The Journal of Infectious Diseases

BRIEF REPORT

Differences in Antibody Kinetics and Functionality Between Severe and Mild Severe Acute Respiratory Syndrome Coronavirus 2 Infections

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We determined and compared the humoral immune response in patients with severe (hospitalized) and mild (nonhospitalized) coronavirus disease 2019 (COVID-19). Patients with severe disease (n = 38) develop a robust antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including immunoglobulin G and immunoglobulin A antibodies. The geometric mean 50% virus neutralization titer is 1:240. SARS-CoV-2 infection was found in hospital personnel (n = 24), who developed mild symptoms necessitating leave of absence and self-isolation, but not hospitalization; 75% developed antibodies, but with low/absent virus neutralization (60% with titers <1:20). While severe COVID-19 patients develop a strong antibody response, mild SARS-CoV-2 infections induce a modest antibody response. Long-term monitoring will show whether these responses predict protection against future infections.

Keywords. SARS-CoV-2; COVID-19; antibody response; IgA antibodies; virus neutralization.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in the human population at the end of 2019, had reached pandemic proportions by March 2020 [1, 2]. The host defense against this new member of the coronavirus family will depend on innate immunity and humoral and cellular immune responses of yet-unknown relative importance [3, 4]. For these reasons, the level of protection and longevity of protection cannot be established or predicted.

Similar to other respiratory viruses, the primary diagnosis of coronavirus disease 2019 (COVID-19) is routinely made by reverse-transcription polymerase chain reaction (RT-PCR) detection of SARS-CoV-2. As the sensitivity of molecular detection relies on severity of illness, sample type, and timing of sampling in the course of infection [5, 6], serology has important additional value to establish a recent infection and to support patient care. It is therefore important to carefully determine the kinetics, magnitude, and functionality of the humoral immune response in COVID-19 patients with different disease severity.

Here, we have analyzed the type and functionality of the humoral immune response of PCR-confirmed hospitalized COVID-19 patients, both intensive care unit (ICU) and non-ICU admitted, and of nonhospitalized patients with mild disease. We performed a semi-quantitative analysis of total immunoglobulin, immunoglobulin G (IgG), and immunoglobulin A (IgA) antibodies by enzyme-linked immunosorbent assay (ELISA), as well as a functional analysis by virus neutralization assays.

MATERIALS AND METHODS

Patients and Blood Sampling

Serum samples were collected from a prospective cohort of 38 patients with RT-PCR–confirmed COVID-19 [7] (Supplementary Table 1) admitted to the Admiral de Ruyter Hospital in Goes, The Netherlands, in accordance with the local clinical procedures in the period March 2020–May 2020. The criteria for hospital admission were severity and/or progression of clinical symptoms, as assessed by the referring general practitioner. The presenting clinical symptoms included fever (n = 17), cough (n = 18), dyspnea (n = 11), dizziness and/or confusion (n = 4), and general malaise (n = 6). Patients were admitted to the hospital a median of 7 days (range, 1–12 days) after onset of symptoms. Fifteen of 38 patients were admitted to the ICU. The clinical criteria for admission of hospitalized patients to the ICU primarily were respiratory insufficiency, hemodynamic instability, and/or multiorgan failure. RT-PCR was always performed on the first hospital day on nasopharyngeal swabs. Serial blood sampling (3 times per week) was started a median of 2 days (range, 1–7 days) after positive RT-PCR.

The second cohort of 24 patients with mild COVID-19 disease consisted of hospital personnel (both from clinical departments as well as laboratory departments) who developed fever, coughing, and/or dyspnea and tested positive for SARS-CoV-2 by RT-PCR. Their median age was 42 years (range, 21–66 years).

Received 4 June 2020; editorial decision 21 July 2020; accepted 23 July 2020; published online July 29, 2020.

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The Journal of Infectious Diseases® 2020;XX:1–5

DOI: 10.1093/infdis/jiaa463

They were asked to maintain self-quarantine until symptoms resolved. All of them were kept under control of their own general practitioner, and none of them required hospitalization.

The study was performed in accordance with the guidelines for sharing of patient data of observational scientific research in emergency situations as issued by the Commission on Codes of Conduct of the Foundation Federation of Dutch Medical Scientific Societies (https://www.federa.org/federa-english).

SARS-CoV-2 Immunoassays

The Wantai SARS-CoV-2 total antibody ELISA (Beijing Wantai Biological Pharmacy Enterprise, Beijing, China; catalog number WS1096) was performed according to the manufacturer's instructions [8]. This assay is a double-antigen sandwich ELISA using the recombinant receptor binding domain antigen of SARS-CoV-2 as antigen. Optical density (OD) is measured at 450 nm and the antibody titer for each sample is calculated as the ratio of the reading of that sample to the reading of a calibrator (included in the kit):OD ratio. SARS-CoV-2 IgG and IgA antibodies were determined by ELISA using the beta version of the EUROIMMUN immunoassay kit (EUROIMMUN Medizinische Labordiagnostika AG, https://www.euroimmun.com) according to the manufacturer's protocol [8, 9]. In this assay, wells are coated with recombinant structural protein (S1 domain) of SARS-CoV-2. Antibody titers are also expressed as OD ratios (of sample to calibrator) as described above. Sensitivity and specificity of the antibody assays are given in Supplementary Table 2.

Coronavirus Microarray

Sera were tested for the presence of IgG antibodies reactive with the 4 common human coronaviruses OC43, HKU1, NL63, and 229E S1 subunit antigens in a protein microarray, in duplicate 2-fold serial dilutions starting at 1:20, essentially as described previously [10]. For each coronavirus, a 4-parameter log-logistic calibration curve was generated. Antibody titers were defined as the interpolated serum dilution that gave a fluorescence intensity of 50% of the corresponding calibration curve. Raw data were processed with the R 2.12.1 statistical software package as described previously [11].

SARS-CoV-2 Neutralization Assay

Sera were tested by a SARS-CoV-2–specific virus neutralization test (VNT) based on a protocol described previously with some modifications [12] (see also Supplementary Methods). In brief, replicate serial dilutions of heat-inactivated samples (30 minutes at 56°C) were incubated with 100 fold tissue culture 50% infectious dose of SARS-CoV-2 for 1 hour at 35°C. African green monkey (Vero-E6) cells were added in a concentration of 2×10^4 cells per well and incubated for 3 days at 35°C in an incubator with 5% carbon dioxide. The 50% and 90% VNT titer (VNT_{50} and VNT_{90}, respectively), defined as the highest serum dilution that protected more than 50% or 90% of cells from cytopathological effect, was taken as the neutralization titer. Samples with titers ≥10 were defined as SARS-CoV-2 seropositive.

Statistical Analysis

Categorical variables were described as frequency rates and percentages, and continuous variables were described using geometric means, median, and interquartile range. Means for continuous variables were compared using independent group *t* tests. Categorical variables were compared using the χ^2 test. All analyses were done in Excel with the data analysis toolpack. *P* values of < .05 were considered statistically significant.

RESULTS

A prospective cohort of 38 consecutive hospitalized patients with COVID-19 (Supplementary Table 1) was monitored for development of anti-SARS-CoV-2 total immunoglobulin, IgG, and IgA antibodies. Within 2–5 days after onset of symptoms, 4 patients already showed total immunoglobulin responses and a steep increase in IgG ratios (see also below and Figure 1 and Supplementary Figure 1). Most patients responded for IgG and IgA between days 10 and 15 (Supplementary Figure 1). The vast majority of the hospitalized patients developed high levels of antibodies after 3–4 weeks: 100% of patients had detectable (total) antibodies, 84% had detectable IgG antibodies, and 92% had IgA antibodies.

Patients admitted to the ICU (n = 15) showed an antibody response similar to patients in the general COVID-19 ward (n = 23), with an IgG antibody ratio at days 14–21 of 9.9 ± 5.2 (ICU) and 8.7 ± 5.3 (ward) ($P > .05$); the IgA antibody ratio was ≥15 (ICU) and 13.6 ± 2.8 (ward) ($P > .05$).

The functionality of the antibodies in the hospitalized cohort was assessed by VNT_{50} and VNT_{90} (Figure 1 and Supplementary Figure 1). In the first week upon onset of symptoms, 43% of the patients had detectable VNT_{50} (median, 22 [range, 10–640]). At 21–28 days, all patients had detectable neutralizing antibody titers in the VNT_{50} with a median titer of 226 (range, 20–800). Median titer in the VNT_{90} was 50 (range, <10–240).

As indicated above, 4 patients already showed high IgG levels (OD ratio >5) and even plateau levels of IgA antibodies early during the course of disease (ie, within 10 days after onset of symptoms). To exclude cross-reactivity due to (recent) previous infection with 1 of the 4 common human coronaviruses (ie, OC43, NL63, HKU1, and 229E) in these so-called early IgA responders, antibody titers against these common coronaviruses were determined in 2 early responders (Figure 2*A* and 2*B*) and compared with those in 2 other COVID-19 patients (Figure 2*C* and 2*D*). We found minimal and inconsistent fluctuations in IgG against the 4 common coronaviruses across all 4 patients, indicating that the early IgA and total immunoglobulin anti–SARS-CoV-2 responses for some patients were not the result of cross-reactivity due to a previous common coronavirus infection.

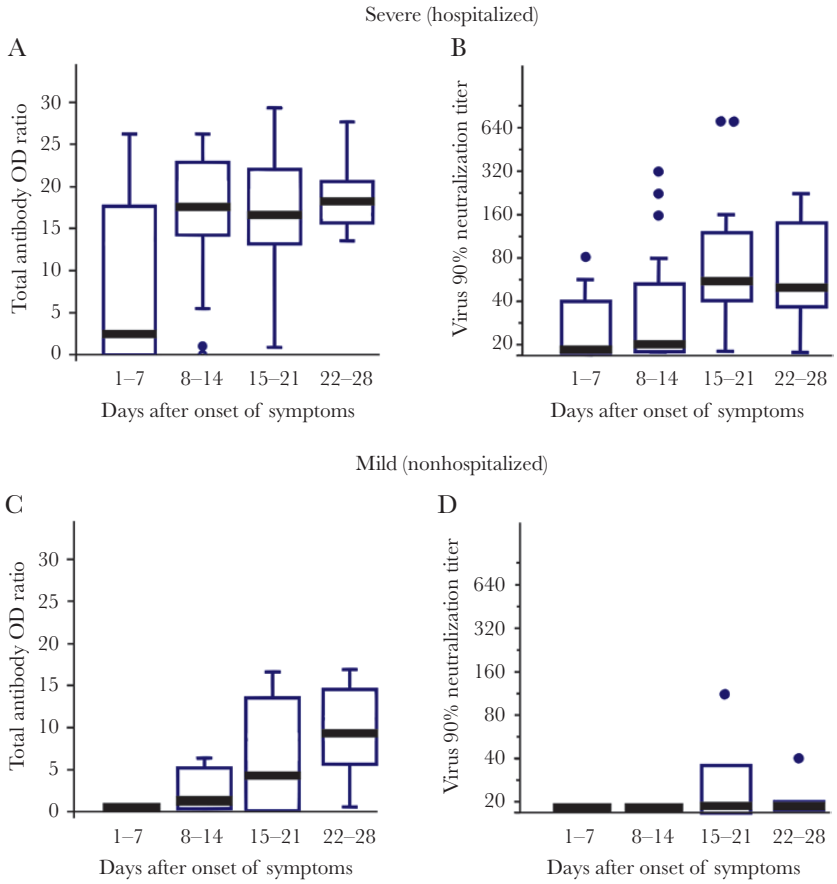

Figure 1. Quantitative and qualitative antibody responses against severe acute respiratory syndrome coronavirus 2 in patients with severe (*A* and *B*) and mild (*C* and *D*) coronavirus disease 2019. Shown are total antibody optical density (OD) ratio (*A* and *C*), as well as virus neutralization titer at 50% neutralization (*B* and *D*). The horizontal line in the middle of each box indicates the geometric mean, the top and bottom borders of the box mark the 75th and 25th percentiles, and the whiskers above and below the box indicate the range. Outliers, with values >1.5 the interquartile range, are indicated with individual dots.

Next, we investigated the antibody responses in a cohort of hospital personnel who had tested positive by SARS-CoV-2 RT-PCR but had only experienced mild clinical symptoms. Eighty-seven percent developed SARS-CoV-2 total antibodies by days 21–28, with a significant lower titer than patients with severe illness (geometric mean titer, 4.9 and 17.3, respectively; $P < .001$). The median titer in the VNT_{50} was 29 (range, <10–640) and in VNT_{90} was 10 (range, <10–12), both significantly lower than in patients with severe illness (P values < .0001) (Figure 1 and Supplementary Figure 1). Thirty-three percent of the mild patients remained negative for the presence of virus neutralizing antibodies.

DISCUSSION

Serial blood sampling of 38 patients with severe (hospitalized) COVID-19 and 24 with mild COVID-19 allowed for detailed analysis of the kinetics and magnitude of the SARS-CoV-2 antibody response in patients with different levels of disease severity. At 2–4 weeks after onset of symptoms, detectable total immunoglobulin, IgG, and IgA antibodies were found in 100%, 86%, and 94% of the severe (hospitalized) patients, and 81%, 81%, and 61% of the mild (nonhospitalized) patients, respectively. Virus neutralizing activity was demonstrable in the vast majority of severe patients (all at VNT_{50}, 95% at VNT_{90}) but only in 65% (VNT_{50}) and 30% (VNT_{90}) in the mild patients. We did not find a significant difference in the kinetics, magnitude, or functionality of the response between hospitalized patients at the general ward or the ICU. This is in accordance with findings in hospitalized patients with SARS coronavirus [13]. It is, however, possible that larger series of hospitalized patients with variable clinical outcome can reveal differences in the nature and kinetics of the immune response, as our sample size was limited.

In 2 of our patients, we had serum samples available from the period before onset of COVID-19. As expected, total antibody, IgG, and IgA anti–SARS-CoV-2 antibodies were not demonstrable at that time.

Early responders (especially levels of IgG and IgA within 5 days after onset of symptoms) were only observed in the hospitalized cohort and could have been due to a prolonged

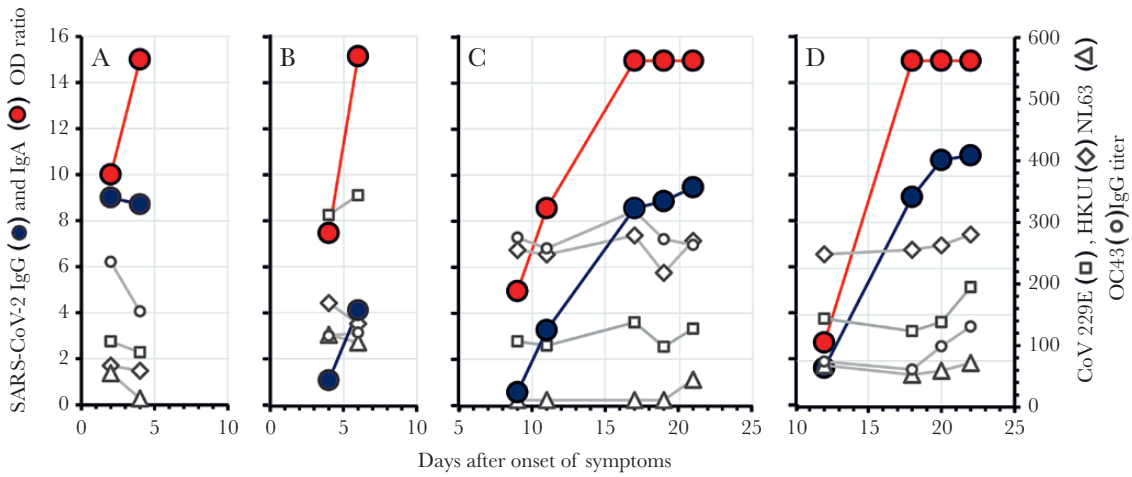

Figure 2. Antibody levels against circulating coronaviruses during the course of coronavirus disease 2019. Each panel (*A*–*D*) represents a single patient. Immunoglobulin G (IgG) and immunoglobulin A (IgA) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies are displayed by blue and red circles, respectively, and expressed as optical density (OD) ratio values on the left y-axis. Human coronavirus (CoV) 229E (□), HKU1 (◊), NL63 (Δ), and OC43 (○) IgG antibody titers are shown as open symbols and expressed as titers.

presymptomatic period, or recall bias with respect to onset of complaints [14]. Antibody levels against human coronaviruses OC43 and HKUI in early responder patients did not differ from other patients (data not shown), and antibody levels against circulating coronaviruses did not change during the course of COVID-19 in both very early and "normal" responders, whereas SARS-CoV-2–directed IgA and IgG levels did (Figure 2).

Two of our patients with severe disease who survived failed to show an IgG response at day 21 after disease onset (although the total antibody assay was positive). These patients, 69 and 87 years of age, had persistently positive PCR test results on day 28 and day 37 after disease onset, respectively. Patients with an inadequate IgG antibody response may exhibit prolonged viral shedding, and thus longer periods of infectivity. Prolonged viral RNA shedding has been reported previously [15], and further studies that include viral cultures are needed to investigate the prolonged infectivity hypothesis. One (other) patient remained negative for IgA antibodies, although this patient, as well as all others, had normal serum IgA immunoglobulin levels (data not shown). Patients with severe COVID-19 remaining seronegative have also been reported by others (eg, [6, 8]). It can be speculated that their antibody response is dominated by epitopes not represented in the immunoassays we have used. Antibody testing against a more extensive array of SARS-CoV-2 peptides will be required to address this possibility.

From our data, it is clear that hospitalized COVID-19 patients mount a robust humoral immune response against SARS-CoV-2, including antibodies with virus neutralizing activity. Mild infections with SARS-CoV-2, with clinical symptoms not requiring hospital admission, do show an antibody response, but delayed in comparison to severe patients and with minimal functional activity. Long-term monitoring will be required to determine whether these quantitative and qualitative parameters of the humoral immune response predict protection against future infection.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank all of the personnel in the laboratory who made this evaluation possible, in particular Fion Brouwer, Gert-Jan Godeke, Gabriel Goderski, Marieke Hoogerwerf, and Ilse Zutt.

Potential conflicts of interest. All authors: No reported conflicts of interest. 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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