

Antibody titers after a third dose of the SARS-CoV-2 BNT162b2 vaccine in immunocompromised adults in Greece: Is a fourth dose necessary?

Konstantina Kontopoulou¹  | Christos T. Nakas^{2,3}  | Chrysoula Belai¹ | Georgios Papazisis^{4,5} 

¹Laboratory of Microbiology, "G. Gennimatas" General Hospital, Thessaloniki, Greece

²Laboratory of Biometry, University of Thessaly, Nea Ionia/Volos, Magnesia, Greece

³University Institute of Clinical Chemistry, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland

⁴Department of Clinical Pharmacology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

⁵Clinical Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

Correspondence

Georgios Papazisis, Clinical Research Unit, Special Unit for Biomedical Research and Education, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Email: papazisg@auth.gr

Abstract

Real-world data suggest that protection against COVID-19 declines a few months after vaccination, particularly in the elderly and immunocompromised individuals. Our study aimed to analyze the humoral response induced by a third supplemental dose of BNT162b2 vaccine in a mixed group of immunocompromised individuals by determining anti-spike (anti-S) IgG antibody titers at baseline (pre-third vaccine dose) and 4 weeks after the dose. Serum samples were obtained from a total group of 85 immunocompromised individuals (history of cancer: $n = 20$, lymphoma: $n = 4$, leukemia: $n = 3$, transplant recipients: $n = 4$, autoimmune disease: $n = 42$, inflammatory disease: $n = 6$, autoimmune diabetes type 1: $n = 6$) all of whom had previously received a two-dose schedule of the vaccine. The average number of days between second and third dose was 139.6145 (± 41.39071). The overall IgG GMCs 4 weeks postvaccination were increased by more than 35 times (fold change = 35.30, $p < 0.001$). Fold changes were not significantly correlated with underlying condition, age, sex nor with days between second and third dose. Considering the predominance of omicron variants in the current period and the results of studies showing a decrease in the effectiveness of the third dose after 10 weeks we highly recommend a fourth dose to this vulnerable population group.

KEYWORDS

antibody titers, BNT162b2 mRNA COVID-19 vaccine, Greece, immunocompromised adults, immunogenicity, SARS-CoV-2, third dose

1 | INTRODUCTION

The rapid development and subsequent authorization of vaccines against SARS-CoV-2 has been an important step toward the management of the COVID-19 pandemic.¹ The efficacy of the vaccines administered so far is beyond doubt managing to reduce, fundamentally, the severe forms of the COVID-19 disease and hence the mortality worldwide. Nevertheless, real-world data suggested that the humoral immune response and protection against SARS-CoV-2 infection and disease seem to be declining a few months after

vaccination, particularly in the elderly and immunocompromised individuals.²

Immunocompromised people, like those with solid or hematological malignancies, hematopoietic stem cell transplantation, and those with autoimmune diseases or receiving immunosuppressive drugs, are among those most susceptible to COVID-19 infection. Studies indicate a reduced antibody response in immunocompromised people following a primary vaccine series, compared to healthy vaccine recipients.^{3,4} Observation of SARS-CoV-2-specific immune response in individuals with defined immunodeficiencies

revealed that defects in humoral immunity have been associated with incomplete immune control of SARS-CoV-2, prolonged viral culture, and shedding.⁵ In addition, studies have shown that patients requiring immunosuppressive treatment for autoimmune and inflammatory rheumatic diseases have worse outcomes from COVID-19 compared with patients without such conditions.⁶

Finally, it is worth mentioning that immunocompromised people are more likely to develop breakthrough infections. Since 40% of hospitalized breakthrough cases are immunocompromised people⁷ to minimize the risk of COVID-19 breakthrough infections among vaccinated immunocompromised people, many countries prioritized this group to receive an additional vaccine dose. The Greek Ministry of Health announced a deployment plan in early autumn to administer a third dose of the BNT162b2 mRNA COVID-19 vaccine, starting with immunocompromised patients on September 15, 2021. The third dose was only given to people who had received the second dose at least 3 months before.

Immune responses following SARS-CoV-2 additional doses vaccination, have not been adequately studied in potentially immune vulnerable patient groups. This study aimed to analyze the humoral response induced by a third supplemental dose of BNT162b2 vaccine in a mixed group of immunocompromised individuals by determining anti-spike (anti-S) IgG antibody titers at baseline (pre-third vaccine dose) and 4 weeks post third BNT162b2 additional dose.

2 | MATERIAL AND METHODS

Serum samples were obtained from a total group of 85 immunocompromised individuals all of whom had previously received a two-dose schedule of the vaccine with a 3-week dosing interval. In particular, on the day of the third vaccination, blood was drawn, before administration of the additional dose, for baseline serology assessment of receptor-binding domain (RBD) IgG antibodies titers. Four weeks following the third dose, testing for RBD IgG titers, was repeated to assess the humoral response to the vaccine. The participants were classified into two groups. In the first group ($n=31$), patients with a history of solid organ or hematologic cancers and recipients of hematopoietic cell or solid organs transplant were included (history of cancer: $n=20$, lymphoma: $n=4$, leukemia: $n=3$, transplant recipients: $n=4$). The second group ($n=54$) composed of patients with autoimmune diseases or patients under immunosuppressive therapy (autoimmune disease: $n=42$, Inflammatory disease: $n=6$, autoimmune diabetes type 1: $n=6$). Written informed consent was obtained from all participants. Approval of the study protocol was obtained by the ethics committee of the scientific council of the G. Gennimatas General Hospital (protocol number:1/13.1.2021), in accordance with the Declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice.

Titers of total RBD-specific IgGs against SARS-CoV-2 were determined using the SARS-CoV-2 IgG II Quant assay on the ARCHITECT System (SARS-CoV-2 IgG II Quant, Abbott Sligo) on participant-derived serum samples. The SARS-CoV-2 IgG II Quant assay is used to monitor antibody response in individuals who have

received the COVID-19 vaccine, by quantitatively measuring IgG antibodies against the spike RBD of SARS-CoV-2.

The geometric mean concentration (GMC) and respective 95% confidence intervals were calculated based on the recorded antibody concentration values.

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on seven participants who became infected with SARS-CoV-2, 2–5 months after the third dose vaccination, found positive by the Allplex™ SARS-CoV-2 Master Assay (Seegene Inc.).

PCR was performed with a CFX96-Dx Touch Real-Time PCR Detection System (Bio-Rad) and results were analyzed with Seegene Viewer V1.0 software (Seegene). Furthermore, detection of Omicron variant (B.1.1.529) was achieved, using Allplex™ SARS-CoV-2 Variants I Assay (HV69/70del E484K N501Y–spike variants) and Allplex™ SARS-CoV-2 Variants II Assay (W152C L452R K417N K417T–spike mutations; Seegene Inc.).

The participants were encouraged to fill and submit spontaneously to the Greek regulatory agency for medicines (EOF) the Individual Case Safety Report (ICSR) form for any suspect adverse event, especially the serious adverse events and the suspected unexpected serious adverse events.

Descriptive statistics were based on GMCs of anti-SARS-COV-2 spike IgG (AU/ml) and the corresponding 95% confidence intervals by groups of interest (Groups 1 and 2, gender, COVID-19 infection after third dose). Independent samples *t*-tests were used for the assessment of differences of log₁₀ IgG levels for two group means comparisons. Correlation analysis was performed for the study of linear relationships between continuous variables of interest (fold changes due to booster dose, age, days between second and third dose). Stata 16.1 (Stata Corp. LLC) was used for data analysis.

3 | RESULTS

Our study included 85 vulnerable participants whose anti-RBD IgG titers were determined just before and 1 month after the third dose administration. Among them, 39 (45.9%) were male with a mean age of 62.72 ± 13.16 years. The average number of days between second and third dose was $139.6145 (\pm 41.39071)$.

At baseline, the measured levels of IgG GMC were 363.87 (185.38, 714.22) AU/ml in the first group, which then increased after the third dose to 12874.01 (9106.99, 18199.2) and the correspondents of the second group were 437.38 (308.09, 620.93) at baseline and 17430.22 (14690.71, 20680.6) after the additional dose. The overall IgG GMCs 4 weeks postvaccination compared to those pre third dose values were increased by more than 35 times (fold change = 35.30, $p < 0.001$; Table 1). After the third booster dose, there were nonsignificant differences between the two groups ($p = 0.338$) (Figure 1). Furthermore, fold changes were not significantly correlated with age ($r = 0.013$, $p = 0.908$) nor with days between second and third dose ($r = 0.167$, $p = 0.131$; Figure 2). Only seven subjects were infected with the Omicron variant (B.1.1.529) 2–5 months after the third dose. All of the patients with subsequent

TABLE 1 Geometric means of concentrations (GMC) and fold changes of anti SARS-CoV-2 Spike IgG antibodies before and 4 weeks after third-dose administration between the two groups and between infected and not infected subjects after third dose. Group 1: cancer, lymphoma, leukemia, transplants. Group 2: autoimmune, type I diabetes, irritated bowel.

Groups	GMC (95% CI) Before booster dose	GMC (95% CI) After booster dose	Fold change	p value
Group 1 (n = 31)	363.87 (185.38, 714.22)	12874.01 (9106.99, 18199.2)	35.38 (25.48, 49.13)	<0.001
Group 2 (n = 54)	495.27 (365.98, 670.24)	17430.22 (14690.71, 20680.6)	35.19 (3.86, 40.14)	<0.001
p value	0.338	0.079	0.903	
No COVID after 3rd dose (n = 72)	437.38 (308.09, 620.93)	14802.39 (12312.52, 17795.76)	33.84 (28.66, 39.96)	<0.001
COVID after 3rd dose (n = 7)	573.53 (261.17, 1259.44)	23211.73 (11537.69, 46697.76)	40.47 (37.08, 44.18)	<0.001
p value	0.643	0.151	0.631	
Female (n = 46)	513.55 (343.81, 767.10)	15947.25 (12619.20, 20153.00)	31.33 (22.66, 43.32)	<0.001
Male (n = 39)	370.87 (228.27, 602.57)	15195.57 (11930.26, 19354.59)	36.04 (26.17, 49.63)	<0.001
p value	0.296	0.775	0.579	
Overall (n = 85)	442.17 (325.40, 600.85)	15606.72 (13229.01, 18411.78)	35.30 (30.64, 40.65)	<0.001

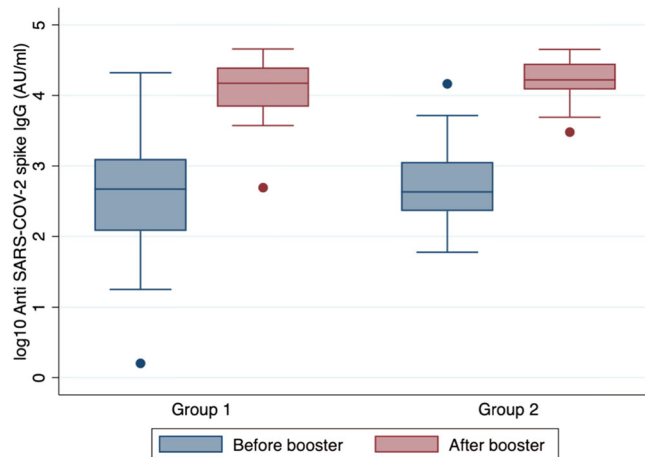


FIGURE 1 Boxplots of antibody levels (logarithmic scale) of the two groups before and 4 weeks after the third dose

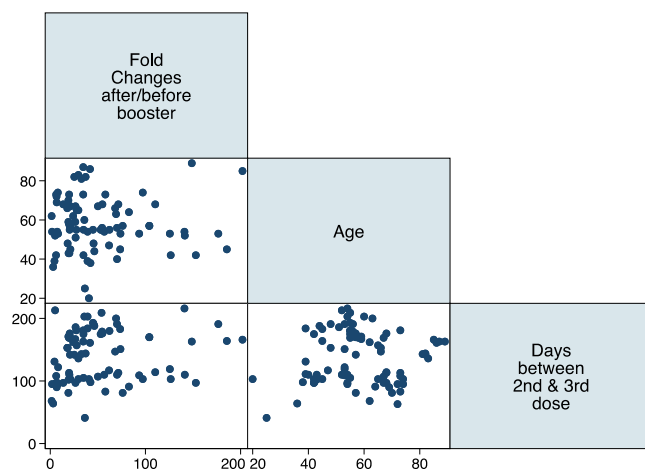


FIGURE 2 Scatterplot matrix depicting the relationship between fold changes in IgG after the booster dose against the subjects' age and days between second and third dose. No significant relationships were found.

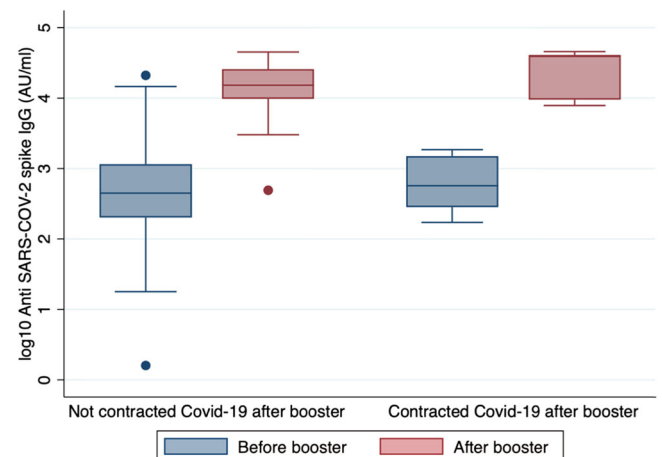


FIGURE 3 Boxplots of antibody levels (logarithmic scale) of subjects infected 2–6 months after the third booster dose versus those not infected. No significant differences were observed.

COVID-19 infection developed only mild disease, and no one required oxygen support. There were consistently nonsignificant differences between antibody levels of those infected after the third dose relative to those noninfected (Figure 3). Regarding reactogenicity, adverse events were not systematically recorded in our study. However, none of our study participants declared an ICSR submission, evidence confirming the absence of serious adverse events in this study.

4 | DISCUSSION

This study evaluated the serologic response to a third BNT162b2 mRNA vaccine dose in a mixed cohort of immunocompromised patients. The main result of our study was that the third dose of vaccine allows

boosting the humoral response to SARS-CoV-2 in this population. Indeed, the humoral response to the third homologous additional dose of BNT162b2 vaccine was found to be significantly higher (about 35 times) than the baseline immune status before the third vaccine. All patients produced sufficient levels of anti-S IgG antibodies except one with lymphoma history. Similar trends were observed in studies where the third dose was administered in patients receiving hemodialysis or peritoneal dialysis.⁸

A significant number of vulnerable people enrolled in our study ($n = 20$) reported a history of cancer. We observed a strong and effective humoral response to the third dose, even the ones with low titles before administration. Shapiro et al.⁹ found that among 88 patients with cancer who received booster vaccinations, 64% were seropositive before booster vaccination, and 36% seronegative and even those patients who received therapy within 30 days of booster vaccination had a statistically significant chance for seroconversion.

Zeng et al. demonstrated that the diagnosis of a solid cancer per se does not appear to negatively impact the humoral immune response to booster-mediated protections against SARS-CoV-2 variants, including Omicron. They also examined the nAb titers against the Omicron, D614G, and Delta variants for patients with cancer who received a third dose of vaccine and overall, booster recipients exhibited dramatically increased nAb titers. Their results indicate a stronger and much broader neutralization even in the Omicron variant after the booster vaccination.¹⁰

In our study were included three patients with a history of leukemia who showed a significant increase in the immune response after third dose administration. Similarly, in a study conducted by Heirishanu et al.¹¹ in patients with chronic lymphocytic leukemia who failed to achieve a humoral response after the standard two-dose vaccination regimen, a significant immune response was observed to a third BNT162b2 mRNA COVID-19 vaccine.

Concerning transplant recipients, vaccination with a third dose elicited significantly higher antibody titers compared with the very low levels observed at baseline to four participants. Similarly, 44% of solid-organ transplant recipients who had been seronegative after two doses of BNT162b2 became seropositive after a third vaccine dose.¹² Likewise, Hod et al.¹³ found that a booster dose elicited a strong and effective humoral response in renal transplant recipients who were either seropositive or seronegative before the administration of the third dose.

Fifty-four patients with chronic autoimmune disease were involved in our study, who developed a significant higher immune response compared to that at baseline. These results demonstrate that a third dose may be extremely useful, for the prevention of severe disease, hospitalization, and deaths of infections caused by SARS-CoV-2, in this patient group. This is of utmost importance since patients with autoimmune disease are more at risk of serious COVID-19 infections. Indeed, Papagoras et al.¹⁴ proved that the hospitalization and mortality rates were higher in unvaccinated (29% and 4%) than the fully vaccinated rheumatic patients (10% and 0%).

In accordance with our study are the results of a recently published very interesting network meta-analysis which found that the effect of

two-dose mRNA vaccines is weaker in those who are immunocompromised than in those who are non-immunocompromised but a three-dose mRNA regimen works comparably well in the non-immunocompromised and immunocompromised individuals. Further, that a three-dose regimen is similarly effective in all age groups, even in people over 65.¹⁵

Finally, in our study seven participants were infected with the Omicron variant (B.1.1.529), 2–5 months after the third dose. Owing to the large number of mutations in the spike protein,¹⁶ there is concern that this variant will exhibit substantial escape from vaccine-elicited immunity. Although data suggest that three doses of heterologous or homologous booster vaccination had a 25–100-fold-increase in neutralizing titers against Omicron variant, compared to two-dose vaccinations,¹⁷ recent studies demonstrate a decrease in the effectiveness of the third dose after 10 weeks¹⁸ and that antibody titers diminish more rapidly in immunocompromised patients than healthy individuals¹⁹ while a study in Israel provided evidence for the effectiveness of a fourth vaccine dose in subjects having received a third dose more than 4 months earlier.²⁰

5 | LIMITATIONS

Limitations of this study include a small and heterogeneous convenience sample and the absence of assays for neutralizing antibody, B-cell memory, and T-cell responses. Furthermore, measurement of antibody titers 6 months after the third dose is pending; however, it has already been planned. These limitations may be addressed in further studies.

6 | CONCLUSIONS

Overall, our findings verify the strong immunogenicity elicited by the third dose of BNT162b2 vaccine in all categories of immunocompromised participants, highlighting the importance of the third dose. Continued monitoring of vulnerable patient groups is of paramount importance for deciding the vaccination scheme for this population. Considering the predominance of omicron variants in the current period and the results of studies showing a decrease in the effectiveness of the third dose after 10 weeks we highly recommend a fourth dose to this vulnerable population group.

AUTHOR CONTRIBUTIONS

Konstantina Kontopoulou: conceptualization, project administration, investigation, writing—original draft; **Christos T. Nakas:** methodology, formal analysis; **Chrysoula Belai:** project administration, investigation; **Georgios Papazisis:** conceptualization, supervision, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Not applicable.

ETHICS STATEMENT

The study was approved by the ethics committee of the scientific council of the G. Gennimatas General Hospital (protocol number:1/13.1.2021). Informed consent was obtained from all subjects involved in the study.

ORCID

Konstantina Kontopoulou  <http://orcid.org/0000-0003-0121-8688>

Christos T. Nakas  <http://orcid.org/0000-0003-4155-722X>

Georgios Papazisis  <http://orcid.org/0000-0003-1641-9095>

REFERENCES

- Haas EJ, Angulo FJ, McLaughlin JM, et al. Impact and effectiveness of mRNA BNT162b2 vaccine against SARS-CoV-2 infections and COVID-19 cases, hospitalisations, and deaths following a nationwide vaccination campaign in Israel: an observational study using national surveillance data. *Lancet*. 2021;397(10287):1819-1829.
- Levin EG, Lustig Y, Cohen C, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 months. *N Engl J Med*. 2021;385(24):e84.
- Deepak P, Kim W, Paley MA, et al. Effect of immunosuppression on the immunogenicity of mRNA vaccines to SARS-CoV-2: a prospective cohort study. *Ann Intern Med*. 2021;174(11):1572-1585.
- Scarfò L, Chatzikonstantinou T, Rigolin GM, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. *Leukemia*. 2020;34(9):2354-2363.
- Tarhini H, Recoing A, Bridier-Nahmias A, et al. Long-term severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infectiousness among three immunocompromised patients: from prolonged viral shedding to SARS-CoV-2 superinfection. *J Infect Dis*. 2021;223(9):1522-1527.
- Curtis JR, Johnson SR, Anthony DD, et al. American College of Rheumatology guidance for COVID-19 vaccination in patients with rheumatic and musculoskeletal diseases: Version 3. *Arthritis Rheumatol*. 2021;73:e60-e75.
- Brosh-Nissimiv T, Orenbuch-Harroch E, Chowers M, et al. BNT162b2 vaccine breakthrough: clinical characteristics of 152 fully-vaccinated hospitalized COVID-19 patients in Israel. *Clin Microbiol Infect*. 2021;27:1652-1657.
- Bensouna I, Caudwell V, Kubab S, et al. SARS-CoV-2 antibody response after a third dose of the BNT162b2 vaccine in patients receiving maintenance hemodialysis or peritoneal dialysis. *Am J Kidney Dis*. 2022;79:185-192.e1.
- Shapiro LC, Thakkar A, Campbell ST, et al. Efficacy of booster doses in augmenting waning immune responses to COVID-19 vaccine in patients with cancer. *Cancer Cell*. 2022;40(1):3-5.
- Zeng C, Evans JP, Chakravarthy K, et al. COVID-19 mRNA booster vaccines elicit strong protection against SARS-CoV-2 Omicron variant in patients with cancer. *Cancer Cell*. 2022;40(2):117-119.
- Herishanu Y, Rahav G, Levi S, et al. Efficacy of a third BNT162b2 mRNA COVID-19 vaccine dose in patients with CLL who failed standard 2-dose vaccination. *Blood*. 2022;139(5):678-685.
- Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three doses of an mRNA COVID-19 vaccine in solid-organ transplant recipients. *N Engl J Med*. 2021;385(7):661-662.
- Hod T, Ben-David, Olmer L, et al. BNT162b2 third booster dose significantly increases the humoral response assessed by both RBD IgG and neutralizing antibodies in renal transplant recipients. *Transpl Int*. 2022;35:10239.
- Papagoras C, Fragoulis GE, Zioga N, et al. Better outcomes of COVID-19 in vaccinated compared to unvaccinated patients with systemic rheumatic diseases. *Ann Rheum Dis*. 2022;8:10-13. doi:10.1136/annrheumdis-2021-221539.
- Au WY, Cheung PP. Effectiveness of heterologous and homologous covid-19 vaccine regimens: living systematic review with network meta-analysis. *BMJ*. 2022;377:e069989. doi:10.1136/bmj-2022-069989
- Wang L, Cheng G. Sequence analysis of the emerging SARS-CoV-2 variant Omicron in South Africa. *J Med Virol*. 2022;94:1728-1733.
- Stowe J, Andrews N. Covid-19 vaccine effectiveness against the Omicron (B.1.1.529) variant. *N Engl J Med*. 2022;386:1532-1546.
- Iacobucci G. Covid-19: fourth vaccine doses—who needs them and why? *BMJ*. 2022;376:o30.
- Geisen UM, Berner DK, Tran F, et al. Immunogenicity and safety of anti-SARS-CoV-2 mRNA vaccines in patients with chronic inflammatory conditions and immunosuppressive therapy in a monocentric cohort. *Ann Rheum Dis*. 2021;80:1306-1311.
- Bar-On YM, Goldberg Y, Mandel M, et al. Protection by a fourth dose of BNT162b2 against Omicron in Israel. *N Engl J Med*. 2022;386:1712-1720.

How to cite this article: Kontopoulou K, Nakas CT, Belai C, Papazisis G. Antibody titers after a third dose of the SARS-CoV-2 BNT162b2 vaccine in immunocompromised adults in Greece: Is a fourth dose necessary? *J Med Virol*. 2022;94:5056-5060. doi:10.1002/jmv.27954