

Antibody response and self-reported adverse reactions following vaccination with Comirnaty: a pilot study from a Croatian university hospital

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ABSTRACT

This study aimed to determine antibody responses against SARS-CoV-2 spike (S) after both BioNTech–Pfizer Comirnaty vaccine doses and study the correlation with self-perceived adverse reactions. Antibodies determination with Elecsys anti-SARS-CoV-2 S assay was performed a day prior to or just before administration of the second dose and 8–13 days after the second dose. Participants selected from a predefined list of the experienced local (injection site reactions) and/or systemic (fatigue, headache, myalgia, arthralgia, chills and fever) post-vaccination adverse reactions. An average 100-fold increase in antibody titre in naive vaccinees was observed between the two time points (median 67 U/mL vs 2841 U/mL, $p < 0.001$). Participants aged below 50 had higher antibody titres (median 99 U/mL vs 26 U/mL, $p = 0.003$ after the first dose; median 3617 U/mL vs 2556 U/mL, $p = 0.026$ after the second dose). All reported adverse reactions were mild-to-moderate, with more participants declaring systemic reactions after the second dose ($p = 0.001$), without a clear correlation with antibody titre.

INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the major public health concern nowadays, with tremendous healthcare, social and economical consequences worldwide. Widespread vaccination is promoted as the single most effective response strategy and enormous efforts have been made to rapidly develop safe and effective vaccines.^{1 2} Vaccination stimulates immune response against SARS-CoV-2 and provides individual immune prophylaxis, as well as limits further community spread of the disease through providing herd immunity once a critical mass of people becomes immune.¹ Earlier studies showed that immunoglobulins G (IgG) targeted against the SARS-CoV-2 spike (S) glycoprotein have neutralising activity and therefore SARS-CoV-2 S was used as the primary candidate antigen for vaccine development.³

The first vaccine to be licensed by the US Food and Drug Administration and by the European Medicines Agency was tozinameran, sold under the brand name Comirnaty, and developed by BioNTech (Mainz, Germany) in cooperation with Pfizer (New York, USA). At the beginning of 2021, Comirnaty became available in Croatia and the initial vaccine supplies were prioritised to categories at high risk

of SARS-CoV-2 infection, including long-term care facility residents and healthcare professionals.

Comirnaty is a messenger RNA vaccine that encodes a mutated form of the SARS-CoV-2 S protein, which in turn stimulates production of targeted antibodies. As attested in clinical trials, such activation of the immune system can be accompanied by short-term adverse reactions of mild-to-moderate intensity, the most frequently being local reactions at injection site, followed by fatigue, headache, myalgia, chills, arthralgia and fever.^{4–7} For a complete immune response, a two-dose vaccination regimen is recommended, with applications at least 21 days apart. The extent of the induced humoral immunity with Comirnaty has already been largely reported and studies provide evidence of an efficient and robust antibody response.^{8–10} However, the possible relationship between the antibody titre and adverse reactions remains unknown. Given that stronger antibody response was shown to correlate with disease severity in patients with COVID-19,¹¹ we hypothesised that more prominent post-vaccination adverse reactions might be associated with a stronger immune response reflected by higher antibody titres. Additionally, recent studies suggest that ageing is accompanied by a lower immune response to COVID-19 vaccines.^{9 12}

Therefore, the aim of this study was to determine the antibody response following administration of both doses of Comirnaty in healthy individuals among healthcare workers, to record the experienced adverse reactions after the first and the second vaccine dose and to assess whether antibody titres correlate with the severity of self-reported post-vaccination adverse reactions, as well as differ according to age and gender.

MATERIALS AND METHODS

Setting and study protocol

The study was performed at the Department of Laboratory Diagnostics, University Hospital Centre Zagreb, and included healthy adult laboratory and clinical staff volunteers with no known serious underlying comorbidities, who did not experience any COVID-19-related symptoms and were not in known close contact with COVID-19 positive persons from the beginning of the pandemic, therefore supposedly not previously being affected by COVID-19 and underwent voluntary vaccination with Comirnaty (Pfizer/BioNTech). None of the study participants were involved in direct care of patients with COVID-19 and strictly adhered to the



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recommended preventive measures at the workplace, including use of personal preventive equipment, social distancing and hygiene measures. Additionally, to suppress virus spreading within the hospital setting, patient triage at hospital entrance was applied without exception, as well as nasopharyngeal swab PCR testing in cases of hospitalisation.

The vaccine was given by intramuscular injection and a two-dose prime-boost approach was applied to all study participants, each dose (0.3 mL) containing 30 µg of Comirnaty vaccine. Doses were administered 21–24 days apart; the first dose in the period from 21 to 24 January 2021, while the second between 12 and 16 February 2021, with Comirnaty batches EJ6134 and EL0725, respectively.

Blood sampling was performed at two time points, that is, a day prior to or just before administration of the second vaccine dose (20–24 days from the first dose) and 8–13 days after the second dose. Blood was collected in 5 mL serum tubes (Becton Dickinson, Wokingham, UK) and antibody titre measurement was performed within 4 hours from blood collection, using the quantitative electrochemiluminescence immunoassay Elecsys anti-SARS-CoV-2 S (Roche Diagnostics, Mannheim, Germany) applied to Cobas e801 analyser (Roche Diagnostics, Mannheim, Germany) that measures antibodies (including IgG) against the SARS-CoV-2 S glycoprotein receptor binding domain. Results are expressed in units per millilitre (U/mL), and values below 0.8 U/mL are considered negative.

Participants who developed antibody titres above 100 U/mL after the first dose were assigned to additional testing with the qualitative Elecsys anti-SARS-CoV-2 assay, which measures antibodies targeted against the SARS-CoV-2 nucleocapsid antigen. These antibodies are found exclusively in individuals previously infected by SARS-CoV-2 so their determination was used to distinguish whether high anti-SARS-CoV-2 S titre pertains exclusively to vaccine-related antibodies or is partly a result of a resolved asymptomatic COVID-19 infection.

A laboratory staff member (LZ) interviewed each participant, asking them to report self-perceived post-vaccination adverse reactions after administration of each dose. The participants could select multiple answers from the predefined list of adverse reactions that included local reactions (injection site pain, redness and swelling) and systemic reactions (fatigue, headache, myalgia, arthralgia, chills and fever). Any other experienced adverse reactions were also noted. Participants were grouped into three categories according to reported adverse reactions for each vaccine dose: a group with no experienced adverse reactions, a group including participants who reported only local adverse reactions and a group encompassing participants who reported systemic reactions.

All participants signed an informed consent before enrolment.

Statistical analysis

The Shapiro-Wilk test was used to assess the normality of distribution for quantitative data and the results are presented as medians and IQRs. Differences in antibody titres according to age and gender were tested using the Mann-Whitney U test. Spearman's rank correlation coefficient (ρ) was used to determine the correlation between anti-SARS-CoV-2 S antibody titres and participants' age. Given that only active working-age population was included in the study, the cut-off of 50 years was chosen for age stratification. Difference in the distribution of participants reporting no adverse reactions, local or systemic reactions after administration of the first compared with the second vaccine dose was assessed with χ^2 test for comparison of two proportions. Comparison of antibody titres between participants grouped according to reported adverse reactions was tested using the Kruskal-Wallis test. P value less than 0.05 was considered statistically significant. Statistical analysis was performed using MedCalc software, V.19.5.2.

RESULTS

Initially, 68 participants were enrolled in the study. Four female participants tested positive for antibodies against the nucleocapsid antigen after the first dose, indicating a previous asymptomatic COVID-19 infection and were therefore excluded from the study. Finally, a total of 64 participants (median age 52 years, from 25 to 63) were enrolled in the study, of whom 49 (77%) were women. Five study participants pertain to clinical staff, while all others were laboratory staff members. Median time of blood sampling after the first vaccine dose was 22 days (from 20 to 24 days), whereas it was 9 days (from 8 to 13 days) after the second vaccine dose. Median time from the first to the second vaccine dose was 23 days (from 21 to 24 days). An expected, substantial increase, however largely heterogeneous among participants, was observed after administration of the second dose (median 2841 U/mL, IQR: 1890–4745 U/mL) compared with the first time point (median 67 U/mL, IQR: 20–129 U/mL), $p < 0.001$. When evaluating raw data, antibody titres were highly heterogeneous, that is, ranging after the first dose from borderline 0.9 to 547 U/mL, while after the second dose from 731 to 19 743 U/mL. The average observed increase in antibody titre between the first and second time point was 100-fold, ranging from 11-fold to a 754-fold increase.

Distribution and comparison of the number of participants reporting no adverse reactions, local or systemic reactions after the first and the second dose of Comirnaty vaccine are presented

Table 1 Distribution and comparison of the number of participants reporting no adverse reactions, local or systemic reactions after the first and the second dose of Comirnaty vaccine

| | After the first Comirnaty dose | After the second Comirnaty dose | P value |
|---|--|--|---------|
| Participants reporting no adverse reactions, N (proportion) | 29 (0.45) | 17 (0.26) | 0.025 |
| Participants reporting only local reactions, N (proportion) | 31 (0.49) | 30 (0.47) | 0.822 |
| Participants reporting systemic reactions,* N (proportion) | 4 (0.06): ► Myalgia (2/4). ► Fatigue (1/4). ► Chills, fever, myalgia, headache (1/4). | 17 (0.27): ► Chills, fever, myalgia and headache (7/17). ► Fatigue and myalgia (6/17). ► Chills (3/17). ► Headache (1/17). | 0.001 |

*All participants reported local reactions coupled with at least one systemic adverse reaction. Additionally, after the second vaccine dose two participants reported lymphadenopathy and one high blood pressure (not presented in the table); $p < 0.05$ was considered statistically significant.

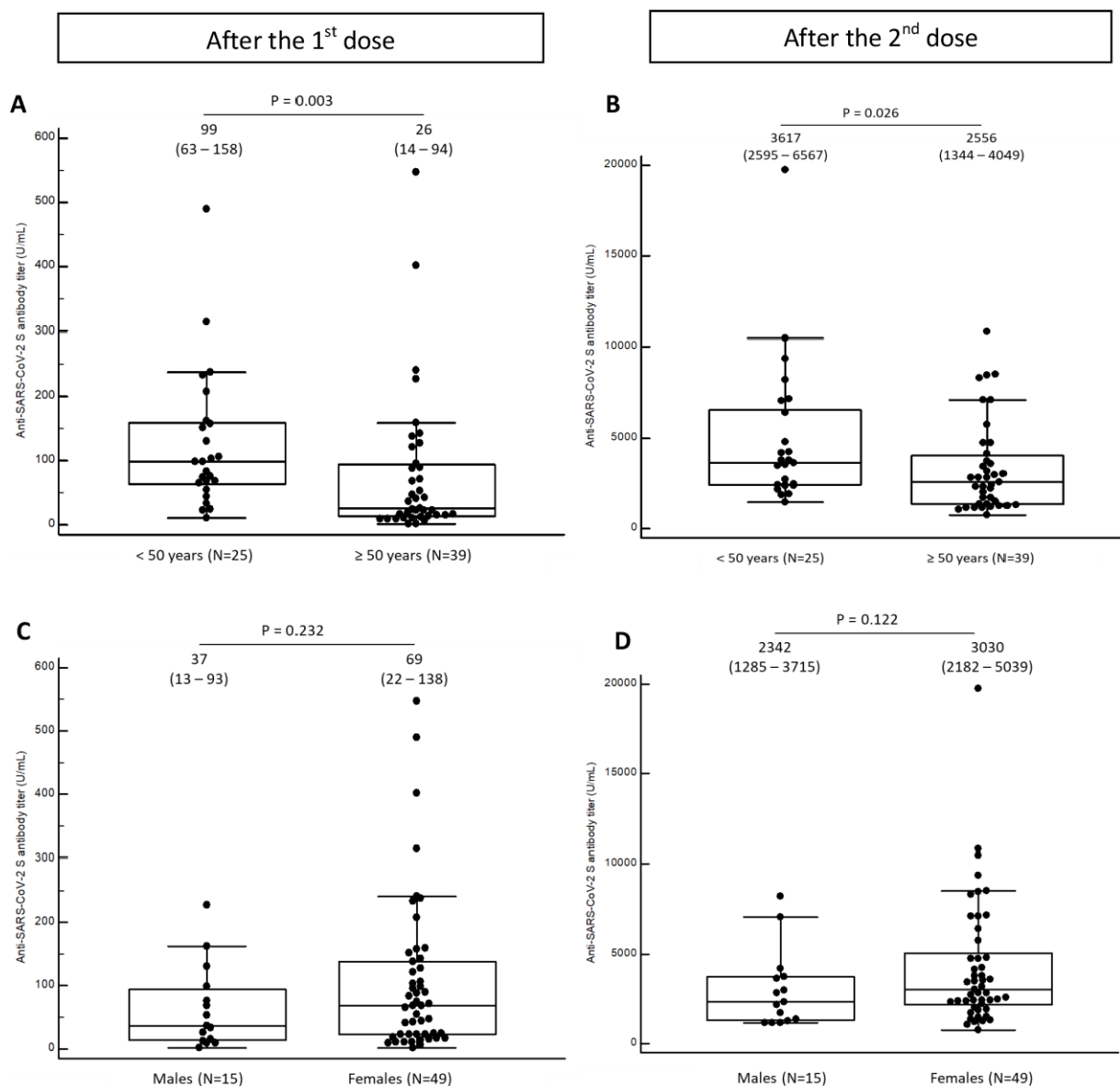


Figure 1 Comparison of antibody titres between participants younger than 50 years and equal or above 50 years after the first (A) and the second vaccine dose (B), and between men and women after the first (C) and the second vaccine dose (D). Results are presented as medians and IQRs; $p < 0.05$ is considered statistically significant.

in table 1. More participants reported adverse reactions after the second dose, compared with the first dose. However, in all cases the reported adverse reactions were of mild or moderate intensity and resolved within a day or two. Some cases of fever, chills, headache and myalgia were treated with antipyretics and/or anti-inflammatory drugs. The four participants who were found to have been previously affected by COVID-19 reported adverse reactions after the first vaccine dose only. One case was associated with fever, chills, lymphadenopathy and short-term hemiparesis, another with lymphadenopathy while the remaining two reported only injection-site pain. All four revealed antibody titres higher than 2500 U/mL after the first dose.

Figure 1 shows the comparison of antibody titres per age and gender. Statistically significant difference between antibody titres according to participants' age was observed at both assessed time points, with participants younger than 50 years developing a stronger immune response with higher antibody titres. Spearman's ρ indicated a moderate inverse correlation of antibody

titres with participants' age at both time points ($\rho = -0.609$ and $\rho = -0.439$, respectively) (figure 2).

The comparison of antibody titres among participants grouped according to self-reported adverse reactions did not yield a statistically significant difference for either of the administered vaccine doses (figure 3). However, a trend towards higher antibody titre values can be observed after the second dose in participants reporting systemic adverse reactions.

DISCUSSION

The present study reports that 20–24 days from administration of the priming Comirnaty dose there was in all vaccinated participants a detectable but modest antibody response, followed by a substantial increase 8–13 days after the boost dose. Our study confirms the presence of more adverse reactions after administration of the second dose, however only of mild or moderate intensity. Importantly, there was no statistically significant

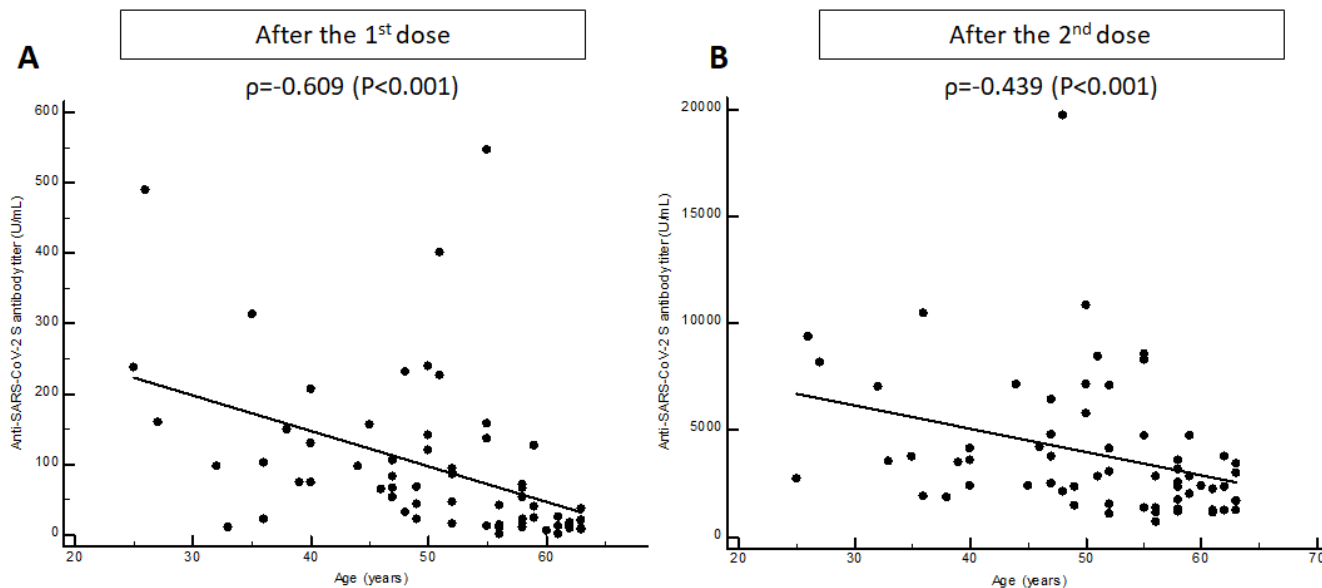


Figure 2 Correlation of antibody titres with participants' age after the first (A) and the second vaccine dose (B). ρ : Spearman's rank correlation coefficient; $p < 0.05$ is considered statistically significant.

difference in antibody titres among participants with or without self-reported adverse reactions. While safety, reactogenicity and immunogenicity of the Comirnaty vaccine were extensively evaluated through rigorous and comprehensive, large-scale clinical trials,^{5–7} this is, to the best of our knowledge, the first study to investigate the possible relationship between induced immune response against SARS-CoV-2 reflected by antibody titre and self-reported adverse reactions from vaccination with Comirnaty.

The observed antibody response mirrors the pattern obtained during clinical trials,^{6,7} and once again indicates the importance of booster vaccination for reaching optimal immune response in individuals previously not affected by COVID-19. However, the individual antibody response rate to vaccination was not

consistent among participants, with largely variable magnitudes of response and highly heterogeneous antibody titres which were significantly higher in persons below 50 years of age. This finding, along with the inverse correlation of antibody titre with increasing age, supports data from previous studies that reveal age-dependent immune response to vaccination with lower neutralising response in older compared with younger vaccinated adults, presumably due to immunosenescence which causes decline of immune efficiency.^{6,7,9,12,13} The magnitude of antibody response is known to correlate with the efficacy of neutralising activity and duration of immune protection.^{5–7,14} Therefore, further longitudinal monitoring of antibody titre is required to assess the duration of vaccine-induced immunity

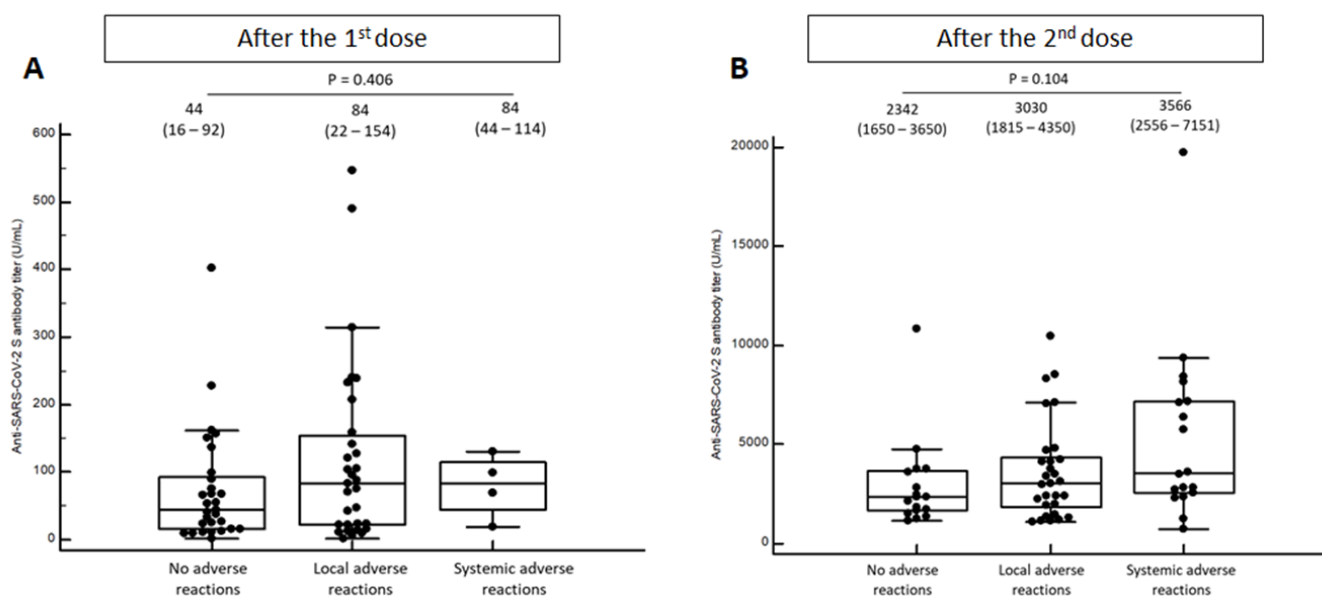


Figure 3 Comparison of antibody titres according to self-reported adverse reactions after the first (A) and the second vaccine dose (B). Results are presented as medians and IQRs; $p < 0.05$ is considered statistically significant.

and the dynamics of antibody titre decline over time, in order to timely identify conversion to seronegativity and the need for re-vaccination.

The self-reported adverse reactions were without exception mild to moderate in intensity, and consisted exclusively of the most common and well-documented adverse effects of vaccination with Comirnaty.^{4,5} The most frequently observed adverse reactions were local reactions at injection site which were reported by 55% and 74% participants after the first and the second dose, respectively. All adverse reactions were transient and resolved spontaneously within 2 days after vaccination, supporting the safe application of Comirnaty in apparently healthy adult individuals. Several cases of fever, chills, headache and myalgia were managed with symptomatic therapy. The incidence of systemic adverse reactions was significantly higher after administration of the second vaccine dose, which can be attributed to the induced boost of the immune system. The proportion of participants reporting local, injection-site reactions, either being the only adverse reaction or coupled with systemic reactions, increased significantly after the second dose, which is contrary to the results obtained from the clinical trial where local reactions were equally experienced after both doses.⁵ Although not reaching statistical significance, there was a trend of higher antibody titres after the second dose in participants reporting systemic adverse reactions. Notably, one participant with the highest antibody titre of 19 743 U/mL reported extreme fatigue lasting for 2 weeks after the second dose, which raises suspicion of an exaggerated immune response. Similar adverse reaction presentation coupled with an antibody titre comparable to the ones seen after the second dose in naive vaccinees, was observed after the first dose in one participant with prior COVID-19 infection, indicating a rapid and intense immune response in seropositive individuals. High antibody titres after the first dose in such individuals were already documented and imply that a single-dose regimen might indeed be sufficient in individuals with pre-existing immunity.^{15,16}

The main limitation of the study pertains to self-reporting of adverse reactions, which might have introduced a bias due to subjective perceptions. The study is also limited by the small number of participants, especially men, which is due to the predominance of women within our laboratory staff but also voluntary participation in the study. Moreover, age-related effects on antibody titres should be assessed on age subgroups other than only the working-age population. Although no serious comorbidities are present among study participants, we are unaware of the possible unknown underlying comorbidities that could have affected the development and intensity of both adverse reactions and the immune response.

CONCLUSION

In conclusion, the results presented herein unequivocally confirm that the two-dose Comirnaty vaccine regimen elicits high anti-SARS-CoV-2 S titres, with only minor or no adverse reactions, as commonly seen after any kind of vaccination. These findings point to the obvious conclusion once again: the long-term benefits of vaccination greatly surpass the possible short-term discomforts. Although clear correlation between adverse reactions and antibody titres was not confirmed, the increasing trend in antibody titres observed in participants who reported systemic adverse reactions might be indicative of a more prominent immune response and clearly deserves further investigation on a larger study population. Assessment of anti-SARS-CoV-2

S antibodies following vaccination provides a valuable insight into the vaccine-mediated immune response against COVID-19. Further studies are necessary to better elucidate the duration of the immune protection induced by vaccination. Additional studies should also focus on the relationship between adverse reactions and antibody titre in individuals with prior COVID-19 infection, as well as in subgroups with different comorbidities.

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REFERENCES

- Lippi G, Sciacovelli L, Trenti T, et al. Kinetics and biological characteristics of humoral response developing after SARS-CoV-2 infection: implications for vaccination. *Clin Chem Lab Med* 2021;59:1333–5.
- Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021;384:403–16.
- Bennet BM, Wolf J, Laureano R, et al. Review of current vaccine development strategies to prevent coronavirus disease 2019 (COVID-19). *Toxicol Pathol* 2020;48:800–9.
- Comirnaty, INN-COVID-19 mRNA vaccine (nucleoside-modified). Annex I – summary of product characteristics. Available: <https://ema.europa.eu> [Accessed 7 Mar 2021].
- Skowronski DM, De Serres G. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2021;384:1576–7.
- Sahin U, Muik A, Derhovanessian E, et al. COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses. *Nature* 2020;586:594–9.
- Mulligan MJ, Lyke KE, Kitchin N, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 2020;586:589–93.
- Salvagno GL, Henry BM, di Piazza G, et al. Anti-SARS-CoV-2 receptor-binding domain total antibodies response in seropositive and seronegative healthcare workers undergoing COVID-19 mRNA BNT162b2 vaccination. *Diagnostics* 2021;11:832.
- Padoan A, Dall'Omo L, Rocca FD, et al. Antibody response to first and second dose of BNT162b2 in a cohort of characterized healthcare workers. *Clin Chim Acta* 2021;519:60–3.
- Favresse J, Bayart J-L, Mullier F, et al. Early antibody response in health-care professionals after two doses of SARS-CoV-2 mRNA vaccine (BNT162b2). *Clin Microbiol Infect* 2021;27:1351.e5–1351.e7.
- Imai K, Kitagawa Y, Tabata S, et al. Antibody response patterns in COVID-19 patients with different levels of disease severity in Japan. *J Med Virol* 2021;93:3211–8.
- Prendecki M, Clarke C, Brown J, et al. Effect of previous SARS-CoV-2 infection on humoral and T-cell responses to single-dose BNT162b2 vaccine. *Lancet* 2021;397:1178–81.
- Müller L, André M, Moskorz W, et al. Age-Dependent immune response to the Biontech/Pfizer BNT162b2 COVID-19 vaccination. *Clin Infect Dis* 2021. doi:10.1093/cid/ciab381. [Epub ahead of print: 27 Apr 2021].
- Röltgen K, Powell AE, Wirz OF, et al. Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. *Sci Immunol* 2020;5:eabe0240.
- Saadat S, Rikhtegaran Tehrani Z, Logue J, et al. Binding and neutralization antibody titers after a single vaccine dose in health care workers previously infected with SARS-CoV-2. *JAMA* 2021;325:1467–9.
- Krammer F, Srivastava K. The Paris team, et al. robust spike antibody responses and increased reactogenicity in seropositive individuals after a single dose of SARS-CoV-2 mRNA vaccine. *medRxiv*. doi:10.1101/2021.01.29.21250653