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A single dose of BNT162b2 vaccine elicits strong humoral response in SARS-CoV-2 seropositive individuals

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To the Editor:

The high demand for COVID-19 vaccines, combined with a significant lack of supply, leaves smaller and developing countries behind in mass immunization. This prompts the question whether administering a single vaccine dose in SARS-CoV-2 seropositive individuals could be a method for rationing available vaccine doses.

We report results from a prospective study on Macedonian health care workers who received two doses of the Pfizer/BioNTech BNT162b2 mRNA vaccine, comparing antibody titers and frequency of side effects after vaccine administration between individuals who were SARS-CoV-2 seropositive (SeroPOS group) and seronegative (SeroNEG group) prior to immunization. The study included 226 participants recruited through convenience sampling, of whom 41 were SeroPOS (73.17% female; mean age 43 years, SD: 10.571), and 185 were SeroNEG (68.11% female, mean age 46 years, SD: 10.523). Baseline patients' characteristics are provided in the Supplementary Appendix (Table S1). Blood samples were collected 18-21 days after the first vaccine dose and 25-28 days after the second dose. Baseline antibody levels were obtained from patient records. All participants gave

blood samples after the first dose and filed a questionnaire for side effects, and 189 participants (83.63%) returned for assessment four weeks after the second dose. Serological testing was performed using the commercially available quantitative CLIA anti-SARS-CoV-2 RBD kit (Snibe, Shenzhen, China)¹, which targets the S1 subunit of the viral spike protein. More details on methods are available in the Supplementary Appendix.

Anti-SARS-CoV-2 RBD IgG antibody levels after the first dose of BNT162b2 were on average 11.7-times higher in SeroPOS individuals (mean: 923.40 AU/ml, SD: 948.119, range 15.04-5034.70) compared to SeroNEG individuals (mean: 79.06 AU/ml, SD: 253.243, range 0.912-1867.30; Wilcoxon rank-sum test, $p < 2e-16$, Figure 1A). After the second dose, anti-SARS-CoV-2 RBD IgG antibody levels were still higher in SeroPOS individuals (mean: 602.59 AU/ml, SD: 511.545, range 25.41-1986.00) compared to SeroNEG individuals (mean: 375.567 AU/ml, SD: 437.088, range 9.617-3704.40; Wilcoxon rank-sum test, $p = 0.006$, Figure 1B). SeroNEG individuals had on average a 5.35-fold increase in anti-SARS-CoV-2 RBD IgG antibody levels after the second dose (Wilcoxon signed-rank test, $p < 2e-16$, Figure 1B), whereas SeroPOS individuals had no benefit of increased antibody levels after the second dose (Wilcoxon signed-rank test, $p = 0.529$, Figure 1B). SeroPOS individuals had higher antibody levels after the first dose than SeroNEG individuals after the second dose (Wilcoxon rank-sum test, $p = 0.0039$, Figure 1B). Exploratory analysis of the influence of sex and age on antibody response showed that older age had a reducing effect on antibody levels after the first and second vaccine dose (Supplementary Appendix, Table S2). The vast majority of the study participants reported at least one side effect after the first dose (91.15%, Figure 1C), mostly minor local pain (69.47%). A higher proportion of study participants reported at least one side effect after the second dose (97.35%, Figure 1D), again mostly minor local pain (53.97%).

Our findings are in line with previous reports of higher antibody levels in SeroPOS individuals after a single dose of BNT162b2 compared to SeroNEG individuals²⁻⁵, and support the hypothesis that a single dose of BNT162b2 in SARS-CoV-2 seropositive individuals might provide sufficient humoral immunity towards SARS-CoV-2. These findings should be validated in a clinical trial setting as soon as possible, due to direct implications for public health policy in developing countries with limited access to vaccines. Future investigations should incorporate analyses of the cellular immunity and take into consideration the duration of the immune response, which have not been evaluated in this study. The more rational use of vaccines could accelerate the attainment of collective immunity at reduced costs.

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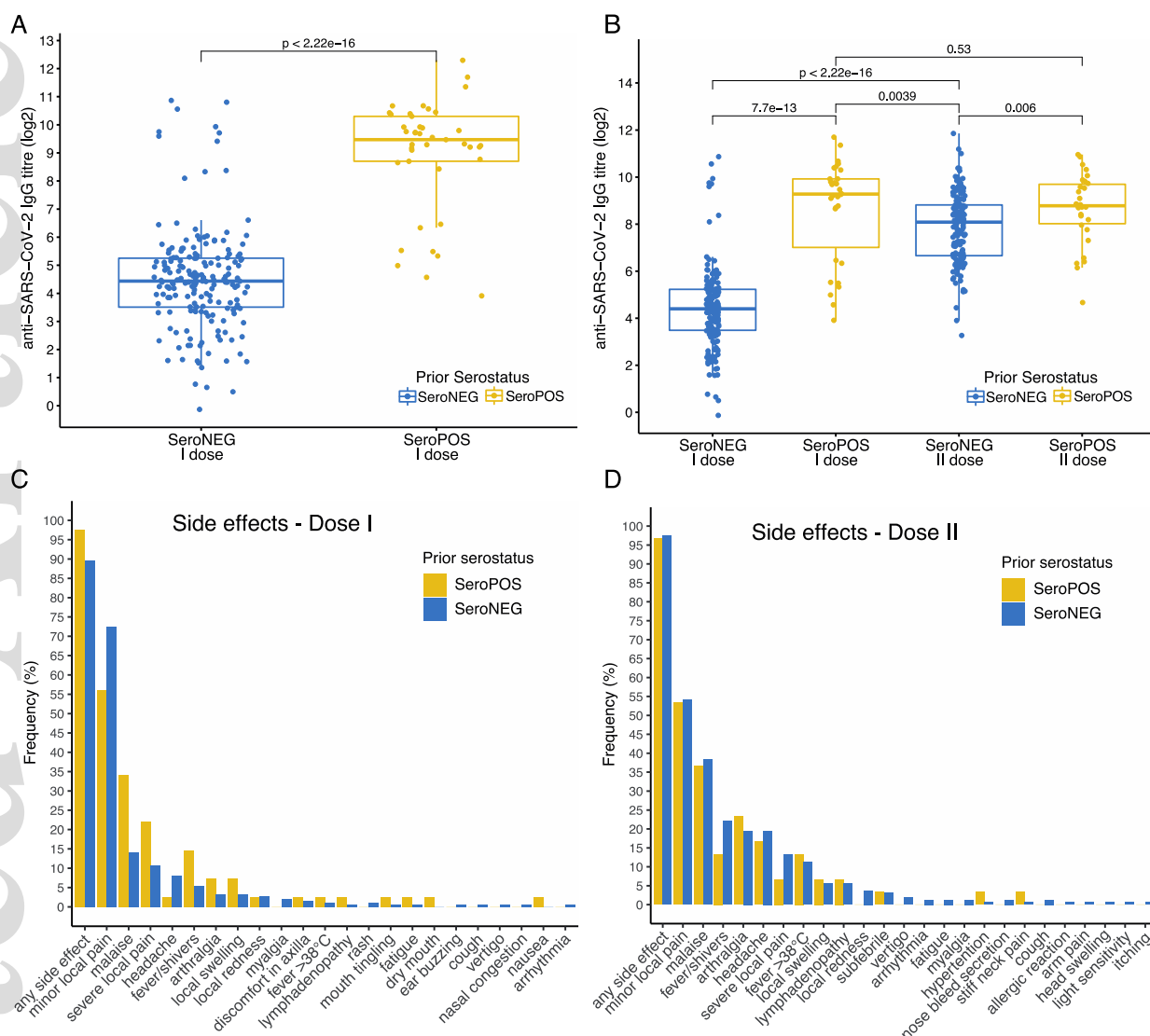


Figure 1. Antibody titers (log2-transformed) and side effects after the first and second dose of the Pfizer/BioNTech BNT162b2 vaccine in patients with (SeroPOS) and without (SeroNEG) anti-SARS-CoV-2 antibodies before vaccination.

A. Anti-SARS-CoV-2 RBD antibody titers after the first dose of BNT162b2 in SeroPOS individuals (n = 41) versus SeroNEG individuals (n = 185), full cohort;

B. Anti-SARS-CoV-2 RBD antibody titers after the first and second dose of BNT162b2 in SeroPOS individuals (n = 30) versus SeroNEG individuals (n = 159), participants who returned for analysis after the second dose;

C-D. Frequency of self-reported side effects in SeroPOS and SeroNEG individuals after the first and second dose of BNT162b2;

Supplementary Appendix

Brnjarchevska-Blazhevskaja T, Babačić, H, Sibinovska O, Dobrevski B, Kirijas M, Milanovski G, Arsov T and Petlichkovski A. A single dose of BNT162b2 vaccine elicits strong humoral response in SARS-CoV-2 seropositive individuals. 2021

Materials and methods

The study was performed according to the Declaration of Helsinki on Ethical principles for medical research involving human subjects, and approved by the Ethics Committee of the Faculty of Medicine, University Ss Cyril and Methodius, Skopje, North Macedonia. All participants provided informed consent and completed a questionnaire on age, sex, prior COVID-19 disease, and side effects experienced after the first dose of the BNT162b2 vaccine.

The participants received the first BNT162b2 dose in the period between 16 February and 17 March 2021. The second dose was administered three weeks after the first dose. A SARS-CoV-2 seropositive individual (SeroPOS) was defined as a participant who had anti-SARS-CoV-2 IgG antibodies prior to vaccination, based on patients' records. A SARS-CoV-2 seronegative individual (SeroNEG) was defined as a participant who did not have anti-SARS-CoV-2 IgG antibodies prior to vaccination.

The SARS-CoV-2 RBD IgG assay is an immunoassay based on indirect chemiluminescence. It is a fully automated quantitative serology test with a strong correlation to the levels of neutralizing antibodies. The linear range of detection is from 0.180 – 100 AU/ml. Samples with antibody concentration above the measuring range, were automatically diluted by the analyzer. The cut-off value for a positive antibody test is 1 AU/ml.

Statistical analysis

Descriptive statistics was calculated for each variable. The antibody titer levels were normalized with a log2-transformation for visualization and statistical testing.

We used two-sided Wilcoxon rank-sum tests for comparing anti-SARS-CoV-2 IgG antibody titers and age between SeroPOS and SeroNEG individuals, at a significance level of $\alpha = 0.05$. Matched comparisons before and after the vaccine doses within the groups were performed with a two-sided Wilcoxon signed-rank test, at a significance level of $\alpha = 0.05$. The categorical variables were compared between the groups with a two-sided Fisher's exact test, at a significance level of $\alpha = 0.05$.

The effect of serological status prior to vaccination, age (in years) and sex on antibody levels after the first and second vaccine dose was explored with a linear regression model, following the equation:

$$\log_2(\text{IgG levels}) = 0 + \beta_1 \times \text{SeroStatus} + \beta_2 \times \text{age} + \beta_3 \times \text{sex}.$$

All analyses were performed in R, version 4.0.3.

Table S1. Baseline patients' characteristics, stratified according to serological status.

Variable	Value	All (<i>n</i> = 226)	SeroPos (<i>n</i> = 41)	SeroNEG (<i>n</i> = 185)
Age (years)	Mean	45.137	43.195	45.568
	Median	45	42	46
	Range	18 to 68	23 to 68	18 to 67
	SD	10.548	10.572	10.523
	Missing	0	0	0
	<i>P</i>			0.217
Sex	Female, <i>n</i> (%)	156 (69.027%)	30 (73.171%)	126 (68.108%)
	Male, <i>n</i> (%)	70 (30.973%)	11 (26.829%)	59 (31.892%)
	<i>P</i>			0.580
Positive PCR before vaccination	Yes, <i>n</i> (%)	35 (15.487%)	32 (78.048%)	3 (1.621%)
	No, <i>n</i> (%)	191 (84.513%)	9 (21.95%)	182 (98.378%)
	<i>P</i>			< 2.2e-16

Table S2. Effect of age and sex on anti-SARS-CoV-2 IgG antibody levels. Dose I and dose II linear models refer to linear regression models predicting log2 IgG antibody levels after the first and second vaccine dose, respectively, with prior seropositive/seronegative status, age in years, and sex (Female = 0, Male = 1). Abbreviations: SE = standard error; DF = degrees of freedom.

	Variable	β estimate	SE	<i>t</i> value	<i>P</i> value	
DOSE I LINEAR MODEL	SeroPOS - Yes	10.6688	0.5841	18.2669	<0.0001	
	SeroNEG - Yes	6.2796	0.5568	11.2784	<0.0001	
	Age (years)	-0.0398	0.0116	-3.4349	0.0007	
	Sex - Male	0.0594	0.2630	0.2259	0.8215	
Model Characteristics	Multiple R ²	Adjusted R ²	F statistic	DF	<i>P</i> value	Residual SE
	0.9059	0.9042	531.6	4 and 221	< 2.2e-16	1.82
	Variable	β estimate	SE	<i>t</i> value	<i>P</i> value	
DOSE II LINEAR MODEL	SeroPOS - Yes	9.5082	0.5312	17.8984	<0.0001	
	SeroNEG - Yes	8.7750	0.5015	17.4992	<0.0001	
	Age (years)	-0.0204	0.0103	-1.9735	0.0499	
	Sex - Male	0.1021	0.2294	0.4452	0.6567	
Model Characteristics	Multiple R ²	Adjusted R ²	F statistic	DF	<i>P</i> value	Residual SE
	0.9685	0.9678	1422	4 and 185	< 2.2e-16	1.458