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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulins using chemiluminescence immunoassay and its correlation with neutralizing antibodies

Yasmine Gaber [a,*], Shereen Abdel Alem [a], Sherief Musa [a], Khaled Amer [b], Tarek Elnagdy [b], Wael A. Hassan [b], Raafat Zaher Abdelrahman [c], Ahmed Gad [c], Mohamed A. Ali [d], Hedy A. Badary [a], Shereen Shawky [e], Hala Talaat [f], Abdel Meguid Kassem [a], Rabab Fouad [a]

[a] Endemic Medicine Department, Kasr Al-Aini Faculty of Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt
[b] Egypt Center for Research and Regenerative Medicine (ECRRM), Cairo, Egypt
[c] Armed Forces Laboratories for Medical Research and Blood Bank, Egypt
[d] Center of Scientific Excellence for Influenza Viruses, National Research Center, Giza, Egypt
[e] Clinical Pathology Department, Kasr Al-Aini Faculty of Medicine, Cairo University, Cairo, Egypt
[f] Department of Pediatrics, Kasr Al-Aini Faculty of Medicine, Cairo University, Cairo, Egypt

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ABSTRACT

Background: Neutralizing antibodies (NAbs) against SARS-CoV-2 infection have a pivotal role in protective immune response; however, their measurement requires specialized facilities. We evaluated the degree of correlation between NAbs and anti-SARS-CoV-2 IgG/total Ig antibodies detected by chemiluminescent immunoassay in asymptomatic and previously symptomatic SARS-CoV-2 patients.

Methods: A total of 1241 participants (previously symptomatic patients and asymptomatic individuals), who were screened for SARS-CoV-2 infection by RT-PCR or serology, were enrolled in our study. Sera were analyzed for the presence of anti-spike-1(S1)-SARS-CoV-2 IgG/total Ig antibodies, using Ortho Clinical Diagnostics, USA. A signal/cut-off value (S/CO) $\geq$ 1 was considered reactive. NAbs were measured in 103 random samples from groups using microneutralization assay, with titer $\geq$ 1:10 being considered positive.

Results: Asymptomatic (n = 229) and 261 previously symptomatic individuals with positive serology and negative RT-PCR were finally included. Significant higher anti-S1-IgG titers were seen in asymptomatic individuals ($P < 0.0001$). Conversely, anti-S1-total Ig titers were significantly higher in previously symptomatic ($P < 0.0001$). NAbs were detected in both groups, however, higher titers were seen in previously symptomatic patients. There is a correlation between NAbs and both IgG/total anti-S1-SARS-CoV-2 antibodies ($r = 0.47$, $P < 0.0001$ and $r = 0.49$, $P < 0.0001$, respectively). IgG and total Ig could predict a neutralization titer of $\geq$ 1:160 at S/CO >4.44 and >65 with AUC 0.69 and 0.67, respectively.

Conclusion: Asymptomatic SARS-CoV-2 infection can produce comparable antibodies response to previously symptomatic individuals, however higher neutralization activity was seen in the previously symptomatic. Anti-S1-SARS-CoV-2 IgG/total Ig antibodies showed a correlation with neutralization activity and can be used to estimate the presence of protective immunity.

1. Introduction

Coronavirus disease 2019 (COVID-19) which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic by World Health Organization (2020b). COVID-19 has a wide spectrum of disease severity, ranging from asymptomatic, and mild to a life-threatening disease, with significant morbidity and mortality (Huang et al., 2020).

Confirmed SARS-CoV-2 infection depends on the detection of viral nucleic acid by real-time reverse transcription-polymerase chain reaction (RT-PCR) in pharyngeal or respiratory specimens, which is considered the reference standard method for diagnosis (Mohit et al.,

* Corresponding author.
E-mail address: yasmin.mohamed@cu.edu.eg (Y. Gaber).

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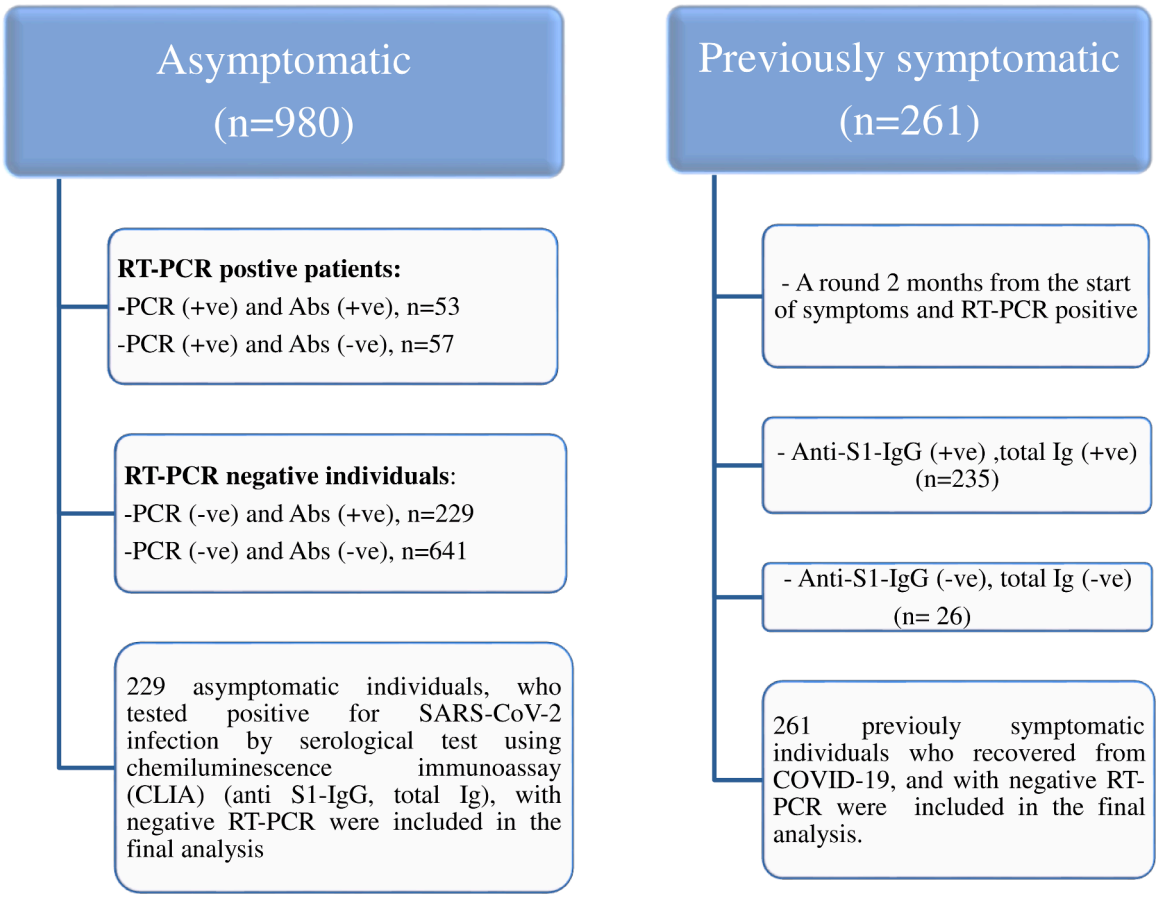

Fig. 1. Diagram outlines the virological and serological characteristics of asymptomatic and previously symptomatic SARS-CoV-2 infection.

2021; Zou et al., 2020). However, this underestimates the true prevalence, as the sensitivity of testing by RT-PCR is only around 50–70% (Stites and Wilen, 2020).

On the other side, different serological tests that have been commercialized, can detect the antibody response against the SARS-CoV-2 virus, including enzyme-linked immunosorbent assays (ELISAs), lateral flow immunoassays (LFIAs), and chemiluminescent immunoassays (CLIAs). Some of these assays depend on the whole inactivated virions, while others determine viral subunits such as the spike or nucleocapsid proteins (Padoan et al., 2021). Chemiluminescent immunoassays are the most sensitive in terms of methodology, produce extremely accurate and precise results, and are mainly used to detect viral nucleocapsid (N) and spike (S) antigens (S1, S2, or receptor-binding domain (RBD) of S1) of SARS-CoV-2, or a combination of them (Shaffaf and Ghafar-Zadeh, 2021). Serological tests are more beneficial in being faster, with lower cost, having a complementary role to RT-PCR in patients with low viral load, and estimating the seroprevalence of the disease (Wolff et al., 2020). On the other side, infection time course can affect the accuracy of serological tests (GeurtsvanKessel et al., 2020), with seroconversion usually occuring 3–14 days after onset of symptom, so early diagnosis of COVID-19 using only serological tests may not be accurate (Wang et al., 2020). Furthermore, serological assays can determine the immunity and the possibility of protection against a re-infection; however, most of the commercially available serological assays detect binding antibodies, but not neutralizing antibodies (Huang et al., 2020; GeurtsvanKessel et al., 2020).

Neutralization assays detect neutralizing antibodies that can effectively bind to the virus and render it incapable of infection, with specificity to the receptor-binding domain of the viral spike protein (Grenache et al., 2021). Viral neutralization assay is the gold standard to determine the presence of protective immunity against SARS-CoV-2 (Huang et al., 2020; GeurtsvanKessel et al., 2020), so it can evaluate vaccine effectiveness, and also identify eligible donors for convalescent plasma therapy (Bonanni et al., 2021). However, neutralization assays for SARS-CoV-2 are of limited availability, requiring biosafety level 3 facilities and skilled personnel (Lee et al., 2021). Therefore, whether antibody levels measured by the commercially available serological assays can be used instead of serum neutralizing activity is an important issue. WHO stated that antibodies detected against SARS-CoV-2 by serological tests, did not grant the presence of protective immunity (Huang et al., 2020; Phelan, 2020). Therefore, we aimed to assess whether antibodies to SARS-CoV-2 infection detected by chemiluminescent immunoassays correlate with viral neutralization in asymptomatic and previously symptomatic SARS-CoV-2 infection.

2. Materials and methods

2.1. Study population

The participants were enrolled consecutively in the study from Kasr Al-Aini hospital during the period from June 1st to June 14th, 2020, during the first wave of the COVID-19 pandemic and before starting vaccination campaigns, where the total number of cases reported in Egypt in this period was around 26,521 at 1st of June (World Health Organization, 2020a). Participants included two cohorts (asymptomatic individuals who did not report symptoms suggestive of or proof of SARS-CoV-2 infection during the last two months, and previously symptomatic patients with confirmed SARS-CoV-2 infection by positive RT-PCR during the last two months, who recovered and turned RT-PCR negative).

The Ethical Committee approval was obtained before starting the study. All the participants were informed of the study and voluntarily

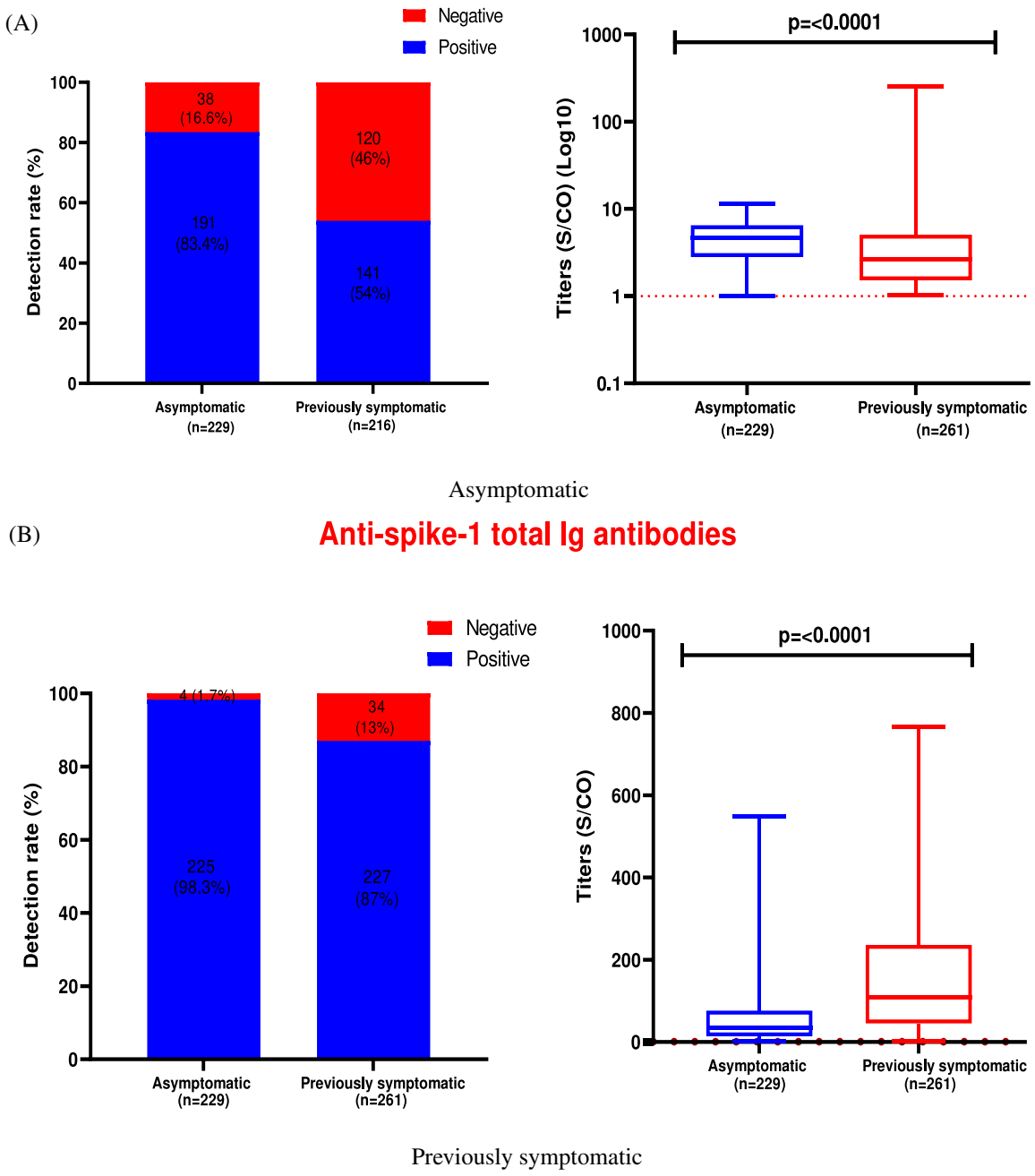

Fig. 2. Anti-SARS-CoV-2 IgG/total Ig antibodies against spike (S1) glycoprotein in sera of asymptomatic and previously symptomatic participants with SARS-CoV-2 infection.

(A) Percentage of detection rate and measurements of anti-spike-1 IgG antibodies in asymptomatic and previously symptomatic SARS-CoV-2 infection. A solid horizontal line indicates the median titers of positive results; a red dotted horizontal line indicates the cutoff for positivity of anti-S1-SARS-CoV-2 IgG antibodies assay. Statistical significance was assessed using the Mann-Whitney U test.

(B) Percentage of detection rate and measurements of anti-spike-1 total Ig antibodies in asymptomatic and previously symptomatic SARS-CoV-2 infection. Solid horizontal line indicates the median titers of positive results; red dotted horizontal line indicates the cutoff for positivity of anti- S1-SARS-CoV-2 total Ig antibodies assay. Statistical significance was assessed using the Mann-Whitney U test.

(C) Venn diagram displaying the proportion of asymptomatic and previously symptomatic individuals who exhibited positive results with anti-S1-SARS-CoV-2 IgG/total Ig antibodies. Values are in n (%).

agreed to participate, providing written consent. After that, all participants fulfilled a questionnaire composed of demographics, occupation, history of comorbid conditions, history of previously confirmed SARS-CoV-2 infection by RT-PCR, and potential exposure to confirmed COVID-19 cases. Following the interview, a nasopharyngeal swab for SARS-CoV-2 nucleic acid detection was taken from all the studied groups, followed by withdrawal of venous blood samples for measuring immunoglobulins using chemiluminescence immunoassay for detection of anti-SARS-CoV-2 total and IgG immunoglobulins, as well as detection of neutralizing antibodies using microneutralization assay that was done for random samples from some participants.

Fig. 2. (*continued*).

2.2. Detection of SARS-CoV-2 RNA by RT-PCR

Nucleic acid was extracted from the upper respiratory specimens (nasopharyngeal, oropharyngeal swabs) and transmitted using Viral Transport Media at designated sites by experienced personnel (VTM). QUIAGEN columns RNA Isolation Kit was used to extract SARS-CoV-2 RNA. The isolated RNA was reverse transcribed into cDNA and amplified in one step using Thermofisher Scientific's TaqPathTM COVID-19 CE-IVD RT-PCR ComboKit, Revision D.0 (Cat.# A48067). Amplification was done using Fast Dx Applied Biosystems 7500 real-time thermal cycler. Probes were annealed to three unique SARS-CoV-2 target sequences: ORF1ab, nucleocapsid (N), and spike (S) primers/probes for bacteriophage MS2. The MS2 (internal process control) and two of the three genes must all be positive for the result to be regarded as conclusive.

2.3. SARS-CoV-2 total and IgG by chemiluminescence immunoassay (CLIA)

After centrifugation for 5 min at 2000 g at room temperature, plasma samples were separated from whole blood. Until further analysis, all plasma samples were kept at 80 °C. Anti-SARS-CoV-2 antibodies against spike glycoprotein were detected using VITROS Total & IgG anti-SARS-CoV-2 test (Ortho Clinical Diagnostics, USA). According to the manufacturer, these tests are chemiluminescent immunoassays for the qualitative detection of serum total (comprising IgG, IgM, and IgA) or IgG antibodies against the spike-1 (S1) of SARS-CoV-2, with a sensitivity and specificity of 100% and 90%, respectively. Antibodies to SARS-CoV-2 found in the sample bind to SARS-CoV-2, spike protein S1 antigen coated on wells in the first step. The conjugate reagent is then supplemented with horseradish peroxidase (HRP)-labeled murine monoclonal anti-human antibodies in the second stage. The conjugate binds to the antigen-antibody complex's antibody component specifically. A luminous reaction is used to determine the amount of SARS-CoV-2 antibody present in the bound HRP conjugate. For both antibodies, a signal/cut-off value (S/CO) of 1 was considered reactive according to manufacturer's instructions.

2.4. Neutralizing antibodies by microneutralization assay

Heat inactivated serum samples for 30 min at 56 °C. Starting at 1:10, twofold serial dilutions were combined with an equal volume of viral solution containing 100 TCID50 of SARS-CoV-2. The serum-virus mixture was incubated in a humidified environment with 5% CO_2 for 1 h at 37 °C. Following incubation, 35 ul of each dilution of the mixture was put in duplicate to a cell plate containing a semi-confluent vero E6 cell monolayer and incubated for 2 h under the same conditions. After aspirating the inoculum, each well received 150 ul of the medium. Before the cultures were examined under a light microscope for the existence of a cytopathic effect (CPE), the plates were incubated for 5 days at 37 °C in a CO_2 incubator. The neutralizing antibody titer was calculated using the reciprocal of the last highest serum dilution that totally prevents the virus from developing CPE (Kandeil et al., 2020).

3. Statistical analysis

Graphpad Prism software, version 9.0 (San Diego, USA), and the MedCalc software (version 20) were used for statistical analyses and graphical presentations. Results were expressed as frequencies and percentages for categorical variables or medians and interquartile ranges (IQR) for continuous variables. Chi-squared or Fisher's exact tests were used for comparisons of categorical variables. The normality was determined using the Shapiro-Wilk normality test. Significance was assessed using the Mann-Whitney U test, which was used for comparisons of continuous variables. The Spearman's rank correlation was used to evaluate the correlation between the obtained different antibody titers, a two-tailed p-value with 95% confidence intervals. The predictive power of the IgG/total Ig antibodies was assessed by the area under the curve (AUC); receiver operating characteristic (ROC) curves were plotted, and cut-off levels, sensitivities, and specificities were calculated. *P*-values < 0.05 were considered significant.

4. Results

4.1. Participants' characteristics

A total of 1241 participants, comprising 980 asymptomatic individuals and 261 previously symptomatic SARS-CoV-2 individuals, were consecutively enrolled in the study from June 1st to June 14th, 2020. All the previously symptomatic individuals were tested negative for RT-PCR at the time of the study and included in the analysis. Out of 980 asymptomatic individuals, 339/980 were tested positive for SARS-CoV-2 infection either by serology or RT-PCR, (32.4% (110/339) were RT-PCR positive for SARS-CoV-2 infection (presumed to have an acute infection) and 67.6% (229/339) were RT-PCR negative with positive serology results) as shown in Fig. 1. For the subset of asymptomatic participants, individuals who tested positive for SARS-CoV-2 infection by serology, and with negative RT-PCR were only included in the final analysis, together with the previously symptomatic group. The median (IQR) age of asymptomatic participants was 30 (22–36) years and 68.1%

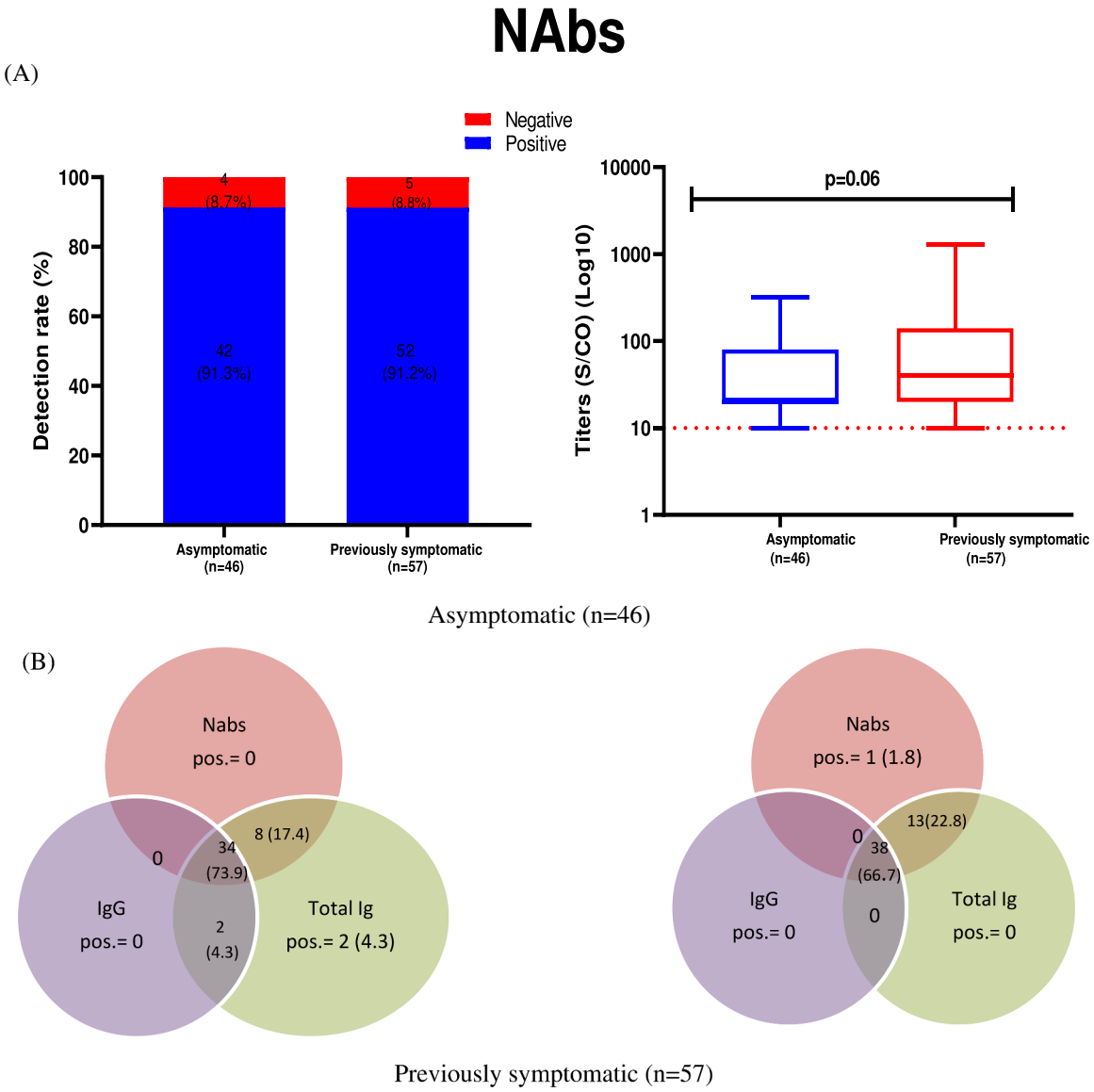

Fig. 3. Neutralization activity in sera of asymptomatic and previously symptomatic participants with SARS-CoV-2 infection.
(A) Percentage of detection rate and antibodies measurements in asymptomatic and previously symptomatic SARS-CoV-2 infection. A solid horizontal line indicates the median titers of positive results; red dotted horizontal line indicates the limit of detection (LOD) of anti-S1-SARS-CoV-2 neutralizing antibodies assay. Statistical significance was assessed with the Mann-Whitney U test.
(B) Venn diagram displaying the proportion of asymptomatic and previously symptomatic individuals who exhibited positive results with anti-SARS-CoV-2 neutralizing antibodies. Values are in n (%).
(C) Distribution of NTs falling to various quantitative categories in asymptomatic and convalescent SARS-CoV-2 infection.

(156/229) were male, whereas the median (IQR) age of previously symptomatic patients was 25 (22–30) years and 51.3% (134/261) were female.

4.2. SARS-CoV-2-specific antibodies in sera from asymptomatic and previously symptomatic individuals

We used Ortho Clinical assay to measure SARS-CoV-2-specific antibodies targeting the S1 subunit of the spike protein of SARS-CoV-2 in sera from asymptomatic and previously symptomatic individuals. Fig. (2, panel A) showed that asymptomatic and previously symptomatic individuals had detectable levels of anti-S1-SARS-CoV-2-specific IgG and total Ig. The positive rate of IgG antibodies was higher among the asymptomatic group compared to the previously symptomatic group, the positive rate was 83.4% (191/229), and 54% (141/261) in asymptomatic and previously symptomatic individuals, respectively. Consequently, the positive rate of total Ig antibodies was higher among the asymptomatic compared to the previously symptomatic group, total Ig-positive rates were detected in 98.3% (225/229), and 87% (227/261), of the asymptomatic group, compared to a previously symptomatic group, respectively. The used serological assay enables the semi-quantitative measurement of antibody titers as well as a threshold-based positive/negative result. We next measured the median titers of positive results for IgG/total Ig antibodies for each category as shown in Fig. 2 (Panel A). The median titers of IgG antibody showed significantly higher levels in asymptomatic individuals compared to previously symptomatic (4.66 (2.8-6.5) S/CO vs. 2.6 (1.5-5.1) S/CO, $P < 0.0001$, respectively). In contrast, the corresponding median titers of total Ig antibody displayed significantly higher levels in previously symptomatic compared to asymptomatic individuals (109 (45.1-236) S/CO vs. 32.9

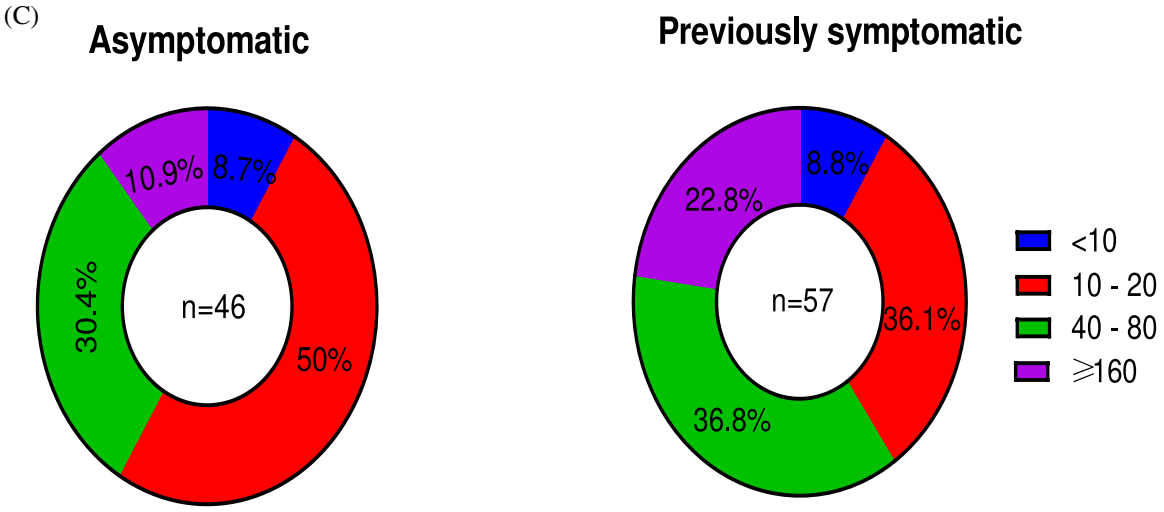

Fig. 3. (*continued*).

(13.6-72.6) S/CO, $P < 0.0001$, respectively). Subsequently, we try to explore the proportion of individuals in each category who had positive results with anti-SARS-CoV-2 IgG/total Ig antibodies as shown in Fig. 2 (Panel B). Notably, the percentage of asymptomatic individuals who had positive results with both anti-SARS-CoV-2 IgG/total Ig antibodies was significantly higher than previously symptomatic individuals (81.7% (187/229) vs. 51% (133/261), $P = 0.001$, respectively).

4.3. Anti-SARS-CoV-2 neutralizing antibody responses

To measure neutralizing antibody activity, a total of 103 serum samples were randomly assessed using a microneutralization assay (46 serum samples from asymptomatic, and 57 samples from previously symptomatic individuals). Fig. 3 (Panel A) showed the detection rate of neutralization titers (NTs) in each category. Forty-two out of 46 (91.3%) asymptomatic individuals had detectable SARS-CoV-2 neutralizing antibodies (NAbs) but at heterogeneous titers ranging from 1:10-1:320, as 8.6% (4/46) and 2.1% (1/46) had titers 1:160 and 1:320, respectively. None of the asymptomatic individuals had a titer ≥1:640. However, SARS-CoV-2 NAbs were generated in 91.2% (52/57) of previously symptomatic within the range of 1:10–1:1280, whereas 13.5% (7/52), 7.7% (4/52), 1.9% (1/52), and 1.9% (1/52) of them exhibited titers 1:160, 1:320, 1:640, and 1:1280, respectively. As observed in Fig. 3 (Panel A), the median value of neutralization in previously symptomatic exhibited higher titers than in asymptomatic individuals, (1:40 (1:20–1:120) vs. 1:20 (1:20–1:80), $P = 0.06$, respectively). Again, we evaluated the proportion of individuals in each category who had positive results with anti-S1-SARS-CoV-2 IgG/total Ig/NAbs as shown in Fig. 3 (Panel B). The proportion of individuals who had positive results with anti-S1-SARS-CoV-2 IgG/total Ig/NAbs was higher in asymptomatic than previously symptomatic individuals (73.9% (34/46) vs. 66.7% (38/57), respectively). Furthermore, the distribution of NT values in each category was plotted in Fig. 3 (Panel C).

4.4. Comparison between anti-SARS-CoV-2 IgG/total Ig antibodies by Ortho Clinical assay and neutralization activity

First, IgG antibody titers were evaluated for correlation with total Ig antibody titers then both antibodies were evaluated for correlation with NTs, which is currently the gold standard for determining anti-SARS-CoV-2 protective immunity. We observed a correlation between total Ig and IgG antibodies titers (r=0.35, $P = < 0.0001$). Moreover, a significant correlation was found between NTs and both total Ig and IgG antibodies titers ($r = 0.49$, $P = < 0.0001$ and $r = 0.47$, $P = < 0.0001$, respectively) (Fig. 4). Second, to predict neutralization activity, we calculated cut-off values for anti-S1 IgG/total Ig antibodies. Using the ROC curves in predicting neutralizing antibodies, we identified an IgG S/Co cutoff value of > 1.5 which gave a sensitivity of 73.1%, a specificity of 66.7%, and the AUC of 0.74 while a total Ig S/Co cutoff value > 4.97 had a sensitivity of 94.7%, a specificity of 66.7%, and the AUC was 0.83 (Fig. 5, Panel A). Furthermore, we also suggested other cut-off values for each antibody corresponding to the NAbs titer ≥ 1:160. IgG antibody at cut-off value > 4.44 S/Co yielded a sensitivity of 72.2% and a specificity of 70.2% with the AUC was 0.69. For total Ig, the cut-off value > 65 S/Co had a sensitivity of 83.3% and a specificity of 55.2% with the AUC was 0.67 (Fig. 5, Panel B).

5. Discussion

Serological tests of SARS-CoV-2 are very informative and important owing to their ability to determine the current immune response of the infected patients. We still need to know more about the extent and duration of immunity induced by SARS-CoV-2 infection, especially in comparing symptomatic and asymptomatic patients (Shaffaf and Ghafar-Zadeh, 2021; Zhao et al., 2020). In some situations, serologic antibody testing may aid in the establishment or confirmation of a diagnosis, as well as in the prediction of clinical course and clinical decision-making, particularly in the era of SARS-CoV-2 vaccinations and the emergence of new viral variants (Bonanni et al., 2021; Winter and Hegde, 2020).

In the present study, we evaluated Ortho Clinical anti-Spike-1 IgG/total Ig antibodies responses to SARS-CoV-2 that have not been extensively studied in asymptomatic and previously symptomatic SARS-CoV-2 infection. IgG antibodies are the major antibodies that induce a long-term immune response, showing that the disease has recovered or that there was a previous infection. (Jacofsky et al., 2020). In our study, the median titers of IgG were seen higher in asymptomatic than previously symptomatic individuals. Different studies showed that the reported time to IgG positivity ranges from 13 to 21 days following disease onset (Long et al., 2020; Guo et al., 2020), and can appear as early as 5 to 7 days, also IgG titer can reach the peak levels at 3–4 weeks (Maeda et al., 2021). Studies showed that there is a strong correlation between the clinical severity of COVID-19 and the detected antibody signal, with severe COVID-19 patients having a stronger humoral immune response than non-severe cases (Nakano et al., 2021; Chen et al., 2020). However, other studies showed that asymptomatic infection can also mount a humoral immune response, consistent with our results. Dwyer et al., showed that asymptomatic individuals and convalescent patients have

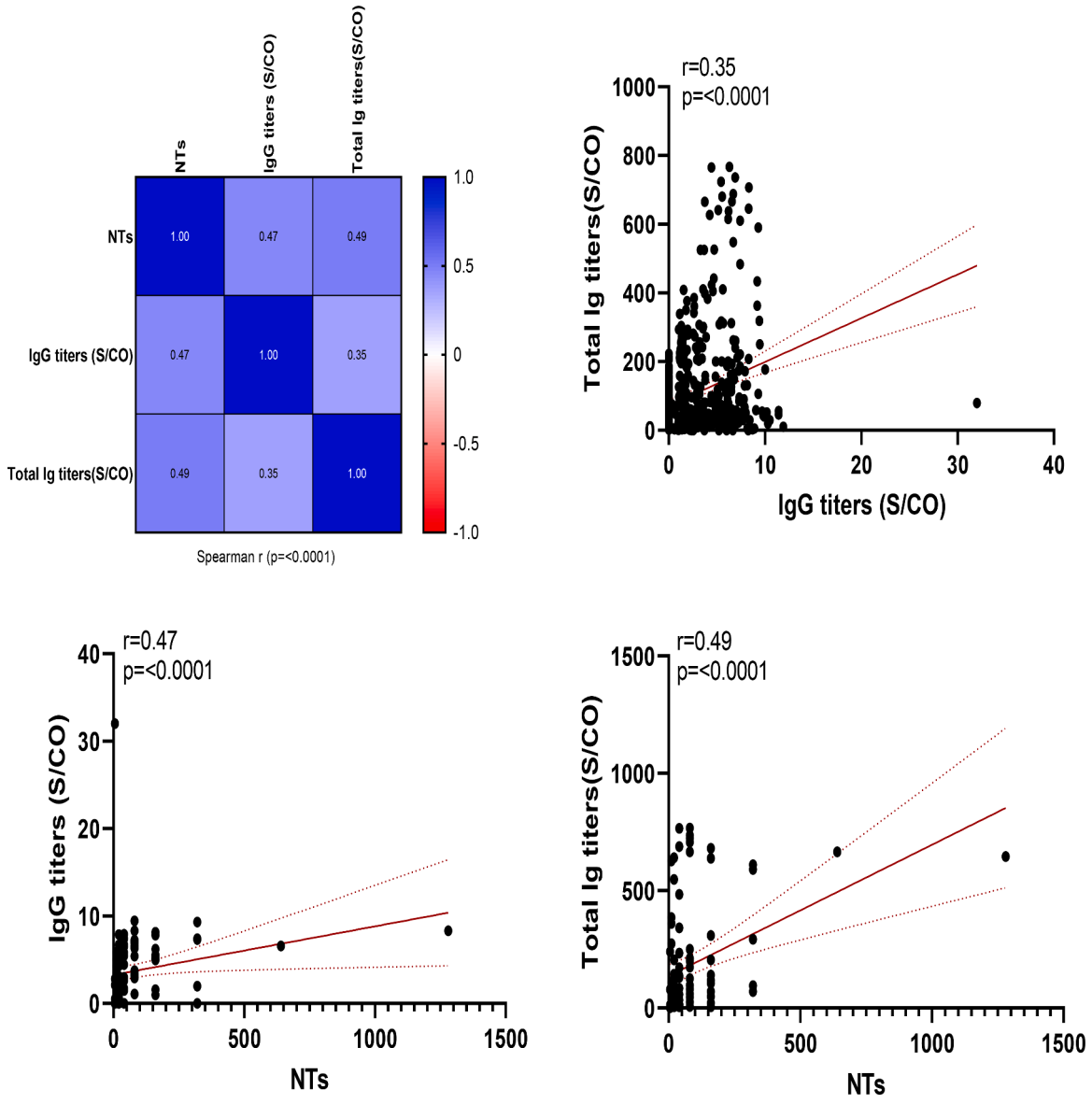

Fig. 4. Correlation analysis between Ortho Clinical IgG/Total Ig antibodies titers and neutralization titers (NTs) in asymptomatic and previously symptomatic SARS-CoV-2 infection; regression line [solid] and 95% confidence interval [dotted] are given in red.

comparable levels of IgG antibodies against SARS-CoV-2 S and RBD after conducting over 60,000 antibody tests to determine IgG antibodies against S protein in community serum samples (Dwyer et al., 2021). Conversely to IgG, we observed a higher titer of anti-S1 total Ig in previously symptomatic than asymptomatic SARS-CoV-2 individuals. Anti-S1 total Ig antibodies (IgG, IgM, IgA, and other isotypes) are less commonly used in clinical practice. In general, serum anti-SARS-COV-2 IgM and IgA titers decline after approximately 28 days from the onset of symptoms (Stephens and McElrath, 2020). The higher titers of anti-S1 total Ig and lower IgG observed in previously symptomatic individuals in our study would depend on the measured subclass of IgG, there are 4 subclasses of IgG (IgG1, IgG 2, IgG3, IgG 4) (Jacofsky et al., 2020). Goh et al. demonstrated that patients with COVID-19 showed isotype switching of all IgG subclasses against the S protein over time, with IgG1 being the most dominant IgG subclass, and all individuals having a positive IgG1 response by a median of 23 days post-illness onset. However, in our study, we were not able to detect IgG subclasses (Goh et al., 2021).

Neutralizing antibodies are vital in virus clearance and have long been regarded as a critical immunological product for viral illness prevention and treatment, while it is not yet clear if NAbs are the predominant mechanism conferring immunity to SARS-CoV-2 (Wu et al., 2020). The presence of symptoms and the timing post-SARS-CoV-2 infection had a major impact on the degree and level of NAbs as reported in previous studies. SARS-CoV-2 NAbs responses are most abundant at 31–35 days post symptoms onset (PSO) (Lee et al., 2021), but later than the 2 to 3 weeks, PSO was also reported (Duan et al., 2020; Wölfel et al., 2020). Patients with severe or moderate SARS-CoV-2 infection had the earlier appearance of NAbs at higher levels compared to those with mild or asymptomatic illness (Jeewandara et al., 2021). In line with this, we found that 91.2% (52/57) of previously symptomatic individuals developed SARS-CoV-2-specific NAbs. The median titer of NAbs was 1:40 ranging from 1:10-1:1280, with the highest titer encountered seen in one individual (1:1280). This was that all individuals in this group were symptomatic at the time of SARS-CoV-2 diagnosis with varying degrees of severity and samples were taken around 2 months from onset of infection. On the other hand, asymptomatic individuals were capable of generating NAbs but at low neutralization titers. The median titer of NAbs was 1:20 ranging from 1:10-1:320 and none of them had a titer $\geq$ 1:640. The absence of

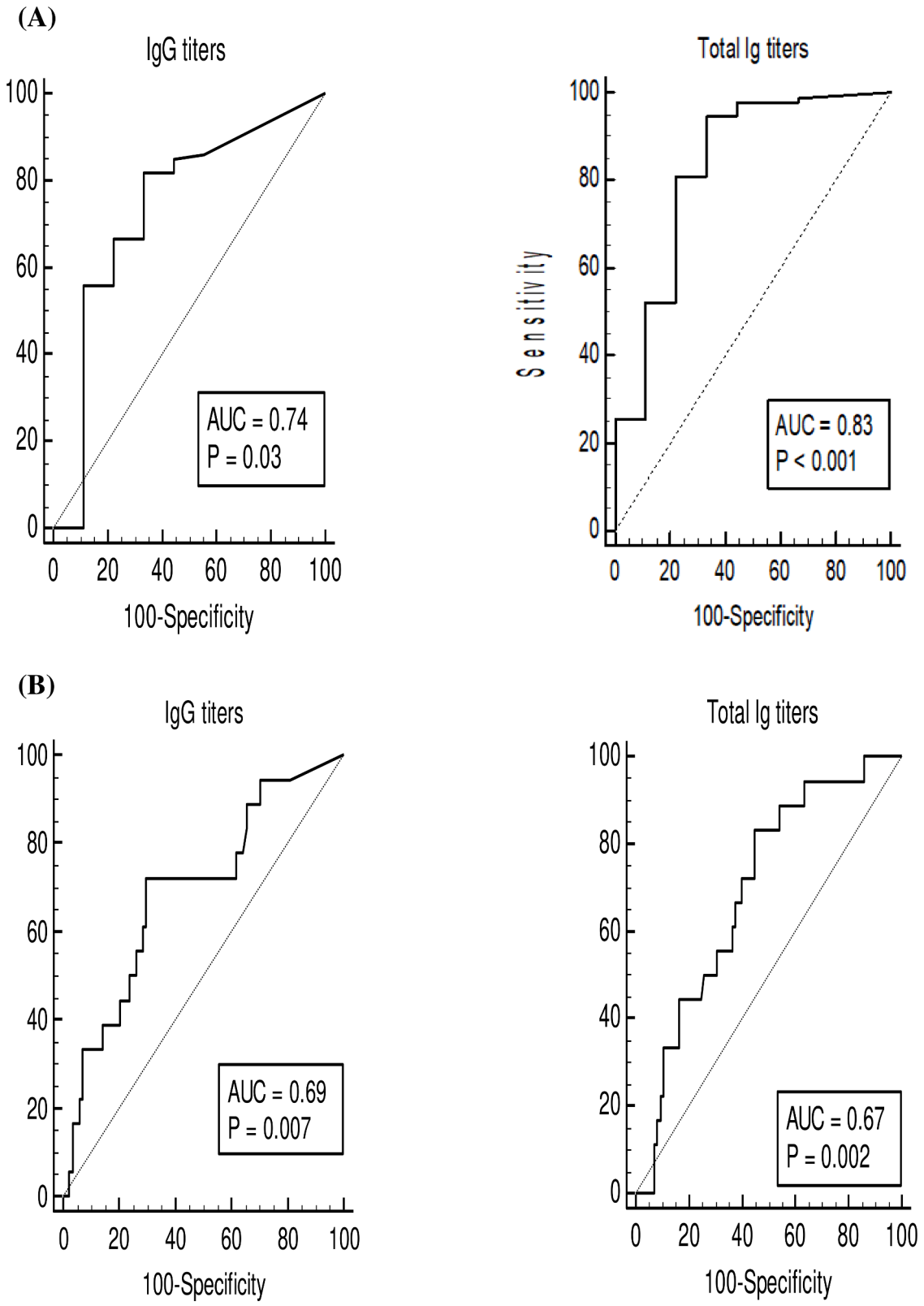

Fig. 5. The receiver-operating characteristic curve (ROC curve) of Ortho Clinical IgG/total Ig antibodies for predicting (A) the presence of neutralizing antibodies and (B) the presence of a neutralization titer of 160.

symptoms and the timing of the sample collection could affect the interpretation of the neutralization test in asymptomatic individuals.

Despite neutralization assays being considered the gold-standard tests for identifying NAbs, they are not feasible for routine laboratories and cannot be performed for large-scale testing of populations (Tan et al., 2020). Therefore, commercial serological SARS-CoV-2 assays have emerged as an intriguing alternative to be used as "correlate" or "surrogate" for protection if they correlate with the

result of the neutralization test. Antibodies against SARS-CoV-2 can target several proteins such as nucleocapsid proteins and spike proteins. Ortho Clinical assay targets the spike-1 protein of SARS-CoV-2, the primary target of neutralizing antibodies (Wrapp et al., 2020). Here, we evaluated the correlation between Ortho Clinical assay and neutralizing antibodies to set cut-off values of this assay to predict the level of antibody neutralization. Interestingly, we have demonstrated a correlation of Ortho Clinical IgG/total Ig against spike-1 of SARS-CoV-2 virus assay with the neutralizing assay in agreement with other studies using anti-spike protein assays (Šimánek et al., 2021; Salazar et al., 2020; Suhandynata et al., 2021; Musa et al., 2021). Subsequently, we assessed the ability of our serology assay to predict the presence of neutralizing antibodies and to establish a high titer of 1:160 using ROC curves. First, we found that Ortho Clinical IgG/total Ig assay against S1 of SARS-CoV-2 virus could predict neutralizing antibodies at cut-off values of >1.5 S/Co and >4.9 S/Co, respectively. It was notable that the current recommendation has issued a minimum titer of 1:160 for the neutralizing antibodies as a criterion for passive antibody therapy (FDA, 2020), although a titer of 1:80 is still acceptable as a minimum threshold value (Freedenberg et al., 2021). Second, an important finding from this study is that Ortho Clinical IgG/total Ig assay against S1 of SARS-CoV-2 virus could predict titer 1:160 of the neutralizing antibodies at cut-off values of > 4.44 S/Co and > 65 S/Co, respectively. Little published literature is available to correlate the cut-off for Ortho Clinical assay and neutralizing antibody titers. The published cut-off for Ortho Clinical IgG to predict NTs ≥1:80 was 16.2 S/CO (Moscato et al., 2021). To our knowledge, the cut-off for Ortho Clinical assay to correlate neutralization titer of ≥1:160 has not been determined. The cut-off for the Ortho Clinical anti-S1 IgG assay established here (> 4.44 S/Co) to predict a neutralization titer of 1:160 is closer to the cutoff of the Euroimmun IgG assay (3.06) and Abbott IgG assay (6 S/C) (Šimánek et al., 2021). Based on our results, Ortho Clinical IgG/total Ig assay against S1 of SARS-CoV-2 virus could serve as a surrogate test to detect the presence of neutralizing antibodies and high titer of neutralization to SARS-CoV-2, particularly in the vaccinated population, without the cost, hazards, time, and requirement of any specific equipment.

The limitation of our study is that we could not follow up our participants for serial measurements of antibodies to detect the durability of immune response especially NAbs. Also, we lack the time of onset of infection in asymptomatic individuals, and the degree of severity of the previously symptomatic group.

6. Conclusion

Asymptomatic SARS-CoV-2 infection can mount comparable IgG response compared to previously symptomatic, however higher neutralization activity was seen in previously symptomatic individuals, detonating presence of symptoms and time post-illness needed to develop protective immune response; Ortho Clinical IgG/total Ig antibodies showed a correlation with neutralization activity and can be used as a surrogate test allowing estimates on the presence of protective antibody response, and high titer of neutralization to SARS-CoV-2 to assess immunity to re-infection and to support vaccination programs or antibodies-based therapeutic trials.

Declarations

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Competing interests: The authors report no declarations of interest.

Availability of data and material: The data supporting the results are available from the corresponding author upon reasonable request.

Ethics approval: The study was conducted according to the principle of the Declaration of Helsinki. Written informed consent was obtained from each participant before sample collection. Ethical Committee approvals: Egypt Center for Research and Regenerative Medicine (ECRRM) and Faculty of Medicine, Cairo University, approval number IRB00012517.

Consent to participate: The consent form is available if needed.

CRediT authorship contribution statement

Yasmine Gaber: Conceptualization, Writing – original draft. **Shereen Abdel Alem:** Formal analysis, Writing – review & editing. **Sherief Musa:** Writing – review & editing. **Khaled Amer:** Investigation. **Tarek Elnagdy:** Conceptualization, Investigation. **Wael A. Hassan:** Investigation, Formal analysis. **Raafat Zaher Abdelrahman:** Methodology. **Ahmed Gad:** Methodology. **Mohamed A. Ali:** Methodology. **Hedy A. Badary:** Investigation. **Shereen Shawky:** Investigation, Resources, Methodology. **Hala Talaat:** Supervision. **Abdel Meguid Kassem:** Conceptualization, Funding acquisition. **Rabab Fouad:** Supervision.

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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