BPR MCS Bulletin of Pioneering Researches of Medical and Clinical Science

Bulletin of Pioneering Researches of Medical and Clinical Science

Available online: https://bprmcs.com
2025 | Volume 4 | Issue 2 | Page: 115-125

Epidemiological Determinants and Trends of SARS-CoV-2 Antibody Seroprevalence in Poland

Katarzyna Szum[1], Wiktoria Suchy[2], Rafal J. Wiglusz[2], Karolina Nieoczym[2*], Iwona Duraj[2]

[1] Department of Infectious Diseases and Neuroinfections, Medical University of Bialystok, Bialystok, Poland.
[2] Department of Population Medicine and Lifestyle Diseases Prevention, Medical University of Bialystok, Bialystok, Poland.

Abstract

This study aimed to evaluate the presence of anti-SARS-CoV-2 antibodies in the general population and in COVID-19 convalescents six months post-infection. The study included two groups: Group I comprised 232 individuals recovering from COVID-19, and Group II included 544 participants from a population-based cohort. Anti-nucleocapsid (anti-N) antibodies were measured using the Elecsys Anti-SARS-CoV-2 assay, while anti-spike (anti-S) antibodies were assessed using the LIAISON SARS-CoV-2 S1/S2 IgG test. Following the Omicron wave, the general population showed a gradual increase in antibody prevalence, reaching 92.5% for anti-S and 69.7% for anti-N antibodies. Among COVID-19 convalescents, 6 months post-infection, 4.3% and 3.7% failed to develop detectable anti-S and anti-N antibodies, respectively. Among vaccinated individuals, 1% did not produce anti-S antibodies. Non-responders were generally older than responders, while sex had no significant effect. Comparisons of antibody levels six months post-infection revealed higher anti-N titers in previously infected patients compared to the general population. Notably, 17.4% of the general population without prior COVID-19 or vaccination had anti-N antibodies, and 9% had anti-S antibodies. The high prevalence of anti-N antibodies among individuals without reported COVID-19 history, even after the official end of the pandemic, indicates widespread SARS-CoV-2 exposure and frequent asymptomatic or undiagnosed infections. These findings have important implications for public health surveillance.

Keywords: Anti-SARS-CoV-2, COVID-19 serology, Anti-N antibodies, Anti-S antibodies, Population study

Corresponding author: Karolina Nieoczym
E-mail: Karolinanieoczym@yahoo.com

Received: 08 June 2025
Revised: 06 October 2025
Accepted: 12 October 2025

How to Cite This Article: Szum K, Suchy W, Wiglusz RJ, Nieoczym K, Duraj I. Epidemiological Determinants and Trends of SARS-CoV-2 Antibody Seroprevalence in Poland. Bull Pioneer Res Med Clin Sci. 2025;4(2):115-25. https://doi.org/10.51847/X18cKjheZm

Introduction

By late 2022, the SARS-CoV-2 pandemic had reached its seventh wave in several European countries. In Poland, despite public health interventions between 2020 and 2022—including quarantine measures and mass vaccination—COVID-19 accounted for approximately 120,000 deaths (https://stat.gov.pl/covid/). From a travel medicine perspective, detection and monitoring of the disease were challenging, as diagnostic strategies largely relied on identifying clinical symptoms and confirming infection via real-time reverse transcription polymerase chain reaction (qRT-PCR). Both methods have limitations: asymptomatic or mild infections can evade clinical detection, and qRT-PCR tests may yield false negatives.

Like other viruses, SARS-CoV-2 enters host cells to replicate. The viral spike (S) protein binds to angiotensin-converting enzyme 2 (ACE2) receptors, which are expressed in multiple organs, including the lungs, heart, kidneys, and liver. Proteins such as TMPRSS2, Neuropilin-1 (NRP1), and cathepsins B and L facilitate viral entry, with TMPRSS2 cleaving the S protein into S1 and S2 subunits, enabling membrane fusion and viral RNA release [1, 2]. Following infection, viral replication triggers inflammatory responses, including the release of cytokines like TNFα, IL-1β, IL-6, and the production of specific antibodies [3]. Seroconversion kinetics in SARS-CoV-2 infection mirror those observed in other acute viral infections, with IgM antibodies detectable as early as 3 days post-symptom onset, followed by IgG antibodies, typically measurable from day 7 and peaking around day 25 [4]. However, negative serology does not exclude infection [5]. While serological tests are less useful for early diagnosis compared with PCR, they are essential in later stages when viral RNA may be undetectable, providing insights into population-level exposure.

Wastewater-based genomic surveillance has emerged as a complementary tool for tracking SARS-CoV-2 variants and mutations. By analyzing wastewater, researchers can monitor the circulation of different viral lineages and assess their prevalence over time. Pilapil *et al.* [6] demonstrated this approach by examining 8,511 wastewater-derived genome sequences from nine countries, documenting the successive dominance of B.1 (2020), Alpha and Delta (2021), and Omicron lineages (2022), along with 5,031 unique amino acid substitutions, some associated with increased transmissibility.

Population-based seroprevalence studies have been crucial in understanding SARS-CoV-2 spread, including asymptomatic infections. These studies provide insight into antibody prevalence across different demographics and regions [7]. In our region, accounting for vaccination rates and prior waves of infection, we established a cohort to assess COVID-19 seroprevalence in the population of Bialystok, Poland, after four pandemic waves.

It is important to consider methodological challenges affecting serological and PCR testing. Cross-contamination during qRT-PCR can produce false positives; strict laboratory protocols, including frequent glove changes, aseptic handling, and equipment sterilization, are critical [8]. Additionally, extensive viral mutations can result in mismatches with primers and probes, potentially impacting detection accuracy [9].

The aim of the present study was to evaluate anti-SARS-CoV-2 antibody prevalence following successive waves of the pandemic. Specific objectives included: (1) assessing antibody prevalence in the general population across four pandemic waves, (2) comparing antibody responses between COVID-19 convalescents and the general population, (3) estimating asymptomatic infection rates using anti-N antibody status, and (4) analyzing differences in antibody production across pandemic waves.

Materials and Methods

Study population

The study included two distinct groups. **Group I** comprised 232 patients who had previously tested positive for SARS-CoV-2 by PCR and required hospitalization due to COVID-19. These individuals were assessed approximately six months post-infection. Many participants had comorbidities: 99 (42.7%) with hypertension, 3 (1.3%) with heart failure, 19 (8.2%) with diabetes, 102 (44%) with obesity, 17 (7.3%) with renal insufficiency, 24 (10.3%) with cancer, 8 (3.4%) with chronic obstructive pulmonary disease (COPD), and 17 (7.3%) with asthma. Pneumonia history was available for 190 patients, of whom 155 had developed pneumonia and 35 had not.

Group II included 544 participants from the **Bialystok PLUS** population cohort [10], a community-based study designed to provide a representative overview of the health, psychosocial, and demographic characteristics of Bialystok residents. Participants were randomly selected to reflect the population distribution of the city. In this group, 157 (28.9%) had hypertension, 12 (2.2%) had heart failure, 39 (7.2%) had diabetes, 133 (24.5%) had obesity, 30 (5.5%) had renal insufficiency, 31 (5.7%) had cancer, 14 (2.6%) had COPD, and 26 (4.8%) had asthma.

Group II was further stratified into:

- **Group IIa:** 151 participants who reported a prior COVID-19 diagnosis
- **Group IIb:** 393 participants who reported no previous COVID-19 diagnosis

The distribution of participants across study groups is presented in **Figure 1**.

Definition of pandemic waves

Pandemic waves were categorized based on the locally dominant SARS-CoV-2 variants:

- Wave 1 (29 February 2020 – 31 December 2020): Wild-type variants
- Wave 2 (1 January 2021 – 30 April 2021): Wild-type and Alpha variants
- Wave 3 (1 May 2021 – 31 December 2021): Delta variants
- Wave 4 (1 January 2022 – 31 March 2022): Omicron variants [6, 11, 12]

Exclusion criteria

Participants were excluded if they had active COVID-19 infection or declined to provide informed consent.

Antibody assessment

Anti-N and anti-S antibody titers were measured six months post-infection in Group I, and at relevant time points in the general population cohort. Anti-S antibodies provide neutralizing activity against SARS-CoV-2, whereas anti-N antibodies indicate prior viral exposure. Antibody profiles differ between convalescent and vaccinated individuals: convalescents typically generate both anti-S and anti-N antibodies, mRNA vaccine recipients produce only anti-S antibodies, and recipients of inactivated vaccines may develop both types. This study compared the prevalence of both antibody types, with a particular focus on the general population.

Laboratory methods

- **Anti-N antibodies:** Measured using the Elecsys Anti-SARS-CoV-2 assay (Roche Diagnostics, Rotkreuz, Switzerland) on the fully automated COBAS platform. Samples were classified as reactive or non-reactive, with a cutoff index (COI) ≥1.0 considered reactive.
- **Anti-S IgG antibodies:** Measured using the LIAISON SARS-CoV-2 S1/S2 IgG assay (DiaSorin, Saluggia, Italy) on the automated LIAISON XL platform. The assay detects neutralizing antibodies with 94.4% agreement with Plaque Reduction Neutralization Test (PRNT90). Results were expressed in AU/mL, with >15.0 AU/mL considered positive.

Survey data

Both groups completed surveys capturing vaccination status and health history to complement serological analyses.

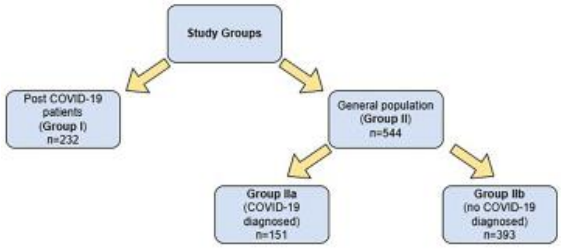


Figure 1. Distribution of study population

Table 1. Demographic characterisation and vaccination status in total and in each wave

Empty Cell	Group I			Group II		
	Me	**Min**	**Max**	**Me**	**Min**	**Max**
			Age (years)			
Total	54	23	78	50	20	80
Wave 1	54	27	78	40.5	29	66
Wave 2	57	34	78	51	21	79
Wave 3	54	23	76	50	20	80
Wave 4	49	36	73	51	20	79
			Vaccination status			
	Vaccinated patients, n (%)	**Unknown status of patients, n (%)**	**Total**	**Vaccinated patients, n (%)**	**Unknown status of patients, n (%)**	**Total**
Total	113 (48.7 %)	119 (51.3 %)	232	319 (58.7 %)	224 (41.3 %)	543
Wave 1	50 (48.1 %)	54 (51.9 %)	104	0 (0 %)	10 (100 %)	10
Wave 2	19 (73.1 %)	7 (26.9 %)	26	11 (11.6 %)	84 (88.4 %)	95
Wave 3	37 (40.7 %)	54 (59.3 %)	91	86 (65.6 %)	45 (34.4 %)	131
Wave 4	7 (63.6 %)	4 (36.4 %)	11	222 (72.3)	85 (37.7 %)	307
			Sex			
	Males, n (%)	**Females, n (%)**	**Total**	**Males, n (%)**	**Females, n (%)**	**Total**
Total	117 (50.4 %)	115 (49.6 %)	232	250 (46 %)	293 (54 %)	543
Wave 1	49 (47.1 %)	55 (52.9 %)	104	4 (40 %)	6 (60 %)	10
Wave 2	13 (50 %)	13 (50 %)	26	56 (58.9 %)	39 (41.1 %)	95
Wave 3	51 (56 %)	40 (44 %)	91	63 (48.1 %)	69 (51.9 %)	131
Wave 4	4 (36.4 %)	7 (63.6 %)	11	127 (41.4 %)	180 (58.6 %)	307

Abbreviations: ME – median; Min – minimum; Max – maximum.

Statistical analysis

All statistical analyses were conducted using Statistica software (version 13.0, StatSoft, Inc., https://www.statsoft.com/). Data were summarized as medians with minimum and maximum values, as appropriate. The normality of data distribution was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using the Kruskal-Wallis test for continuous variables and the Chi-squared test for categorical variables. A p-value of <0.05 was considered statistically significant. Figures were generated using the ggstatsplot package in R [13].

Ethical considerations

The study received ethical approval from the Ethics Committee of the Medical University of Bialystok, Poland (approval number: APK.002.346.2020). All procedures adhered to the principles of the 1964 Declaration of Helsinki and its subsequent amendments. Participation was voluntary, with written informed consent obtained from all participants, and study procedures were fully explained prior to enrollment.

Results

Seroprevalence in the general population (Group II)

Figure 2 illustrates the prevalence of anti-N and anti-S IgG antibodies in the Bialystok PLUS cohort (group II) six months after each pandemic wave. Both antibody types demonstrated a gradual increase, reaching 92.5% for anti-S and 69.7% for anti-N antibodies following the Omicron wave.

Notably, 17.4% of participants had anti-N antibodies despite reporting no prior COVID-19 diagnosis, suggesting possible asymptomatic infections, and 9% had anti-S antibodies despite neither vaccination nor confirmed COVID-19.

Seroprevalence in post-hospitalized COVID-19 patients (Group I)

Six months post-infection, 4.3% and 3.7% of previously hospitalized patients lacked detectable anti-S and anti-N antibodies, respectively. Among vaccinated patients, 1% did not generate anti-S antibodies. Non-responders were older than responders (median age 63.5 years [range 47–76] versus 51 years [range 20–80]; $p < 0.05$). Vaccinated individuals were also older than non-vaccinated participants (median age 55 versus 45 years; $p < 0.05$). Among participants without confirmed COVID-19, those with positive anti-S titers were significantly older. No significant sex-related differences were observed for antibody prevalence across waves.

Comparisons of antibody titers

Anti-N titers were significantly higher in hospitalized patients (median COI 97.34; range 0.078–275.3) than in the general population (median COI 0.091; range 0.05–293.3; $p < 0.05$). Anti-S titers, however, did not differ significantly between group I (median 1190 AU/mL; range 16.3–98,900 AU/mL) and group II (median 1090 AU/mL; range 4.81–53,936 AU/mL).

Comparing previously hospitalized patients (group I) with non-hospitalized COVID-19 cases from the general population (group IIa), anti-N titers were higher in hospitalized individuals (median COI 97.34 versus 41.54), while anti-S titers showed no significant difference (median 1190 AU/mL versus 1290 AU/mL).

Additional analyses evaluating the association between acute-phase symptoms (e.g., fever, cough, dyspnea, desaturation, fatigue, myalgia, chest pain, anosmia, headache, shivers) and post-infection sequelae at six months (e.g., dyspnea, cough, memory and concentration impairment, headaches, blood pressure and heart rate abnormalities) are presented in **Tables 2 and 3**.

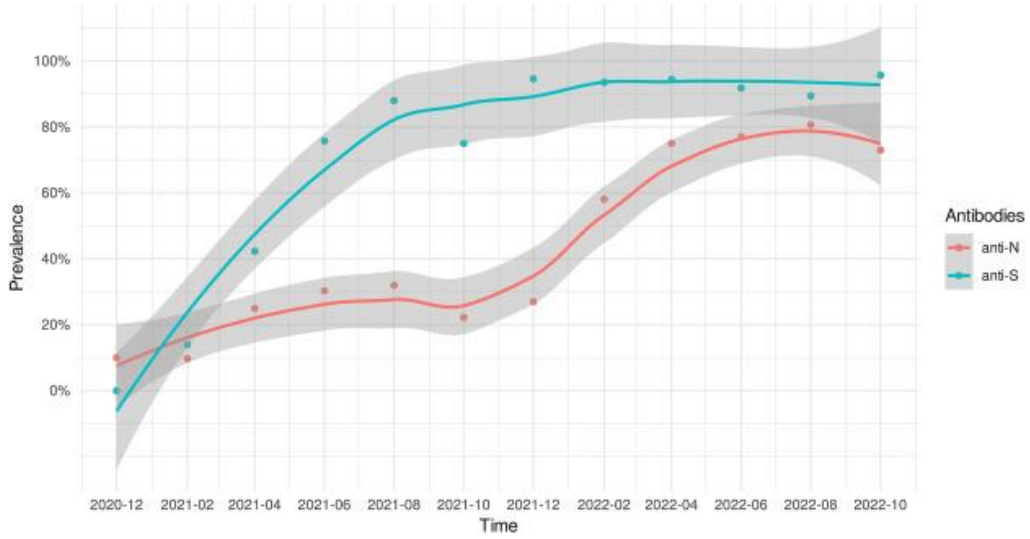


Figure 2. Prevalence of anti-N and anti-S antibodies 6 months after each wave of COVID-19 pandemics

Table 2. Comparison of anti-N and anti-S antibodies titers 6 months after COVID-19 infection by symptoms in patients in the acute phase of the disease

Symptom	Antibodies	Present			Absent			p value
		Me	Min	Max	Me	Min	Max	
Fever	anti-N	84.26	0.08	275.3	22.55	0.061	239.9	0.05
	anti-S	1770	4.81	98900	799	4.81	74400	0.000004

Cough dry	anti-N	88.79	0.061	275.3	43.495	0.062	259	0.000393
	anti-S	1630	4.81	74400	1160	4.81	98900	0.014365
Cough wet	anti-N	104.1	0.48	275.3	61.34	0.061	259	0.023386
	anti-S	1490	19.4	39900	1420	4.81	98900	NS
Dyspnea	anti-N	98.2	0.062	256	41.27	0.061	275.3	0.001
	anti-S	1650	4.81	98900	1255	4.81	39500	0.003383
Desaturation	anti-N	109.5	0.906	247.2	42.88	0.061	275.3	0
	anti-S	1960	49.9	98900	1185	4.81	39900	0.000904
Fatigue	anti-N	85.31	0.062	275.3	10.6	0.061	253.8	0
	anti-S	1630	4.81	98900	787.5	4.81	28100	0.000685
Runny nose	anti-N	82.29	0.08	259	66.66	0.061	275.3	0.042404
	anti-S	1505	4.81	98900	1420	4.81	74400	NS
Muscle pain	anti-N	88.79	0.062	275.3	40.13	0.061	253.8	0.000005
	anti-S	1390	4.81	74400	1605	4.81	98900	NS
Sore throat	anti-N	67.2	0.061	259	70.87	0.062	275.3	NS
	anti-S	1720	5.5	54200	1300	4.81	98900	NS
Chest pain	anti-N	93.9	0.062	256	64.64	0.061	275.3	0.013142
	anti-S	1690	4.81	39900	1365	4.81	98900	NS
Anosmia	anti-N	85.87	0.061	259	52.03	0.062	275.3	0.00075
	anti-S	1475	5.5	98900	1180	4.81	70400	NS
Headache	anti-N	93.92	0.062	275.3	38.045	0.061	253.8	0.00002
	anti-S	1420	4.81	98900	1430	4.81	70400	NS
Chills	anti-N	84.18	0.062	259	53.26	0.061	275.3	0.001321
	anti-S	1670	4.81	39900	1240	4.81	98900	0.030677
Thromboembolic disorders	anti-N	144.7	0.541	235.4	66.66	0.061	275.3	0.017942
	anti-S	1630	98.2	70400	1425	4.81	98900	NS
Diarrhea	anti-N	84.77	2.53	244.1	67.74	0.061	275.3	NS
	anti-S	1215	19.4	74400	1450	4.81	98900	NS
Skin lesions	anti-N	121.5	0.184	235.4	67.74	0.061	275.3	NS
	anti-S	1130	33.8	4290	1430	4.81	98900	NS

Abbreviations: NS – non-significant; ME – median; Min – minimum; Max – maximum.

Table 3. Comparison of anti-N and anti-S antibodies titers 6 months after COVID-19 infection by symptoms in patients 6 months after the disease

Symptom	Antibodies	Present			Absent			p value
		Me	Min	Max	Me	Min	Max	
Cough dry	anti-N	88.79	0.184	275.3	65.71	0.069	259	0.020305
	anti-S	1980	18.1	74400	1250	4.81	98900	0.018231
Cough wet	anti-N	100.3	0.184	156.9	70.57	0.069	275.3	NS
	anti-S	1120	19.4	37800	1620	4.81	98900	NS
Dyspnea	anti-N	103.05	0.078	256	57.84	0.069	275.3	0.001504
	anti-S	1955	19.4	74400	1350	4.81	98900	0.00767
Fatigue	anti-N	92.05	0.078	259	16.01	0.069	275.3	0
	anti-S	1810	18.1	98900	747	4.81	37800	0.000035
Memory disorders	anti-N	88.32	0.53	253.8	59.82	0.069	275.3	0.007626
	anti-S	1720	31.3	74400	1315	4.81	98900	0.027407
Headache	anti-N	85.31	0.184	256	66.28	0.069	275.3	NS
	anti-S	1620	19.4	74400	1510	4.81	98900	NS
Concentration problems	anti-N	92.09	0.184	253.8	59.82	0.069	275.3	0.015798
	anti-S	1570	19.4	98900	1505	4.81	70400	NS
Anosmia	anti-N	93.98	0.184	259	58.83	0.069	275.3	0.012901
	anti-S	1300	23	98900	1690	4.81	70400	NS
Blood pressure disturbances	anti-N	137.4	2.18	247.2	65.9	0.069	275.3	0.007526
	anti-S	2045	155	70400	1460	4.81	98900	NS
Heart rate disturbances	anti-N	73.82	2.97	203	67.74	0.069	275.3	NS
	anti-S	1245	19.4	26800	1625	4.81	98900	NS

Anxiety	anti-N	142.5	2.53	253.8	65.805	0.069	275.3	0.005955
	anti-S	1760	19.4	37800	1490	4.81	98900	NS
Thromboembolic disorders	anti-N	139.7	19.94	203	67.5	0.069	275.3	NS
	anti-S	1270	181	38000	1570	4.81	98900	NS
Joints pain	anti-N	98.07	0.66	256	65.805	0.069	275.3	NS
	anti-S	1190	19.4	37800	1650	4.81	98900	NS
Skin lesions	anti-N	57.38	0.53	121.5	70.655	0.069	275.3	NS
	anti-S	4090	33.8	6580	1490	4.81	98900	NS

Abbreviations: NS – non-significant; ME – median; Min – minimum; Max – maximum.

Comparison of Anti-N antibodies across COVID-19 waves

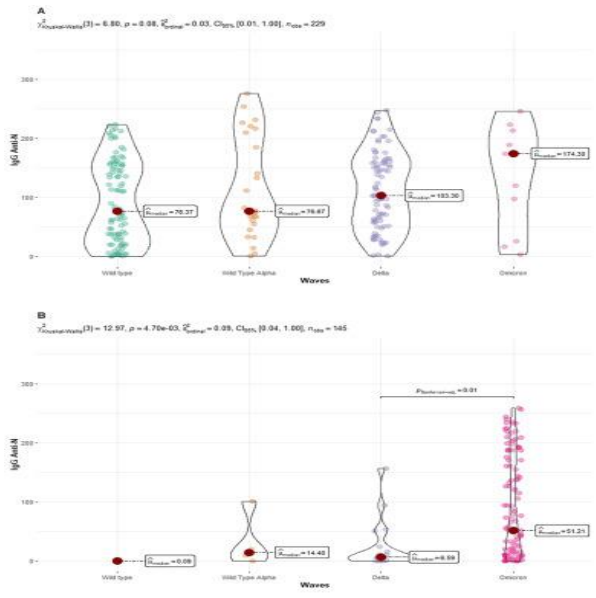
In group I (post-hospitalized COVID-19 patients), no statistically significant differences in anti-N antibody titers were observed across the four pandemic waves **(Figure 3a)**.

Within group IIa (general population participants with prior COVID-19), anti-N titers differed significantly between individuals infected during wave 3 compared with wave 4 **(Figure 3b)**.

For group IIb (general population participants without a reported history of COVID-19), statistically significant differences in anti-N antibody levels were found between wave 1 and waves 2, 3, and 4 **(Figure 3c)**.

Specifically, among group IIb participants who did not report infection, anti-N antibodies were detected in 2/11 (18%) after wave 1, 14/85 (16.5%) after wave 2, 22/132 (16.7%) after wave 3, and 97/307 (31.6%) after wave 4 **(Figure 4)**.

Comparisons across groups revealed significant differences in anti-N titers between group I and IIa, group I and IIb, and group IIa and IIb during wave 3. For wave 4, significant differences were noted between group I and IIb, as well as between group IIa and IIb.



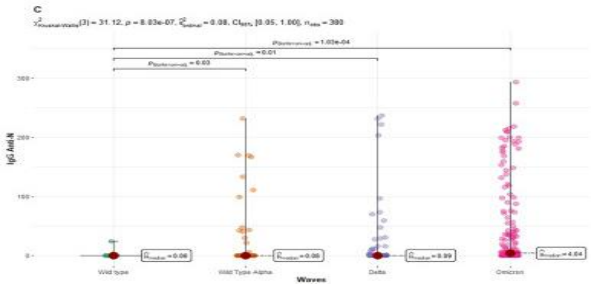


Figure 3. Comparison of anti-N antibodies between the COVID-19 waves in groups I (A), IIa (B), and IIb (C)

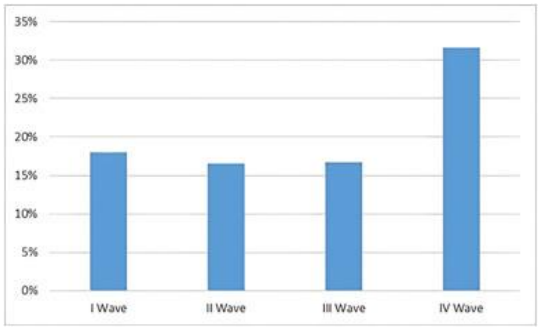


Figure 4. The percentage of individuals in the general population (group IIb) with anti-N antibodies who were not diagnosed with COVID-19 after each wave. Antibody levels were 18 % after wave 1, 16.5 % after wave 2, 16.7 % after wave 3, and 31.6 % after wave 4

Comparison of Anti-S antibodies across COVID-19 waves

Analysis of anti-S antibody titers during wave 1 revealed statistically significant differences between group I (hospitalized COVID-19 patients) and group IIb (general population without reported infection; $p < 0.05$). Similarly, in wave 2, significant differences were observed between group I and IIb, paralleling the pattern seen for anti-N antibodies. During wave 3, group I showed significantly higher anti-S titers compared with both group IIa (general population with prior COVID-19) and group IIb. By wave 4, no statistically significant differences were detected between the groups, reflecting the evolving serological landscape over the course of the pandemic.

Within group I, comparison of anti-S titers across all waves did not demonstrate statistically significant differences **(Figure 5a)**. In contrast, group II showed significant differences in anti-S titers when comparing wave 4 with the preceding waves ($p < 0.05$).

Subgroup analysis of group IIa demonstrated statistically significant increases in anti-S titers after wave 4 compared with wave 2 and wave 3 **(Figure 5b)**. In group IIb, significant differences were observed between wave 1 and waves 3 and 4, as well as between wave 2 and waves 3 and 4 **(Figure 5c)**.

Overall, comparisons of anti-S titers across waves in group II indicated significant differences between waves 1 and 3, waves 1 and 4, waves 2 and 3, and waves 2 and 4 ($p < 0.05$). These trends correspond to the timeline of mass vaccination campaigns, which began after wave 1, and the substantial surge in infections observed during wave 4.

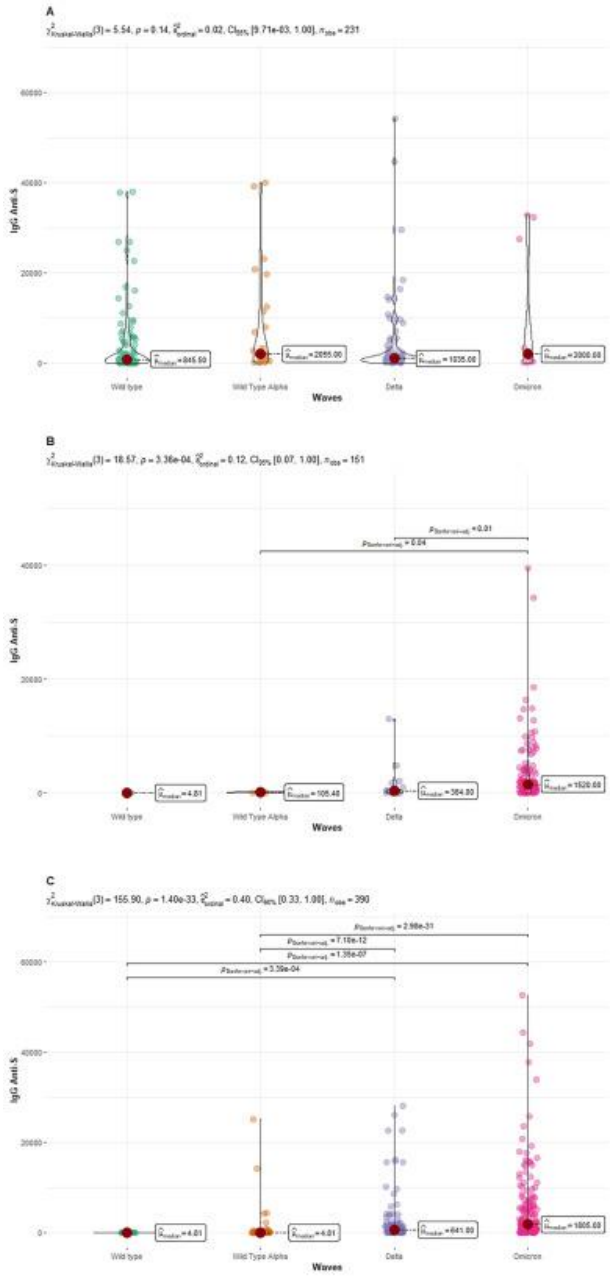
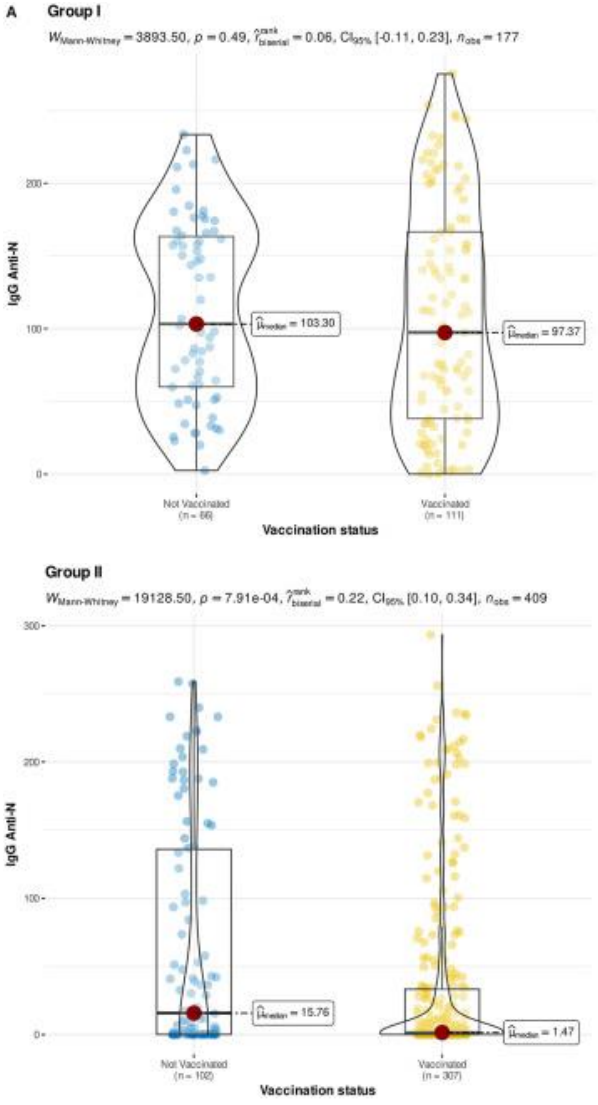


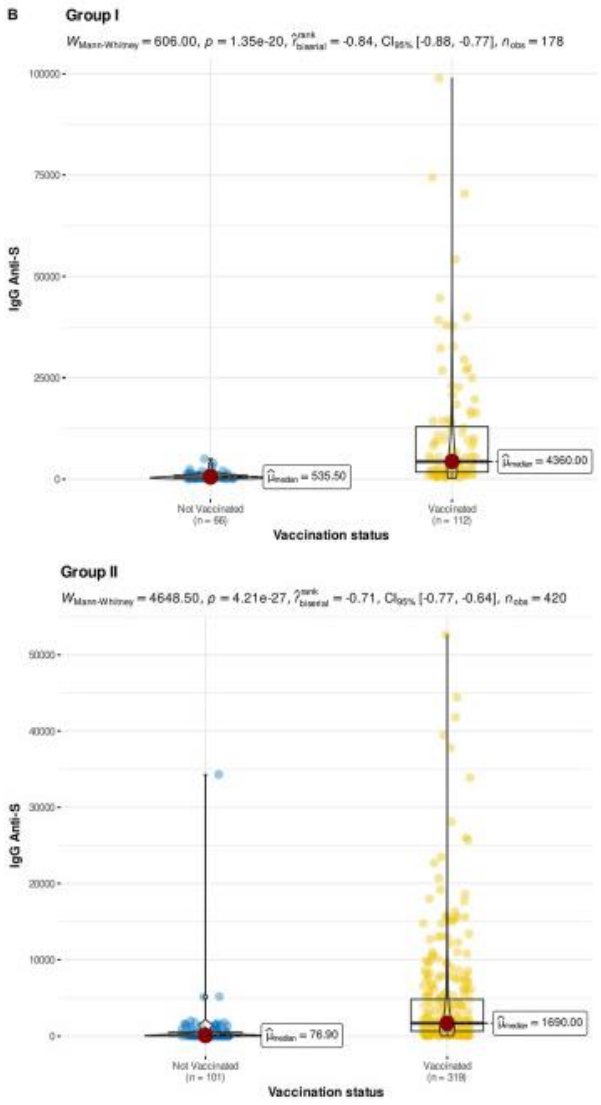
Figure 5. Comparison of anti-S antibodies between the COVID-19 waves in groups I (A), IIa (B), and IIb (C)

Comparison of Anti-N and Anti-S antibody titers between vaccinated and unvaccinated participants

Analysis of antibody responses revealed statistically significant differences in anti-S titers between vaccinated and unvaccinated individuals in both group I (hospitalized patients) and group II (general population) **(Figure 6a and 6b)**. In group II, anti-N titers also differed significantly between vaccinated and unvaccinated participants. However, in group I, no significant difference was observed in anti-N titers between vaccinated and unvaccinated patients.



A



B

Figure 6. Comparison of anti-N (A) and anti-S (B) antibody titers in vaccinated vs. non-vaccinated patients between groups I and II

Comparison of Anti-N and Anti-S antibody titers in patients with and without pneumonia

Patients who developed pneumonia exhibited significantly higher titers of both anti-S antibodies (median: 1390 AU/mL, range: 0.682–253.8 AU/mL vs. median: 610 AU/mL, range: 16.3–54,200 AU/mL) and anti-N antibodies (COI median: 114.6, range: 19.4–98,900 vs. median: 74.3, range: 0.078–233.6) compared to those without pneumonia ($p < 0.05$).

Discussion

Our study demonstrated a progressive increase in the seroprevalence of anti-N and anti-S antibodies within the general population: after the first three waves, anti-N prevalence rose from 10% to 27%, and anti-S from 0% to 82.4%. Six months following the fourth wave, seroprevalence reached 69.7% for anti-N and 92.7% for anti-S (group II).

Over the course of the pandemic, differences in antibody prevalence between previously infected individuals (group I) and the general population (group II) diminished, likely reflecting the effects of widespread vaccination and asymptomatic infections. Following vaccine rollout after the second wave, disparities in anti-S antibody levels largely disappeared. Our findings align with global reports; for instance, Bergeri *et al.* [7] noted a sharp rise in seroprevalence in 2021, driven by regional infections and vaccination campaigns, with variations across continents. Comparable observations were made in Spain (Castilla *et al.* [14]) and Japan (Ren *et al.* [15]), where seroprevalence of anti-N and anti-S antibodies exceeded 90% by early 2022. In African populations, seroprevalence increased from 26–41% pre-third wave to 60–70% post-third wave [16].

In our study, 17.4% of participants and 9% of the general population exhibited anti-N and anti-S antibodies, respectively, despite reporting no prior infection or vaccination. The proportion of asymptomatic infections, especially after the fourth (Omicron) wave, reached 31.6%, emphasizing the importance of surveillance to prevent unnoticed viral spread. Wang *et al.* [17] estimated that asymptomatic infections account for at least one-third of cases globally.

By October 2022, Poland's overall vaccination rate was 56.75%, comparable to our cohort (58.63%). Analysis revealed higher anti-S and anti-N titers among vaccinated participants, with vaccinated individuals generally older than their unvaccinated counterparts. Notably, 1% of vaccinated participants, primarily older adults, failed to produce detectable anti-S antibodies, consistent with findings by Hägg *et al.* [18] that vaccine-induced immunity wanes with age. Reduced humoral responses were also noted in patients with comorbidities, echoing observations in hemodialysis patients reported by Notarte *et al.* [19].

Sex did not influence antibody prevalence, consistent with other studies [20–26]. Globally, higher antibody titers correlate with disease severity [27, 28]. In our cohort, hospitalized patients demonstrated higher anti-N titers than non-hospitalized individuals. Interestingly, elevated anti-S titers were observed in severe cases during the first three waves but not after the fourth. Furthermore, correlations between antibody levels and acute-phase symptoms may relate to ongoing post-COVID-19 syndrome manifestations, consistent with findings that long COVID can occur even in asymptomatic infections but is more prevalent in severe cases (~70%) [29].

Impact of spike protein mutations on antibody response

Mutations in the spike protein of dominant SARS-CoV-2 variants have been suggested to facilitate evasion from population-level immunity. He *et al.* [30] reported that specific substitutions, including L452R in the Delta variant, disrupt interactions involving the VH1-69 hydrophobic HCDR2 region, weakening antibody-antigen binding and allowing the virus to escape immune detection. Initially, early Omicron variants were sensitive to antibody R1-32; however, subvariants carrying L452R rapidly emerged and spread [30, 31]. In our cohort, higher antibody titers were observed following the fourth wave, likely reflecting repeated exposures from reinfections and vaccination, which boosted immune responses.

Chansaenroj *et al.* [32] observed a gradual decline in anti-N IgG seropositivity over time in COVID-19 convalescent patients, with rates decreasing at 6, 9, and 12 months post-symptom onset compared to 3 months. Patients who had experienced pneumonia displayed higher seropositivity at all time points, suggesting that severe infection may prolong the immune response. In a subsequent study [33], they measured anti-S1 IgG, total immunoglobulin against the receptor-binding domain (RBD), and neutralizing antibodies, reporting a time-dependent decline in antibody levels across both mild and severe cases. Higher initial IgG titers were associated with longer-lasting immunity. These findings inform vaccination strategies for individuals recovering from COVID-19.

Our results align with these studies, indicating that patients who developed pneumonia during infection tend to produce higher antibody levels and maintain seropositivity longer than those with milder or asymptomatic disease.

Limitations and strengths

The study had several limitations. The rapidly changing epidemiological context resulted in relatively small sample sizes for each time period, limiting the ability to fully analyze the interactions between clinical variables and antibody titers. Data on reinfections were not available, which may have influenced results, particularly during the Omicron wave. Additionally, information regarding the specific types of vaccines administered was unavailable, which could have provided deeper insights into immune responses.

The study's novelty lies in its timing, capturing immune responses across different waves dominated by distinct SARS-CoV-2 variants. Access to a large population cohort alongside COVID-19 patient samples allowed a unique comparison of antibody prevalence.

The strength of this work is its comprehensive approach, enabling the assessment of both anti-N and anti-S antibodies in the general population and in COVID-19 convalescents six months post-infection. Furthermore, antibody levels were analyzed in relation to post-COVID-19 symptoms, providing a detailed picture of immunity dynamics within the Polish population during the pandemic. This data contributes valuable information on seropositivity trends and may inform future public health strategies.

Conclusions

Symptomatic COVID-19 infections are associated with higher anti-N antibody production compared to oligosymptomatic or asymptomatic cases. While differences in anti-S antibody levels between previously infected patients and the general population have diminished over time, anti-N antibodies continue to reflect prior infection history. Seroprevalence of anti-S antibodies remains substantial among both vaccinated individuals and the general population. Asymptomatic infections likely contribute to ongoing viral circulation, representing a persistent public health concern. The high prevalence of anti-N antibodies in individuals without reported COVID-19, even after the official end of the pandemic, suggests widespread environmental exposure and frequent asymptomatic or non-specific infections, underscoring the need for continuous monitoring. Despite widespread anti-S antibody presence during the Delta and Omicron waves, the observed increase in new infections highlights ongoing susceptibility and transmission risk.

Acknowledgments: None.

Conflict of interest: None.

Financial support: None.

Ethics statement: None.

References

1. Jiang Y, Rubin L, Peng T, Liu L, Xing X, Lazarovici P, et al. Cytokine storm in COVID-19: From viral infection to immune responses, diagnosis and therapy. Int J Biol Sci. 2022;18:459–72. https://doi.org/10.7150/ijbs.59272.
2. Sodhi P, Sidime F, Tarazona DD, Valdivia F, Levano KS. A closer look at ACE2 signaling pathway and processing during COVID-19 infection: identifying possible targets. Vaccines (Basel). 2022;11:13. https://doi.org/10.3390/vaccines11010013.
3. Du L, Yang Y, Zhang X. Neutralizing antibodies for the prevention and treatment of COVID-19. Cell Mol Immunol. 2021;18:2293–306. https://doi.org/10.1038/ s41423-021-00752-2.
4. Liu X, Wang J, Xu X, Benameur K, Bassit LC, Ramonell R, et al. Patterns of IgG and IgM antibody response in COVID-19 patients. Emerg Microb Infect. 2020;9:1269–74. https://doi.org/10.1080/22221751.2020.1773324.

5. Quinti I, Mortari EP, Fernandez Salinas A, Milito C, Carsetti R. IgA antibodies and IgA deficiency in SARS-CoV-2 infection. Front Cell Infect Microbiol. 2021;11. https://doi.org/10.3389/fcimb.2021.655896
6. Pilapil J, Notarte K, Yeung K. The dominance of co-circulating SARS-CoV-2 variants in wastewater. Int J Hyg Environ Health. 2023;253:114224. https://doi.org/ 10.1016/j.ijheh.2023.114224
7. Bergeri I, Whelan MG, Ware H, Subissi L, Nardone A, Lewis HC, et al. Global SARS- CoV-2 seroprevalence from January 2020 to April 2022: a systematic review and meta-analysis of standardized population-based studies. PLoS Med. 2022;19: e1004107. https://doi.org/10.1371/journal.pmed.1004107
8. Albano P, Notarte K, Macaranas I, Maralit B. Cross-contamination in molecular diagnostic laboratories in low- and middle-income countries: a challenge to COVID- 19 testing. PJP. 2020;5(2):7–11.
9. Sharma D, Notarte KI, Fernandez RA, Lippi G, Gromiha MM, Henry BM. In silico evaluation of the impact of Omicron variant of concern sublineage BA.4 and BA.5 on the sensitivity of RT-qPCR assays for SARS-CoV-2 detection using whole genome sequencing. J Med Virol. 2023;95(1):e28241. https://doi.org/10.1002/jmv.28241
10. Chlabicz M, Jamiołkowski J, Sowa P, Zalewska M, Kiszkiel Ł, Ciołkiewicz M, et al. Multimorbidity patterns in the urban population in Poland. J Clin Med. 2023;12: 5860. https://doi.org/10.3390/jcm12185860
11. Worldometers. https://www.worldometers.info/coronavirus/country/poland/.[Accessed 23 January 2023].
12. Charkiewicz R, Niklin´ski J, Biecek P, Ki´sluk J, Pancewicz S, Moniuszko- Malinowska AM, et al. The first SARS-CoV-2 genetic variants of concern (VOC) in Poland: the concept of a comprehensive approach to monitoring and surveillance of emerging variants. Adv Med Sci. 2021;66(2):237–45. https://doi.org/10.1016/ j.advms.2021.03.005
13. Patil I. Visualizations with statistical details: the „ggstatsplot" approach. J Open Source Softw. 2012;6(61):3167. https://doi.org/10.21105/joss.03167
14. Castilla J, Lecea O´, Salas CM, Quílez D, Miqueleiz A, Trobajo-Sanmartín C, et al. Seroprevalence of antibodies against SARS-CoV-2 and risk of COVID-19 in Navarre, Spain, may to july 2022. Euro Surveill. 2022;27. https://doi.org/10.2807/1560-7917.ES.2022.27.33.2200619
15. Ren Z, Nishimura M, Tjan LH, Furukawa K, Kurahashi Y, Sutandhio S, et al. Large- scale serosurveillance of COVID-19 in Japan: acquisition of neutralizing antibodies for Delta but not for Omicron and requirement of booster vaccination to overcome the Omicron's outbreak. PLoS One. 2022;17. https://doi.org/10.1371/journal.pone.0266270
16. Kleynhans J, Tempia S, Wolter N, von Gottberg A, Bhiman JN, Buys A, et al. SARS- CoV-2 seroprevalence after third wave of infections, South Africa. Emerg Infect Dis. 2022;28:1055–8. https://doi.org/10.3201/eid2805.220278
17. Wang Y, Zheng K, Gao W, Lv J, Yu C, Wang L, et al. Asymptomatic and pre- symptomatic infection in Coronavirus Disease 2019 pandemic. Med Rev. 2022;2: 66–88. https://doi.org/10.1515/mr-2021-0034
18. H€agg S, Religa D. COVID vaccination in older adults. Nat Microbiol 2022;7:1106–7. https://doi.org/10.1038/s41564-022-01166-0
19. Notarte K, Catahay J, Peligro P, Velasco JV, Ver AT, Guerrero JJ, et al. Humoral response in hemodialysis patients post-SARS-CoV-2 mRNA vaccination: a systematic review of literature. Vaccines 2023;11(4):724.
20. Havers FP, Reed C, Lim T, Montgomery JM, Klena JD, Hall AJ, et al. Seroprevalence of antibodies to SARS-CoV-2 in 10 sites in the United States, March 23-may 12, 2020. JAMA Intern Med. 2020;180:1776–86. https://doi.org/10.1001/jamainternmed.2020.4130
21. Lai CC, Wang JH, Hsueh PR. Population-based seroprevalence surveys of anti-SARS- CoV-2 antibody: an up-to-date review. Int J Infect Dis. 2020;101:314–22. https://doi.org/10.1016/j.ijid.2020.10.011
22. Shrivastava S, Palkar S, Shah J, Rane P, Lalwani S, Mishra AC, et al. Early and high SARS-CoV-2 neutralizing antibodies are associated with severity in COVID-19 patients from India. Am J Trop Med Hyg. 2021;105:401–6. https://doi.org/10.4269/ajtmh.21-0014
23. Chen W, Zhang J, Qin X, Wang W, Xu M, Wang LF, et al. SARS-CoV-2 neutralizing antibody levels are correlated with severity of COVID-19 pneumonia. Biomed Pharmacother. 2020;130. https://doi.org/10.1016/j.biopha.2020.110629
24. Schlickeiser S, Schwarz T, Steiner S, Wittke K, Al Besher N, Meyer O, et al. Disease severity, fever, age, and sex correlate with SARS-CoV-2 neutralizing antibody responses. Front Immunol. 2021;11. https://doi.org/10.3389/fimmu.2020.628971
25. Trinit´e B, Tarre´s-Freixas F, Rodon J, Pradenas E, Urrea V, Marfil S, et al. SARS-CoV- 2 infection elicits a rapid neutralizing antibody response that

correlates with disease severity. Sci Rep. 2021;11. https://doi.org/10.1038/s41598-021-81862-9

26. Wang P, Liu L, Nair MS, Yin MT, Luo Y, Wang Q, et al. SARS-CoV-2 neutralizing antibody responses are more robust in patients with severe disease. Emerg Microb Infect. 2020;9:2091–3. https://doi.org/10.1080/22221751.2020.1823890
27. Ro€ltgen K, Powell AE, Wirz OF, Stevens BA, Hogan CA, Najeeb J, 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. https://doi.org/10.1126/ SCIIMMUNOL.ABE0240.
28. Kurano M, Ohmiya H, Kishi Y, Okada J, Nakano Y, Yokoyama R, et al. Measurement of SARS-CoV-2 antibody titers improves the prediction accuracy of COVID-19 maximum severity by machine learning in non-vaccinated patients. Front Immunol. 2022;13. https://doi.org/10.3389/fimmu.2022.811952
29. Perumal R, Shunmugam L, Naidoo K, Abdool Karim SS, Wilkins D, Garzino-Demo A, et al. Long COVID: a review and proposed visualization of the complexity of long COVID. Front Immunol. 2023;14. https://doi.org/10.3389/fimmu.2023.1117464
30. He P, Liu B, Gao X, Yan Q, Pei R, Sun J, et al. SARS-CoV-2 Delta and Omicron variants evade population antibody response by mutations in a single spike epitope. Nat Microbiol. 2022;7:1635–49. https://doi.org/10.1038/s41564-022-01235-4
31. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol. 2021;19:409–24. https://doi.org/10.1038/s41579-021-00573-0
32. Chansaenroj J, Yorsaeng R, Posuwan N, Puenpa J, Wanlapakorn N, Sudhinaraset N, et al. Long-term specific IgG response to SARS-CoV-2 nucleocapsid protein in recovered COVID-19 patients. Sci Rep. 2021;11:23216. https://doi.org/10.1038/ s41598-021-02659-4
33. Chansaenroj J, Yorsaeng R, Puenpa J, Wanlapakorn N, Chirathaworn C, Sudhinaraset N, et al. Long-term persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein-specific and neutralizing antibodies in recovered COVID-19 patients. PLoS One. 2022;17(4):e0267102. https://doi.org/ 10.1371/journal.pone.0267102