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Clinical application of Chemiluminescence Microparticle Immunoassay for SARS-CoV-2 infection diagnosis

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ABSTRACT

Background: The unsatisfactory accuracy and capacity of real time RT-PCR depends on several unavoidable reasons, which cannot meet the demands for COVID-19 diagnosis.

Methods: 206 serum samples were collected from patients who were treated in the General Hospital of the Central Theater Command of the PLA between January 18 and April 4, 2020. 270 serum samples from healthy blood donors were used as control. IgM and total antibodies (Ab) against SARS-CoV-2 were detected by Chemiluminescence Microparticle Immunoassay (CMIA).

Results: Among the 206 patients, the positive rate of IgM and Ab were 149/206 (72.3 %) and 187/206 (90.8 %), respectively. And the specificity of IgM and Ab detection were 99.3 % and 98.9 %, respectively. The sensitivity of CMIA for Ab detection was significantly higher than that of IgM. An increase of the positive rate and S/CO value for detecting IgM and Ab accompanied with the increasing of days post-disease onset (d.p.o.) were observed. The positive rate of Ab detected by CMIA increased rapidly after 7 d.p.o., while that of IgM was obviously increased after 14 d.p.o.. In addition, the age and gender of these patients did not affect the seroconversion and titer of antibodies during the whole course. The disease-severity of patients had no effect on the seroconversion of antibodies. However, the critical patients possessed a much higher antibody titers than the no-critical cases after 14 d.p.o..

Conclusions: The CMIA can provide important complementation to nucleic acid assay and help to enhance the accuracy and capacity of diagnosis of SARS-CoV-2 infection.

1. Introduction

The recent outbreak of coronavirus infectious disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been classified as a global pandemic on March 12, 2020 [1]. The disease rapidly spreads all over the world and results in more than 4,098,000 cases to be infected and over 283,000 deaths up to May 12, 2020 [2]. So far, the number of infected people is still rapidly growing.

To identify infected-patients as early as possible is the first line of epidemic disease control. Currently, laboratory diagnosis of SARS-CoV-2 infection has been predominantly carried out by detecting viral RNA in nasal or pharyngeal swab samples based on real-time reverse transcription polymerase chain reaction (RT-PCR) assay [3,4]. However, viral loads mainly in lower respiratory tract and specimen collection in upper respiratory tract caused a high false negative rate of RT-PCR diagnosis [5,6]. Mainly caused by low quality specimen collection, the overall positive rate of RNA testing is estimated to be around 30–60 % in COVID-19 patients [7]. Therefore, a rapid and accurate detection method for SARS-CoV-2 infection is urgently needed.

Another most widely used method serological assay is supposedly a powerful approach for timely diagnosis of COVID-19 and detection of antibody against SARS-CoV-2, which was recommended to clinical diagnosis according to the New Coronavirus Pneumonia Diagnosis and Treatment Program (7th edition) published by the National Health
Commission of China [8]. The serological assays used for diagnosis are mainly based on specific antibodies against SARS-CoV-2 proteins. Genomic analysis reveals that SARS-CoV-2 has four major structural proteins including Spike (S) protein, Nucleocapsid (N) protein, Envelope (E) protein, and Membrane (M) protein, as well as a number of accessory open reading frame (ORF) proteins [3,9]. In this study, we evaluated the performance of Chemiluminescence Microparticle Immunoassay (CMA) which was developed based on recombinant spike protein for detecting IgM and total antibodies against SARS-CoV-2 in human serum. A total of 206 serum samples from confirmed COVID-19 patients and 270 serum samples from healthy blood donors were tested by CMA in the study. In addition, the potential influence factors of antibody production were analyzed.

2. Material and methods

2.1. Patients and samples

A total of 206 serum samples were collected from patients who were treated in the General Hospital of the Central Theater Command of the People’s Liberation Army (PLA) between January 18 and April 4, 2020. One sample was collected from each patient. All the patients were laboratory-confirmed cases with SARS-CoV-2 infection, who were tested positive for viral RNA by real time RT-PCR assay on pharyngeal swab specimens. Real time RT-PCR was performed using the nucleic acid testing kit (Daan, Guangzhou, China) for SARS-CoV-2 detection as previously described [10]. A patient was categorized as critical case if any of the bellowed clinical scenes appeared: 1) with Acute Respiratory Distress Syndrome or oxygen saturation < 93 % and needing mechanical ventilation either invasively or non-invasively; 2) shock; and 3) complication of organ functional failure and need intensive care unit support. A patient did not meet the above criteria was defined as non-critical case. The general information was extracted from electronic medical records. The control serum samples were randomly collected from 270 healthy blood donors who donated blood in May 2019, in Wuhan, China. The healthy blood donors were healthy people without other infection and autoimmune diseases. One serum sample was collected from each healthy donor. The demographics (including age and gender) of patients and healthy donors were compared, with no significant differences. This study was approved by the Hospital Ethics Committee of the General Hospital of the Central Theater Command of the PLA ((2020)003-1). The written informed consent was waived by the Ethics Commission of the designated hospital for emerging infectious diseases.

2.2. Evaluation of the chemiluminescence microparticle immunoassay

The IgM and total antibodies (Ab) against SARS-CoV-2 in serum samples was tested using chemiluminescence microparticle immunoassays. The CMA reagents were supplied by Xiamen InnoDx Biotech Co., Ltd., China (Xiamen, China). The receptor binding domain (RBD) of the SARS-CoV-2 spike protein was expressed by mammalian cell and used to develop serological assays. The CMA for IgM antibodies detection was based on μ-chain capture immunoassay (IgM-CMA), while that for total antibodies detection was based on double-antigens sandwich immunoassay (Ab-CMA). The measurement process of CMA was conducted with automatic analyzer Caris 200 (Xiamen UMIC Medical Instrument Co. Ltd., China), and 200 tests per hour can be completed by the analyzer. And the cutoff value of IgM and total antibodies were calculated according to the manufacturer’s instructions. A test was determined as positive if the signal/cutoff (S/CO) ratio > 1.0. The antibody lever was positively associated with relative light unit (RLU) detected by Caris 200 system, and was shown using the S/CO value of each assay.

2.3. Statistical analysis

Categorical variables were expressed as the counts, percentages and compared using the chi-square test, while the Fisher exact test was used when the data were limited. Continuous variables were described as the means and standard deviations or medians and interquartile ranges (IQR) values. Independent group tests were applied to continuous variables that were normally distributed; otherwise, the Mann-Whitney test was used. Statistical analyses were performed using SPSS version 22.0. A two-sided P-value < 0.05 was considered statistically significant.

3. Results

3.1. Performance of serological assays for COVID-19 diagnosis

The serum samples were collected from 206 admitted hospital patients with confirmed SARS-CoV-2 infection in General Hospital of Central Theater Command of PLA from January 18 to April 4, 2020. One sample was collected from each patient. Each serum sample of 206 patients were respectively tested for IgM and total antibodies (Ab) against SARS-CoV-2 by using CMA based on S protein of SARS-CoV-2. The overall positive rate of IgM and Ab were 149 (72.3 %) and 187 (90.8 %), respectively (Table 1). The result demonstrated that the sensitivity of Ab was significantly higher than that of IgM (P < 0.001). Moreover, we analyzed the seroconversion of IgM and Ab in different groups of patients who with different days of post onset (d. p. o.). According to the days from symptom onset to serum collection, patients were divided into four groups, including 0–7, 8–14, 15–21 and more than 21 d.p.o.. The positive rates and S/CO values of IgM and Ab in different groups were shown in Table 1. For IgM detection, the positive rate was low before 14 d.p.o. and increased to 86.1 % rapidly after 14 d.p.o.. For Ab detection, the positive rate detected by CMA was merely 53.8 % at the early stage of 0–7 d.p.o. but rapidly increased to 91.7 % after 7 d.p.o.. The durations of illness onset for detecting Ab were shorter than that of IgM. For both IgM and Ab detection, the positive rate and S/CO value simultaneously showed a significant upward trend.

| Table 1 | Performance of Chemiluminescence Microparticle Immunoassay for detection of IgM and Ab in serum samples of patients at different stages after disease onset. |
|---------|-------------------------------------------------------------------------------------------------|
| Days    | No. of serum samples | No. (%) of positive | Median (IQR) S/CO of positive |
|         |                     | IgM | Ab   | IgM | Ab   |
| 0–7     | 26                   | 9(34.6) | 14(53.8) | 1.8(1.5–3.6) | 95.6(2.9–244.5) |
| 8–14    | 70                   | 45(64.3) | 67(95.7) | 4.1(2.3–9.7) | 272.7(35.0–550.1) |
| 15–21   | 72                   | 62(86.1) | 69(95.8) | 5.2(2.0–10.5) | 395.4(149.6–672.3) |
| > 21    | 38                   | 33(86.8) | 37(97.4) | 5.2(2.5–9.1) | 462.9(179.5–788.9) |
| Total   | 206                  | 149(72.3) | 187(90.8) | 4.8(2.1–9.1) | 321.9(101.3–633.8) |

Days: Days post-disease onset.  
No.: Number.  
IQR: Interquartile range.
with the increasing of d.p.o.. These results demonstrated that course of disease is an important factor that has effects on the seroconversion and titer of antibodies against SARS-CoV-2. To verify the specificity of the CMIA, 270 samples from healthy blood donors were analyzed. The specificity of IgM and Ab were 99.3 % (268/270) and 98.9 % (267/270), respectively. Taken together, we can give an idea that CMIA simultaneously has a high sensitivity and specificity in diagnosis of SARS-CoV-2 infection.

3.2. Influence factors of antibody production

Among the enrolled patients, 126 (61.1 %) were males, and 80 (38.8 %) were females. The median age of these patients was 57 years (IQR, 43–68 years), ranging from 17 to 91 years. And 54 patients were critical cases, 152 patients were no-critical cases according to the severity of clinical symptoms as described above. To study the factors associated to the production of antibodies in COVID-19 patients, we investigated the characteristics of antibody responses in these patients. Several potential factors (sex, age, and condition) that may have effects on the seroconversion and titer of antibodies against SARS-CoV-2 were analyzed according to different stages of disease onset. The seroconversion and titer of antibodies (IgM and Ab) against SARS-CoV-2 was respectively displayed as positive rate and S/CO values of positive results.

During whole stage of disease onset, no significant differences were observed in the positive rates and S/CO values between males and females for IgM and Ab detection (Table 2). Similarly, no significant differences were observed between the groups of age less than 60 years and above 60 years for detecting IgM and Ab (Table 3). In addition, the positive rates for IgM and Ab tests in critical cases were respectively equal to those in no-critical cases during whole stage of disease onset. Nevertheless, the S/CO values of critical cases for IgM and Ab detection were significantly higher than those of no-critical cases after 14 d.p.o., while the S/CO values of IgM and Ab were not statistically different between the two groups before 14 d.p.o. (Table 4).

4. Discussion

The ongoing worldwide pandemic of COVID-19 caused by SARS-CoV-2 poses a great threat to the world. The rapid and accurate diagnostic methods are the crucial strategy for recognizing this disease, and contribute to public health surveillance, prevention and control measures. Real time RT-PCR is the primary method for COVID-19 diagnosis at present [4]. However, in the early stage of the outbreak, the inadequate access to reagents and equipment, needing of skillful operators and high rate of false negative results have resulted in low efficiency of in-time detection of this disease. Serological assays are urgently needed for diagnosis, contact tracing, epidemiologic and vaccine evaluation studies. Although, some serological assays have been developed regarding this virus [11–15], which were mainly based on enzyme linked immunosorbent assays and colloidal-gold lateral-flow immunoassays. In this study, we evaluated the serological assay based on Chemiluminescence Microparticle Immunoassay (CMIA) for detecting antibodies against SARS-CoV-2. 206 patients with confirmed SARS-CoV-2 infection were enrolled to investigate the diagnostic value of CMIA. Meanwhile, we also researched the dynamic variance of IgM and Ab, and the influence factors of antibody production.

The study found that the sensitivity of Ab (90.8 %) was significantly higher than that of IgM (72.3 %). We speculate the difference is due to the relatively high sensitivity of Ab that detected all antibodies (including IgM) and IgM detected immunoglobulin M only. Moreover, that possibly attributed to the double-antigen sandwich assay that used for detecting Ab usually show much higher sensitivity than capture assay that used for detecting IgM. The positive rate for IgM detected by CMIA was equal to that for IgM detected by recombinant S protein (rS)-based ELISA (165/214, 77.1 %) that we evaluated previously [14]. In our previous study [14], the rS-based ELISA combined IgM or IgG detection has identified 176 patients with positive results and the total positive rate for antibodies (IgM and/or IgG) detection reached 82.2 % (176/214). The positive rate of CMIA (90.8 %) for Ab was significantly higher than that (82.2 %) of ELISA (P < 0.05), which further demonstrate the high sensitivity of CMIA for total antibodies against SARS-CoV-2.

We divided the patients into different groups according to d.p.o. and observed a gradually increased positive rate and S/CO values accompany with the increase of d.p.o for IgM and Ab detection. This difference of positive rate and S/CO values between the groups is consistent with the regular of antibody production. The positive rates of IgM and Ab were 34.6 % and 53.8 %, respectively, at the early stage of 0–7 d.p.o., which may be explained by that serum antibodies in most of COVID-19 patients have not been produced at 0–7 d.p.o.. Hence, the SARS-CoV-2 infection cannot be excluded on account of negative results at the early stage of illness onset. The positive rate for Ab detection was rapidly increased to above 90 % after 7 d.p.o. However, the positive rate for IgM and/or IgG detection by rS-based ELISA was extremely increased after 10 d.p.o., we evaluated previously [14]. The durations of illness onset for detecting Ab by CMIA were shorter than that by ELISA, which indicates that Ab detected by CMIA can shorten the “window period” for COVID-19 diagnosis compared with that detected by ELISA. The study has reported that the overall RNA positive rate was lower than 70 % in COVID-19 patients on the first week post symptoms onset and fall to 50 % on the next week [11]. Therefore, the serological assay especially CMIA assay could be an exact complement for RNA detection.

We also investigated the influences of antibody production. In order to exclude the influence of onset time, we divided the patients into two groups: the 0–14 d.p.o. group and the >14 d.p.o. group. The results revealed that age and gender of the patients did not affect the production of antibodies. The positive rate for IgM and Ab test was not significantly different between critical case and no-critical case. But the S/CO value of critical case for IgM and Ab detection was significantly higher than no-critical case at >14 d.p.o. That possibly attributed to the critical patients have a stronger immune response at the stage of >14 d.p.o.. The study showed that the production of antibodies mainly affected by the onset time, the longer period post onset suggests the higher seroconversion rate and antibody levels. And the severity of disease also affects the antibody levels when the onset time >14 d.p.o..

This study demonstrated that the CMIA of serological assay could be

Table 2

The effect of gender on antibody detection.

| Days | No. (%) of positive for IgM | P | Median (IQR) S/CO of positive for IgM | P | No. (%) of positive for Ab | P | Median (IQR) S/CO of positive for Ab |
|------|-----------------------------|---|--------------------------------------|---|---------------------------|---|-------------------------------------|
|      | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 0−14 | 33/57 | 23/29 | 0.695 | 3.1 (1.8−7.0) | 4.8 (2.3−10.9) | 0.252 | 48/57 | 33/39 | 0.957 | 181.2 (36.9−415.7) | 161.2 (22.8−944.4) | 0.408 |
| >14  | 62/69 | 33/41 | 0.166 | 4.8 (2.3−8.9) | 6.1 (1.8−11.4) | 0.432 | 67/69 | 39/41 | 0.628 | 371.2 | 481.3 | 0.658 |

Note: P < 0.05 was considered significant difference.
an important complementary detection of RNA assay for COVID-19 diagnosis. And CMIA has high sensitivity and specificity for the detection of serum samples, especially the durations from symptom onset to this serological test was more than 7 days. CMIA also takes the advantages of automatic-operation, high-throughput and rapid, but the instrument was costly. This method provides us a flexible choice of serological immunoassays. It should be recommended to apply in the clinical management and epidemiological surveillance. We also found that the age and gender do not affect the production of antibodies, the days of post onset was the main factor to influences the antibodies production for COVID-19 patients. The disease-severity of COVID-19 patients affect the antibodies levels. The specific mechanisms and whether there are other factors affecting antibody production need further study.

Authors’ contributions
S.Z. and W.L. conceived the study and designed experimental procedures. L.L., G.K., Y.D., Y.Z., Y.D., W.N., W.W., S.T., Z.X., and Y.Z. collected patients’ samples. L.L. and G.K. established the CMIA and performed serological assays. W.L., S.Z., and L.L. wrote the paper. All authors contributed to data acquisition, data analysis, and/or data interpretation, and reviewed and approved the final version.

CRedIT authorship contribution statement
Wanbing Liu: Formal analysis, Data curation, Writing - original draft. Guomei Kou: Formal analysis, Data curation, Writing - original draft. Yaoyu Dong: Investigation, Formal analysis, Resources. Yaqiong Zheng: Investigation, Formal analysis, Resources. Yinjing Dian: Investigation, Formal analysis, Resources. Wenwu Ni: Investigation, Formal analysis, Resources. Wanlei Wu: Investigation, Formal analysis, Resources. Shitang: Investigation, Formal analysis, Resources. Yiming Zang: Investigation, Formal analysis, Resources. Lei Li: Conceptualization, Methodology, Project administration, Writing - review & editing. Shangen Zheng: Conceptualization, Methodology, Project administration, Writing - review & editing.

Table 3
The effect of age on antibody detection.

| Days | No. (%) of positive for IgM | P | Median (IQR) S/CO of positive for IgM | P | No. (%) of positive for Ab | P | Median (IQR) S/CO of positive for Ab | P |
|------|---------------------------|---|--------------------------------------|---|---------------------------|---|--------------------------------------|---|
| ≤60  | > 60                      | ≤60| > 60                                 | ≤60| > 60                      |
| 0–14 | 25/50 (50.0)              | 29/46 (63.0) | 0.198 (3.5 (2.0–8.6) | 3.0 (1.8–8.2) | 0.621 (41/50 (82.0) | 40/46 (87.0) | 0.504 (135.8 (22.9–651.9) | 284.8 (60.9–445.8) | 0.699 |
| >14  | 60/68 (88.2)              | 35/42 (83.3) | 0.467 (4.8 (2.2–9.0) | 5.4 (2.7–10.8) | 0.603 (65/68 (95.6) | 41/42 (97.6) | 0.977 (413.5 | 393.2 (172.9–729.5) | 4.0 (142.2–690.0) | 0.473 |

Table 4
The effect of disease severity on antibody detection.

| Days | No. (%) of positive for IgM | P | Median (IQR) S/CO of positive for IgM | P | No. (%) of positive for Ab | P | Median (IQR) S/CO of positive for Ab | P |
|------|---------------------------|---|--------------------------------------|---|---------------------------|---|--------------------------------------|---|
| ≤60  | > 60                      | ≤60| > 60                                 | ≤60| > 60                      |
| 0–14 | 13/22 (59.1)              | 41/74 (55.4) | 0.760 (2.6 (1.6–12.1) | 4.1 (2.0–8.6) | 0.379 (20/22 (90.9) | 61/74 (82.4) | 0.531 (215.3 | 685.7 (61.1–685.7) | 178.4 (22.9–486.1) | 0.701 |
| >14  | 28/32 (87.5)              | 67/78 (85.9) | 0.003 (8.7 (4.9–13.3) | 4.4 (2.0–8.2) | 0.457 (32/32 (100.0) | 74/78 (94.9) | 0.457 (538.6 | 436.0 (229.6–1057.7) | 0.043 |

Declaration of Competing Interest
The authors declare that no conflict of interest exists.

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