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Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019

Yujiao Jin[a,1], Miaochan Wang[a,1], Zhongbao Zuo[a], Chaoming Fan[b], Fei Ye[c], Zhaobin Cai[d], Ying Wang[a], Huaizhong Cui[a], Kenu Pan[a], Aifang Xu[a,*]

[a] Department of Clinical Laboratory, Xixi Hospital of Hangzhou, Hangzhou 310023, Zhejiang, China
[b] Department of Clinical Laboratory, The Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, Zhejiang, China
[c] Department of Infectious Diseases, Xixi Hospital of Hangzhou, Hangzhou 310023, Zhejiang, China
[d] Department of Cardiovascular Medicine, Xixi Hospital of Hangzhou, Hangzhou 310023, Zhejiang, China

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ABSTRACT

Objective: To investigate the diagnostic value of serological testing and dynamic variance of serum antibody in coronavirus disease 2019 (COVID-19).
Methods: This study retrospectively included 43 patients with a laboratory-confirmed infection and 33 patients with a suspected infection, in whom the disease was eventually excluded. The IgM/IgG titer of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was measured by chemiluminescence immunoassay analysis.
Results: Compared to molecular detection, 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 the COVID-19 group, the IgM-positive rate increased slightly at first and then decreased over time; in contrast, the IgG-positive rate increased to 100% and was higher than IgM at all times. The IgM-positive rate and titer were not significantly different before and after conversion to virus-negative. The IgG-positive rate was up to 90% and not significantly different before and after conversion to virus-negative. However, the median IgG titer after conversion to virus-negative was double that before, and the difference was significant.
Conclusions: Viral serological testing is an effective means of diagnosis for SARS-CoV-2 infection. The positive rate and titer variance of IgG are higher than those of IgM in COVID-19.

Introduction

In December 2019, a group of patients with pneumonia of unknown cause were identified in Wuhan, Hubei Province, China (Huang et al., 2020). The pathogen was identified as a novel coronavirus and named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as it has a phylogenetic similarity to SARS-CoV (Zhu et al., 2020). Since then, SARS-CoV-2 has spread rapidly and the resulting coronavirus disease 2019 (COVID-19) has been declared a public health emergency of international concern (PHEIC) by the World Health Organization (WHO) (WHO, 2020a). As of March 4, 2020, 93,091 laboratory-confirmed cases and 3198 deaths have been documented globally (WHO, 2020b).

Coronaviruses are enveloped non-segmented positive-sense RNA viruses belonging to the *Orthocoronavirinae* subfamily. Although most human coronavirus infections are mild, SARS-CoV and the Middle East respiratory syndrome coronavirus (MERS-CoV) – betacoronaviruses zoonotic in origin – have been associated with potentially fatal disease, particularly during the outbreaks in 2003 and 2012, respectively (Zaki et al., 2012; Zhong et al., 2003). Currently, the mortality rate of SARS-CoV-2, a novel betacoronavirus, is about 3.4%, which is lower than the rate of 10% for SARS-CoV and 34% for MERS-CoV (WHO, 2020b,c,d). However, SARS-CoV-2 has potentially higher transmissibility than both SARS-CoV and MERS-CoV (Chen, 2020).

The rapid and accurate diagnosis of COVID-19 contributes to disease and outbreak management by enabling prompt and accurate public health surveillance, prevention and control measures. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) has been the primary means for diagnosing SARS-CoV-2 (Huang et al., 2020). However, molecular detection carries the risk of false-negatives because of low viral loads in

* Corresponding author.
E-mail address: xuaifangxxh@163.com (A. Xu).
[1] These authors contributed equally to this work.

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specimens (Pang et al., 2020). Serological testing, another common laboratory diagnostic, can diagnose illness by detecting antibodies. Serological studies on SARS-CoV-2 appear to be scarce. The aim of this study was to investigate the diagnostic value of serological detection to COVID-19 and the dynamic variance of viral antibodies in SARS-CoV-2 infection.

Methods

Data sources

A retrospective study was conducted at Xixi Hospital of Hangzhou, a designated hospital for emerging infectious disease in Zhejiang Province, China, from January 2020 to March 4, 2020. Case definitions of confirmed COVID-19 are based on the WHO interim guidance (WHO, 2020e).

Forty-three patients with a laboratory-confirmed infection and at least one viral serological test performed in the hospital were enrolled in this study. Thirty-three patients with suspected SARS-CoV-2 infection, in whom the disease was eventually excluded in the hospital and who quarantined at home, were included as a control group. The definition of suspected SARS-CoV-2 infection included a fever or any respiratory symptoms, especially in those with a history of travel to Wuhan or exposure to an infected case within 2 weeks before the onset of disease since January 2020 (Xu et al., 2020). Patients who were suspected to be infected were discharged from hospital once the results of two separate molecular tests performed with an interval of 24 h were negative. The demographic and clinical data of these patients were extracted from their medical records.

Twenty-four patients received laboratory confirmation at other hospitals and were transferred to Xixi Hospital of Hangzhou. Oral swab or sputum specimens collected from the remaining 19 cases at admission were sent to the Center for Disease Control of Hangzhou and tested by real-time RT-PCR for SARS-CoV-2 RNA. Laboratory confirmation of the virus was based on the result of real-time RT-PCR (Huang et al., 2020). Virus detection was repeated twice, every 24 h. Fitness for discharge of COVID-19 patients was based on a normal body temperature for at least 3 days, with improvement of chest radiographic evidence and viral clearance in respiratory samples from the upper respiratory tract on two occasions.

The study was approved by the Ethics Committee of Xixi Hospital of Hangzhou and written informed consent was obtained from each participant.

Serological test

Serum was separated by centrifugation at 2500*g* for 5 min within 12 h of collection. The SARS-CoV-2 IgM and IgG chemiluminescence immunoassay (CLIA) kits used in this study were supplied by Shenzhen YHLO Biotech Co., Ltd (China); the magnetic beads of these CLIA assays are coated with two antigens of SARS-CoV-2 (nucleocapsid protein or N protein, spike protein or S protein). All serum antibody tests were performed with an iFlash3000 fully automated CLIA analyzer from Shenzhen YHLO Biotech Co., Ltd (China). SARS-CoV-2 IgM/IgG titers (in arbitrary units, AU/ml) were calculated automatically by the immunoassay analyzer on the basis of relative light units (RLU), because the viral antibody titer is positively associated with RLU. According to the manufacturer's instructions, the cut-off value for a positive SARS-CoV-2 IgM/IgG result is 10 AU/ml.

Statistical analysis

Continuous variables were expressed as the mean ± standard deviation (SD) if normally distributed, or as the median (interquartile range, IQR) if not; categorical variables were described as the count (%). Serum antibody titers before and after conversion to virus-negative were compared using the Wilcoxon matched-pairs signed-ranks test. Proportions for categorical variables were compared using Fisher's exact test. All analyses were done with IBM SPSS Statistics software (version 23.0). A two-sided *p*-value of less than 0.05 was considered statistically significant.

Results

Forty-three patients with a laboratory-confirmed SARS-CoV-2 infection were included in the study. Among them, 24 patients were transferred to Xixi Hospital of Hangzhou after laboratory confirmation elsewhere and the remaining 19 patients were confirmed in the hospital. Thirty-three patients with suspected COVID-19, in whom the disease was finally excluded, were chosen as the control group.

The demographic and clinical characteristics of the COVID-19 and control groups are shown in Table 1.The median age of the COVID-19 patients was 47.0 years (IQR 34.0–59.0 years), ranging from 7 years to 74 years, and 39.5% were male. Among both groups, less patients had chronic disease, including hypertension, diabetes, and liver disease. Fever was present in 62.8% of COVID-19 patients before or on admission. The second most common symptom was cough (60.5%). Similarly, fever and cough were also the most common symptoms in the control group. The duration from first symptoms to hospital admission, to laboratory confirmation, and to first serological test in the COVID-19 group patients was 3 days (IQR 2–7 days), 3 days (IQR 2–7 days) and 18 days (IQR 11–23 days), respectively. Among these patients, two with a history of exposure to an infected case presented without any symptom until the first serological test.

In the control group, the IgM and IgG positive rates were 0 (0/33) and 9.1% (3/33), respectively. The IgG titers of the three positive patients were all less than 15 AU/ml.

In the COVID-19 group, 27 patients were tested for viral antibody before becoming virus-negative (including oral swabs, anal swabs, or sputum). The median duration from first symptoms to serological testing in these 27 patients was 16 days (IQR 9–20 days). Among these people, 13 were IgM-positive (48.1%) and 24 were IgG-positive (88.9%). Three IgG-negative patients were also IgM-negative. According to molecular detection as the gold standard, the sensitivities of serum IgM and IgG antibodies to diagnose COVID-19 were 48.1% (13/27) and 88.9% (24/27), respectively, and the specificities were 100% (33/33) and 90.9% (30/33), respectively. Moreover, the positive predictive values (PPVs) of IgM and IgG antibodies were 100% (13/13) and 88.9% (24/

Table 1
Demographic and clinical characteristics of the COVID-19 and control groups; data are presented as the median (IQR), or number (%).

Characteristic	COVID-19 group (*n* = 43)	Control group (*n* = 33)
Age, years	47.0 (34.0–59.0)	31.0 (25.5–37.5)
Male sex	17 (39.5%)	22 (66.7%)
Smoking history	6 (14.0%)	6 (18.2%)
Diabetes	3 (7.0%)	1 (3.0%)
Hypertension	10 (23.3%)	4 (12.1%)
Liver disease	2 (4.7%)	0
Fever	27 (62.8%)	24 (72.7%)
Cough	26 (60.5%)	15 (45.5%)
Fatigue or myalgia	9 (20.9%)	3 (9.1%)
Sputum production	8 (18.6%)	6 (18.2%)
Onset of symptoms to (in days):		
Hospital admission	3.0 (2.0–7.0)	1.0 (1.0–7.0)
Laboratory confirmation	3.0 (2.0–7.0)	–
First serological test	18.0 (11.0–23.0)	3.0 (2.0–8.0)

IQR, interquartile range.

27), respectively, and the negative predictive values (NPVs) were 70.2% (33/47) and 90.9% (30/33), respectively.

After the 43 cases in the COVID-19 group were laboratory-confirmed, 98 serological tests were performed. Figure 1A shows that the IgM-positive rate increased slightly at first and then decreased as the number of days from laboratory confirmation to serological detection increased; in contrast, the IgG-positive rate increased to 100% and was higher than IgM at all times. Meanwhile, the virus-positive rate tended to decrease over time. Before laboratory confirmation, two serological tests were performed in one case, and viral IgM and IgG were both positive in two results. Figure 1B shows a similar trend as the duration from symptom onset to serological testing increased. It was also found that both IgM and IgG levels were not high during the first 5 days following symptom onset. In the COVID-19 group, 34 patients were tested for viral antibody after two oral swabs taken 24 h apart tested negative, without other molecular detection positive. The IgM and IgG positive rates were 55.9% (19/34) and 94.1% (32/34), respectively. Two IgG-negative patients were also IgM-negative. The median IgM and IgG titers among these 34 people were 12.1 AU/ml (IQR 5.2–46.6 AU/ml) and 132.2 AU/ml (IQR 65.5–179.1 AU/ml), respectively. All patients were discharged and the median time from this serological test to discharge was 2 days (IQR 1–3 days).

Among these 34 people who were tested after they were virus-negative, 20 also had serological testing performed before they became virus-negative. Table 2 shows that the IgM-positive rate and titer were not significantly different before and after conversion to virus-negative. The IgG-positive rate was up to 90% and also not significantly different before and after conversion to virus-negative. However, the median IgG titer after testing virus-negative was double that before, and the difference was statistically significant. The median interval between these two serological tests was 6 days (IQR 4–9 days).

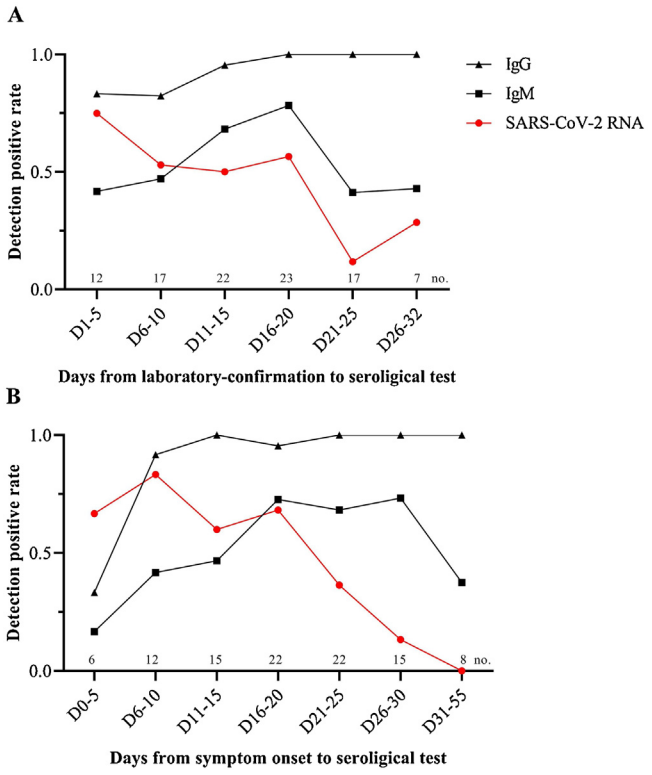

Figure 1. Serological test positive rates and SARS-CoV-2 viral load at different intervals: (A) days from laboratory confirmation to serological testing; (B) days from symptom onset to serological testing.

Table 2
Comparison of SARS-CoV-2 antibodies in 20 patients before and after conversion to virus-negative; data are presented as the median (IQR), or number (%).

	Before negative (n = 20)	After negative (n = 20)	p-Value
IgM-positive	10 (50.0%)	10 (50.0%)	1
IgM titer, AU/ml	8.8 (4.2–14.7)	7.8 (4.3–27.8)	0.198
IgG-positive	18 (90.0%)	19 (95.0%)	1
IgG titer, AU/ml	78.6 (56.5–107.9)	161.2 (102.6–184.3)	<0.001

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IQR, interquartile range.

Discussion

As the diagnosis of COVID-19 is complicated by the diversity of symptoms and imaging findings, molecular and serological detection tools are rapidly being developed. Laboratory confirmation of COVID-19 has been based on a positive real-time RT-PCR result. However, molecular and serological studies on this virus appear to be scarce. In this study, 43 patients with a laboratory-confirmed SARS-CoV-2 infection and 33 suspected patients in whom the disease was finally excluded by nucleic acid test twice and who were rapidly discharged, were included to investigate the diagnostic value of serological detection to COVID-19. In addition, the dynamic variance of viral antibodies during SARS-CoV-2 infection was also examined.

Most of the infected patients in this study were female. The age range of individuals was wide, with children and those older than 65 years also being infected. The proportion of patients with any co-existing illness among the infected individuals was low in this study, consistent with the non-severe patients in the study by Guan et al. (Guan et al., 2020). Through the media and national advocacy, people who had been in contact with an infected case, as well as those with a suspected infection, were asked to go to the hospital at an early stage–as soon as possible. Furthermore, Xixi Hospital of Hangzhou was a designated tertiary hospital and mainly admitted patients with mild to moderate symptoms in Hangzhou. Fever and cough were the dominant symptoms, in concert with recent studies (Huang et al., 2020; Xu et al., 2020; Guan et al., 2020). In the control group, the patients were generally younger, more often male, and less commonly presented any co-existing illness when compared to those in the infected group.

In this study, it was found that the specificities of serum IgM and IgG to diagnose COVID-19 were both more than 90% when compared to molecular detection. Moreover, the IgG titers of the three positive patients in the control group were only weakly positive. In the COVID-19 group, IgM and IgG were also both found to be positive in one case before laboratory confirmation for the first time. Therefore, COVID-19 should be considered when serum IgM or IgG is positive. In the COVID-19 patients, the IgG-positive rate (88.9%) was found to be higher than the IgM-positive rate (48.1%) before conversion to virus-negative by molecular detection. Interestingly, three IgG-negative patients were also IgM-negative in this study. The duration from symptom onset to this serological test in these three patients was 0 days, 5 days, and 8 days, respectively. Furthermore, it was observed that serum viral antibodies increased only slightly in the early stage of the disease. Hence, it may be that serum viral antibodies have not been produced yet and could be undetectable. Zhang et al. found that the IgM and IgG positive rates were 50% and 81% on day 0 (the day of first sampling) and increased to 81% and 100%, respectively, on day 5 (Zhang et al., 2020). Thus, COVID-19 cannot be excluded at an early stage when viral serological testing is negative.

The IgM-positive rate showed a trend to increase at first and then decline; however, the IgG-positive rate increased and then became stable over time. Furthermore, the IgG-positive rate was consistently higher than the IgM-positive rate, and this phenomenon was also observed in the study by Zhang et al. (2020). There were two patients with both IgM and IgG negative after oral swabs were negative twice. The interval between laboratory confirmation and serological testing was 10 days for one of these patients and 14 days for the other. A lag period was found, as antibodies specifically targeting MERS-CoV would normally appear between 14 and 28 days after the illness onset (Al Johani and Hajeer, 2016). Therefore, we cannot infer whether viral antibodies have not been produced yet or have turned negative, because of the use of only one serological test. As well as the high positive rate, we found that the IgG titer after conversion to virus-negative was double that before. In this study, these COVID-19 patients were discharged soon after oral swabs were negative on two occasions. Whether higher antibodies titers in SARS-CoV-2 infection are associated with better outcomes needs to be studied further.

This study has several limitations. First, only 43 laboratory-confirmed COVID-19 patients and 33 controls were included; therefore, due to the small sample size, the study results should be interpreted with caution. Second, the time to viral molecular detection and to serological testing was variable and depended on clinician judgement, as this was a retrospective study. Third, since the infected patients included in this study were non-severe cases, the value of serological testing in patients with severe cases of the disease needs to be assessed. Fourth, the median duration from symptom onset to serological testing was long, as viral serological detection kits were available late. Finally, follow-up data on these individuals who were discharged are scarce.

In conclusion, viral serological testing is an effective means of diagnosis for SARS-CoV-2 infection. The positive rate and titer variance of IgG are higher than those of IgM in the course of COVID-19.

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Ethical approval

The study was approved by the Ethics Committee of Xixi Hospital of Hangzhou and written informed consent was obtained from each participant.

Conflict of interest

The authors declare no conflicts of interest. The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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