable and ranged from 2 to 50 days. SARS-CoV2 infection was confirmed in each case by reverse-transcription PCR detection of viral RNA from oropharyngeal or nasopharyngeal

Table 1. Patient Characteristics of Patients in Coronavirus Disease 2019 Cohort

Characteristic	Blood Donors (n = 32)	Suspected Cases (EH; n = 10)	UCSD (n = 3)	UW (n = 13)	EH (n = 13)	NIH (n = 6)
Sex, no. male/no. female	ND	4/6	2/1	10/3	3/10	5/1
Age mean (range), y	ND	32 (7–49)	73 (59–84)	66 (43–95)	59 (19–88)	45 (22–67
≥1 Risk factor[a]	ND	0 (0)[b]	2 (66)	13 (100)	6 (46)	5 (83)
PCR positive for SARS-CoV-2	ND	0 (0)[c]	3 (100)	13 (100)	13 (100)	6 (100)
Time from symptom onset to 1st blood sample, mean (range), d	ND	47.1 (26–79)[b]	7.8 (5–14)	13.2 (4–24)	18 (2–50)[b]	5.5 (0–11)
Ventilator, no. (%)	ND	0 (0)	3 (100)	4 (31)	3 (23)	3 (50)
Death, no. (%)	ND	0 (0)	1 (33)	5 (38)	3 (23)	1 (17)

Abbreviation: EH, EvergreenHealth, Kirkland, Washington; ND, not determined; NIH, National Institutes of Health; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; UCSD, University of California, San Diego; UW, University of Washington, Seattle.

[a]Risk factors including heart disease, lung disease, diabetes, obesity, and immunocompromise. None of 3 patients from UCSD, 4 of 13 from UW, 1 of 13 from EH, and 3 of 6 from NIH were immunocompromised.

[b]Unknown for 1 subject.

[c]PCR negative in 2 and ND in 8.

swab samples obtained at clinical laboratories at each location. For the NIH samples, serial daily blood samples (n = 68) were available for 0–20 days from symptom onset.

Storage and Heat Inactivation

Plasma and serum samples were collected and stored frozen at −80° C, except for the heat-inactivated samples from the NIH that were not previously frozen. In light of previous studies that demonstrated a marked loss in infectivity of SARS-CoV-1 [23] and MERS-CoV [24] with heating, we adopted a precautionary safety protocol performed before analysis. Aliquots of plasma or serum from each patient sample were first incubated at 56° C for 30 minutes and then used for testing, as described below.

LIPS Assays for Measurement of SARS-CoV-2 Antibodies

LIPS assays, in which viral proteins fused to light-emitting luciferase are immunoprecipitated, were essentially performed as described elsewhere [25]. The SARS-CoV-2 nucleocapsid was constructed in the standard pREN2 vector as a C-terminal *Renilla* luciferase fusion protein. The spike protein, owing to its signal peptide sequence, was generated as an N-terminal fusion with a different luciferase, *Gaussia* luciferase, in the pGAUS3 vector for better expression of the fusion protein [26]. The nucleocapsid sequence of SARS-CoV-2 (GenBank MN908947) was generated as a synthetic DNA (Twist Biosciences) and then cloned into pREN2. A spike protein construct of SARS-CoV-2 (amino acids 1–538 of GenBank MN908947) was generated by PCR from a plasmid containing a prefusion form of the spike protein (2019-nCoV-2_S-2P) [27] and subcloned to generate pGAUS3-Spike. A second spike construct, pGAUS3-Spike-Δ2 (amino acids 1–513) was constructed in the pGAUS3 vector in the same way. Preliminary tests comparing antibody detection using pGAUS3-Spike-Δ2 and pGAUS3-Spike showed similar results and the former construct was not used further.

Nucleocapsid and spike protein-light emitting plasmid constructs were transfected into Cos1 cells and lysates were harvested 48 hours later to obtain crude cell extracts. For testing, heat-inactivated serum/plasma samples were diluted 1:10 in assay buffer A (20 mol/L Tris, pH 7.5, 150 mmol/L sodium chloride, 5 mmol/L magnesium chloride, and 1% Triton X-100), and 10 μL of the diluted sample was then tested in a 96-well microtiter plate, as described elsewhere [25]. After incubation for 1 hour, the mixture was transferred to a microtiter filter plate containing protein A/G beads and incubated for 1 hour. Protein A/G beads efficiently bind IgG and, to a much lesser extent, IgM, to capture the antibody-antigen-bead complexes. Microtiter plates containing the beads were then washed 8 times with buffer A and twice with phosphate-buffered saline to remove unbound antigens. After the final wash, coelenterazine substrate (Promega) was added to detect the luciferase activity, and light units (LU) were measured using a Berthold LB 960 Centro microplate luminometer (Berthold Technologies).

Percentages for categorical variables, means and ranges, and geometric means with 95% confidence intervals (CIs) were used to describe some of the data. Antibody levels were reported as geometric mean levels with 95% CIs. Cutoff limits for determining positive spike and nucleocapsid antibodies in the SARS-CoV-2–infected samples were based on the mean plus 3 and 4 standard deviations (SDs), respectively, of the serum values derived from the 32 uninfected blood donor controls. Based on the typical 2-week period between when a virus is initially encountered and the time to generate virus-specific IgG antibody, intervals of >14 or ≤14 days were chosen to calculate the sensitivity of the LIPS assays. Wilcoxon signed rank and Fisher exact tests were used for statistical analysis.

RESULTS

Characteristics of Patients with COVID-19

Patients with COVID-19 were located in 4 geographically distinct locations across the United States and included 35 with SARS-CoV-2 confirmed by PCR and10 who had COVID-19–like symptoms or were household contacts of persons with COVID-19 (not tested with PCR); 32 blood donors who donated samples before 2018 were used as controls (Table 1). The majority of the patients with PCR-confirmed SARS-CoV-2 were male (87%), and the median age was 44 years (range, 32–50 years). A subset of the patients with PCR-confirmed SARS-CoV-2 had ≥1 risk factor, including heart disease, lung disease, diabetes, and an immunocompromising condition. Two plasma samples, drawn 2–3 days apart, were available for each of the 3 patients with COVID-19 from the UCSD, and multiple daily samples were available from the NIH patients with COVID-19. Combining the cross-sectional and longitudinal studies resulted in 100 samples from PCR-positive patients.

Sensitivity in Detection of Antibodies to Nucleocapsid and Spike Proteins in Patients With COVID-19

LIPS assays for detecting antibodies were developed using SARS-CoV-2 nucleocapsid and spike antigens produced in mammalian cells. Pilot experiments using nucleocapsid-*Renilla* luciferase and spike protein–*Gaussia* luciferase fusion proteins were conducted with serum or plasma from blood donor controls collected before 2018. Results showed a low background with little or no antibody immunoreactivity against the spike protein, but there was a higher background against the nucleocapsid protein (data not shown). The specificity of the 2 SARS-CoV-2 LIPS assays was also tested with 4 serum samples from patients who had recently tested positive by PCR of oropharyngeal or nasopharyngeal samples for other coronaviruses. Serum samples from 2 patients infected with the HKU1 betacoronavirus and 2 infected with the NL63 alphacoronavirus were seronegative by LIPS assays for SARS-CoV-2 nucleocapsid and spike antibodies (data not shown).

In addition, using our previous LIPS test for MERS-CoV nucleocapsid protein [21], which has higher amino acid homology with the SARS-CoV-2 nucleocapsid than the seasonal coronaviruses [28], we found no seropositivity for the MERS-CoV nucleocapsid protein in COVID-19–positive patients, highlighting the specificity of the SARS-CoV-2 LIPS assays (data not shown). To develop highly specific serological tests without potential false-positives, stringent cutoff values from the blood donor controls were assigned based on statistical methods and/or receiver operator characteristic curves. Cutoff values for the nucleocapsid and spike proteins were derived from the mean plus 4 SDs (125 000 LU) and the mean plus 3 SDs (45 000 LU) of the blood donor controls, respectively.

These cutoff values were used to evaluate plasma and serum samples from the COVID-19 cohort with the 2 LIPS assays. For safety reasons, all samples were heated at 56°C for 30 minutes to reduce the likelihood of infectious virus in the samples. Anonymized samples from EH patients with suspected COVID-19 were then tested, as well as noncoded samples from pre-2018 blood donors, and samples from patients with PCR-positive SARS-CoV-2 from the UCSD, the University of Washington, EH, and the NIH Clinical Center. A wide dynamic range of antibody levels against the nucleocapsid and spike protein was observed, with levels differing by up to 100-fold between samples (Figure 1).

To compare the sensitivity of the nucleocapsid and spike LIPS assays, a minimum interval of >14 days between onset of symptoms and time of blood collection was used to determine the number of seropositive serum or plasma samples in the SARS-CoV-2 PCR-positive group. Among the PCR-positive patient samples collected >14 days after onset of symptoms (Figure 1), seropositive nucleocapsid antibodies were detected in all 35 samples, yielding both a sensitivity and specificity of 100%. A similar analysis of the spike antibody in samples collected >14 days after onset of symptoms showed a slightly lower sensitivity of 91% (32 of 35) with 100% specificity, but this was not significantly different from the nucleocapsid result (Fisher exact test). Comparison of antibody levels for nucleocapsid and spike antibodies showed that they tracked with each other well ($r_s = 0.72$; $P < .001$).

Evaluation of samples collected ≤14 days after onset of symptoms showed reduced sensitivity, but specificity was maintained. The sensitivity for antibody to the nucleocapsid protein at this time point was 51% (33 of 65) (Figure 1). Analysis of spike antibodies of samples collected ≤14 days after onset of symptoms showed a sensitivity of 43% (28 of 65). Taken together, our findings indicate that detection of antibodies against the nucleocapsid protein is more sensitive than detection of antibodies against the spike protein, and that nucleocapsid antibodies generally appear earlier than spike antibodies.

In addition to the patients with PCR-confirmed SARS-CoV-2, patients with suspected COVID-19 from EH were also analyzed for seropositivity. Nine of the 10 patients with suspected COVID-19 without viral PCR confirmation, some with symptoms of fever, cough, and/or fatigue compatible with COVID-19 and others with household exposure to COVID-19, in whom samples were collected in January and February 2020, were seronegative for both nucleocapsid and spike antibodies (Figure 1). Interestingly, 1 patient from March 2020, a household contact of a patient with PCR-positive SARS-CoV-2, was seropositive for both nucleocapsid and spike antibodies.

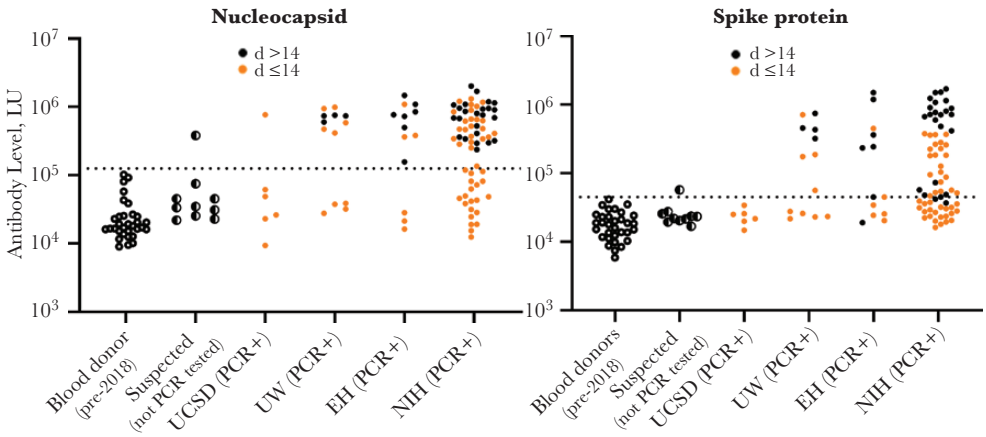

Figure 1. Detection of antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleocapsid and spike protein in patients with coronavirus disease 2019 (COVID-19). Antibody levels against SARS-CoV-2 nucleocapsid and spike protein were determined in 32 pre-2018 blood donors, 10 patients with suspected COVID-19 (not confirmed with polymerase chain reaction [PCR]) from EvergreenHealth, Kirkland, Washington (EH) and patients PCR positive (PCR+) for COVID-19, including 3 from the University of California, San Diego (UCSD), 13 from the University of Washington (UW), 13 from EH, and 6 from the National Institutes of Health Clinical Center (NIH). Each symbol represents a sample from an individual patient or different time point from an individual patient. Antibody levels are plotted in light units (LU) on a $\log_{10}$ scale. Black circles represent plasma or serum samples obtained >14 days after symptom onset; orange circles, samples obtained ≤14 days after symptom onset; and dashed lines, cutoff levels for determining positive antibody titers, as described in Methods.

Because there is interest in using serological assays to assess current and historical infections, we evaluated the robustness of the LIPS assay for detecting SARS-CoV-2 antibodies by analyzing the level of antibodies in all PCR-confirmed samples collected >14 days after symptom onset. The geometric mean level of nucleocapsid antibody levels in the 35 seropositive samples was 694 600 LU (95% CI, 570 000–844 600 LU), which was approximately 32 times higher than the geometric mean level of the blood donor controls of 21 356 LU (95% CI, 17 032–26 752 LU). Antibodies against spike protein showed a similar discriminatory potential for the seropositive samples with a geometric mean level of 346 800 LU (95% CI, 218 800–550 000 LU), which was approximately 21 times higher than the blood donor controls with 16 843 LU (95% CI, 14 172–20 007 LU).

Different Time Courses in the Appearance of Antibodies Against SARS-COV-2 in Immunocompetent and Immunocompromised Patients

To elucidate the timing and trajectory of SARS-CoV-2 antibodies against nucleocapsid and spike proteins, serial daily blood samples from the 6 NIH patients with COVID-19 were studied. In all 6 subjects, SARS-CoV-2 antibody levels rose with time in both the 3 immunocompetent (Figure 2A; NIH patients 1–3) and the 3 immunocompromised patients (Figure 2B; NIH patients 4–6). The latter 3 patients had chronic lymphocytic leukemia, metastatic chordoma, or had received a hematopoietic stem cell transplant. All 3 immunocompetent patients with COVID-19 showed a rapid rise in antibody to nucleocapsid that began within 10 days of symptom onset in 2 patients (no samples were available before day 11 for the third patient, Figure 2A). Antibodies against the spike protein in these 3 immunocompetent patients generally tracked with the nucleocapsid antibodies, but in 1 case seropositivity appeared 2 days later than nucleocapsid antibody. The third patient, NIH patient 3, with a history of hypertension and heart disease, died of cardiovascular shock and hypoxemia 13 days after onset of symptoms.

Antibody profiles in 3 immunocompromised NIH patients showed more blunted responses against the SARS-CoV-2 antigens (Figure 2B). NIH patient 4 became seropositive for both nucleocapsid and spike antibodies on day 14, and these antibodies then plateaued at these low levels for the next 7 days. Similarly, NIH patient 5 did not become seropositive until day 13 for spike antibody and day 14 for nucleocapsid antibody. NIH patient 6 was both PCR positive for SARS-CoV-2 and seropositive on the day of symptoms, suggesting that he had an asymptomatic infection for several days before diagnosis. Review of the non-NIH patients showed that of the 5 immunocompromised patients in this group, 1 had low levels of spike antibody and 2 were negative for spike antibody but positive for nucleocapsid antibody.

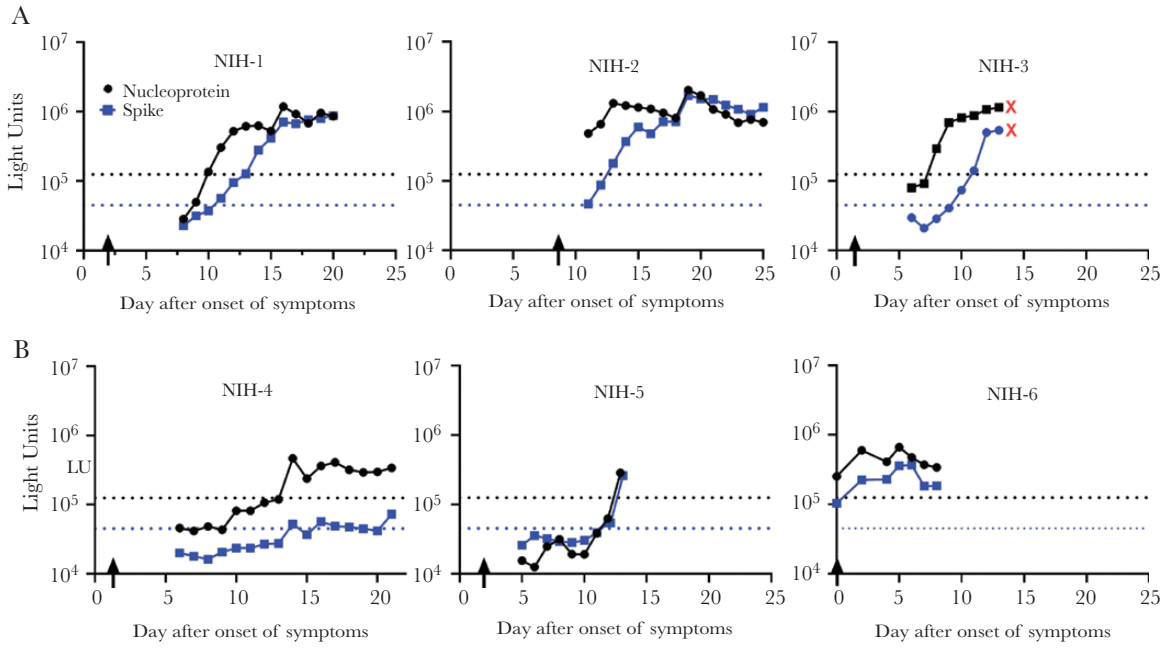

Figure 2. Longitudinal profile of antibodies against nucleocapsid and spike proteins in immunocompetent and immunocompromised patients with coronavirus disease 2019 (COVID-19) from the National Institutes of Health (NIH). Antibody levels were determined in daily blood samples from 6 patients with COVID-19. Three patients (NIH patients 1–3 [NIH-1, NIH-2, and NIH-3]) were immunocompetent (*A*) and 3 (NIH patients 4–6 [NIH-4, NIH-5, and NIH-6]) were immunocompromised (*B*). The levels of antibody to the nucleocapsid (*black lines*) and to spike (*blue lines*) proteins over time were plotted on the y-axis, using a $\log_{10}$ scale. Time 0 represents the day symptoms appeared; arrows, the time of diagnosis with polymerase chain reaction; dotted lines, cutoff values for determining seropositivity; and red *X*'s, the day after onset of symptoms that NIH patient 3 died.

Effect of Heat Inactivation of Plasma on the Detection of SARS-COV-2 Antibodies With LIPS Assays

Although heating plasma to 56° C for 30 minutes has been shown to reduce the titer of coronaviruses, heating might reduce or eliminate IgM and IgG responses [29]. Therefore, we performed LIPS assays in a subset of the patients with known or suspected COVID-19 (n = 38), with and without heat inactivation, to evaluate its impact on nucleocapsid antibody levels and seropositivity status. Evaluation of antibody responses in heated versus unheated plasma samples showed that antibody levels were mostly unchanged (Figure 3). In a single sample from a patient with COVID-19, antibody to SARS-CoV-2 was not detected after heat inactivation. Of note, this sample came from an NIH patient with COVID-19 who was antibody positive at day 7 with nonheated plasma and who became seropositive with heat-inactivated plasma from day 8. Statistical analysis showed no significant difference in antibody levels between heated and unheated plasma samples (Wilcoxon signed rank test), and the values were highly correlative ($r_s = 0.913$; $P < .001$). These findings indicate that the heat inactivation process is diagnostically suitable for LIPS testing of SARS-CoV-2 antibodies.

DISCUSSION

The fluid-phase LIPS assay was used to investigate antibodies to the SARS-CoV-2 nucleocapsid and spike protein in patients with COVID-19 after infection. The LIPS assay demonstrates high sensitivity and a wider dynamic range for antibody detection compared with other published assays [11, 16–19]. Analysis of longitudinal plasma samples showed that antibodies against the nucleocapsid and spike proteins appeared about the same time between days 8 and after the onset of symptoms. Only one other study to date has examined antibodies separately against the nucleocapsid and spike proteins [11], and our findings are in general agreement. Plasma samples from patients with COVID-19 obtained ≥14 days after symptom onset showed that the LIPS assay for antibodies against the nucleocapsid and spike protein had 100% and 94% sensitivity, respectively, with 100% specificity for both antibodies. Additional studies using this highly quantitative LIPS assay may help determine whether the relative levels of antibodies observed in convalescent patients with COVID-19 or uninfected vaccinated persons are correlated with prevention of reinfection or primary infection, respectively. Following humoral response profiles over time from convalescent patients with COVID-19 should provide important insights into the half-life of these antibodies.

Our studies with serial patient samples from the NIH cohort showed the temporal relationship between antibody dynamics with onset of symptoms and PCR positivity for SARS-CoV-2. Cutoff values for a positive result were based on pre-2018 blood donors and may underestimate the number of seropositive persons because some individual patients showed low antibody values initially that gradually rose before exceeding the cutoff value. The 3 immunocompetent patients with COVID-19 showed rapid seroconversion within 10 days of onset of symptoms for nucleocapsid antibody, and then spike antibodies appeared a day or so later. In contrast, the immunocompromised NIH patients exhibited a slower rise in antibody levels with a plateau at lower levels compared to the immunocompetent patients, and 2 patients did not become seropositive until 14 days after onset of symptoms. Despite the blunted antibody responses in the immunocompromised patients, they had a favorable clinical outcome. The NIH patient who died (NIH patient 3) was not immunocompromised and had a rapid rise in antibody production, reaching levels comparable to the other immunocompetent patients. In addition, 1 of the 2 EH patients who died showed the highest antibody levels in that cohort of patients. Although excessive proinflammatory responses to the virus have been reported to contribute to poor outcomes [30–32], larger studies of patients with COVID-19 are required to determine whether antibody levels are directly correlated with disease severity.

Prior studies have shown high levels of SARS-CoV-2 RNA in blood from some patients with COVID-19 [11, 13, 33]. At present, it is not certain whether infectious virus might be circulating in the blood early during infection. Accordingly, plasma or serum was heated to 56° C for 30 minutes to reduce the titer of SARS-CoV-2 before performance of the LIPS assays, because prior studies have shown a marked loss in infectivity of SARS-CoV-1 [23, 34] and MERS-CoV [24] with heat treatment. Whereas impaired detection of viral IgM and IgG antibody

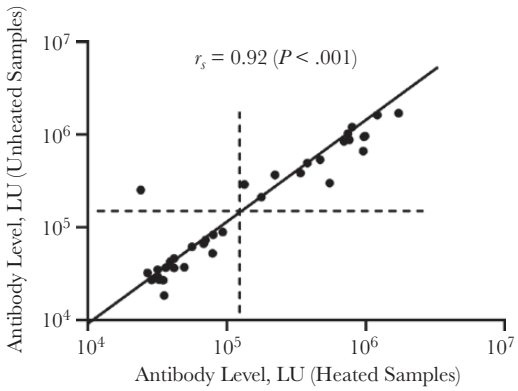

Figure 3. Heat inactivation of plasma or serum samples has no significant impact on detection of nucleocapsid antibodies. A subset (n = 38) of plasma samples from patients with coronavirus disease 2019 (COVID-19) was analyzed, including samples from polymerase chain reaction–positive patients during very early infection (<8 days after onset of symptoms) and at later times after initial infection. Levels of antibody to the nucleocapsid protein were determined using luciferase immunoprecipitation system assays for aliquots of paired unheated and heated plasma or serum samples. Antibody levels were plotted. The horizontal and vertical dotted lines represent cutoff values for seropositivity, and the diagonal line represents theoretically identical antibody levels for heated and unheated samples. Antibody values strongly correlated for heat-treated and unheated samples as shown by the Spearman rank correlation ($r_s = 0.92$; $P < .001$); only 1 sample showed a significant decrease with heating.

responses to viruses after heating of samples to 56°C has been reported [29] and several abstracts report similar findings with SARS-CoV-2 samples, our direct comparison of untreated and treated samples found high concordance of the antibody values, revealing the suitability of heat inactivation. Serological studies with other antigens have shown that the activity of IgM is markedly reduced or destroyed by 56°C heating, while IgG is often preserved [29, 35]. Thus, the lack of difference in antibody levels we observed after heating to 56°C suggested that the antibodies measured were primarily IgG.

A limitation of our study was the focus on more severe COVID-19 cases rather than asymptomatic cases for assay validation. Asymptomatic cases may show lower antibody responses. Further modification of LIPS assays for detection of SARS-CoV-2 antibodies, including the use of other immunoglobulin capture reagents (eg, anti-IgA, anti-IgG, or anti-IgM beads) for measuring isotype-specific antibodies, different SARS-CoV-2 protein fragments, or different luciferase reporters, may further expand or improve assay performance. Nonetheless, our current assay provides quantitative results with a high degree of sensitivity and specificity and should be useful for larger seroepidemiological studies.

Notes

Acknowledgements. We thank Kizzmekia S. Corbett, Barney Graham, and Jason S. McClellan for the gift of plasmid 2019-nCoV-2_S-2P, Jocelyn Voell for assistance with sample collection, Marie K. Hensley, Jemilin E. Mendoza, and Kristine A. Vander Hart for clinical data management, Jason Van Winkle, and Diego Lopez de Castilla, for clinical support, and the John and Mary Tu foundation for support. We thank Joana Klubo-Gwiezdzinska, Mary Walter, Christopher Melani, Steven Rosenberg, Yaron Rotman, and Anna Lau for providing human serum samples from patients.

Financial support. This work was supported by the intramural research programs of the National Institute of Dental and Craniofacial Research, the National Institute of Allergy and Infectious Diseases, and the National Institutes of Health Clinical Center.

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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