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Letter
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Third Dose of the BNT162b2 Vaccine Results in Sustained High Levels of Neutralizing Antibodies Against SARS-CoV-2 at 6 Months Following Vaccination in Healthy Individuals

Ioannis Ntanasis-Stathopoulos[1], Vangelis Karalis[2], Aimilia D. Sklirou[3], Maria Gavriatopoulou[1], Harry Alexopoulos[3], Panagiotis Malandrakis[1], Ioannis P. Trougakos[3], Meletios A. Dimopoulos[1], Evangelos Terpos[1]

Correspondence: Evangelos Terpos (eterpos@med.uoa.gr; eterpos@hotmail.com).

The waning of humoral immunity post coronavirus disease 2019 (COVID-19) vaccination necessitated the implementation of booster doses. BNT162b2 administered as first and second booster dose following initial full vaccination leads to significant humoral responses against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2] In Israel, a third BNT162b2 vaccine shot significantly reduced the risk of SARS-CoV-2 among 1650 triple-vaccinated health care workers compared with 278 double-vaccinated individuals.[3] Importantly, a third dose of the BNT162b2 vaccine improves the protection against severe COVID-19 hospitalizations.[4] Recently, a fourth dose has been offered in many developed countries to maintain an adequate level of protection against COVID-19.[2] However, the optimal time interval between the booster doses remains under question. The purpose of this study was to examine the kinetics of SARS-CoV-2 neutralizing antibodies (NAbs) after vaccination with the BNT162b2 mRNA vaccine (Pfizer-BioNTech) for a period up to 6 months after the third vaccination (booster dose). The potential impact of gender, age, and body mass index (BMI) on NAbs level was also investigated.

This study enrolled 100 healthy participants, all of whom were vaccinated with 3 doses of the BNT162b2 mRNA vaccine. NAbs were measured on the first day (immediately before the first vaccination), 1 week later (day 8), on the day of the second vaccination (ie, day 22), and 2 weeks (day 36), 1 month, 3 months, 6 months, and 9 months after the second dose. NAbs measurements were also performed at 1 month, 3 months, and 6 months after the third dose of vaccine. It should be mentioned that none of the participants was tested positive for COVID-19; therefore, NAbs values reflect immunization dynamics.

SARS-CoV-2 NAbs were measured using Food and Drug Administration-approved techniques. To detect SARS-CoV-2 NAbs in blood, the cPass SARS-CoV-2 NAbs Detection Kit (GenScript, Piscataway, NJ) was used to test antibody-mediated suppression of SARS-CoV-2 receptor-binding domain binding to the human host receptor angiotensin-converting enzyme 2. After venipuncture, serum was isolated within 4 hours and refrigerated at −80°C until measurement. Parallel experiments were performed with stored samples from the same donor.

Participants had to be over 18 years of age, able to sign an informed consent form, and eligible for the national COVID-19 vaccination program. Individuals receiving immunosuppressive medications, as well as those with active malignancies and/or end-stage renal disease, were excluded. Subject data were kept confidential in accordance with the Basic Data Protection Regulation (Regulation 2016/679 of the European Parliament 2016). To prevent identification of patients, all names were immediately removed and replaced by a random number. The Alexandra Hospital Ethics Committee approved the study in accordance with the Declaration of Helsinki and the International Conference on Harmonization of Good Clinical Practice.

The analysis included both descriptive statistics (eg, median, mean, and variability estimates) and statistical group comparisons. Prior to the statistical comparisons, the Shapiro-Wilk criterion was used to test the normality of the data distributions. In all cases in this study, the variables (ie, NAbs titers) were found to deviate from the normality distribution, so nonparametric methods were used. Independent 2-group analyses (eg, men versus women) were performed using the nonparametric Mann-Whitney *U* test. Pairwise comparisons between time points were performed with the Wilcoxon test. Analysis of more than 2 independent groups was performed with the Kruskal-Wallis test. The significance level was set at 5%, and a result was considered significant if the estimated *P* value was below the significance level. All statistical analysis was implemented in Python v.3.9.2.

[1]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Greece
[2]Section of Pharmaceutical Technology, Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Greece
[3]Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Greece

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Overall, 100 healthy participants were included in the study. The median age was 51 years, the median BMI was 26.0 kg/m^2, and the male-to-female ratio was 1:1. Figure 1 shows NAbs values at 1, 3, and 6 months after the third vaccination (ie, 1 month post 3rd dose [M1P3D], 3 months post 3rd dose [M3P3D], and 6 months post 3rd dose [M6P3D], respectively). Figure 1 also depicts the percent inhibition of NAbs on days 1, 8, and 22 after the first vaccination and 2 weeks (D36), 1 month (D50), 3 months (M3), 6 months (M6), and 9 months (M9) after the second dose. Visual inspection of Figure 1 shows that NAbs levels increase and then decrease after each vaccination dose. However, after the third dose, the increase in NAbs is very rapid, whereas the decrease is much slower compared with the results after the second dose. Previous studies have also shown that the humoral response after the first booster dose of BNT162b2 is sustained for at least 4 months, which may be more prolonged among nursing home residents.[5–7] Special populations such as patients who have undergone kidney transplant may present with a waning antibody response over time after the third BNT162b2 dose and should be prioritized for additional booster doses.[8] However, patients on dialysis or those with a prior stem cell allogeneic transplant have persistent protection against COVID-19 for up to 6 months from the third vaccine dose.[9,10]

Because the purpose of this study was to examine NAbs titers 6 months after the third dose, all comparisons were made with reference to M6P3D values. The median NAbs titers 6 months after the third dose were 95.5%, which was lower than the median values for M3P3D (97.2%) and M1P3D (97.8%). These 2 differences (M6P3D versus M3P3D, M6P3D versus M1P3D) were found to be statistically significant ($P < 0.001$). However, it is interesting to compare the inhibition of NAbs 6 months after the third dose with the neutralizing inhibitory activity a similar time interval after the second dose (ie, M6). It is noteworthy that the inhibitory titers on M6P3D remained very high (95.5%) compared with the M6 median values (57.3%). As expected, this difference proved to be statistically significant ($P < 0.001$). It is worth noting that NAbs values 6 months after the third vaccination were comparable only to those 2 weeks (median 96.5%) and 1 month (96.3%) after the second vaccination; P values were 0.250 and 0.529 for the comparison with D36 and D50, respectively. In all other cases, percent inhibition values were significantly higher (D1, D8, D22, M3, M6, M9) or lower (M1P3D, M3P3D) for M6P3D than for NAbs at the abovementioned time points, respectively.

The higher NAbs titers after the third dose compared with those after the second vaccination were also reflected in the higher proportions of participants with moderate, high, or very high protection. Specifically, 6 months after the third dose, 96% of subjects had inhibition levels above 50% and 75%, estimating that they were moderately or highly protected. One and 3 months after the third vaccination, the corresponding percentages were 100% in both cases. Interestingly, 6 months after the second dose, 60% of the subjects had NAbs greater than 50% and only 20% of the participants managed to enter the high protection range.

A further analysis was conducted with respect to gender to uncover possible gender differences in the development of NAbs against SARS-CoV-2 for a period of up to 6 months after the third dose. However, no statistically significant differences were found for any time point; the P values of the

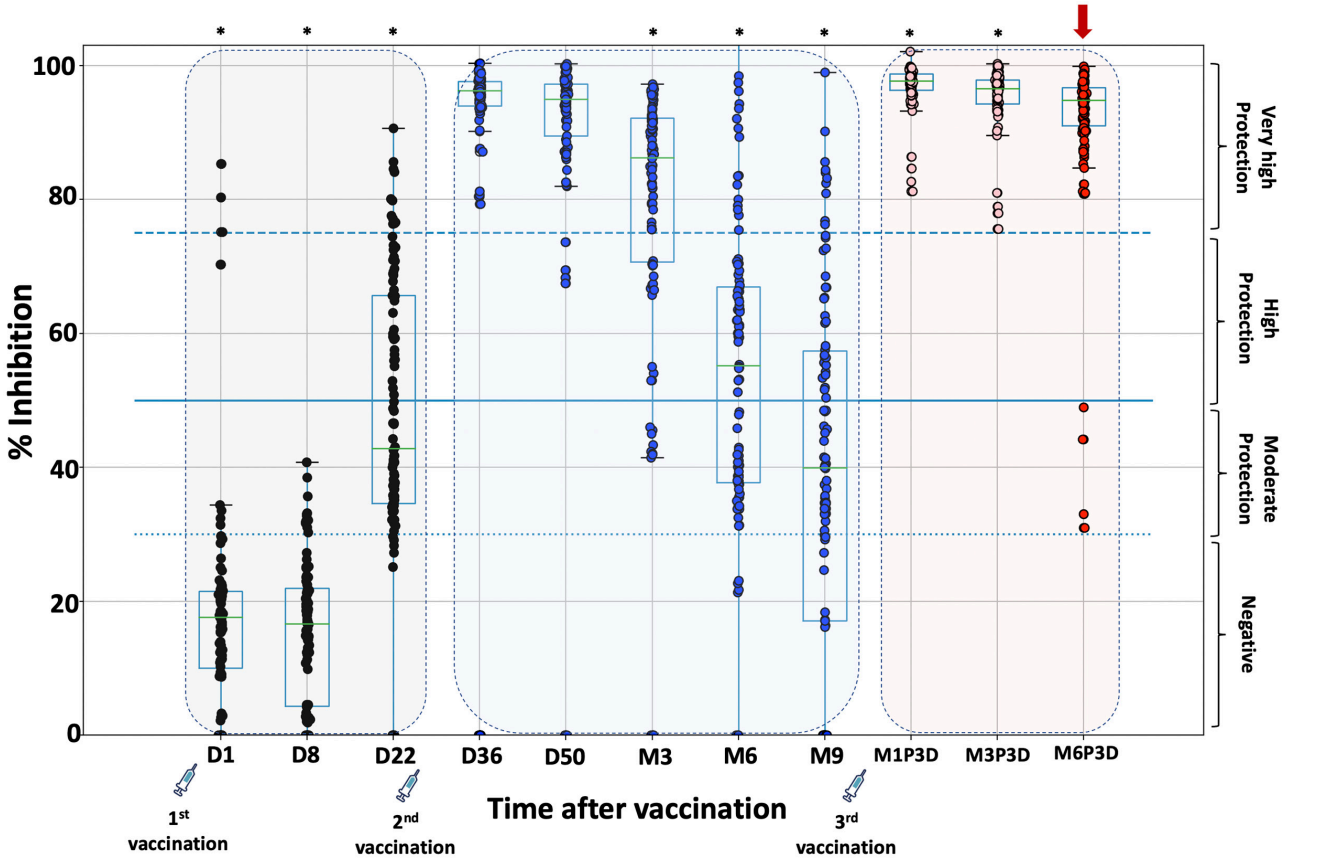

Figure 1. Inhibition (percentage) of SARS-CoV-2 binding to the human host receptor angiotensin-converting enzyme 2 after immunization with BNT162b2 mRNA vaccine (Pfizer-BioNTech). NAbs were measured at the following time points: the first day (D1), 1 wk later (day 8), the day of the second vaccination (D22) and 2 wk (day 36), 1 mo (D50), 3 mo (M3), 6 mo (M6), and 9 mo (M9) after the second dose. In addition, NAbs were measured 1 mo (M1P3D), 3 mo (M3P3D), and 6 mo (M6P3D) after the third dose. The box plot boundaries show the quartiles of the distribution, while the superimposed dots represent the individual levels of NAbs inhibition. The asterisk (*) indicates a statistically significant difference ($P < 0.05$) in the NAbs of the highlighted (with the arrow) case compared with M6P3D. M1P3D = 1 month post 3rd dose; M3P3D = 3 months post 3rd dose; M6P3D = 6 months post 3rd dose; NAbs = neutralizing antibodies; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Mann-Whitney comparisons were 0.804, 0.972, and 0.414 for M1P3D, M3P3D, and M6P3D, respectively. Thus, gender did not affect the degree of immunization or decline after the third dose. Similarly, the possible influence of the age of the participants was also evaluated. Subjects were divided into groups according to the estimated median age of 51 years, namely ≥51 years and <51 years. Again, no statistically significant differences were found, neither for M1P3D, nor for M3P3D, nor for M6P3D (P = 0.301, 0.860, 0.871, respectively). Another study has also shown that age is not a key predictive factor of NAbs production against the Omicron SARS-CoV-2 variant following a third BNT162b2 dose.[1] Finally, the effect of BMI on NAbs titers was examined. The median BMI was 26, while a quarter of the participants had a BMI of less than 23 and another 25% of the subjects had a BMI of more than 28.9. Application of the Kruskal-Wallis test resulted in no significant differences between groups (P = 0.433). However, it should be noted that the relatively small study population may have led to underpowered subgroup analyses.

In conclusion, these results indicate the sustained humoral response against SARS-CoV-2 in healthy individuals even at 6 months following the first booster dose of BNT162b2. Our data advocate for an extended time between the first and the second booster dose. A shorter interval can be considered for immunocompromised patients and the elderly, depending on the epidemic dynamics.

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AUTHOR CONTRIBUTIONS

IPT, MAD, and ET participated in research design. IN-S, VK, and ET participated in the writing of the article. IN-S, ADS, MG, HA, PM, IPT, MAD, and ET participated in the performance of the research. ADS, HA, IPT, and ET contributed new reagents or analytic tools. IN-S, VK, and ET participated in data analysis. All authors reviewed and provided approval for the final draft for submission.

DISCLOSURES

ET is a HemaSphere editor. The authors have no relevant conflicts of interest to disclose.

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