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Clinical Validity of Serum Antibodies to SARS-CoV-2

A Case–Control Study

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Background: The clinical utility of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies remains undefined.

Objective: To determine the clinical validity and utility of SARS-CoV-2 antibodies.

Design: Case–control study.

Setting: First month of testing for coronavirus disease 2019 (COVID-19) by using a nucleic acid amplification test (NAAT) on nasopharyngeal swabs at the Johns Hopkins Hospital, Baltimore, Maryland (11 066 persons).

Participants: Of the 11 066 tested persons, 115 (1%) were hospitalized adults investigated for COVID-19. Clinical record review was performed to classify them into a COVID-19 case group (n = 60) or a non–COVID-19 control group (n = 55). The laboratory control groups comprised 513 persons not tested by NAAT: 160 healthy laboratory employees, 101 persons positive for IgG antibodies against Epstein-Barr virus capsid antigen, 215 positive for thyroperoxidase antibody, and 37 positive for rheumatoid factor.

Measurements: Serum IgG and IgA antibodies against SARS-CoV-2 spike protein were detected by using enzyme-linked immunosorbent assay.

Results: Sensitivity and specificity of the SARS-CoV-2 IgG assay were 0.976 (95% CI, 0.928 to 0.995) and 0.988 (CI, 0.974 to 0.995), respectively, when performed 14 days or later after symptom onset, but sensitivity decreased at earlier time points. Immunoglobulin G developed rapidly and was sustained at high levels throughout follow-up (up to 58 days). Antibodies to SARS-CoV-2 predicted the odds of developing acute respiratory distress syndrome, which increased by 62% (CI, 48% to 81%; $P <$ 0.001) for every 2-fold increase in IgG. Of 11 066 NAAT-tested patients, 457 were repeatedly NAAT-negative, and serum samples were obtained for 18 such patients: 6 COVID-19 case patients and 12 non–COVID-19 control patients. Antibodies were present in 5 of 6 case patients and none of the 12 control patients (P = 0.001).

Limitations: The study was retrospective and performed at a single-center; the sample was small; follow-up was limited; and selection bias may have occurred.

Conclusion: Antibodies to SARS-CoV-2 demonstrate infection when measured at least 14 days after symptom onset, associate with clinical severity, and provide valuable diagnostic support in patients who test negative by NAAT but remain clinically suspicious for COVID-19.

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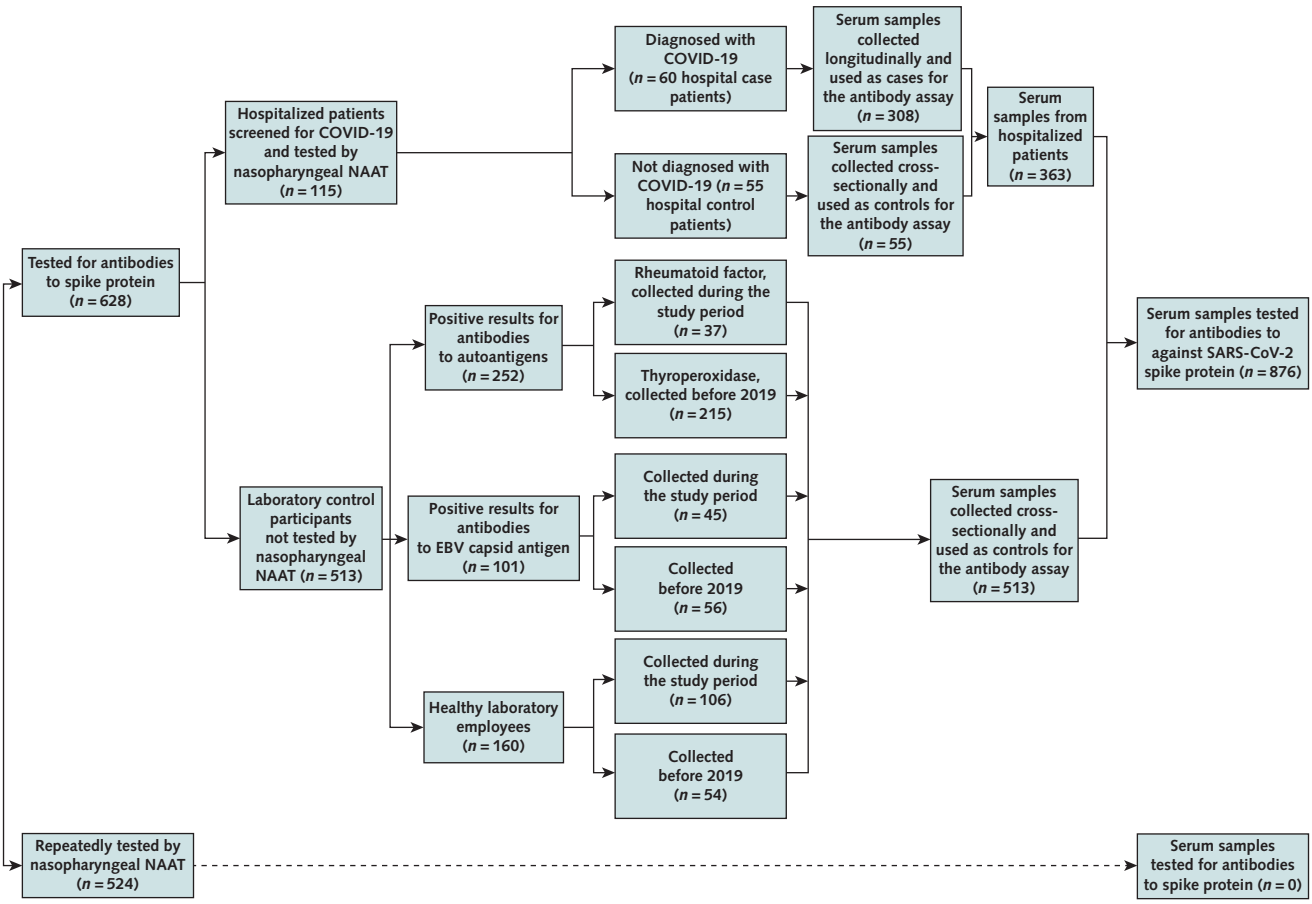
Serum antibodies are the component of the adaptive immune system used most frequently and to greatest effect by clinicians and epidemiologists. Antibodies have accompanied immunology since its inception as an academic discipline in the late 19th century (also enjoying numerous Nobel Prize recognitions), and are once more brought to center stage by the coronavirus 2019 (COVID-19) pandemic. First reported in Wuhan, China, in December 2019, severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) has infected 10 424 992 persons as of 30 June 2020 (1), causing severe disease in about 15% (2) and death in approximately 0.4% (3), due to diffuse alveolar damage featuring intra-alveolar edema and lymphoplasmacytic infiltrate (4).

SARS-CoV-2 is a single-stranded, positive-sense RNA, enveloped, helical virus that synthesizes 4 structural proteins: spike (S), nucleocapsid, matrix, and envelope (5). Spike is a trimeric protein that protrudes from the envelope, giving the virus its "crown" (6). Its S1 subunit mediates cell entry by binding to angiotensin-converting enzyme 2 after "priming" by transmembrane protease serine S2 (7). Given its size, location, and essential function, spike is predicted to be a key target of antibodies (8, 9).

Serologic testing for COVID-19 is considered at all levels of society for many purposes, from diagnosis and management of individual patients (10) to selection of convalescent patients as donors for antibody transfer to critically ill patients (11) and screening of blood or organ donors (12). Serology facilitates assessment of prevalence in at-risk communities (such as health care workers, homeless people, and assisted living residents, among others) and the general population—a prevalence which, as demonstrated in previous viral pandemics, is typically higher than expected (13–16).

Clinical applications of COVID-19 serologic testing remain to be defined. A possible use is to complement the laboratory gold standard of COVID-19 diagnosis: reverse-transcriptase polymerase chain reaction assay, commonly referred to as "nucleic acid amplification test" (NAAT). These tests are predominantly performed on nasopharyngeal swabs, although samples from other anatomical sites, such as bronchoalveolar lavage, sputum, and endotracheal aspirate, are also tested. With increased use, NAAT begins to show limitations (17) arising from intermittent viral shedding (18), time since exposure (19), and nasopharyngeal swab technique (20). Cases where clinical suspicion remains high despite repeated negative NAAT results could espe-

Figure 1. Study flow diagram.



The study included 628 participants tested for serum antibodies against spike protein. The study also included clinical record review of all 558 patients with repeated nucleic acid amplification testing of nasopharyngeal swabs (34 were tested for antibodies, whereas 524 were not). COVID-19 = coronavirus disease 2019; EBV = Epstein–Barr virus; NAAT = nucleic acid amplification test; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

cially benefit from serologic testing. Several recent studies have described the technical performance of antibody assays (8, 18, 20–27), but data on clinical sensitivity and specificity are scarce (15). We report the performance of a serum assay for SARS-CoV-2 spike protein, providing insights into antibody kinetics and clinical uses.

METHODS

This study was approved by the institutional review board of the Johns Hopkins Hospital (IRB 00247645).

Study Design and Participants

We performed a case–control study designed to assess the clinical validity and utility of a serum antibody test for SARS-CoV2.

The study included 628 persons (876 serum samples) tested for SARS-CoV-2 antibodies: 115 hospitalized adults (363 serum samples) investigated for COVID-19 by using NAAT of nasopharyngeal swab, and 513 persons (513 serum samples) not tested by NAAT who provided a serum sample to the immunology laboratory (Figure 1). Clinical record review (without antibody testing) of all 558 patients who had more than 1 nasopharyngeal NAAT was also performed.

Patients Investigated for COVID-19 and Tested by NAAT for SARS-CoV-2

First, we assembled a data set of NAATs on nasopharyngeal swabs performed by the Johns Hopkins microbiology laboratory during the first month of COVID-19 testing (11 March to 12 April 2020), and tallied a total of 11 699 tests. We then stratified the NAATs according to whether they were performed only once (n = 10 508) or more than once (1191 tests in 558 persons) in a given person, for a total of 11 066 persons. We then stratified those 11 066 persons by NAAT result and frequency, yielding 3 groups: NAAT single-positive (1453 [13%]), NAAT single-negative (9055 [82%]), and repeated NAATs (558 [5%]).

We next searched the clinical pathology laboratories for residual serum samples (samples retrieved from the laboratory after being ordered and tested for rou-

tine patient care) with an earliest collection date within the study period. A convenience sample featuring 115 hospitalized patients of the total 11 066 tested by NAAT was obtained (1%), representing the 3 groups indicated above. In particular, this sample included 207 serum samples for 38 single-positive patients, 43 serum samples for 43 single-negative patients, and 113 serum samples for 34 repeatedly tested patients (thus, a total of 363 serum samples from 115 hospitalized patients).

Persons With Laboratory Samples Tested for SARS-CoV-2 Antibodies, but Not by NAAT

The 513 persons in the laboratory control group were 160 healthy laboratory employees and 353 patients with a polyclonal activation of the antibody response (28). The latter subset was composed of 101 persons positive for IgG antibodies against Epstein–Barr virus (EBV) capsid antigen (the most prevalent viral antibody reported in our hospital population), 215 persons positive for thyroperoxidase antibody, and 37 persons positive for rheumatoid factor (humoral markers of the 2 most prevalent autoimmune diseases: Hashimoto thyroiditis and rheumatoid arthritis, respectively). Persons in the laboratory control group were further stratified by time of sample collection, distinguishing the study period from pre-2019 (thus preceding the emergence of COVID-19).

Patients Investigated for COVID-19 and Tested by NAAT, but Without Antibody Measurement

For the 558 patients with repeated NAAT, we reviewed clinical records, irrespective of whether serum was available for antibody assessment. This review aimed to identify the subset of patients for whom diagnostic application of SARS-CoV-2 serology could be most useful, considering that patients with repeated NAAT would be those for whom a clinical diagnosis of COVID-19 is most uncertain.

Detailed Clinical Record Review and Case Definition of the 115 Hospitalized Patients

Extensive review of electronic health records was performed, following each patient from admission to discharge. This review collected risk factors (travel history, sick contacts, occupation), presentation (symptom onset, fever, cough, sputum production, dyspnea, fatigue, chills, myalgia, headache, sore throat, rhinorrhea, nausea, emesis, diarrhea, anorexia, anosmia, dysgeusia), basic chemistry and hematologic analytes (complete blood cell count, complete metabolic panel), radiologic findings (chest plain radiography and computed tomography), comorbidities (hypertension, diabetes, chronic obstructive pulmonary disease, chronic kidney disease, congestive heart failure, cancer), smoking and alcohol history, body mass index, reason for repeated NAAT testing (as applicable), and complications (length of stay, intubation, acute respiratory distress syndrome [ARDS], acute kidney injury, shock).

Since NAAT results alone cannot incontrovertibly establish a COVID-19 diagnosis (29), we integrated the judgment of the clinical team with the collected variables to classify the 115 hospitalized patients as COVID-19 case patients (n = 60) or non–COVID-19 control patients (n = 55).

Serum Antibody Testing

The 628 study participants contributed a total of 876 residual serum samples: 308 obtained longitudinally from 60 COVID-19 case patients, and 568 cross-sectionally from control persons (**Figure 1**). All serum samples were tested for the presence of IgG and IgA antibodies against the S1 domain of the SARS-CoV-2 spike protein by using a manual commercial enzyme-linked immunosorbent assay (ELISA) (Euroimmun), which received an Emergency Use Authorization by the U.S. Food and Drug Administration on 5 May 2020 (30). The assay yields a unitless ratio calculated by dividing the absorbance (optical density) of antibody reactivity in patient serum by that of an assay calibrator (a proprietary humanized monoclonal antibody to the SARS-CoV-2 spike protein). The assay was performed according to the manufacturer's recommendations, testing the serum samples at a 1:100 dilution and respecting specified incubation temperatures, with 2 main modifications. First, we extensively honed the assay to select an in-house threshold value of the ratio that best distinguished COVID-19 case patients from control persons, in addition to applying the cutoff suggested by the manufacturer (1.1 units for both IgG and IgA isotypes). We also serially diluted (1:1000 and 1:2000) samples that violated the Beer–Lambert law, which describes the linear relationship between absorbance and concentration (31), to obtain a quantitative value instead of a "greater than" estimate for samples with high antibody reactivity.

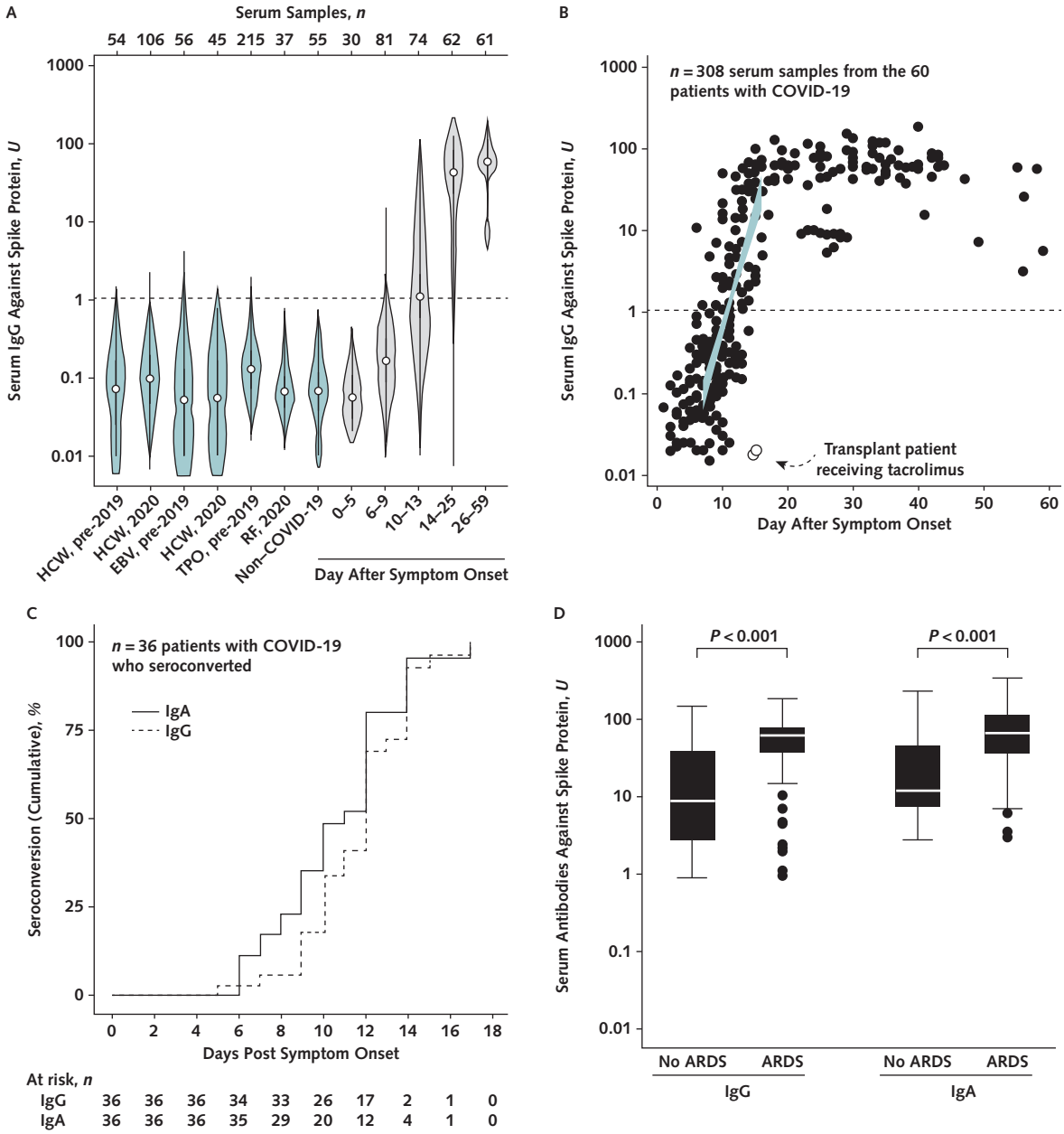
Statistical Analysis

Univariate logistic regression was used to select the antibody level that best classified clinical diagnosis into COVID-19 and non–COVID-19. As COVID-19 case patients for this analysis, we created 5 different scenarios based on the day the antibody was measured after recorded onset of symptoms. As non–COVID-19 control patients, we used all other control groups combined, among whom antibody levels did not differ. After each regression, we calculated the area under the receiver operating characteristic curve for the probability of having a COVID-19 diagnosis. We then used the closest-to-(0,1) method to select the antibody cutoff.

Multiple linear regression with generalized estimating equations (32) was used to analyze how antibodies in COVID-19 case patients evolved longitudinally after the onset of symptoms, considering that measures repeated over time in the same patient are likely to be correlated with one another. In addition to day after symptom onset, predictors featured in the final model included antibody isotype (IgG or IgA) and use of immunosuppressive drugs at admission. Kaplan–Meier nonparametric survival function was used to calculate the seroconversion rate of IgG and IgA after symptom onset in COVID-19 case patients.

Clinical characteristics of COVID-19 case patients and persons in the control group were compared by us-

Figure 2. Biological characteristics of serum IgG antibodies against SARS-CoV-2 spike protein in the COVID-19 case group and the laboratory control groups.



A. Violin plot showing the distribution of IgG levels, indicating median and interquartile range. The horizontal dashed line represents the manufacturer cutoff (1.1 units). B. Overall relationship between IgG levels and day post symptom onset. The white circles represent a hypogammaglobulinemic patient receiving immunosuppressive therapy due to kidney transplant. C. Kaplan–Meier survival function of IgG and IgA seroconversion. D. Serum IgG and IgA antibody levels, stratified by the presence or absence of ARDS. ARDS = acute respiratory distress syndrome; COVID-19 = coronavirus disease 2019; EBV = Epstein–Barr virus; HCW = health care worker; RF = rheumatoid factor; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; TPO = thyroperoxidase.

ing nonparametric tests (Fisher exact χ^2 test for low incidences and Wilcoxon rank-sum test) and were presented as proportions or medians with interquartile ranges. Because the antibody result was not normally distributed, results were transformed to natural logarithm for all analyses. Differences in mean antibody levels among groups were assessed by analysis of variance.

Data were analyzed by using Stata statistical software, release 16.1.

Role of the Funding Source

The study was funded internally by the Clinical Immunology Laboratory of the Department of Pathology, Johns Hopkins Hospital. The funder had no role in the

design or conduct of the study, analysis of the data, or the decision to submit the manuscript for publication.

RESULTS

The serum ELISA for SARS-CoV-2 antibodies performs well when used 14 days or more after symptom onset. Serum IgG antibodies against SARS-CoV-2 were significantly higher in COVID-19 case patients (median, 2.01 units [interquartile range, 0.16 to 44.33 units]) than in all persons in the control groups (median, 0.10 unit [interquartile range, 0.05 to 0.19 unit]; $P < 0.001$) (**Figure 2**, *A*). Time of assessment, however, was critical to the interpretation of antibody results: After time since symptom onset was grouped into 5 intervals (0 to 5 days, 6 to 9 days, 10 to 13 days, 14 to 25 days, and 26 to 59 days), median IgG increased from 0.06 units to 0.17, 1.11, 44.33, and 59.08 units, respectively (**Figure 2**, *A*). When measured at least 14 days after symptom onset, serum IgG to SARS-CoV-2 spike protein distinguished COVID-19 case patients from persons in the control group with a sensitivity of 0.976 (95% CI, 0.928 to 0.995) and a specificity of 0.988 (CI, 0.974 to 0.995), using the manufacturer's cutoff of 1.1 units (**Table 1**). Similar sensitivity (0.976 [CI, 0.928 to 0.995]) and specificity (0.989 [CI, 0.977 to 0.996]) were obtained by using the internally derived IgG cutoff (1.23 units). Serum IgA antibodies against SARS-CoV-2 spike protein measured at the same time point yielded similar sensitivity (0.984 [CI, 0.939 to 0.999]) but lower specificity (0.880 [CI, 0.851 to 0.905]) when the manufacturer cutoff (1.1 units) was used (**Appendix Table 1**, available at Annals.org). Sensitivity decreased to 0.951 (95% CI, 0.895 to 0.980) and specificity increased to 0.961 (95% CI, 0.942 to 0.975) when the internally derived IgA cutoff (2.26 units) was used (**Appendix Table 1**).

When measured before day 14, IgG and IgA antibodies yielded substantially lower sensitivity (**Table 1** and **Appendix Table 1**). Positivity rates for the 2 isotypes in each control group are reported in **Appendix Table 2** (available at Annals.org).

We found that SARS-CoV-2 antibodies rapidly reached high levels in COVID-19 case patients and remained elevated up to 2 months after symptom onset. From being undetectable in all patients until day 5 after symptom onset, IgG levels increased 1.78 units (95% CI, 1.62 to 1.97 units; $P < 0.001$) per day until 16 days after symptom onset (**Figure 2**, *B*). By that point, all case patients except 1 had elevated antibody results; the exception was a hypogammaglobulinemic patient receiving immunosuppressive therapy (tacrolimus and prednisone) for kidney transplant (**Figure 2**, *B*). Similarly, rapid kinetics was observed for the IgA isotype (data not shown). As the humoral response to SARS-CoV-2 matured, spike antibodies reached levels approximately 100-fold greater than those in the control group; these levels were sustained for the duration of follow-up, up to 59 days after symptom onset.

Immunoglobulin A seroconversion anticipates IgG by 2 days. Of 60 COVID-19 case patients, 49 had longitudinal serum collection, with a follow-up time ranging from 5 to 59 days after symptom onset (median, 23 days [interquartile range, 12 to 29 days]) (**Appendix Figure** [available at Annals.org], *top*). Of these, 36 patients had baseline (preseroconversion) serum collection. On time-to-event analysis, median time to seroconversion was 12 days after symptom onset for IgG, and all patients developed IgG by day 17 (**Figure 2**, C). Serum IgA levels correlated well with those of IgG: For every unit increase in the natural logarithm of IgG, the natural logarithm of IgA increased by 0.73 units (adjusted $R^2 = 0.83$; $P < 0.001$) (**Appendix Figure**, *middle*). On average, IgA was approximately 14 units higher than IgG ($P < 0.001$) and appeared earlier, with a median time to seroconversion of 10 days after symptom onset (**Figure 2**, C).

We noted that SARS-CoV-2 antibody levels strongly predicted development of ARDS, after adjustment for G or A isotype and time since symptom onset. Both IgG and IgA levels were significantly elevated in COVID-19 case patients with ARDS than in less severe cases ($P < 0.001$) (**Figure 2**, *D*). The odds of ARDS increased by 62% (CI, 48% to 81%; $P < 0.001$) or 76% (CI, 54% to 111%; $P < 0.001$) for every 2-fold increase in IgG or IgA, respectively. In addition to a significantly higher incidence of intubation and ARDS, COVID-19 case patients were more frequently male and symptomatic (fever, cough, myalgia, fatigue, rhinorrhea, chills, anorexia, and headache), and stayed longer than non–COVID-19

Table 1. Performance Characteristics of Serum IgG Against SARS-CoV-2 Spike Protein for the Diagnosis of COVID-19*

Day Post Symptom†	Sensitivity			Specificity			LR+	LR-
	Positive Serum Samples From COVID-19 Group, *n*	Total Serum Samples From COVID-19 Group, *n*	Point Estimate (95% CI)	Negative Serum Samples From Control Group, *n*	Total Serum Samples From Control Group, *n*	Point Estimate (95% CI)		
≥14	120	123	0.976 (0.928–0.995)				79.2	0.02
10–13	38	74	0.514 (0.402–0.624)				42.7	0.5
6–9	6	81	0.074 (0.031–0.155)	561	568	0.988 (0.974–0.995)	6.0	0.9
0–5	0	30	0 (0–0.135)				0	1.0

COVID-19 = coronavirus disease 2019; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
* The manufacturer (Euroimmun) cutoff of 1.1 units was used for the antibody result. Confidence intervals for sensitivity and specificity were calculated by using the Agresti–Coull method.
† Time between serum collection and the onset of symptoms, as documented in the clinical record.

control patients (**Appendix Table 3**, available at Annals .org).

In patients who repeatedly test negative on nasopharyngeal NAAT but the index of clinical suspicion is still high, SARS-CoV-2 antibodies support COVID-19 diagnosis. Of 11 066 persons who underwent nasopharyngeal NAAT during the study period, 1517 (14%) tested positive and 9549 (86%) negative (**Figure 3**, *top*), a relatively high prevalence reflective of the referral nature of the Johns Hopkins Hospital. In 10 508 (95%) persons, NAAT was performed once, whereas it was repeated 2, 3, 4, or 5 times in 558 (5%) persons (**Figure 3**, *top*). The interval between first and last NAAT ranged from 1 to 28 days (median, 6 days [interquartile range, 2 to 12 days]), with 8% of tests repeated within 24 hours (**Appendix Figure**, *bottom*). Most repeated tests (457 of 558 [82%]) were consistently negative (**Figure 3**, *middle*), whereas the remaining ones were consistently positive (42 [8%]), negative followed by positive (36 [6%]), positive followed by negative (17 [3%]), or inconsistent (6 [1%]) (**Figure 3**, *middle*). Clinical record review demonstrated that the predominant reason for repeating NAAT was clinical suspicion of COVID-19 (325 of 558 [58%]), followed by administrative requirements, such as discharge placement requirement or patient request (87 [16%]); belonging to an at-risk population, such as health care employees or homeless (79 [14%]); or unknown (67 [12%]).

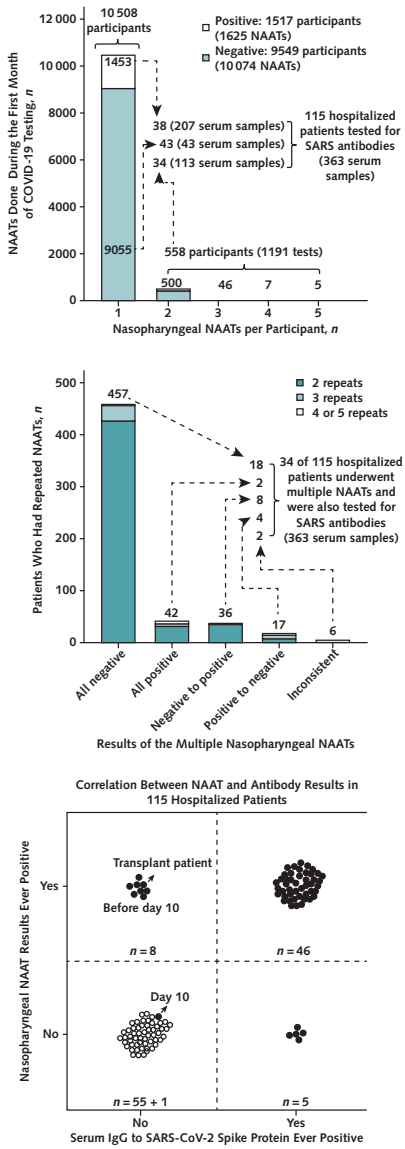
Review of clinical records showed that 13 of 457 (3%) consistently NAAT-negative patients were clinically diagnosed with COVID-19 (**Table 2**). We obtained residual serum samples for 18 of these 457 patients: 12 were clinically classified as non–COVID-19 control patients and 6 as COVID-19 case patients. Antibodies for SARS were present in 5 of 6 (83%) COVID-19 case patients (**Figure 3**, *bottom*) and absent in all 12 non–COVID-19 control patients (**Figure 3**, *bottom*) (P = 0.001). The antibody- and NAAT-negative patient with COVID-19 (**Figure 3**, *bottom*) contributed only an early serum sample (day 10 after symptom onset), which was a premature time frame for adequate assessment of seroconversion. This patient was also the only of the 6 COVID-19 case patients who had NAAT of a sample from an additional anatomical site (bronchoalveolar lavage), which was positive.

Correlating NAAT and antibody results (both dichotomously categorized as ever positive or negative) in all 115 study patients showed agreement in 102 patients, 46 both positive (**Figure 3**, *bottom*) and 56 both negative (**Figure 3**, *bottom*), for an overall concordance of 89%. Of 8 patients who tested positive by NAAT and negative by antibodies (**Figure 3**, *bottom*), 1 was the immunosuppressed transplant recipient described above, and the other 7 had only an early serum sample collection (between 2 and 9 days after symptom onset).

DISCUSSION

We report SARS-CoV-2 antibody characteristics in an early cohort of patients with COVID-19 from the Johns Hopkins Hospital, highlighting sensitivity and

Figure 3. Integration of clinical data and molecular testing with serum antibodies to SARS-CoV-2 spike protein.



Top. Classification of 11 066 patients undergoing nasopharyngeal NAAT during the first month of COVID-19 at the Johns Hopkins Hospital, by NAAT result and number per patient. Middle. Classification of NAAT results in 558 repeatedly tested patients. Bottom. Correlation between antibody and NAAT results, classified dichotomously as ever positive or negative, in 115 hospitalized patients. Closed circles indicate COVID-19 case patients; open circles indicate non-COVID-19 hospital control patients. The top left quadrant shows the 8 patients who tested positive by NAAT and negative by antibodies (the immunosuppressed transplant patient and 7 patients with serum collection between 2 and 9 day after symptom onset). The top right and bottom left quadrants show the correlation between NAAT and antibody results, which agreed in 102 patients (46 both positive and 56 both negative). In the lower left quadrant, the closed circle indicates the antibody- and NAAT-negative patient with COVID-19 who contributed a serum sample at day 10. The lower right quadrant indicates patients with false-negative results on NAAT who tested positive for SARS-CoV-2 antibodies. COVID-19 = coronavirus disease 2019; NAAT = nucleic acid amplification test; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

specificity, seroconversion kinetics, correlation with disease progression, and diagnostic potential in NAAT-negative cases. Besides epidemiologic and therapeutic applications, the study shows the potential contribution of serology to COVID-19 diagnosis, which currently relies on integrating symptom surveillance, radiographic findings, and NAAT results.

Antibodies to SARS-CoV-2 begin to appear approximately 10 days after symptom onset and rapidly increase to a high and sustained plateau, in keeping with IgG and IgA seroconversion reported in other infectious diseases and with SARS-CoV-2 incubation (33–35). When chronology is taken into account by measuring at least 14 days after symptom onset, serologic testing has excellent specificity and sensitivity. Conversely, premature testing can result in false-negative results and probably explains the suboptimal assay performance reported elsewhere (24). A wide analytical measuring range (0.01 to 300 units) coupled with proper serum dilution allows assay results to be reported as continuous numbers rather than titers, enabling precise monitoring of humoral kinetics and accurate stratification of convalescent plasma donors. Selecting the appropriate time to first measure antibodies is challenging because nonspecific and mild symptoms, altered mental status, language barriers, and other patient or physician factors can obfuscate the clinical history.

Patients with a clinical diagnosis of COVID-19 who do not have antibodies can be considered to have false-negative results, which probably arise from inaccurate estimation of infection time but also antigenic variation in spike protein or immunosuppression (19, 27, 36). In our cohort of 60 hospitalized patients with COVID-19, in fact, the 8 false-negative results were in patients with immunosuppression or follow-up not beyond day 10. Conversely, false-positive results are seen those who have antibodies in the absence of clinically diagnosed COVID-19. In our cohort, 5 of 513 (1%) persons in the laboratory control groups had false-positive results, but their antibody level was low and remained stable over time, in sharp contrast to the humoral kinetics observed in COVID-19 case patients. Two of these persons with false-positive results were hospital employees with serum collected during the study period and could thus represent asymptomatic infection, especially prevalent in younger healthy individuals (37). The other 3 results predated COVID-19 and could represent cross-reactivity with other human betacoronaviruses (38) or conserved viral antigens. For instance, EBV induces a robust polyclonal antibody response and predisposes to autoimmunity, possibly via molecular mimicry (39). Of 101 EBV-positive persons in our study, however, only 2 (2%) had SARS-CoV-2 antibodies of low titer, a proportion not significantly different from that seen in the healthy laboratory employees and non–COVID-19 patients.

In addition to clinical implications of antibody kinetics and levels, our study revealed a strong relationship between antibodies and development of ARDS in COVID-19. This novel finding provides further insights on the contribution of the immune system to COVID-19 pathogenesis, as demonstrated in cytokine storm (35, 40, 41).

An important application of serology is to support diagnosis in patients who test negative by NAAT but remain at high clinical suspicion of COVID-19. Since its inception just a few months ago, NAAT has been rapidly scaled up, but like any new test, is starting to show its limitations, possibly arising from intermittent viral shedding and nasopharyngeal swab technique (18, 20). Recent reports estimate false-negative results to be as frequent as 20%, depending on timing of measurement (29). Considering the risks of disease progression and further contagion, identification of false-negative results is imperative, especially given the prevalence of the pandemic. Until the virus becomes endemic, antibodies can play a useful role in the diagnosis of acute illness.

The observation that 5% of patients underwent NAATs repeatedly (as many as 5 times) and frequently (median interval of 6 days between first and last test, with 8% repeated within 24 hours) suggested diagnostic uncertainty. Review of clinical record for all repeatedly tested patients indeed confirmed that clinical suspicion of COVID-19 was the most common reason for repetition. Applying antibody determination to the NAAT false-negative subset revealed the remarkable utility of serology: SARS-CoV-2 infection was demonstrated in all but 1 patient, for whom serum collection was limited to 10 days after symptom onset.

Our study has limitations, which include a retrospective design, a relatively small number of cases from a single center, convenience sampling of hospitalized patients, a relatively brief duration of follow-up, imprecision in what represents the true time zero for anti-

Table 2. Diagnosis of COVID-19 in the 558 Patients Who Had Repeated NAAT of Nasopharyngeal Swabs

Group	Nasopharyngeal NAAT Result, *n*					
	All Negative	All Positive	Negative to Positive	Positive to Negative	Inconsistent	Total
No chart available	30	8	3	0	1	42
Unknown diagnosis	9	0	4	0	0	13
Non–COVID-19 patient	405	0	0	1	1	407
COVID-19 patient	13	34	29	16	4	96
Total	457	42	36	17	6	558

COVID-19 = coronavirus disease 2019; NAAT = nucleic acid amplification test.

body response, and incomplete isotype characterization owing to lack of IgM assay. Convenience sampling of hospitalized patients (Berkson bias) limits conclusions about pretest characteristics of case patients and the control group. Extended follow-up is always desirable but is currently precluded by the recent emergence of COVID-19. Nevertheless, every patient who seroconverted maintained antibodies for the duration of follow-up. An accurate determination of time of infection can rarely be evinced on clinical grounds, but its estimation by time of symptom onset revealed itself to be precise and clinically applicable. As recently reported, IgM appears and wanes rapidly, thus limiting diagnostic utility and appropriate characterization of convalescent plasma donors (42).

In conclusion, we report the clinical utility of SARS-CoV-2 antibodies by describing their kinetics, association with disease severity, and utility in diagnosing COVID-19 in patients with false-negative results on NAAT.

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Reproducible Research Statement: *Study protocol, statistical code, and sample data set:* Available upon reasonable request to Dr. Patrizio Caturegli (e-mail, pcat@jhmi.edu).

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Current author addresses and author contributions are available at Annals.org.

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Appendix Table 1. Performance Characteristics of Serum IgA Against SARS-CoV-2 Spike Protein for Diagnosis of COVID-19*

Day Post Symptom†	Cutoff‡	Sensitivity			Specificity			LR+	LR-
		Positive Serum Samples From COVID-19 Group, *n*	Total Serum Samples From COVID-19 Group, *n*	Point Estimate (95% CI)	Negative Serum Samples From Control Group, *n*	Total Serum Samples From Control Group, *n*	Point Estimate (95% CI)		
≥14	1.1	121	123	0.984 (0.939–0.999)				8.2	0.02
10–13	1.1	57	74	0.770 (0.662–0.852)				6.4	0.3
6–9	1.1	39	81	0.481 (0.376–0.589)	500	568	0.880 (0.851–0.905)	4.0	0.6
0–5	1.1	4	30	0.133 (0.047–0.303)				1.1	1.0
≥14	2.26	117	123	0.951 (0.895–0.980)				24.6	0.1
10–13	2.26	47	74	0.635 (0.521–0.736)				16.4	0.4
6–9	2.26	21	81	0.259 (0.176–0.365)	546	568	0.961 (0.942–0.975)	6.7	0.8
0–5	2.26	117	123	0.951 (0–0.181)				1.0	1.0

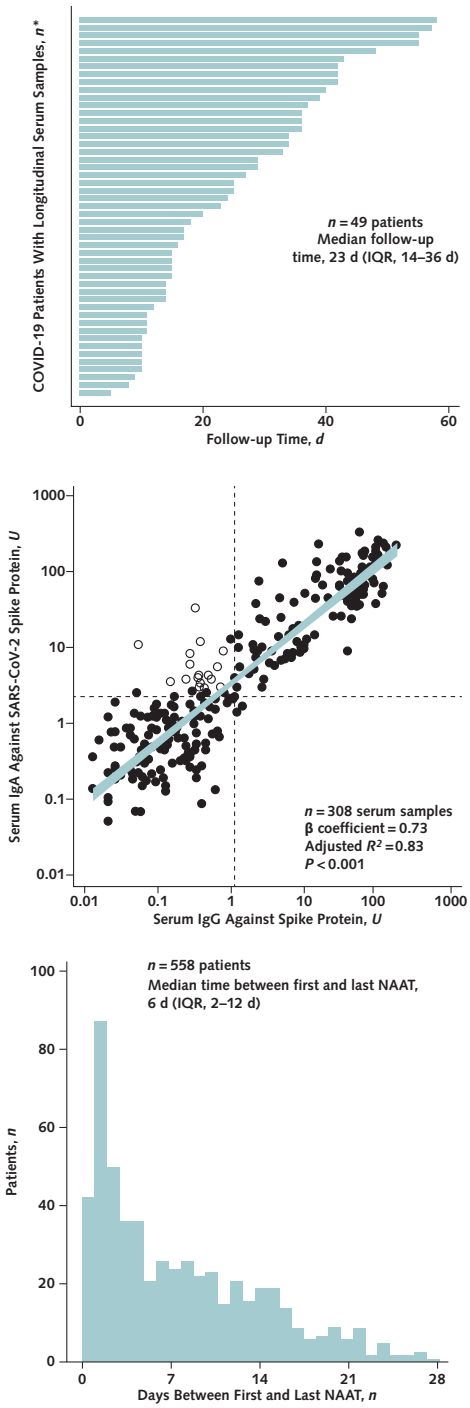
COVID-19 = coronavirus disease 2019; LR+ = positive likelihood ratio; LR- = negative likelihood ratio; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
* Confidence intervals for sensitivity and specificity proportions were calculated by using the Agresti–Coull method. Confidence intervals for area under the receiver operating characteristic curve were calculated by bootstrapping with bias-corrected estimates.
† Time between serum collection and the onset of symptoms, as documented in the clinical record.
‡ The manufacturer (Euroimmun) cutoff of 1.1 units and an internally derived cutoff (2.26 units) were used for the antibody result.

Appendix Table 2. Positivity Rates of IgG and IgA Antibodies Against SARS-CoV-2 Spike Protein in the Control Groups*

Analyte	Group	Total Serum Samples, *n*	Positive Serum Samples, *n*	Proportion of Positive Serum Samples (95% CI)
IgG	Healthy laboratory employees, pre-2019	54	0	0
IgG	Healthy laboratory employees, study period	106	2	0.019 (0.001–0.070)
IgG	EBV capsid antigen positive, pre-2019	56	1	0.018 (0–0.103)
IgG	EBV capsid antigen positive, study period	45	0	0
IgG	Thyroperoxidase positive, pre-2019	215	4	0.019 (0.006–0.049)
IgG	Rheumatoid factor positive, study period	37	0	0
IgG	Non–COVID-19 hospitalized patients, study period	55	0	0
IgA	Healthy laboratory employees, pre-2019	54	1	0.019 (0–0.107)
IgA	Healthy laboratory employees, study period	106	1	0.009 (0–0.057)
IgA	EBV capsid antigen positive, pre-2019	56	5	0.089 (0.035–0.197)
IgA	EBV capsid antigen positive, study period	45	2	0.044 (0.004–0.156)
IgA	Thyroperoxidase positive, pre-2019	215	8	0.037 (0.018–0.073)
IgA	Rheumatoid factor positive, study period	37	0	0
IgA	Non–COVID-19 hospitalized patients, study period	55	4	0.072 (0.024–0.177)

COVID-19 = coronavirus disease 2019; EBV = Epstein–Barr virus; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.
* The manufacturer (Euroimmun) cutoff of 1.1 units was used for IgG, and an internally derived cutoff of 2.26 units was used for IgA. Confidence intervals for sensitivity and specificity were calculated by using the Agresti–Coull method.

Appendix Figure. Additional study characteristics.



Top. Distribution of follow-up time in 49 of the 60 COVID-19 patients for whom longitudinal serum samples were available. Middle. Linear relationship between serum IgG and IgA levels. Open circles indicate patients who exclusively converted IgA but not IgG before day 12 post symptom onset. Bottom. Distribution of time between first and last NAAT in 558 patients tested repeatedly. COVID-19 = coronavirus disease 2019; IQR = interquartile range; NAAT = nucleic acid amplification test; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Appendix Table 3. Clinical Characteristics of the COVID-19 Case Group and the Non–COVID-19 Control Group From the First Month of the Epidemic in Baltimore (March–April 2020)*

Characteristic	Non–COVID-19 Control Patients (n = 55)	COVID-19 Case Patients (n = 60)	*P* Value
Demographic			
Age, *y*	61 (47–69)	59 (48–70)	0.84
Sex			0.001
Female	33 (60)	17 (18)	
Male	22 (40)	43 (72)	
Race			0.025
White	27 (49)	27 (45)	
African American	26 (47)	20 (33)	
Asian	2 (4)	7 (12)	
Other	0	6 (10)	
Medical history			
Hypertension	34 (62)	30 (50)	0.20
Diabetes mellitus	20 (36)	12 (21)	0.050
COPD	8 (15)	4 (7)	0.167
Asthma	6 (11)	11 (18)	0.26
Congestive heart failure	6 (11)	4 (7)	0.42
Chronic kidney disease	10 (18)	4 (7)	0.059
Cancer	11 (20)	11 (18)	0.82
Alcohol use	14 (25)	19 (32)	0.46
Smoking	26 (47)	12 (20)	0.002
BMI, kg/m^2	26.8 (22.9–33.6)	27.2 (23.3–31.1)	0.53
Risk factors			
Travel history	6 (11)	21 (35)	0.002
Sick contacts	0	20 (33)	<0.001
Healthcare worker	3 (5)	5 (8)	0.54
Clinical presentation			
Duration of symptoms, *d*	3 (1–7)	7 (4–7)	0.042
Fever	17 (31)	55 (92)	<0.001
Cough	30 (55)	48 (80)	0.004
Shortness of breath	26 (47)	33 (55)	0.41
Myalgia	11 (20)	32 (53)	<0.001
Fatigue	12 (22)	25 (42)	0.023
Rhinorrhea	7 (13)	20 (33)	0.009
Chills	6 (11)	17 (28)	0.020
Sputum	6 (11)	12 (20)	0.180
Anorexia	6 (11)	23 (38)	<0.001
Nausea/emesis	10 (19)	18 (30)	0.155
Headache	5 (9)	17 (28)	0.009
Diarrhea	8 (15)	13 (22)	0.32
Sore throat	7 (13)	9 (15)	0.73
Dysgeusia	2 (4)	2 (3)	0.93
Anosmia	2 (4)	0 (0)	0.136
Laboratory and radiologic investigations			
Positive first NAAT of nasopharyngeal swab	0	50 (83)	<0.001
Hemoglobin level, *g/L*	109 (96–128)	131 (114–141)	<0.001
Leukocyte count, × 10^9 *cells/L*	9.2 (6.7–12.6)	6.0 (4.2–8.2)	<0.001
Lymphocyte count, × 10^9 *cells/L*	1.4 (0.9–1.9)	0.9 (0.6–1.2)	<0.001
Platelet count, × 10^9 *cells/L*	228 (195–280)	191 (143–241)	0.015
Creatinine concentration			0.64
μmol/L	88.4 (70.7–123.8)	88.4 (79.6–114.9)	
mg/dL	1.0 (0.8–1.4)	1.0 (0.9–1.3)	
Aspartate aminotransferase level, *U/L*	24 (17–55)	35 (28–52)	0.011
Alanine aminotransferase level, *U/L*	22 (12–46)	33 (21–46)	0.018
Chest radiography consistent with COVID-19†	9 (19)	26 (57)	<0.001
Chest CT consistent with COVID-19†	10 (32)	19 (90)	<0.001
Outcomes			
Length of stay, *d*	4 (1–9)	12 (5–23)	<0.001
Intubation	5 (9)	23 (38)	<0.001
ARDS	3 (6)	23 (38)	<0.001
Acute kidney injury	14 (25)	22 (37)	0.195
Shock	3 (5)	5 (8)	0.54
Death	1 (2)	7 (12)	0.07

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Appendix Table 3–Continued

Characteristic	Non-COVID-19 Control Patients (*n* = 55)	COVID-19 Case Patients (*n* = 60)	*P* Value
Discharge diagnosis			
COVID-19	0	60 (100)	NA
Respiratory	21 (38)		
Pneumonia (non–COVID-19)	9 (43)		
COPD	7 (33)		
Interstitial lung disease	4 (19)		
Asthma	1 (5)		
Drug toxicity	8 (15)		
Cardiovascular	6 (11)		
Neurologic	5 (9)		
Sepsis	5 (9)		
Renal	3 (5)		
Rheumatologic	2 (4)		
Trauma	1 (2)		
Obstetric	1 (2)		
Unknown	3 (5)		

ARDS = acute respiratory distress syndrome; BMI = body mass index; COPD = chronic obstructive pulmonary disease; COVID-19 = coronavirus disease 2019; CT = computed tomography; NA = not applicable; NAAT = nucleic acid amplification test.
* Data are presented as the number (percentage) for categorical variables or as the median (interquartile range) for continuous variables.
† Chest radiography was available for 46 COVID-19 patients and 46 non–COVID-19 patients. Chest CT was available for 21 COVID-19 patients and 31 non–COVID-19 patients.