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Evaluation of the auxiliary diagnostic value of antibody assays for the detection of novel coronavirus (SARS-CoV-2)

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Running head: The auxiliary diagnostic value of antibody assays for SARS-CoV-2

Abstract

The spread of SARS-CoV-2 has taken on pandemic proportions, affecting over 100 countries in a matter of weeks. The goal of this study was to assess the diagnostic values of different methods of detecting and estimating the SARS-CoV-2 infection, and the auxiliary diagnostic potential of antibody assays. By retrospectively analyzing the data of viral RNAs and serum IgM-IgG antibodies against SARS-CoV-2 from 38 cases with confirmed COVID-19 in the Second People's Hospital of Fuyang, we found that, in the early phase of the illness, the viral RNA was most abundant in the sputum specimens, followed by that in the throat swabs, while the antibody assays identified fewer positive cases at this stage. However, the sensitivity of the antibody assays overtook that of RNA test from eighth day of disease onset. Simultaneous use of antibody assay and RT-qPCR improved the sensitivity of the diagnoses. Moreover, we found that most of these cases with no detectable viral RNA load during the early stages were able to be seropositive after 7 days. Our findings indicate that the antibody detection could be used as an effective supplementary indicator of SARS-CoV-2 infection in suspected cases with no detectable viral RNA, and in conjunction with nucleic acid detection in confirming the infection.

KEYWORDS

SARS-CoV-2; COVID-19; Nucleic acids detection; Antibody test

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1. INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues throughout the world. As of March 21, 2020, it has reached more than 115 countries, with 266073 cases and 11184 deaths.[1,2] The World Health Organization has declared the COVID-19 outbreak a pandemic and rates the global risk assessment as very high, making robust global response an imperative to prepare the health systems worldwide.[3] Timely and accurate diagnosis of the suspected SARS-CoV-2 infection, and early isolation of these patients, are of great importance in interrupting human-to-human transmission, and in limiting the further spread of the virus. Common diagnostic features of the COVID-19 include decreased counts of white blood cells, lymphocytes, and platelets, and an increased levels of serum lactate dehydrogenase (LDH), creatine kinase (CK), and C-reactive protein (CRP).[4,5] The reported common clinical symptoms include fever, cough, myalgia and fatigue.[6] However, these abnormalities and symptoms are not unique to COVID-19, but are common to several other viral diseases. Moreover, some COVID-19 patients can be asymptomatic, yet a source of infection, which makes the early diagnosis essential.

The quantitative real-time PCR (RT-qPCR) assay has become the primary and crucial diagnostic tool to identify the SARS-CoV-2 infection, but it has some limitations in clinical practice.[7-9] The RNA-based diagnostic tests show a positive result only when the virus is still present. The tests cannot identify the people who were previously infected, recovered, and have cleared the virus from their bodies. In addition, false-negative results of PCR test were reported and the positive rates varied among different specimens from the COVID-19 patients. Respiratory tract specimens of a few patients, who were epidemiologically linked to SARS-CoV-2

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exposure, and with typical lung CT images, were still negative for SARS-CoV-2 RNA detection.[10-12]

Presence of anti-SARS-CoV-2 IgG/IgM antibody is included as one of the diagnostic criteria in China's updated version of the guidelines for diagnosis and treatment of COVID-19 (3rd, March 2020). It will help to trace, in a much more population-based way, whether a person has been infected in the past, as the body normally retains for varied durations, the antibodies against the virus it has already overcome. Such assays still need to be carefully validated to ensure that they detect antibodies against only SARS-CoV-2. The extensive similarity between SARS-CoV-2 and related viruses may lead to cross-reactivity. Also false positive and false negative results have been reported for IgG/IgM antibody tests.[13-15] In this context, there is an urgent need for determining the diagnostic accuracies of different methods for different types of specimens, to devise preventive plans against virus transmission and to design the most optimum treatment regimen.

2. MATERIALS AND METHODS

2.1. Study Design and Participants

Medical records of 38 patients of COVID-19, aged between 15 years and 75 years, admitted to the Second People's Hospital of Fuyang between January 22, 2020 and February 28, 2020, were collected and retrospectively analyzed. Diagnosis of COVID-19 was based on the New Coronavirus Pneumonia Prevention and Control Program (5th edition) published by the National Health Commission of China. Specimens, including throat swabs, sputum and serum were collected during the period of hospitalization. Viral RNA and serum IgM-IgG antibodies against SARS-CoV-2 were measured by RT-qPCR(reverse

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transcription-quantitative real-time polymerase chain reaction) and GICA (colloidal gold immunochromatographic assay), respectively. This study was approved by the National Health Commission of China and Ethics Commission of the Second People's Hospital of Fuyang. Written informed consent was waived by the Ethics Commission of the designated hospital for studies of emerging infectious diseases, with an urgent need to collect data.

2.2. RT-qPCR Assay for SARS-CoV-2

Respiratory specimens, including throat swabs and sputum, were collected; the throat swabs were placed in sterile test tubes, each with 1 mL sterile saline, and the sputum samples were added to an equal volume of acetylcysteine and shaken at room temperature for 30 min to fully and homogenously liquefy it. Total RNA was extracted from both the samples independently, using a viral nucleic acid isolation kit (Jiangsu Bioperfectus Technologies Company Ltd.), and the RT-qPCR assay was performed using a SARS-CoV-2 nucleic acid detection kit (Shanghai BioGerm Medical Biotechnology Co. Ltd.) according to the manufacturer’s instructions. Genes for the open reading frame 1ab (ORF1ab) and nucleocapsid protein (N) of SARS-CoV-2, were simultaneously amplified and tested. Following criteria were used for interpreting the test results: 1) FAM channel was used for detecting ORF1ab gene, and HEX/C Channel for N gene; 2) results were scored as negative when the Ct value > 37 or not detected; 3) positive results were scored when the amplification curve was S-shaped, with Ct value ≤ 37; 4) results were classified as suspicious, when the amplification curve was S-shaped, with Ct value >37 and <40.

Following criteria were used for interpreting the SARS-CoV-2-infection: 1) both the genes (ORFla/b, and N) of SARS -CoV-2 were positive in the specimen; 2) cases with a single positive gene were confirmed by retesting. If it was still

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positive for the same single target, it was classified as positive. If not, it was deemed to be negative. These diagnostic criteria were based on the recommendations of the National Institute for Viral Disease Control and Prevention of China (http://ivdc.chinacdc.cn/kyjz/202001/t20200121_211337.html).

2.3. Colloidal Gold Antibody Test for SARS-CoV-2

Serum IgG and IgM antibodies from patients against SARS-CoV-2 were tested using GICA kits (Beijing Innovita Biological Technology Co. Ltd.), according to the manufacturer's protocol. Briefly, for each test, 10 μL of serum sample and 80 μL of sample diluent were added onto the pad of the test strip, and the strip was placed flat at room temperature for 15 min and then the result were scored according to the color of the test and control lines. 1) When both sample band and the control band turned red, the sample was be scored as positive; 2) When only the control band turned red, and the sample band did not, it was classified as negative; 3) When neither band was colored, the test was deemed to be failed and retesting was required for confirmation.

2.4. Statistical Analysis

All analyses were performed using SPSS 19.0. Continuous variable data were in the median (Interquartile range, IQR), categorical variables were expressed as frequencies (percentages), chi-square test with Yates's correction or Fisher's exact test was used for comparison between groups. $P<0.05$ was scored as statistically significant.

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3. RESULTS

3.1. Clinical characteristics and seropositive rates of antibodies against SARS-CoV-2

Medical records from 38 patients with COVID-19 were collected and retrospectively analyzed. The median age of this cohort was 40.5 years (IQR, 31.0-49.5 years) and 55.3% were males. Of these patients, 3 were in severe or critical conditions, and the rest were mild cases. The median number of specimens collected from each patient was 8. A total of 76 serum samples, collected from these patients during hospitalization, were tested for IgM and IgG antibodies against SARS-CoV-2. The total seropositive rate for IgM and IgG was 50.0% (19/38) and 92.1% (35/38), respectively.

Two cases, who were reported to be close contacts of previously confirmed COVID-19 patients, remained seronegative for antibodies during hospitalization. Case 1 was a female aged 15, with no fever or fatigue, no digestive system morbidity, and no significant changes in the counts of lymphocyte subsets, during the course of the disease. She continued to be seronegative in the antibody test for 14 days following the discharge from hospital. Case 2 was a female aged 40, with fever (body temperature up to 38.2℃ at the onset of illness). CT scan of chest showed signs of inflammation, accompanied by increased count of T-lymphocyte subsets and decreased NK cells. The antibody test showed a seroconversion of IgG in the 14 days following the discharge from hospital.

3.2. The detectability of viral RNA and antibody against the virus in patients at different time-points after the onset of infection

We analyzed the detectability of the viral RNA and the antibody in the cohort during the time course of infection, since its onset. As shown in Table 1, in the

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early phase of illness i.e., within 7 days after onset (dao), the viral RNA in the sputum had the highest detectability at 92.3%, followed by the RNA from the throat swabs (69.2%), while the antibodies showed lower positive rates (IgM, 23.0%; IgG, 53.8%) at this timepoint. However, after 8 dao, the antibody titer increased (IgM, 50.0%; IgG, 87.5%) and became more detectable than RNA. Significantly, the detection rate of RNA in the throat swabs was only 13.0% at the later phase (≥15 dao), while the sensitivities of IgM and IgG rose to 52.2% and 91.3%, respectively.

3.3. The auxiliary diagnostic potential of antibody assays for suspected cases with no detectable load of viral RNA

Based on the above-mentioned findings, we aimed to assess the auxiliary diagnostic potential of antibody assays for suspected cases, with negative nucleic acid detection. First, the antibody was tested in patients with no detectable viral RNA in the respiratory tract specimens. As shown in Table 2 and Table 3, simultaneous tests for antibody and RNA improved the sensitivity of detection of SARS-CoV-2 infection, particularly for the throat swabs group at the later stages of infection. Then, we further analyzed the antibody test data of cases with no detectable viral RNA in their throat swabs specimens at the early stages of illness. Most of them were shown to be IgM/IgG seropositive, 7 days after the negative nucleic acid test (IgM^+ 47.1%, IgG^+ 91.1%), suggesting an auxiliary diagnostic value for antibody assays.

4. DISCUSSION

Recently, SARS-CoV-2 is identified as the cause of acute respiratory disease. It is the third highly pathogenic and transmissible coronavirus, after the severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory

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syndrome coronavirus (MERS-CoV) in humans.[16] Accurate identification of SARS-CoV-2 infection is essential for an effective diagnosis of COVID-19 in people, and this is of great importance not only for the individual patients, but also for public health efforts. Clinical manifestations of SARS-CoV-2 infection include fever, cough, dyspnea, myalgia, fatigue, and radiographically detectable pneumonia. Diagnosis based on clinical history, laboratory results, and CT images, need to be confirmed with detection of viral nucleic acid. Yet many cases of pneumonia cannot be diagnosed owing to the negative viral nucleic acid test. For example, throat swab is commonly used for nucleic acid test, but in COVID-19 cases, the viral load is usually much less in the upper respiratory tract than in the lower respiratory tract, and this may lead to a gross under-estimation of the viral load. Moreover, in the same patients, viral load varies in different stages of infection.[17] These problems necessitate a reliable clinical auxiliary diagnosis, to improve the sensitivity and accuracy of virus detection and to provide timely treatment, and to impose preventive quarantine.

The human immune system can produce specific IgM and IgG antibodies against viral infection. IgM is the earliest antibody that appears as the first immune response. Serological presence of IgM indicates a recent infection and it may be used for auxiliary diagnosis of early infection. IgG is produced later and lasts long, which can be used as an indicator of previous or secondary infection.[18,19] Several serological assays that can detect IgM only or IgM-IgG antibodies against SARS-CoV-2 have been developed recently,[20-23] while their results are not completely consistent. Due to the emergency of the outbreak of COVID-19 and limited time, researchers do not have complete detailed information for each patient and could not carry out enough tests to compare different kits about their technical performance. In this study, throat swabs were positive for SARS-CoV-2 only in 13% of the later phase of infection (≥15 dao),

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and the sensitivities of IgM and IgG against SARS-CoV-2 were 52.2% and 91.3%, respectively at this timepoint. Combining the antibody assay with nucleic acid detection greatly improved the sensitivity of diagnosis of SARS-CoV-2 infection, particularly for the throat swabs group at later stages of infection. Moreover, our analyses suggest that, when viral RNA is not detectable in throat swabs at the early stage of illness, most of the cases may turn IgM/IgG seropositive after 7 days, indicating an auxiliary diagnostic potential of the antibody assays.

Nevertheless, it is noteworthy that antibody assays too have false positive and false negative results. When IgM and IgG levels are below the detection limit, the test results would be negative. As they gradually decrease and disappear after 14 days, IgM molecules against SARS-CoV-2 may not be detectable later during infection. Difference in the individual immune response may also lead to false negative results in suspected cases.[23,24] Patients who do not produce sufficient antibodies, or who produce antibodies relatively late, might have a relapse, once their immunity is reduced.

5. CONCLUSION

The test of IgM and IgG antibodies against SARS-CoV-2 provides important immunological evidence of infection, and it can be an effective supplementary indicator in diagnosing the suspected cases with no detectable viral RNA, or in conjunction with nucleic acid detection in the diagnosis of suspected cases. Combination of viral RNA RT-qPCR and IgM-IgG antibody test can provide more accurate diagnosis of SARS-CoV-2 infection. Development of test kits with IgG-IgM antibodies against SARS-CoV-2, supported with further research, will improve the diagnostic sensitivity and specificity.

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AVAILABILITY

All data generated in this work is freely available to the research community via the corresponding authors (lxy8726@126.com; fyhmf@163.com).

AUTHOR CONTRIBUTIONS

Gao Y, Yuan Y, Wang XW, Li A and Han MF were involved in designing the study and preparing the manuscript; Gao Y and Li TT performed most of the experiments; Gao Y, Yuan Y, Li TT and Wang XW analyzed the data; Gao Y, Yuan Y, Li A, Li XY and Han MF contributed to critical revision of the manuscript. The corresponding authors were responsible for all aspects of the study, and ensured that issues related to the accuracy or integrity of any part of the work were investigated and resolved. All authors reviewed and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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TABLE 1. Detection in different samples during SARS-CoV-2 infection

Days after onset	n	RNA from sputum		RNA from throat swabs		IgM		IgG	
		n^+	Sensitivity	n^+	Sensitivity	n^+	Sensitivity	n^+	Sensitivity
Total	38	29	76.3%	14	36.8%	19	50.0%	35	92.1%
0-7	13	12	92.3%	9	69.2%	3	23.0%	7	53.8%
8-14	8	3	37.5%	2	25.0%	4	50.0%	7	87.5%
≥15	23	14	60.8%	3	13.0%	12	52.2%	21	91.3%

TABLE 2. Presence of antibodies against SARS-CoV-2 in patients with no detectable viral RNA in sputum

Days after onset	No of cases with no detectable RNA	IgM		IgG	
		n^+	Sensitivity	n^+	Sensitivity
0-7	1	0	0.0%	0	0.0%
8-14	5	3	60.0%	5	100.0%
≥15	9	4	44.4%	9	100.0%

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TABLE 3. Presence of antibodies against SARS-CoV-2 in patients with no detectable viral RNA in throat swabs

Days after onset	No of cases with no detectable RNA	IgM		IgG	
		n^+	Sensitivity	n^+	Sensitivity
0-7	4	2	50.0%	4	100.0%
8-14	6	2	33.3%	6	100.0%
≥15	20	9	45.0%	18	90.0%

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