Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Systemic COVID-19 vaccination also enhances the humoral immune response after SARS CoV-2 infection. An approach to criteria for COVID-19 re-immunization is needed. Do we need a third dose?

Piotr Kosiorek (pkosiorek@onkologia.bialystok.pl)
Oncology Center Bialystok
Dorota Kazberuk
Oncology Center Bialystok
Anna Hryniewicz
Medical University of Białystok
Robert Milewski
Medical University of Białystok
Samuel Stróż
Medical University of Białystok
Anna Stasiak-Barmuta
Medical University of Białystok

Article

Keywords: humoral response, COVID-19, SARS CoV-2, re-immunization

Posted Date: August 31st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-858160/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Systemic vaccination of the BNT162b2 mRNA stimulates humoral response. The aim of our study was to compare the intensity of humoral immune response, measured by SARS CoV-2 IgG, SARS CoV-2 IgM, and neutralization S-RBD IgG antibodies level, post COVID-19 vaccination versus post SARS COV-2 infection. We analysed 1060 people in the following groups: convalescents, healthy vaccinated, vaccinated with COMIRNATY, AstraZeneka, Moderna, Johnson&Johnson and vaccinated SARS CoV-2 convalescents. A concentration of SARS CoV-2 IgG, SARS CoV-2 IgM, and neutralizing S-RBD IgG was estimated in Bialystok Oncology Center laboratory by chemiluminescent immunoassay- CLIA, MAGLUMI. Results: 1. We observed a raise of antibodies response in both, convalescent SARS CoV-2 and COVID-19 vaccinated groups 2. The level of all antibodies' concentrations in vaccinated COVID-19 convalescents was significantly higher. 3. We differentiated an asymptomatic SARS CoV-2 convalescents from control group. Based on our analysis we suggest that it is important to monitor SARS CoV-2 antibodies concentrations as an indicator of asymptomatic COVID-19 infection, and as an equivalent of effectiveness of humoral response in convalescents and vaccinated people. Taking into consideration the time-limited nature of the effects of post infection SARS CoV-2 recovery or vaccination, among others physiological half-life, we suggested monitoring IgG antibodies level as a criterium for next vaccination.

Introduction

Vaccination is the best form of prevention of infectious diseases. The Pfizer-BioNTech vaccine BNT162b2 (COMIRNATY)[1] has been recognized so far as the most effective vaccine in preventing SARS Co-V-2 infection and severe COVID-19 disease [2,3,4]. A rising level of specific antibodies – IgM and IgG – and neutralizing S-RBD IgG as an immune humoral response is an effect of vaccination. A similar effect is observed post SARS CoV-2 exposure (COVID-19)[5]. It has been proven that in symptomatic SARS CoV-2 infections, the level of neutralizing S-RBD antibodies correlates with the severity of COVID-19 disease and with hospitalisation[5,6]. Such a correlation was observed neither in asymptomatic convalescent COVID-19 patients nor in healthy vaccinated individuals after the first dose of systemic double vaccination[7,8]. There was evidence that after a second dose of systemic vaccinations with mRNA Pfizer-BioNTech, the antibody neutralizing level was lower than that in vaccinated convalescent COVID-19 patients at regular times8. Higher levels of naturalizing antibodies after SARS CoV-2 exposure and COVID-19 vaccinations are seen in autoimmune disease[9,10]. Seroconversion to neutralizing antibodies is mostly gradual and dependent on a patient's clinical state resulting from immune defence mechanisms, such as neutralization, complement activation, and cell cytotoxicity (ADCC)[7,11,12]. In extremely rare situations, SARS CoV-2 infection gives rise to systemic inflammatory response syndrome (SIRS) with systemic inflammatory multiorgan dysfunction (MODS)[13]. Depending on the phase and intensity of the inflammatory response, giving convalescents serum plasma antibodies for COVID-19 treatment may reduce or strengthen the inflammation[14]. There is much controversy involving convalescent-derived serum plasma for COVID-19 therapy[26]. Clearly, serum plasma antibodies are very effective at an early

stage of COVID-19 disease, when the inflammation process has not yet involved elements of the humoral response[12,15,26].

The evaluation of systemic vaccinations against SARS CoV-2 invariably raises questions about seroprotective antibody concentration levels and their half-life[11]. Aging of the immune system is, on the one hand, a risk factor for contracting COVID-19 and, on the other hand, determines poor postvaccination response[16]. The question then arises whether elderly individuals require an additional dose of the vaccine[16,17]. The same question can be asked about immunosuppression, people with immunodeficiency syndromes, post transplantation patients, or oncologic or dialysis patients. Many published papers prove the safety of BNT162b2 vaccination in dialysis patients[18], patients after lymphoma therapy[19], and the safety of a third dose in kidney transplant recipients[20]. In healthy individuals, partial seroprotection, a mean of approximately 53% (32-68%, confidence interval 95%), is reached 14 days after the first dose of BNT162b2 vaccine. Seven days after the second dose, seroprotection reached 95%[16]. Seroprotection is even higher for SARS CoV-2 variants B.1.1.7 and B.1.351, 75% and 97% after the first and second doses, respectively[21].

Materials And Methods

In our study, we observed a group of 1063 people, 783 females (74%) and 277 males (26%), in the age bracket between 18 and 89 years old. People over 50 years old constituted 45% (N=479) of the group. Because of different postvaccination reactions, three subjects were considered early hype responders IgM and IgG and were excluded from further evaluation. Final group of 1060 subjects (Table 1.) was divided into 5 subgroups: Control group G0 (N=15) was unvaccinated persons with no clinical signs of COVID-19 disease who tested negative for SARS CoV-2 with RT-PCR. Group G1 (N=76) included fully symptomatic COVID-19 patients who tested positive for SARS CoV-2 by RT-PCR. Group G2 included 472 healthy individuals vaccinated with both doses of COMIRNATY. Group G3 included 42 persons vaccinated with AstraZeneca (N=21), Moderna (N=19), and Johnson & Johnson (N=2) according to the vaccination scheme. Group G4 included 312 COVID-19 convalescents vaccinated with COMIRNATY. Group G5 consisted of 4 individuals with COVID-19 convalescents infected with SARS CoV-2 after a full systemic vaccination cycle. The infection was confirmed by two positive RT-PCR tests.

After a retrospective analysis of concentrations of SARS CoV-2 IgG antibodies in group G0, we identified subgroup G01 of 43 persons who had higher than cut-off value (0,2 AU/ml) concentrations of specific IgG antibodies. The existence of specific SARS CoV-2 antibodies was recognized as evidence of viral contact, and the patients were qualified as SARS CoV-2 convalescents with no symptomatic COVID-19 history. G0=111. Among 43 analysed cases (G01), 13 persons were in the seroconversion phase (both positive IgM>1,0 AU/ml and IgG>0,2 AU/ml), 30 persons were in a late phase producing secondary antibodies (positive IgG, negative IgM), but 8 persons were in early phase of humoral viral response (positive IgM, negative IgG). Table 1.

Materials

Our study materials were blood specimens taken through venepuncture sampling. The concentration of antibodies was evaluated 4 hours after blood collection. If immediate assessment was not possible, the serum was collected and stored at -80 °C.

Methods

All 1063 participants had elevated levels of IgM and IgG antibodies oriented specifically towards SARS CoV-2; in 546 subjects, IgG anti-S (S-RBD) antibodies were detected by chemiluminescent immunoassay-CLIA (MAGLUMI, Snibe Diagnostic, Shenzhen China).

The results greater or equal to 1.00 AU/ml SARS CoV-2 IgG, IgM and S-RBD were considered to be reactive and recognized as positive according to the manufacturer's protocol.

The Bioethics Commission of Medical University of Bialystok gave consent for our research.

Statistical Analysis

For the case-control analyses, nonparametric statistical methods were applied because of a lack of considered variables with a normal distribution. Comparing two groups to each other, the Mann-Whitney test was adopted, whereas to assess more than two groups, the Kruskal-Wallis test was applied. The results that were statistically essential were assumed to be $p<0,05$. PQStat Software 2021 and Tibco statistic 13.3 was used.

Results

After evaluating the concentrations of specific antibodies in the control group of healthy unvaccinated subjects, we identified a subgroup of SARS CoV-2 convalescents (G01) after an asymptomatic COVID-19 infection. The concentration of antibodies from IgG class in that subgroup was comparable to the concentration observed in COVID-19 convalescents with fully symptomatic disease confirmed by a positive RT PCR test.

Our research showed the presence of SARS CoV-2 antibodies in both the convalescent and COVID-19 vaccinated groups, wherein the concentration of antibodies decreased with time in both groups. Figure 1.

Significantly higher IgG and IgM specific antibody concentrations were observed in vaccinated COVID-19 convalescents (G4). Figure 2.

After we analysed the strength of specific IgG and IgM anybody generation after vaccination, we have not found any significant difference between studied vaccines (Pfizer, AstraZeneca, Moderna). The sample size of the Johnson & Johnson vaccine recipients (N=2) was too small to render a meaningful result; hence, we excluded it from further analysis.

Figure 3 shows the levels of antibodies in the following groups: (a) evaluated in individual groups at time intervals after full vaccination and (b) after a positive RT PCR test result for SARS CoV-2 (COVID-19). The completed analysis showed a linear dependence over time for both vaccination response and response naturally taken from viral infection in the group after clinically mild or moderate COVID-19 disease. We observed the abovementioned effect in each studied group up to 180 days post exposure and up to 90 days post vaccination.

Discussion

The evaluation of concentrations of IgG and IgM antibodies specific to SARS CoV-2 that we conducted for this study allowed us to identify a new subgroup among the healthy, unvaccinated controls: subjects who had a previous asymptomatic SARS CoV-2 infection. Every patient in this subgroup presented lower concentrations of specific SARS CoV-2 IgG antibodies than the cut-off assumed for a positive result by laboratory tests, between 0,2 and 1,0 AU/ml.

Based on the concentration of neutralizing antibodies, we propose the following hierarchy of activation of the humoral response: unvaccinated (G0, where antibody concentrations were below the cut off <1,0) < convalescents (G1) < vaccinated (G2, G3) < vaccinated convalescents (G4) < patients with COVID-19 infection after being fully vaccinated (G5).

Group G4 was exposed to the virus three times: the first time when they were infected with SARS CoV-2 virus and the next two times when they were infected through immunization. The triple exposure of this group caused the largest rise in neutralizing antibodies among all groups observed. Every contact with antigen altered the immune system, causing a burst of antibody production lasting up to 14 days[22,23].

Although group G5 (N=4), vaccinated people who developed fully symptomatic COVID-19 infection, was excluded from further statistical analysis due to the group's small size, the results we obtained for that group correlate with literature data, which shows the largest growth in antibody concentration in vaccinated and infected people[8,21].

Who needs the third does of the vaccine?

Synthesis of antibodies from the IgM class is the first phase of the humoral response after initial contact with the antigen. After the second or repeated contact with the same antigen, IgG antibody synthesis occurs. Conducted analysis of concentrations of specific SARS CoV-2 antibodies IgG and IgM and their mutual dependence let us discover early and late phases of humoral response to SARS CoV-2 infection as well as to COVID-19 vaccine[17,23]. Indirectly, it allowed us to identify individuals who had asymptomatic COVID-19 infections. Of course, such an evaluation should be based on clinical examinations, identification of infection symptoms of lack of thereof, serological investigation – presence or lack of virus antigens. One should remember that timing of blood sample collection is fundamental for correct evaluation of concentrations of antibodies IgM and IgG. This timing depends on the patient's clinical

status and dynamic changes in the immunologic system, e.g., after SARS COV-2 infection, and can effectively optimize planning of the next vaccine dose against Delta variant[27].

In Israel, an increase in SARS infection was observed among people vaccinated with the BNT1262b vaccine 146 days post vaccination[24]. This increase was the most pronounced in people over 60[24]. In our opinion, it is reasonable to start measuring specific antibodies IgG (late effect) and IgM (early effect) for vaccination evaluation at serological window neutralizing antibodies. As we know so far, a humoral response to neutralizing antibodies post vaccination is rapid and decreasing. In addition, in some people without exposure to SARS CoV-2, humoral and cellular responses are detected late, which indicates widespread immunity to SARS in the population[25]. In summary, our results indicate the importance of evaluating and monitoring immunological response parameters in vaccinated healthy people and convalescents, which will help to establish the necessity for the third dose of can vaccine against SARS CoV-2.

Declarations

Acknowledgements

We thank all health-care workers involved in examinations in Bialystok Oncology Center.

Conflict of interest

All the authors declare that there are no conflicts of interest.

Author contributions

Piotr Kosiorek, Anna Hryniewicz, and Anna Stasiak-Barmuta analysed the data and drafted the manuscript. Robert Milewski participated in data analysis and extensively reviewed the manuscript. Other authors contributed to clinical and laboratory data acquisition and reviewed the manuscript.

References

1 Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270-273, doi:10.1038/s41586-020-2012-7 (2020).

2 Arunachalam, P. S. *et al.* Systems vaccinology of the BNT162b2 mRNA vaccine in humans. *Nature*, doi:10.1038/s41586-021-03791-x (2021).

3 Koch, T., Mellinghoff, S. C., Shamsrizi, P., Addo, M. M. & Dahlke, C. Correlates of Vaccine-Induced Protection against SARS-CoV-2. *Vaccines (Basel)* **9**, doi:10.3390/vaccines9030238 (2021).

4 Polack, F. P. *et al.* Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med* **383**, 2603-2615, doi:10.1056/NEJMoa2034577 (2020).

5 Long, Q. X. *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* **26**, 1200-1204, doi:10.1038/s41591-020-0965-6 (2020).

6 Lucas, C. *et al.* Delayed production of neutralizing antibodies correlates with fatal COVID-19. *Nature Medicine* **27**, 1178-1186, doi:10.1038/s41591-021-01355-0 (2021).

7 Dufloo, J. *et al.* Asymptomatic and symptomatic SARS-CoV-2 infections elicit polyfunctional antibodies. *Cell Rep Med* **2**, 100275, Doi: 10.1016/j.xcrm.2021.100275 (2021).

8 Vicenti, I. *et al.* The second dose of the BNT162b2 mRNA vaccine does not boost SARS-CoV-2 neutralizing antibody response in previously infected subjects. *Infection*, doi:10.1007/s15010-021-01680-z (2021).

9 Wang, E. Y. *et al.* Diverse functional autoantibodies in patients with COVID-19. *Nature* **595**, 283-288, doi:10.1038/s41586-021-03631-y (2021).

10 Robbiani, D. F. *et al.* Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* **584**, 437-442, doi:10.1038/s41586-020-2456-9 (2020).

11 Becker, M. *et al.* Immune response to SARS-CoV-2 variants of concern in vaccinated individuals. *Nature Communications* **12**, 3109, doi:10.1038/s41467-021-23473-6 (2021).

12 Tan, L. *et al.* Plasma therapy cured a COVID-19 patient with long duration of viral shedding for 49 days: The clinical features, laboratory tests, plasma therapy, and implications for public health management. *MedComm (Beijing)*, doi:10.1002/mco2.2 (2020).

13 Jang, J. G., Hur, J., Hong, K. S., Lee, W. & Ahn, J. H. Prognostic Accuracy of the SIRS, qSOFA, and NEWS for Early Detection of Clinical Deterioration in SARS-CoV-2 Infected Patients. *J Korean Med Sci* **35**, e234, doi:10.3346/jkms.2020.35. e234 (2020).

14 Arvin, A. M. *et al.* A perspective on potential antibody-dependent enhancement of SARS-CoV-2. *Nature* **584**, 353-363, doi:10.1038/s41586-020-2538-8 (2020).

15 Mungmunpuntipantip, R. & Wiwanitkit, V. COVID-19 convalescent plasma therapy and immunodeficiency. *Transfus Clin Biol* **28**, 306-307, doi: 10.1016/j.tracli.2021.05.007 (2021).

16 Kow, C. S. & Hasan, S. S. Real-world effectiveness of BNT162b2 mRNA vaccine: a meta-analysis of large observational studies. *Inflammopharmacology* **29**, 1075-1090, doi:10.1007/s10787-021-00839-2 (2021).

17 Kontopoulou, K. *et al.* Second dose of the BNT162b2 mRNA vaccine: Value of timely administration but questionable necessity among the seropositive. *Vaccine* **39**, 5078-5081, Doi: 10.1016/j.vaccine.2021.07.065 (2021).

18 Polewska, K. *et al.* Safety and Tolerability of the BNT162b2 mRNA COVID-19 Vaccine in Dialyzed Patients. COViNEPH Project. *Medicina (Kaunas)* **57**, doi:10.3390/medicina57070732 (2021).

19 Perry, C. *et al.* Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with B-cell non-Hodgkin lymphoma. *Blood Adv* **5**, 3053-3061, doi:10.1182/bloodadvances.2021005094 (2021).

20 Stumpf, J. *et al.* Cellular and Humoral Immune Responses after Three Doses of BNT162b2 mRNA SARS-Cov-2 Vaccine in Kidney Transplant. *Transplantation*, doi:10.1097/TP.0000000000003903 (2021).

21 Abu-Raddad, L. J. *et al.* Pfizer-BioNTech mRNA BNT162b2 Covid-19 vaccine protection against variants of concern after one versus two doses. *J Travel Med*, doi:10.1093/jtm/taab083 (2021).

22 Lou, B. *et al.* Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. *European Respiratory Journal* **56**, 2000763, doi:10.1183/13993003.00763-2020 (2020).

23 Gobbi, F. *et al.* Antibody response in individuals infected with SARS-CoV-2 early after the first dose of the BNT162b2 mRNA vaccine. *J Infect*, doi: 10.1016/j.jinf.2021.08.008 (2021).

24 Israel, A. *et al.* Elapsed time since BNT162b2 vaccine and risk of SARS-CoV-2 infection in a large cohort. *medRxiv*, doi:10.1101/2021.08.03.21261496 (2021).

25 Grifoni, A. *et al.* SARS-CoV-2 human T cell epitopes: Adaptive immune response against COVID-19. *Cell Host Microbe* **29**, 1076-1092, doi: 10.1016/j.chom.2021.05.010 (2021).

Table 1

Table 1 is available in the Supplementary Files section.

Figures

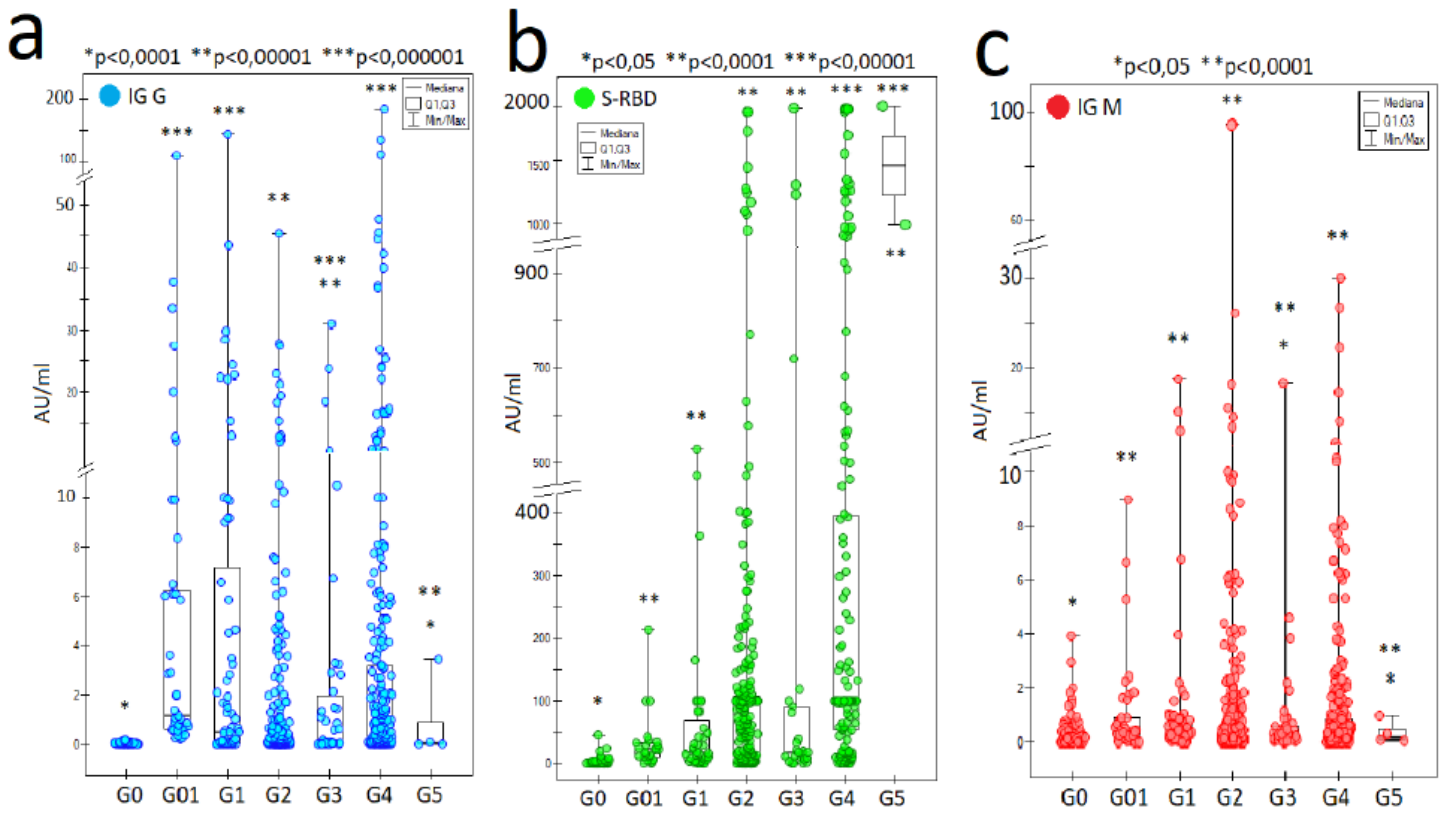

Figure 1

presented concentrations (AU/ml) (a) antibodies SARS CoV-2 IgG (aquamarine) (b) neutralizing S-RBD IgG (green) (c) antibodies SARS CoV-2 IgM (red) in control-study group; G0, G01 controls, G1 convalescent, G2 vaccinated COMIRNATY, G3 vaccinated other vaccines, G4 vaccinated convalescent group, G5 twice passed COVID-19 vaccinated COMIRNATY (*p, ANOVA Kruskala-Wallisa)

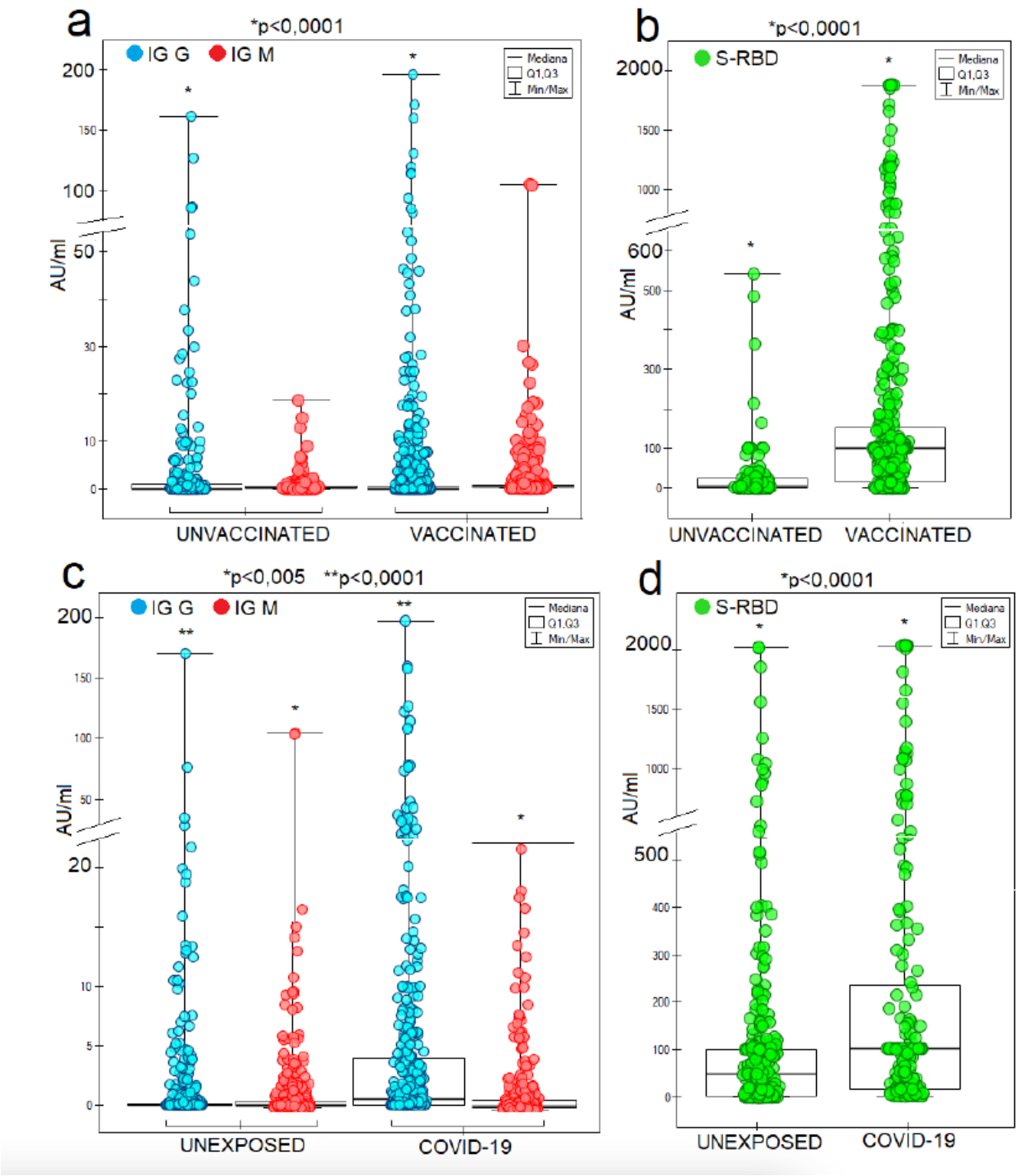

Figure 2

presented concentrations (AU/ml) (a,c) antibodies SARS CoV-2 IgG (aquamarine) and IgM (red) (b,d) neutralizing S-RBD IgG (green) in vaccinated groups and post infection SARS CoV-2 (COVID-19) with compare to unvaccinated and unexposed groups respectively (*p, Mann-Whitney).

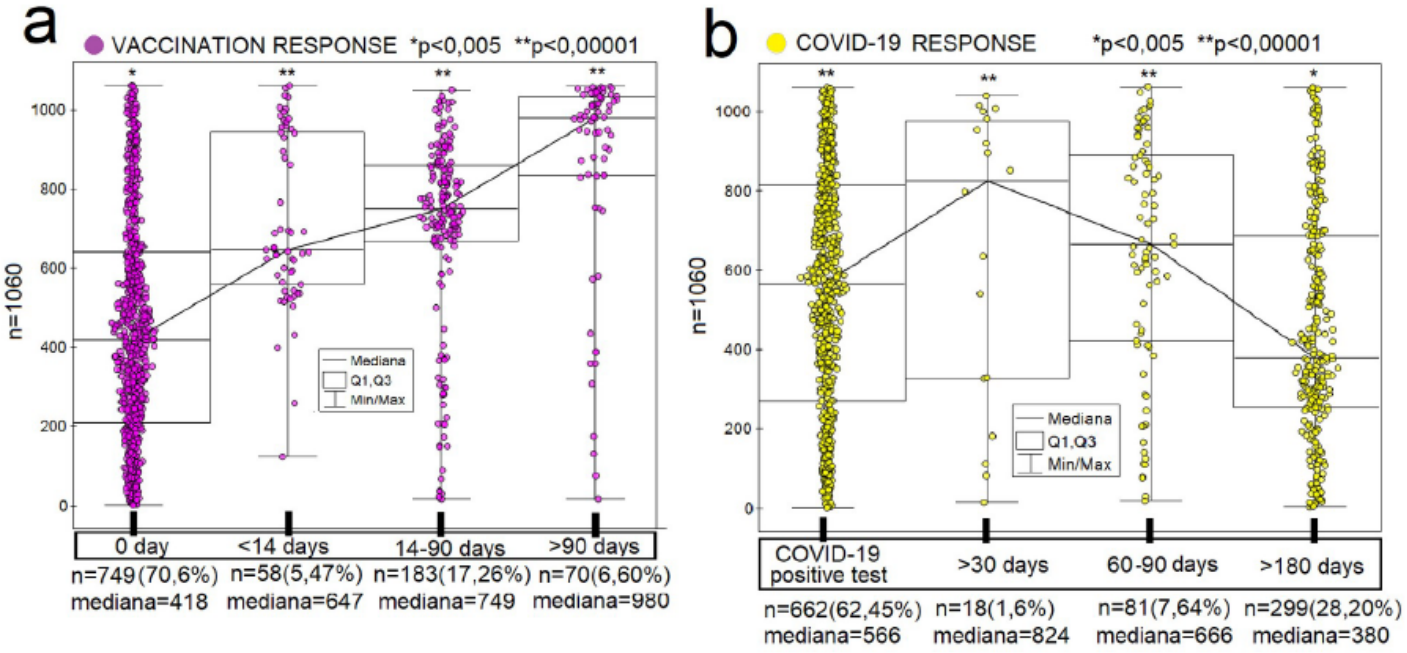

Figure 3

presented results (n=1060) in next refined time selected groups (a) after fully vaccination (Vaccination response) measured in time compartments (0 day, <14 days, 14-90 days, >90 days), (b) after tested positive SARS CoV-2 (COVID-19 response) measured in time compartments (test day, >30 days, 60-90 days, >180 days) (*p, ANOVA Kruskal-Wallis).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 30.08.2021tabela1ENGzopisem.pdf