LETTER TO THE EDITOR
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Long-term antibody-response monitoring following primary exposure to SARS-COV-2 and afterward mRNA COVID-19 vaccination: A case report

Dugoročno praćenje odgovora posredovanog antitelima posle primarne ekspozicije SARS-COV-2 i posle mRNA COVID-19 vakcinacije: Prikaz slučaja

Key words:
antibodies; covid-19; covid-19 serotherapy; infections; vaccination.

Ključne reči:
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To the Editor:

The majority of individuals infected by the novel coronavirus, or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), typically have a mild/moderate form of the resulting "coronavirus disease 2019" (COVID-19). However, the current pandemic counts of COVID-19 cases are higher than 111 million infected individuals worldwide, with approximately 2.5 million deaths. The most important preconditions for COVID-19 "expansion" are: 1) high potential of "human-to-human" virus transmission and 2) existence of an "immunologically-naive" background, i.e., population [1–4].

Rapid and almost limitless spreading of the disease has inspired the emergence of intense fundamental and preclinical studies, as well as initial clinical trials. The aim of these investigations was: 1) to determine multiple immune-mediated and other morphological, functional, and molecular "damaging-events" in targeted tissues; 2) to improve diagnostic tools in order to verify or exclude SARS-CoV-2 infection; 3) to "update" available therapy and improve newly-developed treatment options in order to reduce global healthcare-system crisis and decrease morbidity/mortality rate [3–5]. However, multiple prospective studies are needed to determine treatment directions, dosing, and side-effects of these medications.

Antibody-response to the receptor-binding domain (RBD) of the spike (S) protein of SARS-CoV-2 after infection remains incompletely evaluated. Dynamics/kinetics intensity and duration of antibody production, as well as anti-SARS-CoV-2 cross-reactivity with other coronaviruses and antibody-mediated protection after infection, are still undetermined [6,7]. Potential treatments incorporate medicaments, such as antiviral drugs, anti-interleukin-6 receptor monoclonal antibodies (mAbs), and allogeneic convalescent plasma with neutralizing anti-SARS-CoV-2 antibodies, which have been used for some earlier indications and innovative therapeutic approaches/strategies [4–9]. Finally, numerous safe, well-tolerated, and immunogenic COVID-19 vaccines have been developed. Thus, to this day, different vaccines have been already certified or are still progressing through phase-3-trials [10, 11]. Although researchers are not absolutely sure whether the infection itself or the use of vaccines generate a more powerful antibody-response, one fact is undoubtedly evident – the use of vaccines is much safer [7, 10, 11].

The purpose of this letter is to present our results of a 10-month continuous anti-SARS-CoV-2 antibody level monitoring in serum/plasma by enzyme-linked immunosorbent assay (ELISA), after the "initial/natural" exposure to SARS-CoV-2 (infection), followed by the application of the mRNA COVID-19 vaccine. Moreover, some "diagnostic-steps" and data concerning convalescent plasma collection by apheresis – designed for upcoming basic studies and/or potential therapeutic use – will be summarized.

On April 6, 2020, a 67-year-old male was diagnosed with COVID-19, owing to positive molecular testing, using the quantitative Polymerase Chain Reaction (qPCR) technique. SARS-CoV-2 RNA, isolated from nasopharyngeal/oropharyngeal swabs, was reversely transcribed to cDNA

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and subsequently amplified using QuantStudio-5 Real-Time PCR-System (Thermo-Fisher Scientific; USA). The results of relevant laboratory testing were the following: white blood cells (WBC) = 7.4 × 10^9/L, lymphocytes = 2.9 × 10^9/L, C-reactive protein (CRP) = 6.9 mg/L (normal ≤ 5.0 mg/L), D-dimer = 0.35 μg/mL (normal ≤ 0.5 μg/mL), and chest radiography was without signs of a pathological process. The patient was self-isolated at home according to the regulations at that time (28-days quarantine) with mild symptoms of COVID-19, such as rare subfebrility (up to 37.3°C), dry cough, and throat scratching. On April 20 and May 5, 2020, the results of the PCR-testing were negative. After that, the patient's quarantine was canceled.

On June 9, 2020, the results of rapid-antibody-testing were negative for IgM and positive for IgG, using Vazyme 2019-nCoV IgG/IgM Detection-Kit (Vazyme Biotech Co. Ltd.; China).

For detecting IgG antibodies to SARS-CoV-2, sera samples were firstly inactivated at 56 ºC for 30 minutes. These assays detected IgG antibodies, targeting the spike (S1) and nucleocapsid (N) proteins. Positive, negative, and cut-off controls were run with each test run. The cut-offs were calculated according to the manufacturer, as well as the antibody index, which was estimated as the ratio of sample and mean cut-off optical densities. Sera displaying antibody indices < 4 are considered as negative, those from 4–6 as equivocal, and those > 6 were presented as positive.

Simultaneously with the rapid-antibody-testing (June 9, 2020), the results of the first IgG anti-SARS-CoV-2 investigations were: 47.5 (positive > 6) by ELISA, using VirClia device (Vircell, Spain), and 7.4 (positive > 1.4) by two-step chemiluminescent microparticle immunoassay (CMIA) using Architect i2000SR system (Abbott, Germany), respectively. After that, levels of IgG antibodies were monitored only by ELISA. Kinetics of antibody levels was nearly constant in the first 6 months after the onset of the symptoms – on August 5, 2020, antibody index was 47.9, and on October 15, 2020, it was 33.3, respectively. These results were comparable with the data from literature [6, 7]. Afterward, progressive decreases in IgG levels were shown on November 30, 2020, and January 11, 2021 – indexes were 18.8 and 17.3, respectively (Figure 1).

On June 15, 2020, convalescent plasma was collected from the investigated person by apheresis. Plasmapheresis was performed by Spectra Optia device (Terumo BCT; USA). The total volume of collected plasma was 960 mL: 360 mL for studies and 750 mL for potential therapeutic use. The patients was non-reactive for hepatitis B and C viruses (HBV and HCV, respectively), human immunodeficiency virus (HIV), and syphilis (lues) markers. Plasma samples (10 mL per tubes; 6 samples) and units (150 mL per bag; 6 units) were cryopreserved with uncontrolled-rate technique ("dump-freezing"; cooling rate: 1–2°C/min) by simple placing of tubes/units into a mechanical freezer ULT C75 (Nordic Lab; Denmark). They will be stored at –90 ± 5°C thawing and investigation (or potential therapeutic use).

On January 13, 2021, the investigated person received the first dose of the mRNA COVID-19 vaccine (Pfizer-BioNTech; USA). The first vaccination was well tolerated, without adverse events or complications. Afterward, the IgG antibody level rapidly increased – the IgG index was 67.87 on January 27, 2021. Finally, the second dose of the same vaccine was applied on February 03, 2021. Following the second dose of vaccine, transient chills manifested 6–8 hours after application. On February 18, 2021, the IgG index (70.3) was also high, as shown in Figure 1. The presented data generally agree with those reported in the literature on both the antibody levels and the duration of their presence in human plasma [6–10, 12].

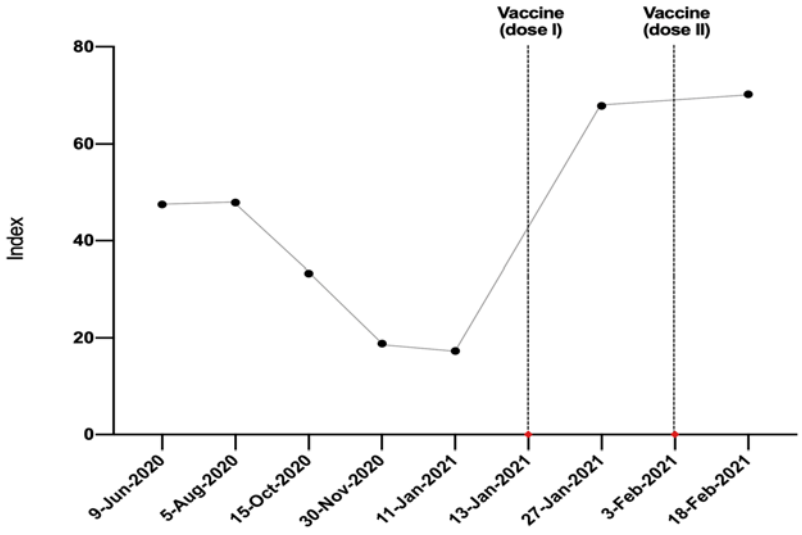

Fig. 1 – Antibody plasma levels in the investigated person before and after application of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine.

As recently verified, immune-response mediated by specific antibodies to RBD epitopes of the SARS-CoV-2 S protein positively and closely correlates with their neutralizing-capacity because RBD is responsible for binding to angiotensin-converting enzyme 2 (ACE2). Thus, the synthesis and elevated plasma level of these antibodies could make an effective platform for SARS-CoV-2 elimination and corre-

late with a milder course of the disease, as well as superior clinical recovery [2, 7]. Antibody-response correlates clearly with SARS-CoV-2 neutralizing activity (virus deactivation/elimination) [6, 7]. Furthermore, as presented, antibody titers remain relatively stable for several (6–8) months after the primary exposure to SARS-CoV-2 [6, 12]. Although the titer of antibodies may significantly decline with time in some persons, the specific T and B memory cells remain [12].

The SARS-Cov-2 infection could be treated by allogeneic plasma collected from recovered COVID-19 patients, typically simultaneously with antiviral agents, steroids, and other medication [4, 8, 9]. Although polyclonal antibodies (existing in collected plasma) are already in routine therapeutic use, further controlled clinical trials are needed to confirm the concept of COVID-19 treatment by convalescent plasma infusion [8, 9]. There are also data concerning the production of mAbs for treating COVID-19. A major disadvantage of this therapeutic approach is an insufficient mAb quantity for expected oversize requests in healthcare systems and their high cost [5]. Finally, since the RBD-region is a potent immunogenic epitope, it is most likely an ideal "antigen-candidate" for vaccine design [7].

In conclusion, the anti-SARS-CoV-2 antibodies detected in this pilot study, particularly their increased plasma level after vaccination, could be protective enough against a possible new COVID-19. We speculate that they could provide a more effective virus elimination following a (re)infection. Besides, in presenting this case, we point out that vaccination (particularly the use of the first dose) to date has demonstrated neither critical side-effects nor inferiority in antibody-response when compared to the infection itself. The results presented require further basic studies and prospective clinical studies.

Conflict of interests

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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