



OPEN ACCESS

EDITED BY

Venkatesh Kumaresan,
University of Texas at San Antonio,
United States

REVIEWED BY

Blanka Wolszczak Biedrzycka,
University of Warmia and Mazury in
Olsztyn, Poland
Kannan Thangamani,
Northeastern University, United States

*CORRESPONDENCE

Pilar Requena
✉ prequena@ugr.es

[†]These authors share first authorship

[‡]These authors share senior authorship

RECEIVED 12 March 2025

ACCEPTED 08 May 2025

PUBLISHED 30 May 2025

CITATION

Sarabia de Ardanaz L, Serrano-Conde E,
Fuentes A, Leyva A, García F and Requena P
(2025) Negative associations of age
and lifestyle factors with the antibody
response to the COVID-19 vaccine
BNT162b2 in health workers from Spain.
Front. Immunol. 16:1590939.
doi: 10.3389/fimmu.2025.1590939

COPYRIGHT

© 2025 Sarabia de Ardanaz, Serrano-Conde,
Fuentes, Leyva, García and Requena. This is an
open-access article distributed under the terms
of the [Creative Commons Attribution License
\(CC BY\)](#). The use, distribution or reproduction
in other forums is permitted, provided the
original author(s) and the copyright owner(s)
are credited and that the original publication
in this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

Negative associations of age and lifestyle factors with the antibody response to the COVID-19 vaccine BNT162b2 in health workers from Spain

Luis Sarabia de Ardanaz^{1,2†}, Esther Serrano-Conde^{3†},
Ana Fuentes^{3,4,5}, Alba Leyva⁶, Federico García^{3,4,5‡}
and Pilar Requena^{1,4,7**}

¹Universidad de Granada, Departamento de Medicina Preventiva y Salud Pública, Granada, Spain,

²Servicio de Reumatología, Hospital Universitario de Jaén, Jaén, Spain, ³Servicio de Microbiología, Hospital Universitario Clínico San Cecilio, Granada, Spain, ⁴Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), Granada, Spain, ⁵Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain, ⁶Vircell, S.L., Granada, Spain, ⁷Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain

Introduction: Despite the high efficacy of the anti-coronavirus disease 2019 (COVID-19) BNT162b2 vaccine (Comirnaty[®], Pfizer-BioNTech), variability in the antibody titers following vaccination has been described. However, little is known about the risk factors that are associated with a poorer antibody response to the BNT162b2 vaccine.

Methodology: We studied the determinants of the humoral response to the anti-COVID-19 vaccine BNT162b2 in 200 healthcare workers followed up for 2 years. Serum samples were tested for the anti-spike immunoglobulin G (IgG) levels and neutralizing antibody titers against selected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants at different time points after primary and booster vaccinations. Anthropometric data and clinical and lifestyle information were also collected. Statistical analyses consisted of linear and logistical regressions for point estimations and the Mann–Whitney, Friedman, and generalized estimating equations for repeated measures.

Results: After the primary vaccination, the antibody titers and the percentage of seroconverted individuals peaked at 5 weeks but declined after 1 year; however, they remained high after the booster administration. After the first dose of the vaccine, negative associations of the anti-spike IgG levels with age ($\beta = -0.01$, 95%CI = -0.03 to -0.003), smoking habit ($\beta = -1.08$, 95%CI = -1.70 to -0.46), and alcohol consumption ($\beta = -1.43$, 95%CI = -2.20 to -0.65) were found. With regard to the booster vaccine, the following associations were retained in the stepwise multivariate model: anti-Delta neutralizing antibodies with hip

circumference (OR = 1.07, 95%CI = 1.01–1.12, p = 0.008), anti-Delta-K antibodies with hip circumference (OR = 1.06, 95%CI = 1.01–1.11, p = 0.007), and anti-Omicron antibodies with the Mediterranean diet score (OR = 0.74, 95%CI = 0.58–0.96, p = 0.023).

Conclusion: Lifestyle habits and age had an association with the humoral response to the BNT162b2 vaccine.

KEYWORDS

COVID-19, SARS-CoV-2, vaccines, BNT162b2, anti-spike IgG, neutralizing antibodies

Introduction

From the beginning of the coronavirus disease 2019 (COVID-19) pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has remained an extraordinary health risk based on the reported rates of infection, disease, and mortality (1). Throughout the pandemic, several variants of the virus have emerged, and some of them have been designated by the World Health Organization as “variants of concern,” i.e., Alpha, Beta, Gamma, Delta, and Omicron.

The genome of SARS-CoV-2 encodes several proteins. Among them, the spike (S) glycoprotein is expressed in the virus surface and is responsible for the viral attachment, the fusion, and the endosome-mediated host entry (2). Therefore, some of the antibodies generated against this protein have the ability to block the entry of the virus into the target cells and are so-called neutralizing antibodies.

Mass vaccination plays a crucial role in the prevention and management of infectious diseases. In the case of SARS-CoV-2, the S protein is the target of most commercialized vaccines. This is the case with BNT162b2 (Comirnaty®, Pfizer-BioNTech), which contains messenger RNA (mRNA) encoding the S protein, and in clinical trials provided approximately 95% protection against COVID-19 when two doses were administered (3). Research indicates that the maximum humoral immune response occurs between 21 and 28 days following the administration of the second vaccine dose. Subsequently, there is a gradual reduction in the serum antibody levels at 4–6 months post-vaccination, irrespective of factors such as age, gender, initial serostatus, or the presence of other health conditions (4). However, we and others have shown variability in the antibody titers after vaccination or natural disease, including individuals who did not develop a humoral response at all (5–7). This does not necessarily mean a lack of immunity since cellular responses could equally confer protection in the absence of antibodies. However, it is necessary to examine what the determinants of this lower humoral response may be. It has been noted that the levels of neutralizing antibodies or their decline is associated with the comorbidities of the patient. Thus, individuals of older age and those with immunosuppression produced a significantly lower antibody response after the first

vaccine dose compared with healthy adults (8). This has also been observed in obese individuals regardless of sex, previous infection status, or the time elapsed since the last vaccination dose (9). However, in these studies, the neutralizing antibodies for variants other than the wild type were not considered. Furthermore, additional socio-demographic, clinical, or lifestyle factors could affect the immune response after vaccination.

Therefore, the aim of this study was to assess the determinants of a lower humoral response to the BNT162b2 vaccine, analyzed as the total anti-S immunoglobulin G (IgG) levels and neutralizing antibodies against different variants.

Materials and methods

Study design and population

This is an ambispective internal comparison cohort study performed in two stages. Initially, 147 workers (cohort 1) from the Hospital Universitario Clínico San Cecilio who received two doses of BNT162b2 and were under 65 years of age were recruited. The study participants were enrolled in January 2021, in order of arrival to the vaccination event, and were followed up until December 2021. In a second stage and coinciding with the administration of the third dose of vaccine in December 2021, participants were invited to continue the follow-up for an additional year and to reply to an auto-administered questionnaire, with 99 out of 147 individuals providing their consent. In addition, 54 more individuals were recruited to compensate for the losses at follow-up (estimated to be 10% every 6 months due to the high turnover of healthcare personnel in the hospital). Those 99 plus 54 individuals comprised cohort 2.

Study samples and data collection

Blood samples were collected from the initial cohort at the following times: during administration of the first dose (t_0) and the second dose (3 weeks later, t_1), as well as 5 weeks (t_2), 3 months (t_3), and 8–9 months (t_4) after administration of the first dose.

Abbreviations: MedD, Mediterranean diet; SD, standard deviation; CI, confidence interval; β , beta coefficient; OR, odds ratio; BMI, body mass index.

Moreover, coinciding with the administration of the third dose of the vaccine, additional samples were collected (t_5), as well as 1 month (t_6), 4–6 months (t_7), and 9 months (t_8) after the third dose. For the 54 additional subjects recruited in the second stage, only samples collected from t_5 to t_8 were obtained.

Blood samples were obtained at each time point, and serum was obtained by centrifugation of the blood samples at $600 \times g$ for 5 min, aliquoted, and kept at -20°C until use.

At t_5 , an interview was performed and anthropometric data [body mass index (BMI) and waist and hip perimeters] were determined by a dietician–nutritionist. Moreover, a questionnaire was auto-filled with regard to information about the times of administration of the first and third doses. The information collected included the following: 1) socio-demographic, including sex, age, and level of education; 2) lifestyle habits, with the 14-item PREDIMED adherence to Mediterranean diet (MedD) questionnaire (10), smoking habit (collected into categories of never smoker, ex-smoker, or active smoker), and alcohol consumption (informed as never, low consumption of ≤ 7 alcoholic drinks spread throughout the week or concentrated in 1–2 days, or high consumption of > 7 drinks spread throughout the week or concentrated in 1–2 days); and 3) auto-reported clinical information with regard to prevalent diseases at the time of vaccination, classified as a) immune-mediated diseases (i.e., hematological neoplasms, primary or secondary immunodeficiencies, autoimmune diseases, and/or transplant recipients), b) cardiometabolic diseases (e.g., heart diseases, dyslipidemia, and/or diabetes mellitus, and c) other diseases (e.g., cancer and/or respiratory diseases).

Infections prior to vaccination were auto-reported by the study subjects.

Antibody determination

The serum anti-S IgG antibodies were analyzed in all samples using the COVID-19 VirClia[®] IgG mono test (Vircell, S.L., Granada, Spain) following the manufacturer's instructions. This is a qualitative indirect chemiluminescent immunoassay with a manufacturer-declared sensitivity of 98%. Individuals were considered as seroconverted when their antibody levels were higher than the mean plus 3 standard deviations of the levels observed before vaccination.

A full-virus assay was used to test the neutralization capacity of the serum of the study participants against the wild type and the Alpha (B.1.1.7), Beta (B.1.351), Delta (B.1.617), Delta_K (B.1.617+E484K), and Omicron (B.1.1.529) variants of SARS-CoV-2. Briefly, 70 μl of serum was twofold serially diluted in a culture medium with 2% fetal bovine serum, starting at 1:20 dilution until 1:2,560, and added into microplates. Thereafter, 70 μl of the cell culture medium containing $100\times$ of the median tissue culture infectious dose of the virus was added to every well containing the diluted sera of the participants. The plates were incubated at room temperature for 1 h, and 100 μl of each mixture of serum plus virus was added into a plate containing Vero E6 cells. After a 5-day incubation period at 37°C in 5% CO_2 , the full cytopathic effect was evaluated by microscopic examination. The highest serum dilution that completely inhibited the cytopathic effect was considered as the neutralization titer.

Samples below or over the limit of detection were assigned the value of the detection limit.

Statistical analysis

A descriptive analysis of the variables was carried out. The variables were presented as follows: mean \pm standard deviation (SD) for normally distributed continuous variables, median for non-normally distributed variables, and percentages and frequencies for categorical variables.

The differences in the levels of anti-S between time points ($t_0 - t_5$ and $t_5 - t_8$) were analyzed by means of the Kruskal–Wallis test followed by individual comparisons *versus* t_0 , corrected with the Dunn's test for multiple comparisons. It was not possible to perform a paired test due to the losses at follow-up. The differences in the neutralizing antibody titers before/after the booster administration ($t_5 - t_6$) were assessed with the Wilcoxon matched-pairs signed-rank test, as in this case there were no losses at follow-up. Furthermore, the differences between the different variant neutralizing antibody titers were analyzed with the Friedman's test followed by Dunn's multiple comparisons test using the Omicron variant as a reference. These analyses were only performed here for the Omicron, Delta, and Delta_K variants after the booster administration as the analysis of the wild-type and the Alpha and Beta variants after primary vaccination had already been published in a previous article (5).

Univariate linear regression models for the anti-S IgG levels and univariate ordinal logistic regression models for the neutralizing antibody titers were estimated to identify the determinants of antibody levels after the primary and booster vaccinations. Beta coefficients (β) or odds ratios (ORs), as well as the 95% confidence intervals (CIs) and p -values, were estimated. Subsequently, those variables with $p < 0.2$ were included in the stepwise multivariate model.

Finally, generalized estimating equation models for repeated measures were utilized to evaluate the determinants of the average antibody levels over the follow-up. Initially, univariate analyses were conducted, and variables with $p < 0.2$ were subsequently included in the multivariable analysis.

Statistical significance was indicated as $p < 0.05$. Stata program v.17 and GraphPad Prism v.10.2.3 were used for the statistical analysis and graphs.

Results

Description of the study cohort

As shown in Table 1, the overall mean age was 46.52 years (SD = 11.86), 20.90% were men, and most individuals completed higher education studies. The average BMI was 25.37, indicating a slightly overweight population. With regard to lifestyle habits, the adherence to MedD was high, and most study subjects did not smoke or drink alcohol.

In terms of health conditions, 4.96% of the study population had immune-mediated diseases, while cardiovascular diseases were

TABLE 1 Baseline demographic and clinical variables.

	Cohort 1	Cohort 2	Total
Age, mean (SD) [n]	45.31 (11.92) [147]	47.71 (11.69) [153]	46.52 (11.86) [200]
Sex (men), n (%)	33 (22.45)	30 (19.48)	42 (20.90)
Higher education studies (yes), n (%)	70 (79.55)	104 (77.04)	107 (76.98)
Waist circumference, mean (SD) [n]	83.87 (12.49) [88]	83.66 (12.96) [131]	83.64 (12.86) [135]
Hip circumference, mean (SD) [n]	101.35 (9.56) [82]	100.26 (9.71) [131]	101 (9.75) [135]
BMI, mean (SD) [n]	25.22 (4.30) [81]	25.63 (4.81) [128]	25.37 (4.54) [134]
MedD score, mean (SD) [n]	8.92 (1.71) [88]	9.03 (1.81) [135]	9.05 (1.81) [139]
Smoking status, n (%)			
- Smoker	10 (11.36)	19 (13.77)	21 (14.79)
- Ex-smoker	23 (26.14)	34 (24.64)	35 (24.65)
- Non-smoker	55 (62.50)	85 (61.59)	86 (60.56)
Alcohol consumption, n (%)			
- Nothing	44 (50)	69 (50.74)	70 (50)
- ≤7 drinks/week	38 (43.18)	60 (44.12)	62 (44.29)
- >7 drinks/week	6 (6.82)	7 (5.15)	8 (5.71)
Immune disease (yes), n (%)	5 (5.68)	7 (5.11)	7 (4.96)
Cardiovascular disease (yes), n (%)	16 (18.18)	22 (16.06)	24 (17.02)
Other diseases (yes), n (%)	6 (6.82)	12 (8.76)	13 (9.22)

BMI, body mass index; MedD, Mediterranean diet.

present in 17.02% of the study subjects. Only five participants with available anti-S IgG data reported a SARS-CoV-2-positive diagnostic test between 3 and 10 months prior to vaccination. The antibody titers were similar to those with no previous infection (Supplementary Figure S1).

Antibody levels after vaccination

In the primary vaccination, the highest levels of serum anti-S IgG were observed 5 weeks after the administration of the first dose and then declined progressively (Figure 1). Of note is that, although all individuals seroconverted after the vaccination, there was a high variability in the antibody titers observed. By the end of the initial follow-up, approximately 1 year after the primary vaccination, only 64% of individuals were still seroconverted and the antibody titers were much lower (Figure 1). With regard to the vaccine booster, all individuals seroconverted 1 month later, and the anti-S IgG levels were even higher than that after the primary vaccination. The titers and percentage of seroconverted individuals remained high during the follow-up (Figure 1).

We had previously analyzed the titers of neutralizing antibodies against some of the initial SARS-CoV-2 variants after primary vaccination. In this research, we analyzed the titers of neutralizing

antibodies against the Omicron, Delta, and Delta_K variants after the booster administration. The titers significantly increased after the booster administration for all variants (Figure 2). Moreover, the highest titers were observed against the Delta variant, both before and after the booster administration.

Effect of exposure variables on the antibody levels after the primary vaccination

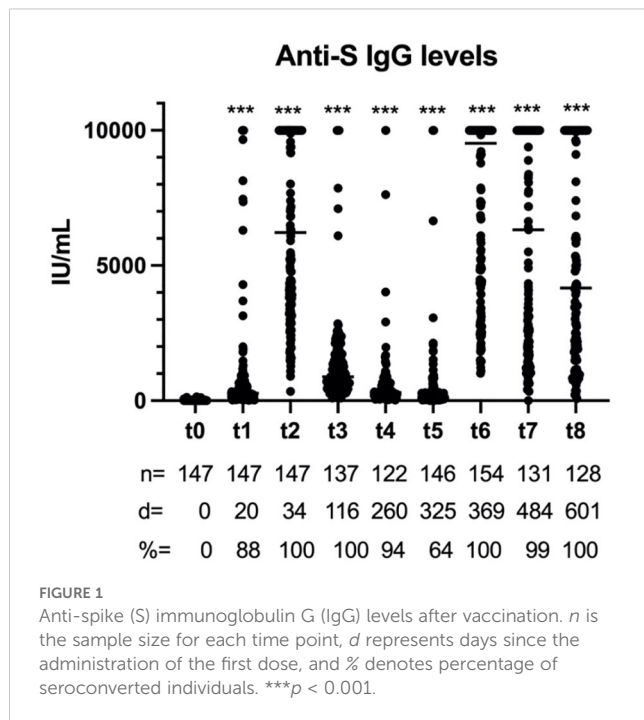
Several socio-demographic, lifestyle, and health variables were evaluated in relation to the anti-S IgG levels at the peak of the humoral response (5 weeks after vaccination). In the univariate regressions, a trend toward lower levels was observed in individuals with immune-mediated and cardiovascular diseases compared to those without disease (Table 2). None of these variables were retained in the stepwise multivariate models.

The effect of the exposure variables on the mean anti-S levels during 1 year of follow-up was also assessed (Table 3). Negative significant associations were found with age, smoking habit, and alcohol consumption. Moreover, positive and negative trends were observed for education level and immune-mediated diseases, respectively (Table 3). Considering the sex differences in alcohol metabolism, a stratified analysis was performed for the effect of alcohol on the antibody levels separately in men and women. A negative association was observed in both groups when no alcohol consumption was compared with >7 drinks/week (women: $\beta = -1.28$, 95%CI = -2.33 to -0.23 , $p = 0.017$, $n = 114$; men: $\beta = -1.87$, 95%CI = -3.26 to -0.47 , $p = 0.009$, $n = 22$). In the multivariate models (data not shown), the statistical significance was maintained for the smoking variable ($\beta = -0.67$, 95%CI = -1.34 to -0.00 , $p = 0.048$) and alcohol consumption ($\beta = -1.31$, 95%CI = -2.15 to -0.47 , $p = 0.002$). Furthermore, there was a trend toward statistical significance in the MedD score ($\beta = -0.11$, 95%CI = -0.24 to -0.00 , $p = 0.054$).

With regard to the neutralizing antibody titers, the following results were observed (Table 2): 1) for the Alpha variant, there was a negative association with age and smoking, although this did not reach statistical significance; 2) for the Beta variant, there was a borderline non-significant negative association with smoking habit; and 3) for the wild-type variant, there was no association. In the multivariate models (data not shown), the only association retained was between the neutralizing antibodies against the Alpha variant and age ($\beta = -0.05$, 95%CI = -0.10 to -0.00 , $p = 0.046$).

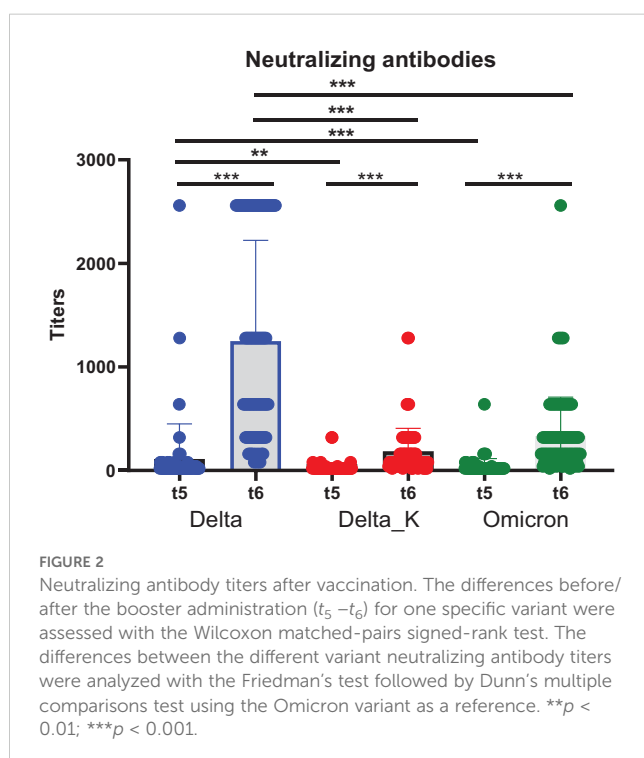
Effect of the exposure variables on the antibody levels after the booster vaccination

In the univariate regressions after the booster vaccination, the anti-S IgG levels measured at the peak of the response (t_6) showed a borderline non-significant positive association with the hip and waist circumference (Table 4). None of these variables were retained in the stepwise multivariate models (data not shown). At the follow-



up, no significant association was observed between the mean antibody levels and the different determinants measured (Table 3).

With respect to the neutralizing antibodies for the predominant variants in that phase of the pandemic, the following associations were observed (Table 4): 1) for the Delta variant, there was a positive association with hip circumference; 2) for the Delta_K variant, there were positive significant associations with the hip and waist circumference; and 3) for the Omicron variant, there was a significant



negative association with the MedD score. In the multivariate models, the following associations were retained (not shown): anti-Delta antibodies with hip circumference (OR = 1.07, 95%CI = 1.01–1.12, *p* = 0.008); anti-Delta_K antibodies with hip circumference (OR = 1.06, 95%CI = 1.01–1.11, *p* = 0.007); and anti-Omicron with the MedD score (OR = 0.74, 95%CI = 0.58–0.96, *p* = 0.023).

Discussion

In the present study, factors that influence the BNT162b2 vaccine response of both the initial vaccination and the booster dose were explored.

In our cohort, the vaccine seroconversion curve was highest at 5 weeks after the first dose; however, the titers and the percentage of seroconverted individuals declined over time, prior to the administration of the booster dose, and a high variability on the antibody titers was observed. This is consistent with previous results in other cohorts (11–14). In contrast to these studies, some of them performed in health workers, higher antibody titers were not observed in individuals with a previous history of COVID-19 infection, nor even before the vaccine administration. This could be due to the low number of pre-exposed subjects in our cohort and the long period of time between the infection and the measurement of antibodies. However, other studies did not show differences in the vaccine-induced antibody levels between unexposed and previously SARS-CoV-2-exposed health workers (15).

After the booster administration, the antibody titers increased and remained high during the 9 months of follow-up, in consonance with other studies (11, 14, 16). These results suggest that a third dose is necessary to maintain the high titers of antibodies and, presumably, the protection in the population. The possible impact of the third dose on disease transmission has yet to be investigated. However, currently, in Spain and many other countries, a third dose is only recommended for risk groups.

Although the booster dose administered had the sequence of the wild-type variant (17–19), the neutralizing antibody levels increased significantly after this dose, even against the more recent and different variants, e.g., Omicron and Kappa, as previously reported (20). However, the titers were significantly higher for the earlier variant, Delta.

In our cohort, the mean antibody levels at follow-up had a negative association with age after the initial dose, and a similar trend was observed after the booster dose. Moreover, age was negatively associated with the neutralizing antibody titers against the Alpha variant. This result is in consonance with other cohorts (8, 12, 21–23). However, we did not find any association with age at the peak of the antibody response, i.e., 5 weeks after the first vaccination. This may be due to the fact that our study subjects were health workers, with an age range of 24–66 years, meaning the extreme age groups were excluded from our cohort. Of note is that, in most studies, the strongest age-related association with antibody levels has been observed in the age group >65 years (8, 12). Furthermore, we did not find any association of the antibody levels with sex. This is in consonance with previous studies (11,

TABLE 2 Association between specific determinants and antibody levels at the peak of the humoral response after primary vaccination.

	Anti-S IgG			Neutralizing WT			Neutralizing Alpha			Neutralizing Beta		
	Beta	95%CI	p	OR	95%CI	p	OR	95%CI	p	OR	95%CI	p
Age	-0.00	-0.01 – 0.00	0.256	0.97	0.94 – 1.00	0.112	0.96	0.93 – 0.99	0.020	0.99	0.96 – 1.02	0.845
Sex (ref. female)	-0.21	-0.46 – 0.04	0.108	1.09	0.48 – 2.47	0.819	1.00	0.44 – 2.24	1.000	1.05	0.43 – 2.55	0.902
Waist circumference	-0.00	-0.01 – 0.01	0.850	1.00	0.96 – 1.03	0.951	0.98	0.94 – 1.01	0.273	0.99	0.95 – 1.03	0.728
Hip circumference	0.01	-0.00 – 0.02	0.263	1.01	0.97 – 1.06	0.467	1.00	0.96 – 1.05	0.784	1.01	0.96 – 1.06	0.631
BMI	-0.01	-0.04 – 0.02	0.700	1.00	0.89 – 1.11	0.967	0.96	0.87 – 1.06	0.465	0.98	0.87 – 1.10	0.785
Higher Education studies (ref. no)	-0.03	-0.38 – 0.31	0.861	1.15	0.39 – 3.38	0.791	0.51	0.16 – 1.61	0.254	1.96	0.60 – 6.41	0.264
MedD Score	-0.03	-0.11 – 0.04	0.435	0.97	0.74 – 1.26	0.842	1.02	0.78 – 1.33	0.863	0.88	0.67 – 1.15	0.364
Smoking status (ref. never smoker)												
- Ex-smoker	-0.20	-0.52 – 0.12	0.216	0.50	0.17 – 1.47	0.209	0.37	0.12 – 1.14	0.084	0.68	0.22 – 2.12	0.511
- Smoker	-0.34	-0.79 – 0.09	0.126	0.44	0.12 – 1.59	0.217	0.44	0.11 – 1.65	0.227	0.27	0.06 – 1.25	0.095
Alcohol consumption (ref. no consumption)												
- ≤ 7 drinks/week	0.12	-0.16 – 0.41	0.387	1.16	0.44 – 3.02	0.756	1.11	0.42 – 2.92	0.832	1.29	0.48 – 3.49	0.608
- > 7 drinks/week	-0.29	-0.86 – 0.27	0.303	0.54	0.11 – 2.56	0.446	0.76	0.16 – 3.61	0.736	2.31	0.52 – 10.28	0.269
Immune disease (ref. no)	-0.56	-1.16 – 0.31	0.063	2.26	0.38 – 18.65	0.324	1.82	0.29 – 11.15	0.515	0.40	0.03 – 4.78	0.471
Cardiovascular disease (ref. no)	-0.31	-0.67 – 0.04	0.086	0.77	0.25 – 2.30	0.641	0.61	0.20 – 1.82	0.381	0.40	0.11 – 1.39	0.152
Other diseases (ref. no)	-0.25	-0.80 – 0.30	0.372	1.00	0.22 – 4.45	0.990	1.54	0.29 – 7.99	0.607	2.29	0.48 – 10.92	0.296

Ref., reference category. Highlighted in bold if p-value<0.1. CI, confidence interval; BMI, Body Mass Index; MedD Score, Mediterranean Diet Score; WT, wild-type.

TABLE 3 Association of specific determinants with median antibody levels at follow-up after first and third vaccine doses.

	First dose			Third dose		
	Coef	95% CI	p	Coef	95% CI	p
Age	-0.01	-0.03 – 0.00	0.017	-0.00	-0.01 – 0.00	0.081
Sex (ref. female)	0.09	-0.36 – 0.55	0.696	0.10	-0.18 – 0.38	0.488
Waist circumference	-0.01	-0.03 – 0.00	0.231	0.00	-0.00 – 0.01	0.200
Hip circumference	0.00	-0.02 – 0.02	0.959	0.00	-0.00 – 0.01	0.456
BMI	-0.03	-0.08 – 0.01	0.131	0.00	-0.01 – 0.03	0.443
Higher Education studies (ref. no)	0.45	-0.05 – 0.95	0.082	0.01	-0.33 – 0.36	0.948
MedD Score	-0.10	-0.24 – 0.03	0.130	-0.01	-0.08 – 0.05	0.675
Smoking status (ref. never smoker)						
- Ex-smoker	-0.31	-0.86 – 0.24	0.270	0.00	-0.32 – 0.34	0.953
- Smoker	-1.08	-1.70 – -0.46	0.001	-0.05	-0.39 – 0.29	0.772
Alcohol consumption (ref. no consumption)						
- ≤ 7 drinks/week	-0.03	-0.49 – 0.42	0.881	0.17	-0.07 – 0.43	0.172
- > 7 drinks/week	-1.43	-2.20 – -0.65	0.000	0.40	-0.08 – 0.89	0.109
Immune disease (ref. no)	-0.85	-1.86 – 0.16	0.099	-0.33	-1.13 – 0.45	0.400
Cardiovascular disease (ref. no)	-0.45	-1.06 – 0.16	0.150	-0.02	-0.40 – 0.36	0.904
Other diseases (ref. no)	-0.37	-1.28 – 0.52	0.412	0.05	-0.38 – 0.48	0.813

Values are highlighted in bold if p < 0.1.

ref., reference category; BMI, body mass index; MedD Score, Mediterranean diet score; CI, confidence interval.

TABLE 4 Association between specific determinants and antibody levels after third vaccine dose.

	Anti-S IgG			Neutralizing delta			Neutralizing delta_K			Neutralizing omicron		
	Beta	95% CI	p	OR	95% CI	p	OR	95% CI	p	OR	95% CI	p
Age	-0.00	-0.01 –0.00	0.414	0.98	0.95 – 1.01	0.406	1.00	0.97 –1.03	0.789	1.00	0.96 –1.03	0.964
Sex (ref. female)	0.16	-0.09 –0.41	0.213	0.85	0.33 – 2.14	0.735	1.65	0.62 –4.39	0.314	1.40	0.53 –3.68	0.494
Waist circumference	0.00	-0.00 –0.01	0.083	1.02	0.98 – 1.06	0.173	1.04	1.00 –1.08	0.014	1.00	0.97 –1.04	0.718
Hip circumference	0.01	-0.00 –0.02	0.053	1.06	1.01 –1.12	0.009	1.06	1.01 –1.11	0.010	1.00	0.96 –1.04	0.861
BMI	0.01	-0.00 –0.03	0.173	1.10	0.98 – 1.24	0.103	1.10	0.97 –1.24	0.106	1.02	0.91 –1.13	0.712
Higher Education studies (ref. no)	0.02	-0.23 –0.29	0.839	1.55	0.54 – 4.46	0.409	0.94	0.32 –2.69	0.909	1.00	0.34 –2.90	0.994
MedD Score	-0.03	-0.09 –0.02	0.248	0.84	0.66 – 1.07	0.177	0.94	0.74 –1.18	0.613	0.74	0.58 –0.96	0.023
Smoking status (ref. never smoker)												
- Ex-smoker	0.19	-0.06 –0.45	0.140	0.78	0.31 – 1.94	0.599	1.39	0.55 –3.48	0.481	1.14	0.44 –2.91	0.784
- Smoker	0.11	-0.20 –0.44	0.476	1.13	0.32 – 3.96	0.837	0.84	0.27 –2.59	0.770	1.37	0.44 –4.22	0.579
Alcohol consumption (ref. no consumption)												
- ≤ 7 drinks/week	0.02	-0.19 –0.25	0.801	0.86	0.33 – 2.23	0.767	1.74	0.67 –4.52	0.252	1.28	0.49 –3.34	0.602
- > 7 drinks/week	0.17	-0.37 –0.72	0.525	0.26	0.02 – 2.57	0.251	0.95	0.14 –6.40	0.965	5.5	0.56 –53.98	0.143
Immune disease (ref. no)	0.06	-0.43 –0.55	0.807	2.48	0.38 – 15.92	0.338	1.00	0.20 –4.81	1.000	1.75	0.39 –7.79	0.459
Cardiovascular disease (ref. no)	-0.01	-0.31 –0.28	0.938	0.95	0.33 – 2.73	0.937	1.18	0.40 –3.46	0.759	1.11	0.39 –3.14	0.841
Other diseases (ref. no)	0.29	-0.08 –0.67	0.131	0.63	0.15 – 2.52	0.515	1.11	0.25 –4.85	0.888	0.79	0.18 –3.44	0.754

Values are highlighted in bold if $p < 0.1$.

ref., reference category; CI, confidence interval; BMI, body mass index; MedD Score, Mediterranean diet score.

14, 22), but in contrast to others (8, 12, 21, 23) that found men to have lower antibody titers than women. Thus, more studies are necessary to confirm this finding.

Lifestyle habits, smoking status, and, to a lesser extent, alcohol consumption were negatively associated with the antibody response to the BNT162b2 vaccine in our cohort, as previously reported for COVID-19 (21, 22) and other vaccines [reviewed in (24)]. Smoking reduced the percentages of cytotoxic CD8⁺ T cells, total lymphocytes with granzyme B and perforin, and lymphocytes with KLRB1 (CD161) expression (25). Similarly, some studies suggest that alcohol consumption has deleterious effects on the response to infection, with potential mechanisms that include alterations in the migration of leukocytes to sites of infection, functional abnormalities in T and B cells and other leukocytes, and changes in cytokine expression [reviewed in (26)]. Notably, it has been reported that women are more susceptible to alcohol-related effects on the inflammatory and immune responses [reviewed in (27)]. However, a negative association was observed in both sexes, which was actually slightly stronger in men.

Several studies have directly examined gender differences in the effects of alcohol on inflammatory and immune responses, reporting that women exhibit greater sensitivity to alcohol than men.

We found that those with previous immune-mediated diseases had borderline non-significant lower levels of anti-S IgG levels at the peak of the seroconversion curve, as reported

previously in individuals taking immunosuppressants (8, 21, 28) or in those with immunosuppression (12). The deleterious effect of comorbidities related to the immune system on the vaccine response appears quite logical. However, we did not observe such an effect during follow-up or after the booster dose, nor with regard to the neutralizing antibody titers, suggesting that, overall, the vaccine response may be sufficient or not different from the response in individuals without those diseases, at least after a booster dose has been administered. This is in consonance with previous studies (29, 30).

No significant association was found between the humoral response to the vaccine and BMI; however, after the booster dose, a consistent positive association was observed between the hip or waist circumference and the levels of anti-S IgG or neutralizing antibodies. In agreement with this, a negative association was observed between the titers of the anti-Omicron neutralizing antibodies and the MedD score, a healthy nutritional pattern largely seen as a protective factor for obesity (31). Notably, a recent cross-sectional study has shown that the direction of the association of body fat with the COVID-19 vaccine-induced antibody response is dependent on sex, being negative in men and positive in women (32). Around 80% of our cohort were female health workers, which could probably explain our result. In this regard, in another cohort of women receiving a different anti-SARS-CoV-2 vaccine, a positive association of the antibody titers with obesity has been shown (33).

Among the limitations of this study are the relatively small sample size and the choice of health workers as the subjects, who may not be representative of the general population as they have generally better health and lifestyle, resulting in a more robust immune response. In addition, only antibodies were measured; however, it is well accepted that the cellular response plays an important role in vaccine-induced immunity. In addition, clinical and lifestyle information was collected by means of retrospective auto-administered questionnaires, with a potential information bias. This study also has important strengths. Our data provided information on antibody dynamics in a controlled population, with similar blood extraction timings and a reasonable amount of follow-up. In addition, both anti-S and neutralizing antibodies were measured against different variants, providing a good picture of the immunogenicity of BNT162b2.

Conclusions

In health workers vaccinated with BNT162b2, the antibody titers and the percentage of seroconverted individuals declined 1 year after the primary vaccination; however, they remained high after the booster administration. Age, immune-mediated comorbidities, smoking habit, and alcohol consumption were negatively associated with the antibody response. In contrast, waist and hip circumferences were positively associated with the antibody response after vaccination.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study was approved by the Andalusian Ethics Committee (opinions 0670-N-21 for initial recruitment and 2406-N-21 for the second stage of the study). This research was conducted in accordance with the ethical standards of the Declaration of Helsinki and all participants signed the informed consent to participate in the study. The studies were conducted in accordance with their local legislation and institutional requirements.

Author contributions

LS: Formal analysis, Writing – original draft, Data curation, Investigation. ES-C: Investigation, Methodology, Resources, Writing – review & editing. AF: Investigation, Writing – review & editing. AL: Methodology, Writing – review & editing. FG:

Conceptualization, Funding acquisition, Visualization, Writing – review & editing. PR: Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was funded by Consejería de Salud, Junta de Andalucía (Spain) under Grant PI-0198-2021. The funder played no role in study design, data collection, analysis and interpretation of data, or the writing of this manuscript.

Acknowledgments

The authors thank all the healthcare professionals in the HUSSC for their help with sample collection, as well as all the participants in the study.

Conflict of interest

Author AL was employed by company Vircell, S.L.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fimmu.2025.1590939/full#supplementary-material>

References

- McVernon J, Liberman J. WHO keeps covid-19 a public health emergency of international concern. *BMJ*. (2023) p504. doi: 10.1136/bmj.p504
- Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. (2020) 367:1260–3. doi: 10.1126/science.abb2507
- Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J Med*. (2020) 383:2603–15. doi: 10.1056/NEJMoa2034577
- Notarte KI, Guerrero-Arguero I, Velasco JV, Ver AT, Santos de Oliveira MH, Catahay JA, et al. Characterization of the significant decline in humoral immune response six months post-SARS-CoV-2 mRNA vaccination: A systematic review. *J Med Virol*. (2022) 94:2939–61. doi: 10.1002/jmv.27688
- Serrano-Conde E, Leyva A, Fuentes A, de Salazar A, Chueca N, Pérez-Castro S, et al. *In vitro* neutralizing activity of BNT162b2 mRNA-induced antibodies against full B.1.351 SARS-CoV-2 variant. *Transbound Emerg Dis*. (2022) 69:2649–55. doi: 10.1111/tbed.14417
- Petersen LR, Sami S, Vuong N, Pathela P, Weiss D, Morgenthau BM, et al. Lack of antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a large cohort of previously infected persons. *Clin Infect Dis*. (2021) 73:e3066–73. doi: 10.1093/cid/ciaa1685
- Dobaño C, Ramírez-Morros A, Alonso S, Vidal-Alaball J, Ruiz-Olalla G, Vidal M, et al. Persistence and baseline determinants of seropositivity and reinfection rates in health care workers up to 12.5 months after COVID-19. *BMC Med*. (2021) 19:155. doi: 10.1186/s12916-021-02032-2
- Lustig Y, Sapir E, Regev-Yochay G, Cohen C, Fluss R, Olmer L, et al. BNT162b2 COVID-19 vaccine and correlates of humoral immune responses and dynamics: a prospective, single-centre, longitudinal cohort study in health-care workers. *Lancet Respir Med*. (2021) 9:999–1009. doi: 10.1016/S2213-2600(21)00220-4
- Faizo AA, Qashqari FS, El-Kafrawy SA, Barasheed O, Almashary MN, Alfalali M, et al. A potential association between obesity and reduced effectiveness of COVID-19 vaccine-induced neutralizing humoral immunity. *J Med Virol*. (2023) 95. doi: 10.1002/jmv.28130
- Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvado J, et al. A Short screener is valid for assessing mediterranean diet adherence among older spanish men and women. *J Nutr*. (2011) 141:1140–5. doi: 10.3945/jn.110.135566
- Desmecht S, Tashkeev A, El Moussaoui M, Marechal N, Perée H, Tokunaga Y, et al. Kinetics and persistence of the cellular and humoral immune responses to BNT162b2 mRNA vaccine in SARS-CoV-2-naïve and -experienced subjects: impact of booster dose and breakthrough infections. *Front Immunol*. (2022) 13:863554. doi: 10.3389/fimmu.2022.863554
- Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 Covid-19 vaccine over 6 Months. *N Engl J Med*. (2021) 385:e84. doi: 10.1056/NEJMoa2114583
- Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science*. (2021) 371:eabf4063. doi: 10.1126/science.abf4063
- Lo Sasso B, Agnello L, Giglio RV, Gambino CM, Ciacchio AM, Vidali M, et al. Longitudinal analysis of anti-SARS-CoV-2 S-RBD IgG antibodies before and after the third dose of the BNT162b2 vaccine. *Sci Rep*. (2022) 12:8679. doi: 10.1038/s41598-022-12750-z
- Wolszczak-Biedrzycka B, Bieńkowska A, Cieślakiewicz B, Smolińska-Fijołek E, Biedrzycki G, Dorf J. The effect of the third dose of the BNT162b2 vaccine on anti-SARS-CoV-2 spike antibody levels in healthcare workers with and without COVID-19 infection. *Ann Med*. (2023) 55:722–32. doi: 10.1080/07853890.2023.2182907
- Biedrzycka BW, Bieńkowska A, Smolińska-Fijołek E, Biedrzycki G, Dorf J. The influence of two priming doses of different anti-COVID-19 vaccines on the production of Anti-SARS-CoV-2 antibodies after the administration of the Pfizer/BioNTech booster. *Infect Drug Resist*. (2022) 15:7811–21. doi: 10.2147/IDR.S390351
- Clairon Q, Prague M, Planas D, Bruel T, Hocqueloux L, Prazuck T, et al. Modeling the kinetics of the neutralizing antibody response against SARS-CoV-2 variants after several administrations of Bnt162b2. *PLoS Comput Biol*. (2023) 19:e1011282. doi: 10.1371/journal.pcbi.1011282
- Noori M, Nejadghaderi SA, Arshi S, Carson-Chahhoud K, Ansarin K, Kolahi A-A, et al. Potency of BNT162b2 and mRNA-1273 vaccine-induced neutralizing antibodies against severe acute respiratory syndrome-CoV-2 variants of concern: A systematic review of *in vitro* studies. *Rev Med Virol*. (2022) 32:e2277. doi: 10.1002/rmv.2277
- Matula Z, Bekő G, Király V, Gönczi M, Zóka A, Baráth A, et al. Long-term SARS-CoV-2-specific humoral and t cell responses after the BNT162b2 or BBIBP-CorV booster and the incidence of breakthrough infections among healthcare workers. *Vaccines*. (2023) 12:3. doi: 10.3390/vaccines12010003
- Gir P, Von Buttlar H, Mantel E, Antwerpen MH, Wölfel R, Müller K. Comparative analysis of vaccine-induced neutralizing antibodies against the alpha, beta, delta, and omicron variants of SARS-CoV-2. *Vaccines*. (2024) 12:515. doi: 10.3390/vaccines12050515
- Kageyama T, Ikeda K, Tanaka S, Taniguchi T, Igari H, Onouchi Y, et al. Antibody responses to BNT162b2 mRNA COVID-19 vaccine and their predictors among healthcare workers in a tertiary referral hospital in Japan. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis*. (2021) 27:1861.e1–1861.e5. doi: 10.1016/j.cmi.2021.07.042
- Watanabe M, Balena A, Tuccinardi D, Tozzi R, Risi R, Masi D, et al. Central obesity, smoking habit, and hypertension are associated with lower antibody titres in response to COVID-19 mRNA vaccine. *Diabetes Metab Res Rev*. (2022) 38:e3465. doi: 10.1002/dmrr.3465
- Tribin FE, Williams E, Testamarck V, Carreño JM, Bielak D, Yellin T, et al. Determinants of health as predictors for differential antibody responses following SARS-CoV-2 primary and booster vaccination in an at-risk, longitudinal cohort. *PLoS One*. (2024) 19:e0292566. doi: 10.1371/journal.pone.0292566
- Zimmermann P, Curtis N. Factors That Influence the Immune Response to Vaccination. *Clin Microbiol Rev*. (2019) 32:10. doi: 10.1128/cmr.00084-18
- Tran QT, Arimilli S, Scott E, Chen P, Prasad GL. Differences in biomarkers of inflammation and immune responses in chronic smokers and moist snuff users. *Cytokine*. (2021) 137:155299. doi: 10.1016/j.cyt.2020.155299
- Solopov PA. COVID-19 vaccination and alcohol consumption: justification of risks. *Pathogens*. (2023) 12:163. doi: 10.3390/pathogens12020163
- Romeo J, Wörnberg J, Nova E, Díaz LE, Gómez-Martínez S, Marcos A. Moderate alcohol consumption and the immune system: A review. *Br J Nutr*. (2007) 98:S111–5. doi: 10.1017/S0007114507838049
- Van Der Heyden J, Leclercq V, Duysburgh E, Cornelissen L, Desombere I, Roukaerts I, et al. Prevalence of SARS-CoV-2 antibodies and associated factors in the adult population of Belgium: a general population cohort study between March 2021 and April 2022. *Arch Public Health*. (2024) 82:72. doi: 10.1186/s13690-024-01298-1
- Azzolini E, Pozzi C, Germagnoli L, Oresta B, Carriglio N, Calleri M, et al. mRNA COVID-19 vaccine booster fosters B- and T-cell responses in immunocompromised patients. *Life Sci Alliance*. (2022) 5:e202201381. doi: 10.26508/lsa.202201381
- Ollila TA, Masel RH, Reagan JL, Lu S, Rogers RD, Paiva KJ, et al. Seroconversion and outcomes after initial and booster COVID-19 vaccination in adults with hematologic malignancies. *Cancer*. (2022) 128:3319–29. doi: 10.1002/cncr.34354
- Barrea L, Muscogiuri G, Frias-Toral E, Laudisio D, Pugliese G, Castellucci B, et al. Nutrition and immune system: from the Mediterranean diet to dietary supplementary through the microbiota. *Crit Rev Food Sci Nutr*. (2021) 61:3066–90. doi: 10.1080/10408398.2020.1792826
- Perales-Torres AL, Perez-Navarro LM, Garcia-Oropesa EM, Diaz-Badillo A, Martinez-Lopez YE, Rosas M, et al. Influence of adiposity and sex on SARS-CoV-2 antibody response in vaccinated university students: A cross-sectional ESFUERSO study. *PLoS Glob Public Health*. (2024) 4:e0002686. doi: 10.1371/journal.pgph.0002686
- Golec M, Zembala-John J, Fronczek M, Konka A, Bochenek A, Wystyrk K, et al. Relationship between anthropometric and body composition parameters and anti-SARS-CoV-2 specific IgG titers in females vaccinated against COVID-19 according to the heterologous vaccination course: A cohort study. *PLoS One*. (2023) 18:e0287128. doi: 10.1371/journal.pone.0287128