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ORIGINAL ARTICLE

Clinical Evaluation of Serum Levels of SARS-CoV-2 Anti-Spike Protein IgG Antibodies in Infected Patients and Vaccinated Subjects

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SUMMARY

Background: **The aim was clinical evaluation of immune response against SARS-CoV-2, analyzing serum levels of IgG antibodies against the SARS-CoV-2 protein S in infected and vaccinated patients, as well as in subjects with and without frequent comorbidities (arterial hypertension, diabetes mellitus, heart disease, and chronic respiratory disease).**
Methods: **Patients infected by SARS-CoV-2 confirmed by RT-PCR and subjects vaccinated with vaccines based on the mRNA encoding the SARS-CoV-2 protein S were studied. SARS-CoV-2 anti-S IgG serum levels were quantified by chemiluminescent microparticle immunoassay.**
Results: **There were 79 infected patients with a median age of 53.0 years; 35 women and 44 men; 42 patients with any comorbidities and 37 without comorbidities. The median of SARS-CoV-2 anti-S IgG serum level was 203.4 BAU/mL (11.6 - 5,620.6). The median antibody level in the infected patients with any comorbidities was higher than those without comorbidities. The group of vaccinated subjects included 96 subjects with a median age of 49.5 years; 77 women and 19 men; 31 subjects with any comorbidities and 65 without comorbidities. The median of SARS-CoV-2 anti-S IgG serum levels was 1,145.6 BAU/mL (138.3 - 4,828.1). No significant differences were found in terms of specific or global comorbidities in the vaccinated subjects.**
Conclusions: **SARS-CoV-2 anti-S IgG serum levels were 5.6 times higher in vaccinated subjects than infected patients. The vaccination produces higher serum antibody levels than SARS-CoV-2 infection. This reinforces the indication for the vaccine in infected patients. These antibodies did not decrease significantly in patients with frequent comorbidities such as hypertension, diabetes, heart disease or chronic respiratory disease.**
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KEY WORDS

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INTRODUCTION

SARS-CoV-2 is a new human beta coronavirus that appeared in late 2019 in Wuhan (China). It is an enveloped virus, which has one of the largest genomes among all RNA viruses (about 30 kb), like other members of its

family. These viruses are mainly associated with respiratory and enteric diseases, both in animals and humans [1]. The SARS-CoV-2 infection, also called COVID-19, causes very heterogeneous symptoms, ranging from asymptomatic forms, key in the silent transmission of the virus, to severe pneumonia with a fatal outcome [2, 3]. The microbiological diagnosis of the acute disease is based on the detection of the genetic material of the virus by real-time reverse transcription-polymerase chain reaction (RT-PCR) or viral antigen by rapid immunochromatographic tests in nasopharyngeal and other respiratory samples [4,5]. The immune response to this infection mainly involves memory B cells, through the production of antibodies, as well as CD4+ and CD8+ T cells. The demonstration of the presence of antibodies by indirect methods is a fundamental tool for the knowledge of the prevalence of the infection and the confirmation of cases with a negative molecular diagnosis. Most tests evaluate both IgG and IgM antibodies. IgM generally rises rapidly, while IgG rises more slowly and can persist over time reflecting longer-term immunity [6]. Two main types of antibodies are used in the serological study of SARS-CoV-2 infection: i) against the nucleocapsid (N) protein which are induced early in most infected individuals (it is unlikely that these antibodies are functionally relevant to confer protection or immunity); ii) against the spike (S) protein that probably provides immunity by neutralizing the virus [7]. Serological studies are also necessary for the evaluation of the immune status in vaccinated people. The vaccination process stimulates the immune system to generate memory B cells producing neutralizing antibodies, as is the case with mRNA-based COVID-19 vaccines encoding the S protein of SARS-CoV-2 [8].

Clinical studies evaluating serological tests are necessary to determine the strength of immunity against SARS-CoV-2 after infection or vaccination. The aim of this study was the clinical evaluation of the immune response against SARS-CoV-2, analyzing the levels of IgG antibodies against the SARS-CoV-2 protein S in infected patients and vaccinated subjects, as well as in subjects with and without frequent comorbidities (hypertension, diabetes, heart disease and chronic respiratory disease).

MATERIALS AND METHODS

Study design

The study is a descriptive, retrospective, observational and cross-sectional study and complies with the ethical recommendations of the Declaration of Helsinki [9], the EC Directive 86/609/EEC for animal experiments, and with the Uniform Requirements for manuscripts submitted to biomedical journals. This work was approved by the Cadiz Research Ethics Committee (registration number 80.21).

Subjects

Patients from the Bahia de Cadiz Health Area, treated at the Puerto Real University Hospital (HUPR), were studied. Two groups of patients were analyzed according to the mechanism of generation of antibodies against SARS-CoV-2:

1. Patients who have generated antibodies innately (infected patients). Electronic medical records of all patients with SARS-CoV-2 infection from November 1, 2020, to March 31, 2021, were reviewed. We included patients infected by SARS-CoV-2, confirmed by RT-PCR, aged 18 years and over, and with a serological study against SARS-COV-2 after at least 21 days following the onset of symptoms or molecular diagnosis in asymptomatic patients. We excluded infected patients without a molecular diagnosis and patients who received a vaccine against SARS-CoV-2 after recovering from the infection.
2. Subjects who have acquired antibodies (vaccinated subjects). Electronic medical records of vaccinated subjects between January 1 and March 31, 2021, were reviewed. We included subjects aged 18 years and over who were vaccinated against SARS-CoV-2 with two doses of the Pfizer-BioNTech vaccine (based on the mRNA encoding the protein S), with a serological study at least 21 days after the second dose. Subjects vaccinated with vaccines other than Pfizer-BioNTech, subjects that did not receive the full vaccination schedule, or subjects previously diagnosed with COVID-19 were excluded.

Methods

All venous blood samples were obtained by venipuncture, collected in standard serum tubes (VACUETTE® TUBE CAT 5 mL CAT Serum Separator Clot Activator), and centrifuged at 3,500 x g for 7 minutes at 20°C. IgG antibodies against SARS-CoV-2 protein S were quantified by chemiluminescent microparticle immunoassay (CMIA) in the Alinity i autoanalyzer (ref. 06S 6122, Abbott Laboratories, Chicago, IL, USA), with a cutoff value of 50.0 AU/mL provided by the manufacturer, equivalent to 7.1 BAU/mL (unit recommended by the World Health Organization: BAU/mL = 0.142 x AU/mL). Antibody levels below 7.1 BAU/mL were considered as negative results, and higher than 7.1 BAU/mL as positive. Antibody quantification was performed between 21 days and 6 months after infection or the last dose of vaccination to obtain maximum sensitivity [7,8].

The molecular diagnosis of SARS-CoV-2 was carried out in samples of nasopharyngeal exudate using the Allplex™ SARS-CoV-2/FluA/FluB/RSV Assay kit (ref. RV10259X, Seegene, St. Ingbert, Germany). This is a multiplex real-time RT-PCR that simultaneously detects three specific SARS-CoV-2 genes (N gene, S gene, and RdRP gene) along with influenza and respiratory syncytial virus.

Variables

a) Dependent variable: Serum levels of IgG antibodies against SARS-CoV-2 protein S. Continuous quantitative variable in BAU/mL.

b) Independent variables: demographic data (age and gender); common comorbidities (diabetes mellitus, arterial hypertension, heart disease (coronary syndromes, cardiac arrhythmias, cardiomyopathies) or chronic respiratory diseases (bronchial asthma, chronic obstructive pulmonary disease)); time to serology in infected patients (days elapsed between the appearance of symptoms and the determination of antibodies, or between the molecular diagnosis and the quantification of antibodies in asymptomatic patients); and time to serology in vaccinated subjects (days elapsed between the administration of the second dose and the antibody quantification).

Sample size

The sample size was calculated to obtain a significant difference between the means of infected patients and vaccinated subjects. Higher levels of antibodies were expected in vaccinated subjects than in infected patients. Based on our experience, with an expected mean of approximately 500 BAU/mL in infected patients and 1,500 BAU/mL in vaccinated subjects, and standard deviation of 1,000 BAU/mL in both groups, a minimum of 26 participants was required in each group to obtain a significance level $p < 0.05$ and a power of 95%.

Statistical analysis

Statistical analysis was performed using the statistical software MedCalc 13.0 (MedCalc Software, Ostend, Belgium). We performed a descriptive analysis: qualitative variables through frequencies; quantitative variables with normal distribution with arithmetic mean and standard deviation; and quantitative variables with non-Gaussian distribution using the median and interquartile range (IQR: difference between the 75th and 25th percentiles). The D'Agostino-Pearson test was used to determine the distribution of quantitative variables. Statistical association tests included: a) Comparison between groups of patients using the chi-squared test for qualitative variables, Student's *t*-test for quantitative variables with normal distribution, and the non-parametric Mann-Whitney U test for variables quantitative with non-Gaussian distribution; and b) Correlation between quantitative variables, such as Pearson's correlation coefficient for variables with normal distribution and Spearman's Rho correlation coefficient for non-Gaussian variables.

RESULTS

A total of 175 subjects were studied with ages between 19 and 89 years (median = 51.0 years), 112 (64%) were women and 63 (36%) were men. The anti-S SARS-CoV-2 IgG serum levels ranged between 11.6 and 5,620.6 BAU/mL (median = 781.2 BAU/mL). Time to serology ranged between 21 and 167 days (median = 41 days).

The group of infected patients consisted of 79 patients aged between 19 and 89 years (median = 53.0 years), 35 (44.3%) women and 44 (55.7%) men, 68 (86.1%) symptomatic and 11 (13.9%) asymptomatic. The anti-S SARS-CoV-2 IgG serum levels ranged from 11.6 to 5,620.6 BAU/mL (median = 203.4 BAU/mL). The time from the onset of symptoms or molecular diagnosis in asymptomatic patients to antibody quantification ranged between 21 and 167 days (median = 57.0 days). Regarding the comorbidities, 32 (40.5%) were hypertensive, 13 (16.4%) were diabetic, 18 (22.8%) had a history of heart disease, and 8 (10.1%) had chronic respiratory diseases. A directly proportional correlation was observed between serum antibody levels and time to serology (Spearman's coefficient of rank correlation (rho) = 0.458 ($p = 0.0001$)). Then, according to time to serology, we divided infected patients into two groups; Group A, before 60 days; and Group B, after 60 days. The descriptive statistics of serum antibody levels in both groups are shown in Table 1. Group A consisted of 40 patients with a median age of 43.5 years (19 to 81 years) and a time to serology between 21 and 57 days (median = 27 days). Group B consisted of 39 patients with a median age of 62 years (30 to 89 years) and a time to serology between 65 and 167 days (median = 105 days). No differences were found between genders. We found a direct proportional correlation between serum antibody levels and age in group A (Spearman's coefficient of rank correlation (rho) = 0.441 ($p = 0.0059$), but no correlation was found in group B. In group A, we only found significant statistical differences according to the presence of symptoms, arterial hypertension or diabetes mellitus ($p < 0.05$). No significant differences were found in group B with the presence of symptoms or comorbidities ($p > 0.05$). The median of antibody levels in the infected patients with any comorbidities studied in this work was higher than those without comorbidities (193.6 BAU/mL vs. 60.8 BAU/mL in group A ($p = 0.0175$) and 464.2 BAU/mL vs. 322.5 BAU/mL in group B ($p > 0.05$), respectively).

The group of vaccinated subjects included 96 subjects treated with the two doses of the vaccine, with a median age of 49.5 years (25 to 68 years), 77 (80.2%) women and 19 (19.8 %) men, and with anti-S SARS-CoV-2 IgG serum levels between 138.3 and 4,828.1 BAU/mL (median = 1,145.6 BAU/mL). The time from the administration of the last dose of the vaccine to antibody quantification ranged between 24 and 61 days (median = 38 days). Nineteen subjects (19.8%) were hypertensive, four (4.2%) were diabetic, 10 (10.4%) had a history of heart disease, and six (6.2%) had chronic respiratory disease. No correlation was found between serum antibody levels and time to serology or age. The descriptive statistics of serum antibody levels in vaccinated subjects are shown in Table 2. No significant differences were found between gender or comorbidities ($p >$

Table 1. Descriptive statistic of SARS-CoV-2 anti-S IgG serum levels in SARS-CoV-2 infected patients (n = 79).

		Group A		Group B	
		n	anti-S IgG (BAU/mL) median (IQR)	n	anti-S IgG (BAU/mL) median (IQR)
All infected patients		40	72.1 (171.7)	39	401.5 (599.7)
Gender	female	19	108.4 (454.8)	16	405.1 (622.8)
	male	21	60.83 (120.2)	23	401.5 (563.7)
Asymptomatic	yes	10	58.9 (82.4)	1	1,082.7
	no	30	98.2 (425.9)	38	375.6 (580.4)
Arterial hypertension	yes	10	358.2 (1,737.3)	22	406.9 (658.1)
	no	30	63.0 (114.1)	17	401.5 (403.7)
Diabetes mellitus	yes	5	628.8 (3,070.2)	8	405.1 (624.1)
	no	35	65.3 (147.7)	31	401.5 (578.4)
Heart disease	yes	4	58.9 (28.77)	14	484.9 (617.4)
	no	36	96.1 (304.9)	25	349.6 (597.2)
Chronic respiratory disease	yes	3	158.4 (393.5)	5	290.32 (667.6)
	no	37	66.81 (170.53)	34	432.9 (580.4)
Any comorbidity	yes	15	193.6 (1051.0)	27	464.2 (612.8)
	no	25	60.8 (114.5)	12	322.5 (349.6)

Group A - Serology before 60 days of SARS-CoV-2 infection, Group B - Serology after 60 days of SARS-CoV-2 infection, IQR - interquartile range.

Table 2. Descriptive statistic of SARS-CoV-2 anti-S IgG serum levels in vaccinated subjects with vaccines based on the mRNA encoding the SARS-CoV-2 protein S (n = 96).

		n	Anti-S IgG (BAU/mL) median (IQR)
All vaccinated subjects		96	1,145.6 (1,033.6)
Gender	female	77	1,276.7 (1,151.7)
	male	19	809.6 (978.9)
Diabetes mellitus	yes	4	539.4 (517.0)
	no	92	1,154.9 (1,026.0)
Arterial hypertension	yes	19	870.9 (861.3)
	no	77	1,149.4 (1,118.0)
Heart disease	yes	10	834.35 (1267.7)
	no	86	1,154.9 (1,008.8)
Chronic respiratory disease	yes	6	747.7 (525.0)
	no	90	1,173.2 (1,016.1)
Any comorbidity	yes	31	856.9 (971.9)
	no	65	1,276.7 (1,062.0)

IQR - interquartile range.

0.05).

Comparing the infected patients with the vaccinated subjects, we observed that the age and the time to serology were slightly higher in infected patients ($p = 0.0212$ and $p = 0.0129$, respectively). Inversely, the antibody levels were lower in infected than in vaccinated ($p <$

Table 3. Comparison of SARS-CoV-2 anti-S IgG serum levels in SARS-CoV-2 infected patients and vaccinated subjects with vaccines based on the mRNA encoding the SARS-CoV-2 protein S (n = 175).

	Anti-S IgG (BAU/mL) in infected patients (n = 79)	Anti-S IgG (BAU/mL) in vaccinated subjects (n = 96)
Range	**11.6 - 5,620.6**	**138.3 - 4,828.1**
Mean (95% CI)	**549.9 (340.3 - 759.6)**	**1,425.3 (1,222.9 - 1,627.8)**
Median (95% CI)	**203.4 (149.6 - 348.0)**	**1,145.6 (879.8 - 1,382.6)**
Standard deviation	**936.1**	**999.3**
Interquartile range	**542.5**	**1,033.6**
5th percentile (95% CI)	**19.2 (12.05 - 35.7)**	**361.0 (203.1 - 503.9)**
10th percentile (95% CI)	**25.7 (17.5 - 47.1)**	**409.8 (346.5 - 574.0)**
25th percentile (95% CI)	**61.9 (40.6 - 144.4)**	**765.7 (539.1 - 857.3)**
75th percentile (95% CI)	**604.4 (378.0 - 908.5)**	**1,799.3 (1,522.7 - 2,256.5)**
90th percentile (95% CI)	**1,218.9 (784.6 - 3,014.2)**	**2,936.2 (2,168.1 - 3,720.1)**
95th percentile (95% CI)	**2,390.4 (968.8 - 5,475.9)**	**3,544.9 (2,624 - 4,638.4)**

CI - confidence interval.

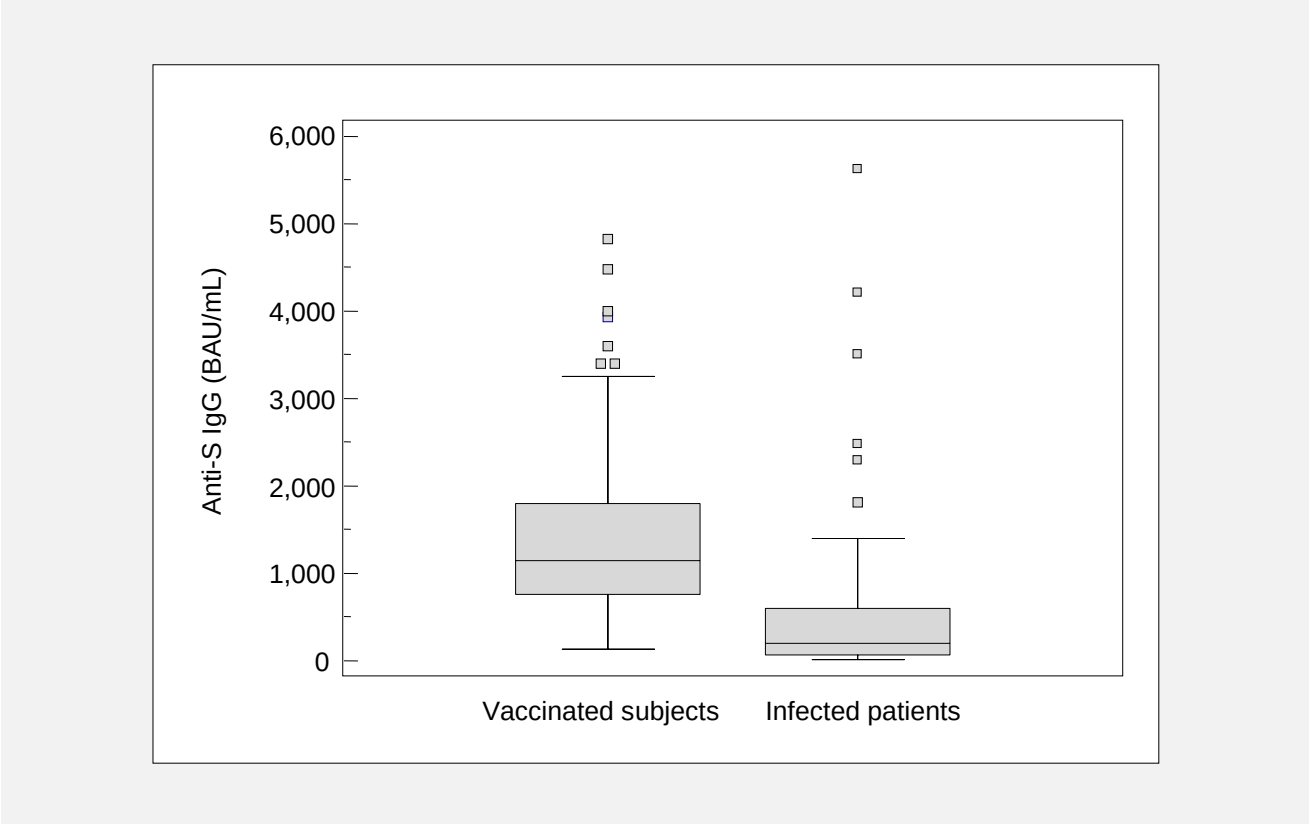

Figure 1. Box and whisker of SARS-CoV-2 anti-S IgG serum levels in SARS-CoV-2 infected patients (n = 79) and vaccinated subjects with vaccines based on the mRNA encoding the SARS-CoV-2 protein S (n = 96).

0.0001). Figure 1 and Table 3 compare the serum levels of SARS-CoV-2 anti-S IgG antibodies in infected patients and vaccinated subjects.

DISCUSSION

In this study, IgG antibodies against SARS-CoV-2 protein S have been detected in all infected patients at least 21 days after the positivity of the RT-PCR against SARS-CoV-2. Antibody levels were > 11.6 BAU/mL in all infected patients, much higher than the cutoff point provided by the manufacturer (7.1 BAU/mL), which implies 100% sensitivity of the test for the diagnosis of SARS-CoV-2 infection in the study population after 21 days of infection. Recently, Narasimhan et al. [8] analyzed the anti-S SARS-CoV-2 IgG levels using the same methodology, 20 days after the positivity of the RT-PCR, and they also obtained a high sensitivity (97.6%). Other works that studied antibodies against the SARS-CoV-2 N protein, reported sensitivities between 84% and 100% [10-15].

Serum antibody levels showed a directly proportional correlation with time to serology in infected patients. The antibody levels determined from 60 days after infection (group B) were significantly higher than those determined before 60 days (group A). No differences were found in serum antibody levels between infected men and women. In terms of age, although we found a correlation with serum antibody levels in group A, this was not observed in group B. Therefore, gender or age seems to have no influence on the long-term serum antibody levels. Asymptomatic patients had lower antibody levels than symptomatic patients. It seems that the infection with clinical manifestations could increase the immune response. This fact was found in group A. Due to the small sample size of asymptomatic patients (11 patients), this assumption must be taken with caution. According to published studies [16-19], hypertension was the most frequent comorbidity in COVID-19 patients, 40.5% of patients in our study. Patients with arterial hypertension in group A presented higher antibody levels than non-hypertensive patients, although the older age of hypertensive patients could justify this difference (serum levels of antibodies were correlated with age in this group). We could find the same justification for diabetic patients. On the other hand, no significant differences were found in group B regarding comorbidities. The medians of antibody levels in infected patients with some of the comorbidities studied in this work were higher than those without comorbidities. This means that the presence of hypertension, diabetes, heart or respiratory diseases in infected patients seems not to produce a decrease in the serum levels of IgG antibodies against SARS-CoV-2 protein S.

In the group of vaccinated subjects, serum levels of IgG antibodies against SARS-CoV-2 protein S did not correlate with age or time to serology. Although serum antibody levels were higher in women than in men (1,276.7 BAU/mL vs. 809.6 BAU/mL), no statistically significant differences were found ($p > 0.05$) and the number of vaccinated males was low ($n = 19$). We did not find significant differences regarding specific or global comorbidities, although the median of antibody levels was lower in subjects with any comorbidity than without comorbidities (856.9 BAU/mL vs. 1,276.7 BAU/mL, respectively). The median of anti-S SARS-CoV-2 IgG serum levels was slightly higher in our study than that reported by Narasimhan et al. (1,145.6 BAU/mL vs. 908.2 BAU/mL, respectively) [8]. In another work using the same methodology, the sera of 69 subjects were analyzed 21 days after vaccination with a single dose of Pfizer-BioNTech obtaining median of anti-S SARS-CoV-2 IgG levels of 155.7 BAU/mL, much lower than that obtained in our study after complete vaccination with the two doses [7]. This highlights the importance of full-schedule vaccination.

Regarding the comparison of infected patients and vaccinated subjects, we found approximately 5.6 times higher antibody levels in vaccinated subjects than in infected patients ($p < 0.0001$). Strikingly, if we compare similar time to serology, this difference was much higher (15.8 times compared with group A of infected patients; time to serology between 21 and 60 days). These results reveal a greater immunity acquired by vaccination than immunity generated innately after infection by SARS-CoV-2. These results reinforce the recommendation of the vaccination of infected patients, in order to increase protection against possible reinfections.

On the other hand, we know that most of the patients infected by a microorganism produce an innate immunity against that microorganism. If we consider that at least 95% of patients infected by SARS-CoV-2 can generate immunity, we could establish the 5th percentile of antibody serum level (19.2 BAU/mL) as the level from which a person could have an immune response with high efficacy. All vaccinated people had serum antibody levels higher than 138.3 BAU/mL, much higher than the 5th percentile of infected patients. This means that all vaccinated people included in this study acquired immunity against SARS-CoV-2.

In conclusion, SARS-CoV-2 anti-S IgG serum levels were significantly higher in vaccinated subjects than infected patients (5.6 times higher). The vaccination produces higher serum antibody levels than SARS-CoV-2 infection. This reinforces the indication for the vaccine in infected patients. Serum antibody levels in infected patients were directly correlated with time after infection, increasing significantly after 2 months. Asymptomatic infected patients could have lower levels of anti-SARS-CoV-2 IgG antibodies than symptomatic patients. Serum antibody levels did not decrease significantly in patients with frequent pathologies such as arterial hypertension, diabetes mellitus, heart disease or chronic respiratory diseases. Thus, patients vaccinated or infected by SARS-CoV-2 with any of these comorbidities will develop a humoral immunity against SARS-CoV-2 similar to those without comorbidities.

Declaration of Interest:
None declared.

References:

1. Kaur N, Singh R, Dar Z, Bijarnia RK, Dhingra N, Kaur T. Genetic comparison among various coronavirus strains for the identification of potential vaccine targets of SARS-CoV2. Infect Genet Evol 2021;89:104490. (PMID: 32745811)

2. Fu L, Wang B, Yuan T, et al. Clinical characteristics of coronavirus disease 2019 (COVID-19) in China: a systematic review and meta-analysis. J Infect 2020;80:656-65. (PMID: 32283155)

3. Martin S, Fuentes S, Sanchez C, et al. Development and validation of a laboratory-based risk score to predict the occurrence of critical illness in hospitalized patients with COVID-19. Scand J Clin Lab Invest 2021;81:282-9. (PMID: 33974458)

4. Kabir MA, Ahmed R, Iqbal SMA, et al. Diagnosis for COVID-19: current status and future prospects. Expert Rev Mol Diagn 2021;21:269-88. (PMID: 33621145)

5. Khandker SS, Nik Hashim NHH, Deris ZZ, Shueb RH, Islam MA. Diagnostic Accuracy of Rapid Antigen Test Kits for Detecting SARS-CoV-2: A Systematic Review and Meta-Analysis of 17,171 Suspected COVID-19 Patients. J Clin Med 2021;10:3493. (PMID: 34441789)

6. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Science 2021;371(6529):eabf4063. (PMID: 33408181)

7. Perkmann T, Perkmann-Nagele N, Koller T, et al. Anti-Spike Protein Assays to Determine SARS-CoV-2 Antibody Levels: a Head-to-Head Comparison of Five Quantitative Assays. Microbiol Spectr 2021;9:e0024721. (PMID: 34190591)

8. Narasimhan M, Mahimainathan L, Araj E, et al. Clinical Evaluation of the Abbott Alinity SARS-CoV-2 Spike-Specific Quantitative IgG and IgM Assays among Infected, Recovered, and Vaccinated Groups. J Clin Microbiol 2021;59:e0038821. (PMID: 33827901)

9. World Medical Association. World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. JAMA 2013;310:2191-4. (PMID: 24141714)

10. Van Elslande J, Hijjit S, De Vusser K, et al. Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by four automated immunoassays and three ELISAs. Clin Microbiol Infect 2020 Nov;26(11):1557.e1-1557.e7. (PMID: 32745595)

11. Bryan A, Pepper G, Wener MH, et al. Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. J Clin Microbiol 2020;58:e00941-20. (PMID: 32381641)

12. Paiva KJ, Grisson RD, Chan PA, et al. Validation and performance comparison of three SARS-CoV-2 antibody assays. J Med Virol 2021;93:916-23. (PMID: 32710669)

13. Theel ES, Harring J, Hilgart H, Granger D. Performance Characteristics of Four High-Throughput Immunoassays for Detection of IgG Antibodies against SARS-CoV-2. J Clin Microbiol 2020;58: e01243-20. (PMID: 32513859)

14. Manalac J, Yee J, Calayag K, et al. Evaluation of Abbott anti-SARS-CoV-2 CMIA IgG and Euroimmun ELISA IgG/IgA assays in a clinical lab. Clin Chim Acta 2020;510:687-90. (PMID: 32910980)

15. Weber MC, Risch M, Thiel SL, et al. Characteristics of Three Different Chemiluminescence Assays for Testing for SARS-CoV-2 Antibodies. Dis Markers 2021;2021:8810196. (PMID: 33532006)

16. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054-62. (PMID: 32171076)

17. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med 2020;382:1708-20. (PMID: 32109013)

18. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. BMJ 2020;368:m1091. (PMID: 32217556)

19. Santotoribio JD, Nunez-Jurado D, Lepe-Balsalobre E. Evaluation of Routine Blood Tests for Diagnosis of Suspected Coronavirus Disease 2019. Clin Lab 2020;66(9). (PMID: 3290237)