Correlation of sample-to-cut-off ratio of anti-SARS-CoV-2 IgG antibody chemiluminescent assay with neutralization activity: a prospective multi-centric study in India

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Background and Objectives There are limited published data on association of results from commercial serological anti-SARS-CoV-2 IgG antibody CLIA (chemiluminescent immunoassay) assays with neutralizing antibodies. This study was undertaken with an objective to correlate sample-to-cut-off (S/Co) ratio of CLIA antibody tests with inhibition activity, which may then serve as a valuable guide for labelling plasma as COVID convalescent plasma (CCP) for therapy and assessing vaccine efficacy.

Materials and Methods A total of 139 donor serum samples who were previously RT-PCR positive and had recovered completely from COVID-19 at least 28 days prior to collection of samples were recruited at three sites. The samples were analysed for S/Co ratio and per cent inhibition activity with VITROS SARS-CoV-2 IgG chemiluminescent assay and GenScript cPass SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT) kit, respectively. Linear regression equation and receiver operating characteristic (ROC) curve were used to check the proposed model of comparing S/Co with per cent inhibition.

Results The results indicate very good correlation between the S/Co ratio of the chemiluminescent IgG assay and the neutralization activity depicted by per cent inhibition on sVNT assay. S/Co ratio of 4/C04 (low-titre) and 8/C19 (high-titre) correlated with 30% and 68% inhibition, respectively.

Conclusion Chemiluminescent SARS-CoV-2 IgG assay can be used as a semi-quantitative test, with a cut-off of >8/C19S/Co ratio for selecting donors for convalescent plasma therapy and assessing efficacy of vaccination.

Key words: anti-SARS-CoV-2 IgG antibodies, convalescent plasma, correlation, immunoassay, neutralization.
These tests are able to detect total antibodies but are unable to differentiate between binding antibodies (BAbs) and neutralizing antibodies (NAbs). Measuring NAbs, however, is important to ensure that qualified CCP is used to achieve desired therapeutic outcome in patients with a moderate-to-severe COVID-19 disease and for the development and assessment of COVID-19 vaccine efficacy [3].

There are three types of tests one can use for measuring NAbs. The first type is the conventional virus neutralization test (cVNT), which detects neutralizing antibodies (NAbs) in patient’s blood and is considered as the gold standard. The cVNT requires handling live SARS-CoV-2 virus, in a specialized biosafety level 3 (BSL 3) containment facility, which is limited to very few laboratories. Moreover, cVNT is tedious and time-consuming, taking 2–4 days to complete. The second type of test is pseudovirus-based neutralization test (pVNT), which, on the other hand, can be performed in a BSL 2 laboratory, and does not require the use of live viruses and cells. The third type is surrogate virus neutralization test (sVNT) using purified receptor-binding domain (RBD) from the S protein of virus and the host cell receptor angiotensin-converting enzyme-2 (ACE2), and this test is designed to mimic the virus-host interaction in an EIA (enzyme immuno-assay) plate well. This RBD-ACE2 interaction can be neutralized (i.e. blocked or inhibited) by specific NAbs in human sera, in the same manner as in cVNT or pVNT [4]. This sVNT test is easily available and can be performed in any laboratory with EIA set-up.

There are limited published data on association of results from commercial CLIA assays (detecting total antibodies) with neutralizing antibodies [5, 6]. We, therefore, undertook a study to correlate CLIA assay sample-to-cut-off ratio (S/Co) with neutralization activity, using EIA based sVNT. This would then serve as a valuable guide for the interpretation of commonly used CLIA tests for SARS-CoV-2 for selecting suitable plasma donors for CCP therapy and assessing vaccine efficacy.

Materials and methods

Settings

This was a prospective multi-centric, cross-sectional analytical study where three blood centre laboratories in north India contributed donor samples for the study. All three centres followed same standard operating procedure (SOP) for donor selection, donor testing and CCP collection. This study was performed over a period of 3 months from October 2020 to December 2020. Retesting of anti-SARS-CoV-2 antibody immunoassay (VITROS anti-SARS-CoV-2 IgG antibody, Ortho Clinical Diagnostics {OCD}, Raritan, NJ, USA), EIA neutralization test, correlation between BAbs and NAbs was conducted at one of the participating blood-centre laboratories.

Donor selection

A total of 139 donors were selected with a prior history of positive real-time polymerase chain reaction (RT-PCR) and who volunteered for CCP donation. Donors presenting to the blood centre either 28 days after complete cessation of symptoms or at least 14 days after complete cessation of symptoms in the presence of negative RT-PCR report, were included in the study [7]. An informed consent was obtained from the donors stating that their blood samples would be tested for anti-SARS-CoV-2 IgG antibodies (BAbs) and neutralizing antibodies (NAbs). Donor selection included mandatory administration of health history questionnaire and brief physical examination comprising of weight, blood pressure, pulse, temperature and haemoglobin in accordance with national guidelines [8].

Donor testing

Donor samples at all three participating laboratories were tested for anti-SARS-CoV-2 IgG antibody using same assay (VITROS anti-SARS-CoV-2 IgG antibody, Ortho Clinical Diagnostics {OCD}) and same equipment, VITROS ECiQ (Ortho Clinical Diagnostics Inc.). Only serum samples were used for testing. Donors with S/Co of more than 1-00 were accepted for CCP donation. Other tests conducted on donor samples included serum protein (>6 g/dl) beside the mandatory transfusion transmissible infections (TTI) like HIV, HBV, HCV, syphilis and malaria. These tests were also done on the same platform.

COVID convalescent plasma donation

Plasma donations were done on apheresis equipment (Amicus, Fresenius Kabi AG, Bad Homburg, Germany/Terumo Penpol Pvt. Ltd., Thiruvananthapuram, Kerala, India). Four hundred twenty five milliliters of plasma was collected from each donor. Two therapeutic doses of around 200 ml were used in patients. Twenty-five milliliters of serum sample was frozen at temperature lower than –30°C for neutralization test (EIA) to be performed (batch analysis) at a later point-in-time.

Re-analysis for anti-SARS-CoV-2 IgG antibody and neutralization test

Since participating laboratories used different lots of the same assay for donor selection, all 139 serum samples were re-analysed on the VITROS kit, Lot # 210.
(Ortho Clinical Diagnostics {OCD}) to ensure uniformity in S/Co signals and interpretation of results. All these samples were further analysed in batches using SARS-CoV-2 surrogate virus neutralization test (sVNT) (GenScript, USA). S/Co signal of chemiluminescent assay was compared with inhibition percentage of neutralization test.

Type of immunoassays used
In this study, two immunoassays were used as follows: CLIA and EIa (neutralization test).

Chemiluminescent immunoassay
Anti-SARS-Cov-2 IgG CLIA (Vitros, OCD) was used as the primary serological immunoassay, which uses the enhanced chemiluminescence technology for the detection of IgG antibodies. The results were expressed as S/Co ratio, which served as a semi-quantitative index. As per kit instructions for use [9], the sensitivity with RT-PCR at >15 days after symptoms was 90% and clinical specificity was 100%. Similar serology test performance was mentioned in the U.S. FDA release notification (90% sensitivity, 100% specificity, negative predictive value (NPV) of 99.5%-50% and positive predictive value (PPV) of 100%) [10].

Enzyme immuno-assay (Neutralization test)
SARS-CoV-2 Surrogate Virus Neutralization Test (sVNT; cPass, GenScript Inc., Piscataway, NJ, USA) detects circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the receptor binding domain of the viral spike glycoprotein (RBD) with the ACE 2 cell surface receptor. The assay detects any antibody in serum that neutralize the RBD-ACE 2 interaction. The test is both species and isotype independent and is qualitative. The results were expressed as percentage inhibition. A ≥30% inhibition on cPass sVNT kit, as per version 4.0 of instructions for use, indicated a positive result for neutralizing antibodies and was indirectly equivalent to 90% plaque reduction in viral plaque reduction neutralization test (PRNT). This cPass kit had a sensitivity of 93.3% when compared with RT-PCR and specificity of 100% [11]. Only serum samples were used for neutralization test. S/Co corresponding to 30% inhibition on cPass sVNT kit was calculated to qualify plasma as CCP as this S/Co would indirectly equate with 90% plaque reduction on PRNT. This was labelled as ‘low-titre’ CCP. Additionally, S/Co corresponding to 68% inhibition on cPass sVNT kit was calculated to label CCP as ‘high-titre’ in accordance with US FDA emergency use authorization [6].

Quality assurance of equipment and reagents used in the study
Daily quality control (QC) samples were run at all three centres, and Levy-Jenning’s graph was maintained. The samples were run only when all QC samples passed and Westgard rules were adhered to according to SOP.

Ethical clearance
The institutional ethics committee approved the study.

Statistical analysis
Test for normality was performed to see the characteristics of distribution of data for both VITROS SARS-Cov-2 IgG (CLIA) assay and cPass sVNT % neutralization assay. Spearman’s correlation was done since the data were skewed. Axis reversal for correlation was also carried out to test appropriateness of residual distribution in addition to residuals plot for residual behaviour. Correlation of CLIA S/Co ratio was done with the percentage inhibition on neutralization test. Linear regression equation \( y = mx + c \) was used to check the proposed model of comparing S/Co with per cent inhibition. Receiver operating characteristic (ROC) curve was generated for the proposed model of comparing S/Co with % inhibition, and area under curve (AUC) was calculated. SPSS software (Version 26, IBM India Pvt. Ltd., Bengaluru, Karnataka, India) was used for statistical evaluations.

Results

Demographic data
The gender ratio was 27:1 (males = 134 and females = 5). The average age of men was 34.5 (range = 19–59) years, while that of women was 25.6 (range = 25–27) years. The blood groups of donors in decreasing order were B (\( n = 58; 41.7\% \)), O (\( n = 43; 30.9\% \)), A (\( n = 23; 16.6\% \)) and AB (\( n = 15; 10.8\% \)), respectively. All 139 CCP donors presented 28 days (range=28-156 days) after complete cessation of symptoms at the blood centre. One hundred twenty three donors had history of mild-to-moderate COVID-19 illness, and 16 donors did not have any symptoms. All donors were first-time CCP donors.

Chemiluminescent immunoassay (Sample/Cut-off \{S/Co\})
Upon re-testing, S/Co of all 139 samples was ≥1.00 and ranged between 1.12 and 14.10.
Neutralization test (inhibition percentage)
The inhibition percentage of 139 samples ranged from 4.08% to 96.96%, sorted by results (Fig. 1).

Comparison of S/Co and inhibition percentage
The inter-quartile range (Q2 and Q3) of S/C ratio (50%) was between 4.64 and 9.74 with median at 7.43 (Fig. 1) on the SARS-Cov-2 IgG assay corresponding to 41.4% inhibition to 84.2% inhibition with a median inhibition % of 65.27% on the sVNT kit. It can be noticed that results on both the IgG assay and inhibition assay are skewed to lower side of the median in the boxplot, indicating that majority of samples in the mid inter-quartile range fall between S/Co ratio of 4.64 and 7.43 with percent inhibition between 41.4% to 65.27%.

Calculation of low-titre CCP S/Co (30% inhibition)
In the linear regression analysis, the residual distribution was appropriate and this validated the proposed model of comparing S/Co with the inhibition percentage. When data of 139 samples were sorted in ascending order as per S/Co ratio, and applying the linear regression equation, the S/Co ratio at and above which there was 30% inhibition (90% PRNT) was 4.04. Hence, this S/Co ratio can be considered as the cut-off using the VITROs CLIA assay at which there was minimum 30% inhibition, which was indirectly equivalent to 90% PRNT. This S/Co ratio of 4.04 was slightly lower than 20th percentile, when samples were arranged in ascending order of S/Co ratio (Table 1). The Spearman’s correlation coefficient between S/Co ratio of SARS-Cov-2 IgG assay and the per cent inhibition of neutralization test was 0.922. The diagnostic accuracy of this S/Co ratio of 4.04 was further confirmed by plotting a ROC. The area under curve (AUC) of the ROC curve was 0.977 (95% confidence interval: 0.955–0.999, sensitivity: 94.8%, specificity: 91.3%) (Fig. 2). S/Co of 4.04 corresponding to 30% inhibition on cPass sVNT kit was used to qualify plasma as 'low-titre' CCP.

Calculation of high-titre S/Co (68% inhibition)
Similarly, S/Co ratio at which there was 68% inhibition was 8.19. This S/Co corresponded to ‘high-titre’ antibodies for qualifying CCP in clinical therapy. The diagnostic accuracy of this S/Co ratio of 8.19 was further confirmed by plotting a ROC. The area under curve (AUC) of the ROC curve was 0.953 (95% confidence interval: 0.955–0.999, sensitivity: 80.9%, specificity: 90.1%).

Discussion
cPass sVNT EIA vs. live cell viral neutralization test (pVNT)
It has been postulated and shown that the cPass sVNT gives comparable results and can potentially be used in lieu of cVNT or pVNT [12]. In pseudovirus viral neutralization test (pVNT), the numbers of plaques are counted 48–72 h after initial inoculation. The highest dilution of
### Table 1 Percentile arrangement (20th to 95th percentile) of 139 samples according to their ascending S/Co ratio

| Total number of samples | Percentile Slab | k value (percentile in decimals) | % percentile number | Rounded-off | S/Co |
|-------------------------|----------------|----------------------------------|--------------------|-------------|------|
| 139                     | 20             | 0.20                             | 27.8               | 28          | 0.41 |
|                         | 30             | 0.30                             | 41.7               | 42          | 0.68 |
|                         | 40             | 0.40                             | 55.6               | 55          | 0.07 |
|                         | 50             | 0.50                             | 69.5               | 70          | 0.08 |
|                         | 60             | 0.60                             | 83.4               | 83          | 0.30 |
|                         | 70             | 0.70                             | 97.3               | 97          | 0.26 |
|                         | 80             | 0.80                             | 111.2              | 111         | 0.40 |
|                         | 90             | 0.90                             | 125.1              | 125         | 1.60 |
|                         | 95             | 0.95                             | 132.05             | 132         | 12.30|

**Fig. 2** Scatter plot and ROC* curves. Figure a and figure b is scatter plot and ROC curve for 30% inhibition on cPass sVNT kit (sensitivity: 94.8%, specificity: 91.3%, CI 95%: 0.955–0.999, AUC**: 0.977). Figure c and figure d is scatter plot and ROC curve for 68% inhibition on cPass sVNT kit (sensitivity: 80.9%, specificity: 90.1%, CI 95%: 0.955–0.999, AUC: 0.953). *ROC receiver operator characteristics; **AUC, area under curve.

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serum that inhibits (reduces) plaque (plaque reduction neutralization test {PRNT}) formation by 50%, 75% or 90% is called PRNT50, PRNT75 and PRNT90, respectively. In a study by Taylor et al. [13] compared PRNT50, 75 and 90 values with sVNT on 66 well-characterized samples. This gave high correlation in delineating positive and negative samples for PRNT75 and 90 where only one samples did not corroborate. When using a lower stringency analysis for the PRNT, PRNT50 (i.e. the reciprocal dilution that inhibited 50% of infection), two samples found to be negative by the PRNT75 and PRNT90 had detectable PRNT50 titres. As described in the present article, therefore, cPass sVNT, which is known to give comparable results [12], was used in lieu of pVNT.

Semi-quantitative assay

The present study used a surrogate viral neutralization test (sVNT; GenScript) for assessing S/Co of commonly available CLIA assay (VITROS anti-SARS-CoV-2 IgG assay). Sorting the data for percentage inhibition and results from the SARS-CoV-2 IgG assay, it was seen that S/Co result of 4:04 and above on the IgG assay definitely reflected ≥30% inhibition, which was indirectly equivalent to ≥90% PRNT titres. Correlation coefficient of 0.922 indicated very good correlation and confirmed the semi-quantitative nature of this CLIA assay. As cited by Akoglu [14], Spearman’s correlation coefficient of >0.8 represents very strong correlation.

Comparison with published reports

Moderate positive correlation of 0.71 (95% CI: 0.58-0.81) was reported in a recent study using VITROS anti-SARS-CoV-2 IgG antibody assay vis-a-vis Reporter Viral Particle Neutralization (RVPN) assay, by EG Meyer et al. [15]. They attributed use of spike glycoprotein as the reason for high correlation between both assays used in their study. In the present study, also both VITROS anti-SARS-CoV-2 IgG antibody and GenScript sVNT test targeted the spike glycoprotein and therefore had strong positive correlation (correlation coefficient = 0.922).

In a large multi-centric study by Joyner et al. [12], at Mayo clinic, USA, the recommended cut-offs for VITROS anti-SARS-CoV-2 IgG antibody assay were based on 20th and 80th percentiles of distribution of S/Co ratios. These were classified according to levels of protective antibodies in CCP; low level of protective antibodies was defined when the S/Co was 1:0–4:62, medium level of protective antibodies when the S/Co was 4:62–18:45 and high level of protective antibodies when the S/Co was more than 18:45. Even in the present study, the S/Co cut-off value for effective neutralization, using VITROS anti-SARS-CoV-2 IgG assay, was 4:04, which is very close to 4:62 that Joyner et al. recommended as cut-off value for low level of protective antibodies, using the same immunoassay.

S/Co ratio of 8:19 corresponding to 68% inhibition on cPass sVNT was close to S/Co of 9:5, defined by US FDA emergency use authorization [6], S/Co of 9:5 was considered ‘high-titre’ antibodies for qualifying CCP in clinical therapy. Likewise, the present study indicates that S/Co of 8:19 or more is needed for labelling CCP as ‘high-titre’.

Antibody titre

There are reports comparing titre (ELISA titre or neutralizing antibody titre) with patients’ clinical outcome. Li et al. [16] transfused CCP with an ELISA titre >1:640 and reported 51.9% in test arm as compared to 43.1% in the control arm. In another trial, Gharbharan et al. [17] treated patients with CCP with neutralization titre of 1:80. It is acknowledged that detecting nAb via surrogate tests is not the same as detecting high titre nAb. Performing titres was beyond scope of the present study and it can be low throughput, subjective and cumbersome to perform titre in a routine laboratory. The authors, therefore, chose sVNT to determine S/Co of the CLIA assay.

Conclusion

It can be concluded from the present study that S/Co of 8:19 on CLIA can be used for selecting suitable plasma donors for CCP therapy.

Conflict of Interest

The authors declare no conflict of interests.

Author contributions

AKT, GN, RMJ and GA were involved in concept, design and literature search. NY and VV did data acquisition and analysis. KK prepared the draft manuscript, which was edited by AKT and GA. All authors have reviewed the final draft.

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