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INFECTIOUS DISEASE

Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset

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Antibody responses were induced after SARS-CoV-2 infection, and the complementary diagnostic value of the antibody test to the RNA test was observed. Antibody tests are critical to the clinical management and control of SARS-CoV-2 infection and COVID-19. https://bit.ly/3fQZwZp

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ABSTRACT

Background: Timely diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a prerequisite for treatment and prevention. The serology characteristics and complement diagnosis value of the antibody test to RNA test need to be demonstrated.

Method: Serial sera of 80 patients with PCR-confirmed coronavirus disease 2019 (COVID-19) were collected at the First Affiliated Hospital of Zhejiang University, Hangzhou, China. Total antibody (Ab), IgM and IgG antibodies against SARS-CoV-2 were detected, and the antibody dynamics during the infection were described.

Results: The seroconversion rates for Ab, IgM and IgG were 98.8%, 93.8% and 93.8%, respectively. The first detectible serology marker was Ab, followed by IgM and IgG, with a median seroconversion time of 15, 18 and 20 days post exposure (d.p.e.) or 9, 10 and 12 days post onset (d.p.o.), respectively. The antibody levels increased rapidly beginning at 6 d.p.o. and were accompanied by a decline in viral load. For patients in the early stage of illness (0–7 d.p.o), Ab showed the highest sensitivity (64.1%) compared with IgM and IgG (33.3% for both; $p<0.001$). The sensitivities of Ab, IgM and IgG increased to 100%, 96.7% and 93.3%, respectively, 2 weeks later. When the same antibody type was detected, no significant difference was observed between enzyme-linked immunosorbent assays and other forms of immunoassays.

Conclusions: A typical acute antibody response is induced during SARS-CoV-2 infection. Serology testing provides an important complement to RNA testing in the later stages of illness for pathogenic-specific diagnosis and helpful information to evaluate the adapted immunity status of patients.

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Data availability: We will share individual participant data that underlie the results reported in this article after de-identification (text, tables, figures and appendices). The data will be available beginning 6 months after the major findings from the final analysis of the study were published, ending 2 years later. The data will be shared with investigators whose proposed use of the data has been approved by an independent review committee identified for individual participant data meta-analysis. Proposals should be directed to chenyuzy@zju.edu.cn. To gain access, data requestors will need to sign a data access agreement.

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Introduction

In early December 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) was first reported to cause lethal pneumonia in humans, and person-to-person transmission was demonstrated soon in Wuhan, the capital city of Hubei Province, China [1]. The virus rapidly spread through China and then many other countries globally. By 6 May 2020, the virus had resulted in >3.5 million laboratory-confirmed cases of coronavirus disease 2019 (COVID-19) and >243 000 deaths in 215 countries [2]. The World Health Organization (WHO) declared COVID-19 a public health emergency of international concern and gave it a "very high" risk assessment on a global level [3]. A recent report from China showed that the median (interquartile range (IQR)) incubation period of COVID-19 infection was 4 (2–7) days [4]. Fever, cough and fatigue are the most common symptoms [1]. Severe cases could rapidly progress to acute respiratory distress syndrome and septic shock. Abnormalities on chest computed tomography, particularly ground-glass opacity and bilateral patchy shadowing, were found in >80% of patients [4]. >80% of patients had lymphopenia, and ∼60% of patients had elevated C-reactive protein [5]. However, the clinical and laboratory findings of COVID-19 infection are not distinguishable from pneumonia caused by infection of some common respiratory tract pathogens, such as influenza virus, *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* [6]. Hence, the timely diagnosis of SARS-CoV-2 infection is important for providing appropriate medical support and preventing the spread by quarantining.

Currently, the diagnosis of SARS-CoV-2 infection almost solely depends on the detection of viral RNA using PCR-based techniques [7]. Unfortunately, the sensitivity of the RNA test in the real world is not satisfactory, particularly when samples collected from the upper respiratory tract are used [6, 8–10]. In Wuhan, the overall positive rate of RNA testing is estimated to be ∼30–50% in patients with COVID-19 when they come to the hospital [11]. Furthermore, the overall throughput of available RNA tests is highly limited by the fact that they require high workload, need skilful operators for testing and sample collection, and need costly instruments and special operation places [12]. As a result, convenient serological detection is expected to be helpful. However, current knowledge of the antibody response to SAR-CoV-2 infection is very limited. The diagnostic value of the antibody test remains to be clearly demonstrated. How many patients would raise an antibody response, and how long will it take for the antibody to convert to positive since the exposure? Are there any meaningful differences between patients with short and long incubation periods? What are the sensitivities of antibody detection for patients in different illness stages? Is there any temporal association between the antibody response and the decline in viral load? To answer some of these questions, we investigated the characteristics of antibody responses in 80 patients with COVID-19 during their hospitalisation periods by detecting total antibodies, IgM and IgG using immunoassays.

Methods

Study design and participants

A confirmed COVID-19 case was defined based on the New Coronavirus Pneumonia Prevention and Control Program (6th edition), published by the National Health Commission of China [13]. Briefly, a confirmed case should meet three criteria: 1) fever and/or respiratory symptoms; 2) abnormal lung imaging findings; and 3) a positive result of the nucleic acid of SARS-CoV-2. The degree of severity of the patient was categorised as critical if any of the following clinical findings appeared: 1) acute respiratory distress syndrome or oxygen saturation <93% and requiring mechanical ventilation either invasively or noninvasively; 2) shock; and 3) complications of organ functional failure and requiring intensive care unit support. A COVID-19 patient who did not meet the above criteria was defined as a non-critical case.

The study enrolled a total of 80 COVID-19 cases, where all patients were admitted to the hospital between 19 January and 9 February 2020, and were willing to donate their blood samples. All enrolled cases were

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confirmed to be infected by SARS-CoV-2 through quantitative real-time reverse transcriptase PCR (qRT-PCR) testing. The date of illness onset, clinical classification, RNA testing results during the hospitalisation period, and personal demographic information were obtained from the clinical records. A total of 300 healthy people were enrolled from the local community during the circulation of the virus. All the controls had not reported close contact with any confirmed COVID-19 patient. The study was reviewed and approved by the Medical Ethics Committee of the First Affiliated Hospital of Zhejiang University (Hanzhou, China; approval number 2020-IIT-47). Written informed consent was obtained from each enrolled subject.

Laboratory confirmation of COVID-19 by reverse-transcription polymerase chain reactions

For each patient, the sputum specimen was collected preferentially. Where sputum was not produced, saliva after deep cough was collected, as previously reported [14]. The sputum was first treated with an equal volume of protease K solution (0.4 $mg{\cdot}mL^{-1}$) at room temperature for 20 min. 200 µL of treated sputum or saliva was subjected to total nucleic acid extraction by MagNA Pure LC 2.0 (Roche, Basel, Switzerland), and each sample obtained 50 µL elution. A commercial one-step real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay (Bio-Germ, Shanghai, China) targeting the nucleocapsid gene and open reading frame lab gene was performed with 5 µL of total nucleic acid according to the manufacturer's instructions.

Antibody measurement

The total antibody (Ab), IgM antibody and IgG antibody against SARS-CoV-2 in plasma samples were tested using three enzyme-linked immunosorbent assays (ELISA-Ab, ELISA-IgM and ELISA-IgG), three colloidal-gold lateral-flow immunoassays (LFIA-Ab, LFIA-IgM and LFIA-IgG) and two chemiluminescence microparticle immunoassays (CMIA-Ab and CMIA-IgM). The ELISA reagents and LFIA reagents were supplied by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd (Beijing, China), and the CMIA reagents were supplied by Xiamen InnoDx Biotech Co., Ltd (Xiamen, China). Total Ab detection was based on a double-antigen sandwich immunoassay, and IgM antibody detection was based on a µ-chain capture immunoassay. Mammalian cell-expressed recombinant antigens containing the receptor binding domain (RBD) of the SARS-CoV-2 spike protein were used to develop total Ab and IgM antibody assays. The IgG antibody kits were indirect immunoassays, and a recombinant nucleoprotein of SARS-CoV-2 expressed in *Escherichia coli* was used as the coating antigen. The antigens used in these assays were in-house preparations by Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, and the same antigen or antigen pairs were used for ELISA, CMIA and LFIA assays.

The measurement processes of ELISA and CMIA were conducted with automatic ELISA analyser HB-300E (Jiaxing CRED Medical Equipment Co. Ltd, Zhejiang, China) and automatic CMIA analyser Caris 200 (Xiamen UMIC Medical Instrument Co. Ltd, Xiamen, China) according to the manufacturers' instructions. The details of the procedures are described in the supplementary material. When LFIA-Ab and LFIA-IgM were performed, 10 µL of sample was pipetted onto the sample receiving zone, followed by two drops of sample buffer. For LFIA-IgG, the sample was diluted 1000-fold with sample buffer, and then 80 µL of dilution was added onto the sample receiving area. 15 min after the sample was added, the LFIA results were observed by eye and recorded. It takes 75 min, 29 min and 15 min to get the results back for ELISA, CMIA and LFIA assays, respectively.

Statistical analysis

The incubation period was defined as the interval between the earliest date of SARS-CoV-2 exposure and the earliest date of symptom onset. Non-normally distributed continuous data were described as the median (IQR) and compared using the Wilcoxon test. Categorical data were summarised as counts and percentages. The 95% confidence intervals of sensitivity and specificity were estimated using the binomial exact test. Categorical data were compared using the Chi-squared test or Fisher's exact test for unpaired proportions and McNemar's test for paired proportions. Cumulative seroconversion rates were calculated using the Kaplan–Meier method. All statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC, USA).

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in the design and implementation of the study.

Dissemination declaration

Dissemination to these groups is not possible/applicable.

Results

Characteristics of enrolled patients with COVID-19

Of the 81 COVID-19 patients admitted to the hospital (before 9 February 2020), 80 (99%) were enrolled in the study (table 1). The median (IQR) age of the patients was 55 (45–64) years, and 38.7% were females. Critical patients were significantly older than non-critical patients ($p<0.001$). The time of SARS-CoV-2 exposure before onset in 45 patients (15 critical cases) was determined according to unambiguous close contact with confirmed patients with COVID-19 through epidemiological inspection upon admission to the hospital. The incubation period ranged 0–23 days with a median (IQR) of 5 (2–10) days. By 15 February 2020, a total of 32 (40%) patients (all non-critical cases) were recovered and discharged from hospital; none died.

The performance of SARS-CoV-2 antibody detection kits

A total of 80 patients with COVID-19 and 100–300 healthy people were tested for antibodies against SARS-CoV-2 using different immunoassays. The seroconversion rates for Ab, IgM and IgG in patients were 98.8% (79 out of 80), 93.8% (75 out of 80) and 93.8% (75 out of 80), respectively (table 2 shows the combined sensitivity of three methods). The last blood sample from the only patient who had not seroconverted was collected at 7 days post onset (d.p.o.). For the Ab, IgM and IgG tests, ELISAs seemed to perform the best, although the differences were generally not significant. Therefore, the following serological analyses were all based on the results of ELISAs, unless specifically noted.

Seroconversion sequentially appeared for Ab, IgM and IgG, with median onset times of 9, 10 and 12 days, respectively (figure 1a). No significant difference of the time of seroconversion was observed between critical and non-critical patients (supplementary figure E1). The seroconversion of Ab was significantly quicker than that of IgM and IgG ($p<0.001$). The cumulative seroconversion curve showed that the rate for Ab and IgM reached 100% and IgG reached 97.1% on days 16, 21 and 29 post symptom onset, respectively. The antibody levels increased rapidly starting at 6 d.p.o. (figure 1b). The decline in viral load co-occurred with increasing antibody levels. For patients in the early stage of illness (0–7 d.p.o.), ELISA-Ab showed the highest sensitivity (64.1%) compared with ELISA-IgM and ELISA-IgG (33.3% for both; $p<0.001$) (table 3). The sensitivities of Ab, IgM and IgG detection increased significantly when the patient entered the later stage and reached 100%, 96.7% and 93.3%, respectively, 2 weeks later ($p<0.05$).

The antibody dynamics after exposure to SARS-CoV-2

Because of the difficulty of determining the exact infection date for patients with prolonged exposure, the antibody dynamics after the earliest date of exposure instead of those after infection were investigated in 45 patients whose exposure times had been determined (figure 2). Seroconversion appeared sequentially for Ab, IgM and IgG, and the levels increased rapidly, with median days post exposure (d.p.e.) of 15, 18

TABLE 1 Demographics and clinical characteristics of enrolled patients with coronavirus disease 2019 (COVID-19)

	Total	Non-critical	Critical	p-value
Subjects n	80	54	26	
Sex				
Female	31 (38.7)	24 (44.4)	7 (26.9)	0.132
Male	49 (61.3)	30 (55.6)	19 (73.1)	
Age years	55 (45–64)	51 (38–39)	65 (52–74)	<0.001
Clinical outcome				
Recovery	26 (32.5)	26 (48.1)	0 (0.0)	<0.001
Still in hospital	54 (67.5)	28 (51.9)	26 (100.0)	
Incubation period[#]	5 (2–10)	4 (2–12)	7 (1–10)	1.000
Day of first positive SARS-CoV-2 RNA finding	4 (1–6)	4 (2–6)	3 (1–6)	0.179
Day when first antibody testing sample was collected	8 (6–10)	7 (5–10)	8 (7–10)	0.215
Samples tested for antibodies for each case	4 (3–5)	4 (3–4)	4 (3–5)	0.888
Total plasma samples n	305	205	100	

Data are presented as n (%) or median (interquartile range), unless otherwise stated. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2. [#]: for patients who had no travel history to Wuhan, China, but had close contact with a confirmed COVID-19 patient (index patient) within 14 days before symptom onset, the exposure time was defined as the first day of close contact with the index patient. The time of SARS-CoV-2 exposure before onset in 45 patients was determined through epidemiological inspection; among them, 15 were critical cases.

TABLE 2 Sensitivity and specificity of different immunoassays to detect antibodies against severe acute respiratory syndrome coronavirus 2

Type of immunoassay[#]	Sensitivity			Specificity[¶]		
	Patients n	Positive n	% (95% CI)	Uninfected n	Negative n	% (95% CI)
Ab						
ELISA-Ab	80	78	97.5 (91.3–99.7)	300	300	100.0 (98.8–100.0)
CMIA-Ab	80	77	96.3 (89.4–99.2)	300	298	99.3 (97.6–99.9)
LFIA-Ab	80	78	97.5 (91.3–99.7)	209	199	95.2 (91.4–97.7)
Combined	80	79	98.8 (93.2–100.0)	209	197	94.3 (90.2–97.0)
IgM						
ELISA-IgM	80	74	92.5 (84.4–97.2)	300	300	100 (98.8–100)
CMIA-IgM	80	69	86.3 (76.7–92.9)	300	298	99.3 (97.6–99.9)
LFIA-IgM	80	71	88.8 (79.7–94.7)	209	205	98.1 (95.2–99.5)
Combined	80	75	93.8 (86.0–97.9)	209	203	97.1 (93.9–98.9)
IgG						
ELISA-IgG	80	71	88.8 (79.7–94.7)	100	100	100.0 (96.4–100.0)
LFIA-IgG	80	69	86.3 (76.7–92.9)	209	208	99.5 (97.4–100.0)
Combined	80	75	93.8 (86.0–97.9)	100	99	99.0 (94.6–100)

Ab: total antibody; CMIA: chemiluminescence microparticle immunoassay; LFIA: lateral flow immunoassay. [#]: the combined sensitivities were calculated based on positive findings by any of the assays; the combined specificities were calculated based on negative findings for all assays. [¶]: none of the controls reported close contact with any confirmed coronavirus disease 2019 patients, and individuals who were positive for any of the antibody tests were tested to be PCR negative with sputum or saliva samples collected later. With the very limited local community spread of the virus during the period in mind, and for more rigorous evaluation of an assay's specificity, the community controls with positive results in antibody assays were considered false-positive.

and 20, respectively (figure 3). The median seroconverting times post exposure in critical patients were not different from those in non-critical patients (supplementary figure E2). The decline in viral load co-occurred with increasing antibody levels. The cumulative positive rates for Ab, IgM and IgG separately reached 100%, 94.2% and 96.7% at 37 d.p.e. Patients who reported symptoms within 5 days since exposure were assigned to the short incubation period group (0–5 days), and the remaining patients were assigned

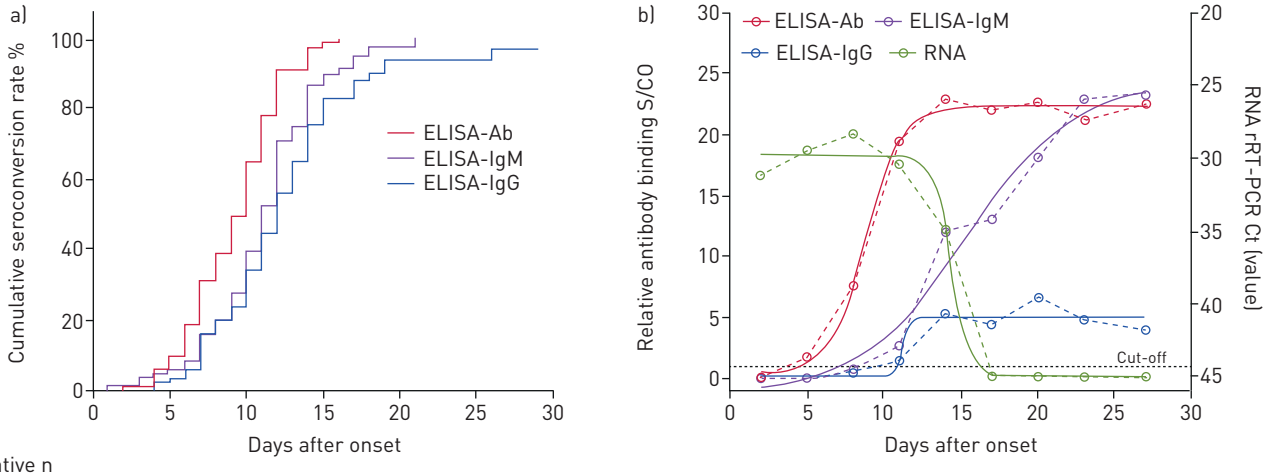


FIGURE 1 Cumulative seroconversion rates and the dynamics of antibody levels since the onset of illness in 80 patients with coronavirus disease 2019. a) Curves of the cumulative seroconversion rates for total antibody (Ab), IgM and IgG detected by ELISAs plotted according to Kaplan–Meier methods. The serological status of patients was assigned to be negative before the time that the first sample was collected. b) The antibody levels were surrogated and expressed using the relative binding signals compared with the cut-off value of each assay (signal to cut-off (S/CO)). Four parametric logistic fitting curves (solid lines) were used to mimic the trends of antibody levels. rRT-PCR: real-time reverse transcriptase PCR; Ct: cycle thresholds

TABLE 3 Performance of different detection methods in different periods post onset

Days after onset	Patients n	RNA[#]		ELISA-Ab		ELISA-IgM		ELISA-IgG	
		Positive n	Sensitivity %	Positive n	Sensitivity %	Positive n	Sensitivity %	Positive n	Sensitivity %
0–7	39	36[¶]	100.0	25	64.1	13	33.3	13	33.3
8–14	75	64[¶]	90.1	74	98.7	65	86.7	57	76.0
15–29	60	41[¶]	70.7	60	100.0	58	96.7	56	93.3

Ab: antibody. [#]: tested using deep sputum samples; [¶]: 36, 71 and 58 patients underwent RNA testing during the periods 0–7, 8–14 and 15–29 days post onset, respectively.

to the long incubation period group (6–23 days) (table 4). There was no significant difference in age or viral load in the early stage of illness, or the risk of critical status between the groups. However, the median seroconversion time was shorter for the short incubation period group than for the long incubation period group (13 d.p.e. *versus* 21 d.p.e.; p<0.001). In contrast, the median seroconversion days

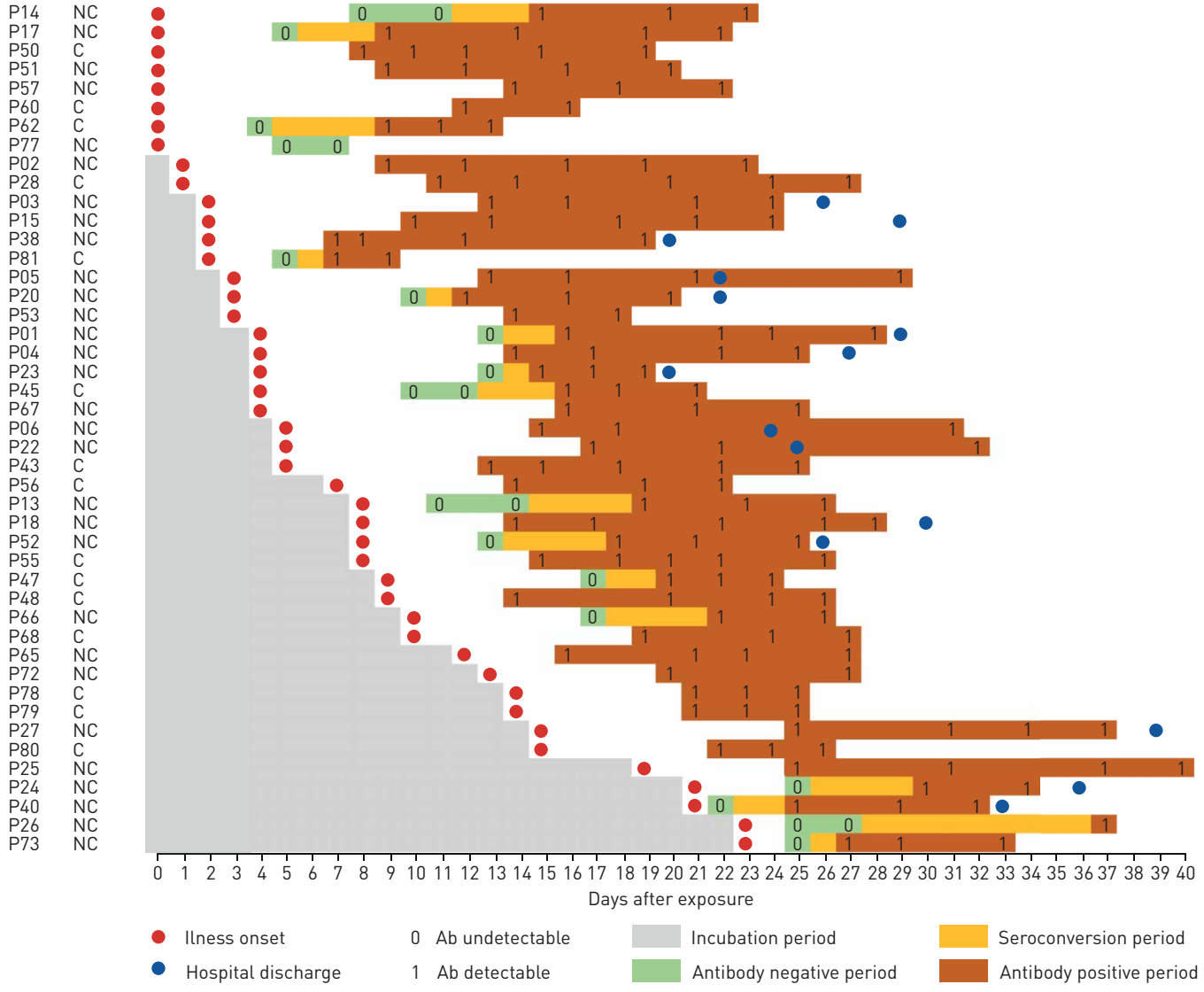
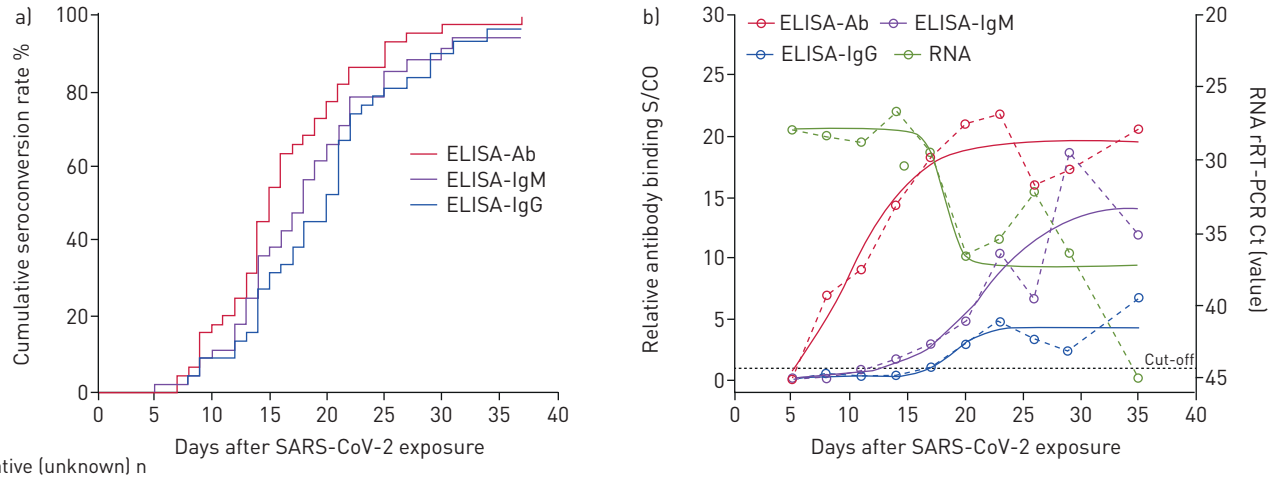


FIGURE 2 The total antibody dynamics of 45 patients with determined exposure time. The patients are listed according to their lasting time of incubation period, from shorter to longer. The clinical severity of each patient is described beside the patient ID. The serological status of each patient is presented only during the period of sample collection, without backward or forward extension. NC: non-critical; C: critical; Ab: antibody.



Seronegative (unknown) n

Ab	45	45 (28)	37 (24)	24 (12)	12 (6)	6 (2)	2 (0)	1 (0)	0
IgM	45	45 (18)	40 (15)	28 (7)	16 (4)	9 (1)	4 (0)	1 (0)	0
IgG	45	45 (12)	40 (11)	32 (7)	23 (5)	9 (1)	3 (0)	1 (0)	0

FIGURE 3 Cumulative seroconversion rates and the dynamics of antibody levels since the onset of illness in 45 patients with coronavirus disease 2019. a) Curves of the cumulative seroconversion rates for total antibody (Ab), IgM and IgG detected using ELISAs were plotted according to Kaplan–Meier methods. The serological status of patients was assigned to be negative before the time that the first sample was collected. For patients with a positive result when the first sample was collected, the sero-status before the first sample was collected was unknown and was assigned as negative. The number of these patients at the indicated days after exposure is shown in brackets. b) The antibody levels were surrogated and expressed using the relative binding signals compared with the cut-off value of each assay (signal to cut-off (S/CO)). Four parametric logistic fitting curves were used to mimic the trends of antibody levels (solid lines). SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; rRT-PCR: real-time reverse transcriptase PCR; Ct: cycle thresholds.

post onset were longer for the short incubation period group than the long incubation period group (10 d.p.o. *versus* 7 d.p.o.; p<0.05).

Discussion

The present study showed that nearly all (98.8%, 79 out of 80) patients with COVID-19 converted to be seropositive during the illness course. Seroconversion was first observed on 7 d.p.e. The first detectible antibody was total Ab, followed by IgM and IgG, with a median seroconversion time of 15 d.p.e. (9 d.p.o.), 18 d.p.e. (10 d.p.o.) and 20 d.p.e (12 d.p.o.). It was very similar to that observed in SARS-CoV-1 infection [15, 16]. Interestingly, the increase in antibody levels was accompanied by a decline in viral load.

Currently, the quarantine period is set as 14 days after close contact with a confirmed SARS-CoV-2 infection case. The proportion of infection that could not be screened out through RNA testing during the quarantine period remains unknown. However, it has been documented that some close contactors present symptoms and cause transmission after de-isolation [17]. It is believed that the antibody test might improve the sensitivity of detecting infections, but no evidence has been shown before. Our data showed

TABLE 4 Comparison of patients with short and long incubation periods

	Short incubation period[#]	Long incubation period[¶]	p-value
Patients n	25	20	
Incubation period days	2 (0–4)	13 (9–17)	<0.001
Age years	51 (36–63)	54 (46–65)	0.784
Critical cases	7 (28.0)	8 (40.0)	0.396
Viral load in early stage of illness[+]	27.8 (22.6–34.9)	27.0 (24.1–30.1)	0.599
Days post exposure when antibody converted	13 (9–15)	21 (17–25)	<0.001
Days post onset when the antibody converted	10 (9–12)	7 (6–10)	0.013

Data are presented as median (interquartile range) or n (%), unless otherwise stated. [#]: 0–5 days; [¶]: 6–23 days; [+]: measured using the cycle threshold value of real-time reverse transcriptase PCR when detecting the first available sputum samples collected after onset.

that the cumulative seroconversion rate was 45.5% on 14 d.p.e. This result indicated that approximately half of the carriers could be screened out if antibodies were tested before de-isolation. The cumulative seroconversion rate reached 75% on 20 d.p.e. (11 d.p.o.). Seven (15.6%) patients did not present any symptoms before 14 d.p.e., among them, four patients remained seronegative at 22 d.p.e. (one patient) and 25 d.p.e (three patients). Hence, follow-up antibody testing and monitoring of respiratory symptoms for 2 weeks after de-isolation would be helpful to further reduce the risk of spread.

The median (IQR) incubation period was 5 (2–10) days, very similar to that previously reported [4, 18]. The time required for seroconverting since exposure is significantly longer for patients with a long incubation period than for patients with a short incubation period (21 days *versus* 13 days; $p<0.001$). In contrast, the antibody of patients with a longer incubation period appeared on the earlier days post onset (7 d.p.o. *versus* 10 d.p.o.; $p<0.05$). The difference does not seem to be biased by the competence of host immunity because of the similar age distribution between groups. Hence, it is more likely to be due to the lower intake dose of virus or less efficiency of virus reproduction in the host. Nevertheless, the longer incubation period does not change the initial viral load when the symptom onset occurs or the risk of experiencing critical status. Therefore, in addition to the onset time, the interpretation of the negative findings of antibodies in suspected infections should also take the exposure time into consideration. The faster seroconversion post onset suggests a longer incubation period.

Recently, Guan *et al.* [4] reported that the median time from the onset of symptoms to needing mechanical ventilator support was 9.3 days. Therefore, timely diagnosis and admission to the hospital within 7 d.p.o. might be crucial to lowering the fatality of COVID-19 infection. Zhao *et al.* [19] reported that the overall RNA positive rate was <70% in patients in the first week post symptom onset and fell to 50% in the next week. Several reports have indicated that many patients were finally diagnosed by RNA testing through daily repeat sampling and testing, and many cases that were strongly epidemiologically linked to SARS-CoV-2 exposure and with typical lung radiological findings remained RNA negative [6]. In our study, the RNA positive rate was 100% when admitted to the hospital, but it cannot be excluded that some patients were not diagnosed due to their undetectable viral RNA. Another reason for the relatively high RNA positive rate in our study is that we used a deep sputum sample for RNA testing, in contrast to the more convenient and popular throat/nasal swabs in many other hospitals. This suggests that lower respiratory samples, such as deep sputum and bronchoalveolar lavage, might be more reliable for SARS-CoV-2 RNA detection.

Similar to the RNA test, the negative antibody finding in the early stage of illness could not exclude the possibility of infection. Our study showed that the seroconversion rate at 7 d.p.o. reached 64.1% and then increased to >90% in the next week. The relatively long period of seroconversion indicates that, for searching previously exposed patients or subclinical carriers, the specific serology should not replace RNA detection but could be an important complement. Furthermore, the fact that nearly all patients will convert to antibody positive and that the titre increased rapidly underlines the usage of antibody tests in confirming or excluding the diagnosis of SARS-CoV-2 infection if the convalescent sample was tested.

The present data show that the sensitivity of total antibody detection was higher than that of IgM and IgG ($p<0.001$), while the specificities were comparable overall when the same testing technique (ELISA, CLMA or LFIA) was used. The detection of total Ab is based on double-antigen sandwich methodology. It can detect any type of antibody, including IgM, IgG and IgA, in principle. In addition, the two Fab arms of the same antibody molecule need to bind to the coated antigen and the enzyme-conjugated antigen, which guarantees the specificity of the test and then allows a high concentration of antigens to be used for coating and second binding to increase the sensitivity of the assay. Therefore, it was not unexpected that the sensitivity of total Ab detection would be superior to that of IgM and IgG detection in our study. Usually, IgM is considered a marker of current or recent infection, while IgG is considered a marker of post or recent infection. The implications of total Ab are not so straight and are less commonly used in clinical practice. However, the total Ab test outweighs IgM and IgG if the sensitivity is the top priority and has been frequently used in blood transfusion product screening, such as HIV and hepatitis C virus. For the current urgently required sensitive diagnosis for SARS-CoV-2 infection, in order to contain the spread, the Ab test might be a better choice than IgM or IgG, particularly considering that the virus invaded human society <4 months ago and the prevalence of antibody induced post infection is nearly zero. Recently, similar findings were reported by Lassaunière *et al.* [20], who showed that total Ab testing using the same Wantai SARS-CoV-2 Ab ELISA as the present study outperformed the assays detecting specific IgG or IgA only in sensitivity and specificity. A Hong Kong group also reported that antibody seroconversion occurred ∼10 days after onset, and the patients showed lower viral load in seroconverted stages than earlier stages [21]. Interestingly, in their study, more patients had earlier seroconversion for IgG than IgM. We noted that the indirect ELISA methodology was applied for IgG and IgM antibody

detection in their study, despite the fact that the μ-chain capture methodology is preferred for IgM testing. This might be explained by the different analytical sensitivity between the assays for detection of IgG and IgM.

In our study, antibody tests based on ELISA, CMIA and LFIA were validated and showed good performance overall. CMIA has the advantages of automatic operation, having a high-throughput, and being rapid, objective and quantitative, but it requires a costly specific instrument. ELISA is low-cost, objective and has a high-throughput and is widely used in most medical laboratories worldwide. LFIA is a rapid point-of-care test that does not require special instrumentation, is very convenient and is easy to operate, but the reading of the results is subjective. The establishment of different immunoassays provides flexible choices for users.

To date, several reports on antibody response to SARS-CoV-2 infection post illness have been published [19–23]. Although the profiles of antibody response presented in these reports are generally the same, the orders of IgM and IgG seroconversion that are reported are conflicting. This may be because of differences in the sensitivity of the assays used in these studies.

The limitations of our study include the following. 1) Only symptomatic infections were enrolled; therefore, whether the antibody response to asymptomatic infection follows similar features remains to be determined. 2) Most blood samples were collected at 1 month post onset, so the duration of antibodies cannot be estimated. 3) The antibody levels were not exactly titrated, and different antigens were used in total antibody (RBD), IgM (RBD) and IgG (nucleoprotein), hence the correlation of the degree of antibody response with clinical severity is not investigated. 4) No blood sample was collected during the incubation period, and the fact that the serological status before onset was artificially assigned to be negative might overestimate the time needed for seroconversion. Future studies are needed to better understand the antibody response profile of SARS-CoV-2 infection and to precisely interpret the clinical meaning of serology findings.

In conclusion, a typical acute antibody response is induced during SARS-CoV-2 infection. Serology testing provides an important complement to RNA testing for pathogen-specific diagnosis and helpful information to evaluate the adapted immunity status of patients. It should be strongly recommended that well-validated antibody tests be applied in clinical management and public health practice to improve the control of COVID-19 infection.

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