

# The Healthcare Study Examines the Humoral Anti-S1 Antibody Response Following mRNA Vaccination, Comparing Individuals with and without Prior SARS-CoV-2 Infection

Małgorzata Staruszkiewicz<sup>1</sup> · Anna Pituch-Noworolska<sup>2</sup> · Mohamad Skayne<sup>3</sup> · Torsten Matthias<sup>2</sup> · Szymon Skoczen<sup>4,5</sup>✉

## Abstract

Vaccines targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been pivotal in curtailing the spread of infection. Health care workers, as frontline responders, were among the first to receive vaccination to mitigate coronavirus disease in 2019 (COVID-19) transmission. This study aimed to assess the humoral response elicited by mRNA vaccines, specifically measuring antibodies against the spike S1 protein, a marker of immune response. A cohort of 649 health care workers received three doses of mRNA vaccine, with antibody levels evaluated before and after each dose within a 2- to 3-week interval. Participants were stratified into groups based on prior exposure to the virus: those without prior contact (440 individuals) and those with a history of infection (209 individuals). Among the latter, cases of SARS-CoV-2 infection ranged from asymptomatic (92 individuals) to mild symptomatic (117 individuals). Participants with a history of infection exhibited elevated levels of IgG antibodies against the S1 protein prior to vaccination. Notably, both immunoglobulin IgA class (IgA) and immunoglobulin IgG class (IgG) antibody responses increased significantly post-vaccination, peaking after the second dose for IgG and after the third dose for IgA. Interestingly, the immune response to the vaccine did not vary significantly based on the symptomatic or asymptomatic nature of prior infection. Furthermore, the study findings indicate that completion of the vaccination regimen led to sustained antibody production lasting between 6 months and 9 months. This study underscores the robust and enduring humoral response elicited by mRNA vaccines, particularly among health care workers, irrespective of prior SARS-CoV-2 exposure.

## Keywords

SARS-CoV-2 · COVID-19 · S1 protein · Vaccine · Antibodies · Immunoglobulins

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## 1. Introduction

### 1.1. SARS-CoV-2 immunology and immune response

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the RNA coronaviridae family, emerged as the causative agent of the coronavirus disease in 2019 (COVID-19). This virus exhibits a notable affinity for the angiotensin-converting enzyme 2 (ACE2) receptor found on epithelial cells, particularly in organs like the lungs, facilitating infection and subsequent tissue damage. Upon infection, the innate and adaptive immune systems mount responses to combat the virus (Catanzaro et al. 2020; Esmaelizadeh and Elahi 2020; Mortaz et al. 2020; Vabret et al. 2020; Sette and Crotty 2021).

The adaptive immune response initiates with antigen-presenting cells recognizing and presenting viral antigens, thereby stimulating and orchestrating cellular immune

responses against SARS-CoV-2. Activation of T helper (Th) cells prompts the secretion of Th1 cytokines such as interferon gamma (IFN- $\gamma$ ), tumor necrosis factor (TNF), and interleukin 2 (IL-2). Notably, cytotoxic T lymphocytes (CD8<sup>+</sup>) play a pivotal role in viral clearance through targeted elimination of infected cells. Among the most immunogenic antigens fostering the induction of memory T and B cells are the spike (S) protein and the matrix (M) protein from the viral capsule. Longitudinal studies have demonstrated a decline of approximately half in the memory CD8<sup>+</sup> T-cell population within 6 months post-SARS-CoV-2 infection, contrasting with a similar decline in memory CD4<sup>+</sup> T cells observed 3 months after patient recovery (Dan et al. 2021).

### 1.2. B lymphocytes and antibodies

T follicular helper cells specific to SARS-CoV-2 within lymph nodes play a crucial role in stimulating B lymphocytes to generate antigen-specific antibodies and establish a memory B-cell subset. Notably, seroconversion from immunoglobulin IgM class (IgM) to IgG antibodies occurs in approximately 90% of patients infected with SARS-CoV-2, often within 10 days post-onset of clinical symptoms. Many of these

<sup>1</sup> Department of Pathology, University Children's Hospital, Krakow, Poland

<sup>2</sup> Department of Immunology, University Children's Hospital, Krakow, Poland

<sup>3</sup> AESKU. Diagnostics GmbH & Co. KG, Sales and Marketing Department, Wendelsheim, Germany

<sup>4</sup> Department of Pediatric Oncology and Hematology, Institute of Pediatrics, Jagiellonian University, Medical College, Krakow, Poland

<sup>5</sup> Department of Oncology and Hematology, University Children's Hospital, Krakow, Poland

✉ szymon.skoczen@uj.edu.pl

patients demonstrate neutralizing activity against the spike (S) protein and its receptor binding domain (RBD), crucial for blocking viral attachment to the ACE2 receptor. However, the production kinetics and titers of antibodies vary considerably among symptomatic SARS-CoV-2 cases.

In a cohort study involving 6062 healthcare workers exposed to SARS-CoV-2, specific antibodies were detected in 212 individuals, particularly among those of younger age who exhibited typical symptoms such as anosmia, dry cough, and myalgia with fatigue (Ebinger et al. 2020; Garcia-Beltran et al. 2021).

The significance of neutralizing antibodies in combating SARS-CoV-2 is evident, as they aid in reducing viral load through cell-mediated mechanisms. Nevertheless, observations from patients with X-linked agammaglobulinemia, who recovered from SARS-CoV-2 infection without antibody production, underscore the essential role of antibodies in virus clearance. Additionally, concerns have been raised regarding the potential adverse effects of antibodies, including inflammation enhancement (antibody-dependent enhancement) and organ damage, particularly in the lungs (Vabret et al. 2020; Bettini and Locci 2021; Fraley et al. 2021; Garcia-Beltran et al. 2021; Sette and Crotty 2021; Vashishtha and Kumar 2021).

### 1.3. Vaccine and antibody responses

Antibodies targeting the S1 protein serve as vital indicators for evaluating the efficacy of immunization strategies (Tregoning et al. 2020; Bettini and Locci 2021; Vaquero et al. 2021; Vashishtha and Kumar 2021; Melgoza-González et al. 2022; Salleh et al. 2022). Following administration of mRNA vaccines, individuals not previously infected experience a robust germinal center response, accompanied by increases in plasma cell numbers and expansion of the memory B-cell population. Conversely, individuals with prior SARS-CoV-2 infection exhibit heightened responses following the initial vaccine dose, with a majority of antibodies demonstrating neutralizing capabilities. Notably, the magnitude of antibody production correlates with the proliferation of specific B memory cells (Laidlaw and Ellebedy 2022; Padoan et al. 2022; Roltgen et al. 2022; Van Elslande et al. 2022).

Studies have scrutinized antibody synthesis during SARS-CoV-2 infection, post-vaccination in non-infected individuals and post-infection followed by vaccination. Sequential vaccine doses consistently elevate antibody production, with a notable prevalence of the IgG1 subclass. Vaccination induces a response primarily in the IgA1 subclass, with no significant increase observed in IgM levels post-vaccination (Vaquero et al. 2021; Salleh et al. 2022). Furthermore, individuals recovering from SARS-CoV-2 infection exhibit enhanced vaccine responses compared to those without prior infection,

particularly when following similar vaccination schedules. Incorporating a third dose into the vaccination regimen significantly boosts specific antibody production, regardless of prior infection status (Ebinger et al. 2021; Fraley et al. 2021; Vaquero et al. 2021; Padoan et al. 2022; Salleh et al. 2022). Enhanced vaccination schedules, including third and fourth doses, prompt robust responses, stimulating the production of memory T and B cells alongside a substantial proportion of neutralizing antibodies (Amanat et al. 2021; Padoan et al. 2022; Roltgen et al. 2022).

This study aimed to analyze the titer and kinetics of anti-S1 protein antibodies (including IgG, IgA, and IgM) across various vaccination regimens (from first to third dose), particularly in relation to prior SARS-CoV-2 infection. Conducted among healthcare workers at a pediatric hospital, the research seeks to provide insights into antibody responses among this specific population.

## 2. Materials and Methods

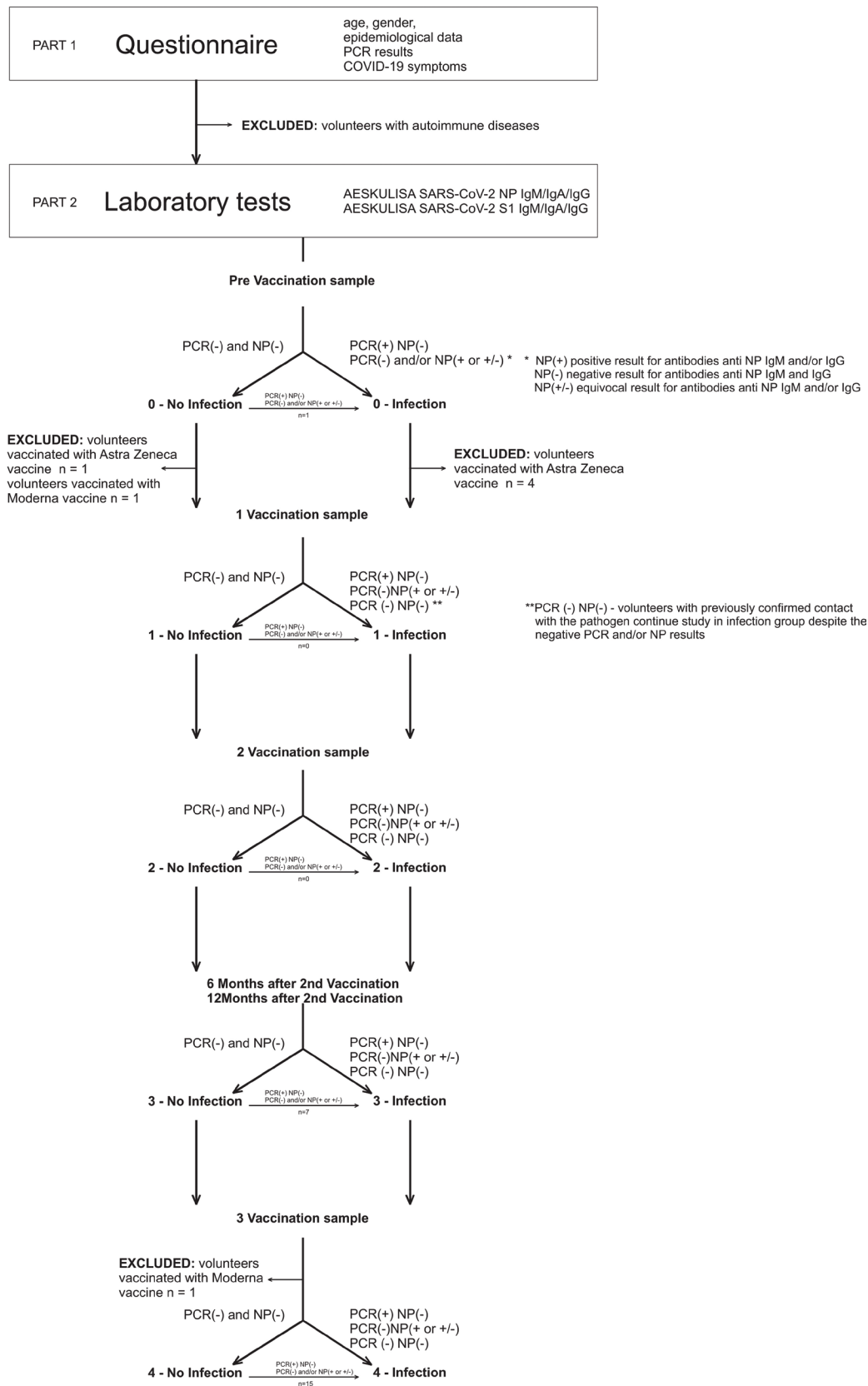
### 2.1. Material

Under “The Staff Vaccination Program,” employees from the University Children Hospital in Krakow participated in a study evaluating antibody production targeting the SARS-CoV-2 spike S1 protein following vaccination with the BioNTech-Pfizer BNT162b2 vaccine in two doses (later adjusted to include a third dose by the government). The cohort comprised 43 men and 606 women, totaling 649 participants. Data on participants’ SARS-CoV-2 exposure history, PCR results, COVID-19 symptoms, comorbidities, medical treatment, vaccination tolerance, and complications were self-reported. Ethical approval was obtained from the Jagiellonian University Medical College Ethics Committee.

### 2.2. Methods

Serum samples were collected from the participants according to the following schedule:

- Before the administration of the first vaccine dose in select participants.
- Within 3–4 weeks after the first vaccine dose.
- About 2–4 weeks after the administration of the second or third vaccine dose. The second dose was administered approximately 3 weeks after the first, with the final dose given 3–5 months later. The vaccination program commenced in late December 2020, initially utilizing the Pfizer-BioNTech BNT162b2 vaccine. In March 2021, some participants received the AstraZeneca ChAdOx1 nCoV-19 vaccine and were subsequently excluded from the study (Figure 1). Samples characteristics are shown in Table 1.



**Fig 1.** Study design schema. COVID-19, coronavirus disease in 2019; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2.

**Table 1.** Characteristics of the study samples

Groups	Infection	No infection
<b>Pre vaccination samples</b>		
N	17	13
Age (years), mean (SD)	42.1 (10)	43.7 (13)
Race %	White 100%	White 100%
Gender M/F	2/15	2/11
<b>1 Vaccination samples</b>		
N	40	62
Age (years), mean (SD)	45.8 (10)	42.3 (11)
Race %	White 100%	White 100%
Gender M/F	5/35	5/57
<b>2 Vaccination samples</b>		
N	101	221
Age (years), mean (SD)	48.9 (11)	45.6 (11)
Race %	White 100%	White 100%
Gender M/F	16/85	29/192
<b>3 Vaccination samples</b>		
N	15	40
Age (years), mean (SD)	44.4 (12)	42.3 (12)
Race %	White 100%	White 100%
Gender M/F	3/12	9/31

SD, standard deviation.

### 2.3. Serological analysis

Serological analyses were conducted at AESKU.Diagnostics GmbH & Co. KG's facilities in Wendelsheim, Germany, using AESKULISA® SARS-CoV-2 nucleocapsid protein (NP) IgM, IgA and IgG, as well as AESKULISA® SARS-CoV-2 S1 IgM, IgA and IgG immunoassays. Tests were performed following the manufacturer's instructions. Test results >12 U/mL were considered positive, while results <8 U/mL were deemed negative. Antibody activities were quantified using recommended dilutions, with results referenced to the international standard preparation for easier comparison with other studies.

### 2.4. Statistical analysis

Participants were divided into two groups:

- Group 1 comprised individuals with evidence of prior infection, confirmed by positive SARS-CoV-2 polymerase chain reaction (PCR) or serological test results prior to vaccination, and positive serological test results after the second dose.
- Group 2 included staff without evidence of prior infection, including those with negative PCR or serological test results.

## 3. Results

The COVID-19 pandemic has presented unique challenges for medical professionals, particularly in pediatric hospitals, where asymptomatic children pose a risk of infection to hospital staff. University Children's Hospital in Krakow responded by implementing PCR tests for children and their parents upon admission, while testing hospital staff only after exposure to infected individuals.

During the pandemic's onset, the hospital lacked the capability to develop COVID-19 antibody tests. In collaboration with AESKU Diagnostics GmbH & Co. KG, Jagiellonian University facilitated a study to monitor antibody production post-infection and post-vaccination among hospital staff. Participation in the study was voluntary.

The participants were divided into two groups according to the information available from the questionnaire and the results of SARS-CoV-2 tests prior to immunization. The first group included 440 persons (M:F – 29:411, mean age: 46.9 years, 20–74 years) without evidence of prior contact with SARS-CoV-2 (negative PCR, negative test results with AESKULISA® SARS-CoV-2 NP or S1 immunoassays and no clinical symptoms of SARS-CoV-2 infection) prior to the first dose of vaccination. The second group consisted of 209 persons (M:F – 14:195, mean age: 47.88 years, 22–84 years) with evidence of prior contact with SARS-CoV-2: positive PCR and/or positive or borderline test results (>8 U/mL) with AESKULISA® SARS-CoV-2 NP immunoassays prior to the first dose of vaccination. In this cohort, asymptomatic SARS-CoV-2 disease was noted or reported in 92 individuals (M:F – 5:87, mean age: 49 years, 22–84 years), and symptomatic disease was observed in 117 individuals (M:F – 9:108, mean age 47 years, 23–71 years). Symptomatic infections were generally mild without the need for hospitalization. Symptoms of the SARS-CoV-2 infections were as follows: headache n=84 (71.8%), anosmia n=69 (58.9 %), loss of taste n=67 (57.2 %), diarrhea n=29 (24.8%), stomach pain n=27 (23.0%), muscle pain n=17 (14.5%), weakness n=15 (12.8%), fever n=14 (11.9%), cough n=8 (6.8%), tiredness n=8 (6.8%), sore throat n=6 (5.12 %), common cold syndrome n=5 (4.27%), sinusitis, breathing difficulties, flu-like symptoms, hoarseness n=2 (1.70%), dizziness, smell irritation, metallic taste, back ache (kidneys?) n=2 (1.70%), hair loss, somnolence, skin hypersensitivity, lymph node swelling, sweating, eye pain and problems with vision, rash, shivers n=1 (0.85%).

The time between infection and immunization was no shorter than 3 months, or special indications were established separately. The study began when the vaccination protocol was ongoing, which resulted in different numbers of people being tested before, after the first, and following vaccination doses. Serum samples for subsequent analysis with AESKULISA®

SARS-CoV-2 NP or S1 immunoassays after the first and third doses of vaccination were collected 19–25 days post immunization, and serum samples after the second dose of vaccination were collected 14–60 days after vaccination.

### 3.1. IgM, IgA, and IgG antibodies directed against SARS-CoV-2 NPs

The average concentration of IgG anti-NP antibody in participants with prior SARS-CoV-2 infection was greater after the first and third doses of vaccination than before vaccination (mean values 27.4 U/mL and 24.8 U/mL, respectively, compared to 20.6 U/mL) but lower after the second dose (mean value 16.6 U/mL) (Table 2 and Figure 2). GraphPad Prism version 6.01 was used for statistical analyses. Kruskal-Wallis test was conducted to identify any significant changes in categorical variables over time and between groups. Non-parametric Mann-Whitney (Wilcoxon) test was used to compare quantitative data over time or between groups, respectively. Participants without SARS-CoV-2 contact had low antibody levels (below the limit of positive results). There was a statistically significant difference between participants after contact with SARS-CoV-2 and those without infection at all the assayed points. Positive or borderline anti-NP IgG antibody activity was considered a marker for previous infection with SARS-CoV-2 (Salleh et al. 2022). Individuals from the non-infected group with positive or borderline anti-NP IgG antibody levels at the time of vaccination were included in the “infection” group and were tested for reinfection with SARS-CoV-2. In patients without SARS-CoV-2 infection, the increase in the anti-NP antibody levels after vaccination may be the result of the period between infection and vaccination, persistent immune memory of the virus proteins, and nonspecific stimulatory activity of the vaccine. The production of this type of antibody by memory B cells against the NP protein is stimulated by vaccination as an effect of the general immune system being stimulated by the vaccines. Observation of antibody production in the majority of patients recovered from severe infection 1 year after infection and in patients after a mild course supported the induction of a persistent memory B-cell population (Van Elslande et al. 2022). The mean IgA antibody level against the NP was negative in both groups during the study. In some individuals from the group after prior infection, IgA antibodies were detected, with the highest value of 39.7 U/mL after the second dose of vaccination. The presence of anti-NP IgM antibodies was observed in individual participants, although the mean value remained below the cutoff point throughout the study. Despite this, the group of individuals with prior evidence of SARS-CoV-2 infection exhibited a slightly elevated average level of anti-nucleoprotein IgM. The maximum IgM antibody activity recorded was 36.2 U/mL in one participant after the second dose of vaccination.

Interestingly, participants with detectable IgA and IgM antibodies, above the cutoff point, did not display any symptoms of disease and did not develop COVID-19 during the 6 months observation period following the last dose of vaccination. This suggests that the presence of these antibodies may confer some level of protection against SARS-CoV-2 infection or disease manifestation.

### 3.2. IgG, IgA, and IgM antibodies directed against SARS-CoV-2 S1 after vaccination

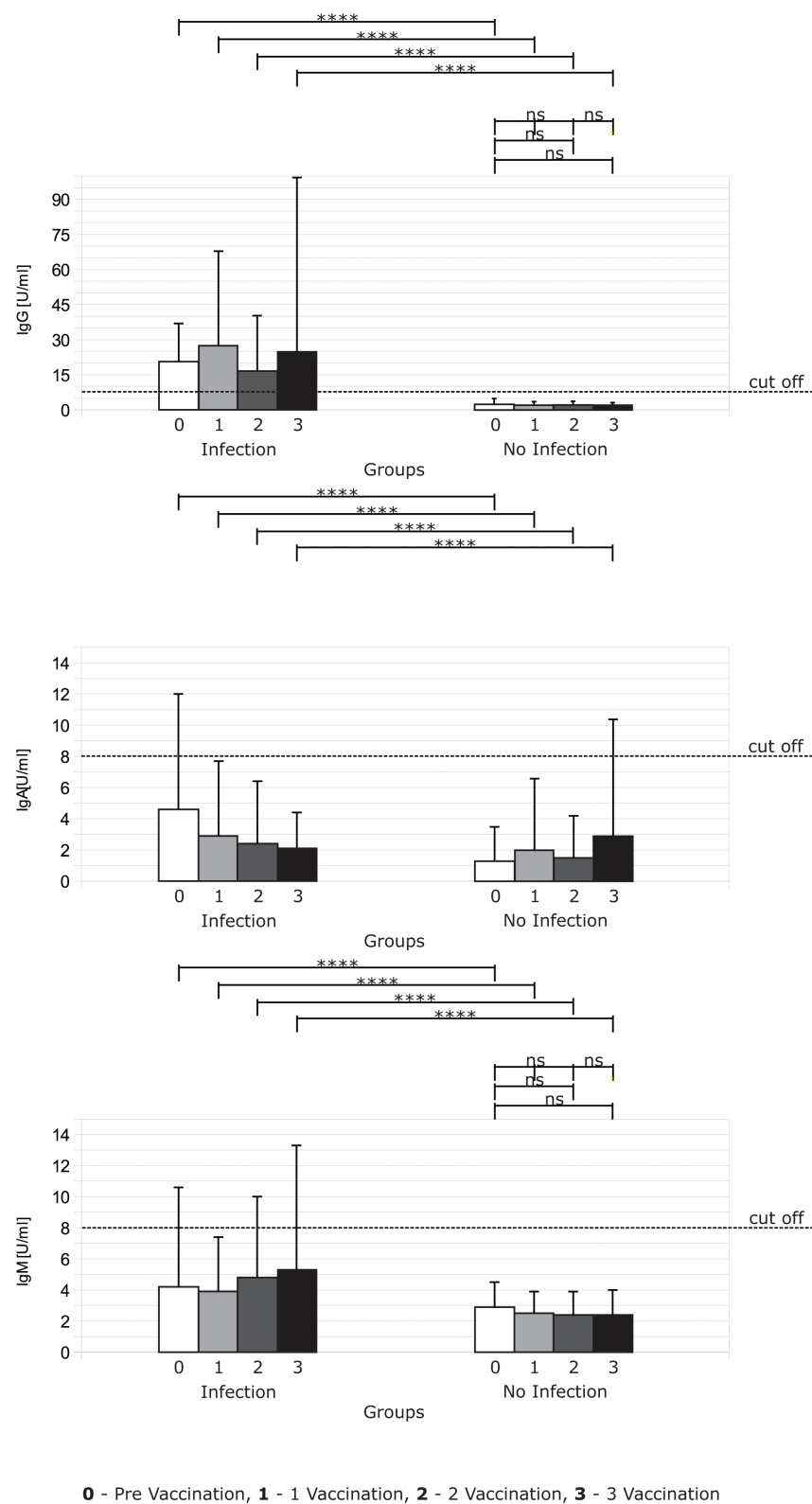
The most interesting observation was the antibody profile of the anti-S1 IgG immune response to the vaccination (Table 3 and Figure 3). The anti-S1 IgG antibody level in the group without prior SARS-CoV-2 infection was high after the first dose of vaccination (347.1 BAU/mL from 12.2 BAU/mL) and further increased after the second dose of vaccination, which was expected (mean value 2329.5 BAU/mL). The final dose of vaccination did not increase the mean IgG antibody level (1522.6 BAU/mL) in the noninfected group of participants. The highest individual anti-S1 IgG antibody levels after subsequent doses of the vaccine were 2672.8 BAU/mL after the first dose, 7937.0 BAU/mL after the second dose and 5304.3 BAU/mL after the final dose. The anti-S1 IgG antibody activity was already high before vaccination in the group with prior SARS-CoV-2 infection (symptomatic or asymptomatic), with a mean value of 88.3 BAU/mL (maximal value—456.2 BAU/mL) and an increase of up to 1696.2 BAU/mL after the first dose of vaccination. The individual maximal values were 7281.4 BAU/mL. However, the average anti-S1 IgG antibody level after the second dose was slightly greater than that after the first dose (mean value—1969.2 BAU/mL), with a decrease (mean value—1152.1 BAU/mL) after the final dose of vaccination. An explanation for this decrease might be the long duration (up to 6 months) between the second and third doses (final) and the still high level of IgG before the final dose (infection group 1, 164 participants; mean value, 330.6 BAU/mL; no-infection group 2, 377 participants; mean value, 163.7 BAU/mL). According to a separate analysis (data not shown), IgG antibody production after vaccination in participants with asymptomatic SARS-CoV-2 infection in comparison to that in the symptomatic group (although with mild symptoms) did not significantly differ.

The systematic increase in IgG antibody concentration after two doses of vaccination in the group without prior contact with the virus aligns with expectations for a robust immune response following vaccination. However, the lack of a further increase in IgG antibody levels after the third dose, coupled with a decrease compared to the response after the second dose, suggests a potential plateau or diminishing returns in antibody production with additional doses. Despite variations in individual responses within both groups, all participants

**Table 2.** Changes of IgM, IgA and IgG antibodies levels against NP produced post vaccination according to prior infection of SARS- Cov-2.

IgA											
Group	Before vaccine			After first vaccine			After second vaccine			After third vaccine	
	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL
Infection	17	4.6 0.4–30.7 7.4	4.8 1.8 1.1	40	2.9 0.3–28.9 4.8	2.7 1.4 0.8	101	2.4 0.1–39.7 4.0	2.3 1.2 0.8	15	2.1 0.2–15.8 2.3
No infection	13	1.3 0.3–8.7 2.2	1.1 0.7 0.5	62	2.0 0.2–36.0 4.6	1.4 0.9 0.6	221	1.5 0.0–33.1 2.7	1.3 0.8 0.6	40	2.9 0.3–43.1 7.5
IgM											
Group	Before vaccine			After first vaccine			After second vaccine			After third vaccine	
	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL
Infection	17	4.2 0.6–28.5 6.4	3.5 2.6 1.7	40	3.9 0.4–17.4 3.5	4.8 2.8 1.7	101	4.8 0.2–36.2 5.2	6.5 7.8 1.6	15	5.3 0.6–47.2 8.0
No infection	13	2.9 0.7–5.8 1.6	3.8 3.3 1.7	62	2.5 0.4–6.6 1.4	3.4 2.2 1.6	221	2.4 0.1–7.9 1.5	3.2 2.0 1.3	40	2.4 0.0–7.4 1.6
IgG											
Group	Before vaccine			After first vaccine			After second vaccine			After third vaccine	
	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL
Infection	17	20.6 0.4–54.0 16.3	27.9 16.7 8.4	40	27.4 0.3–175.9 40.6	28.8 12.4 8.3	101	16.6 0.3–168.7 23.7	18.0 9.5 4.0	15	24.8 1.1–462.7 74.5
No infection	13	2.3 0.6–7.6 2.3	1.8 1.4 1.1	62	1.9 0.5–7.2 1.5	2.2 1.4 1.1	221	2.0 0.4–8.2 1.5	2.4 1.4 1.0	40	1.9 0.3–5.0 1.1





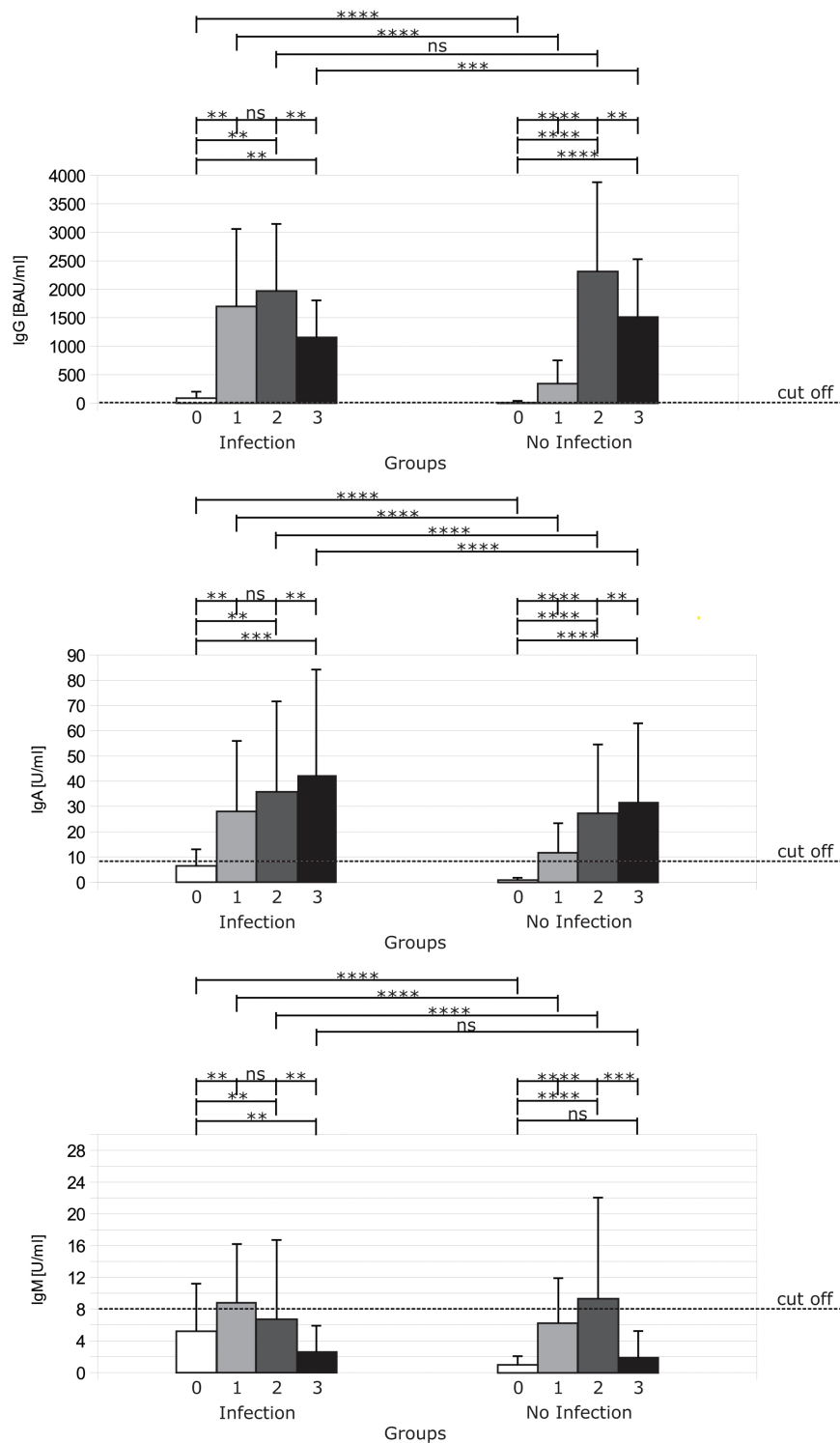
\* P<0,05, \*\*P<0,01, \*\*\*P<0,001, \*\*\*\*P<0,0001, nsP>0,05 Mann - Whitney (Wilcoxon) test

**Fig 2.** Changes of IgM, IgA and IgG antibodies levels against NP produced post vaccination according to prior infection of SARS- Cov-2.

**Table 3.** Changes of IgM, IgA and IgG antibodies levels against S1 protein produced post vaccination according to prior infection of SARS- Cov-2.

IgA											
Group	Before vaccine			After first vaccine			After second vaccine			After third vaccine	
	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL
Infection	17	6.5 0.4–28.1 7.6	5.5 3.6 2.6	40	28.0 0.8–103.5 23.7	37.9 19.6 12.0	101	35.8 1.0–165.9 32.4	43.9 24.7 14.9	15	42.1 2.4–169.5 43.1
No infection	13	0.9 0.2–2.8 0.9	1.0 0.5 0.3	62	11.7 1.0–88.4 13.7	12.8 7.8 4.6	221	27.3 1.8–262.4 28.7	31.0 19.9 12.5	40	31.5 1.9–155.1 31.6
IgM											
Group	Before vaccine			After first vaccine			After second vaccine			After third vaccine	
	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL	Q3 Median Q1	No	Mean value Min–Max ±SD U/mL
Infection	17	5.2 0.6–19.2 6.0	7.2 1.8 1.2	40	8.8 0.5–29.3 7.4	13.1 7.7 2.4	101	6.7 0.1–116.1 10.0	8.3 3.9 2.0	15	2.6 0.2–13.4 3.3
No infection	13	1.0 0.2–4.3 1.1	1.0 0.7 0.5	62	6.3 0.3–25.3 5.7	7.8 4.0 2.1	221	9.4 0.0–105.6 12.8	11.2 4.9 2.1	40	1.9 0.1–20.5 3.4
IgG											
Group	Before vaccine			After first vaccine			After second vaccine			After third vaccine	
	No	Mean value Min–Max ±SD BAU/mL	Q3 Median Q1	No	Mean value Min–Max ±SD BAU/mL	Q3 Median Q1	No	Mean value Min–Max ±SD BAU/mL	Q3 Median Q1	No	Mean value Min–Max ±SD BAU/mL
Infection	17	88.3 0.0–456.6 113.5	87.1 62.2 21.3	40	1696.2 23.9–7281.4 1361.4	2368.2 1498.5 782.8	101	1969.2 124.8–6101.4 1177.8	2646.7 1569.4 1148.9	15	1152.1 81.0–2698.3 650.1
No infection	13	12.2 0.0–88.8 28.7	3.9 0.0 0.0	62	347.1 30.9–2672.8 411.8	371.3 267.3 124.7	221	2329.5 123.4–7937.0 1579.4	3170.0 1821.8 1118.0	40	1522.6 349.6– 5304.3 1026.3





0 - Pre Vaccination, 1 - 1 Vaccination, 2 - 2 Vaccination, 3 - 3 Vaccination

\*  $P < 0,05$ , \*\*  $P < 0,01$ , \*\*\*  $P < 0,001$ , \*\*\*\*  $P < 0,0001$ , ns  $P > 0,05$  Mann - Whitney (Wilcoxon) test

**Fig 3.** Changes of IgM, IgA and IgG antibodies levels against S1 protein produced post vaccination according to prior infection of SARS-Cov-2.

exhibited antibody production against the S1 spike protein, indicating a general immune response to the vaccination.

Significant differences in IgG antibody response were observed between the group after infection and the noninfected group, highlighting the impact of prior exposure to the virus on vaccination outcomes. Similarly, comparisons within the noninfected group revealed differences in IgG antibody responses to the first and second doses of vaccination. These findings underscore the importance of considering individuals' prior infection status when assessing vaccine efficacy and designing vaccination strategies.

The production of IgA antibodies against the S1 protein was studied due to the specific role of IgA in mucous defense against pathogens, including viruses. In the group after prior infection, the response to vaccination was greater than that in the noninfected group. In contrast to the IgG profile, which shows a response in IgA antibody production, a systematic increase in antibody levels after each dose of vaccination was noted, with the highest mean value occurring after the final dose. Similar to IgG antibody production, statistically significant differences were noted between the group after prior infection and the noninfected group. The level of IgA antibodies was low, but the response to the vaccination was proportional and even greater than that of IgG (Staruszkiewicz et al. 2022).

The anti-S1 IgM antibody activity in all participants was assessed before vaccination and was generally below the cutoff value, with individual positive findings (up to 19.2 U/mL) within the group of participants after prior infection. After the first dose of vaccination, an increase in the average anti-S1 IgM antibody level was observed in both groups (6.3 BAU/mL in the group without prior infection and 8.8 BAU/mL in the group after prior infection), but a systematic increase was noted in the group without prior infection (0.7, 6.3 and 9.6 U/mL). Despite a slight increase in the IgM antibody level after the second dose of vaccination within the group without prior infection, the mean value of IgM antibody level was close to the cutoff level in both studied groups. The individual anti-S1 IgM antibody level after vaccination was high, up to 116.1 BAU/mL in one participant with prior infection and 105.6 BAU/mL in one participant without prior infection. The results showed that the vaccination induced a weak and short-lasting response in the IgM immunoglobulin class.

#### 4. Discussion

The study findings on antibody production against the S1 spike protein of SARS-CoV-2 following three doses of the mRNA vaccine in a cohort of 649 health care workers showed a robust response to vaccination. The first dose of vaccination induced the synthesis of IgG-specific antibodies in both studied groups, with a much greater level in the group with

prior infection. The second dose of vaccination increased the level of antibodies in the group without infection, as expected. The observed increase in IgG antibody production after the second dose of vaccination, followed by a decrease after the final dose, suggests a dynamic response to the vaccination regimen. This pattern was consistent across both participants with prior SARS-CoV-2 infection and those without prior contact with the virus. The temporary decline in IgG antibody levels after the final dose may be linked to the duration between the second and third doses, which could result in a long-lasting immune response lasting up to 6 months. This highlights the complexity of immune responses to vaccination and underscores the importance of further research to understand the optimal timing and dosing of COVID-19 vaccines.

Specific IgA antibodies showed a systematic increase in the mean value following subsequent steps of the vaccination program. In both groups of participants, the highest value was observed after three doses of the vaccination. The changes in IgM antibody levels were very discrete, often on the cutoff line. The response to vaccination in individuals with symptomatic and asymptomatic courses of SARS-CoV-2 infection was similar as a result of contact with the virus without affecting the occurrence of clinical symptoms. In our study, participants were tested within a range of 4–10 weeks after the second dose of the vaccine; however, the results were similar to those of studies in which IgG antibody production was detected precisely 7 days and 14 days after a single dose of the mRNA vaccine was administered (Ebinger et al. 2021; Kim et al. 2021; Saadat et al. 2021; Vaquero et al. 2021). To avoid long-lasting effects of symptomatic SARS-CoV-2 infection in the Saadat study, vaccination was administered 8 months after recovery from infection. There was no difference in antibody levels between the symptomatic and asymptomatic infection groups (Saadat et al. 2021). The range of antibody levels was wide, from a low level to overcoming the upper limit of the measurable range of the test. We observed that the occurrence of antibodies against the spike S1 protein in the IgG class after two doses of vaccination was rapid, i.e., within 2 weeks, and >95% of the efficacy was reached (Saadat et al. 2021). The response of participants with prior SARS-CoV-2 infection to the first dose of vaccination was rapid and high, increasing from the baseline level and usually approaching or exceeding the upper limit of the test. Moreover, the occurrence of specific memory B cells was detected (Goel et al. 2021). The production of antibodies in the IgA class after infection or after vaccination is thought to be a marker of increased mucous membrane defense due to dimeric IgA antibodies produced by specific B memory cells. The neutralizing activity of IgA antibodies in the recalculation of the level of antibodies in the IgG class was shown to be greater than that of IgG antibodies (Sterlin et al. 2021).

The effectiveness of the vaccines was assayed by evaluating the neutralization activity against different types of mutated viruses. Like antibody decline kinetics, neutralization activity lasts longer (up to 10 months) in patients with hybrid immunity due to a faster decrease in participants without contact with the virus. The third dose of vaccination involves the use of a booster to support the production of specific antibodies (Falsey et al. 2021; Decru et al. 2022; Maltezou et al. 2022). The production of antibodies, especially recall products, depends on the development of an antigen-specific, long-lived memory B-cell population. Symptomatic infection or asymptomatic contact with the SARS-CoV-2 virus is a signal for the development of specific memory B cells as a result of an induced immune response to the pathogen. The study of the memory B-cell population showed the possibility of expansion after vaccination in participants with prior infection; however, further doses of vaccination did not improve the memory B-cell subpopulation. At the end of the study, both groups of people had a memory-specific B-cell population comprising a comparable percentage of the total B-cell population (Goel et al. 2021). Moreover, this observation seemed to be important not only for IgG-producing memory B cells but also for all classes of specific antibodies, including IgA, leading to an increase in mucous membrane defense after vaccination. The induction of IgA-induced memory B cells specific for the S1 protein of SARS-CoV-2 was significant for participants without prior immunization after contact with the virus.

The low but visible response in the IgM class suggested the induction of IgM-specific memory B cells. A similar proportion of specific memory B cells IgG<sup>+</sup> to specific memory B cells IgM<sup>+</sup> was noted 15 days after the first dose of vaccination (Goel et al. 2021).

These findings underscore the importance of vaccination in generating an immune response against SARS-CoV-2, particularly for healthcare workers who face heightened exposure risks. The study highlights the effectiveness of vaccination in inducing antibody production even in individuals without prior exposure to the virus, as well as those with mild or asymptomatic infections. Long-term monitoring of immune responses, including the role of antigen-specific T and B memory cells, may provide valuable insights into combating mutated forms of SARS-CoV-2. Given the ongoing risk of infection, especially among healthcare workers, vaccination remains a crucial strategy until effective antiviral medications are developed.

Currently, the decision about obligatory or voluntary vaccination is still open and individual (Sterlin et al. 2021).

## 5. Conclusions

The study revealed robust antibody production across the IgG, IgA, and IgM immunoglobulin classes following three doses of mRNA vaccination in both previously infected and noninfected participants. Specifically, IgG antibodies exhibited the highest mean concentration after the second vaccine dose, indicating a robust response to vaccination. In contrast, the IgA class displayed a steady increase in antibody production post-vaccination, highlighting the importance of these antibodies in mucous membrane defense against pathogens. This antibody production suggests the activation of a specific memory B lymphocyte population, crucial for generating an effective humoral response against the pathogen.

## Statements and Declarations

### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Author Contributions

MSt and SS designed and performed research. MSt collected data. MSt and TM analyzed and interpreted data. MSt and AP-N wrote the manuscript. All authors read and approved the final manuscript.

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### Ethics Approval

The study was approved by the Ethics Committee of the Jagiellonian University (1072.6120.61.2021) and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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