

Humoral immune response following the third dose of BNT162b2 received by employees at a Slovak cancer healthcare facility

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Abstract. BNT162b2 is a mRNA vaccine, approved by the US Food and Drug Administration and the European Medicines Agency that was designed to target Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the causative agent of Coronavirus disease 2019 (COVID-19). Despite its widespread use, predictors of humoral immune responses after BNT162b2 administration remain uncertain. Consequently, a case-control study was conducted involving 273 employees at the National Cancer Institute (Bratislava, Slovakia) for the present study. Specifically, the present study aimed to determine the incidence of symptomatic COVID-19. In addition, the association between the safety of the third-dose BNT162b2 and sex and body mass index (BMI) was assessed. The present study also explored the potential determinants of plasma IgG antibody levels following vaccination. In total, 294 participants were recruited and 273 met the inclusion criteria. At a median follow-up of 4.7 months, 38 participants had symptomatic SARS-CoV-2 infection after vaccination. The incidence of adverse events (AEs) was found to be significantly higher in female patients compared with that in male patients. Furthermore, patients with a high BMI (≥ 30 kg/m²) had higher number of AEs compared with those with low BMI ($P < 0.05$). IgG levels were observed to be significantly higher in male patients compared with those in female patients, in participants with high BMI compared with those with low BMI, in those experiencing three or more AEs compared with those with fewer AEs and in participants who had contracted COVID-19 compared with those who had not ($P < 0.05$ for all). Multivariate analysis identified sex, BMI, number of AEs and

prior COVID-19 infection as independent predictors of IgG plasma level ($P < 0.05$). Therefore, the present study found the low number of COVID-19 in the subjects vaccinated with the third dose of BNT162b2 and revealed that the safety profile of the vaccine is associated with sex and BMI. Higher IgG levels, indicating more efficient protection against SARS-CoV-2, were observed in male patients, in participants with high BMI, those experiencing a higher frequency of AEs and those who had previously contracted COVID-19 despite vaccination against SARS-CoV-2. These findings could lead to the stratification of subjects within future clinical studies and the development of specific vaccination protocols in regard to sex, BMI and history of SARS-CoV-2.

Introduction

Coronavirus disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), necessitates urgent preventive measures and vaccination, particularly in high-risk populations (1,2), such as individuals aged >60 years and those with lung or heart disease or diabetes. Among the currently known mRNA vaccines developed, BNT162b2 encodes the SARS-CoV-2 spike protein (S protein) and was authorized in 2021 by the U.S. Food and Drug Administration and recommended by the European Medicines Agency for medical use following a large, placebo-controlled, randomized phase III study, which demonstrated its efficacy in preventing COVID-19 and safety of two 30- μ g doses administered 21 days apart (3,4). A third dose of BNT162b2, tested to boost its efficacy against SARS-CoV-2 in a placebo-controlled, randomized, phase III trial (5), was administered at a median of 10.8 months after the second dose (5). At a median follow-up of 2.5 months, the third dose showed 95.5% effectiveness against COVID-19 without any corresponding new unexpected adverse events.

COVID-19 infection typically starts with flu-like symptoms (6) and can either remain asymptomatic or has a mild to severe disease course (7). The infection is characterized by a significant burden of inflammation (8). The association between inflammatory parameters, such as neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, mean platelet volume (MPV), red

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cell distribution width (RDW), monocyte to lymphocyte ratio (MLR) and COVID-19 infection, has been previously established (9). In addition, decreased hemoglobin and increased red cell distribution width have been associated with recurrent hospitalizations by patients with COVID-19 (10). A hemoglobin A1C (HbA1C) test, MPV and MLR were introduced as possible predictors of frailty, a geriatric state of reduced functional reserve and vulnerability, in patients with diabetes during COVID-19 (11). Considering the association between COVID-19 vaccines and increased levels of inflammatory biomarkers, such as TNF- α , IL-1 β , IL-6, IL-8 and IL-10 (12), the study of their effects in a health-care setting is reasonable.

Healthcare facilities pose a high risk for infection acquisition (13). Therefore, personal protective equipment combined with vaccination is crucial for COVID-19 protection. Real world data from US and Israel (14,15) confirmed the high efficacy of BNT162b2 in healthcare workers (HCWs), consistent with findings from previous randomized trials (16,17). Despite the high effectiveness of this vaccine, new COVID-19 cases have increased worldwide over 1-12 months (18,19), raising concerns of waning immunity. A prospective study involving vaccinated HCWs with BNT162b2 previously reported a consistent decline in IgG and neutralizing antibody titers over 6 months. In addition, 6 months after the second vaccine dose, significant declines in neutralizing antibodies were observed in male patients, in individuals aged >65 years and in those with immunosuppression (20). However, another previous report (21) showed a low rate (2.1%) of BNT162b2 failure (defined as ratio of subjects diagnosed with COVID-19 to overall study population) despite a rapid decline in IgG antibodies and an absence of cellular immune response after 6 months in the majority of enrolled HCWs. In addition, at 6 months, IgG antibodies and the levels of T-cell activation had significantly declined in individuals with obesity (21).

The purpose of the present study was to determine the incidence of symptomatic COVID-19 following the third dose of BNT162b2 and to assess its safety profile, with focus on sex and BMI, in a real-world medical facility setting (National Cancer Institute, Bratislava, Slovakia). Additionally, IgG levels following vaccination were explored, which were then used to assess their possible association with sex, BMI, number of adverse events (AEs) and the presence of COVID-19.

Materials and methods

Study design and end points. The present study is a case-control study with the primary objective of determining the incidence of symptomatic COVID-19, defined as onset within ≤ 7 days after the third dose of BNT162b2. Another objective of the present study was to assess vaccine safety with focus on sex and BMI. As in a real-world scenario, a symptomatic SARS-CoV-2 infection was confirmed using rapid antigen tests, rapid antigen real-time reverse transcription PCR (RT-PCR) or loop-mediated isothermal amplification tests. In the present study, patients only reported symptomatic infection and that they had tested positive for SARS-CoV-2. The investigators did not provide precise data on the test used, rendering it impossible to state the criteria used to define infection using each of these kits. A secondary objective was to explore potential differences in plasma IgG levels following

vaccination and their association with sex, BMI, AEs and positivity for SARS-CoV-2. The recruitment period was between October and December 2021. All subjects were recruited at the National Cancer Institute (NCI; Bratislava, Slovakia).

Enrollment criteria. Participants had to be current employees of the NCI, vaccinated with three doses of BNT162b2 and aged ≥ 18 years old. Exclusion criteria included the use of corticosteroids, such as prednisone, prednisolone, methylprednisolone and hydrocortisone, COVID-19 diagnosed between the second and third vaccine doses, cigarette smoking and concomitant vaccines administered.

Data validation. Data were entered into electronic forms by EM, KR, AS, which were subsequently validated by LS and JO to ensure accuracy.

Process of vaccination. All individuals were vaccinated with the dilution of 0.3 ml BNT162b2 (batch no. FE7010 01/01/2022; supplied by Pfizer, Inc. and BioNTech) at the NCI. The process of the vaccine preparation, as previously described (13), was conducted in accordance with the product license and manufacturer recommendations. Briefly, the vials of BNT162b2 were thawed and diluted with 1.8 ml sodium chloride 9 mg/ml (0.9%) solution for injection. After dilution, 6 doses were extracted from each vial and administered intramuscularly.

Measurement of IgG antibodies. Blood samples were obtained from fasting participants by 6 ml venipuncture in the morning using BD Vacutainer[®] tubes with citrate (BD Biosciences). IgG antibodies to the receptor-binding domain (RBD) of the S1 antigen of SARS-CoV-2 were determined using the Atellica[®] IM SARS-CoV-2 IgG assay (cat. no. 11207388 Siemens Healthineers). Results were reported qualitatively as non-reactive (< 1.00 index; IgG negative) or reactive (≥ 1.00 index; IgG positive) and quantitatively within a measuring interval of 1.0-150.0 index (22).

Statistical analysis. All statistical analyses were performed using NCSS 24 Statistical Software Version 24.0.3 (NCSS) (23). Data were summarized as mean $\pm$ standard deviation (SD) and range for continuous variables, or as frequencies for categorical variables. For some continuous variables, data were also analyzed using the median. For normally distributed continuous variables, defined by Shapiro-Wilk test, P-values were determined using the paired t-test and unpaired t-test when comparing two independent groups (IgG levels in males vs. females or high vs. low BMI groups). When the distribution of values was found to be non-normal, the Wilcoxon rank-sum test was used to calculate the P-values. For categorical variables, P-values were determined using Fisher's exact test or the χ^2 test.

To examine predictors of adverse events (AEs) and immune response, multiple logistic regression analysis and multiple linear regression analysis were performed. The models included the following: i) Headache occurrence as a dependent variable (yes vs. no) with sex (male vs. female), BMI (continuous, kg/m²), IgG index (continuous), prior COVID-19 infection (yes vs. no) and the number of AEs (continuous count) as independent variables; ii) presence of AEs as a binary dependent variable

(yes vs. no) with sex (male vs. female), BMI (continuous, kg/m²) and IgG index (continuous) as independent variables; and iii) IgG levels as a continuous dependent variable, with sex (male vs. female), BMI (continuous, kg/m²), number of AEs (continuous count) and prior COVID-19 infection (yes vs. no) as independent variables. In addition, a comprehensive multivariate analysis was conducted to evaluate the combined effects of multiple predictors on single outcome variables. The logistic regression model included sex, BMI and IgG levels as independent variables, with presence of AEs as the dependent variable evaluated as binary outcome (yes vs. no). Additionally, linear regression was used to assess IgG levels (continuous variable) as the dependent variable, with sex (male vs. female), BMI (continuous, kg/m²), number of AEs (continuous count) and prior COVID-19 (yes vs. no) infection as independent variables. $P \leq 0.05$ was considered to indicate a statistically significant difference. Multicollinearity was assessed using variance inflation factors (VIFs) (24), where the variable BMI ≥ 30 kg/m² was removed due to collinearity issues. Briefly, VIF was measured, and the threshold was defined as $VIF \leq 10$ to detect multicollinearity among independent variables. If a variable had $VIF > 10$, it was considered highly collinear and potentially redundant in the model. As a result, BMI ≥ 30 kg/m² was removed due to excessive collinearity with other predictors, ensuring that the regression analysis provided stable and interpretable estimates.

Results

Subjects. In total, 273 eligible individuals who met the inclusion criteria were enrolled into the present study. Of this cohort, 232 were HCWs (48 doctors, 103 nurses, 8 pharmacists, 52 medical technicians, 1 psychologist, 2 physiotherapists 18 other HCWs) and 41 were non-HCWs in administration. The mean age of the study cohort was 47 ± 11 years, with a median of 47 years (range, 22–80 years). The majority of the participants were female individuals ($N=233$; 85.3%). The median of BMI was 24.8 kg/m² (95% CI, 23.7–25.7; range, 16.3–44.6 kg/m²), 53 participants had a high BMI of ≥ 30 kg/m². All individuals had been vaccinated with three doses of BNT162b2. The median time between second and third vaccine dose was 8.5 months (range, 4.9–10.9 months). Baseline characteristics are shown in Table I.

Incidence of symptomatic SARS-CoV-2 infection following vaccination. At the median follow-up of 4.7 months (range, 2.9–5.0 months), 38 participants contracted COVID-19 following vaccination, resulting in an incidence rate of 13.9%. During the spring of 2022, the predominant circulating SARS-CoV-2 strain in Slovakia was the Omicron variant (BA.5) (25). The median time from receiving the third dose of BNT162b2 to the diagnosis of COVID-19 was 2.8 months (range, 0.2–4.4 months). All patients who contracted COVID-19 experienced a mild course of infection and survived.

AEs following BNT162b2 third dose. At least one AE was reported by 258 participants (94.5%), with the median number of BNT162b2 AEs being 3 (range, 0–13). In total, 143 individuals (52.4%) experienced three or more AEs. The most frequently reported AEs were pain at the injection site (200,

Table I. Characteristics of subjects.

Total population	N (%)	Median (range)
Overall	273 (100.0)	
Healthcare workers	232 (85.0)	
Doctors	48 (17.6)	
Nurses	103 (37.7)	
Pharmacists	8 (2.9)	
Medical technicians	52 (19.0)	
Psychologist	1 (0.4)	
Physiotherapists	2 (0.7)	
Other HCWs	18 (6.6)	
Non-HCWs	41 (15.0)	
Age, years		47 (22–80)
Sex		
Male	40 (14.7)	
Female	233 (85.3)	
Body mass index, kg/m ²		24.8 (16.3–44.6)
<30	220 (80.6)	
≥ 30	53 (19.4)	

N, number; HCWs, healthcare workers.

73.3%), fatigue (120, 44.0%) and pain in extremity (90, 33.0%). A comprehensive summary of all AEs is presented in Tables II and III.

In addition to the common AEs, 17 participants (6.2%) reported other AEs possibly associated with the vaccination, including herpes infection (3, 1.1%), shivering (3, 1.1%), vomitus (2, 0.7%), vomitus and diarrhea (1, 0.4%), diarrhea (1, 0.4%), tinnitus (1, 0.4%), dry cough (1, 0.4%), abdominal pain (1, 0.4%), loss of appetite (1, 0.4%), lower back pain (1, 0.4%), tachycardia (1, 0.4%), and eyelid edema (1, 0.4%). The median number of AEs in all participants was 3 (0–13) and it was significantly higher in female individuals compared with that in male individuals (3.0 ± 2.8 vs. 1.5 ± 2.6 ; $P < 0.05$) and in participants with high BMI compared to those with low BMI (4.0 ± 3.0 vs. 2.0 ± 2.8 ; $P < 0.05$). No serious AEs (SAEs), such as myocarditis, anaphylaxis or severe thromboembolic events, were reported following vaccination.

In terms of sex, the incidence of joint pain and pain in extremity was statistically significantly higher in female patients compared with those in their male counterpart ($P < 0.05$; Table II). In addition, participants with high BMI more frequently reported muscle pain, joint pain, swelling, redness and itching at injection site ($P < 0.05$; Table III).

Plasma IgG following vaccination. The median time to plasma IgG measurement after the third dose of the vaccine was 3.4 months (range, 2.1–4.8 months), with a median plasma IgG level of 114.9 index (range, 4.7–150.0 index).

IgG antibody levels were found to be significantly higher in male patients compared with those in female patients, in participants with high BMI compared with those with low BMI, in individuals experiencing a high number of AEs of

Table II. Adverse events of the BNT162b2 third dose by sex (N=273).

Study population	Total incidence N (%)	Female (n=233), n (%)	Male (n=40), n (%)	P-values
Any adverse events	256 (93.8)	220 (94.4)	36 (90.0)	
Headache	62 (22.7)	56 (24.0)	6 (15.0)	0.2086
Muscle pain	68 (24.9)	60 (25.8)	8 (20.0)	0.4381
Joint pain	47 (17.2)	45 (19.3)	2 (5.0)	0.0271
Pain at injection site	200 (73.3)	175 (75.1)	25 (62.5)	0.0967
Fatigue	120 (44.0)	105 (45.1)	15 (37.5)	0.3741
Chills	55 (20.1)	49 (21.0)	6 (15.0)	0.3806
Pyrexia	51 (18.7)	43 (18.5)	8 (20.0)	0.8172
Swelling at injection site	36 (13.2)	33 (14.2)	3 (7.5)	0.2508
Nausea	25 (9.2)	22 (9.4)	3 (7.5)	0.6946
Redness at injection site	29 (10.6)	27 (11.6)	2 (5.0)	0.2125
Swollen lymph nodes	47 (17.2)	42 (18.0)	5 (12.5)	0.3933
Insomnia	19 (7.0)	15 (6.4)	4 (10.0)	0.4143
Pain in extremity	90 (33.0)	84 (36.1)	6 (15.0)	0.0091
Itching at injection site	12 (4.4)	12 (5.2)	0 (0.0)	0.1429
Lethargy	23 (8.4)	32 (13.7)	3 (7.5)	0.2769
Hypoaesthesia	9 (3.3)	8 (3.4)	3 (7.5)	0.2278

Table III. Adverse events of the third dose of the BNT162b2 by BMI (N=273).

Study population	N (%)	BMI <30 kg/m ² (n=233), n (%)	BMI ≥30 kg/m ² (n=40), n (%)	P-values
Any adverse event	256 (93.8)	220 (94.4)	36 (90.0)	
Headache	62 (22.7)	46 (20.9)	16 (30.2)	0.1485
Muscle pain	68 (24.9)	48 (21.8)	20 (37.7)	0.0164
Joint pain	47 (17.2)	33 (15.0)	14 (26.4)	0.0486
Pain at injection site	200 (73.3)	160 (72.7)	40 (75.5)	0.6859
Fatigue	120 (44.0)	91 (41.4)	29 (54.7)	0.0793
Chills	55 (20.1)	41 (18.6)	14 (26.4)	0.2059
Pyrexia	51 (18.7)	41 (18.6)	10 (18.9)	0.9691
Swelling at injection site	36 (13.2)	24 (10.9)	12 (22.6)	0.0237
Nausea	25 (9.2)	20 (9.1)	5 (9.4)	0.9382
Redness at injection site	29 (10.6)	18 (8.2)	11 (20.8)	0.0078
Swollen lymph nodes	47 (17.2)	39 (17.7)	8 (15.1)	0.6492
Insomnia	19 (7.0)	14 (6.4)	5 (9.4)	0.4313
Pain in extremity	90 (33.0)	70 (31.8)	20 (37.7)	0.4116
Itching at injection site	12 (4.4)	6 (2.7)	6 (11.3)	0.0061
Lethargy	23 (8.4)	28 (12.7)	7 (13.2)	0.9254
Hypoaesthesia	9 (3.3)	10 (4.5)	1 (1.9)	0.3778

BMI, body mass index.

≥3 compared with those with a low number of AEs and in participants who tested positive for COVID-19 compared with those who did not ($P<0.05$; Fig. 1 and Table IV).

Multivariate regression results from the model explained 28.03% of the variance in headache occurrence ($R^2=0.2803$;

adjusted $R^2=0.2612$). The F-statistic ($F=14.741$; $P<0.0001$) confirmed that at least one predictor significantly contributed. All adverse events (not only headache) were included in the multivariate analysis, which showed that the only AE of significance was headache occurrence. Table V only shows

Table IV. Plasma IgG antibodies following the BNT162b2 third dose.

Study population	N	Mean $\pm$ SD (Index)	P-values
Sex			0.0005
Female	233	97.48 $\pm$ 49.98	
Male	40	126.99 $\pm$ 39.37	
Body mass index			0.0001
<30 kg/m ²	220	96.25 $\pm$ 50.47	
$\geq$ 30 kg/m ²	53	124.84 $\pm$ 38.44	
Number of adverse events			0.0003
<3	130	90.56 $\pm$ 50.83	
$\geq$ 3	143	112.03 $\pm$ 46.35	
Presence of Coronavirus disease 2019			0.0006
No	235	97.69 $\pm$ 49.92	
Yes	38	127.27 $\pm$ 39.52	

SD, standard deviation.

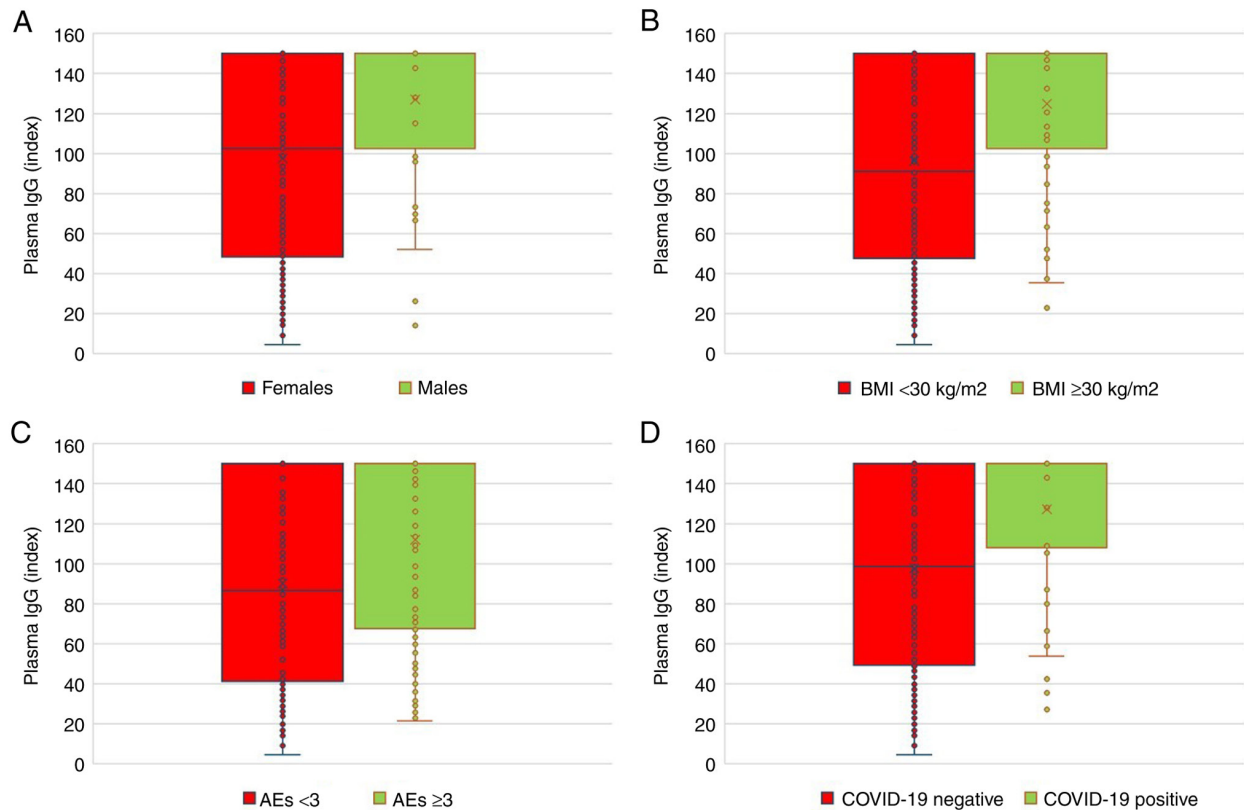


Figure 1. Plasma IgG antibodies following the BNT162b2 third dose. (A) Sex, (B) body mass index, (C) number of AEs and (D) COVID-19-positive participants were compared. AEs, adverse events; BMI, body mass index; COVID-19, Coronavirus disease 2019.

the multivariate analysis (multiple regression) for headache occurrence for transparency. The number of AEs was the only significant predictor of headache occurrence ($\beta=0.0789$; $P<0.0001$). Other factors, including IgG index, sex, BMI and prior COVID-19 infection, were not significant predictors (Table V). The comparison calculations were not performed for the BMI ≤ 30 kg/m² due to collinearity issues, whereas other predictors had acceptable VIFs.

The univariate analyses were not performed as they could provide biased data in this case. Univariate analysis does not control the confounding variables, meaning the observed associations may be misleading. If the number AEs is associated with both IgG levels and prior COVID-19 infection, univariate analysis may wrongly suggest that AEs alone are driving IgG changes, when in fact prior COVID-19 infection is a confounding factor. Additionally, reporting univariate

Table V. Multiple regression analysis for headache occurrence.

Variable	Regression Coefficient	Standard Error	OR (95% CI)	P-value
Sex (male vs. female)	0.006	0.065	1.006 (0.886-1.143)	0.927
Body mass index (continuous)	-0.0049	0.0068	0.995 (0.982-1.008)	0.473
IgG (continuous)	-0.0003	0.0005	0.9997 (0.999-1.001)	0.554
COVID-19 after 3rd dose (yes vs. no)	0.0805	0.0651	1.083 (0.954-1.231)	0.217
Number of AEs (<3 AEs vs. ≥3 AEs)	0.0789	0.0083	1.082 (1.065-1.100)	<0.0001

OR, odds ratio; AEs, adverse events; CI, confidence interval; COVID-19, Coronavirus disease 2019.

results alongside multivariate findings may create confusion by presenting unadjusted associations that do not accurately reflect real-world interactions. Since the primary aim of the present study was to identify independent predictors of IgG levels, univariate analysis would not provide meaningful additional insights. Instead, it could introduce interpretation bias and misrepresentation of causal relationships. Therefore, a multivariate approach was chosen to ensure that results account for multiple influencing factors simultaneously, providing a more accurate and reliable assessment of predictors.

Discussion

In the present study with enrolled >270 individuals, a third 30- μ g dose of BNT162b2, received at a median of 8.5 months after the second dose, was effective in terms of percentage of subjects tested positive to SARS-CoV-2 and safety according to the absence of serious AEs. The incidence of COVID-19 following vaccination was found to be <14%, where ~95% participants reported at least one AE. The most frequent AEs were injection site pain (73.3%), fatigue (44.0%) and extremity pain (33.0%). Statistically significant differences in the incidence of pain (joints and extremity) favored male patients, whilst pain (joints and muscles) with local reactions at the injection site (swelling, itching, and redness) were more common in participants with low BMI. These observations associated with a significantly higher median number of AEs in female patients compared with male patients and in participants with high compared with low BMI.

The use of the BNT162b2 third dose was authorized by European Medicine Agency for individuals aged ≥ 16 years to improve protection against COVID-19 based on the results of a phase III study (5). At a median follow-up of 2.5 months, 7 cases of COVID-19 were identified among 5,056 subjects who received three doses of the vaccine (5). In the present study, the detection of 38 cases of infection in a population of 273 individuals can be explained by the longer follow-up period and the circulation of a different predominant SARS-CoV-2 strain when COVID-19 was diagnosed. Additionally, the fact that the majority of the present study population consisted of HCWs, who tended to be more aware of even clinically minor symptoms and signs, may have led to the more frequent testing for SARS-CoV-2, resulting in a higher proportion of COVID-19 cases diagnosed. The higher overall incidence of

AEs may also be attributed to the constitution of the present study population, mostly comprising of HCWs who are better informed about AEs and are more likely to attribute new symptoms to the administered vaccine.

The present findings demonstrate that the number of AEs was the strongest predictor of headache occurrence, supporting a dose-response relationship between systemic reactogenicity and headache risk. Other demographic and clinical factors, including sex, BMI, IgG levels and prior COVID-19 infection, were not significantly associated with headaches. These results emphasize that immune response intensity (as measured by IgG) does not directly associate with headache occurrence. In addition, the use of multivariate regression revealed a moderate model fit ($R^2=0.2803$; adjusted $R^2=0.2612$), suggesting that additional unmeasured factors may contribute to headache risk. These findings provide potential insights into the reactogenicity of the third BNT162b2 dose and suggest that headache risk is primarily influenced by overall reactogenicity instead of specific demographic or immunological factors. Future studies should consider larger sample sizes, additional confounding factors as pre-existing autoimmune diseases (such as rheumatoid arthritis and lupus), medication use (such as corticosteroids and immunosuppressants), lifestyle factors (including smoking and alcohol consumption) or genetic predisposition to immune response and extended follow-up periods to confirm these findings.

Evidence of the effective protection provided by BNT162b2 has been previously yielded by various countries. In Hungary, the risk of mortality associated with COVID-19 was reduced by 82% in the general population immunized with a booster dose of vaccines, including BNT162b2 (26). Furthermore, the booster dose was found to enhance BNT162b2 vaccine efficacy even in immunocompromised subjects in Hungary (27). Similarly, Berec *et al* (28) provided real-world evidence on the waning protection against COVID-19 at 8 months and the restoration of efficacy following booster dose administration in Czechia (28). In a medical center in Israel, known for its mass vaccination policy during the pandemic (29), the BNT162b2 booster dose was found to significantly reduce the breakthrough infection rate regardless of age in HCWs (29).

The higher incidence of AEs in female patients compared with male counterparts and in those with high BMI compared with low BMI can stem from the recognized germline-encoded differences in innate immune responses, evident

in polymorphisms or variability in sex chromosomes and the autosomal genes encoding immunological proteins (30). These differences include the varying activity and number of innate immune cells, CD4+ and CD8+ T cells, B lymphocyte subsets and the production of cytokines and chemokines. Hormonal mediators, such as estradiol, progesterone and androgens, serve significant roles in innate and adaptive immunity (30). Environmental factors, such as nutrition and microbiota, can also impact vaccine efficacy (30). Results from the present study are consistent with those of a previous meta-analysis including 46 studies, which showed higher rates of AEs in female patients compared with those in male patients following immunization with the seasonal influenza vaccine (31). Differences in the incidence of AEs following the BNT162b2 mRNA vaccine by sex were also shown in a meta-analysis conducted by Green *et al* (32).

The present study revealed statistically significantly higher plasma IgG antibodies following the third vaccine dose measured at a median time of 3.4 months in male patients, participants with high BMI, those with a high number of AEs and those who contracted COVID-19. Multivariate analysis showed all these factors to be independent predictors of high plasma IgG antibodies. Data regarding sex as a predictor of the humoral immune response to vaccination are inconsistent. A number of studies identified males to be low responders (33,34), whilst others did not confirm such an association (35). In addition, Papaioannidou *et al* (36) did not find any significant relationship between BMI and IgG antibodies after two doses of the BNT162b2 vaccine among HCWs in their hospital in Northern Greece (36). However, in a previous study on IgG dynamics following two doses of BNT162b2 (21), high vaccine efficacy despite the rapid decline of plasma IgG and a negative or borderline cellular immune response was observed in a specific population of the NCI employees. There were significant differences in IgG among the BMI subgroups (<18.5 kg/m², 18.5-24.9 kg/m², 25.0-29.9 kg/m², 30.0-34.9 kg/m² and ≥35.0 kg/m²). However, this must be interpreted cautiously due to the small number of subjects in the subgroups with BMI <18.5 kg/m² and ≥35.0 kg/m². Previous reports have shown associations between SARS-CoV-2 IgG antibody titers and the incidence of AEs following two doses of BNT162b2 (37) and greater IgG levels in individuals infected after their booster mRNA vaccine compared with those in uninfected subjects (38-40). It appeared that the level of IgG could also be associated with the type of vaccine used (BNT162b2 vs. ChAdOx1) (41).

Interpreting results from various studies and making indirect comparisons is challenging in the present study due to the use of different methods for determining IgG following vaccination developed by various manufacturers. In a previous report (21) and the present study, the Atellica[®] IM SARS-CoV-2 IgG (sCOVG) assay from Siemens was used with numeric results reported as an index from 1 to 150. Wei *et al* (33) measured IgG antibody levels using an ELISA method developed by the University of Oxford, with results reported in ng/ml and positive samples defined as ≥42 ng/ml (33). Wolszczak-Biedrzycka *et al* (42) used the Elecsys anti-SARS-CoV-2 S assay developed by Roche to determine the total IgG, IgA and IgM antibodies against the spike RBD protein, with results reported in U/ml and positive results defined as ≥0.80 U/ml (42).

COVID-19 has been shown to reduce mitochondrial bioenergetics functions (43), where vaccination against SARS-CoV-2 can prevent declines in energy production and mitochondrial respiration in platelets (44). Hypothetical dysregulation in immune cell mitochondrial bioenergetics may explain the differing plasma IgG levels following vaccination in SARS-CoV-2 infected compared with non-infected participants in the present study.

In the present study, significantly higher IgG levels after vaccination were found in male patients, those with high BMI and the individuals following COVID-19. Therefore, they all could represent the populations of special interest for subject stratifications within future clinical trials with novel vaccines. Once these observations are confirmed and an association with lower incidence of COVID-19 demonstrated in a prospective manner, this may have a potential impact on public health policy due to the reduction of costs incurred for the vaccination of individuals who do not need to be vaccinated repeatedly or for whom a lower dose of vaccine is sufficient. Reducing the number or doses of vaccine may also mitigate the incidence of its late or long-lasting toxicities. In addition, a positive impact on public healthcare and saving resources may accordingly manifest. In the light of the present findings, monitoring of IgG levels following vaccination against COVID-19 seems to be reasonable.

The present study has certain limitations, including the absence of data on IgG levels prior to vaccination due to the time of its designing and the various methods for confirming the diagnosis of symptomatic COVID-19. With the vast majority of the study population being female, such a sex imbalance could render the generalizability of the results in assessing the impact of sex differences on the immune response difficult. The present study also did not account for potential confounding factors, such as comorbidities, medications or lifestyle factors. The comorbidities, such as end stage renal disease, cancer, autoimmune disease or hypertension, are associated with the impairment of macrophage and lymphocyte functions, affecting immune response following vaccination negatively (45). In addition, cigarette smoking can attenuate the immune system by activating MAPK and NF-κB signaling with activation of enzymes regulating posttranslational modifications (methylation, acetylation, phosphorylation and ubiquitination) of histones (46). Nevertheless, taking corticosteroids with a negative impact on humoral immune response were among the exclusion criteria in the present study.

The absence of unvaccinated individuals or those who received only two doses as control groups limits the ability to draw clear conclusions about the effectiveness of the third vaccine dose in the present study. However, the study design was adapted to the overall number of employees who were not vaccinated with three doses of vaccine being <1% in the NCI. In addition, the relatively short median follow-up period limits the ability to provide insights into the long-term effects of the vaccination. A short follow-up period was chosen for the present study, which is indirectly comparable to the median follow-up 2.5 months in a previous phase III study with BNT162b2 (5), predominantly in the context of fast-changing SARS-CoV-2 variants in the population, making the interpretation of the efficacy results difficult. By contrast, the present

results provide real-world evidence of the benefit of vaccination in terms of the low number of COVID-19 cases and the safety of a third dose of the BNT162b2 mRNA vaccine in a specific medical facility providing healthcare for patients with cancer.

The current global context is determined by the background immunity of the population and reduced severity of SARS-CoV-2 causing clinically less serious infectious disease (47). Even though COVID-19 is no longer considered to be a serious threat at present (48,49), it has nevertheless been acknowledged that there is a need for the development of effective preventive strategies, which may influence potential pandemics in future (49,50). The recent developments in a field of preventive measures against COVID-19 are comprised of the research of novel vaccines with different mechanisms of action, including adenovirus vectors, protein subunit, inactivated viruses and mRNAs (51-54). However, several obstacles remain in relation to mRNA vaccines, such as the strict temperature requirements, the delivery systems of high effectiveness and resistance to degradation. In addition, among the problems preventing their spread in low-income countries are the relatively high costs for production (55).

To conclude, the present study showed the low percentage of COVID-19 cases and the safety of a third dose of BNT162b2 in employees of the NCI. In addition, a greater occurrence of vaccine-related AEs was observed in female patients and participants with high BMI. Furthermore, plasma IgG antibody levels were found to be dependent on several factors, including sex, frequency of vaccine adverse events, BMI and COVID-19 diagnosis following vaccination. Understanding the importance of IgG titers against COVID-19 infection may facilitate the setting up of vaccination programs and adaptation of the vaccine dosage and/or dose interval according to the characteristics of individual subjects.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

PP and MV initiated and designed the study concept, and confirm the authenticity of all the raw data. EM, KR, AS, LS and JO participated in data collection and manuscript writing. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study adhered to the principles of the Declaration of Helsinki and the International Council for Harmonization of Good Clinical Practice Guidelines (ICH GCPG). The study protocol (code Covid-SK001; version 3.0, No. 11/2021) was approved by the Ethics Committee of the NCI (Bratislava, Slovakia) on September 30, 2021. All participants provided written informed consent before enrollment; no minors were included.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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