Doctoral (Ph.D.) thesis

Correlation between adverse reactions followed BNT162b2 vaccination against SARS-CoV-2 and the anti-spike protein antibody levels through a 6-month-long follow-up

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1. INTRODUCTION

According to the WHO database, there have been more than 600 million confirmed SARS-CoV-2 infections and more than 6 million deaths worldwide since 2019, and in Hungary, nearly 30,000 deaths related to SARS-CoV-2 infections have been registered. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the cause of the 2019 Coronavirus Outbreak (COVID-19), the third highly pathogenic human beta coronavirus to cause a public health crisis in the last 20 years. Compared to its predecessors, SARS-CoV and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), the virus has shown a more efficient spread in the general population. The World Health Organization (WHO) declared a public health emergency of international concern pandemic on 11 March 2020.

In addition to its devastating health impacts, the pandemic has had negative economic and social consequences. Combating the pandemic required joint and rapid action on behalf of the scientific and pharmaceutical companies, thus accelerating the development of vaccine platforms that were different from classical vaccines. This has led perhaps to the most significant development in the history of human vaccines: the development of mRNA-based vaccines against SARS-CoV-2.

Already during the third wave, in March and April 2021, five different COVID-19 vaccines were available and widely used in Hungary: two mRNA-based vaccines (BNT162b2 - Pfizer-BioNTech and mRNA-1273 - Moderna), two viral vector vaccines(AZD1222 - AstraZeneca and Gam-COVID-Vac - Sputnik-V), and an inactivated virus vaccine (HB02 - Sinopharm).
The two-dose BNT162b2 and mRNA-1273 proved safe and showed more than 90% efficacy to SARS-CoV- 2. However, several systemic side effects have been observed during vaccination, mainly after the second dose. The most common adverse reactions were fatigue (59-65%), headache (52-58%), fever (16%) and chills (44%).

The rapid pace of vaccine development and the potential for long-term adverse effects has called into question the safety of the vaccine, causing some hesitation in the global community regarding vaccination.

In the context of the world's largest-ever vaccination campaign, the side effects of vaccines and immunogenicity have attracted the interest of several studies, but the results are incongruent and contradictory.

2. AIMS

Our goal is to observe the adverse reactions following the SARS-CoV-2 mRNA vaccine, as well as to look for a correlation between the spike antibody level and the number and type of adverse reactions. Another goal of our study series is to follow the reactions observed after the homologous and heterologous booster vaccinations, as well as any correlation between the antibody levels measured after the vaccinations.

The main steps and assumptions of our investigation:

1.Correlation between SARS-CoV-2 spike protein antibody (SARS-CoV-2-S-Ig) levels in peripheral blood samples obtained by vein puncture and adverse reaction detected after the second vaccination is investigated in a half-year follow-up period. We assume that there is a correlation between the detected antibody levels and the intensity and frequency of adverse reaction.

2. Investigation of the possible relationship between the dynamics of the SARS-CoV-2-S-Ig level obtained during the analysis of peripheral blood samples and the COVID-19 infection status. Immunological changes caused by previous COVID-19 may also affect adverse reaction and detected antibody levels.

3. The SARS-CoV-2 S-Ig level of people being infected previously and of those who were not infected but vaccinated and showed adverse reaction, suggest some presence of similar phenomena in both groups, according to which the previous COVID-19 infection and the symptomatic status after vaccination are similar can result in a humoral immune reaction.

4. Adverse reaction observed after the mRNA-based two-dose base vaccination predict the frequency and intensity of the adverse reactions noted even after the booster vaccination.

5. An adverse reaction noticed after the second vaccination may affect the probability of a COVID-19 infection after the booster vaccination.

3. MATERIALS AND METHODS

3.1. Study Design and Population

Health care workers in Szigetvár Hospital were recruited for the present study. The 383 volunteer participants were scheduled to initiate BNT162b2 mRNA (Pfizer/BioNTech, Comirnaty, Reinbek, Germany) vaccination according to the original protocol of two doses, which they received at the prescribed three-week interval between January 27 and May 9, 2021. In our study, both participants with SARS-CoV-2 infection and infection-free volunteers confirmed 3-5 months prior to the study by RT-PCR (reverse transcriptase-polymerase chain reaction) were taken part.

The study protocol included collection of venous blood samples for anti-SARS-CoV-2 spike protein immunoglobulin (SARS-CoV-2-S-Ig) determination at seven different time points (namely 12, 30, 60, 90, 120, 150 and 180 days following the second vaccine dose; designated Day 12, Day 30, Day 60, Day 90, Day 120, Day 150, and Day 180, respectively). Before administration of the first dose, registration of anamnestic data (high blood pressure, diabetes, hypothyroidism, autoimmune diseases, malignancy, smoking, recent flu vaccination and an inquiry allergies, age, sex, height, body weight, use of medications, including non-steroid anti-inflammatory drugs (NSAIDs), statins, antihypertensives, ACE inhibitors, beta blockers, calcium channel blockers, immunosuppressants, platelet inhibitors, steroids was performed using a questionnaire) were recorded.

Based on the presence of vaccination induced adverse reactions; (i) symptomatic (detection of an adverse reaction within 7 days after each vaccination dose) vs. (ii) asymptomatic (no adverse reaction occurred after any dose), and according to the prior COVID-19 infection status, the following subgroups were created:

Group 1: previously COVID-19 negative and asymptomatic individuals (--)

Group 2: previously COVID-19 negative and symptomatic individuals (-+)

Group 3: previously COVID-19 positive, but asymptomatic individuals (+-)

Group 4: previously COVID-19 positive and symptomatic individuals (++)

Adverse reactions were recorded immediately after the first vaccination before the administration of second dose and adverse reactions after the second vaccination session simultaneously with the first blood sampling on Day 12 follow-up visit. Adverse reactions were

investigated in a questionnaire where the volunteer was required to clearly indicate if they experienced an adverse reaction within 1 week after vaccination. Volunteers had to select the symptoms they experienced within 1 week after vaccination from the following list: local pain, fatigue, fever, myalgia, arthralgia, headache, chills, nausea, lymph node swelling or other.

After the booster vaccination plan became available, in the other part of our study, we again contacted the volunteer group of our study, the health care workers of the Szigetvári Hospital, to participate in our follow-up study. In accordance with the methodology used in our previous study, we collected venous blood samples before and after the third vaccination dose for anti-SARS-CoV-2 spike-immunoglobulin determination (14, 60 and 120 days after the administration of the third vaccine dose) and our volunteers recorded the local and systemic adverse reactions after booster vaccination (asymptomatic and symptomatic group) similar to what happened after primary immunization. After the administration, the type of vaccination (heterologous or homologous vaccination scheme) and the SARS-CoV-2 infection status were also recorded.

For the measurements, the blood samples of the volunteers were drawn via venipuncture with a 21-gauge needle into a closed system anticoagulant-free serum separator tube (Vacuette®, Greiner Hungary LTD, Mosonmagyaróvár, Hungary). Peripheral blood samples were evaluated for IgG antibodies against SARS-CoV-2 spike proteins on a fully automated benchtop Access2 analyzer according to the instructions of manufacturer (Beckman Coulter Hungary LTD, Budapest, Hungary). We used the Beckman-Coulter Access SARS-CoV-2 IgG II assay (Beckman Coulter Hungary LTD) for the determination of antibodies against the SARS-CoV-2 spike protein. The test measures IgG antibodies directed to the receptor-binding domain (RBD) of the spike protein of the coronavirus. The two-step enzyme assay is a chemiluminescent immunoassay consisting of paramagnetic particles, which is based on the semiquantitative determination of IgG antibodies against the SARS-CoV-2 virus in human serum. Briefly about the test: the sample is prepared in a reaction vessel filled with buffer and paramagnetic particles coated with recombinant SARS-CoV-2 protein. After incubation in the reaction vessel, materials bound to the solid phase are held in a magnetic field, while unbound materials are washed away. Next, a monoclonal anti-human IgG alkaline phosphatase conjugate is added to the mixture, and the conjugate binds to the IgG antibodies captured on the particles.

A second separation and washing step removes any unbound conjugates. A chemiluminescent substrate is added to the vessel and the light generated by the reaction is measured with a luminometer. Light production is directly proportional to the concentration of SARS-CoV-2 IgG antibody in the sample. The amount of analyte in the sample is determined based on a multipoint calibration curve. The results are given in IU/mL, which are correlated with the First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin (Human), NIBSC code, 20/136, in BAU/mL (BAU: Binding Antibody Units). The conversion of IU/mL concentrations to BAU/mL, can be done by multiplying IU/mL by multiplication factor 1. The results can be interpreted as follows: cut-off index <10 AU/mL as non-reactive and reactive >/=10 AU/mL.

3.2. Ethics Statement

The methodology of the tests listed above corresponds to that described in the literature and was prepared with the approval of the National Public Health Center (40576-8/2021/EÜIG). The research plan was compiled in accordance with the current legislation and the ethical guidelines of the 1975 Declaration of Helsinki. All participants provided written informed consent prior to inclusion in the present study.

3.3. Statistical Analysis

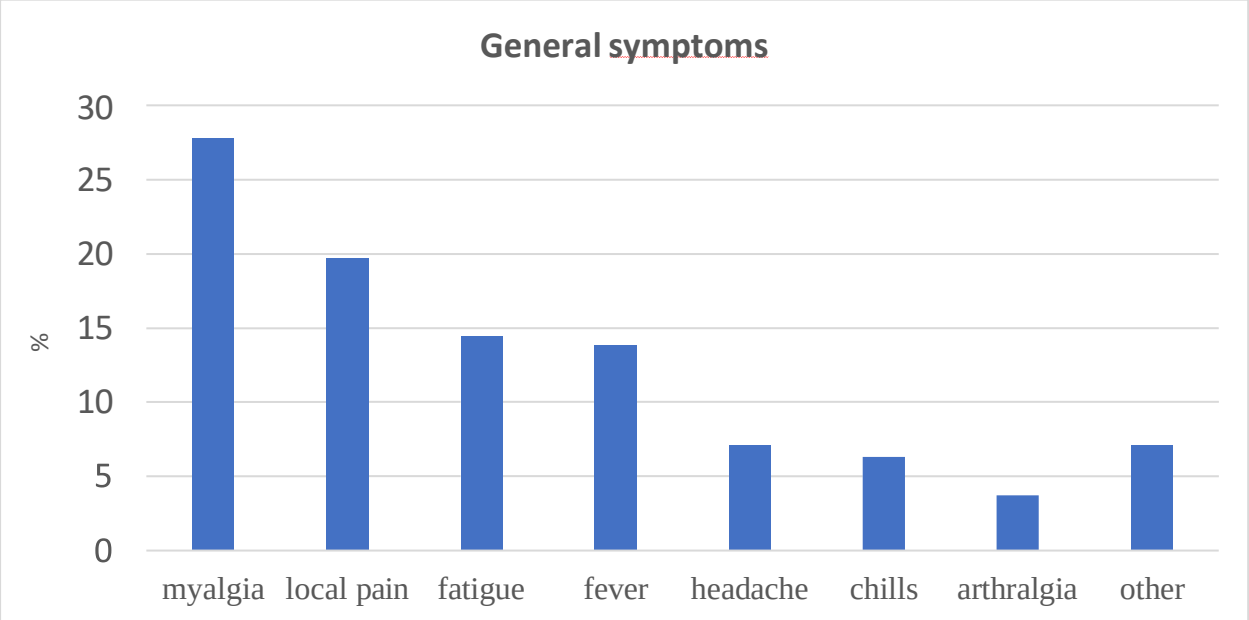
During the analysis of our results, the patient data were anonymized, and, after coding, the data were stored in a database accessible only to the research participants. The summary statistics of the participants were constructed based on the frequency and proportions of the categorical data, and the mean and standard deviation (SD) of the continuous variables. The statistical analysis of the collected data was performed by software version SPSS 23.0 (version 26; IBM Corporation, Armonk, NY, USA). Conformity of data to normal distribution was determined by histogram and Kolmogorov–Smirnov test. The between-group difference was calculated with $\chi 2$, Fisher's exact, Mann–Whitney U, and Kruskal–Wallis tests in line with suitability. The significance level was considered as $p < 0.05$. Data with nonparametric distribution were presented as median and interquartile range (IQR). Correlations of Ig levels with adverse reactions were tested by linear regression using Spearman correlation coefficient (R).

4. RESULTS

4.1. Correlation between adverse reactions, antibody levels and clinical variables after primary immunization

Between 10th of February and 13th of June 2021, a total of 395 people received the second dose of Pfizer-BioNTech vaccine (BNT162b2) and provided informed consent for study enrollment. From these, 383 individuals completed the questionnaire on post-vaccination ARs and gave post-vaccination blood samples at Day 12, 323 at Day 30, 320 at Day 60, 303 at Day 90, 268 at Day 120, 220 at Day 150 and 279 at Day 180. The age of the vaccinated volunteers ranged from 20 to 77 years (mean 46,5±12 years; IQR 39-55). 76.7% were females and 34.7% were current smokers. A total of 169 (44.1%) subjects had at least one AR within 7 days of any vaccination (symptomatic group), and 214 (55.9%) reported no vaccine related ARs (asymptomatic group). There were significantly more patients with history of allergy in the symptomatic group.

ARs occurred in 125 patients after the first dose and in 131 after the second dose. The total number of ARs within 7 days after the first vaccination was 314, while 365 ARs occured within 7 days after the second dose. In 87 participants (22.7%) at least one AR occurred after both vaccinations and in 214 cases (55.9%) no ARs occurred after either dose. The most common ARs during vaccinations were myalgia (27.8%) and local pain (19.7%) (**Table 1.**).



	Number of adverse reactions after 1st dose (N=314)	Number of adverse reactions after 2nd dose (N=365)	Sum of adverse reactions after each dose (N=679)
Myalgia	77	112	189
Local pain	74	60	134
Fatigue	38	60	98
Fever	57	37	94
Headache	18	30	48
Chills	19	24	43
Arthralgia	8	17	25
Other	23	25	48

Table 1. Frequency of adverse reactions after 1st and 2nd vaccination. Only symptoms that occurred immediately after vaccination and for 7 days thereafter were considered vaccination adverse reactions.

Age showed a negative correlation with serum antibody levels at all time points in this follow-up study (**Figure 1**.); data of Day 30, 60, 120 and 150 are not displayed.

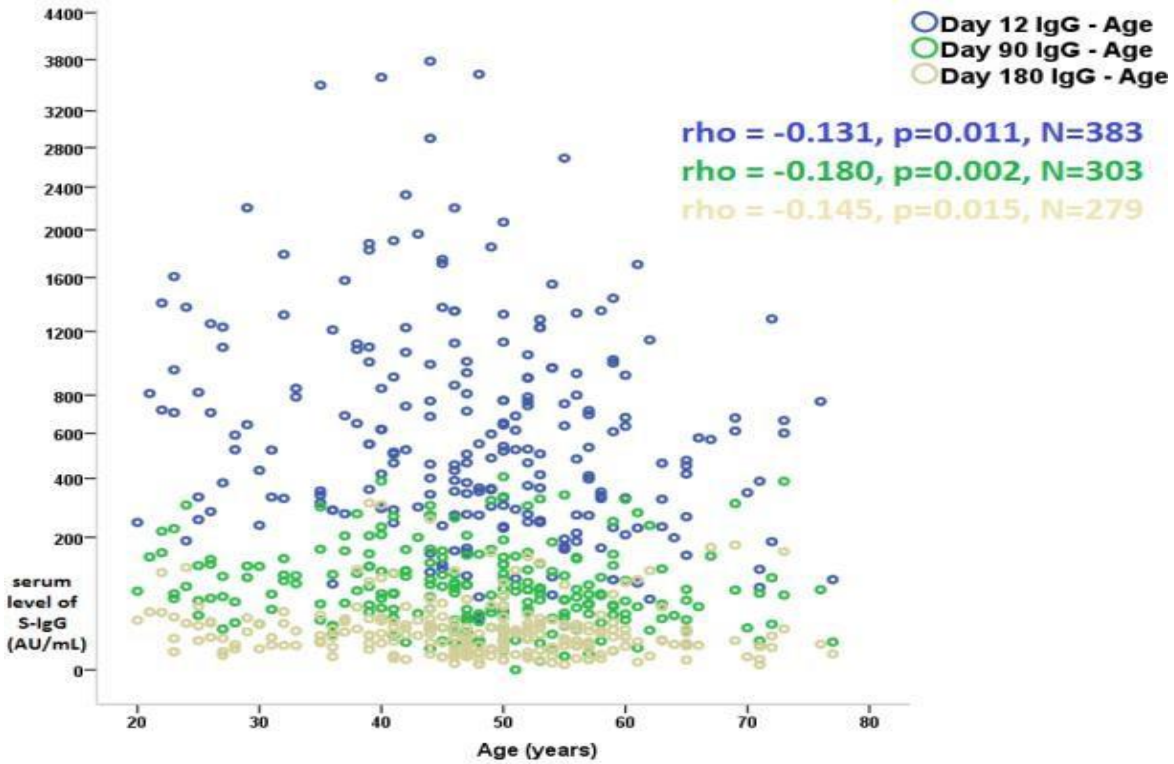


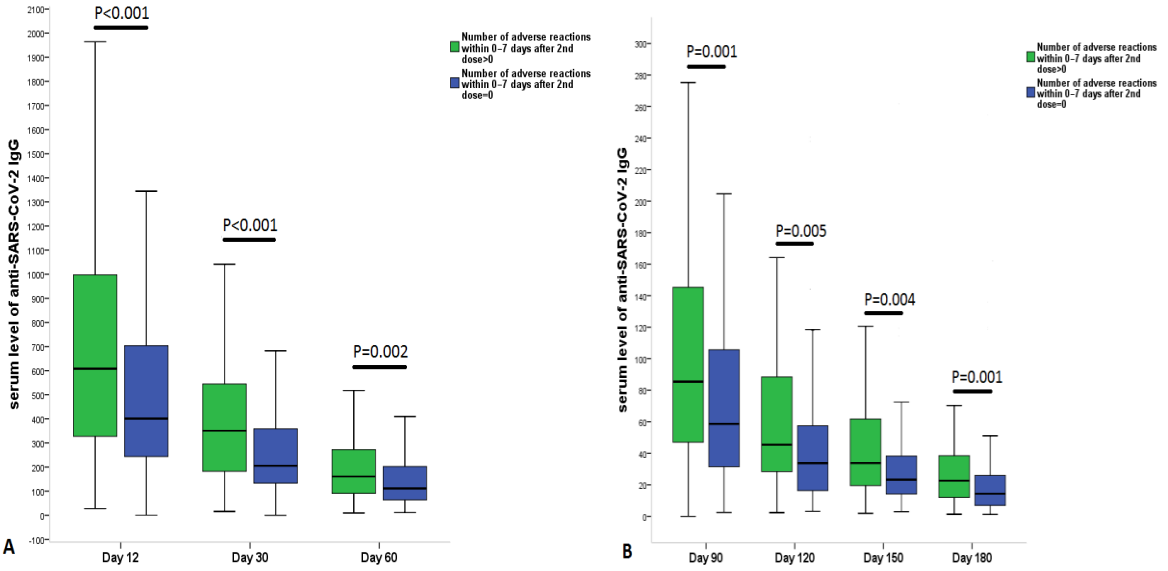
Figure 1. Correlation of serum level of S-IgG and age at Day 12, Day 90, and Day 180 follow-up visit after the 2nd dose of BNT162b2 mRNA (Pfizer/BioNTech, Comirnaty) vaccine. Values are Spearman correlation coefficients (rho). S-IgG; anti-spike immunoglobulin G, mRNA; messenger ribonucleic acid.

Significantly lower serum S-IgG antibody levels were observed in smoking individuals over the entire 6-month study period when compared to non-smokers. Neither female gender nor BMI showed a significant association with antibody production during follow-up. A mild negative correlation was observed between antibody production and ACE inhibitor and statin use respectively, while oral contraceptive treatment was associated with higher antibody levels in the first month (**Table 2.**).

	Day 12 IgG	Day 30 IgG	Day 60 IgG	Day 90 IgG	Day 120 IgG	Day 150 IgG	Day 180 IgG
Gender	0.088	0.050	0.041	0.044	0.002	0.055	0.040
Smoking	-0.107*	-0.134*	-0.177*	-0.142*	-0.164*	-0.091	-0.187**
BMI	-0.006	0.016	-0.024	-0.092	-0.057	-0.018	-0.001
Autoimmunity	-0.121*	-0.119*	-0.167**	-0.138*	-0.153*	-0.122	-0.054
Allergy	0.056	0.111*	0.118*	0.154**	0.106	0.088	0.080
ACE inhibitors	-0.126*	-0.117*	-0.112*	-0.105	-0.087	-0.047	-0.065
Contraceptives	0.121*	0.117*	0.101	0.096	0.114	0.128	0.120
Statins	-0.084	-0.096	-0.095	-0.121*	-0.121*	-0.153*	-0.181**
Hyperlipidaemia	-0.074	-0.085	-0.073	-0.092	-0.124*	-0.151*	-0.081

Table 2. Correlation of S-Ig antibody levels with demographic and clinical factors after 2nd dose of BNT162b2 vaccine manufactured by Pfizer/BioNTech, during the 6-month follow-up period. Values are Spearman correlation coefficients. *p<0.05, **p<0.01

After the first dose fever, chills, and muscle pain showed a strong positive correlation with antibody levels during the 6-month follow-up period. However, after the 2nd dose the strongest positive correlation with antibody titer was observed for fever and chills. Significantly higher serum anti-SARS-CoV-2 spike IgG antibody levels were observed at all time points of the six-month follow-up period in the symptomatic group (**Figure 2.**).



green: symptomatic **blue**: asymptomatic

Figure 2. Comparison of serum level of anti-SARS-CoV-2 IgG at (A) 12, 30, 60 and (B) 90, 120, 150, 180 days after the second dose of vaccination (BNT162b2 mRNA) in patients without or with at least one adverse reaction after each vaccine dose. The data are provided as median and interquartile range. The between-group differences were calculated by the Kruskal-Wallis test.

After grouping patients according to previous COVID-19 infection and adverse reactions after vaccinations, the following results were observed in antibody levels: (i) At the earliest time point at follow-up (Day 12) symptomatic COVID-19 negative patients (Group 2) had the highest antibody levels among the groups; (ii) COVID-19 negative and symptomatic patients (Group 2) had higher antibody levels during the entire 6-month follow- up period than COVID-19 negative and asymptomatic patients (Group 1); (iii) in the first 60 days (Day 12, Day 30 and Day 60) COVID-19 positive status has not led to significantly higher antibody levels in the asymptomatic group compared to COVID-19 negative individuals. This trend was reversed from Day 90, because prior COVID-19 positivity resulted in significantly higher antibody levels at 90-, 120-, 150-, and 180-day follow-up visits in the asymptomatic group. Interestingly, COVID-19 positive but asymptomatic subjects (Group 3) and COVID-19 negative but symptomatic individuals (Group 2) produced similar antibody levels over the 6-month follow-up period, except initial levels at Day 12 (**Figure 3**.).

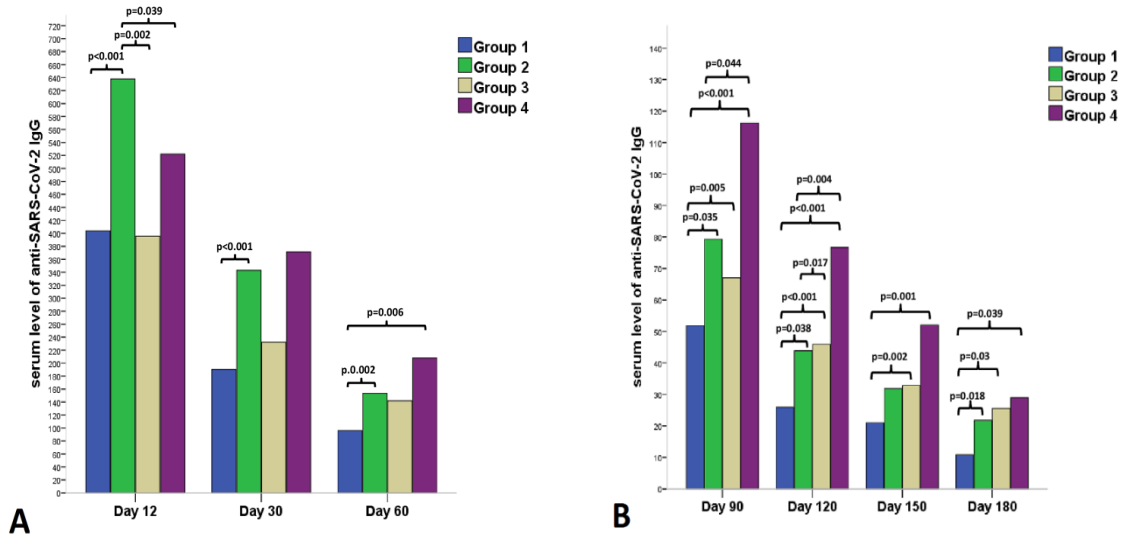


Figure 3. Comparison of serum levels of anti-SARS-CoV-2 IgG at (A) 12, 30, 60 and (B) 90, 120, 150, 180 days after the 2nd dose of vaccination (BNT162b2 mRNA). Healthcare workers were divided into four study groups: Group 1=individuals without prior SARS- CoV-2 infection and with no adverse reaction after vaccination; Group 2=individuals without prior SARS-CoV-2 infection and with at least one adverse reaction after vaccination; Group 3=individuals with prior SARS-CoV-2 infection and with no adverse reaction after vaccination; Group 4=those who had prior SARS-CoV-2 infection and at least one adverse reaction after vaccination. Sample size at each follow-up time point:

	Day 12	Day 30	Day 60	Day 90	Day 120	Day 150	Day 180
Group 1	167	129	136	129	108	90	110
Group 2	131	119	115	115	109	84	107
Group 3	47	42	36	47	26	21	27
Group 4	38	33	33	28	24	24	25

Data are presented as medians and IQR.

4.2. Correlation between adverse reactions, antibody levels and clinical variables after booster vaccination

Collectively, 218 patients were enrolled and underwent blood sampling before and after (Day 14, 60, 120) the 3rd dose of SARS-CoV-2 vaccination. The initial cohort consisted of 383 volunteers, 218 volunteers from the previous cohort were included in the present study. Reasons for dropout: discontinued study (n=101), loss to follow-up (n=25), withdrawal of consent (n=14), physician decision (n=3), dead (n=2), other (n=20). The mean age was 47.6 years, with a prevalence of females (79%). The time difference between the 2nd and 3rd vaccine doses was 249±44 days. 35% (N=77) of participants experienced adverse reactions following the 3rd vaccination. 28% (N=62) of the participants in the study experienced SARS-CoV-2 infection before the 3rd dose, 25% (N=54) of them became positive after the 3rd dose.

The median serum SARS CoV-2 spike Ig level was 72 AU/mL (IQR: 36-135) before the 3rd dose, 639 (424-1100) at 14 days, 413 (215-742) at 60 days, and 268 (128-594) at 120 days after the third dose. In the symptomatic group, the proportion of volunteers receiving the heterologous booster vaccine was significantly higher (16% vs. 3%, p=0.002). The serum SARS-CoV-2 spike Ig level decreases rapidly after a homologous booster dose, after a 14-day peak, while a gradual increase in the antibody level can be seen after a heterologous booster, (**Figure 4.**).

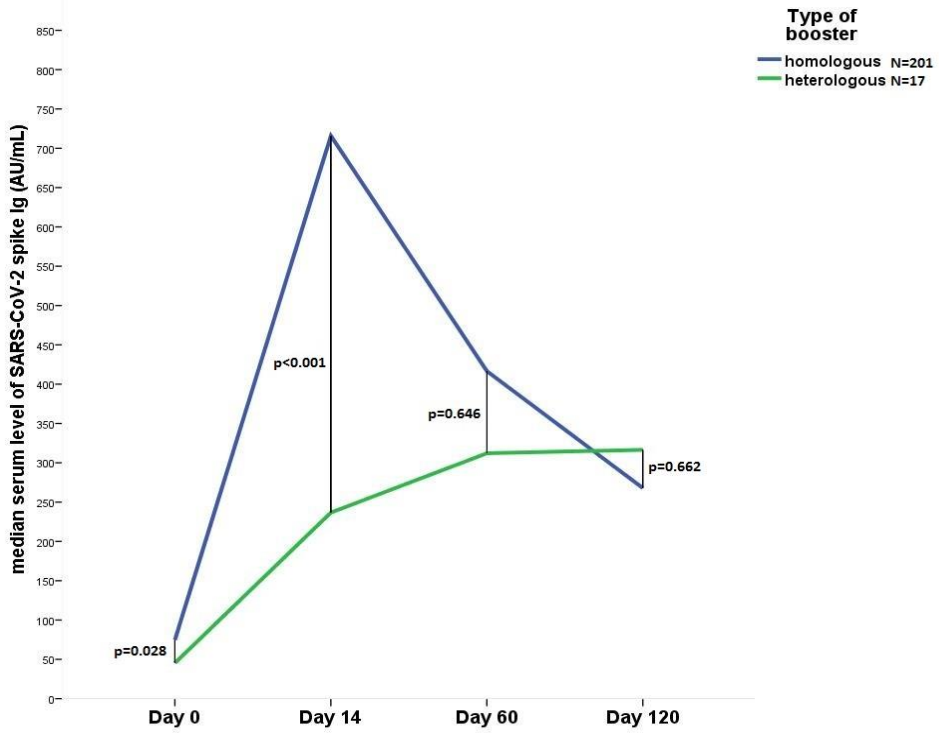


Figure 4. Line diagram shows the change in the median anti SARS-CoV-2 IgG level 14, 60 and 120 days after heterologous (N=17) or homologous (N=201) booster vaccination. Day 0, immediately before 3rd dose.

Adverse reactions occurred in 88/218 patients after the first vaccination, 87/218 after the second vaccination, and 77/218 patients after the third vaccination within 7 days after the dose. The total number of adverse reactions was 234 (first dose), 252 (second dose) and 284 (third dose) after each vaccination. The frequency of fever (N, %) was 27 (13%), 22 (11%) and 36 (17%) after the 1st, 2nd, and 3rd vaccinations, respectively. The most common adverse reaction following the second vaccination was local pain (47%), limb pain (47%), myalgia (36%) and fever (25%) while after the third vaccination local pain was observed in 47%, limb pain in 47%, fever in 46% and chills in 34%. Fever was significantly more frequent after the 3rd vaccination than after the 2nd dose, $p<0.05$.

The serum median SARS-CoV-2 Spike IgG level was significantly higher in the symptomatic group than in the asymptomatic group at all three time points after the second vaccination (Day 14, 60 and 120) (Figure 5A.). After the 3rd vaccination, this correlation disappeared and we did

not detect any significant difference between the serum SARS-CoV-2 spike Ig levels of the two groups (Figure 5B.).

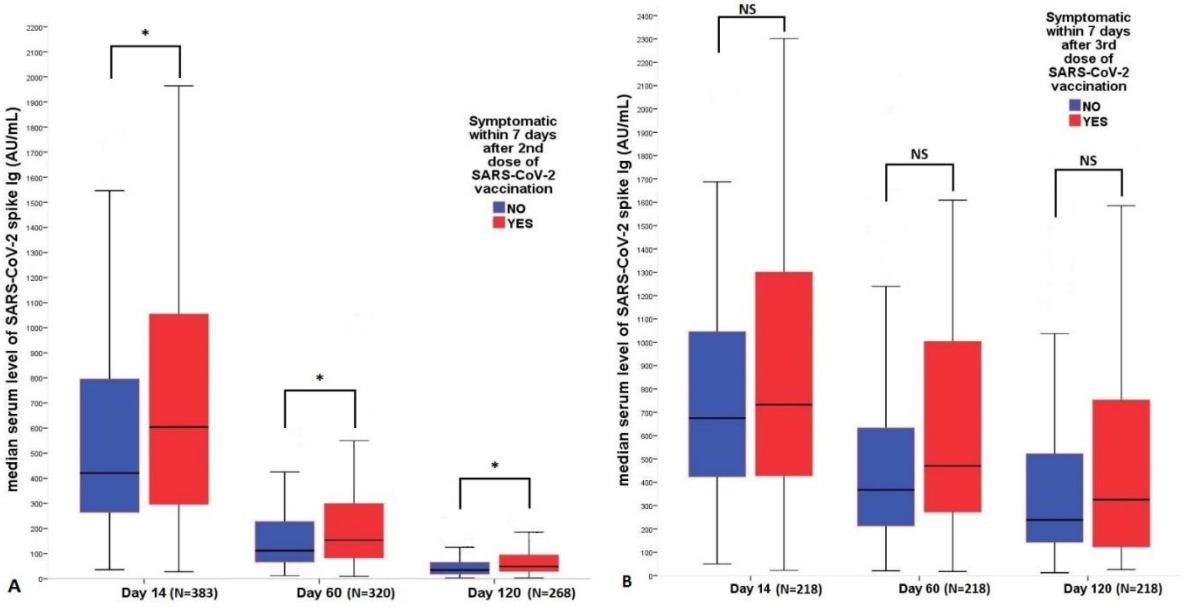


Figure 5. Correlation of antibody titers with symptomatic status after 2nd and 3rd vaccinations. (A) Antibody response of symptomatic versus non-symptomatic patients at 14 (N=383), 60 (n=320) and 120 (N=268) days after the 2nd vaccination, (B) after the 3rd dose (N=218) at all time points. Definition of a symptomatic individual: a local or systematic reaction occurring within 7 days after vaccination. Statistical analysis was performed using Mann-Whitney-U test in each group, respectively. NS, non-significant; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2. * Indicates p<0.05.

If only the serum levels of the volunteers receiving the homologous booster vaccine (N=201) are examined, slightly significantly higher serum levels were observed in the symptomatic patients for the levels measured on days 14, 60 and 120 (p=0.035, p=0.049 and p=0.170 respectively). In the case of volunteers who received a heterologous booster dose (N=17), there was no difference in serum antibody levels between the symptomatic and the asymptomatic group during the studied period. In the case of fever appearing within 7 days after vaccination, significantly higher serum levels were found in the group with fever after both vaccinations,

although the correlation is weaker after the 3rd vaccination, Table 3. We examined the correlations of serum S-IgG levels measured at 4 time points (Day 0, 14, 60 and 120) with demographic and clinical parameters. The Spearman r coefficient of correlation between all these parameters is presented in Table 4.

Fever	Minimum	25%	Median	75%	Maximum	p-Value
			After 2nd dose			
			Day 14 (N=383)			
+ (N=56)	266	689	986	1402	7785	<0.001
- (N=327)	27	262	442	810	655	
			Day 60 (N=320)			
+ (N=49)	96	164	274	457	998	<0.001
- (N=271)	9	70	123	237	655	
			Day 120 (N=268)			
+ (N=45)	28	49	76	148	251	<0.001
- (N=223)	2	19	36	68	379	
			After 3rd dose			
			Day 14 (N=218)			
+ (N=41)	47	388	955	1570	3209	0.045
- (N=177)	22	425	663	1014	5948	
			Day 60 (N=218)			
+ (N=41)	107	331	790	1190	4117	0.002
- (N=177)	17	208	379	670	7101	
			Day 120 (N=218)			
+ (N=41)	56	260	494	815	3005	0.014
- (N=177)	12	124	240	541	2706	

Abbreviations: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ig, immunoglobulin.

Table 3. Changes in serum SARS-CoV-2 spike Ig levels on Days 14, 60 and 120 after the 2nd and 3rd vaccination, depending on whether fever occurred within 7 days after the vaccination. Number of patients with fever (N,%) after 3rd dose: 41/218 (19%).

Variable	Day 0 S-IgG	Day 14 S-IgG	Day 60 S-IgG	Day 120 S-IgG
Age	-0.190**	-0.030	-0.028	-0.089
Smoking	-0.163*	-0.050	-0.128	-0.080
Gender	0.013	0.158*	0.134*	0.149*
mRNS type vaccine	-0.149*	-0.317**	-0.032	0.031
COVID+ before 1st dose	0.293**	0.004	-0.024	-0.042
COVID+between 2nd and 3rd dose	0.144*	0.174*	0.204**	0.116
NSAID	-0.163*	-0.140*	-0.115	-0.145*
Hyperlipidaemia	-0.138*	-0.166*	-0.157*	-0.082
Chills after 2nd dose	0.209**	0.189**	0.138	0.091
Fever after 2nd dose	0.281**	0.261**	0.226**	0.172**
Chills after 3rd dose	N/A	0.138*	0.203**	0.143
Fever after 3rd dose	N/A	0.145*	0.262**	0.189**
Use of beta blocker	-0.169*	-0.054	-0.123	-0.019
Time lag between 2nd and 3rd dose	0.097	0.012	0.118	0.216**

Table 4. Variables associated with levels of S-IgG (AU/mL) in cross-sectional analysis. Day 0, S-IgG measurements immediately before third dose; Day 14, Day 60, and Day 120 S-IgG, S-IgG measurements on 14, 60 and 120 days after third dose. Values are Spearman correlation coefficients. ∗P<0.05, ∗∗P<0.001. S-IgG, anti-spike immunoglobulin; AU, arbitrary unit; COVID+, confirmed corona virus disease-19, mRNS, messenger ribonucleic acid.

SARS-CoV-2 serum spike Ig levels measured before the 3rd dose and 14 days after the 3rd dose were significantly lower in patients who became COVID-19 positive within 120 days after the 3rd dose (before 3rd dose, COVID+: 64 [26-94] vs. COVID-: 79 [38-143], p=0.036, 14 days after 3rd dose, COVID+: 527 [392-778] vs. 734 [461-1203], p=0.015). COVID-19 positivity after 2nd dose and before 3rd dose (OR=2.65; 95%CI=1.05-6.69, p=0.039) was independently associated with COVID-19 positivity within 120 days after booster vaccination, while fever after the 2nd dose (OR=0.14; 95%CI 0.02-0.82; p=0.028) was found to be independently associated with a reduction in the likelihood of COVID-19 positivity within 120 days after the booster dose. The SARS-CoV-2 spike Ig level immediately before the third dose or 14 days after the third vaccination did not prove to be an independent predictor of subsequent COVID positivity.

5. DISCUSSION

In this prospective, single-center follow-up study serum anti-SARS-CoV-2 spike Ig antibody levels were serially recorded in healthcare workers at 12, 30, 60, 90, 120, 150, and 180 days after the 2nd dose of BNT162b2 vaccine.

In our study, we observed significantly lower serum S-IgG antibody titers in older individuals, which is consistent with results previously reported in the literature. A previous study demonstrating that aging decreased antibody response among COVID-19 patients and the fact that aged people demonstrated weaker immunologic responses supports our observations.

Significantly lower serum antibody levels were observed in smoking subjects over the entire 6-month study period when compared to non-smokers. In addition, smoking status was an independent predictor of the median S-IgG level at Day 60, 120 and 180 follow-up visits. However, we have no information on the proportion of seroconversion among smoking and non-smoking volunteers. There is more evidence that smoking lowers serum IgG levels. Smoking was associated with a decrease in serum IgG levels in a small case-control study. In a larger study of 1,787 patients, it was also found that cigarette smoking was associated with reduced IgG median concentrations. There are several explanations for the effect of smoking on the humoral immune response. These might include direct effects on B cells and indirect effects on T cells and antigen-presenting cells, which could affect Ig class switching and/or differential survival of naive B cells or memory B cells. Activity of nicotinic acetylcholine receptors can suppress B-cell activation in response to antigenic challenge. In smokers, we observed significant negative correlation with antibody response to vaccination for a minimum of six months, suggesting that smoking affects the immunogenicity of vaccines in our cohort.

In this study we did not observe a significant difference between genders in terms of antibody response however, the majority of participants were female. Several mechanisms can cause a different antibody response between males and females such as hormonal, genetic, and microbiota differences. Growing body of data provide evidence that sex-specific effects may lead to different outcomes of vaccine safety and efficacy. Therefore, it would be important that

sex-based differences were to be considered and investigated in pre-clinical and clinical trials.

In our study, systemic events such as chills and fever showed a strong correlation with subsequent antibody response against SARS-CoV-2 spike protein. Besides, fever after the second dose proved to be an independent predictor of median S-IgG level at all follow-up time points. The importance of body temperature elevation in an adequate immune response was previously highlighted. Two recent studies found a clear association between systemic adverse events, including fever, and antibody titers after vaccination against COVID-19, which is also consistent with our results.

Physiological temperature change like fever acts to regulate the emergence of new immune responses but does not restrict the activity of existing effector mechanisms once they have been formed. There is a growing body of evidence suggesting that febrile temperatures boost the effectiveness of the immune response during infections by stimulating both the innate and adaptive arms of the immune system. This previous evidence and our results both confirm that the attenuation and elimination of fever in any form (such as the use of NSAIDs) at the beginning of the immune response may adversely affect the immune process, even overall.

Coggins et al. found no correlation between symptom severity following the first or second vaccine doses and IgG reactivity with spike protein, but at the same time a significant correlation was observed with duration of symptoms after the second shot of vaccination and anti-spike IgG titers. *Müller et al.* found that there was not any general correlation between vaccination-induced SARS-CoV-2 spike-specific IgG or neutralizing antibody production and the presence or absence of individual post-vaccination reaction reports. In contrast, a study with the H1N1 vaccine found that titers were 60% higher in children with fever ≥38°C after vaccination, suggesting an enhanced immune response in those who had side effects after vaccination. During the examination of hospital workers who received a prime-boost vaccination with BNT162b2, only a weak but existing correlation was found between the ARs and SARS-CoV-2 antibody levels. In contrast, *Hwang et al.* concluded after vaccination of 135 healthy individuals with either AZD1222 (AstraZeneca) or BNT162b2 (Pfizer/BioNTech) that the local and systemic reactogenicity may not be associated with humoral immunogenicity. However, in two recent studies a clear correlation was found between systemic adverse events

including fever and antibody titer following COVID-19 vaccination, which is also consistent with our results. The literature on the relationship between reactogenicity and immunogenicity of vaccines is limited and contradictory. The inconsistent results shown in the studies are difficult to explain. One possible explanation is that there is no information about the medications taken before and after vaccination, especially regarding the use of NSAIDs. Two (consecutive, randomised controlled, open-label) vaccination studies provided evidence that after vaccination of infants with a ten-valent pneumococcal non-typeable Haemophilus influenzae protein D-conjugate vaccine (PHiD-CV) co-administered with the hexavalent diphtheria- tetanus-3- component acellular pertussis-hepatitis B-inactivated poliovirus types 1, 2, and 3- H influenzae type b (DTPa-HBVIPV/ Hib) and oral human rotavirus vaccines, antibody concentration was significantly lower in the group receiving prophylactic paracetamol than in the group not receiving it. Thus, it is hypothesized that the use of regular or occasional analgesic NSAIDs (or paracetamol) in the peri-vaccination period may affect the production of antibodies. This assumption is supported by several previous evidence NSAIDs suppress T-cell activation by inhibiting p38 MAPK induction, thus the immunosuppressant activity of NSAID on T-cells underlines the role of COX activity in the normal process of lymphocyte activation. *Ryan at al.* found evidence that NSAIDs and the new Cox-2- selective drugs negatively affect B-cell function and attenuate antibody production in humans. NSAIDs are one of the most commonly used drugs; they are recommended for all age categories, are prescribed for relieving transient pain, therefore, their uncontrolled use might affect post-vaccination side effects and may alter the humoral immune response to antigen stimuli. However, the mechanism by which antipyretic analgesics reduce antibody response remains unclear and not fully explained by COX enzyme inhibition, and the involvement of nuclear and subcellular signaling pathways also arises. More detailed immunological studies are needed to accurately determine the effect of NSAIDs or other antipyretic or analgesic drugs on the vaccine-induced immune response. Overall, this evidence may explain the contradictory results in the literature between post- vaccination adverse effects and antibody production.

IgG antibodies in most patients with COVID-19 can last for at least 12 months and the IgG titers decreased significantly in the first 6 months and remained stable in the following 6 months. These results support the findings of our study that previous COVID- 19 infection

compensates for the decrease in antibody levels following vaccination, an effect that occurs primarily in the late phase beyond 90 days. In addition, the correlations observed in our study (correlation between post-vaccination fever and antibody titer) showed a robust association for 6 months.

Our study showed that adverse reactions after the second vaccination against SARS-CoV-2 are associated with a significantly higher serum SARS-CoV-2 spike Ig level for up to 6 months. Other studies have also shown that specific IgG titers after basic BNT162b2 vaccination (2 doses) showed a positive correlation with the occurrence of adverse reactions.

In contrast, in our later study, which primary goal was to investigate whether the adverse reactions following the booster vaccination show a correlation with subsequent antibody levels, we observed that (i), the booster dose in the same cohort no longer shows any correlation between the appearance of adverse reactions and the subsequent antibody level, except a positive correlation with fever. However, this correlation is significantly weaker than observed after the second dose. In contrast to the basic homologous vaccination strategy the booster vaccination also featured a heterologous version, which may have influenced the number and quality of adverse reactions, given the different immunological mechanisms. It is likely that the different immune activation caused by the heterologous immunization is also associated with different adverse reactions, this could be one of the likely mechanisms why the adverse reactions after the 3rd vaccination did not show a correlation with the later antibody response. At the same time, the small number of cases might also explain why we were unable to demonstrate the clear correlation between adverse events and antibody levels that we observed in our previously examined population. Although the difference in the increase of immunoglobulin levels between patients with and without fever is smaller after the third vaccination than after the second vaccination in our study, the booster effect of the vaccine remains evident: it triggered better sustained and higher levels of immunoglobulin compared to those observed following the second vaccination dose. While the second vaccination is part of the basic immunization, where the goal is to form immune memory and teach the immune system to respond to the spike protein, the booster dose is to strengthen the process, making

these mechanisms permanent. Other important findings of our study are the following: (ii) similar to a previous study, we also observed that systemic adverse reactions were reported more often by the younger volunteers than the older ones. This can be explained by the reduced strength of the inflammatory response in the elderly exposed to immune stress. Another potential mechanism of this phenomenon is that older people show a greater tolerance for pain and disease symptoms acquired through life._volunteers in the symptomatic group were younger, (iii) there were more symptomatic patients in the group receiving the heterologous booster vaccine, (iv) those who were symptomatic after the 1st and 2nd dose were more likely to be symptomatic after the third vaccination as well. Finally, (v) fever after second dose was independently associated with a reduction in the likelihood of COVID-19 positivity after booster dose. This can presumably be explained by the fact that fever following the second vaccination induces a stronger humoral immune response and the protective effect of the consequent higher antibody level prevails even after the booster vaccination.

The strength of our results is given by the relatively considerable number of volunteers and the long follow-up period. A significant limitation of our study is that the fact and frequency of NSAID use after vaccination were not recorded in our study questionnaire. Thus, their potential effect on the association between antibody production and adverse events after vaccination cannot be established. Furthermore, the male population was underrepresented and conclusions on gender differences in vaccine response is limited. Some volunteers missed follow-up dates, which reduced the number of participants at consecutive follow-up times, but the statistical power of our results remained strong. To accurately report adverse reactions, volunteers kept a diary. Using this will help reduce the possibility of recall bias, but it cannot be completely ruled out.

Another limitation of our study is that the number of patients receiving a heterologous booster dose is much lower than the number of patients receiving homologous vaccination, which may affect the strength of the statistical results. The follow-up period was only 3 months; a longer observation period would have allowed a better follow-up of the change in antibody levels of the respective groups. We only observed the humoral immune response and not the cellular immune response, so we can only explain the immunological changes that took place after the

third vaccination to a limited extent. At the same time, the strength of our study is that it points out that the type of booster vaccination (heterologous vs. homologous) probably causes different immune activation, which can also be inferred from the different vaccination reactions.

6. CONCLUSION

The overall aim of this thesis was to draw general conclusions that can essentially influence the assessment of adverse effects following the administration of vaccines, especially vaccines against SARS-CoV-2. According to our current knowledge, adverse reactions are the adequate reaction of the immune system to a foreign antigenic stimulus, the strength and pattern of which can also affect the humoral immune response.

As a novelty, we investigated the correlation of fever with antibody levels during prime and boost vaccination. The major findings were the following:

(i) After primary immunization against SARS-CoV-2, serum median S-IgG level measured at all follow-up time points during the examined period (6 months) was significantly higher in the symptomatic group (fever!) compared with asymptomatic.

(ii) Lower S-IgG levels were detected in older individuals, which correlates with the results reported in the literature. Several studies have demonstrated that aged people produce a weaker immunological response.

(iii) Significantly lower serum antibody levels were observed in smoking subjects over the entire 6-month study period compared with non-smokers. There are several evidence for the negativ effect of smoking on the humoral immune response.

(iv) In our study we did not detect a significant difference between genders in the antibody response, which could be caused by several mechanisms.

(v) In this study, systemic events such as chills and fever were strongly correlated with the subsequent antibody response to SARS-CoV-2 spike protein. In addition, fever occurring after the second dose proved to be an independent predictor of median S- IgG level at the follow-up times. We were also able to show that:

(vi) The correlation between the appearance of adverse events and the subsequent antibody level disappeared in the same cohort following the booster vaccination, except in the case of fever.

(vii) The volunteers in the symptomatic groups were younger.

(viii) There were more symptomatic individuals in the heterologous booster group.

(ix) Those who reported some kind of adverse reaction after the first and second dose were more likely to be symptomatic after the third vaccination as well. The type of booster vaccination (heterologous or homologous) theoretically causes different immune activation, which can also be supposed from the different vaccination reactions.

(x) Fever after the second dose was independently associated with a reduced likelihood of COVID-19 positivity after the booster dose.

Several factors have an impact on antibody levels after SARS-CoV-2 vaccination including age, smoking status, prior COVID-19 positivity, and adverse reactions after each dose of vaccines. Fever was associated with higher median S-IgG level during a 6-month follow-up period. These results may convince those who refuse vaccination due to fear of vaccination reactions. In addition, an individual approach that takes all factors influencing antibody levels into account might be useful when developing a vaccination strategy. Large, prospective studies are needed to fully explore the effect of post-vaccination fever on the developing immune response.

7. FURTHER AIMS AND PERSPECTIVES

These previous evidence and results both confirm the role of fever in the process of the immune response, so theoretically the question arises regarding the role of antipyretics and anti-inflammatory drugs. A further study involving volunteers would help to correctly interpret the differences between the quality and quantity of adverse reactions with the use of antipyretics and analgetic, as well as their correlation regarding to the antibody levels, which we observed in our current study. It can be useful to study the effect of these drugs on the immune response, so that their possible antibody level-modifying effect can be interpreted in post-vaccination immunization, thereby improving the effectiveness of vaccinations. During their use, in addition to revealing the long-term effects, their role in modifying the probability of subsequent infection or the course of the disease also arises. An individualized approach that considers all factors affecting antibody levels is important when developing a vaccination strategy. Large, prospective studies are needed to fully explore the effect of post-vaccination fever and associated factors on the developing immune response.

8. LIST OF PUBLICATIONS

Publications related to the thesis:

Kanizsai A, Molnar T, Varnai R, Zavori L, Tőkés-Füzesi M, Szalai Z, Berecz J, Csecsei P. Fever after Vaccination against SARS-CoV-2 with mRNA-Based Vaccine Associated with Higher Antibody Levels during 6 Months Follow-Up. Vaccines (Basel). 2022 Mar 14;10(3):447. doi: 10.3390/vaccines10030447. PMID: 35335080; PMCID: PMC8950492.

IF: 4,961

Kanizsai A, Zavori L, Molnar T, Tőkés-Füzesi M, Szalai Z, Berecz J, Varnai R, Peterfi Z, Schwarcz A, Csecsei P. Adverse Reactions after Booster SARS-CoV-2 Vaccination Have Less Impact on Antibody Response than after Basic Vaccination Scheme. Vaccines (Basel). 2023 Jan 15;11(1):182. doi: 10.3390/vaccines11010182. PMID: 36680026; PMCID: PMC9864401.

IF: 4,961

Cumulative impact factor related to the thesis: **9,922**

Other publications:

Varnai R, Molnar T, Zavori L, Tőkés-Füzesi M, Illes Z**, Kanizsai A**, Csecsei P. Serum Level of Anti-Nucleocapsid, but Not Anti-Spike Antibody, Is Associated with Improvement of Long COVID Symptoms. Vaccines (Basel). 2022 Jan 21;10(2):165. doi: 10.3390/vaccines10020165. PMID: 35214624; PMCID: PMC8924883.

Zavori L, Molnar T, Varnai R, **Kanizsai A**, Nagy L, Vadkerti B, Szirmay B, Schwarcz A, Csecsei P. Cystatin-c May Indicate Subclinical Renal Involvement, While Orosomucoid Is Associated with Fatigue in Patients with Long-COVID Syndrome. J Pers Med. 2023 Feb 19;13(2):371. doi: 10.3390/jpm13020371. PMID: 36836605; PMCID: PMC9958557.

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