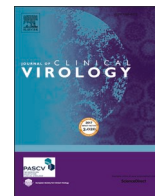




Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



## Evaluation of neutralizing antibodies after vaccine BNT162b2: Preliminary data

Maria Teresa Vietri<sup>a,b,\*</sup>, Luisa Albanese<sup>b</sup>, Luana Passariello<sup>b</sup>, Giovanna D'Elia<sup>b</sup>, Gemma Caliendo<sup>b</sup>, Anna Maria Molinari<sup>a,b</sup>, Italo Francesco Angelillo<sup>c</sup>

<sup>a</sup> Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. De Crecchio, 80138 Naples, Italy

<sup>b</sup> U.O.C. Clinical and Molecular Pathology, A.O.U. University of Campania "Luigi Vanvitelli", 80138 Naples, Italy

<sup>c</sup> Department of Experimental Medicine, University of Campania Luigi Vanvitelli, Via L. Armanni 5, 80138 Naples, Italy

### ARTICLE INFO

#### Keywords:

SARS-CoV-2  
Vaccination  
Humoral immune response  
Neutralizing antibodies  
Immunisation safety  
Surveillance

### ABSTRACT

It is well-known that the Coronavirus Disease 2019, which is caused by the beta-coronavirus severe acute respiratory syndrome (SARS-CoV-2), emerged in December 2019 followed by an outbreak first reported in Wuhan, China. Thus far, vaccination appears to be the only way to bring the pandemic to an end. In the present study, immunogenicity data was evaluated using LIAISON® SARS-CoV-2 TrimericS IgG assay (DiaSorin S.p.A) among a sample of 52 vaccinated healthcare workers, five of whom were previously infected with SARS-CoV-2 and 47 who were seronegative, over a time span of  $\leq 90$  days following the second dose of the BNT162b2 mRNA vaccine. The test detects antibodies against the Trimeric complex (S1, S2 and receptor binding domain). The overall mean value of the serum levels of IgG antibodies to SARS-CoV-2 30 days following the second dose of the vaccine was 1,901.8 binding arbitrary unit (BAU)/ml, after 60 days the mean value declined to 1,244.9 BAU/ml. The antibody levels then reached a plateau, as confirmed by the antibody test carried out 90 days following the second dose, which revealed a mean value of 1,032.4 BAU/ml ( $P < 0.0001$ ). A higher level was observed at all three times in male subjects compared with female subjects, and in younger male participants compared with female participants, although these differences did not reach a statistically significant level. Similarly, no significant difference was found in antibody values at different times according to age. After the second dose of the vaccine, two subjects were infected with SARS-CoV-2, and an increase in antibody values in the third assay was observed in both individuals.

### 1. Introduction

It is well-known that the Coronavirus Disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome (SARS-CoV-2), which first emerged in Wuhan, China, in December 2019, and to date >240 million cases have been confirmed with >4.9 million deaths [1].

Vaccination appears to be one of the most effective tools to control the global COVID-19 pandemic. Several SARS-CoV-2 vaccines have been developed that are currently in use. Two vaccines (Pfizer, Inc./BioNTech SE and Moderna, Inc.) use mRNA, whereas other vaccines (Johnson & Johnson, AstraZeneca, Sputnik V and CanSino Biologics, Inc.) use human and primate adenovirus vectors [2]. The Moderna vaccine mRNA-1273 uses lipid nanoparticle-encapsulated mRNA that encodes

for a full-length, prefusion stabilized S protein of SARS-CoV-2, and a preliminary analysis by the company indicated a 95% efficacy in protecting against COVID-19 [3]. The Pfizer/BioNTech vaccine BNT162, among the four different mRNA vaccines designed by the company, demonstrated a 95% protection rate in a phase III study [4].

To date, there are few studies that have investigated neutralizing anti-SARS-CoV-2 antibodies in subjects not included in clinical trials after mRNA BNT162b2 vaccination, and data on immunogenicity of full-dose administration in real-world scenarios is still incomplete [5]. Understanding the antibody response, including the long-term presence of SARS-CoV-2 antibodies, is essential. Therefore, the purpose of the present study was to evaluate the persistence of antibodies to SARS-CoV-2 over 90 days after the second dose of BNT162b2 mRNA vaccine for

\* Corresponding author at: Department of Precision Medicine, University of Campania "Luigi Vanvitelli", Via L. De Crecchio, 80138 Naples, Italy.

E-mail addresses: [mariateresa.vietri@unicampania.it](mailto:mariateresa.vietri@unicampania.it) (M.T. Vietri), [luisa.albanese@studenti.unicampania.it](mailto:luisa.albanese@studenti.unicampania.it) (L. Albanese), [luana.passariello@studenti.unicampania.it](mailto:luana.passariello@studenti.unicampania.it) (L. Passariello), [giovanna.delia@policliniconapoli.it](mailto:giovanna.delia@policliniconapoli.it) (G. D'Elia), [gemma.caliendo@unicampania.it](mailto:gemma.caliendo@unicampania.it) (G. Caliendo), [annamaria.molinari@unicampania.it](mailto:annamaria.molinari@unicampania.it) (A.M. Molinari), [italof.angelillo@unicampania.it](mailto:italof.angelillo@unicampania.it) (I.F. Angelillo).

<https://doi.org/10.1016/j.jcv.2021.105057>

Received 28 June 2021; Received in revised form 8 December 2021; Accepted 12 December 2021

Available online 14 December 2021

1386-6532/© 2021 Elsevier B.V. All rights reserved.

COVID-19 among a sample of Italian healthcare workers (HCWs).

## 2. Materials and methods

Immunogenicity was evaluated among all 52 vaccinated HCWs (23 men and 29 women), aged 25 to 70 years, in the Clinical Pathology Laboratories at the Teaching Hospital of the University of Campania “Luigi Vanvitelli” of Naples (Italy). Out of the 52 HCWs, 47 were seronegative before the first vaccination dose, according to the result of the SARS-CoV-2 antibodies test (Abbott Architect SARS-CoV-2), and five had been previously infected with SARS-CoV-2 [diagnosed by nasopharyngeal swab polymerase chain reaction (PCR) result and SARS-CoV-2 antibodies test]. None of the enrolled subjects were taking immunomodulatory drugs, whilst lifestyle, diet and level of physical activity remained unchanged throughout the study period and all participants signed an informed consent form. The HCWs received two doses (30 µg each) of the BNT162b2 mRNA COVID-19 vaccine (Comirnaty, Pfizer, Inc.) on January 7, 2021 and after 21 days on January 28, 2021. Venous blood was collected 30, 60 and 90 days after the second dose of the vaccine.

The DiaSorin Liaison SARS-CoV-2 TrimericS IgG (DiaSorin TriS IgG; DiaSorin S.p.A) chemiluminescence immunoassay (CLIA) was used to quantify IgG antibodies in human serum against a trimeric S-protein antigen on a DiaSorin Liaison (DiaSorin S.p.A). Performance of sensitivity and specificity were reported according to the manufacturer’s instructions. Clinical sensitivity of the assay was determined by testing 203 samples collected over a course of time from subjects with a clinical diagnosis of COVID-19, based on a positive SARS-CoV-2 PCR result (sensitivity 0–7, 8–14 and ≥15 days post-RT-PCR, 46.7–82, 74.2–97.7 and 94.5–99.6%, respectively). The clinical specificity was evaluated by testing 1899 presumed SARS-CoV-2 negative samples from US blood donors, collected prior to the COVID-19 outbreak (specificity, 99–99.7%).

The levels of anti-SARS-CoV-2 IgG antibodies were expressed in World Health Organization International Standard (NIBSC code. 20/268) binding arbitrary unit (BAU/ml). Samples with values of ≥33.8 BAU/ml were considered positive.

Descriptive statistics were preliminary used, continuous variables were expressed as the mean and 95% confidence interval (CI), while categorical variables were expressed as frequency. Then, a one-way repeated measures analysis of variance (ANOVA), followed by a Bonferroni’s post hoc test for multiple comparisons, was performed to identify any statistical differences in the serum levels of IgG antibodies to SARS-CoV-2 between the three different time points after the second dose of the vaccination (30, 60 and 90 days) and also according to sex and age categories (27–40, 41–55, 56–70 years) of the individuals. All analyses were based on two-sided P-values,  $P < 0.05$  was considered to indicate a statistically significant difference. The statistical analyses were performed using Stata version 15.1 software (StataCorp LP).

**Table 1**

Mean values of anti-trimeric spike protein specific IgG antibodies (BAU/mL) at 30, 60, and 90 days after the second dose of the vaccine among the 45 seronegative HCWs.

	No.	30 days after dose 2 Mean (95% CI)	60 days after dose 2 Mean (95% CI)	90 days after dose 2 Mean (95% CI)	p-value
All	45	1901.8 (1698.4–2105.1)	1244.9 (1067.3–1422.5)	1032.4 (875.1–1189.6)	<0.0001* <0.0001 <sup>1</sup> 0.29 <sup>2</sup>
Male	19	2024.2 (1683.5–2364.8)	1261.9 (997.4–1526.5)	1051.2 (812.3–1289.9)	0.16*
Female	26	1812.3 (1548.4–2076.2)	1232.5 (977.7–1487.2)	1018.7 (795.6–1241.7)	
27–40 years	14	1740.8 (1334.5–2147.1)	1129 (819.9–1438.1)	1005.3 (705.9–1304.8)	0.16*
41–55 years	13	2116.5 (1670.8–2562.3)	1420.1 (992.7–1847.4)	1049.9 (752.8–1347.1)	
56–70 years	18	1871.8 (1571.3–2172.3)	1208.5 (937.8–1479.2)	1040.8 (754.3–1327.2)	

\* One-way repeated-measures one-way analysis of variance (ANOVA).

<sup>1</sup> Bonferroni’s post-test for multiple comparisons 30 days vs 60 days and 30 days vs 90 days.

<sup>2</sup> Bonferroni’s post-test for multiple comparisons 60 days vs 90 days.

## 3. Results

Of the 47 seronegative HCWs at the beginning of the study, two of them became infected ~70 days after the second dose of the vaccine. Table 1 presents the mean values of the serum levels of IgG antibodies to SARS-CoV-2 at different times, and according to sex and age, among the 45 seronegative HCWs. The overall mean value of the serum levels of IgG antibodies to SARS-CoV-2 30 days after the second dose was 1901.8 BAU/ml, although different kinetics were displayed, and after 60 days the mean value declined to 1244.9 BAU/ml. The antibody levels then reached a plateau, as confirmed by the antibody test carried out 90 days after the second dose, which revealed a value of 1032.4 BAU/ml. The results of the one-way repeated measures ANOVA showed that there was a significant effect of the vaccination on the antibody response. Indeed, the mean value of the antibody levels was statistically significantly different for different time points after the second dose ( $P < 0.0001$ ). Bonferroni’s post hoc analysis identified a statistically significant difference between the three time points and the level of antibody. Indeed, there was a significant difference between time points 1 and 2 ( $P < 0.0001$ ), whereby individuals at 60 days lost on average 656.8 BAU/ml more than those at 30 days, and between time points 1 and 3 ( $P < 0.0001$ ) with individuals at 90 days losing on average 869.4 BAU/ml more than those at 30 days. There was no significant difference between time points 2 and 3 ( $P = 0.29$ ), although those at 90 days lost on average 212.5 BAU/ml more than those at 60 days. There were no associations between the level of antibody response with sex ( $P = 0.16$ ) and age categories ( $P = 0.16$ ). However, a higher level was observed at all three times in male participants with values decreasing from 2024.2 to 1232.5 BAU/ml compared with female participants with values decreasing from 1812.3 to 1018.7 BAU/ml. Furthermore, almost the same value was observed at 90 days in the three different age categories.

Table 2 presents the antibody levels of the five HCWs (three men and two women) previously infected with SARS-CoV-2 and the two HCWs

**Table 2**

The values of anti-trimeric spike protein specific IgG antibodies (BAU/mL) at 30, 60, and 90 days after the second dose of the vaccine in 5 HCWs previously infected by SARS-CoV-2 and in 2 HCWs infected by SARS-CoV-2 after dose 2.

HCWs	Time of infection	30 days after dose 2	60 days after dose 2	90 days after dose 2
1	October 2020	4840	5420	3480
2	October 2020	4600	1640	1360
3	October 2020	3580	1800	1610
4	December 2020	12,660	4400	1660
5	December 2020	20,200	16,720	11,640
6	70 days following the second dose	4760	4300	9040
7	70 days following the second dose	230	141	2200

who tested positive after the second dose. Among those previously infected, asymptomatic or with mild symptoms, three and two of them were infected ~60 days and 15 days before the first dose, respectively. Those who tested positive in December showed higher antibody levels than those who tested positive in October. The two HCWs who tested positive ~70 days after the second dose were among a group of 12 who had been in contact with a SARS-CoV-2-positive individual. An increase in antibody values at 90 days was observed in both HCWs.

#### 4. Discussion

The BNT162b2 mRNA vaccine has been tested in a phase I study among adults aged 18–55 and 65–85 years and all groups but one, received two doses of 10, 20 or 30 µg with a 21-day interval, and one group received one dose of 100 µg. High levels of neutralizing antibodies and substantial T cell responses have been observed in all subjects, with values similar to those of patients previously affected by SARS-CoV-2. Immunogenicity was reduced in those older than 65 years and the vaccine was associated with a lower incidence and severity of systemic reactions [6]. A phase III study, completed in November 2020, involved >43,000 participants who were not immunocompromised and had no previous history of SARS-CoV-2 infection, who received 30 µg mRNA vaccine at an interval of 21 days. The vaccine conferred 95% protection against COVID-19 after ~12 days and the efficacy was similar among groups according to age, sex, race, ethnicity and coexisting conditions. The adverse events were mild in 50% of cases, moderate in 20–30% of cases and severe in a few cases [7]. Danese et al., in a study investigating the antibody response after BNT162b2 vaccination in a three-case series, highlighted an increase of anti-S1/S2 and anti-receptor binding domain (RBD) IgG, which peaked 35 days after the first dose. After this time point, the antibody levels declined, reaching a second plateau 50 days after the first dose, with values still higher than the first peak observed after the first dose [5]. Wang et al., showed that BNT162b2 and mRNA-1273 vaccines, 8 weeks after the second dose, determined high levels of IgM and IgG anti-SARS-CoV-2 spike protein and RBD binding titres [8].

In the present study, the antibody levels were evaluated over a more extended period of time. Antibody levels increased a peak after 30 days and, subsequently, reduced until they reached a plateau at 60 days after the second dose, with similar values observed after 90 days. Moreover, a higher level was observed at all three times in male subjects and in younger male subjects compared with female participants, although these differences did not reach a statistically significant level. Similarly, no significant difference was observed in antibody levels at different times according to age.

In the five HCWs previously infected with SARS-CoV-2, a higher antibody level was observed in those who tested positive in December than those who tested positive in October. Previous studies have shown that mRNA vaccines elicit rapid immune responses in seropositive individuals with post-vaccine antibody titres that are comparable to, or exceed titres in naïve individuals vaccinated with two doses [9,10]. The positive HCWs were infected in the family environment and this confirms that adhering to the workplace preventive measures is crucial to mitigate the risk of COVID-19. Moreover, the two positive HCWs were asymptomatic, confirming the protective effect of the vaccine against the SARS-CoV-2 disease.

In a study investigating COVID-19 mRNA vaccine effectiveness among HCWs between January and March 2021, 3% tested positive ≥7 days after the second dose with one or more symptoms of COVID-19-like illness [11]. In another study among vaccinated HCWs between

December 16, 2020 and February 9, 2021, a SARS-CoV-2 positivity rate of 0.05% has been observed ≥2 weeks after the second dose [12]. Finally, in a prospective cohort study among HCWs who received two doses of BNT162b2 or mRNA-1273, between December 9, 2020 and February 23, 2021, SARS-CoV-2 cases occurred in 0.3% of the sample [13].

The monitoring of humoral immune responses after mRNA COVID-19 vaccination allows us to evaluate the kinetics of anti-SARS-CoV-2 antibodies, paving the way to larger studies to assess the efficacy of different types of vaccines. Moreover, with the appearance of novel SARS-CoV-2 variants that may evade immune recognition, the monitoring of anti-SARS-CoV-2 neutralizing antibodies will provide information on whether changes in the current formulation of vaccines or the administration plans are required to maintain adequate protection against SARS-CoV-2 infection.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### References

- [1] WHO. World Health Organization; Geneva. Coronavirus disease (COVID-19) Dashboard. <https://covid19.who.int> (accessed on October 15, 2021).
- [2] COVID-19 vaccines, No authors listed. *Drugs and Lactation Database (LactMed) [Internet]*, National Library of Medicine (US), Bethesda (MD), 2006 (Accessed on May 17, 2021).
- [3] R. Chakraborty, S. Parvez, COVID-19: an overview of the current pharmacological interventions, vaccines, and clinical trials, *Biochem. Pharmacol.* 180 (2020), 114184, <https://doi.org/10.1016/j.bcp.2020.114184>.
- [4] M.M. Silveira, G.M.S.G. Moreira, M. Mendonça, DNA vaccines against COVID-19: perspectives and challenges, *Life Sci.* 267 (2021), 118919, <https://doi.org/10.1016/j.lfs.2020.118919>.
- [5] Danese E., Montagnana M., Salvagno G.L., Peserico D., Pighi L., De Nitto S., et al. Comprehensive assessment of humoral response after Pfizer BNT162b2 mRNA Covid-19 vaccination: a three-case series. *Clin. Chem. Lab. Med.* 2021. doi: 10.1515/cclm-2021-0339.
- [6] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, et al., Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine, *N. Engl. J. Med.* 383 (2020) 2603–2615, <https://doi.org/10.1056/NEJMoa2034577>.
- [7] S. Pascolo, Synthetic messenger RNA-based vaccines: from scorn to hype, *Viruses* 13 (2) (2021) 270, <https://doi.org/10.3390/v13020270>.
- [8] Wang Z., Schmidt F., Weisblum Y., Muecksch F., Barnes C.O., Finkin S., et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *bioRxiv*. 2021; 2021.01.15.426911. doi: 10.1101/2021.01.15.426911.
- [9] A. Padoan, L. Dall'Olmo, F.D. Rocca, F. Barbaro, C. Cosma, D. Basso, et al., Antibody response to first and second dose of BNT162b2 in a cohort of characterized healthcare workers, *Clin. Chim. Acta* 519 (2021) 60–63, <https://doi.org/10.1016/j.cca.2021.04.006>.
- [10] Krammer F., Srivastava K., Simon V. Robust spike antibody responses and increased reactivity in seropositive individuals after a single dose of SARS-CoV-2 mRNA vaccine. *medRxiv [Preprint]*. 2021. DOI: 10.1101/2021.01.29.21250653.
- [11] T. Piliushvili, K.E. Fleming-Dutra, J.L. Farrar, R. Gierke, N.M. Mohr, D.A. Talan, et al., Interim estimates of vaccine effectiveness of pfizer-BioNTech and moderna COVID-19 vaccines among health care personnel - 33U.S. Sites, January-March 2021, *MMWR Morb. Mortal Wkly Rep.* 70 (20) (2021) 753–758, <https://doi.org/10.15585/mmwr.mm7020e2>.
- [12] J. Keehner, L.E. Horton, M.A. Pfeffer, C.A. Longhurst, R.T. Schooley, J.S. Currier, et al., SARS-CoV-2 infection after vaccination in health care workers in California, *N. Engl. J. Med.* 384 (18) (2021) 1774–1775, <https://doi.org/10.1056/NEJMc2101927>.
- [13] T.C. Bouton, S. Lodi, J. Turcinovic, S.E. Weber, E. Quinn, C. Korn, et al., COVID-19 vaccine impact on rates of SARS-CoV-2 cases and post vaccination strain sequences among healthcare workers at an urban academic medical center: a prospective cohort study, *Open Forum Infect. Dis.* 8 (10) (2021) ofab465, <https://doi.org/10.1093/ofid/ofab465>.