CASE REPORT

COVID-19 disease: monitoring of antibodies against SARS-CoV-2 virus in serum of a vitamin D sufficient male

J. Osredkar[1,5], K. Resman Rus[2], M. Korva[2], T. Kotar[3,6], K. T. Fabjan[1], A. Jerin[1,5], D. Siuka[4] and T. Avšič Županc[2]

[1]University Medical Centre Ljubljana, Institute of Clinical Chemistry and Biochemistry, Slovenia, [2]University of Ljubljana, Institute of Microbiology and Immunology, Faculty of Medicine, Slovenia, [3]University Medical Centre Ljubljana, Infectious Diseases Department, Slovenia, [4]University Medical centre Ljubljana, Division of Internal Medicine, Department of Gastroenterology, Slovenia, [5]University of Ljubljana, Faculty of Pharmacy, Slovenia, [6]University of Ljubljana, Faculty of Medicine, Slovenia

ABSTRACT

Background: COVID-19 presents with a range of symptoms from mild to severe, and some individuals remain asymptomatic. The appearance and decline of antibodies to SARS-CoV-2 varies with disease severity, age, and underlying health conditions. The time course of antibody decline also differs among individuals. Variants of the virus can evade neutralizing antibodies, which can affect vaccine efficacy. SARS-CoV-2 affects biochemical processes, resulting in an overactive immune response, increased risk of blood clots, impaired glucose metabolism, decreased insulin sensitivity, oxidative stress, and neurologic effects.

Case presentation: This study followed antibody levels after SARS-CoV-2 infection and assessed the effects of vaccination on antibody levels over time. A 65-year-old COVID-19 patient was examined and diagnosed by a positive PCR test. The patient was not taking regular medications other than self-administered cholecalciferol. Regular follow-up, including telemetric monitoring and symptom assessment, was performed for one year.

Conclusions: The results showed that antibody levels to SARS-CoV-2 spike protein and neutralizing antibodies were highest after vaccination and booster doses and gradually decreased over time. Of the biochemical parameters studied, only d-dimer and ferritin transiently exceeded reference values. Vitamin D consumption before infection may have had a positive influence on a milder course of the disease, as observed in other viral infections.

Keywords: antibodies against SARS-CoV-2, COVID-19, vaccination

J. Osredkar, K. Resman Rus, M. Korva, T. Kotar, K. T. Fabjan, A. Jerin, D. Siuka and T. Avšič Županc. COVID-19 disease: monitoring of antibodies against SARS-CoV-2 virus in serum of a vitamin D sufficient male. Scientific Chronicles 2024; 29(1): 136-143

INTRODUCTION

A new coronavirus infection cannot be distinguished from other causes of acute respiratory infections, including pneumonia, based on the patient's symptoms and course of illness alone, but should be confirmed by microbiologic testing. Approximately 80% of infected patients have only mild symptoms limited to the upper respiratory tract. However, in severe cases, the disease

progresses to lower respiratory tract infection, resulting in hypoxemia and lung damage.

SARS-CoV-2 infection can trigger an excessive immune system response known as cytokine release syndrome or cytokine storm. The antibody response in severe COVID-19 is well defined, but since most patients have mild or asymptomatic disease, it is important to know the antibody dynamics in this form of COVID-19 as well. According to most observational studies, the risk for severity of COVID-19 correlates with vitamin D deficiency [1]. The antibody response in the mild and asymptomatic form of the disease is important for a better understanding of the role of vitamin D in other viral infections as well.

The main objective of our study was to determine how antibody concentrations change over time depending on infection, vaccination, and booster dose in patients known to have adequate vitamin D levels.

CASE PRESENTATION

The 65-year-old patient was referred to the Infectious Diseases Department on November 4, 2020, because he was ill with COVID-19 illness. The patient became ill a day earlier with a temperature of 37.4˚C and retrosternal pain that felt like someone was sitting on his chest. He also complained of headache, fatigue, and muscle pain. He denied ageusia. He initially presented to ER, where acute ischemic heart disease was ruled out and a SARS-CoV-2 diagnosis was made with a positive real-time PCR test. The patient is not taking any regular medications, except for self-administered cholecalciferol 1000 IU/day as of April 2020, and epidemiologically, he had no known contacts with SARS-CoV-2-positive individuals. On medical examination, he was afebrile, saturation 97%, BP 129/102, heart rate 93/min, temperature 35.70 C, oxygen saturation 97%. The lungs were clear. The patient did not require hospitalization. Because of chest pain, he was monitored telemetrically to check blood pressure, pulse, oxygen saturation, temperature, and all other signs and symptoms twice daily from home.

The patient was examined regularly every two months for one year. Baseline laboratory results and clinical observations during the follow-up period are shown in Tables 1 and 2. In our case report, elevation of D-dimer in acute illness and urea in the first two months after infection were noted. D-dimer, urea, and creatinine were again upregulated one year after acute infection. In the first four months after COVID -19, the main symptoms were chest pain and breathing problems, and one year later the patient had problems with long-term memory. Blood samples were taken at regular monthly intervals to determine the titers of IgG and IgM antibodies to SARS-CoV-2 nucleocapsid (N) and spike (S) protein (Figure 1 A,B) and neutralizing antibodies (Figure 2).

The results showed that the antibody concentration against SARS-CoV-2 S protein is highest after vaccination and buster vaccination, and that the concentration of antibodies decreases with time after vaccination (Figure 1). Similarly, neutralization antibodies to the SARS-CoV-2 B.1 lineage are highest after acute infection and vaccination and decrease with time. The dynamics of neutralization antibodies against delta in the Omicron lineage are similar to the B.1 lineage, but the titer of antibodies is much lower (Figure 2).

Table 1: The basic laboratory results during the observation period. Blood samples with EDTA were measured within 1 hour of collection using the ADVIA 2120 haematology analyser, and coagulation tests were performed using the BCS XP analyzer (both analyzers and reagents Siemens Healthcare Diagnostics, Erlangen, Germany). Biochemical parameters in serum samples were measured using the Advia 1800 analyzer (analyzer and reagents Siemens Healthcare Diagnostics, Erlangen, Germany). An electrochemiluminescence assay (Cobas e411 analyzer, Roche Diagnostics, Mannheim, Germany) was used to measure 25-hydroxyvitamin D in serum.

Laboratory parameter	Acute COVID-19	2 weeks after	2 months after	4 months after	6 months after	1 year after
WBC [10^9/L]	6,4	6,5	7,2	7,1	6,2	7,2
HB [g/L]	163	155	163	158	156	167
CRP [mg/L]	< 5	< 5	< 5	< 5	< 5	< 5
PLATELETS [10^9/L]	171	248	229	208	211	267
D DIMER [µg/L]	**784**	**545**	340	420	391	**516**
LDH [µkat/L]	2.54	2.76	2.80	2.68	2.34	2.86
FERITTIN [µg/L]	274	**300**	242	186	139	184
TROPONIN [ng/L]	< 3	< 3	< 3	< 3	< 3	< 3
UREA [mmol/L]	6.6	**7.9**	**8.4**	7.0	6.8	**8.1**
CREATININE [µmol/L]	92	87	83	84	85	**102**
AST [µkat/L]	0.44	0.45	0.45	0.46	0.32	0.42
ALT [µkat/L]	0.54	0.69	0.51	0.62	0.44	0.49
25-OH-D [nmol/L]	75	60	77	81	71	114

In bold are displayed the results outside the reference range.

Table 2: The clinical observations in the follow-up period.

Date of medical check-up after acute disease	**Observations**
2 weeks	No breathing problems, any pain, afebrile Taste and smell not affected No abnormalities during medical examination
2 months	Occasional shortness of breath Atelectasis or fibrosis in the left laterobasal segment
	Vaccination against Covid-19 in February 2021
4 months	Chest pains similar to those during the acute illness Difficulty breathing Memory problems
6 months	Chest pain during physical activity No breathing problems Memory problems Occasionally problems with balance
1 year	Generally well-being Long-term memory problems

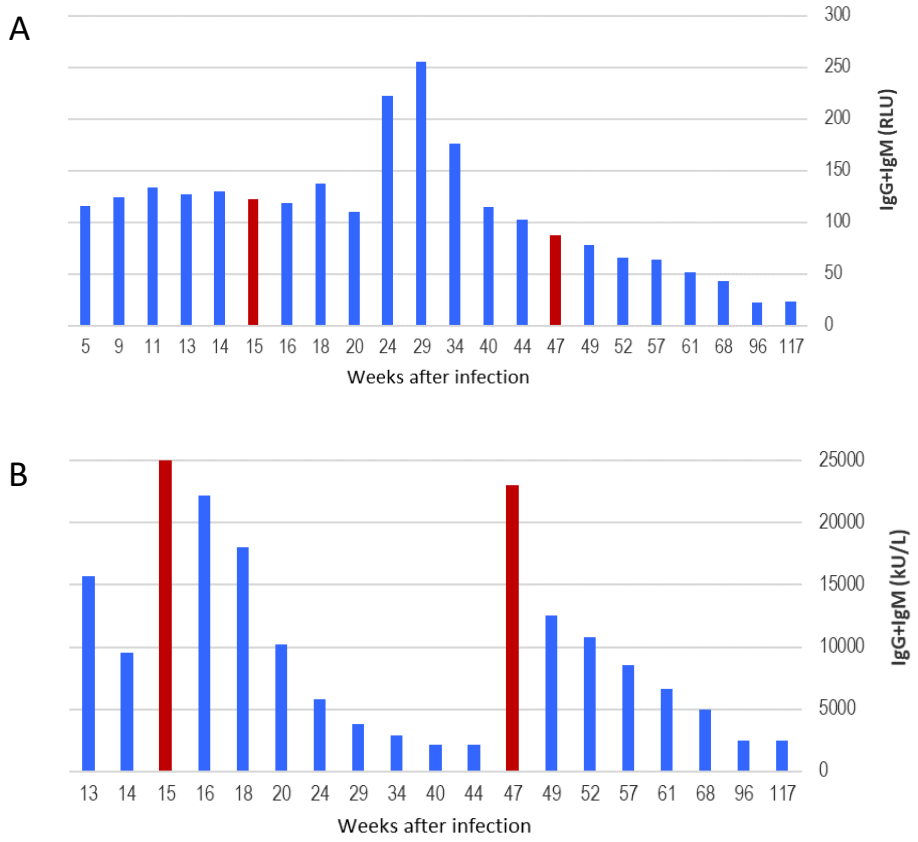

Figure 1: The combined concentration of IgG and IgM antibodies against SARS-CoV-2 nucleocapsid (A) and spike (B) protein. Vaccination and buster vaccination points are coloured in red. (A) An electrochemiluminescence assay (Cobas e411 analyzer, Roche Diagnostics, Mannheim, Germany) was used for qualitative detection of IgG and IgM antibodies to the SARS-CoV-2 nucleocapsid protein; samples with a COI greater than 1.0 were considered positive. (B) The combined concentration of IgG and IgM antibodies against the SARS-CoV-2 spike protein receptor-binding domain was measured in serum samples using an electrochemiluminescence assay (Cobas e411 analyzer, Roche Diagnostics, Mannheim, Germany) with a detection limit of 0.40 kU/L.

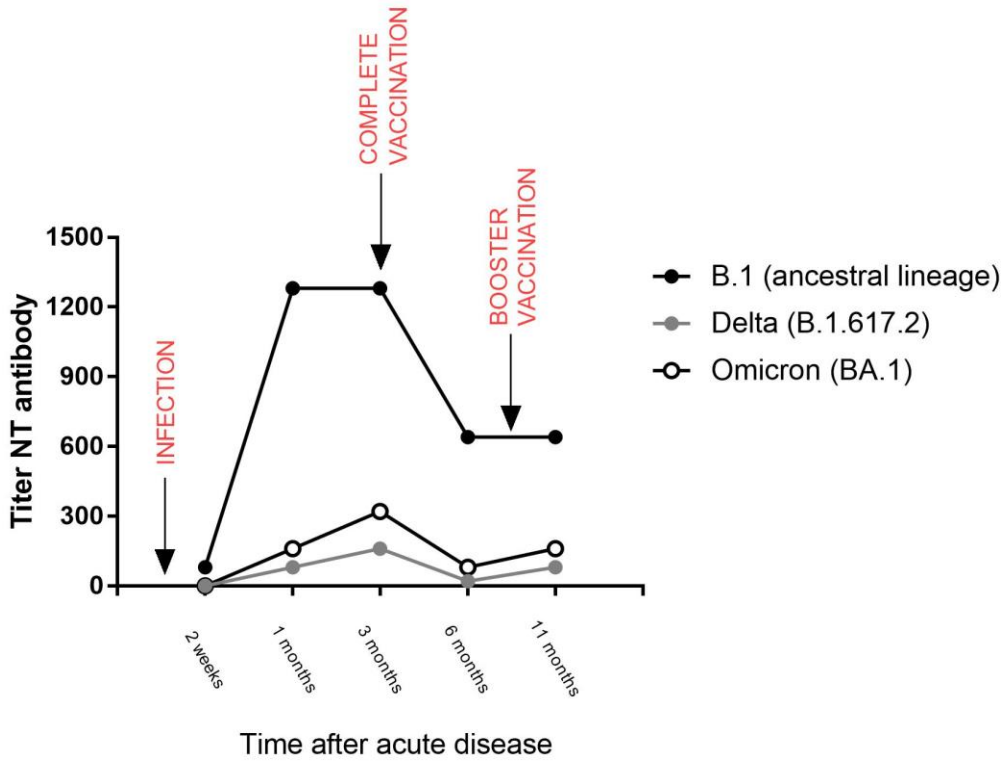

Figure 2: Titer of neutralizing antibodies in the period from November 2020 to November 2021. A neutralization test (NT) was performed in a BSL-3 laboratory. In the test three different SARS-CoV-2 virus isolates were used: ancestral strain D614G (EVA-GLOBAL-Ref-SKU: 005V-03961; B.1), strain Delta (EVA-GLOBAL-Ref-SKU: 005V-04249; B.1.617.2) and strain Omicron (EVA-GLOBAL- Ref-SKU: 005V-04479; BA.1 sublineage of B.1.1.529). Neutralization assay was performed as previously published [2].

COMMENTS

SARS CoV-2 has caused a worldwide pandemic, and symptoms of COVID -19 can range from mild to severe. Most patients develop a mild course of illness with symptoms including fever, cough, fatigue, body aches, loss of smell or taste, sore throat, headache, or nasal congestion and recover within a few weeks. Similar clinical symptoms described in the literature were also experienced by the male patient described in this case report. To find an effective treatment for COVID -19, follow-up of clinical and immune parameters is important even in the mild form of the disease, especially in our case when the patient was taking vitamin D at known concentrations before infection with SARS-CoV-2. Clinical observation during the follow-up period showed that some health problems continued to occur one year after SARS-CoV-2 infection, even though the patient had a mild form of COVID -19 [3].

SARS-CoV-2 can significantly affect various biochemical processes in the body, such as inflammation, blood clotting, metabolism, oxidative stress, and neurological effects. The most common changes are lymphopenia, elevated CRP, LDH, erythrocyte sedimentation and D-dimer levels, and low serum albumin and haemoglobin levels [4]. Of all laboratory parameters followed up, only D-

dimer, urea, and creatinine were elevated in the presenting patient, of which only D-dimer was elevated in the acute COVID -19.

Specific antibodies and a T-cell response are the most important protection against COVID -19. An important target for the induction of antibodies, especially neutralizing antibodies (NAbs), is the SARS-CoV-2 spike protein (S) [5]. However, antibodies to the virus nucleocapsid (N) protein are less likely to directly neutralize the virus [6]. The degree of immune response is related to the severity of disease, with patients with severe disease responding more strongly than patients with mild disease [7]. The results of our serological tests of the combined concentration of IgG and IgM antibodies and the neutralization assay are consistent with previously published results showing that antibody levels decrease approximately exponentially over time [8]. In addition, the results also confirm that titer decreases with time after BNT162b2 vaccination [9].

It is well known that vitamin D plays an important role in innate and adaptive immunity, and at the very beginning of the COVID -19 pandemic, the broad medical community was strongly advised to consider vitamin D supplementation in patients at high risk for adverse COVID -19 outcomes [10]. However, most studies showed no correlation between serum vitamin D levels and SARS-CoV-2 antibody production [11].

CONCLUSIONS

In conclusion, this case report presents a patient who was taking vitamin D before infection with SARS-CoV-2. The patient developed mild COVID -19 symptoms, and an adequate immune response was observed. The case report is consistent with data suggesting that vitamin D may contribute to a milder disease course, as it has previously been shown to stimulate white blood cell production. These immune cells play a critical role in fighting off infections, so it is important to clarify the role of vitamin D in other viral infections as well.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: The authors declare no conflicts of interest.

Research funding: This research was supported by the Institute for Clinical Chemistry and Biochemistry, University Medical Centre Ljubljana and by the Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana; by the Slovenian Research and Innovation Agency (grants P3-0083 and V3-2034); and by the European Virus Archive—GLOBAL project, which has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement no. 871029; Network of infrastructure Centres of University of Ljubljana (MRIC-UL-IC-BSL3+).

Informed consent: The first author was the testing person and he proposed the study. The study was conducted according to the principles expressed in the Declaration of Helsinki.

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Corresponding author: **A. Jerin,** E-mail: ales.jerin@kclj.si