Supplementary Material for the paper "Longitudinal determination of BNT162b2 vaccine induced strongly binding SARS-CoV-2 IgG antibodies in a cohort of Romanian healthcare workers"

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Supplementary discussion, figures and tables

To verify whether the significant difference between antibody levels measured with the N+S1 combined commercial assay at the start of Period 2 (36-65 days after vaccination) is due to high maintained anti-N IgG values in the previously infected group, we remeasured the the majority of samples collected at the baseline and those collected for Period 2, with the ingredients of the commercial assay, except that the plates were coated separately with N and S1 proteins according to the procedure described in the Methods section. We observe an overall decrease in anti-N IgG levels compared to the baseline in the previously infected group (Supplementary

Figure 2, left panel), while the difference of anti-S1 IgG levels between the uninfected and previously infected groups remains significant (p<0.0107, Supplementary Figure 2, right panel).

The reduced sensitivity of the assay for anti-S1 IgG in the previously infected group, compared to that of the anti-N assay, cannot be explained by the difference in antigen size alone, since for a hypothetical case of a patient producing anti-N and anti-S1 antibodies of equal concentration and affinities, the size difference would decrease the signal of anti-S1 antibodies by 40% at most, corresponding to the 1 µg/ml coating solution containing less S1 proteins (1.06 fmol/well N protein *versus* 0.65 fmol/well S1 protein). It is likely therefore that patients produce anti-S1 antibodies that bind with affinities below the threshold our assay can detect. However, high affinity anti-S1 antibodies should have an important role in ensuring protection from infection, since a subset of these would be the most potent in preventing cell attachment.
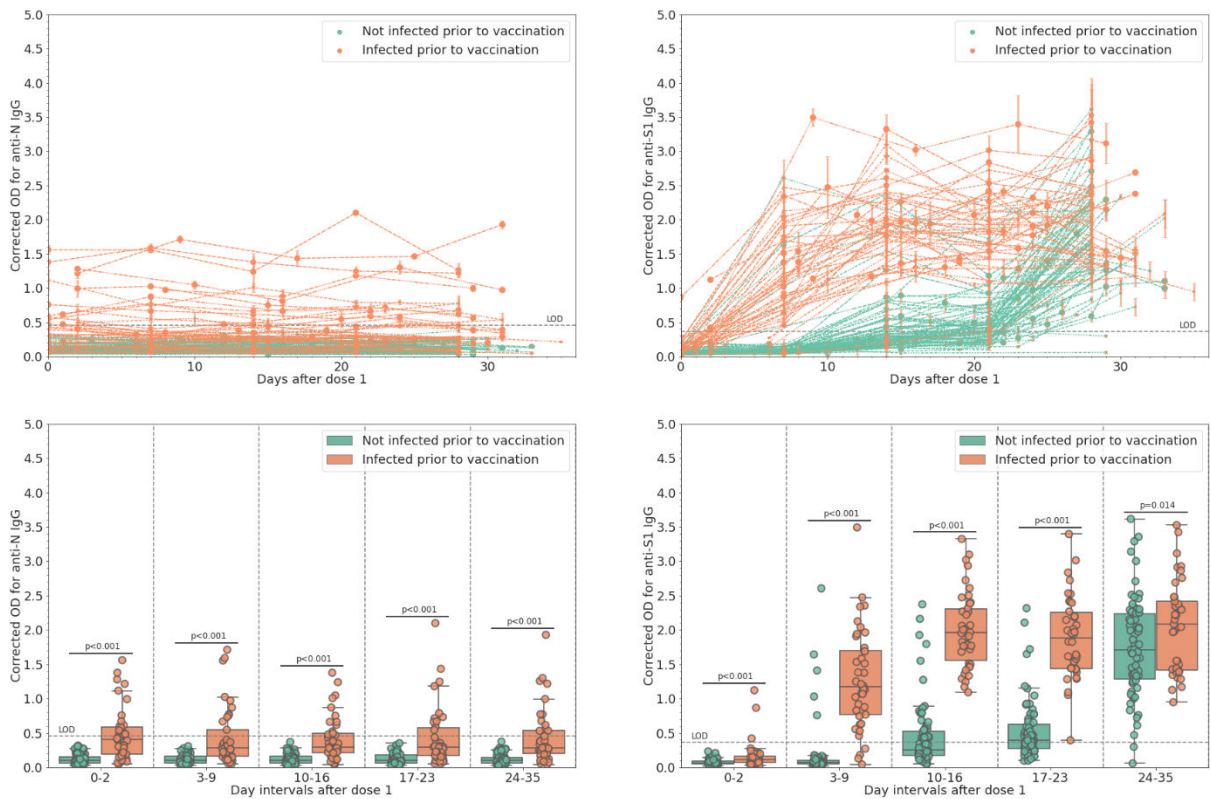

Figure S1. Dynamics of measured SARS-CoV-2 anti-N and anti-S1 IgG levels among the study participants over time for Period 1 of the study (0-35 days post first dose). The limit of detection for each assay was determined as the average IgG values of the uninfected group plus four times their standard deviation. Top column: anti-N IgG levels in optical density units scaled using the average of positive controls on each plate, right column: anti-S1 IgG levels on the same scale. Intergroup p-values were calculated with the Mann-Whitney U test.
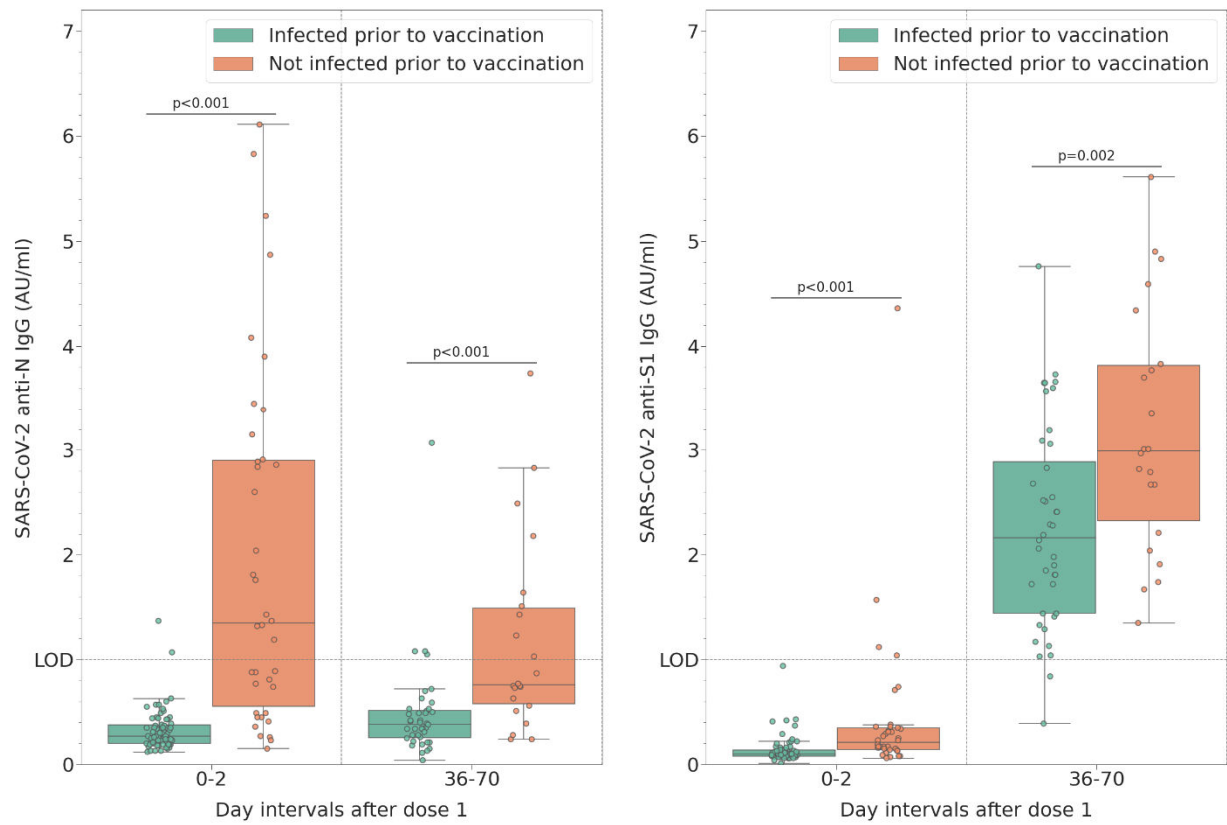
Figure S2. SARS-CoV-2 IgG levels for the uninfected and previously infected groups at the start of vaccination and 36-70 days after the first dose. Left panel: anti-N, right panel: anti-S1 IgG antibodies determined on nucleocapsid-only and S1-only plates. Apart from the concentration of coated antigens, for this measurement we used the same assay ingredients and protocol as that in the CE-marked commercial version. If multiple measurements were done for a participant within these intervals, only the largest value is shown.
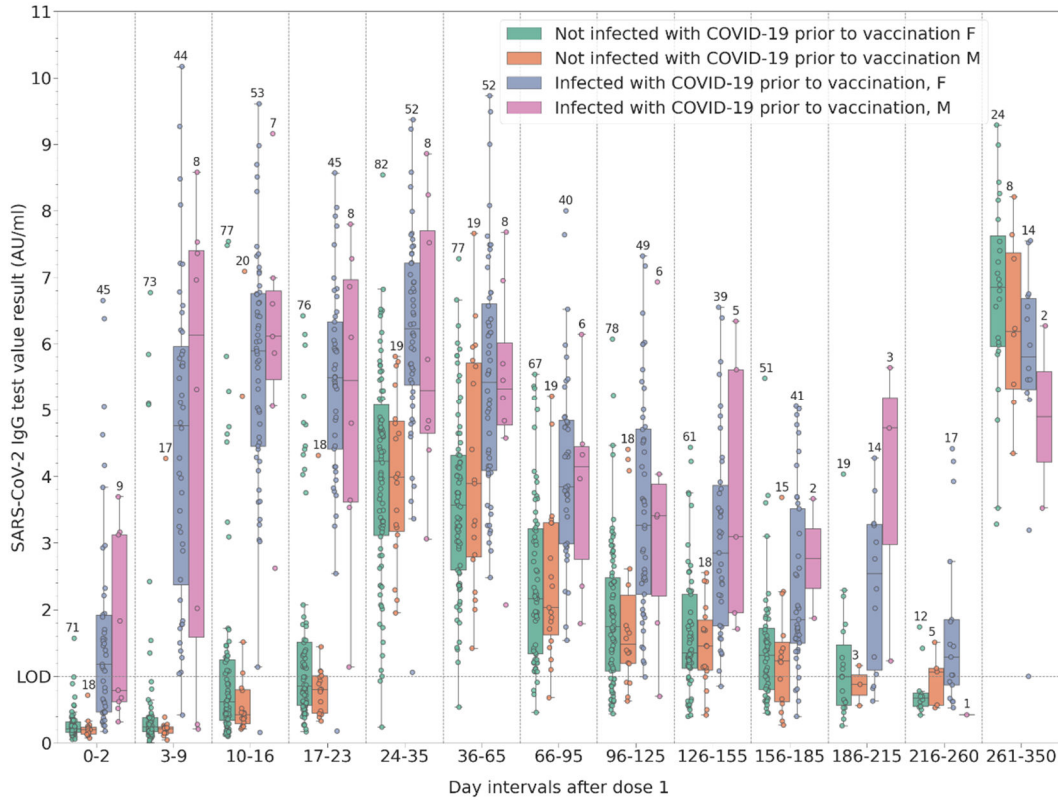
Figure S3. Longitudinal change of determined SARS-CoV-2 IgG values based on the gender and previous infection status of the participants.
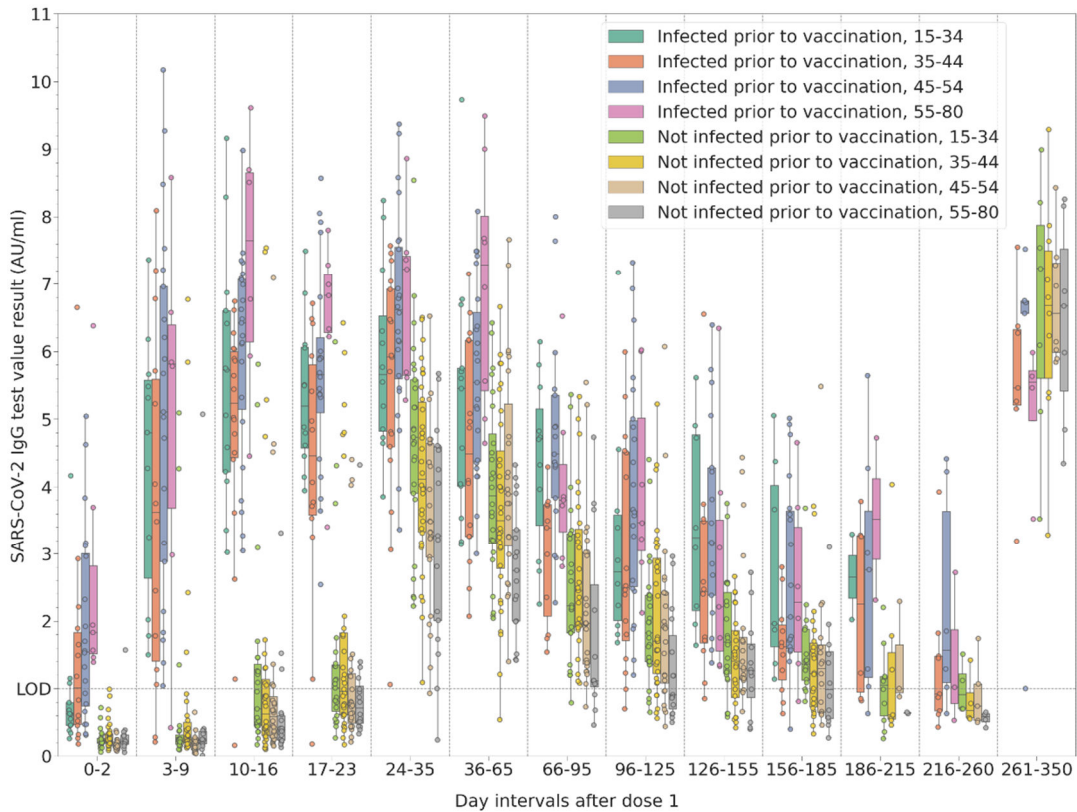
Figure S4. Longitudinal change of determined SARS-CoV-2 IgG values grouped according to the age group and previous infection status of the participants.

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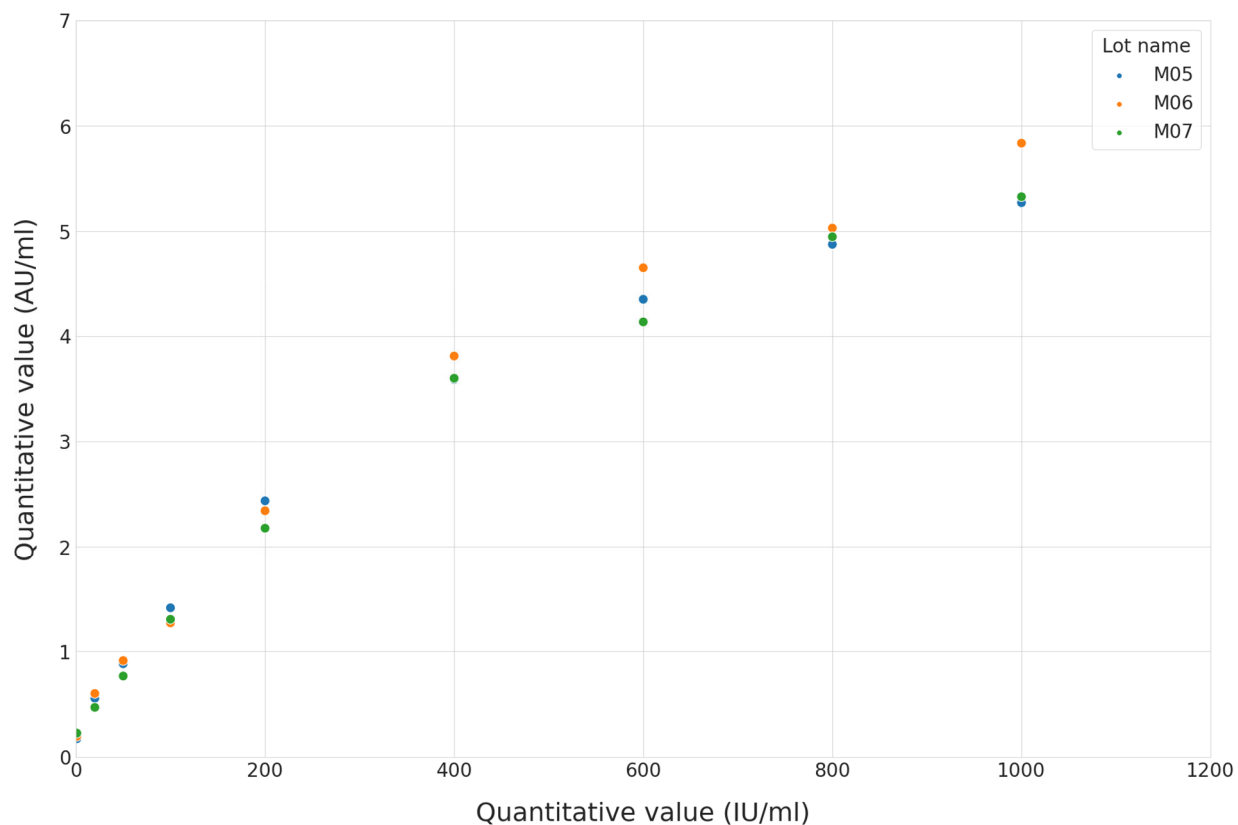
Figure S5. Correlation between SARS-CoV-2 IgG antibody values measured in IU/ml (using the first WHO standard) and AU/ml of the CE-marked version of the assay used in this study.

Supplementary Tables 1 and 2 show the distribution of study participants according to their gender and ages, compared to that of the available statistical data from Romanian healthcare workers.

Table S1. Demographics of the study participants based on their genders and three groups: all patients, the ones with an infection recorded prior to vaccination and the ones with no recorded infection prior to the vaccination. As the p-values show, all groups can be considered as representative of the population of healthcare workers in Romania. The p-values were calculated using the chisq.test function of R.

Group	Sample (Study)	Population (Healthcare statistics)	p-value
Gender (F) all	81.93%	79.30%	0.403
Gender (F) with prior infection	84.38%	79.30%	0.316
Gender (F) with no known prior infection	80.39%	79.30%	0.785

Table S2. Demographics of the study participants based on their ages and three groups: all patients, the ones with an infection recorded prior to vaccination and the ones with no recorded infection prior to the vaccination. As the p-values show all groups can be considered as representative of the population of healthcare workers in Romania. (p-values were calculated using the chisq.test function of R)

Group \ Ages	15 - 34	35 - 44	45 - 54	55 - 80	p-value
Population	20.10%	37.00%	27.00%	15.90%	N/A
All participants	22.89%	31.92%	29.52%	15.66%	0.533
Prior infection	20.31%	29.69%	37.5%	12.5%	0.261
No recorded prior infection	24.51%	33.33%	24.51%	17.65%	0.618

Table S3. P-values between IgG levels measured for infection naïve and previously infected groups, as shown in Figure 2. The comparisons between groups was done using Mann-Whitney U test, p-values were computed using the scipy.stats.mannwhitneyu method of Scipy.

Day interval	p-value (between previously infected - not infected groups)
0-2	<0.001
3-9	<0.001
10-16	<0.001
17-23	<0.001
24-35	<0.001
36-65	<0.001
66-95	<0.001
96-125	<0.001
126-155	<0.001
156-185	<0.001
186-215	<0.001
216-260	0.006
261-350	0.022

Table S4. Median values of the IgG levels measured for infection naïve and previously infected groups and the 95% confidence intervals associated to these values. The confidence intervals were computed using the bootstrap technique by resampling each group 1000 times, calculations were done with Python.

Day interval	Median of previously infected group	95% CI of median of previously infected group	Median of not infected group	95% CI of median of not infected group
0-2	1.18	0.77 - 1.57	0.21	0.19 - 0.23
3-9	4.91	3.36 - 5.69	0.24	0.20 - 0.25
10-16	5.89	5.32 - 6.41	0.52	0.44 - 0.70
17-23	5.5	4.97 - 5.96	0.83	0.74 - 1.00
24-35	6.15	5.70 - 6.78	4.05	3.72 - 4.59
36-65	5.29	4.83 - 5.97	3.58	3.24 - 3.98
66-95	3.9	3.72 - 4.48	2.17	1.94 - 2.53
96-125	3.39	2.55 - 4.03	1.70	1.45 - 1.96
126-155	2.97	2.30 - 3.47	1.38	1.24 - 1.61
156-185	1.87	1.62 - 2.62	1.28	1.04 - 1.45
186-215	2.76	1.23 - 3.29	0.94	0.61 - 1.22
216-260	1.29	0.87 - 1.85	0.70	0.55 - 1.07
261-350	5.8	5.26 - 6.56	6.74	6.06 - 7.28

Data availability

Anonymized sample measurement data is available for download at
https://szilard.ro/files/vaccine_study.csv