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Evaluation of Diagnostic Performance of Severe Acute Respiratory Syndrome Coronavirus 2-Specific Differential Principles Antibody Tests

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Abstract

Objective: We aimed to evaluate the diagnostic performance of tests based on enzyme-linked immunosorbent assay, chemiluminescent microparticle immunoassay, and lateral flow assay methods for detecting severe acute respiratory syndrome coronavirus 2 immunoglobulin G with serum samples of people who received the severe acute respiratory syndrome coronavirus 2 vaccine or have been previously infected.

Methods: The serum samples were taken 28 days after were CoronaVac vaccine, BNT162b2 vaccine, or diagnosed with coronavirus disease 2019, were included in the study (n = 100). Neutralizing antibodies against the receptor-binding region of the spike protein S1 subunit of severe acute respiratory syndrome coronavirus 2, with antibody tests against 3 different principles (chemiluminescent microparticle immunoassay, enzyme-linked immunosorbent assay, lateral flow assay) (ARCHITECT IgG II Quant test, Abbott, USA/SARS-CoV-2 NeutraLISA, Euroimmun, Lübeck, Germany/ NeutraXpress™, JOYSBIO Biotechnology Co. Ltd., Tianjin China) were studied. Statistical Package for Social Sciences v21 package program (IBM Corp., Armonk, NY, USA) was used for the statistical evaluation of the data.

Results: When the surrogate neutralizing antibody enzyme-linked immunosorbent assay was compared with the lateral flow assay neutralizing antibody test, the sensitivity was 74.7% and the specificity was 84%. In the lateral flow assay binding antibody test, the sensitivity was 93.3% and the specificity was 80%. When the chemiluminescent microparticle immunoassay was compared with the lateral flow assay neutralizing antibody test, the sensitivity was 63.2% and the specificity was 100%. In the lateral flow assay binding antibody test, the sensitivity was 78.9% and the specificity was 100%.

Conclusion: According to our study, the diagnostic performance of lateral flow assay-based tests was not satisfactory. However, we think that tests with high sensitivity and specificity and compatible with plaque reduction neutralization test should be preferred in the evaluation of humoral immune response by immunological methods.

Keywords: COVID-19, LFA, CMIA, ELISA, SARS-CoV-2

Introduction

After the administration of the vaccine to avoid coronavirus disease 2019 (COVID-19) infection, both cell-mediated and humoral immune responses develop against specific severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins such as nucleocapsid (N) and/or spike (S) proteins.[1] Immunoglobulin (Ig) M, IgA, and IgG antibody responses are formed in humans against the N and S proteins of SARS-CoV-2 after the natural infection.[1] However, IgM antibody responses are formed first, and then IgG titers reach detectable levels. Also, IgM and IgA antibody titers decline faster than IgG.[1] By using IgG antibody assays, antibodies against S and its subunits can be detected from the serum taken from the patient 2 or 3 weeks after infection or 3 weeks after the first or second dose of the vaccine.[2,3] With SARS-CoV-2-specific antibody assays, epidemiological studies in general and direct detection of COVID-19 seroprevalence in the community can determine whether the person has had COVID-19 disease due to previous contact with the virus.[2,3] It can also be used for diagnosis (indirect), screening, and follow-up (surveillance) purposes. Recently, SARS-CoV-2 IgG tests based on many different methods have been developed commercially due to the increase in use to determine the antibody status after vaccination. Various immunoassays which have different sensitivity and specificity are currently available for SARS-CoV-2 antibody detection, including

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enzyme-linked immunosorbent assay (ELISA), chemiluminescence enzyme immunoassays, and lateral flow tests. In this context, in our study, we aimed to evaluate the diagnostic performance of tests based on ELISA, chemiluminescent microparticle immunoassay (CMIA), and lateral flow assay (LFA) methods for detecting SARS-CoV-2 IgG with serum samples of people who received SARS-CoV-2 vaccine or have been previously infected.

Methods

Our study was planned and carried out as cross-sectional descriptive research. Our study included serum samples taken 28 days after PCR positivity of 30 people diagnosed with COVID-19 clinically and by molecular methods. In addition, blood samples were taken 28 days after the last dose of 35 volunteers who had no previous COVID-19 infection and received 2 doses of inactivated COVID-19 vaccine (CoronaVac, Sinovac Life Sciences, Beijing, China) and 35 volunteers who received 2 doses of messenger ribonucleic acid-based COVID-19 vaccine (BNT162b2, BioNTech SE, Mainz, Germany) were included in the study. SARS-CoV-2 antibody studies for 3 different principles (CMIA, ELISA, LFA) were performed with each serum sample. As the CMIA method, the SARS-CoV-2 IgG test (ARCHITECT IgG II Quant, Abbott, Abbott Park, Ill, USA), which can quantitatively detect IgG antibodies, including neutralizing antibodies against the receptor-binding domain (RBD) of the spike protein's S1 subunit of SARS-CoV-2, was used. Results from all studied sera were evaluated as arbitrary unit/milliliter (AU/mL). The concentrations obtained as AU/mL were multiplied by the correlation coefficient of 0.142 and converted to the "binding antibody unit (BAU/mL)" in the WHO's International Standard for anti-SARS-CoV-2 immunoglobulin.[4] Concentrations of 50 AU/mL or 7.1 BAU/mL and above were considered positive. However, it was reported that the test was 100% compatible with the plaque reduction neutralization test (PRNT), and a concentration of 1050 AU/mL was associated with a 1:80 dilution of PRNT.[5] Neutralizing antibody that inhibits the binding of the RBD of viral SARS-CoV-2 S1 to the angiotensin-converting enzyme 2 receptors of human cells was determined by the competitive ELISA method (SARS-CoV-2 NeutraLISA, Euroimmun, Lübeck, Germany) as semiquantitatively. The %IH (percentage of inhibition) was used as a unit value in the evaluation of the test results. According to the manufacturer's instructions, %IH values below 20% are considered negative, %IH values between 20% (included) and 35% (not included) are considered borderline, and %IH values above 35% are considered positive. The SARS-CoV-2 NeutraLISA test was reported to be 98.6% compatible with PRNT.[6] To detect neutralizing and binding antibodies specific to the SARS-CoV-2 RBD region, lateral-flow-based commercial assay (SARS-CoV-2 IgG/Neutralizing antibody Rapid Test Kit [Colloidal Gold], NeutraXpress™, JOYSBIO Biotechnology Co. Ltd., Tianjin, China) was used, and results are obtained as qualitatively.[7] The results were evaluated by the manufacturer's instructions by adding 15 µL of patient serum and diluent to 1 line of the dual-line (patient and control) and dual-band (T1/T2) cassette test, and only the test diluent to the other line. The formation of any intensity band in the T2 (binding antibody) band was evaluated as reactive in terms of the presence of total IgM+IgG antibody binding to Spike/RBD. The presence of neutralizing antibodies was determined by comparing the color intensity of the lines in the T1 band. If the coloration of the control strip was more intense, the patient sample was considered reactive to the presence of neutralizing antibodies specific to the Spike/RBD region.

Table 1. Evaluation of Demographic and SARS-CoV-II IgG Data of Study Groups

Variables	Study Group (n = 100)
Demographic features	
Age, median (IQR; 25%-75%)	45 (36-53.75)
Sex (female/male)	50/50
SARS-CoV-2 antibody assay	
CMIA AU/mL, median (IQR; 25%-75%)	2635.150 (391.1-8053.425)
CMIA BAU/mL, median (IQR; 25%-75%)	374.191 (55.536-1143.586)
SNA-ELISA; IH%, median (IQR; 25%-75%)	88.411 (32.904-98.832)
LFA; binding antibody (positive/negative)	75/25
LFA; neutralizing antibody (positive/negative)	60/40

CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; LFA, lateral flow assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

For statistical analysis of data, quantitative antibody data were evaluated qualitatively based on the cut-off value. IBM's Statistical Package for Social Sciences version 21.0 (IBM Corp. Armonk, NY, USA) package program was used for statistical analysis. Kappa agreement analysis was used in the evaluation of categorical data. A value of $P < .05$ was considered significant.

Results

Although the mean age of all the cases in the study was 45 (36-53.75), 50 of the cases were female and 50 were male. When the median antibody data of the cases were evaluated, surrogate neutralizing antibody %IH was found to be 88.411% (32.904%-98.832%) and quantitative SARS-CoV-2 IgG was 2635.150 (391.1-8053.425) AU/mL. When the surrogate neutralizing antibody test

Table 2. Sensitivity, Specificity, NPV, PPV, and Kappa Values of SARS-CoV-2 Antibody Tests

Reference Method	Evaluated Method	Sensitivity	Specificity	NPV	PPV	Kappa
Surrogate neutralizing test (ELISA)	LFA binding antibody	93.3%	80%	80%	93.3%	0.733
	LFA neutralizing antibody	74.7%	84%	52.5%	93%	0.489
CMIA-SARS CoV-2 IgG	LFA binding antibody	78.9%	100%	20%	100%	0.273
	LFA neutralizing antibody	63.2%	100%	12.5%	100%	0.146

CMIA, chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; LFA, lateral flow assay; NPV, negative predictive value; PPV, positive predictive value; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

(ELISA) was evaluated according to the cut-off value, which is provided by the manufacturer, it was found positive in 74 people and negative in 26 people, and the presence of SARS-CoV-2 IgG (CMIA) was positive in 95 people and negative in 5 people. When the binding antibody results were evaluated with the LFA method, positivity was found in 75 people, while positivity was found in 60 people for the neutralizing antibodies (Table 1).

When the surrogate neutralizing antibody ELISA was accepted as the reference method and the results of the antibody bound by the LFA method were evaluated, the sensitivity of the LFA method was 93.3%, the specificity was 80%, the negative predictive value (NPV) was 80%, the positive predictive value (PPV) was 93.3%, and the kappa value was 0.733. When the neutralizing antibody results were evaluated with the LFA method, the sensitivity was 74.7%, the specificity was 84%, NPV was 52%, PPV was 93%, and the kappa value was 0.489.

When the binding antibody results were compared with the LFA method, using the CMIA method as a reference, the sensitivity was 78.9%, the specificity was 100%, NPV was 20%, PPV was 100%, and the kappa value was 0.273. When the neutralizing antibody results were evaluated by using the LFA method, the sensitivity was 63.2%, the specificity was 100%, NPV was 12.5%, PPV was 100%, and the kappa value was 0.146 (Table 2).

Discussion

There is great interest in the use of SARS-CoV-2 serological assays as an alternative or adjunct to molecular tests. The ELISA- and CMIA-based methods are preferred because of their high sensitivity, shorter time for results, and easier application.[8] With the recently increasing prevalence of COVID-19 due to its variants, serological tests are frequently used. Among them, lateral flow-based tests, which can detect in vivo binding and neutralizing antibodies and give much faster and qualitative results, have also been commercially available.

The present study evaluated and compared the performances of commercial different method-based assays (ELISA, CMIA, and LFA) that detect specific antibodies to SARS-CoV-2. The evaluation was made concerning the CMI-A and ELISA-based tests included in the "WHO International Standard for Anti SARS-CoV-2 Antibody" guideline. In this context, a low degree of compatibility was found between the results of binding and neutralizing antibodies based on the LFA method and the reference method in our study. Although only 1 test of a commercial company was evaluated in our study, it seems that the LFA method should not be preferred primarily in terms of sensitivity and specificity.

In a study evaluating CMIA and LFA antibody tests concerning RT-PCR, all antibody assays are useful in assessing immune responses to COVID-19 but be insufficient to replace RT-PCR for SARS-CoV-2 as an early detection method. However, it has been emphasized that the diagnostic utility of LFA-based rapid antibody tests is controversial.[9] In a study where they compared SARS-CoV-2 Ig assays belonging to Abbott, Euroimmun, and Roche brands, Tang et al[10] reported that the lowest amounts of false positive and false negative SARS-CoV-2 antibody results were detected in the Abbott assay in the measurements they made 14 days after the onset of the disease. In the study of Kontou et al,[11] it was stated that tests based on ELISA and CMIA methods performed better in terms of sensitivity (90%-94%), followed by LFA and fluorescent immunoassay with sensitivities ranging from 80% to 89%. Although LFA tests are more practical for large-scale seroprevalence studies, it has been emphasized that ELISA- and CMIA-based tests are more reliable because LFA-based assays have low sensitivity.[11] In a review evaluating 12 articles on LFA-based tests, it was demonstrated that in general rapid tests had a lower diagnostic performance compared to ELISA tests.[12] However, it has been reported that this is not only due to technical differences in the 2 methodologies but also due to low antibody concentrations, which may cause false negative results.[12] In the published studies, sensitivity and specificity ranged from 9% to 88.6% and from 88.9% to 91.7%, respectively.[12]

The PRNT could not be used as a reference method in our study due to the need for biosafety level 3, experienced specialists, and special laboratory conditions. However, the test methods taken as a reference in the evaluation have 98%-100% compatibility with PRNT.

As a result, in terms of both determining seroprevalence and evaluating immune response in the general population, large-scale studies should be carried out using test kits that provide quantitative results in accordance with the "binding antibody unit (BAU/mL)" standardization determined by WHO on reference serum, as well as the development of kits that can better detect more optimized, long-term antibody responses, and neutralizing activities.[5] Thus, the fight against the epidemic can be managed more easily if practical, easily applicable tests can be used in routine diagnosis to prevent the spread of fluctuations seen during the epidemic in society. However, the tests can be standardized with studies on the performance of serological tests, and threshold values for the protection of binding or neutralizing antibodies can be determined. In this way, after COVID-19 infection or vaccination, COVID-19 booster vaccine doses can be determined based on this threshold for protection.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of İstanbul University-Cerrahpaşa (Date: August 8, 2022, Number: 449386).

Informed Consent: Written informed consent was obtained from the participants who participated in this study.

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References

1. Qu J, Wu C, Li X, et al. Profile of immunoglobulin G and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis*. 2020;71(16):2255-2258. [CrossRef]
2. Rabi FA, Al Zoubi MS, Kasasbeh GA, Salameh DM, Al-Nasser AD. SARS-CoV-2 and coronavirus disease 2019: what we know so far. *Pathogens*. 2020;9(3):231. [CrossRef]
3. Dinç HÖ, Özdemir YE, Alkan S, et al. SARS-CoV-2 ile İlgili Farklı Prensipli Ticari Antikor Testlerinin COVID-19 Hastalarındaki Tanısal Performanslarının Değerlendirilmesi [Evaluation of the diagnostic performance of different principles of SARS-CoV-2 commercial antibody tests in COVID-19 patients]. *Mikrobiyol Bul*. 2021;55(2):207-222. [CrossRef]

4. Knezevic I, Mattiuzzo G, Page M, et al. WHO International Standard for evaluation of the antibody response to COVID-19 vaccines: call for urgent action by the scientific community. *Lancet Microbe*. 2022;3(3):e235-e240. [CrossRef]
5. Abbott SARS-CoV-2 Immunoassays. Available at: https://www.corelaboratory.abbott/int/en/offerings/segments/infectiousdisease/sars-cov-2.
6. EUROIMMUN, SARS-CoV-2 NeutraLISA- Coronavirus diagnostics. Cited 2022 September 9, Available at: https://www.coronavirus-diagnostics.com/documents/Indications/Infections/Coronavirus/EI_2606_D_UK_F.pdf.
7. SARS-CoV-2 IgG/Neutralizing antibody Rapid Test Kit. Cited 2022 September 9, Available at: https://en.joysbio.com/wp-content/uploads/2021/02/IFU-SARS-CoV-2-IgG-Neutralizing-antibody-Rapid-Test-Kit.pdf.
8. Yan Y, Chang L, Wang L. Laboratory testing of SARS-CoV, MERS-CoV, and SARS-CoV-2 (2019-nCoV): current status, challenges, and countermeasures. *Rev Med Virol*. 2020;30(3):e2106. [CrossRef]
9. Uwamino Y, Wakui M, Aoki W, et al. Evaluation of the usability of various rapid antibody tests in the diagnostic application for COVID-19. *Ann Clin Biochem*. 2021;58(3):174-180. [CrossRef]
10. Tang MS, Hock KG, Logsdon NM, et al. Clinical performance of the Roche SARS-CoV-2 serologic assay. *Clin Chem*. 2020;66(8):1107-1109. [CrossRef]
11. Kontou PI, Braliou GG, Dimou NL, Nikolopoulos G, Bagos PG. Antibody tests in detecting SARS-CoV-2 infection: a meta-analysis. *Diagnostics (Basel)*. 2020;10(5):319. [CrossRef]
12. La Marca A, Capuzzo M, Paglia T, Roli L, Trenti T, Nelson SM. Testing for SARS-CoV-2 (COVID-19): a systematic review and clinical guide to molecular and serological in-vitro diagnostic assays. *Reprod Biomed Online*. 2020;41(3):483-499. [CrossRef]