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Nucleic acid and antibody assay results in Chinese patients with coronavirus disease 2019 (COVID-19)

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Abstract

Aim: To evaluate the nucleic acid and antibody test results of patients with Coronavirus Disease 2019 (COVID-19) in China.

Methods: All patients with laboratory-confirmed SARS-CoV-2 infection from Jan to Apr 2020 were retrospectively analyzed. Clinical characteristics and laboratory test results were obtained from electronic medical records. Patients were divided into three groups based on antibody production, and compared for laboratory test results.

Results: Of 73 patients aged 11–82 years, 12 (16.4%), 28 (38.4%), 25 (34.2%) and 8 (11.0%) were ≤ 30, 31–50, 51–70, and ≥ 71 years old, respectively. Thirty-four (46.6%) patients were male. Most individuals had mild symptoms, and no patient died during treatment. All patients were tested positive for SARS-CoV-2 in sputum and nasopharyngeal samples, and 40 (54.8%) were also tested positive in stool. Nine (12.3%) patients were re-positive for SARS-CoV-2, as assessed by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) after discharge. Sixty-five (89.0%) patients had IgM or IgG antibodies against SARS-CoV-2. Among the four age groups, there was no difference in IgG antibody production (P = 0.664). CD3, CD4, CD8 and CD19 cell counts between the antibody producing and non-producing groups showed no significant differences (all P > 0.05). Lymphocyte count, C-reactive protein, IL-6, lactate dehydrogenase, alanine aminotransferase, creatinine and D-Dimer levels were similar in the three groups (all P > 0.05).

Conclusions: Patients after recovery from COVID-19 can be tested positive for SARS-CoV-2. Some patients may produce antibodies only for a short time, or even no antibodies at all.

1. Introduction

Coronaviruses are non-segmented positive-sense RNA viruses with a roughly 30-kb genome surrounded by a protein envelope [1]. They belong to the family Coronaviridae and the order Nidovirales, and are broadly distributed in humans and other mammals [2]. In the past two decades, two serious betacoronaviruses, severe acute respiratory syndrome coronavirus (SARS-CoV) [3] and Middle East respiratory syndrome coronavirus (MERS-CoV) [4], have caused a large number of deaths.

In December 2019, a series of severe pneumonia cases were reported in Wuhan in Hubei province, China [2]. Further investigation of lower respiratory tract samples revealed the severe acute respiratory syndrome coronavirus SARS-CoV-2 as the causative pathogen.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) was used for the detection of SARS-CoV-2. However, previous studies have found that patients who recovered from COVID-19 can still test positive, although none of the re-positive patients show any clinical symptomatic recurrence [5–7]. Habibzadeh et al. [8] reported that the second RT-PCR test was found to be positive in 9 out of 13 recovered patients after 18 (range 15 to 48) days after complete resolution of symptoms. Antibody detection may offer vital clinical information in the diagnosis and management of COVID-19 patients. Zhao J et al. [9] reported that the median seroconversion times for IgM and IgG were 12 and 14 days, respectively. The presence of antibodies was < 40% among patients within 1-week of onset, and rapidly increased to 94.3% (IgM) and 79.8% (IgG) from day-15 after onset. Kelvin et al. [10] found that inpatients with serum samples available 14 days or more after
symptom onset, the rates of seropositivity were ≥ 94% for IgG and ≥ 88% for IgM.

Thus, the aim of this study was to retrospectively analyze the results of SARS-CoV-2 testing and antibody detection, and to explore the possible reason for differences in antibody detection.

2. Materials and methods

2.1. Patients

The study retrospectively enrolled patients diagnosed with COVID-19 from Jan to Apr 2020 in the affiliated people’s hospital of Ningbo university and Hwa Mei hospital, university of Chinese academy of sciences hospital. The diagnostic criteria were: epidemiological history, fever or other respiratory symptoms, and positive results of RT-PCR for SARS-CoV-2. Infection with influenza virus, avian influenza, parainfluenza virus, and respiratory syncytial virus was excluded by RT-PCR. The patients were discharged from hospitals after meeting the following conditions: normal body temperature for > 3 consecutive days; significantly resolved respiratory symptoms; at least two consecutive negative RT-PCR results in nasopharyngeal swab, sputum, and feces sampled with at least a 24-hour interval. All discharged patients were required 14 days of isolation at home. This study was approved by the Ethics Commission of the affiliated people’s hospital of Ningbo university, and written informed consent was waived by the committee because of the retrospective nature of the study.

Patients were divided into the antibody production, antibody disappearance (within two months), and no antibody production groups, based on antibody test results.

2.2. Detection of coronavirus by real-time RT-PCR

Nasopharyngeal swab, sputum, and fecal specimens were collected from all patients. Eye discharge was collected from patients with elevated eye discharge. In discharged patients, nasopharyngeal swab, sputum and fecal samples were collected for RT-PCR detection of SARS-CoV-2. The RNA Detection Kits (Daan Gene, China), approved by the National Medical Products Administration, were used for virus ORF1ab and N region testing. Amplification was performed at 50 °C for 15 min and 95 °C for 15 min, followed by 45 cycles of 94 °C for 15 s and 55 °C for 45 s.

2.3. Antibody detection

At the early stage after the outbreak of COVID-19, due to the lack of antibody detection reagents, antibody detection could not be conducted on hospital admission in these patients. Since March 1, 2020, even after the patients had been discharged, they returned to the hospital, and antibodies (IgG and IgM) were examined every 3–4 days.

SARS-CoV-2 antibody detection kits (Dongfang Gene, China; Innovita, China), approved by the National Medical Products Administration, were used. In case of positive results, blood samples were tested with another reagent to confirm the finding. No patient specimens were kept because of biosafety concerns.

2.4. Data collection

Clinical characteristics were extracted from the electronic medical records system. The CT values of ORF1ab and N target genes during hospitalization and after discharge were compared in the nine patients who displayed re-positivity for SARS-CoV-2 by RT-PCR after discharge. IgG production rates and CD3, CD4, CD8 and CD19 cell counts were compared between the antibody production and no antibody production groups. Lymphocyte count, C-reactive protein, IL-6, lactate dehydrogenase, alanine aminotransferase, creatinine and D-Dimer levels were examined several times during hospitalization, and the mean values were compared among the three groups. IgM production rates were not recorded because of the short half-life of this antibody.

2.5. Statistical analysis

Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and analyzed by the Student’s t test between two groups. Continuous variables with non-normal distribution were expressed as median (interquartile ranges) and analyzed by non-parametric tests. Categorical variables were expressed as frequency and percentage, and analyzed by the Chi-square test. P < 0.05 was considered statistically significant. Statistical analyses were performed with the SPSS 17.0 software (SPSS Inc., Chicago, USA).

3. Results

3.1. Basic information of the patients

A total of 73 patients were identified with laboratory-confirmed SARS-CoV-2 infection in Ningbo. Some patients were infected by family members; others were infected because of close contact with other patients. None of the patients were medical professionals. Age ranged from 11 to 82 years, including 12 (16.4%), 28 (38.4%), 25 (34.2%) and only 8 (11.0%), who were aged ≤ 30, 31–50, 51–70 and ≥ 71 years, respectively. Only two adolescents (11 and 12 years) were infected. Thirty-four (46.6%) patients were male.

3.2. Detection of coronavirus in different samples

SARS-CoV-2 was detected in sputum and nasopharyngeal swab samples collected from all 73 patients. On admission, each patient had positive results for SARS-CoV-2 in sputum and nasopharyngeal PCR tests. By comparison, only 40 (55%) patients showed SARS-CoV-2 in fecal specimens. Eye secretions were examined for no less than twice in 14 patients who had elevated eye secretions, and only 1 (7.1%) eventually tested positive.

3.3. SARS-CoV-2 testing of discharged patients

SARS-CoV-2 was tested several times during the next 14 days in isolation and possibly longer in discharged patients, depending on the patients’ conditions. PCR assays were positive in 9 (12.3%) discharged COVID-19 Patients. Among the nine cases, SARS-CoV-2 was detected in feces in only two patients; in nasopharyngeal swab, sputum and fecal samples in three patients; and in nasopharyngeal swab and sputum specimens in four patients. Reactivation times in patients with SARS-CoV-2 ranged from 7 to 25 days. In addition, the CT values of ORF1ab (33.52 ± 3.54 vs. 29.37 ± 2.43 copies/ml, P < 0.01) and N (35.28 ± 2.56 vs. 30.43 ± 2.86 copies/ml, P < 0.01) target genes were significantly higher during the post-discharge period compared with hospitalization, indicating that the virus content was significantly lower after treatment (Table 1).

3.4. Antibody detection in COVID-19 patients

All the 73 patients under went antibody detection for 1–5 times. A

| Table 1 | Comparison of CT values at different periods in 9 discharged patients with positive PCR assay. |
|--------|--------------------------------------------------------------------------------------------------|
| CT value (copies/ml) | During hospitalization | During the post-discharge period | P-value |
| ORF1ab | 29.37 ± 2.43 | 33.52 ± 3.54 | < 0.01 |
| N | 30.43 ± 2.86 | 35.28 ± 2.56 | < 0.01 |
total of 65 (89.0%) patients showed IgM or IgG antibodies against SARS-CoV-2. There was no significant difference in IgG antibody production among different age groups (P = 0.664) (Table 2). IgM or IgG antibodies were detected consistently in 61 (83.5%) patients, disappeared within two months in 4 (5.5%) cases, and were never found in 8 (11.0%) patients (Table 3). Lymphocyte count and classification were not significantly altered in the 8 patients who had no antibody production. CD3, CD4, CD8 and CD19 cell counts between the antibody producing and the non-producing groups were also not significantly different (all P > 0.05) (Table 4). In the no antibody production group, only one patient had a history of hypertension, and another had chronic tonsillitis. The remaining 6 patients all reported no previous history of accompanying diseases, and denied immunosuppressive symptoms or tumors. Lymphocyte count, C-reactive protein, IL-6, lactate dehydrogenase, alanine aminotransferase, creatinine and D-Dimer levels were similar among the three groups (all P > 0.05) (Table 5).

4. Discussion

This was a retrospective study based on nucleic acid, antibody production, and laboratory test results in patients with SARS-CoV-2 infection. We report here a cohort of 73 patients with laboratory confirmed SARS-CoV-2 infection. Although COVID-19 patients have a high fatality rate [2,11], all the 73 patients in this study had a good prognosis, with mostly mild symptoms, and none of them died. We found that age, immune cell count, and liver function and inflammation indicators were all similar between patients with and without antibody production.

Table 2
IgG production rates in different age groups.

| Age (years) | N  | Antibody production P  |
|-------------|----|------------------------|
| ≤30         | 12 | 9 (75.0%) 0.664        |
| 31–50       | 28 | 25 (89.3%)            |
| 51–70       | 25 | 23 (92.0%)            |
| ≥71         | 8  | 8 (100.0%)            |

Table 3
Antibody results in the no antibody production and antibody disappearance groups.

| Patients | age | laboratory-confirmed time | Antibody detection time |
|----------|-----|---------------------------|-------------------------|
| No antibody production group | | | |
| 2 | 30 | Jan 23 | IgM Mar 6/- |
| | | | IgG Mar 6/- |
| 71 | 31 | Feb 3 | IgM Mar 8/- |
| | | | IgG Mar 8/- |
| 69 | 23 | Feb 8 | IgM Mar 4/- |
| | | | IgG Mar 7/- |
| 70 | 66 | Feb 8 | IgM Mar 6/- |
| | | | IgG Mar 17/- |
| 72 | 54 | Feb 8 | IgM Mar 4/- |
| | | | IgG Mar 29/- |
| 68 | 45 | Feb 9 | IgM Mar 4/- |
| | | | IgG Mar 11/- |
| 10 | 49 | Mar 10 | IgM Mar 11/- |
| | | | IgG Mar 13/- |
| 73 | 20 | Apr 5 | IgM Apr 6/- |
| | | | IgG Apr 6/- |
| Antibody disappearance group | | | |
| 25 | 31 | Feb 5 | IgM Mar 1/+ |
| | | | IgG Mar 8/+ |
| 65 | 32 | Feb 6 | IgM Mar 3/+ |
| | | | IgG Mar 5/+ |
| 9 | 31 | Feb 7 | IgM Mar 2/+ |
| | | | IgG Mar 6/+ |
| 16 | 57 | Feb 25 | IgM Mar 2/+ |
| | | | IgG Mar 2/+ |

Table 4
Immune function outcomes in the antibody production and no-antibody production groups.

| | Antibody production (n = 61) | No antibody production (n = 8) | P-value |
|----------|-----------------------------|--------------------------------|--------|
| CD3 (60–84)* | 70.4 ± 10.4 | 68.8 ± 10.8 | 0.836 |
| CD4 (29–60)* | 44.5 ± 9.1 | 39.5 ± 7.2 | 0.389 |
| CD8 (12–38)* | 20.7 ± 6.8 | 22.5 ± 4.2 | 0.168 |
| CD19 (7–22)* | 12.2 ± 3.4 | 11.2 ± 2.8 | 0.436 |

* Reference range

Human coronaviruses are the main pathogens responsible for respiratory infections. SARS-CoV and MERSCoV can cause severe respiratory syndrome in humans, and their mortality rates are > 10% and > 35%, respectively [12]. According to current reports, without timely treatment of the disease, the fatality rate of COVID-19 can exceed 10%[2,11]. Research suggests that SARS-CoV-2 is more likely to infect older adult males with chronic comorbidities as a result of weaker immune functions in these patients [11,13–15]. In the present study, most patients were under the age of 50, and only 8 (11.0%) were over 70. Less than a half (34/73, 46.6%) of the patients were male. Differences from previous studies may be related to regional differences or pathways of infection.

The initial symptoms for most COVID-19 patients were fever and cough, and these individuals rarely developed intestinal signs and symptoms (e.g., diarrhea), whereas about 20–25% of patients with MERS-CoV or SARS-CoV infection have diarrhea [16]. However, in this study, SARS-CoV-2 was detected in the feces of 40 (54.8%) COVID-19 patients on the day of hospital admission. Interestingly, the viral nucleic acid was also detected in the stool of 5 out of the 9 patients who displayed re-positivity for SARS-CoV-2 by RT-PCR after discharge. Findings have shown [17] that viral load in feces reaches a peak from the third week to the fourth after onset. In addition, Yuan L et al. [18] found high aerosol viral load in toilets used by COVID-19 patients. Thus, it was suggested that the management of COVID-19 patients’ stool specimens should be strengthened, emphasizing that the medical staff...
should be well protected in contact with patients’ feces. PCR assay was positive in some patients after discharge, indicating that there might be a fluctuating period between apparent improvement in clinic and full recovery from the virus. Xiaohong Y et al. [19] found that SARS-CoV-2 particles still remain in patients’ lungs through autopsy, in individuals meeting discharge standards. Therefore, the negative viral RT-PCR results of two nasopharyngeal swabs or sputum samples may be used as one of the discharge criteria. In some elderly patients with underlying diseases, it may be considered to add alveolar lavage fluid as a nucleic acid test sample for SARS-CoV-2. Meanwhile, repeated viral RT-PCR testing with prolonged duration (such as 48 hours) is essential to assure that the virus has been actually cleared, so that the discharged patients no longer transmit the virus. These asymptomatic carriers brought more challenges to the management and control of the COVID-19 pandemic in China and the other affected countries [6].

Serum antibody detection should play an important role in the identification of asymptomatic infections of SARS-CoV-2. In the present study, no IgM or IgG antibody was detected in 8 patients, and their relevant immune function test was normal; they also did not have a history of tumors or other autoimmune diseases. Only two patients had a slight decrease in CD3 or CD4 count in the early stages of the disease. The reasons for no antibody production need further discussion. According to the antibody detection results of another four patients in the antibody disappearance group, IgG may exist for only 2 months because of chest tightness, and was diagnosed as a new case through antibody detection result of another four patients with SARS-CoV-2. Meanwhile, repeated viral RT-PCR testing with prolonged duration (such as 48 hours) is essential to assure that the virus has been actually cleared, so that the discharged patients no longer transmit the virus. These asymptomatic carriers brought more challenges to the management and control of the COVID-19 pandemic in China and the other affected countries.

One patient in the present study came to the hospital for treatment because of chest tightness, and was diagnosed as a new case through nucleic acid screening, but didn’t produce antibodies. His wife and two sons were also infected and included in this study, and they had no symptoms, including fever, cough, or a sore throat. Only his wife’s serum IgM and IgG antibodies were positive on admission. According to the negative antibody results of the patient and his two sons, the wife might be the first to contact with SARS-CoV-2 but remained asymptomatic. Therefore, antibody detection has certain reference significance in general epidemiological investigation.

This study had several limitations. First, it was a retrospective study with a small sample size. Only 8 patients without antibody production were included. Further large-scale studies are needed to explore the reasons for failed antibody production. Secondly, the mechanism of short-time antibody production and whether it is correlated with immunosuppressive diseases still need further research as well.

In conclusion, patients who have recovered from COVID-19 can be tested positive for SARS-CoV-2. Some patients may produce antibodies only for a short time, or even no antibodies at all.

Authors’ contributions

Wanjun Yu designed the study, Yiping Wang, Jianping Luo collected the samples, detected RT-PCR and antibodies. Yong Lu and Yongyan Li drafted the manuscript and analyzed the data. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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