Serology in COVID-19 – Comparison of Two Methods

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Research Article

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Abstract

The aim of our study was to investigate the difference in the anti-SARS-CoV-2 antibodies assessment with two various assays. 127 patients exposed to SARS-CoV-2 were included. Two serological tests were used: SARS-CoV-2 IgG CMIA on the Alinity system (ABBOTT) and LIAISON® SARS-CoV-2 S1/S2 IgG CLIA (DiaSorin). Both assays exhibited 96.85% (123/127 patients) overall compatibility. In 2 cases the positive results in N-based test, were negative in S-based test, and in 2 cases negative results in S-based test were positive in N-based test. Based on the results of our study we concluded that the assessment of anti-SARS-CoV-2 antibodies against N and S protein in population medicine shows comparable usefulness of both tests.

1. Introduction

COVID-19 pandemics is recently one of the most important subjects of epidemiological investigations throughout the world. Quick identification of viral reservoir hosts, knowledge of virus spread in households, communities and specific settings as well as proper assessment of the rate of asymptomatic infections could significantly influence on establishing proper control measures. Validated serologic tests are crucial in realizing such tasks.

An important application of serological tests is to understand the antibody responses associated with SARS-CoV-2 infection and vaccination. Other important role of serological studies is to provide answers concerning potential reinfection. It is unknown if the presence of binding antibody to the spike (S) protein or receptor-binding domain (RBD) antigens correlates with virus neutralization and whether antibody titers (binding or neutralizing) correlate with protection from reinfection. Serology testing is also useful in the evaluation of antibodies titer in donors for convalescent plasma therapy [1, 2, 3].

More and more commercial tests are available. Evaluation of their usefulness is needed.

2. Aim

We aimed to compare the usefulness of two different immunoassays in anti-SARS-CoV-2 antibodies detection (against N protein and against S protein), because of potential diversity of *Coronavirus* strains and variety of immunological response after infection in population.

3. Material And Methods

Material

In total, 127 patients exposed to SARS-CoV-2 were included in the study. Among them 68 (53.54%) were patients hospitalized the Department of Infectious Diseases and Neuroinfections at the Medical University of Bialystok and 59 (46.46%) were healthcare workers exposed to the virus, but without

symptoms of infection. Blood samples for immunoserological diagnostic were collected from all the patients in the study one month after the exposition.

The study was approved by the Bioethical Commission of Medical University of Bialystok APK.002.259.2020. All patients signed informed consent for participation in the study.

Methods

Two serological tests have been used:

1. The SARS-CoV-2 IgG assay chemiluminescent microparticle immunoassay (CMIA) used for qualitative detection of IgG antibodies to SARS-CoV-2 in human plasma or serum on the Alinity system (ABBOTT) anti-N protein
2. The LIAISON® SARS-CoV-2 S1/S2 IgG chemiluminescence immunoassay (CLIA) technology for the quantitative determination of anti-S1 and anti-S2 specific IgG antibodies to SARS-CoV-2 (DiaSorin)

1. SARS-CoV-2 IgG - ALINITY (ABBOTT)

The SARS-CoV-2 IgG assay is a chemiluminescent microparticle immunoassay (CMIA) used for qualitative detection of IgG antibodies to SARS-CoV-2 in human plasma or serum on the Alinity system (ABBOTT). This assay is an automated two-step immunoassay. Sample SARS-CoV-2 antigen coated paramagnetic microparticles. The IgG antibodies to SARS- CoV-2 present in the sample bind to the SARS-CoV-2 antigen coated microparticles. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated. The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is direct relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics. This relationship is reflected in the calculated index (S/C). The titer above > 1,4 was considered as positive [4].

2. LIAISON® SARS-CoV-2 S1/S2 IgG

The specific recombinant S1 and S2 antigens are used for coating magnetic particles (solid phase) and mouse monoclonal antibodies to human IgG are linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, the SARS-CoV-2 IgG antibodies present in calibrators, samples or controls bind to the solid phase through the recombinant S1 and S2 antigens. During the second incubation the antibody conjugate reacts with IgG to SARS-CoV-2 already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of IgG to SARS-CoV2 concentration present in calibrators, samples or controls. The titer above 15 AU/Ml was considered as positive [5].

All methods were carried out in accordance with relevant guidelines and regulations.

Statistical analysis

Statistical analysis was performed by using STATISTICA Data Miner + QC. For immeasurable features percentage was counted. *P* value <0.05 was considered statistically significant.

4. Results

The anti-SARS-CoV-2 IgG antibodies were matching in 123/127 (96.85%) patients when detecting N or when detecting S protein. Only in 4/127 cases the results differed. In 2 cases the positive results in test based on N, were negative in test based on S, and in 2 cases negative results in test based on S, were positive in test based on N (p=NS).

56/127 (44.09%) patients had anti-SARS-CoV-2 IgG antibodies detected – 55/56 (98.2%) were symptomatic patients. None of healthcare workers was positive. The men titre of anti-SARS-CoV-2 IgG antibodies when detecting N protein was 2.75 ±3.34 (Max – 9.75 S/C), while the men titre of anti-SARS-CoV-2 IgG antibodies when detecting S protein was 90.64 ±68.97 (Max – 330 AU/Ml).

5. Discussion

The majority of serologic assays for anti-SARS-CoV2 are performed in serum or plasma and detect IgG antibodies to spike protein, receptor-binding domain (RBD)), which is part of the spike protein (anti-S1 and anti-S2) and nucleocapsid protein (protein N).

The spike (the main surface glycoprotein that is used to attach and enter cells) and nucleocapsid proteins are major immunogenic components of *Coronavirus* and are produced in abundant quantities during infection. The S protein is the principle determinant of protective immunity and cross-species transmission in *Coronavirus*, monoclonal antibodies against the S protein could neutralize viral infectivity. On this basis, Walls et al. hypothesize that exposure to either SARS-CoV or SARS-CoV-2 would elicit mutually cross-reactive, potentially neutralizing antibodies and demonstrated the ability of plasma from four mice immunized with a SARS-CoV S protein, to bind SARS-CoV-2 S protein and to block SARS-CoV-2 entry into target cells [6].

In the clinical practice the role of serological testing is extremely important, especially in COVID-19 convalescents who are willing to be plasma donors. Sensitivity and specificity of these tests should be as high as possible.

Our study shows, that both tests have comparable value in assessment of the immunity after infection - assays exhibited 96.85% % overall compatibility. The results of our study are in opposition to the study by Burbelo et al., who concluded, that antibody to the nucleocapsid protein of SARS-CoV-2 is more sensitive than spike protein antibody for detecting early infection [7]. We included patients in the convalescent

phase of infection, what influenced on the results and emphasizes role of both tests in evaluation of immunity after SARS-CoV-2 infection.

Conclusions

The assessment of anti-SARS-CoV-2 antibodies in population medicine based on spike protein or on nucleocapsid protein shows comparable usefulness.

Declarations

Conflict of interests:

None

Funding:

No funding was obtained.

Authors contribution:

AMM – planned and conducted the study; wrote manuscript

SP, BM – planned and organized the study

JD, WJ – performed analyses

PCz – collected samples from patients; wrote manuscript

EK - collected samples from patients

Data Availability Statement:

The data that support the findings will be available on request under the Corresponding Autor's e-mail: annamoniuszko@op.pl

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