Performance Evaluation of the Serological Test and Dynamic Changes of Anti-2019-nCoV Antibodies Among COVID-19 Patients

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Abstract

Background

Serological tests for anti-2019-nCoV antibodies have been developed, however, validation with clinical samples was insufficient and mixed.

Methods

A total of 197 patients with COVID-19 hospitalized in the Wuhan Pulmonary Hospital and 114 healthy people were enrolled in this study. The IgM/IgG was measured by chemiluminescence detection kit and the performance of IgM/IgG was assessed through diagnostic test. A smooth spline function was used to fit a possible dynamic changes of antibody content.

Results

The sensitivity and specificity to diagnose COVID-19 were 96.95% and 92.98% for IgG, and 65.99% and 98.25% for IgM, respectively. The period with the highest IgM positive rate (93.75%) was 11 to 13 days after the onset, while the IgG positive rate was almost 100% in the patients whose serum was collected 7 days after onset. Small differences were found in IgM content among mild/regular, severe and critical patients. IgG content in critical patients were highest in the 2-week post-symptom onset group, while the IgG in the severe patients were highest in the 15-28 days and more than 28 days post-symptom onset.

Conclusions

Serological testing performed well in the diagnosis for COVID-19, and the positive rate and variance of IgG are higher than those of IgM.

1. Background

The 2019-nCoV virus poses a constant threat to human health due to its high transmission efficiency, serious infection consequences and unpredictable epidemic time. Although the nucleic acid test based on nasopharyngeal and throat swabs is effective and the standard diagnostic method for COVID-19, it still suffers from some limitations. First, the nucleic acid testing has high requirements on laboratory conditions and personnel technical ability, and the operation is complicated, time-consuming, and the risk of infection of the operators conducting the test is relatively high. Moreover, the results of acid test are greatly affected by the quality of the specimen, the experimental conditions and the personnel operation factors, and are prone to false negatives. Furthermore, whether subjects carry the virus in other organs may still remain unknown even though the nucleic acid testing of pharyngeal swabs suggest negative results, because respiratory tract may not be the only route for 2019-nCoV transmission (1). Thus, detection of serum specific antibodies could be a necessary and effective supplement for nucleic acid test, and the combination of both is the optimal method for diagnosing 2019-nCoV infection (2). Now many laboratories and companies have developed serological tests for anti-2019-nCoV immunoglobulin M (IgM) and immunoglobulin G (IgG), and the 2019-nCoV virus-specific antibody kit has been released, which can be useful in various ways (3).

Although serological tests have been developed rapidly and under urgent market demands, they lacked performance evaluation and validation with clinical samples in daily practice. Several published studies have pointed out that these tests showed inconsistency in results, divergence in sensitivity and specificity that may deviate from what the manufacturers report (2). Furthermore, more studies with larger sample sizes and controls are needed to further verify

the specificity and accuracy of these tests. The sample size for some studies is small and the number of patients included is usually less than 100 (4, 5). Also, as a review pointed out, the duration of IgG response has been yet unknown due to poor specificity of antibody detection tests (6). In addition, many studies used the serological detection methods of which error is relatively greater than that of chemiluminescence, such as the colloidal gold method with low sensitivity and large visual error, or the enzyme-linked immunoassay with high operational details to determine the antibody.

Given the great significance of serum antibody detection in diagnosing infected patients (especially for patients with negative nucleic acid test results) and identifying the stage of infection (when both IgM and IgG antibodies are simultaneously detected) (7), but also given the inconsistency in performance evaluation of antibody tests and longitudinal pattern of the antibodies, we performed this study to evaluate the performance of anti-2019-nCoV antibodies testing in diagnosing COVID-19 and investigate variance of antibodies in different disease progression (including variance in patients with different disease severity).

2. Methods

2.1 Participants and Sample Collection

A total of 197 patients with COVID-19 hospitalized in the Wuhan Pulmonary Hospital from January 3rd, 2020 to February 28th, 2020 were selected as the case group, including 92 male patients and 105 female patients, aged 23 to 87 years old. All the patients enrolled in this study were diagnosed according to the 7th edition of the Guideline on diagnosis and treatment of COVID-19 established by China's National Health Commission, including patient's epidemic history, clinical characteristics, chest computed tomographic (CT) scan and laboratory findings. A total of 114 people with normal physical examination results from August to September 2019 were selected as the control group, including 60 males and 54 females, aged between 18 and 75. Since the blood samples of the control subjects were collected before the COVID-19 outbreak in December 2019, they can all be considered as normal subjects in the control group.

For the patients in the case group, 5 ml fasting venous blood was collected in a non-anticoagulant blood collection tube. All the patients were tested during hospitalization. Then we waited for the blood to coagulate, and centrifuged at 3500 X g for 5 minutes to take serum for testing. The specimen of the control group was taken out of the frozen sample tube from the - 20 °C refrigerator, and then thawed at room temperature before testing.

2.2 Serological Test for anti-2019-nCoV IgM and IgG antibodies

The chemiluminescence detection kit (from Wovent Biotechnology Co., Ltd., Sichuan, China.) and DXI800 automatic chemiluminescence immunoassay analyzer (from Beckman Coulter Co., Ltd., the U.S.) were used to test IgM and IgG antibodies. This chemiluminescence detection kit employed magnetic particles and chemical luminescence technology, according to the principle of indirect method of immunoassays. First, the sample, analysis buffer, biotinylated antigen-coated streptavidin magnetic beads, and alkaline phosphatase-labeled mouse anti-human IgM antibody are added to the reaction tube and incubated to form an "immunomagnetic bead-antibody-enzyme binding" complex, and then wash to remove the unbound enzyme conjugate and other substances. The luminescent substrate is added, and the enzyme conjugate catalyzes the luminescent substrate to emit photons. The number of photons is positively correlated with the amount of virus antibody in the sample.

This kit only can be used for qualitative detection of 2019-nCoV antibodies, not for quantitative detection. The sample measurement result is judged by the S / Co value. Although the level of antibody cannot be clearly detected, the S/Co

value corresponds to the actual antibody level and is directly proportional to the antibody content. The calculation formula of the S / Co value is as follows:

$$S / Co = \frac{RLU_{sample}}{Cutoffvalue}$$

The Cutoff value is the positive judgment value automatically calculated by the automatic chemiluminescence measuring instrument according to the relative luminescence value (RLU) of the calibrator, the calculation formula is:

$$Cutoffvalue = RLU_{calibrator} \times A$$

, where A is the coefficient and RLU is the relative luminescence value measured. If S / Co is less than 1, the result is considered negative, indicating that the 2019-nCoVlgM / IgG antibody was not detected in the sample to be tested. If the S / Co value is greater than or equal to 1, it is considered positive, indicating that the 2019-nCoVlgM / IgG antibody was detected in the sample to be tested.

2.3 Statistic Analysis

The accuracy of diagnostic test was evaluated based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, negative likelihood ratio and the total agreement rate. For evaluation of dynamic changes of anti-2019-nCoV IgG and IgM, the following strategy was used: for each sample included in the study, we acquired number of days from symptom onset to serological test through the corresponding date of symptom onset (i.e. fever) and blood sample collected. Then we divided these days into several categories: ≤ 7 days, 8–10 days, 11–13days, 35–37 days and ≥ 38 days. We fitted a smooth spline function with the S/Co value and the time from the onset of the symptom to serological testing to observe a possible dynamic changes of antibody content with the duration of onset, using “splines” package in R software (version 3.6.3). Additionally, a scatter boxplot was employed to describe variation among patients with different disease severity (mild/regular, severe, critical).

3. Results

3.1 Diagnostic value of serological anti-2019-nCoV IgM and IgG antibodies

Among 197 patients with COVID-19 pneumonia, 132 patients were admitted to Wuhan Pulmonary Hospital from the onset of initial symptoms, and the remaining 65 patients were transferred from Tongji Hospital after laboratory confirmation. Among these 132 patients initially admitted to our hospital, the number of patients classified as mild / regular form, severe form, and critical form patients was 57, 52, and 28, respectively.

The false positive rates (FPR) of IgG and IgM were 7% (8/114) and 1.8% (2/114), respectively. The true positive rates of IgG and IgM were 96.95% (191/197) and 65.99% (130/197). Six IgG-negative patients were also IgM-negative. According to nucleic acid detection, the sensitivity and specificity to diagnose COVID-19 were 96.95% (191/197) and 92.98% (106/114) for anti-2019-nCoV IgG antibodies, and 65.99% (130/197) and 98.25% (112/114) for anti-2019-nCoV IgM antibodies, respectively. In addition, the positive predictive values (PPVs) of IgG and IgM antibodies were 95.98% (191/199) and 98.48% (130/132). Both positive likelihood ratio and negative likelihood ratio of IgM were greater than likelihood ratio of IgG, while total agreement of IgM was smaller than IgG. The sensitivity and specificity of combined detection of serum 2019-nCoV IgM and IgG was 96.95% (191/197) and 91.23% (104/114), which was similar to the results of IgG only. Besides, the positive predictive value, negative prediction value and negative

likelihood ratio of combined detection were all similar to the results of IgG detection only. Positive likelihood ratio and total agreement rate were smaller than results for IgG detection only (Table 1).

Table 1
Diagnostic value of anti-2019-nCoV IgM and IgG antibodies in all participants

	Case	Control	Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	+LR	-LR	Agreement rate (%)
2019-nCoV IgG				96.95	92.98	95.98	94.64	13.81	0.033	95.5
Positive	191	8	199							
Negative	6	106	112							
Total	197	114	311							
2019-nCoV IgM				65.99	98.25	98.48	62.57	37.71	0.343	77.81
Positive	130	2	132							
Negative	67	112	179							
Total	197	114	311							
2019-nCoV IgG and IgM				96.95	91.23	95.02	94.55	11.05	0.033	94.53
Positive	191	10	201							
Negative	6	104	110							
Total	197	114	311							
¹ PPV, Positive predictive value; NPV, Negative predictive value; + LR, Positive likelihood ratio; -LR, Negative likelihood ratio										

The ROC performance curves showed that the area under the curve (AUC) value was 0.987 for anti-2019-nCOVID IgG and 0.899 for anti-2019-nCOVID IgM, respectively (Fig. 1). At the manufacturer's cutoff value of 1, sensitivity and specificity were 97% and 92.1% for IgG, 66.5% and 98.2% for IgM, respectively.

3.2 Dynamic changes of serological anti-2019-nCoV IgM and IgG antibodies

In 197 patients with COVID-19, serum was obtained in different period after symptom onset, and the IgM and IgG antibodies were detected positive as early as on the 3th day after onset. The median duration from first symptoms to serological testing was 25 days, ranging from 3 to 53 days (Table 2). Some patients were observed positive for IgM (1 patient) and IgG (7 patients) within 7 days after symptom onset, respectively. The period with the highest IgM positive rate (93.75%) was 11 to 13 days after the onset. The IgG positive rate was almost 100% in the patients whose serum was collected 7 days after onset (Table 2).

Table 2
Serological test at different intervals (from the symptom onset to serological test) in 197 patients

Days*	No. of patients	Percent (%)	Cumulative percent (%)	IgM		IgG		IgM and IgG	
				No. of positive	positive rate (%)	No. of positive	positive rate (%)	No. of positive	positive rate (%)
≤ 7	10	5.08	5.08	1	10	7	70	7	70
~ 10	5	2.54	7.61	3	60	5	100	5	100
~ 13	16	8.12	15.74	15	93.75	16	100	16	100
~ 16	10	5.08	20.81	6	60	10	100	10	100
~ 19	14	7.11	27.92	10	71.43	13	92.86	13	92.86
~ 22	19	9.64	37.56	12	63.16	19	100	19	100
~ 25	31	15.74	53.30	22	70.97	30	96.77	30	96.77
~ 28	19	9.64	62.94	14	73.68	18	94.74	18	94.74
~ 31	29	14.72	77.66	21	72.41	29	100	29	100
~ 34	22	11.17	88.83	13	59.09	22	100	22	100
~ 37	11	5.58	94.42	8	72.73	11	100	11	100
≥ 38	11	5.58	100	6	54.55	11	100	11	100
* Number of days from the symptom onset to serological test									

Figure 2 showed the dynamic changes of S/Co value of IgM and IgG with days after the onset. The S/Co value of IgM reached peak approximately 12 or 13 days after onset, while the peak S/Co value of IgG appeared approximately 20 or 22 days after symptom onset. After the peak, the S/Co value of IgM decreased faster and more notable than that of IgG.

Among 132 patients initially admitted to our hospital, we found that the difference in the S/Co values of IgM among three types of patients was very small (Fig. 3). However, the S/Co values of IgG in critical patients were highest in the 2-week post-symptom onset group, while the S/Co values of IgG in the severe patients were higher than those in the mild/regular or critical patients in the 15–28 days and more than 28 days post-symptom onset group (Fig. 3).

4. Discussion

Several studies have evaluated the diagnostic performance of serum antibodies in patients with COVID-19, and our results was comparable with them. In 66 confirmed patients with COVID-19, Xiang et al found that sensitivity and specificity of IgM were 77.3% and 100%, and those of IgG were 83.3.3% and 95.0%. Besides, they found that the seroconversion of specific IgM and IgG antibodies were observed as early as the 4th day after symptom onset(4). A study carried out among 397 COVID-19 patients reported that the IgG-IgM combined antibody testing sensitivity was 88.66% and specificity was 90.63%(8). Xie et al found a specificity of 93.75% for IgM 100% for IgM, which suggested that IgM-IgG test is an accurate and sensitive diagnostic method. A combination of nucleic acid and IgM-IgG testing is a more sensitive and accurate approach for diagnosis and early treatment of COVID-19 (2). A study from Italy, including 61 COVID-19 patients and 64 patients from a control group, reported that the ROC performance curves

Loading [MathJax]/jax/output/CommonHTML/jax.js f 0.918 and 0.980 for anti-SARS CoV-2 antibodies IgM and IgG,

respectively(9). Jin et al found that the sensitivities of serum IgM and IgG antibodies to diagnose COVID-19 were 48.1% and 88.9%, and the specificities were 100% and 90.9%, respectively. In addition, the results from these studies suggested that positive rates of IgG were higher than those of IgM in COVID-19, which is consistent with our results. It should be noted that the variation of the methodology and antigens used in the IgM and IgG antibody detection kits are essential for the testing sensitivity and specificity. And the rapid test suggesting a poor sensitivity or specificity could not be recommended for diagnosis of COVID-19 (2).

The findings on dynamic changes were in consistent with some recently published studies. A study reported that proportion of patients with positive virus-specific IgG reached 100% approximately 17–19 days after symptom onset, while the proportion of patients with positive virus-specific IgM reached a peak of 94.1% approximately 20–22 days after symptom onset(10). Another study enrolling 112 patients diagnosed with COVID-19 showed that IgM antibody appeared within a week post-disease onset, lasted for 1 month, and gradually decreased, whereas IgG antibody was produced 10 days after infection and lasted for a longer time (7). In our study, the results showed that the proportion of patients with positive IgM reached a peak value of 91.7% approximately 12–13 days after the onset, and the proportion of patients with positive IgG was almost 100% approximately 7 days after the onset. Furthermore, the S/Co value of IgM, which is positively correlated with concentration, reached peak approximately 12 or 13 days after onset, and the peak S/Co value of IgG appeared approximately 20 or 21 days after symptom onset. Additionally, our results are in line with opinion in a newly published review that specific IgM and IgG antibodies should start to become detectable after 4–5 days, with positive IgM antibodies in 70% of symptomatic patients by days 8–14. This review also pointed out that IgG reactivity was thought to reach > 98% after several more weeks, but duration of this antibody response has not yet known(11).

The sensitivity, negative predictive value and total agreement rate of IgM were lower than that of IgG. The first possible reason may be related to individual difference in immune response and antibody production. Second, it may be related to the fact that there are many critical cases admitted to our hospital. IgM antibody will decrease after two weeks and the IgM below the peak value could not be detectable by this test. In some cases, it is hard to know exactly when the patient was infected or how long the patient was infected. There is long time from onset to serological testing for critical cases, so the 2019-nCoV antibody IgM may decreases or disappear when they are tested.

Antibody detection plays an important role in the diagnosis of COVID-19 as complement approach for viral nuclear acid assays. IgM is the earliest antibody in the initial immune response. IgM appears first during viral infection, but it does not last long and is a marker of recent infection. The short detection window period is conducive to early diagnosis and elimination of suspicious cases. IgG is the main antibody produced by the immune response again. IgG will appear only after the virus continues to be infected for a period of time, and it will last for a long time. IgG detection can improve the accuracy of diagnosis and reduce missed diagnosis. If the new coronavirus (2019-nCoV) antibody diagnostic reagents can be used to screen patients with new coronavirus infection as soon as possible, it will help fight the epidemic spread.

Some limitations in our study should be noted. First, we were unable to perform continuous serological tests on the patients due to the overloaded operation of our hospital and limited medical resources from February to March. Second, the investigation of relationship between antibody levels and disease progression was limited because the kit products and instruments we used cannot provide quantitative data on antibody concentration. Although the S/Co value is positively correlated with antibody concentration, it can only indirectly reflect the relationship between IgG, IgM and the duration of onset. Third, this study cannot exclude the confounding effects of individual characteristics and clinical treatment because of the unavailability of data.

5. Conclusions

In conclusion, serological testing performed well in the diagnosis for 2019-nCoV infection, and can be helpful for the diagnosis of patients with negative nucleic acid test results and for the identification of asymptomatic infections. The positive rate and variance of IgG are higher than those of IgM in patients with COVID-19.

Abbreviations

AUC: Area under the curve

CT: Computed tomographic

FPR: False positive rate

IgM: Immunoglobulin M

IgG: Immunoglobulin G

NPV: Negative predictive value

PPV: Positive predictive value

RLU: Relative luminescence value

+LR, Positive likelihood ratio

-LR, Negative likelihood ratio

Declarations

Ethics approval and consent to participate

This research has been performed in accordance with the Declaration of Helsinki and has been approved by ethics committee of Wuhan Pulmonary Hospital. Written informed consent was taken from participants. Privacy and confidentiality were maintained throughout the study period by excluding personal identifiers during data collection.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LC and HX made substantial contributions to the conception and design of the work. JC and YR made substantial contributions to the acquisition of data. RD made contributions to the funding acquisition. NL made substantial contributions to the analysis of data. LC and NL have drafted the work, NL and HX revised it. All authors have read and approved the manuscript.

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Figures

Figure 1

ROC analysis for 2019-nCoV antibodies detection.

Figure 2

Dynamic changes of antibody content with days from onset to serological testing in 197 patients.

Figure 3

The S/Co values of IgM and IgG among patients with different disease severity in different periods after symptoms.