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Molecular and serological characterization of SARS-CoV-2 infection among COVID-19 patients

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ABSTRACT

Background: SARS-CoV-2 is a novel coronavirus and the cause of COVID-19. More than 80% of COVID-19 patients exhibit mild or moderate symptoms. In this study, we investigated the dynamics of viral load and antibodies against SARS-CoV-2 in a longitudinal cohort of COVID-19 patients with severe and mild/moderate diseases.
Methods: Demographic and clinical information were obtained. Serial samples of blood, nasal and pharyngeal and anal swabs were collected at different time points post-onset. SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies were measured by qRT-PCR and immunoassays, respectively.
Results: Respiratory SARS-CoV-2 RNA was detectable in 58.0% (58/100) COVID-19 patients upon admission and lasted for a median of 13 days post-onset. In addition, 5.9% (1/17) and 20.2% (19/94) of the blood and anal swab specimens were positive for SARS-CoV-2 RNA, respectively. Anal viral RNA was more frequently detected in the patients who were positive for viral RNA in the respiratory samples upon admission. Specific anti-SARS-CoV-2 antibody developed within two weeks after onset, reached peak approximately 17 days post-onset and then maintained at relatively high level up to 50 days we analyzed in most patients. However, the levels of antibodies were variable among the patients. High titers of antibodies appeared to be associated with the severity of the disease. Furthermore, viral proteins from different sources showed significant difference of serological sensitivity especially during the first week post-onset.
Conclusions: Our results indicate rapid clearance or self-elimination of viral RNA in about half of the COVID-19 patients upon admission. Viral RNA shedding of SARS-CoV-2 occurred in multiple tissues including the respiratory system, blood, and intestine. Variable levels of specific anti-SARS-CoV-2 antibody may be associated with disease severity. These findings have shed light on viral kinetics and antibody response in COVID-19 patients and provide scientific evidence for infection control and patient management.

1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, was identified in December of 2019 in Wuhan, Hubei province of China (Chan et al., 2020a, 2020b; Lu et al., 2020; Zhu et al., 2020), and was declared as a pandemic on March 11, 2020 by the World Health Organization (WHO). As of August 31, 2020, there have been 24,854,140 confirmed cases of COVID-19, including 838,924 deaths, reported to WHO (https://covid19.who.int/). In contrast to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), more than 80% of COVID-19 patients exhibit mild or moderate symptoms (Zhu et al., 2020; Guan et al., 2020; Huang et al., 2020; Li et al., 2020). Although it is believed that SARS-CoV-2 is mainly transmitted through respiratory tract, SARS-CoV-2 RNA has been detected in blood and anal swabs, suggesting multiple shedding routes of SARS-CoV-2 (To et al.,

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2020; Zhang et al., 2020).

Since the publication of the full genome of SARS-CoV-2 in the mid-January 2020, several molecular testing protocols for detecting SARS-CoV-2 RNA have been published and recommended by WHO as the only assay that allows the identification of SARS-CoV-2 infection (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance). The FIND Foundation has independently evaluated the molecular assays using clinically and laboratory-confirmed COVID-19 patient samples and reported 92–100% sensitivity and 100% specificity (http://www.finddiagnostics.org/). However, 40–80% sensitivity for the molecular assays have been reported in the real-world application, indicating high false negativity of these assays. False negative results have been observed in the discharged COVID-19 patients. Deng et al. reported that 10.6% of the discharged COVID-19 patients became positive for SARS-CoV-2 RNA again and the positive detection of viral RNA lasted for 3–35 days after discharge from hospital (Deng et al., 2020). Furthermore, they also detected SARS-CoV-2 RNA in the stool or sputum specimens although viral RNA was not detectable in the nasal and pharyngeal swab specimens. Zheng et al. reported that 9.5% of the discharged COVID-19 patients turned back to be positive for SARS-CoV-2 RNA after discharge (Zheng et al., 2020). These results indicate the needs to further characterize the dynamics of virus shedding and to evaluate the detection of multiple tissue specimens to improve the diagnosis, patient management and infection control in COVID-19 patients.

Although serological tests are not suitable for the diagnosis of acute COVID-19 cases, they are useful for epidemiological investigation and characterization of immune responses of SARS-CoV-2-infected individuals. Different from the molecular assays, the FIND Foundation reported significant difference for the serologic assays in which the sensitivity for anti-SARS-CoV-2 IgM/IgG detection ranges from 50% to 87% whereas the specificity is about 82–99% (http://www.finddiagnostics.org/). Previous studies have reported different levels of anti-SARS-CoV-2 antibodies among COVID-19 patients (To et al., 2020; Zhang et al., 2020; Zhao et al., 2020a). Higher titers of anti-SARS-CoV-2 antibodies were more often detected in elderly patients and may be associated with severe disease (Zhao et al., 2020a). However, further characterization of antibody response and evaluation of its clinical value in COVID-19 patients are important for diagnosis, antiviral treatment, epidemiological investigation and vaccine development. In this study, we investigate the dynamics of SARS-CoV-2 RNA detection in multiple tissue specimens and the antibodies against SARS-CoV-2 in a longitudinal cohort of COVID-19 patients with both severe and mild/moderate disease. We evaluate the performance of the serological assays that use different viral antigens of SARS-CoV-2, and an in-house luciferase immunosorbent assay for quantitation of anti- SARS-CoV-2 antibodies.

2. Materials and methods

2.1. Patients and samples

The study included 100 COVID-19 patients (76 mild/moderate cases and 24 severe cases).

All the patients were confirmed to be infected with SARS-CoV-2 by reverse-transcription polymerase chain reaction (RT-PCR) in the respiratory tract samples before being admitted to Guangzhou Eighth People's Hospital. The severity of illness was assessed according to the guideline for COVID-19 (version 6.0) published by the National Health Commission of China (National Health Commissio, 2020) and classified into 1) mild patients who have mild clinical symptoms and no pneumonia on chest image; 2) moderate patients who have clinical symptoms (i.e. fever and respiratory tract symptoms) and pneumonia on chest image; 3) severe patients who meet any one of the following criteria: respiratory rate $\geq$30 breaths/minute; resting oxygen saturation $\leq$93%; arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) $\leq$300 mmHg; disease progression within 24–48 h on chest image; and 4) critical patients who meet any one of the following criteria: developing respiratory failure and requiring mechanical ventilation; occurrence of shock; other organ failure and admission to intensive care unit.

A total of 524 blood specimens (median 5.0 per patient, IQR, 4.0–6.8) were collected for detection of both SARS-CoV-2 RNA and anti-SARS-CoV-2 antibodies. Respiratory and anal swab samples were also collected for detection of SARS-CoV-2 RNA. All the samples were stored at −80 °C before analysis. Demographic, laboratory tests, treatment and clinical outcome data were extracted from the electronic medical records. Written informed consents were obtained from the individuals enrolled in this study, which has been approved by Ethics Committees of Guangzhou Eighth People's Hospital (No. 202002136).

2.2. Detection of SARS-CoV-2 RNA

For the swab samples, 2 ml viral transport medium (DMEM/2% FBS) were added in each tube. Both the supernatant of swab samples and the serum or plasma samples were used for RNA extraction and detection of SARS-CoV-2 RNA using a nested reverse transcription polymerase chain reaction (RT-PCR) assay targeting both nucleocapsid (NP) and ORF1a/b genes of SARS-CoV-2 according to the manufacturer's protocols (DaAn Gene company, Guangzhou, China). A cycle threshold (Ct) value of 40 or more was defined as negative detection for SARS-CoV-2 RNA.

2.3. Detection of anti-SARS-CoV-2 antibody

Luciferase immunosorbent assay (LISA), a semi-quantitative assay for quantitative measurement of the antibody levels has previously been described (Wang et al., 2019a, 2019b) and used with modifications in our study. Detection of IgG, IgM and IgA antibody against SARS-CoV-2 NP protein was done by using the in-house LISA in which white microtiter plates (Corning, New York, USA) were coated with protein G (5ug/ml, Genscript, Nanjing, China), monoclonal goat anti-human IgM (5ug/ml, Boson Biotech, Xiamen, China) and monoclonal mouse anti-human IgA (5ug/ml, Eastmo Biotech, Beijing, China), respectively, followed by incubation with the serum samples (50μl, diluted 1:100) for 1 h at 37 °C, and then Luc-SARS-CoV-2 NP fusion protein (50μl) that contains both luciferase and SARS-CoV-2 NP protein at 37 °C for 30 min. Finally, Nano-Glo Luciferase assay reagent (Promega, Madison, USA) was added to each well to determine the luciferase counts (LU) according to the manufacturer's protocol. The cut-off value was derived from the average value of the negative controls plus 3 standard deviations (SD). The low limit of detection (LOD) was determined to be 0.01 ng/ml with a linear quantitation range of 0.41–75 ng/ml by using monoclonal antibody against SARS-CoV NP, which shares 96% of identity and strong cross-reactivity with SARS-CoV-2 NP (data not shown). The specificity of the assays for IgG, IgM and IgA was determined as 100.0% (88/88) by testing the blood samples of healthy blood donors collected in 2016 when no SARS-CoV-2 infection was identified (data not shown).

For an enzyme-linked immunosorbent assay (ELISA), microtiter plates (Corning, New York, USA) were coated with the recombinant spike protein S1 subunit, receptor-binding domain (RBD) protein and NP of SARS-CoV-2, respectively. Serum samples (50 μl, diluted 1:100) was added to the plates, and incubated at 37 °C for 1 h. After washing, 50 μl horseradish peroxidase (HRP)-conjugated monoclonal goat anti-human IgG antibody (Abcam, Cambridge, USA) was added. Then, the plate was incubated at 37 °C for 30 min, followed by washing. TMB substrate solution (50μl, Beyotime, Shanghai, China) was added and incubated at 37 °C for 15 min. The reaction was terminated by adding 50ul of 2 M sulfuric acid, and the absorbance value at 450 nm (A450) was determined. The cutoff OD value was calculated by the mean value of 53 anonymous archived serum specimens from 2016, plus 3 SDs.

2.4. Statistical analysis

Data were analyzed using R software, version 3.5.2 (R Foundation for Statistical Computing). Continuous variables were summarized as the medians and interquartile ranges (IQR) and compared using Mann-Whitney *U* test whereas categorical variables were expressed as counts and percentages and compared using Chi-square analysis or Fisher's exact test as appropriate. Linear mixed models in which patient was treated as a random factor and the time from illness onset as fixed variate were adapted to estimate the longitudinal slopes of anti-SARS-CoV-2 antibody titers. The time period of viral RNA shedding was defined as the interval from illness onset to the time when two consecutive throat-swab samples were negative for SARS-CoV-2 RNA (at least 24 h apart). Mild and moderate COVID-19 patients were put together as a group for further analysis while severe and critical patients were treated as the patients with severe disease. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Detection dynamics of SARS-CoV-2 RNA

The median age of COVID-19 patients was 51.0 years (IQR 37.8–63.0) and 51.0% of them were men. 77.0% and 55.0% had fever and cough, respectively (Table 1). Among these patients, 69.0% had the history of visiting Hubei province where COVID-19 epidemic started. The median durations from illness onset to hospital admission were 4.5 days (IQR 3.0–7.0) (Table 1). Two patients, #13 and #15, were negative for SARS-CoV-2 RNA upon admission, and did not show any antibody response against both SARS-CoV-2 NP, S1 or RBD proteins thereafter (Fig. 1E and F, Table S1). They may be probably misdiagnosed as SARS-CoV-2 infection due to false positive detection for SARS-CoV-2 RNA or showed delayed antibody response as reported by Zhao et al. (2020b). The two patients were thus excluded from further analysis in our study.

Although all the patients were positive for SARS-CoV-2 RNA before admission, SARS-CoV-2 RNA was detectable in the respiratory swab samples in 59.2% of the COVID-19 patients (58/98) upon admission and lasted for a median of 15.0 (12.0–19.0) days. Viral RNA was detected in the blood of 5.9% (1/17) patients and the anal swab samples of 20.2% (19/94) patients (Table 1). The difference in the clinical findings between the patients with and without respiratory SARS-CoV-2 RNA upon admission included earlier admission (4 vs 6 days), longer hospital stay (24 vs 17 days) and illness duration (28 vs 25 days), extended duration of viral RNA shedding (15 vs 12 days), and earlier IgG seroconversion (12 vs 14 days) for anti-SARS-CoV-2 antibody as well as more frequent detection of viral RNA in the anal swabs (30.2% (16/53) vs 7.7% (3/39), Table 2).

3.2. Temporal profiles of anti-SARS-CoV-2 antibody responses

Based on the quantitative results of LISA, seroconversion of IgM, IgG and IgA antibody against SARS-CoV-2 NP occurred at the median of 10.0, 13.0 and 13.5 days post-disease onset (d.p.o.), respectively, and once seroconverted, the antibodies against SARS-CoV-2 remained positive during the period of our study (Fig. 1A, B and C, Table 3). The percentage of IgM and IgG seroconversion was 25.0% (3/12) and 14.9% (10/67) during 0–7 d.p.o., then increased to 66.7% (10/15) and 75.5% (71/94) 8–15 d.p.o., and 83.3% (10/12) and 93.9% (62/66) 16–20 d.p.o., respectively.

The titers of anti-NP antibody were then used to classify the 98 COVID-19 patients into two groups, i.e., high (>0.5 μg/ml) and low (≤0.5 μg/ml) titers (Table 1, Fig. 1D). Seroconversion for the patients

Table 1
Demographic and clinical characteristics of COVID-19 patients with high or low anti-SARS-CoV-2 antibody titers.

Characteristics	Titers of anti-SARS-CoV-2 NP				p value
	Total (N = 100)	High (N = 58)	Low (N = 40)	No (N = 2)	
Days from onset to admission, median (IQR)	4.5 (3.0–7.0)	6.0 (4.0–7.3)	3.0 (2.0–5.8)	3.0	<0.001
Hospital stay, median days (IQR)	21.0 (15.0–26.0)	23.0 (15.8–27.0)	19.0 (14.0–26.0)	10.5	0.190
Days from onset to discharge, median (IQR)	26.0 (20.0–31.0)	28.0 (22.8–32.0)	22.5 (19.0–28.0)	13.5	0.009
Days of RNA shedding[a], median (IQR)	13.0 (10.0–17.5)	13.5 (10.0–17.3)	13.0 (11.0–18.0)	NA	0.699
Anti-S1 IgG seroconversion time, median days (IQR)	8.0 (6.0–11.3)	9.0 (7.0–12.0)	8.0 (5.0–9.8)	NA	0.028
Anti-RBD IgG seroconversion time, median days (IQR)	8.0 (6.0–11.0)	8.0 (7.0–11.3)	7.5 (4.0–11.3)	NA	0.164
Anti-NP IgG seroconversion time, median days (IQR)	13.0 (9.0–15.3)	12.0 (9.0–16.0)	14.0 (10.3–15.0)	NA	0.213
Anti-NP IgM seroconversion time[b], median days (IQR)	10.0 (8.5–15.0)	10.0 (8.0–11.5)	15.0 (9.0–18.8)	NA	0.260
Anti-NP IgA seroconversion time[b], median days (IQR)	13.5 (8.3–15.0)	13.5 (9.3–15.0)	11.5 (7.3–22.5)	NA	1.000
Age, median years (IQR)	51.0 (37.8–63.0)	54.0 (40.8–65.0)	47.5 (37.8–60.0)	48.5	0.112
Gender					0.411
Male	51 (51.0%)	32 (55.2%)	18 (45.0%)	1 (50.0%)	
Female	49 (49.0%)	26 (44.8%)	22 (55.0%)	1 (50.0%)	
Disease severity					0.002
Severe	24 (24.0%)	21 (36.2%)	3 (7.5%)	0	
Mild/moderate	76 (76.0%)	37 (63.8%)	37 (92.5%)	2 (100.0%)	
Positive nucleic acid test after admission					
In throat swab samples	58 (58.0%)	32 (55.2%)	26 (65.0%)	0	0.405
In blood sample[c]	1/17 (5.9%)	1/9 (11.1%)	0	0	1.000
In anal swab sample[d]	19/94 (20.2%)	10/54 (18.5%)	9/38 (23.7%)	0	0.606
Exposure history					0.659
Recently visited Hubei	69 (69.0%)	39 (67.2%)	29 (72.5%)	1 (50.0%)	
Never been to Hubei	31 (31.0%)	19 (32.8%)	11 (27.5%)	1 (50.0%)	
Symptoms					
Fever	77 (77.0%)	53 (91.4%)	24 (60.0%)	0	<0.001
Cough	55 (55.0%)	38 (65.5%)	17 (42.5%)	0	0.038

NA = not applicable. p values of comparisons between high group and low group were calculated by Mann-Whitney *U* test, χ^2 test, or Fisher's exact test, as appropriate. NP = nucleocapsid proteins.

[a] Days of RNA shedding was defined as intervals from illness onset through two consecutive throat-swab samples negative for SARS-CoV-2 RNA (at least 24 h apart), without converting positive thereafter.

[b] IgM and IgA antibody against nucleocapsid proteins (NP) was tested on 17 patients.

[c] Blood sample was collected from 17 patients for nucleic acid test.

[d] Anal swab sample was collected from 94 patients for nucleic acid test.

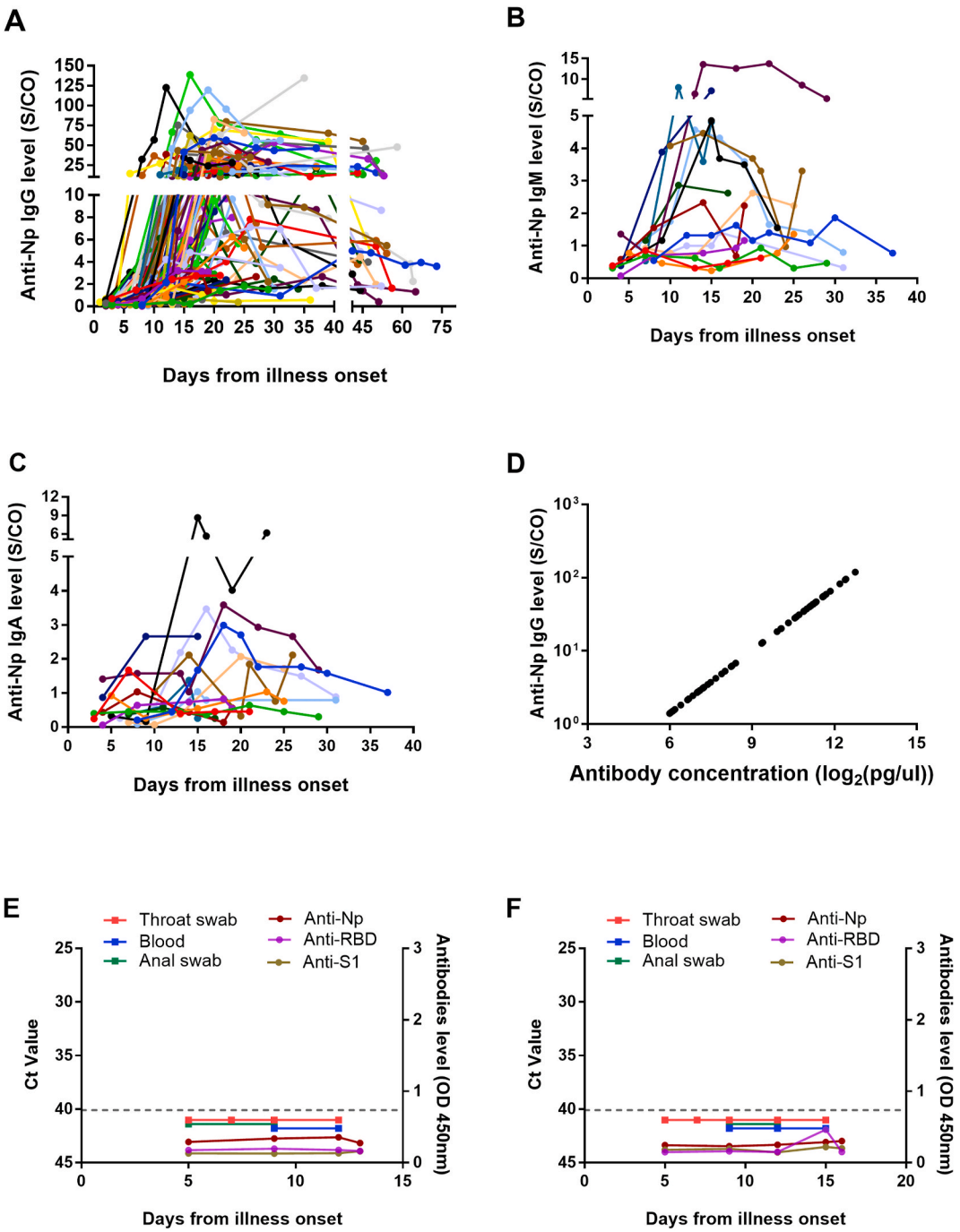

Fig. 1. Temporal profiles of anti- SARS-CoV-2 antibodies. Serum IgG (A), IgM (B) and IgA (C) against SARS-CoV-2 NP were ascertained by LISA in 98, 15 and 15 COVID-19 patients, respectively. Each patient is represented by the lines labelled with different colors. The linear relationship between the luciferase counts of anti-NP and the amount of antibody was presented in panel D. Patient #NCP13 (panel E) and 15 (panel F) were negative for viral RNA in throat (red), blood (blue) and anal swabs (green) by qRT-PCR with cutoff value of 40 cycles (black dash line), and for antibody against SARS-CoV-2 NP (brown), RBD (pink) and S1 subunit (yellow) by ELISA. LISA = luciferase immunosorbent assay. ELISA = enzyme linked immunoassay. NP = nucleocapsid proteins. RBD = receptor-binding domain. S1 = spike protein S1 subunit. S/CO = sample/cutoff. OD450 = optical density at 450 nm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

with high titers of anti-NP antibody occurred at the median of 12.0 d.p.o.. The titers of anti-SARS-CoV-2 antibody rapidly increased with a slope of 0.507 per day (Tables 1 and S2) and reached the plateau at the median of 17.0 (IQR 15.0–20.0) d.p.o.. The patients with low titers of anti-NP antibody became positive for anti-SARS-CoV-2 antibody at the median of 14.0 d.p.o. while the antibody titers increased with a slope of 0.055/day (Tables 1 and S2). The difference in the clinical findings between the two groups includes more frequent major symptoms such as fever (91.4% vs 60%) and cough (65.5% vs 42.5%), delayed admission (6 vs 3 days) and less frequent anal positivity for SARS-CoV-2 RNA (18.5% vs 23.7%, Table 1).

In addition, the antibodies against SARS-CoV-2 NP and S1 or RBD proteins were analyzed by ELISA, and the temporal profiles of anti-SARS-CoV-2 antibodies obtained by ELISA were similar to those of LISA (Figs. 2 and 3, Table 3). Furthermore, the seroconversion for anti-S1 or RBD were earlier and the antibody titers were higher than for anti-

Table 2
Demographic and clinical characteristics of COVID-19 patients who were positive for SARS-CoV-2 RNA in respiratory samples after admission.

Characteristics	Detection of SARS-CoV-2 RNA by NAT[e]		p value
	Positive (N = 58)	Negative (N = 40)	
Days from onset to admission, median (IQR)	4.0 (2.0–7.0)	6.0 (3.3–7.0)	0.086
Hospital stay, median days (IQR)	23.5 (17.0–28.0)	17.0 (13.3–23.8)	0.003
Days from onset to discharge, median (IQR)	28.0 (22.0–34.0)	25.0 (16.3–30.0)	0.050
Days of RNA shedding[a], median (IQR)	15.0 (12.0–19.0)	12.0 (9.0–14.8)	0.001
Anti-NP IgG seroconversion time, median days (IQR)	12.0 (9.0–15.0)	14.0 (10.8–16.3)	0.139
Anti-NP IgM seroconversion time[b], median days (IQR)	10.0 (5.5–19.0)	10.5 (9.0–12.0)	0.833
Anti-NP IgA seroconversion time[b], median days (IQR)	13.0 (5.5–22.5)	14.0 (9.0–15.0)	1.000
Age, median years (IQR)	55.0 (39.3–64.5)	46.0 (37.8–61.5)	0.231
Gender			1.000
Male	30 (51.7%)	20 (50.0%)	
Female	28 (48.3%)	20 (50.0%)	
Disease severity status			0.477
Severe	16 (27.6%)	8 (20.0%)	
Mild/moderate	42 (72.4%)	32 (80.0%)	
Positive nucleic acid test after admission			
In blood sample[c]	1/7 (14.3%)	0	0.467
In anal swab sample[d]	16/53 (30.2%)	3/39 (7.7%)	0.009
Exposure history			0.075
Recently visited Hubei	36 (62.1%)	32 (80.0%)	
Never been to Hubei	22 (37.9%)	8 (20.0%)	
Symptoms			
Fever	46 (79.3%)	31 (77.5%)	1.000
Cough	25 (43.1%)	20 (50.0%)	0.541

p values were calculated by Mann-Whitney *U* test, χ^2 test, or Fisher's exact test, as appropriate.

[a] Days of RNA shedding was defined as intervals from illness onset through two consecutive throat-swab samples negative for SARS-CoV-2 RNA (at least 24 h apart), without converting positive thereafter.

[b] IgM and IgA antibody against nucleocapsid proteins (NP) was tested on 15 patients.

[c] Blood sample was collected from 15 patients for nucleic acid test.

[d] Anal swab sample was collected from 92 patients for nucleic acid test.

[e] NAT, nucleic acid testing.

NP (Figs. 2 and 3). In addition, similar antibody profiles were observed between the patients with severe and mild/moderate diseases although the seroconversion for anti-S1 and anti-RBD antibody was slightly earlier than for anti-NP antibody (Table 4).

3.3. Serological sensitivity for various SARS-CoV-2 antigens

NP, S1 subunit and RBD of SARS-CoV-2 are the most frequently used antigens for detection of anti-SARS-CoV-2 antibodies. We compared the time and percentage of seroconversion for anti-SARS-CoV-2 antibodies determined by different SARS-CoV-2 antigens in ELISA system (Table 3, Fig. 3). The median time of seroconversion ranged from 8 to 14.5 d.p.o. while the anti-S1 and anti-RBD antibody were the earliest antibody detected with a median time of seroconversion of 8 d.p.o. although a significant difference was observed among the different sources of antigens. For the antibody seroconversion, 4 days' difference was observed between the RBD antigens from East-Mab Biomedical Technology (Jiangsu, China) and Darui Biotechnology (Guangzhou, China, P = 0.003). The difference of seroconversion was also observed for the S1 subunit antigens from different vendors especially during the first week post onset. The percentage of the antibody seroconversion ranged from 0 to 61.2%. At the second week post onset, the seropositive rate reached to 90–100% regardless of the antigens used. These results indicate the significant difference of the detection sensitivity determined by different SARS-CoV-2 proteins and the antigens produced by different vendors.

4. Discussion

In the current study, we analyzed the temporal profiles of nucleic acid and antibody of SARS-CoV-2 in the serial samples of both severe and mile/moderate COVID-19 patients. These patients were all diagnosed and confirmed by the specific RT-PCR for SARS-CoV-2 before being admitted to the hospital. However, respiratory viral RNA was only detected in 58.0% of them upon admission. There are several possibilities to explain undetectable SARS-CoV-2 RNA in about 50% of the patients upon admission to the hospital. One possibility is the rapid clearance or self-elimination of SARS-CoV-2 infection within a median of 4–5 days from onset to admission in some COVID-19 patients although in general, viral RNA may last for about 13 days post-onset (Table 1). Another possibility is the difference of the duration of SARS-CoV-2 RNA among different tissue specimens (Wang et al., 2020) and earlier elimination of viral RNA in the respiratory tract since our results and previous studies have confirmed the detection of SARS-CoV-2 RNA in anal swabs but not in the respiratory specimens

Table 3
Temporal profiles of anti-SARS-CoV-2 antibodies.

Anti-SARS-CoV-2 antibody	Vendor of SARS-CoV-2 antigens	Assay	Number of Patient	Seroconversion, median days (IQR)	Positivity (n/N, %) from onset (days)			
					0–7	8–15	16–20	≥21
S1 (IgG)	East-Mab Biomedical Technology, Jiangsu	ELISA	98	8.0 (6.0–11.3)	36/67 (53.7)	90/94 (95.7)	65/66 (98.5)	64/64 (100.0)
S1 (IgG)	Medical institute of oriental ocean, Beijing	ELISA	15	14.5 (13.0–17.7)	0/12	7/15 (46.7)	9/12(75.0)	7/9(77.8)
RBD (IgG)	East-Mab Biomedical Technology, Jiangsu	ELISA	98	8.0 (6.0–11.0)	41/67 (61.2)	89/94 (94.7)	66/66 (100.0)	64/64 (100.0)
RBD (IgG)	Darui Biotechnology, Guangzhou	ELISA	15	12.0 (8.0–15.0)	1/12(8.3)	13/15 (86.7)	12/12 (100.0)	9/9(100.0)
NP (IgG)	Hanrui Biology, Nanjing	ELISA	98	11.0 (8.0–14.5)	21/67 (31.3)	75/94 (79.8)	63/66 (95.5)	62/64 (96.9)
NP (IgM)	in-house	LISA	15	10.0 (8.5–15.0)	3/12 (25.0)	10/15 (66.7)	10/12 (83.3)	7/9(77.8)
NP (IgG)	in-house	LISA	98	13.0 (9.0–15.3)	10/67 (14.9)	71/94 (75.5)	62/66 (93.9)	62/64 (96.9)
NP (IgA)	in-house	LISA	15	13.5 (8.3–15.0)	2/12 (16.7)	10/15 (66.7)	7/12(58.3)	7/9(77.8)

LISA, luciferase immunosorbent assay; ELISA, enzyme linked immunoassay; NP, nucleocapsid proteins.; RBD, receptor-binding domain; S1, spike protein S1 subunit; IQR, interquartile range; NA, not applicable.

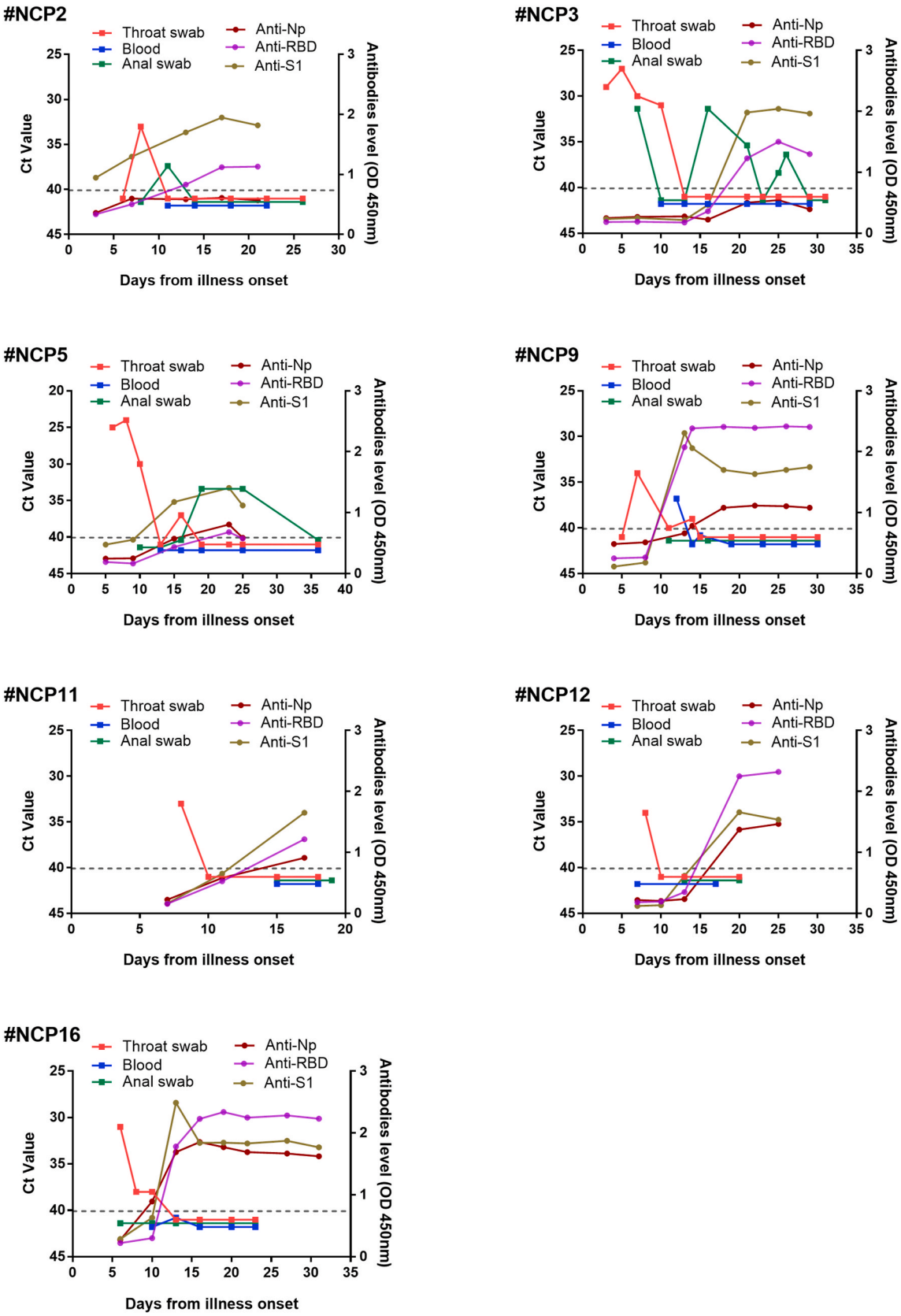

Fig. 2. Temporal profiles of viral RNA and anti-SARS-CoV-2 antibodies for 7 COVID-19 patients with extended viral RNA shedding. Viral RNA in throat (red), blood (blue) and anal swabs (green) was ascertained by qRT-PCR with cutoff value of 40 cycles (black dash line). Serum antibody against SARS-CoV-2 NP (brown), RBD (pink) and spike protein S1 subunit (yellow) was detected by ELISA. Each panel represents each patient. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

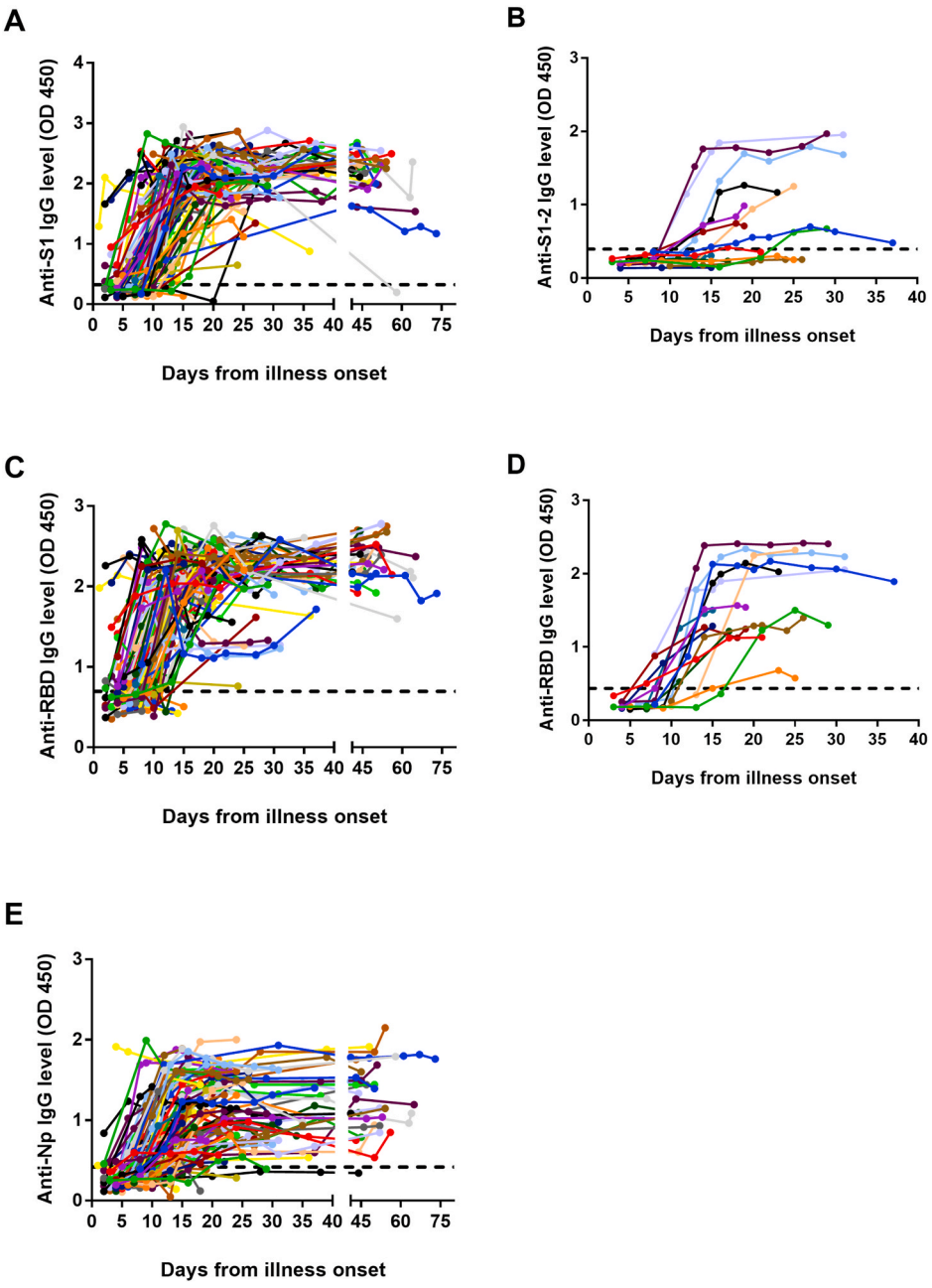

Fig. 3. Temporal profiles of anti-SARS-CoV-2 antibodies among COVID-19 patients. ELISA was adapted for detecting serum IgG antibody against SARS-CoV-2 S1 protein from East-Mab Biomedical Technology, Jiangsu (panel A, N = 98) and Medical institute of oriental ocean, Beijing (panel B, N = 15), and against RBD protein from East-Mab Biomedical Technology, Jiangsu (panel C, N = 98) and Vendor C (panel D, N = 15), as well as NP from Hanrui Biology, Nanjing (panel E, N = 98). Each patient was represented by the line labelled with different color. Dash lines indicate ELISA cutoff values. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(Wang et al., 2020; Cheung et al., 2020; Wu et al., 2020a; Chen et al., 2020a). The third possibility is the false positive detection of SARS-CoV-2 RNA outside of the hospital, which may not be the case in our study since the patients with negative viral RNA detection upon admission developed specific anti-SARS-CoV-2 antibodies. Another possibility is the false negative detection of SARS-CoV-2 RNA after admission due to the low sensitivity of the nucleic acid tests since about 60–80% of sensitivity for detecting SARS-CoV-2 RNA has been reported (Alcoba-Florez et al., 2020). Finally, we would like to emphasize that nucleic acid testing detects viral RNA but not live virus. Therefore, negative detection of SARS-CoV-2 RNA may not mean the eradication of SARS-CoV-2 and viral RNA testing cannot be used as test of clearance or test of cure of SARS-CoV-2. Our results and previous studies support the importance to detect SARS-CoV-2 RNA in multiple tissue specimens especially anal swabs as an aid of patient discharge (Wang et al., 2020; Lan et al., 2020; Wölfel et al., 2020).

Of note, the patients with detectable SARS-CoV-2 RNA upon admission showed one more week longer duration of hospital stay and extended viral RNA detection in respiratory specimens for 3 more days (Table 2). Furthermore, the extended detection of viral RNA in the respiratory samples could further increase the detection of viral RNA in multiple tissues including blood (14.3%) and intestine (30.2%). Compared with the total COVID-19 patients analyzed in our study, SARS-CoV-2 RNA was only detected in 5.9% of the blood samples and 20.2% of the anal swab samples (Table 1). Zhang W et al. also reported the detection of SARS-CoV-2 RNA in 33.3% of anal swabs and 33.3% of blood samples (Zhang et al., 2020). Intestinal infection of SARS-CoV and MERS-CoV have been recorded at later stages of infection (Ding et al., 2004; Shi et al., 2005; Zhou et al., 2017). Lin et al. reported that detection of SARS-CoV-2 RNA in anal swabs were associated with the increase of C-reactive protein, the occurrence of lymphocytopenia and the admission to intensive care unit (Lin and Xie, 2020). Chen et al. found that detection of anal viral RNA detection may be associated with disease severity (Chen et al., 2020b).

Table 4
Serological responses against SARS-CoV-2 among COVID-19 patients with different disease severity.

Disease severity	Anti-SARS-CoV-2 antibody (IgG)	Vendor of SARS-CoV-2 antigens	Testing method	Seroconversion, median days (IQR)	Positivity (n/N, %) from onset (days)			
					0–7	8–15	16–20	≥21
Mild/moderate (N = 74)								
	S1	East-Mab Biomedical Technology, Jiangsu	ELISA	8.0 (6.0–11.0)	29/53 (54.7)	68/72 (94.4)	51/52 (98.1)	43/43 (100.0)
	RBD	East-Mab Biomedical Technology, Jiangsu	ELISA	8.0 (6.0–10.8)	33/53 (62.3)	67/72 (93.1)	52/52 (100.0)	43/43 (100.0)
	NP	Hanrui Biology, Nanjing	ELISA	11.0 (8.0–15.0)	16/53 (30.2)	55/72 (76.4)	49/52 (94.2)	41/43 (95.3)
	NP	in-house	LISA	14.0 (9.8–16.3)	9/53 (17.0)	50/72 (69.4)	48/52 (92.3)	41/43 (95.3)
Severe (N = 24)								
	S1	East-Mab Biomedical Technology, Jiangsu	ELISA	9.0 (6.3–12.0)	7/14 (50.0)	22/22 (100.0)	14/14 (100.0)	21/21 (100.0)
	RBD	East-Mab Biomedical Technology, Jiangsu	ELISA	8.0 (6.3–11.8)	8/14 (57.1)	22/22 (100.0)	14/14 (100.0)	21/21 (100.0)
	NP	Hanrui Biology, Nanjing	ELISA	9.5 (8.0–12.0)	5/14 (35.7)	20/22 (90.9)	14/14 (100.0)	21/21 (100.0)
	NP	in-house	LISA	12.0 (9.0–13.8)	1/14 (7.1)	21/22 (95.4)	14/14 (100.0)	21/21 (100.0)

LISA, luciferase immunosorbent assay; ELISA, enzyme linked immunoassay; NP, nucleocapsid proteins.; RBD, receptor-binding domain; S1, spike protein S1 subunit; IQR, interquartile range; NA, not applicable.

Our results and previous reports demonstrate a specific anti-SARS-CoV-2 antibody response within two weeks after onset (To et al., 2020; Zhang et al., 2020; Zhao et al., 2020a). Once the titers of anti-SARS-CoV-2 antibodies reached their peak at 10–15 days d.p.o., they remained stable in most patients during the period of observation. Similar results have been reported for neutralizing antibodies (NAbs) of SARS-CoV-2 (Wu et al., 2020b) although the levels of antibodies are variable among patients. In our study, 40% of the patients only developed low titers of antibody against SARS-CoV-2. Wu F et al. also found that 30% of patients failed to develop high titers of NAbs against SARS-CoV-2 or showed very low levels of NAbs against SARS-CoV-2 in the convalescent COVID-19 patients (Wu et al., 2020b). We found that high titers of antibodies against SARS-CoV-2 appear to be associated with delayed diagnosis of SARS-CoV-2 infection and admission of COVID-19 patients into hospital, longer duration of illness and severe disease with higher frequency of symptoms (Table 1). Zhao J et al. has also reported the association between the higher titer of total antibodies and severe disease (Zhao et al., 2020a). Wu F et al. observed that the higher titers of NAbs against SARS-CoV-2 were more often detected in elderly patients (Wu et al., 2020b) although it remains to be elucidated whether the high titers of antibody can cause antibody-dependent enhancement (ADE) among the COVID-19 patients with severe disease. These results suggest that titration of anti-SARS-CoV-2 antibody is necessary and useful for monitoring disease progress and severity.

When we originally analyzed 17 patients with mild or moderate COVID-19, we found that anal viral RNA was more frequently detected in the patients with low titer of anti-SARS-CoV-2 antibodies (data not shown). The difference of viral RNA detection was also observed when more COVID-19 patients with both severe and mild/moderate diseases were analyzed. However, the association between viral RNA detection in anal samples and the titers of antibody of SARAS-CoV-2 was not statistically significant (Table 1). Furthermore, anal viral RNA was more frequently detected in the COVID-19 patients who were still positive for SARS-CoV-2 RNA upon admission (Table 2). These results suggest that extended respiratory viral RNA detection may indicate highly efficient virus replication and multiple tissue infection of SARAS-CoV-2. Anal viral RNA detection has been reported to be more often observed in the discharged patients who are positive for SARS-CoV-2 later on (Hu et al., 2020). It has been thus recommended to detect viral RNA in both respiratory and anal swabs to better manage COVID-19 patients (Wu et al., 2020a).

One important role of serological testing is to provide aid for diagnosis of SARS-CoV-2 infection. Our results and previous studies indicate the relatively low seropositive rate of anti-SARS-CoV-2 during the first week post onset (Traugott et al., 2020; Tuaillon et al., 2020). Even IgM detection did not significantly increase the sensitivity at the early stage of disease. Furthermore, our results clearly showed the dramatic difference of detection sensitivity caused by different viral proteins or the antigens from different sources. In general, SARS-CoV-2 S1 subunit or RBD proteins are better than NP for sensitive detection of anti-SARS-CoV-2 antibodies during the early stages of disease.

One major limit of our study is that we only detected the total antibody against SARS-CoV-2. We did not measure the neutralizing antibody and did not evaluate the association between the titers of neutralizing antibody and virus shedding in the COVID-19 patients. Our in-house LISA was better for quantitating the levels of anti-SARS-CoV-2 antibodies than the traditional ELISA. LISA results for anti-SARS-CoV-2 NP antibody was used to classify the COVID-19 patients into high and low titers of antibody in our study. However, we should use both anti-NP and anti-RBD/anti-S1 antibodies for the classification. Unfortunately, we failed to develop LISA for anti-RBD/anti-S1 probably due to the high glycosylation of these two proteins to prevent their successful expression in our system. In addition, in the LISA assay, the total IgM or IgG were captured by anti-IgM antibody or protein G instead of a SARS-CoV-2 protein; therefore, we measured the proportion of a patient's' IgM or IgG that is anti-SARS-CoV-2. The best method to detect antibodies against SARS-CoV-2 proteins is to coat the plate with specific SARS-CoV-2 proteins.

In conclusion, quick clearance of viral RNA was observed in 40% of COVID-19 patients. Extended viral RNA detection existed in multiple tissues including blood and intestine. Our results demonstrated variable levels of specific anti-SARS-CoV-2 antibody among COVID-19 patients. High titers of antibodies appeared to be associated with severe COVID-19. Viral proteins and the sources of reagents are the key facts to affect the sensitivity of serological assays of SARS-CoV-2. These findings have shed light on the viral kinetics and antibody response in COVID-19 patients and provide scientific evidence for infection control, assay development and treatment of convalescent plasma.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contributions

ST and WC designed the study. LL, FH, YL and ZX collected the clinical data. YL, HY, JZ, ZW, HW and JS detected the antibodies and analyzed the data. LL, YL, CW and ST interpreted the results. YL made the tables and figures. ST wrote the manuscript. LL, FH and WC revised the manuscript. LL, YL, FH and HY contributed equally to this work. All authors reviewed, revised, and approved the final report.

CRediT authorship contribution statement

Linghua Li: Investigation, Resources, Writing - review & editing. **Yuanhao Liang:** Investigation, Data curation, Formal analysis, Writing - review & editing. **Fengyu Hu:** Investigation, Resources, Writing - review & editing. **Huanchang Yan:** Investigation, Data curation. **Yueping Li:** Investigation, Resources. **Zhiwei Xie:** Investigation, Resources. **Liping Huang:** Investigation, Data curation. **Jianhui Zhao:** Investigation, Data curation. **Zhengwei Wan:** Investigation, Data curation. **Haiying Wang:** Investigation, Data curation. **Jingwei Shui:** Investigation, Data curation. **Weiping Cai:** Conceptualization, Methodology, Resources, Writing - review & editing. **Shixing Tang:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing.

Declaration of competing interest

We declare no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virol.2020.09.008.

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