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Combination of serological total antibody and RT-PCR test for detection of SARS-COV-2 infections

Pei Wang

Department of Laboratory Medicine, The First People's Hospital of Jingmen, Hubei Province, 448000, China

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ABSTRACT

The purpose of this study was to investigate the feasibility of serological total antibody tests combined with RT-PCR for detection of SARS-COV-2. We conducted a retrospective study in which 375 patients were enrolled during the outbreak of SARS-COV-2 from 25th January to 16th March 2020. Patients were divided into a COVID-19 group (n = 141) and a control group (n = 234). Serum samples and throat swabs were collected from 375 patients for total antibody testing against SARS-COV-2 and RT-PCR analysis, respectively. The results indicated that diagnostic sensitivity and specificity were 95.7 % and 98.7 %, 92.2 % and 100 % by total antibody tests and RT-PCR, respectively. The sensitivity and specificity of total antibody tests combined with RT-PCR were 98.6 % and 98.7 %. The sensitivity of the combined method was significantly higher than RT-PCR (X^2 = 5.16, $P < 0.05$), and similar to that of total antibody tests (X^2 = 1.15, $P > 0.05$). This study supported the advantage of the combined method for detection of SARS-COV-2 with a high degree of sensitivity and specificity, as a useful tool for accurate diagnosis and timely treatment of suspected patients, epidemiological investigation, as well as monitoring ongoing outbreaks of infections with SARS-COV-2.

1. Introduction

As of May 1, 2020, more than 237,000 people died from COVID-19 worldwide, and the estimate of future deaths will number in the millions (Roberton et al., 2020). The SARS-COV-2 is now quickly spreading across the world after being reported in Wuhan first (Zhu et al., 2020), and has become a global health concern (Wang et al., 2020a). SARS-COV-2 is an enveloped non-segmented positive-sense RNA virus, which is highly contagious with high mortality ranging from 3% to 14.6 %, depending on the cohort characteristics (Wang et al., 2020a). Currently, virus RNA detection conducted by RT-PCR has become the standard assessment for the confirmation of SARS-COV-2 infection (Huang et al., 2020). However, virus RNA detection has some limitations in terms of accuracy (Xie and Zhong, 2020). Technically, RNA testing works with throat swabs or nasopharyngeal swabs as testing samples. The RT-PCR test comprises RNA extraction and amplification procedures. It usually takes 2–4 hours to accomplish an RT-PCR testing circle. The RNA detection results rely on the quality of the sample, extracted RNA, the source of the RT-PCR reagents and the multiple steps in RNA preparation. Moreover, different sample types yield different positive detection rates varying from 1% to 93 % (Wang et al., 2020b), and the viral load fluctuates at different infectious phases (Cai et al., 2020).

Taking the above into account, the seventh edition of the medical guideline for SARS-COV-2 infections from the National Health Commission of the People's Republic of China added that serological testing could be used for confirmed diagnosis of COVID-19 (China National Health Commission, 2020). To this end a commercially available kit (Wantai, Xiamen, China) using a chemiluminescence microparticle immunoassay (CMIA) for the determination of total antibody in serum samples can be used. This kit was applied for diagnosing suspected patients of COVID-19 and for epidemiological study (Zhao et al., 2020).

In this study, we presented the results of two diagnostic methods: serum total antibody assays against SARS-COV-2 by CMIA and the RT-PCR for detection of viral RNA. In addition, the combination of the results of the total antibody test and RT-PCR was discussed for detection of SARS-COV-2 infections.

2. Materials and methods

2.1. Study setting

This study was performed in the First People's Hospital of Jingmen, Hubei Province, China, which is a comprehensive public hospital with 2300 beds located in central area of China with the capability of serving 400,000 inhabitants.

Abbreviations: CMIA, Chemiluminescence Microparticle Immunoassay; RT-PCR, Real-time reverse transcriptional polymerase chain; RLU, relative light unit

E-mail address: peiwwien@yahoo.com.

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We retrieved the data of 375 patients which was recorded during the outbreak of SARS-COV-2 from 25th of January to 16th of March 2020. For all enrolled patients, the clinical data included the date of illness onset, clinical features, chest CT during the hospitalization period. Personal demographic information was obtained from the clinical records. The laboratory data included results of total antibody test against SARS-COV-2 and RT-PCR detection.

2.2. Study participants

All 375 patients who visited the hospital with respiratory complaints were included. Of the patients, 141 were confirmed to have SARS-COV-2 infection (COVID-19 group). This group consisted of 65 male and 76 female patients with a median age of 58 years (range 21–95 years). A confirmed COVID-19 case and the clinical classification was defined based on the New Coronavirus Pneumonia Prevention and Control Program (7th edition) published by the National Health Commission of China (China National Health Commission, 2020). A case with a positive SARS-CoV-2 on RT-PCR is defined as a confirmed patient. Despite strong clinical suspicion some patients remained negative with the RT-PCR. In this study are included also as COVID-19 patients, patients with a negative RT-PCR, but with characteristic CT-changes of the lungs.

The other 234 patients with no relevance to COVID-19 were included in a control group consisting of 101 male and 133 female patients with a median age of 42 years (range 16–74 years). The study was performed in accordance with the guidelines approved by the Ethics Committees from The First People's Hospital of Jingmen (No. 202,002,006). The need for informed consent was waived.

2.3. Specimen collection

The throat swab specimens were collected by using a foam swab with transport medium (Sigma Virocult, UK). Specimens were then put in a biosafety transport box and sent to the laboratory located at the hospital for RT-PCR detection immediately.

The blood samples (5 mL) were collected before patients were discharged from the hospital. Sample taking time varied from 0 − 10, 11 − 20, > 20 days after illness onset. Specimens of 141 COVID-19 patients and 234 controls were collected at one of the aforementioned three time periods. The samples were centrifuged at 1500-2000*g* for 10 min, and the serum was aliquoted and tested to determine the total antibody against SARS-COV-2.

2.4. RT-PCR

Virus RNA was extracted from throat swabs with a nucleic acid kit (Roche, Mannheim, Germany) on an automatic workstation MagNA Pure 96 system (Roche, Mannheim, Germany). The whole process of extraction was performed according to the guidelines. Real-time reverse transcriptional polymerase chain (RT-PCR) with Applied Biosystems ViiA7 Dx (Applied Biosystems, Singapore) and RT-PCR reagent BioGerm (Shanghai BioGerm Medical Technology Co., Ltd.) were commercially obtained and used for virus detection. The RT-PCR tests were performed on throat swabs following a previously described method (Wang et al., 2020c). In brief, two target genes, including open reading frame 1ab (ORF1ab) and nucleocapsid protein (N), were simultaneously amplified and tested during the RT-PCR assay. Target 1(ORF1ab): forward primer CCCTGTGGGTTTTACACTTAA; reverse primer ACGATTGTGCA TCAGCTGA; and the probe 5′-VIC − CCGTCTG CGGTAT GTGGAAAGGTTAT GG-BHQ1 − 3′. Target 2 (N): forward primer GGGGAACTTCTCCTGCTAGAAT; reverse primer CAGACATTTT GCTCTCAA GCTG; and the probe 5′-FAM-TTGCTGCT GCTTG ACAG ATT-TAMRA-3′. The RT-PCR assay was performed using a SARS-COV-2 nucleic acid detection kit Bio Germ according to the manufacturer's protocol. The reaction mixture contained 12 μL of reaction buffer, 4 μL of enzyme solution, 4 μL of probe primers solution, 3 μL of diethyl pyrocarbonate treated water, and 2 μL of RNA template. RT-PCR assay was performed under the following conditions: incubation at 50 ˚C for 15 min and 95 ˚C for 5 min, 40 cycles of denaturation at 94 ˚C for 15 s, and extending and collecting fluorescence signal at 55 ˚C for 45 s. A cycle threshold value (Ct-value) less than 37 was defined as a positive test result, and a Ct-value of 40 or more was defined as a negative test. Internal controls, positive and negative controls were routinely performed with each batch of tests.

2.5. Total antibody measurement

The total antibody in against SARS-COV-2 serum samples was determined by chemiluminescence microparticle immunoassay (CMIA) kits (Xiamen Wantai Kairui Biological Technology Co., Ltd, China). According to the manufacturer's instructions. Briefly, like in the Wantai ELISA (GeurtsvanKessel et al., 2020; Lassaunière et al., 2020) the total antibody detection is based on a double-antigen sandwich principle that detects total antibody. Recombinant antigens containing the receptor binding domain (RBD) of the SARS-COV-2 spike protein were utilized to develop a total antibody assay (Lou et al., 2020). The amount of luminescence is quantified by relative light unit (RLU), the amount of RLU can be measured and is proportional to the amount of antibody captured inside the tube. The Carris 200 calculates S/CO (Signal-to-cut off ratio). Values < 1.0, are considered to be negative for SARS-COV-2 antibody, whereas, values ≥1.0, are considered to represent antibody positivity. Both positive and negative controls were routinely performed with each batch of tests.

In addition, next to the Wantai kit two samples were tested with another antibody kit (Shenzhen, YHLO Biotech Co.,Ltd.).

2.6. Statistical analysis

A database was established and statistical analysis was performed with SPSS 19.0. Sensitivity, specificity for detection of SARS-COV-2 by RT-PCR, and the total antibody test method as well as the combined methods were analysed. Sensitivity and specificity were calculated with the rate of positive test results and the rate of negative test results (Krüttgen et al., 2020). Chi-square tests were performed on the numeration data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. RT-PCR of COVID-19 positive patients

Of the 141 COVID-19 patients throat swabs were taken several times till day 20 after admission or until the RT-PCR became positive. Samples were taken for the first time between day 1 − 3 after admission to the hospital (Table 1). At that time patients were waiting for the diagnosis. Only 39.7 % of the samples were positive by RT-PCR. The second samples were taken at day 4 and 5 after admission and only of the patients whose first samples were negative. By then 62.4 % of the 141 patients were positive. After testing the third samples taken at day 6 and 7, still only 86.7 % of the 141 patients were positive. Samples of 5 patients were finally positive with RT-PCR at day 10 and later, resulting in a cumulative positive rate of 92.2 %. Eleven patients were never positive by the RT-PCR test.

3.2. The combination of the total antibody test and the RT-PCR in COVID-19 positive and negative patients

A number of patients was transferred from a local hospital to our hospital. They were admitted a few days after illness onset. The illness onset date was taken from the patients records. The illness period varied from 0 − 10 days, 11 − 20 days, > 20 days. Samples for antibody testing were only taken before discharge of the patients. Data of total

Table 1
Positive RT-PCR for 141 patients of the COVID-19 group at different times after admission.

Test times[a]	Days after admission	Positives per number tested. Positive rate (%) per test time	Cumulative positive results	Cumulative positive rate (%)
1	1 − 3	56/141, (39.7)	56	39.7
2[b]	4 − 5	32/85, (37.6)	88	62.4
3	6 − 7	37/53, (69.8)	125	86.7
4, 5	8 − 20	5/16, (31.3)	130	92.2

***Cumulative number of patients tested positive with the RT-PCR.
[a] ,Samples were taken for the first time between day 1 − 3 (test time 1) after admission to the hospital.
[b] ,The second samples were taken at day 4 and 5 after admission of the patients of whom the first samples were negative. Patients whose samples were negative in the second test were re-tested by samples taken at days 6 or 7. When still negative, a 4th and 5th testing was performed.

antibodies against SARS-COV-2 were grouped according to these three time periods. In a total of the 141 COVID-19 patients, 135 became positive by the total antibody test during the three time periods, 130 out of 141 cases were tested positive by RT-PCR assay and 11 cases were RT-PCR negative. Nine out of the 11 RT-PCR-negative cases were detected positive by the total antibody assay. Of the 234 non − COVID-19 patients, none was positive by the RT-PCR test and 3 were positive by the total antibody assay.

To obtain the results for the combined method, results of the RT-PCR (negative and positive) were supplemented with the positive results of the antibody test (Table 2). For example, for the period 0–10 days, 126 patients were positive and 15 patients were negative in the RT-PCR. In the total antibody test 58 had antibodies against SARS-CoV-2, indicating an infection. Among these 58 antibody positive patients were 6 patients from the group of 15 negative RT-PCR patients. These 6 patients, positive for antibodies and negative in the RT-PCR, were added to the group of 126 RT-PCR-positive patients. This gives a diagnosis of a total of 132 SARS-CoV-2 patients in the period of 0–10 days. The same calculations were applied to the periods of 11–20 days, and more than 20 days. In total, 139 out of 141 cases were SARS-COV-2 positive, and 3 out of the 234 non-COVID-19 (control) samples were tested SARS-COV-2 positive in the total antibody test.

The rate of the positive and the negative test results of the different methods is displayed in Table 2, which gives a sensitivity of 92.2 %, 95.7 % and 98.6 % for the RT-PCR, the total antibody test, and the combined method, respectively. The corresponding results for the specificity are 100 %, 98.7 %, and 98.7 %.

The joint method was found to be more sensitive than RT-PCR alone (98.6 % Vs 92.2 %, $X^2 = 5.16$, $P < 0.05$). There is no significant difference in sensitivity between the joint method and total antibody test alone(98.6 % vs 95.7 %, $X^2 = 1.15$, $P > 0.05$).

4. Discussion

Successful control of SARS-COV-2 spread will need an accurate, rapid and cost-effective detection method. The aim of this study was to evaluate the performance of total antibody test by using CMIA, the RT-PCR, and explore the feasibility of the combination, serological total antibody tests and RT-PCR, as a possible diagnostic tool for detection of SARS-COV-2.

The presence of SARS-COV-2 infection can be detected by RT-PCR in samples from nasopharyngeal or throat swab. A number of patients show progressive multiple peripheral ground-glass opacities in lungs but with negative RT-PCR results (Ai and Yang, 2020). Swabs had to be taken 3–5 times from a number of patients to get a positive RT-PCR, and 11 never had a positive RT-PCR. All 11 RT-PCR negative patients had CT-scan changes of ground-glass opacities in the lungs. A negative RT-PCR for COVID-19 patients is not uncommon. A prior study reported only 57 % positives among specimens from fever clinics (Liu et al., 2020). A number of factors may affect this lack of sensitivity of the RT-PCR testing, like the sample (nose or throat swabs), the sampling procedure, the RNA extraction. Also, the time of sampling may be important. The results showed that detection of SARS-COV-2 by RT-PCR was often late while clinical symptoms were already present. Our study proved that RT-PCR had a high specificity (100 %) but relatively low sensitivity (92.2 %).

To solve this diagnostic problem, the 7th edition of guideline for COVID-19 issued by the National Health Commission of the People's Republic of China, recommends serological testing as supporting proof for COVID-19 diagnosis (China National Health Commission, 2020). Several groups determined the antibody response to SARS-CoV-2 and compared new commercial serologic assays (Zhao et al., 2020; GeurtsvanKessel et al., 2020; Krüttgen et al., 2020; Lassaunière et al., 2020). The total antibody test of Wantai has good sensitivity and specificity as compared to other tests (Lassaunière et al., 2020). The

Table 2
The detection of SARS-CoV-2 infections by the RT-PCR, the total antibody assay and a combination of both methods.

Group	Days after onset	RT-PCR[a]	Total antibody test[b]	Antibody + RT-PCR[c]
	Days	Positive test results	Positive test results	Combined positive test results
COVID (n = 141)	0 − 10	126/141	58/61	132
	11 − 20	3/15	70/72	6
	> 20	1/12	7/8	1
	In total	130	135	139
Control (n = 234)		0	3	3
Sensitivity		92.2 %(130/141)	95.7 %(135/141)	98.6 %(139/141)
Specificity		100 %(234/234)	98.7 %(231/234)	98.7 %(231/234)

[a] RT-PCR. At 0 − 10 days after onset, 126 of 141 tested were RT-PCR positive. 15 negatives were re-tested at 11 − 20 days and 3 out of 15 were positive. Of the remaining 12 only 1 was positive by subsequent testing.
[b] Total antibody test. At days 0 − 10 after onset, 61 patients were discharged and 58 of these were antibody positive. At 11 − 20 days, 70 out of 72 were positive. After > 20 days, 8 were discharged of which 7 were antibody positive.
[c] RT-PCR + Antibody. Per patients the results of the RT-PCR test are supplemented by the results of the antibody test. At 0 − 10 days, 6 out of 58 were antibody-positive and these are RT-PCR-neg. This makes the total 126 RT-PCR-pos. + 6 antibody-pos. = 132 positives. At 11 − 20 days, 3 out of 70 were antibody-pos and RT-PCR-neg. The total is 3 RT-PCR-pos. + 3 antibody-pos. = 6 positives. At > 20 days, 0 out of 7 was antibody-pos. and RT-PCR neg. The total is 0 antibody-pos. + 1 RT-PCR pos. = 1.

Wantai antibody test provides a semi-quantitative result. Total antibodies are determined by CMIA, which is an automated, rapid and high throughput assay, objective and quantitative, but it requires an expensive instrument Carris 200 (Lou et al., 2020). The results show a satisfactory quality for sensitivity (95.7 %) and specificity (98.7 %) of total antibodies against SARS-COV-2 by CMIA.

Of the 141 COVID-19 patients, 135 had antibodies against SARS-CoV-2. Six patients out of the 141 COVID-19 patients were found to be antibody negative, 4 of them were RT-PCR positive. Three of these 4 patients had kidney disease and ongoing hemodialysis therapy. Additional samples were taken from these 3 patients. After discharge from the infectious disease department ward, they were transferred to the hemodialysis ward. Samples were taken before and after hemodialysis. The S/CO values for these three patients before hemodialysis were 180.87, 540.31, and 360.83, respectively. After hemodialysis these values decreased to 0.04, 0.93, and 0.75, respectively. The effect of hemodialysis on the presence and/or absence of antibodies has also been described for the detection of anti-hepatitis－HCV antibodies (El-Sherif et al., 2012). The fourth patient with a positive RT-PCR had severe anemia and his S/CO value was 0.65. The remaining two without antibodies in the Wantai assay were re-tested and found to be positive by using another brand of antibody assay (Shenzhen YHLO Biotech Co., Ltd.).

In the 234 non-COVID patients, 3 were found positive with the total antibody test. Among three positive cases, 2 patients were pregnant, the S/CO values of the antibody test were 1.8 and 2.3, respectively. After birth, the results changed from weak positive to negative. The third patient suffered from colon cancer and the antibody test had an S/CO value 4.8. Heterophile antibodies present in elderly, pregnant women, and cancer patients can interfere with immunoassays by a non－Competitive mechanism and lead to false positive results (Tate and Ward, 2004). The combination of the results of both methods, the RT-PCR and CMIA antibody test, does improve the sensitivity to 98.6 %. A high sensitivity is beneficial for screening and confirming COVID-19 patients. No doubt, for COVID-19 diagnosis, RT-PCR played an important role at an early stage. Antibodies against SARS-CoV-2 appear around 7–14 days after the onset of the disease. Therefore, the total antibody test displays next to the RT-PCR a powerful diagnostic efficiency at a later stage. A combination of the two assays is superior and results in the diagnosis of more COVID-19 patients. The presence of pre-existing antibodies to SARS-COV-2 due to a prior infection cannot be excluded. By testing only one sample, the laboratory should carefully interpret these test results, and use additional blood samples, and/or other criteria like RT-PCR, CT-scans and disease history, to prove a recent infection.

Although the detection of viral RNA by RT-PCR is the standard for the diagnosis of SARS-CoV-2 infections, serological testing maybe used complementary to RT-PCR for the diagnosis of COVID-19.

In conclusion, this study supported the use of a combined method for detection of SARS-COV-2 infections with a high degree of sensitivity and specificity, which was shown to be a useful tool in the diagnosis of suspected patients. Next to diagnostic purposes, serological testing will be needed for epidemiological investigations, as well as monitoring of ongoing outbreaks of infection with SARS-CoV-2 and testing the effects of future vaccines.

Author statement

The author of this paper has no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

Declaration of Competing Interest

The author declares that there is no conflict of interest related to this article content.

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