Antibody Detection and Dynamic Characteristics in Patients with COVID-19

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Summary: The antibodies against SARS-CoV-2 can be detected in the middle and later stage of the COVID-19. Antibody detection may play an important role in the diagnosis of COVID-19 as complement approach for viral nucleid acid assays.

Abstract

Background. The corona virus disease 2019 (COVID-19) caused by the corona virus 2 (SARS-CoV-2) has been rapidly spreading nationwide and abroad. A serologic test to identify antibody dynamics and response to SARS-CoV-2 was developed.

Methods. The antibodies against SARS-CoV-2 were detected by an enzyme-linked immunosorbent assay (ELISA) based on the recombinant nucleocapsid protein of SARS-CoV-2 in patients with confirmed or suspected COVID-19 at 3-40 days after symptom onset. The gold standard for COVID-19 diagnosis was nucleic acid testing for SARS-CoV-2 by RT-PCR. The serodiagnostic power of the specific IgM and IgG antibodies against SARS-CoV-2 was investigated in terms of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and consistency rate.

Results. The seroconversion of specific IgM and IgG antibodies were observed as early as the 4^{th} day after symptom onset. In the confirmed patients with COVID-19, sensitivity, specificity, PPV, NPV, and consistency rate of IgM were 77.3% (51/66), 100%, 100%, 80.0%, and 88.1%, and those of IgG were 83.3.3% (55/66), 95.0%, 94.8%, 83.8%, and 88.9 %. In patients with suspected COVID-19, sensitivity, specificity, PPV, NPV, and consistency rate of IgM were 87.5% (21/24), 100%, 100%, 95.2%, and 96.4%, and those of IgG were 70.8% (17/24), 96.6%, 85.0%, 89.1%, and 88.1%. Both antibodies performed well in serodiagnosis for COVID-19 rely on great specificity.

Conclusions. The antibodies against SARS-CoV-2 can be detected in the middle and later stage of the illness. Antibody detection may play an important role in the diagnosis of COVID-19 as complement approach for viral nucleid acid assays.

Keywords. SARS-CoV-2; COVID-19; serological test; ELISA; diagnosis

Introduction

A novel corona virus (SARS-CoV-2) disease (COVID-19) was first identified and outbroke in Wuhan City, Hubei Province, China. A total of 93, 090 patients with COVID-19 had occurred globally by March 5, 2020 [1]. Accurate and fast diagnosis of the causative SARS-CoV-2 is important to isolate the patients with COVID-19 timely and stop the epidemics, as well as save people's lives. Viral nucleic acid detection using real-time polymerase chain reaction (RT-PCR) assay, which has been developed and used for detection of SARS-CoV-2 rapidly, remains the standard diagnosis of COVID-19 [2]. While a large number of the "suspected" cases with typical clinical COVID-19 features and/or identical specific computed tomography (CT) scan were not diagnosed [3,4]. Moreover, RT-PCR assay which was time consuming and laborious needed special equipment resulted to limit its usage especially in remote areas. The human antibody response which is crucial for the clearance of the initial virus infection has been widely used to help diagnosis virus infection. Compared to RT-PCR assays, the detection of antibody assays are often faster, less expensive, easy-to-use and accessible to staff without laboratory training. Here, we detected dynamics characteristics and magnitude of antibody response in patients with COVID-19, and evaluated serodiagnostic value of ELISA-based IgM, IgG tests for COVID-19 pneumonia. The sensitivity and specificity of antibody tests for detection of IgM and IgG were presented and the clinical application of these antibody assays for serodiagnosis of COVID-19 was discussed.

METHODS

Patients and Data Sources

Patients at Union Hospital, Tongji Medical College, Huazhong University of Science and Technology were evaluated from January 19, 2020 to March 2, 2020. During this period, IgM and IgG antibody responses to SARS-CoV-2 virus infection were analyzed. The gold standard for diagnosis is nucleic acid testing for SARS-CoV-2 by RT-PCR tests of nasopharyngeal and/or oropharyngeal swabs sample. RT-PCR tests were performed for patients who presented with a history of 1) Travel or residential history in Wuhan or local endemic areas; or 2) The epidemioloical history of contact with patients who have been confirmed with COVID-19 pneumonia or individuals present fever or respiratory symptoms from these areas within 14 days; or 3) A clustering outbreak, combined with clinical manifestation of 1) fever and /or respiratory symptoms, or 2) positive findings similar to COVID-19 pneumonia on chest computerized tomography (CT) scan, or 3) laboratory tests showing reduced lymphocytes and white blood cell counts in the early stage. In the case of an initial negative RT-PCR test, repeated testing was performed at intervals of one day or more. The diagnosis of laboratory confirmed COVID-19 defined as positive nucleic acid tests for SARS-CoV-2 by RT-PCR assays. The diagnosis of suspected COVID-19 was based on one of the epidemiological history and two of the clinical manifestations but the RT-PCR of SARS-CoV-2 was negative. In confirmed cases, the patient showing fever and respiratory symptoms with radiological findings of pneumonia was defined as normal case, while the case meeting any of the following criteria was defined as severe case: 1) Respiratory distress (≧30 breaths/ min); 2) Oxygen saturation≤93% at rest; 3) Arterial partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) ≤ 300mmHg (l mmHg=0.133kPa); 4) Cases with chest imaging that shows obvious lesion progression within 24-48 hours >50% shall be

managed as severe cases. In the control group, samples from healthy blood donors or from patients with other disease hospitalized in the same hospital. The detailed diagnosis standards were presented in online Table S1.

RNA Extraction and Real-time RT-PCR Assay

Total RNAs were extracted from nasopharyngeal and/or oropharyngeal swabs samples of patients suspected of having SARS-CoV-2 infection within 2 hours using the respiratory sample RNA isolation kit. In brief, 40 μL of cell lysates were transferred into a collection tube followed by vortex for 10 seconds. After standing at room temperature for 10 minutes, the collection tube was centrifuged at 1000 rpm/min for 5 minutes. The suspension was used for real-time RT-PCR assay of SARS-CoV-2 RNA. Two target genes, including open reading frame1ab (ORF1ab) and nucleocapsid protein (N), were simultaneously amplified. Target 1 (ORF1ab): forward primer CCCTGTGGGTTTTACACTTAA; reverse primer ACGATTGTGCATCAGCTGA. Target 2 (N): forward primer GGGGAACTTCTCC TGCTAGAAT; reverse primer CAGACATTTTGCTCTCAAGCTG. RT-PCR assay was performed under the following conditions: incubation at 50°C for 15 minutes and 95°C for 2 minutes, followed by 45 cycles of denaturation at 95°C for 3 seconds, then annealing, extending and collecting fluorescence signal at 55°C for 30 seconds. The diagnostic criteria were based on the recommendation by the National Institute for Viral Disease Control and Prevention (China).

ELISA Test

The serum SARS-CoV-2 antibodies (IgM and IgG) of the subjects were detected using a sandwich enzyme linked immunosorbent assay (ELISA kits, Livzon Inc, Zhuhai, P.R.China, lot number of IgM: 20200308, IgG: 20200308). For detection of IgM, 100 μl diluted serum (1:100) was added into the 96-well microplate (coated with N protein) and then incubated for 1 h at 37℃. After washing, 100 μl secondary antibody (against human IgM) labeled with conjugate was added into the wells and then incubated for 30 min under 37℃. Following the second wash cycle, 100 μl substrate was added into the wells and incubated for 15 min under 37℃. At last, stop solution was added into the wells to terminate the reaction. The optical density of each well was determined by a microplate reader set to 450 nm within 30 min. The ratio of optical density to the cut off value (optical density of the blank well + 0.1) was reported as the antibody concentration. For detection of IgG, the dilution factor was changed (1:20) and the cut off value was modified (optical density of the blank well + 0.13).

Statistical Analyses

All statistical analyses were performed using SPSS 20.0. The diagnostic value of ELISA-based IgM and IgG antibody test for COVID-19 was based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Log-normal distribution was used to fit the time distribution of IgM and IgG antibody seropositve rate, as well as calculative seroconversion rate of the two antiobdies.

Ethic Approval

Ethics approval was exempted from institutional review board of the hospital since we collected and analyzed all data from the patients according to the policy for public health outbreak investigation of emerging infectious diseases issued by the National Health Commission of the People's Republic of China.

RESULTS

Demographic and Clinical Characteristics

85 patients with confirmed diagnosis and 24 patients with suspected diagnosis were recruited in this study. The demographic and clinical characteristics were showed in Tabel 1. Comorbidities in patients were recorded including hypertension, diabetes, surgical operation, malignancies, chronic lung disease, and chronic renal diseases. Healthy donors who were doctors and nurses working in the hospital, and patients with other lung disorders including bacterial pneumonia (n=5), acute exacerbation of chronic obstructive pulmonary disease (AECOPD, n=2), lung cancer (n=3), empyema (n=1), interstitial lung disease (n=2) and pleuritis (n=1) were included as control group.

Dynamics of Antibody Response

Detection of IgM and IgG Antibody on Different Periods (Table 2.)

We evaluated specificity of IgM and IgG antibodies based on ELISA from 216 serum sample of 85 confirmed COVID-19 pneumonia patients. Serum obtained in different periods after

symptom onset. The IgM and IgG antibodies were detected positive as early as on the 4th day after onset (Table 2). Some patients were observed positive for IgM (7 patients) and IgG (6 patients) within 7 days after illness onset, respectively. The seropositive rate of IgM increased gradually and notably. IgG was increased sharply on the 12th day after onset. We used a log-normal distribution to fit the time distribution of IgM and IgG antibody seropositive rate (Figure 1). The results from the 60 samples in the control group showed that 3 cases were positive for IgG, while all cases were negative for IgM.

The Timeline about Initial Seroconversion of IgM and IgG Antibodies.

To monitor the kinetics of serological antibodies within COVID-19 patients, serological antibodies were tested consecutively once the initiative appearance time of IgM and/or IgG antibody were detected in 29 confirmed patients. A log-distribution was used to fit the seroconversion time of the two antibodies (Figure 2). IgM cumulative seroconversion increased quickly from the 9th day as well as IgG increased from the 11th day after symptom onset. In our investigation, both antibodies were seropositive in nearly all the patients within the illness course for more than 30 days.

Diagnostic Role of IgM and IgG Antibodies for COVID-19 Pneumonia

To evaluate the diagnostic potential of serological IgM and IgG antibodies detection, 66 patients with confirmed COVID-19 pneumonia were evaluated and compare to standard RT-PCR assays. Serum sample were obtained from patients with disease course being or more than 13 days and less than 29days. The results of serological test for IgM and IgG were showed in Table 3. Compared with RT-PCR, the sensitivity, specificity, PPV, NPV, and

consistency rate of IgM were 77.3% (51/66), 100% (60/60), 100% [51/(51+0)], 80.0% [60/(15+60)], and 88.1% [(51+60)/(51+15+0+60)], while those of IgG were 83.3.3% (55/66), 95.0% (57/60), 94.8% [55/(55+3)], 83.8% [57/(11+57)], and 88.9 % [(55+57)/(55+11+3+57)], respectively.

In the control group with healthy donors and patients with other disease, there were only 3 healthy donors showed positive for IgG and there were no case observed positive for IgM.

Diagnostic Role of IgM and IgG Antibody Detection in Patients with Suspected COVID-19 Pneumonia

As shown in Table 4, 24 patients had COVID-19 pneumonia manifestations while being negative at least twice for respiratory tract nucleic acid tests. Patients were evaluated independently by two experienced physicians. The serological tests for IgM and IgG showed in Table 5. For patients with suspected COVID-19, diagnostic power of serological antibodies was as follows: the specificities, sensitivity PPV, NPV and consistency rate of IgM is 87.5% (21/24), 100% (60/60), 100% [21/(21+0)] , 95.2% [60/(3+60)], and 96.4% [(21+60)/(21+3+0+60)], while those of IgG is 70.8% (17/24), 96.6% (57/60), 85.0% [17/(17+3)] 89.1% [57/(7+57)], and 88.1% [(17+57)/(17+7+3+57)] respectively.

DISCUSSION

COVID-19 pneumonia was first reported in Wuhan, Hubei Province, China, in December, 2019, followed by an outbreak across Hubei Province and other parts of China [5, 6]. An accurate, rapid and cost-effective laboratory etiologicmethod was urgently needed for the diagnosis of the disease. Serological test is anticipated to work as a compliment approach for diagnosis. Data from the severe acute respiratory syndrome (SARS) epidemic show that serological responses, including viral-specific IgM and IgG, are valid for serologic diagnosis [7, 8]. Our study characterized the dynamics of serum IgM and IgG antibodies against SARS-CoV-2 as well as evaluated the diagnostics potential about this serological test. The results showed that IgM and IgG antibodies against SARS-CoV-2 could be detected in the middle and later stage of the disease and ELISA-based IgM and IgG antibody tests for serodiagnosis of COVID-19 have great specificity for diagnosis of COVID-19.

During the immune responses against pathogens infection, IgM are usually produced earlier than IgG antibody. However, both IgM and IgG antibodies against SARS-CoV-2 were detected as early as the 4^{th} day after symptom onset, and the appearance of IgM and IgG antibodies seems earlier than SARS, another severe coronavirus pneumonia [9]. In our study, the seropositive rate of IgG was observed decreased around the 28^{th} days after illness onset. The seropositive rate should not decrease on this time point, we thought this may be due to the small sample size, there were only 7 serum samples collected from 7 patients. Diagnostic value of serological test was evaluated in patients with confirmed and suspected COVID-19 pneumonia. Depending on patients whose disease course (>=13 days from the disease onset), specificity and positive predictive value of IgM antibody were very high up to 100%, which indicated that the IgM can be used as a good mark for diagnosis of COVID-19. However, the sensitivity, negative predictive value and consistency rate of IgM were 73.2%, 80.0% and 88.1%, respectively, indicating that acute infection may still be missed based on seronegative

IgM. The specificity negative predictive value and consistency rate of IgG were 95.0%, 94.8% and 88.9%, respectively. That means patients can be diagnosed as COVID-19 pneumonia base on seropositive IgG. Both seropositive antibodies demonstrate outstanding specificity and PPV, it suggest that seropositve IgM and/or IgG can help to establish the diagnosis of COVID-19 pneumonia, especially in patients with a long course. To avoid misdiagnosis, patients with early seronegative antibodies should be retested after 10 days of onset. The data acquired from patients with suspected diagnosis demonstrate that the serological tests are reliable as they show high specifity, of which IgM tests were up to 100% and IgG tests were 95.0%. Those testing results were in accordance with physician's judgments of the symptoms. There were 3 controls out of 60 were positive in IgG tests. These 3 controls (one was weak positive) were healthcare providers, they didn't serve patients who diagnosed as confirmed or suspected COVID-19 at that time. We think these should be false positive or community asymptomatic infection.

In summary, detection of antibodies against COVID-19 based ELISA appears to be a valid approach to serodiagnosis of COVID-19 pneumonia. The specific circulating antibody can be uniformly detected, therefore avoiding false-negative results due to sampling or potential absence of viruses in the respiratory system. As an emerging infection disease, the current population is generally susceptible to it and the background levels of serum specific antibodies are low. Therefore, COVID-19 pneumonia can be diagnosed base on seropositive of specific antibodies as an alterative to viral nucleic acid detection with clear advantages.

Notes

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Conflict of Interest. The authors declare that there are no conflicts of interest.

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Table 1. Demographic and Clinical Characteristics of COVID-19 Pneumonia

Clinical characteristics	Age, median (range)--yrs	Female sex-No.%	Disease severity		Comorbidities	
			normal	severe	yes	no
Confirmed group (n=85)	51.0 (32.0-65)	54/85 (63.5)	67 (78.8)	18 (21.2)	33 (38.8)	52 (61.2)
suspected group (n=24)	44.0 (35.5-60.5)	12/24 (50.0)	22 (91.2)	2 (83.3)	4 (16.7)	20 (83.3)
Contoll group (n=60)	34 (29.0-51.0)	35/60 (58.3)	N/A	N/A	N/A	N/A

Data are presented as medians (interquartile ranges, IQR) and n/N (%).

Table 2. Detection of IgM and IgG Antibodies in Different Periods *

Course (ds)	Number of patients	IgM against SARS-CoV-2		IgG against SARS-CoV-2	
		Number of positive	Positive rate (%)	Number of positive	Positive rate (%)
≤5	5	3	60.0	2	40.0
-7	9	4	44.4	4	44.4
-9	19	9	47.4	8	42.1
-11	23	13	56.5	9	39.1
-13	20	15	75.0	10	50.0
-15	24	20	83.3	20	83.3
-17	20	16	80.0	16	80.0
-19	16	15	93.8	14	87.5
-21	21	14	66.7	17	81.0
-23	15	12	80.0	12	80.0
-25	12	8	66.7	10	83.3
-27	11	7	63.6	10	90.9

-29	7	4	57.1	4	57.1
≧30	14	12	85.7	14	100

* Specific IgM and IgG antibodies against SARS-CoV-2 from 216 serum samples of 85 confirmed COVID-19 pneumonia cases were tested with ELISA. Serum samples were obtained at different time points after symptom onset.

Table 3. Diagnostic Roles of IgM and IgG Antibodies for COVID-19 Pneumonia *

	IgM against SARS-CoV-2		IgG against SARS-CoV-2	
	Confirmed case group	Control group	Confirmed case group	Control group
Positive	51	0	55	3
Negative	15	60	11	57
total	66**	60	66**	60

***** Diagnostic value of serological IgM and IgG antibodies for COVID-19 pneumonia was evaluated in 66 patients with positive nucleic acid tests. Serum samples were obtained from patients with disease courses greater than or equal to 13 days and less than 29 days.

** 66 patients were included in the 85 confirmed cases, and their disease courses were greater than or equal to 13 days and less than 29 days.

Table 4. Detection of IgM and IgG Seropositivity for Suspected COVID-19 Pneumonia Patients *

	IgM against SARS-CoV-2		IgG against SARS-CoV-2	
	Suspected case group	Control group	Suspected case group	Control group
Positive	21	0	17	3
Negative	3	60	7	57
total	24	60	24	60

***** Diagnostic value of serological IgM and IgG antibodies for suspected COVID-19 pneumonia patients was evaluated in 24 patients with negative nucleic acid tests.

Figure legends

Figure 1. Detection of IgM and IgG antibodies in different periods. A log-distribution was used to describe the distribution time period of seropositive rate of the two types of antibodies. Serological IgM and IgG antibodies tested by ELISA in patients with confirmed diagnosis. The IgM and IgG antibodies were detected as positive as early as on the 4th day after onset, the seropositive rate of IgM increased gradually; however, IgG was increased sharply on the 12th day after onset.

Figure 2. The timelines of initial seroconversion of IgM and IgG antibodies. A log-distribution was used to describe the distribution time period of cumulative seroconversion rate of the two antibodies. IgM cumulative seroconversion increased quickly from nearly the 8th day as well as IgG increased from nearly the 10th day after symptom onset.

Figure 1

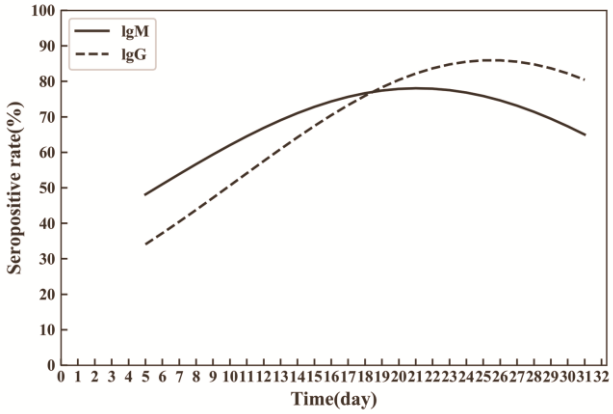
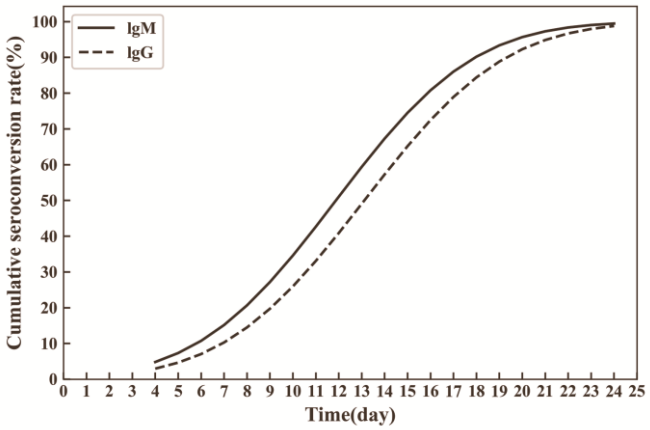


Figure 2


IgM
IgG
Cumulative seroconversion rate(%)
Time(day)
0
10
20
30
40
50
60
70
80
90
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25