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Colloidal gold immunochromatographic assay (GICA) is an effective screening method for identifying detectable anti-SARS -CoV-2 neutralizing antibodies

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ABSTRACT

Introduction: A large number of COVID-19 patients are in recovery, and millions of people are vaccinated for COVID-19 globally. This calls for a rapid screening strategy of SARS-CoV-2 protective antibodies, generated in rehabilitated and vaccinated populations.
Methods: Serum samples collected over a follow-up period of six months from 306 COVID-19 cases discharged from Wuhan Tongji Hospital were analyzed. Anti-S Abs were detected by colloidal gold immunochromatographic assay (GICA), and neutralizing antibodies (nAbs) were detected by chemiluminescent microparticle immunoassay (CMIA).
Results: Most COVID-19 survivors tested positive for anti-S Abs (83.7%) and nAbs (98.0%) 6 months after being discharged from the hospital, and the levels of anti-S Abs in the blood were highly positively correlated with nAbs (r = 0.652, P < 0.0001). The positivity rate of nAbs for patients with anti-S Abs positive was 100%.
Conclusions: There is a good agreement between anti-S Abs detected by GICA and nAbs detected by CMIA. It indicates that anti-S Abs detected by GICA may be used as a cheaper screening strategy for detectable SARS-CoV-2 nAbs in COVID-19 convalescent individuals.

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Introduction

The ongoing global SARS-CoV-2 pandemic has placed an enormous burden on the global public health system and the economy at large. Each day, hundreds of thousands of new confirmed cases are recorded worldwide (WHO, 2021). Concurrently, many patients are in recovery, some of whom are still at risk of reinfection after rehabilitation due to the lack of or inability to produce adequate protective antibodies (Hall et al., 2021; Lumley et al., 2021). Therefore, there have been efforts to develop effective vaccines as the primary means to curtail the detrimental effects of the SARS-CoV-2 infection; so far, millions of people have been vaccinated globally (Chung et al., 2021; Mohammad, 2021). To inform vaccination and optimize immunization strategies, rapid assessment of the level and duration of protective antibodies in natural post-infected and recovered patients make sense.

However, the plaque reduction neutralization test (PRNT) and the microneutralization assay (NT), the gold standards for determining antibody neutralizing activity against SARS-CoV-2, cannot be widely adopted because of their low flux, time-consuming, and fussy operation (CDC, 2021). Recently, several companies have developed reagents such as chemiluminescent microparticle immunoassay (CMIA) kits based on the principle of competitive inhibition to detect SARS-CoV-2 nAbs. The reagents could be widely used because of their high throughput and sensitivity properties (Bonelli et al., 2020; Taylor et al., 2021). However, limited by expensive instruments, it is hard to widely serve undeveloped countries and regions with backward economies. Therefore, cheap, accurate, simple, and rapid methods for quantifying serum nAbs in recovered patients are valuable to determine the duration of antibody response after infection, which

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can guide the development and refinement of vaccine and public immunization strategies. Herein, we reported a good agreement between anti-S Abs detected by colloidal gold immunochromatographic assay (GICA) and nAbs detected by CMIA. Furthermore, all positive anti-S Abs results identified by GICA were also nAbs positive, indicating that this method can be used as a cheaper screening strategy for SARS-CoV-2 nAbs.

Methods

Study participants

In this study, 306 patients recovered from COVID-19 admitted at the Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China, were enrolled. The patients were admitted to the hospital between January and February 2020 and had no reinfection during the follow-up period (Li et al., 2020). The diagnosis was confirmed based on positive high-throughput sequencing of nasopharyngeal swab specimens or nucleic acid detection using real-time reverse transcription-polymerase chain reaction (RT-PCR), according to interim guidelines of the World Health Organization (WHO, 2020). All patients were tested multiple times throughout to ensure diagnostic accuracy. All patients were followed up after discharge to ensure no reinfection occurred. Serum samples were collected six months after discharge for assessment of protective antibody levels.

Disease grading

The grading of disease on admission was based on the Chinese management guidelines for COVID-19 (version 7.0) (NHC, 2020). Mild cases were defined as patients with mild clinical symptoms and pneumonia manifestation undetectable by imaging examination. Moderate cases were defined as patients presenting with clinical symptoms and pneumonia features detectable by imaging tools. Severe cases were those presenting with any of the following: respiratory distress with RR $\geq$ 30 times/min; pulse oxygen saturation (SpO_2) $\leq$ 93% at rest; arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) $\leq$ 300 mmHg (1 mmHg = 0.133 kPa). Critical cases were those showing one of the following features: respiratory failure that needs mechanical ventilation, shock, multiple organ failures that call for monitoring of intensive care unit (ICU), or death.

Detection of total antibodies

Six months after discharged from the hospital, blood samples were collected from patients undergoing rehabilitation by experienced doctors. The blood samples were centrifuged to obtain serum which was stored at 4 °C. All antibody tests were performed within 24 h after sample collection.

The colloidal gold immunochromatographic assay (GICA) reagents (Wondfo, Guangzhou, China, batch number: W19501002), which specifically target the spike glycoprotein (S-protein) antibody, were used to measure the total antibody of SARS-CoV-2 in serum (IgM and IgG). Briefly, 10 µl serum samples and 80 µl diluting water (about 2–3 drops) were added to the hole of the test card, and the test results were observed after15–20 min. If the sample contained IgM or/and IgG antibodies of COVID-19, the antibodies would bind to the recombinant antigen encapsulated in colloidal gold particles to form a composite substance. Anti-S Abs positive produces a red reaction line in the test area (T) and the quality control area (C). The sensitivity, specificity, positive predictive value, and negative predictive value of total antibody were 86.43%, 99.57%, 93.41%, and 100%, respectively.

A colloidal gold immunochromatographic card reader (Hurray Star, Beijing, China) was used to identify the C and T-lines, calculate antibody concentration, and determine negative and positive rates. Antibody levels were graded into five levels (from negative to +++ +), where a negative result was defined as the presence of the C-line and the absence of the T-line, + was a detectable T-line but faint; ++ was defined as a T-line $\leq$50% intensity of the C-line; +++ was defined as the T-line >50%, but $\leq$100% intensity of the C-line and ++++ was defined as the T-line >100% intensity of the C-line. A typical grading diagram of anti-S Abs detected by GICA is shown in Figure 1.

Detection of neutralizing antibodies

NAbs were assayed using COVID-19 neutralizing antibody detection kits (Hotgen, Beijing, China, batch number: 21010115) based on chemiluminescent microparticle immunoassay (CMIA). The serum was collected and prepared in the same way as before. All operations were carried out in strict accordance with the instructions of the reagent manufacturer. According to the competing strategies, in short, the test was performed by reacting the sample with alkaline phosphatase (ALP)-labeled S-RBD antigen. The mixture was incubated to form a neutralizing antibody S-RBD antigen complex. Then, biotin-labeled receptor protein ACE2 and magnetic microspheres encapsulated with streptavidin were added to promote attachment of the ACE2-S-RBD antigen complex to the magnetic microspheres by specific binding of biotin and streptavidin. A matched high throughput automatic chemiluminescence immunoassay analyzer was used to analyze nAb levels which were presented as the chemiluminescence signal values divided by the cutoff (absorbance/cutoff, S/CO). Based on the scanned parameter information in the kit, the analyzer automatically calculated the cutoff (CO) after calibration of Calibration 1 and Calibration 2. Each test consumed about 50 µl of serum samples, and the result was considered a positive test when the S/CO level was lower than 1. To better display the difference of nAb levels between the recovered COVID-19 patients and healthy people, we included the nAb test results of 200 healthy individuals as healthy controls. The healthy controls had never been exposed to SARS-CoV-2 and were negative for both nucleic acid and antibody tests.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared with the χ^2 test or Fisher's exact test as appropriate and were expressed as counts or percentages. Correlation analysis was performed using the Spearman analysis. Variables that did not conform to a normal distribution were presented as the
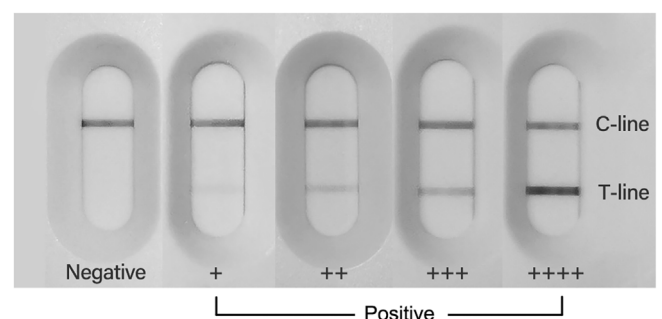
Figure 1. A typical grading diagram of anti-S Abs detected by GICA. Antibody levels of anti-S Abs were graded into five levels (from negative to ++++), and positive test results included +, ++, +++, and ++++.

interquartile range (IQR). Figures were plotted using Graphpad Prism 8.0.

Results

Demographic characteristics among 306 discharged COVID-19 patients

The patients' median age was 62 years (interquartile 53–68 years), and 45.1% (138/306) were male. Among them, 40.5% (124/306) were graded as severe cases, whereas 2.6% (8/306) were diagnosed as critical cases; these two categories were equally distributed between sexes (Table 1).

Relationship between anti-S Abs and nAbs in recovered COVID-19 patients

Six months after being discharged from the hospital, 83.7% (256/306) of COVID-19 survivors tested positive for anti-S Abs and 98.0% (300/306) positive for nAbs. Of the 256 patients that tested positive for anti-S Abs, all of them had detectable nAbs; the positivity rate of nAbs for patients with anti-S Abs positive was 100%, whereas the positivity rate of nAbs in patients with anti-S Abs negative was 88.0% (44/50). No patient tested positive for anti-S Abs and had a negative nAbs result (Table 2). The level of anti-S Abs in the blood was highly positively correlated with nAbs (r = 0.652, P < 0.0001) (Figure 2). As the level of anti-S Abs fell from +++ + to negative, the S/CO value of nAbs in recovered patients increased progressively, indicating a downward trend in nAbs levels. The S/CO values for healthy controls (HC) were all above 1, which denoted a negative nAbs.

Distribution characters in different antibody subgroups

Variances in the severity of disease, age, and gender were also observed among different antibody subgroups. In terms of disease severity, patients who tested negative for anti-S Abs and nAbs all presented with moderate disease, while in anti-S Abs and nAbs positive group, patients were mainly severe (54.2%), followed by moderate (42.6%) and critical (3.2%) (Figure 3b). Compared to anti-S Abs and nAbs positive group, anti-S Abs and nAbs negative patients were predominantly young and middle-aged adults (66.7% vs. 16.8%) (Figure 3a) and male (66.7% vs. 46.9%) (data not shown).

Discussion

After SARS-CoV-2 infection or vaccination, the human immune system produces neutralizing antibodies (mainly anti-RBD) that bind to the S1 RBD epitopes, which hinders the ability of the virus to infect human cells (Garcia-Beltran et al., 2021). Earlier studies indicated that serum levels of protective antibodies drop sharply during the rehabilitation of patients (Gaebler et al., 2021; Isho

Table 1
Demographic information of 306 discharged COVID-19 patients.

| Variables	Discharged patients (n = 306)
Sex, n (%)	
Male	138 (45.1)
Age (yrs), median (IQR) or n (%)	62 (53, 68)
>50	253 (82.7)
Severity, n (%)	
Moderate	174 (56.9)
Severe	124 (40.5)
Critical	8 (2.6)

Data are shown as the median (IQR) or n (%). IQR, Interquartile range.

Table 2
The positivity agreement between anti-S Abs and nAbs among 306 discharged COVID-19 patients' serum.

	Nabs (P)	Nabs (N)	Total
Anti-S Abs (P)	256	0	256
Anti-S Abs (N)	44	6	50
Total	300	6	306

Data are shown as n. P, positive test results, anti-S Abs (P) included +, ++, +++, and ++ ++; N, negative test results.
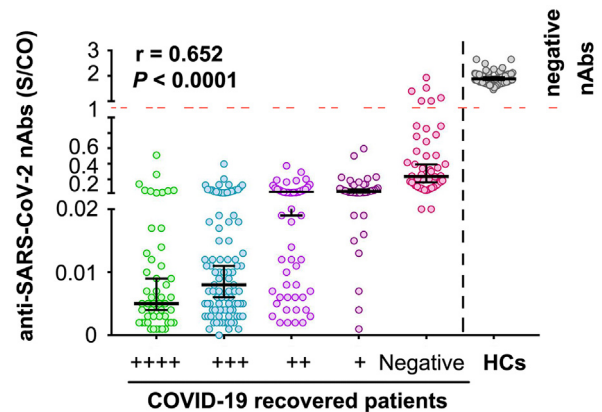
Figure 2. Detection and analysis of the relationship between anti-S Abs and nAbs in samples from 306 recovered COVID-19 patients. HCs, healthy controls, are healthy people who have not been exposed to SARS-CoV-2 and have tested negative for both nucleic acid and antibodies.
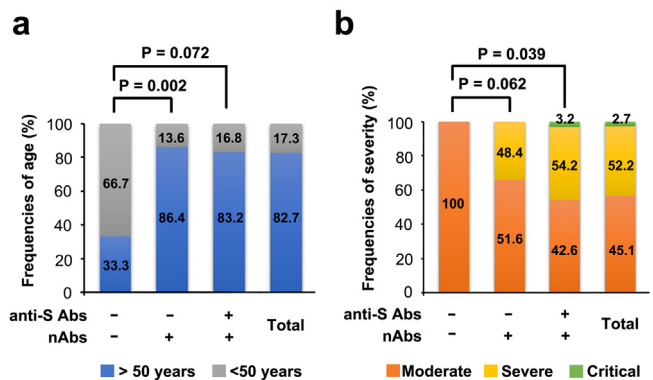
Figure 3. Distribution of age and disease severity in different antibody subgroups. +, positive test results; −, negative test results.

et al., 2020). This demonstrated that the protective power of antibodies may decrease over time. Although it has been suggested that vaccination results in a higher nAb titer compared to titers generated from recovery sera, the actual efficacy and duration of vaccine-induced antibody response are unclear (Jackson et al., 2020). The WHO recommends that the duration of protection conferred by the COVID-19 vaccine exceeds six months. Consequently, clinical and public health surveillance is required to monitor plasma nAb levels in individuals who have recovered from COVID-19 or have been vaccinated, to develop more effective strategies against reinfection and improve the available vaccines.

Currently developed methods and commercially available kits for the evaluation of nAb levels can be classified as fluorescence-based assays, lateral flow immunoassays (LFA), enzyme-linked immunosorbent assays (ELISA), and chemiluminescent immuno-assays (CLIA. Most of these assays have the advantage of high throughput and are dependent on professionals and large, costly

equipment (A et al., 2020; Bonelli et al., 2020; Muruato et al., 2020; Taylor et al., 2021). As a point of care testing (POCT) method, the colloidal gold method has been applied in clinical practice for diagnosing and post-recovery detection of COVID-19. It also features the advantages of sensitive, specific, time-saving, it consumes a small amount of specimen, and is easy to perform without the requirement for special equipment, compared with various other serological testing methods (Fu et al., 2020; Huang et al., 2020).

Previous studies have demonstrated a correlation between IgG titers and nAb titers in the convalescent plasma of COVID-19-recovered patients, suggesting that IgG levels could predict nAb levels to some extent (Klein et al., 2020; Ng et al., 2020). In our study, anti-S Abs detected by GICA and nAbs detected by CMIA presented a clear correlation. This is presumably because the S1-specific antibodies detected by GICA are intimately related to the virus neutralization response. Conversely, N-specific antibodies detected by other serological assays showed a relatively poor correlation with nAb levels (Luchsinger et al., 2020).

Given the close association between anti-S Abs detected by GICA with nAbs, this method could be suitable for primary screening periodic self-evaluation of the protective effects of nAbs in COVID-19 convalescent individual and post-vaccination populations. The latest research has revealed that low levels of nAb titers in convalescents are sufficient to prevent infection, and even lower titers are required to prevent severe symptoms (Khoury et al., 2021). For patients who tested negative for anti-S Abs, this result does not mean that the protection power disappeared, and additional detections should be considered to further improve the efficiency of tests. When judiciously combined, these methods promise reliable monitoring of protective antibody levels, which will help develop strategies to minimize the risk of infection and reinfection.

Author contributions

TS and XL designed the whole study. XL and YY collected and analyzed the data. XL and YY took the lead in drafting and interpreting the manuscript. TS and DX revised the manuscript. All authors participated in the development and revision of statistical methods. Finally, all authors reviewed and approved the manuscript for publication.

Conflict of interest

None declared.

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Ethical approval

The study was approved by the Institutional Review Board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. In addition, the ethics committee of the designated hospital waived the requirement for informed consent.

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