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Dynamics of anti-SARS-CoV-2 antibodies in the Albanian population: Impact of infection- and vaccine-induced immunity during the COVID-19 pandemic

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ABSTRACT

Objectives: Understanding immune response dynamics during the COVID-19 pandemic is crucial for optimizing future vaccine strategies. This study investigated the infection- and vaccine-induced SARS-CoV-2 antibody responses in the Albanian population from August 2021 to August 2022.

Methods: This used a cross-sectional approach, analyzing two independent, randomly selected population samples over 1 year. Participants' demographic, health, vaccination, and COVID-19 data were collected, with blood samples assessed via enzyme linked immunosorbent assay for immunoglobulin G class anti-spike and anti-nucleocapsid antibodies.

Results: By August 2022, all individuals receiving one vaccine dose achieved antibody levels comparable to those receiving two doses (median 7.71 index ratio [IR] vs 7.00 IR). In August 2021, those with previous COVID-19 infection receiving one vaccine dose showed median anti-spike immunoglobulin G levels of 7.22 IR compared with 4.84 IR in those without previous infection receiving two doses. However, individuals aged ≥61 years required two vaccine doses to achieve similar immune responses as younger individuals with one dose.

Conclusions: These findings underscore the importance of hybrid immunity, suggesting one vaccine dose may suffice for individuals with previous COVID-19 infection, whereas older adults require additional doses for optimal protection. This study provides insights into humoral immune response dynamics, which is crucial for refining COVID-19 vaccination strategies in middle-income countries with low vaccination coverage and high infection rates.

Introduction

The onset of the COVID-19 pandemic resulted in significant challenges to the human immune system [1]. The emergence of the novel SARS-CoV-2 virus in late 2019 unleashed a worldwide crisis on a vulnerable population that lacked preexisting immunity to this new infectious agent [2]. Over the subsequent 3 years (2020-2022), a dynamic interplay occurred between the viral agent and the evolving protective immunity of the population brought by infections and vaccinations. This interaction resulted in a progressive increase in the population's immunity against the virus, starting from nearly zero in early 2020 and reaching over 90% by the end of 2022 [3,4]. During the first year of the pandemic, the population's immune protection against the SARS-CoV-2 virus relied exclusively on natural infection. However, from the early months of 2021, mass vaccination campaigns using vaccines developed in record time in 2020 played a pivotal role in rapidly augmenting the protective immunity against the virus [5].

The relationship between vaccination, natural immunization from infections, and the technical aspects of vaccination, such as the number of vaccine doses and their administration intervals, have been the subject of many studies, highlighting their importance in delineating optimal vaccine strategies [5–7]. The contributions of infection- and vaccine-induced immunization varied across different countries and populations, contingent upon vaccine availability, the intensity of vaccination campaigns, and the extent of population exposure to the virus [8–10]. Studying these indicators in different countries and populations

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is essential, and, in Eastern European countries, there is a gap in studies scrutinizing the anti–SARS-CoV-2 antibody response amid variability in vaccine uptake and vaccine doses in a variety of sub-populations during more advanced stages of population immunity [11].

Albania is a middle-income country in Eastern Europe, with a comparatively low SARS-CoV-2 vaccination coverage and a relatively high pandemic toll [12]. Since the onset of 2020, our research has been focused on studying the immune response to SARS-CoV-2 in the Albanian population, and we have closely monitored the population's seropositivity progression throughout the 3 years of the pandemic [13,14]. In this study, we analyzed the humoral immune responses against SARS-CoV-2 spike 1 (S1) and anti-nucleoprotein (N) antigens, and the function of vaccination status, vaccine doses, previous COVID-19 infection and age in the general Albanian population.

The primary objective of this study was to investigate the interaction between vaccination status (including the number of vaccine doses) and natural immunization in generating a seropositivity rate and antibody levels. We applied these antibody parameters as indicators of immunity in the general population and observed them in different exposure categories. These findings can contribute to developing vaccination strategies necessary to sustain durable COVID-19 protective immunity within the Albanian population. In addition, these patterns can serve as a reference for middle-income societies with comparable immunization profiles.

Materials and methods

Study design and data collection procedures

This study used a repeated cross-sectional design. Two independent samples from the Albanian general population, including all age groups, were studied between August 2021 and August 2022 to assess seroprevalence. Participants were randomly enrolled from digital population registries associated with four primary health care centers (HCs) in Tirana and one in Berat City, collectively providing health care services to approximately 281,600 residents.

Health care professionals of these five HCs were instructed to randomly identify 400 persons from their catchment area registries (approximately 100 individuals from each age group [0-15 years, 16-30 years, 31-45 years, 46-60 years, and ≥61 years]) for a total sample of approximately 2000 individuals each year to be representative of the Albanian general population. The sample size was intended to allow meaningful comparisons between categories of interest (vaccination status, previous COVID-19 infection, and age). Individuals were invited via a phone call to participate in the study. They were asked to visit the HCs, provide a blood sample, and complete an interview after they consented to study participation and laboratory testing.

For this study, participants were included based on the following criteria: they had to be part of the Albanian general population, representing all age groups, and registered with one of the four primary HCs in Tirana or Berat. They were recruited if they had the ability to consent to study participation and laboratory testing. Parental consent was required for minors.

Health care professionals employed at the participating HCs and immediate family members of the participants were excluded from the study. If an individual from these categories was initially selected, they were substituted with the next eligible person from the list. This approach was taken to maintain the integrity of the sample and avoid potential biases. The response rate was high among adults over 30 years old, ranging from 90% to 95%, but was lower for children and adolescents.

A standardized questionnaire gathered information concerning participant demographics, health status, symptoms, data about COVID-19 infection in the past, and vaccination history. For participants younger than 18 years, parents provided the necessary information. Blood samples were sent to the Laboratory of Immunology of the University Hospital Center of Tirana for serological analysis.

Serological assessment of immunoglobulin G class anti-spike and anti-nucleoprotein SARS-CoV-2 antibodies

Immune response was measured by anti-SARS-CoV-2 antibody parameters. Each blood sample underwent serological testing through the enzyme linked immunosorbent assay method, where two commercially available diagnostic kits (immunoglobulin [Ig] G anti-S [Spike] 1 SARS-CoV-2 and IgG anti-NCP [Nucleoprotein] SARS-CoV-2 enzyme linked immunosorbent assay, Euroimmun, Luebeck, Germany) were utilized for the identification of SARS-CoV-2 anti-S1 and anti-N IgG antibodies, respectively. Per manufacturer specifications, these kits demonstrate a sensitivity and specificity of 94.4% and 99.6% for IgG anti-S1 SARS-CoV-2 and 94.6% and 99.8% for IgG anti-N SARS-CoV-2, respectively. Results from both kits were evaluated quantitatively by calculating the optical density ratio of the sample compared with a calibrator (index ratio [IR]), per manufacturer's guidelines. A serum sample was considered seropositive if the IR was higher than 1.10 for either antibody type. The primary end points included seropositivity rates (applying the 1.10 IR cutoff) and IR levels of IgG anti-S1 SARS-CoV-2 antibodies in serum samples.

Statistical analysis

Antibody response rates (measured as proportions and medians) were the primary outcome. Predictors included any history of COVID-19 infection and vaccination status (including the number of vaccine doses). Differences in antibody response rates between categories of predictors were examined using chi-square tests for categorical variables and Student's *t* test for continuous variables. The Mann–Whitney U test was used when a variable was not normally distributed. Spearman rank correlation coefficients were used to measure the correlation between two variables. Each analysis was stratified by age ≤60 years or ≥61 years and the interview year. The significance level for all statistical analyses was set at 0.01 after Bonferroni correction for multiple comparisons. All data were analyzed using MedCalc Statistical Software version 20.210 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2022).

Ethics statement

The study protocol was approved by the ethics committee of the Albanian Academy of Sciences (Project number 33-07-05-2020). All participants provided informed written consent before enrollment in the study.

Results

From July to August 2021, 1682 individuals were included in the study, with an age range of 4-97 years and a median age of 52 years (95% confidence interval 51-52 years). From July to August 2022, another cohort of 1899 individuals was analyzed using the same methodology, with a median age of 48 years (95% confidence interval 47-50) and an age range of 1-87 years. Demographic characteristics and information on SARS-CoV-2 infection and vaccine-induced immunization are summarized in Table 1.

Anti-spike 1 and anti-nucleoprotein immunoglobulin G antibody response among unvaccinated individuals in 2021 and 2022

In August 2021, 56.5% of the individuals included in the study were unvaccinated, whereas in August 2022, 31.2% reported no previous vaccination. The rates of previous COVID-19 infection were similar among unvaccinated individuals in both years, with 54.1% and 51.4% in 2021

Table 1
General characteristics of all individuals studied classified according to their vaccination status in August 2021 and August 2022.

Parameters	Years	Not vaccinated	One vaccine dose	Two vaccine doses	Three vaccine doses
Individuals studied	2021	970/57.7%	135/8.9%	577/34.3%	NA
Nr/%, (95% CI)		(54.1-58.9)	(6.70-9.30)	(31.4-35.9)	
	2022	593/31.2%	69/3.6%	875/46.0%	362 (19.1%)
		(29.2-33.2)	(2.82-4.40)	(44.1-47.9)	(17.5-20.6)
P-value	-	<0.0001	<0.0001	<0.0001	-
Sex	2021	600/61.9%	93/68.9%	329/57.0%	NA
Nr. females/%, (95% CI)		(58.8-65.0)	(60.4-76.6)	(52.8-61.1)	
	2022	324/54.6%	43/62.3%	548/62.5%	218/60.2%
		(50.5-58.7)	(49.8-73.7)	(59.2-65.7)	(55.0-65.3)
P-value	-	0.0044	0.3453	0.0361	-
	2021	48.0	57.0	59.0	NA
Age in years		(47.0-49.0)	(54.0-59.0)	(57.0-61.0)	
(median, 95% CI)	2022	38.0	48.5	47.0	62.0
		(35.0-40.0)	(42.0-55.0)	(45.0-48.0)	(61.0-64.0)
P-value	-	<0.0001	0.0002	<0.0001	-
Days after vaccination	2021	NA	36.0	46.0	N.A.
(median, 95% CI)			(27.4-46.6)	(43.0-52.0)	
	2022	NA	330.0	299.0	169.0
			(288.0-367.9)	(288.0-305.0)	(155.8-186.1)
P-value	-	NA	<0.0001	<0.0001	NA
Days after COVID-19 infection	2021	219.0	220.0	254.5	NA
(median, 95% CI)		(213.0-225.0)	(193.4-249.2)	(249.0-257.0)	
	2022	298.0	482.5	482.0	492.0
		(261.0-339.3)	(431.8-497.6)	(456.0-495.0)	(450.0-532.2)
P-value	-	<0.0001	<0.0001	<0.0001	-
Individuals with previous	2021	525/54.1%	63/46.7%	238/40.7%	NA
COVID-19 infection		(50.9-57.3)	(38.1-55.5)	(37,2-45,3)	
Nr/%,	2022	305/51.4%	42/60.9%	517/59.5%	221/62.3%
(95% CI)		(42.3-55.5)	(48.4-72.4)	(56.2-62.8)	(57.0-67.4)
P-value	-	0.2995	0.095	<0.0001	-

CI = confidence interval; NA = not available.

and 2022, respectively. However, the average interval time since the previous COVID-19 infection was 36.1% higher in 2022 than in 2021 (P <0.0001) (Table 1).

In 2022, the rate of anti-S1 seropositivity among unvaccinated individuals was 18.7% higher than in 2021 (P <0.0001), and the IR level of these antibodies was 2.28 times higher (Table 2). Similarly, the rate of anti-N seropositivity and antibody levels in 2022 was 14.5% and 70% higher (P <0.0001), respectively, than in 2021 (Table 3).

Vaccination data in all individuals receiving at least one vaccine dose in 2021 and 2022

In August 2022, there was a 27.3% increase in the number of individuals who had received at least one vaccine dose compared with the same period in 2021 (Table 1). In terms of the type of vaccines received, in 2021, 43.9% of them had received the CoronaVac vaccine, 39.9% BioNTech/Pfizer (Comirnaty), 18.0% AstraZeneca (Vaxzevria), and 2.1% Sputnik V. A year later, 64.8% of individuals had been vaccinated with BioNTech/Pfizer, 19.6% with CoronaVac, 13.8% with AstraZeneca, and 0.9% with Sputnik V. Among those vaccinated in 2022, the time interval since the previous COVID-19 infection was approximately twice as long compared with 2021 (Table 1).

Anti-spike 1 and anti-nucleoprotein immunoglobulin G antibody response among individuals who had received only one vaccine dose

In August 2021, 7.9% of the individuals included in the study had received only one first dose of the vaccine, on average, 36 days earlier (Table 1). A year later, in August 2022, only 3.6% of the cohort received only one vaccine dose, on average, 330 days earlier.

The 2022 group reported 14.2% more previous COVID-19 infections than those who received only one vaccine dose in 2021; however, the difference was not statistically significant (P = 0.095). This group had 18.6% higher anti-S1 seropositivity (P = 0.0007) and 53.0% higher antibody levels (P <0.0001) than the same 2021 group (Table 2). Similarly, the anti-N seropositivity was 24.4% higher (P <0.0001), and the level of these antibodies was 2.3 times higher than in 2021 (Table 3).

Anti-spike 1 and anti-nucleoprotein immunoglobulin G antibody response among individuals who had received two and three vaccine doses

In 2022, the percentage of individuals who had received two vaccine doses was 12.4% higher than in 2021 (P <0.0001). The average time interval from receiving the second dose was 299 days in 2022 compared with 46 days in 2021 and the same time interval since the previous COVID-19 infection was 90% higher in 2022. Also, the rate of individuals reporting past symptomatic COVID-19 infection was 18.8% higher (P <0.0001) (Table 1).

The anti-S1 seropositivity rate in the two doses vaccine group of the 2022 cohort was higher than in 2021 (95.2% vs 91.7%, P = 0.0015), as well as the mean antibody level (7.1 vs 6.3, P <0.0001) (Table 2). Also, the anti-N seropositivity and antibody levels were consistently increasing in 2022 compared with 2021; on average, they were 28.6% and 3.7 times higher, respectively (P <0.0001) (Table 3).

In August 2022, 19.1% of the individuals in this cohort had received a third vaccine dose 169 days earlier on average. The rate of previous COVID-19 infection was similar to that of those who had received the second dose (P = 0.384) (Table 1). Also, the anti-S1 and anti-N seropositivity rates and antibody levels showed no significant changes (P = 0.218) (Table 3).

Comparison of the dynamics of anti-spike 1 seropositivity and antibody levels among groups of individuals with different vaccination statuses in 2021 and 2022

Although anti-S1 seropositivity rates and antibody levels in 2022 were higher than levels in 2021, regardless of vaccination status, there were differences in immunity profiles in the 2021 and 2022 cohorts. In the 2021 cohort, anti-S1 seropositivity and the average antibody levels

Table 2
Anti-S1 IgG SARS-CoV-2 seropositivity rates and antibody levels in Albanian individuals studied in August 2021-2022 by COVID-19 infection and age, stratified by vaccination status.

		Vaccination status			
Parameters studied	Years	Not vaccinated	One vaccine dose	Two vaccine doses	Three vaccine doses
All individuals					
Anti-S1 IgG positivity	**2021**	596/61.4%	106/78.5%	530/91.7%	NA
Nr/%		(58.3-64.5)	(70.6-85.1)	(89.0-93.9)	
(95% CI)	**2022**	475/80.1%	67/97.1%	831/95.2%	358/98.9%
		(76.6-83.2)	(89.6-99.4)	(93.6-96.5)	(97.2-99.7)
P-value	-	<0.0001	0.0007	0.0015	-
Anti-S1 IgG level	**2021**	1.89	4.80	6.30	NA
Median IR,		(1.67-2.12)	(3.02-6.32)	(5.86-6.66)	
(95% CI)	**2022**	4.30	7.33	7.10	7.70
		(3.89-4.70)	(6.76-7.7)	(6.93-7.3)	(7.49-7.92)
P-value	-	<0.0001	<0.0001	<0.0001	-
COVID-19–positive individuals					
Anti-S1 IgG positivity	**2021**	403/76.8%	60/95.2%	230/97.5%	NA
Nr/%,		(72.9-80.3)	(86.7-99.0)	(94.6-99.1)	
(95% CI)	**2022**	258/84.6%	42/100%	500/96.9%	218/99.1%
		(80.1-88.5)	(91.6-100.0)	(95.0-98.2)	(96.8-99.9)
P-value	-	0.0071	0.1516	0.650	-
Anti-S1 IgG level	**2021**	2.90	7.22	7.48	NA
Median IR, (95% CI)		(2.63-3.23)	(6.57-7.74)	(7.05-7.88)	
	2022	4.65	7.40	7.38	7.60
		(4.00-5.20)	(6.80-8.19)	(7.07-7.50)	(7.30-7.80)
P-value	-	<0.0001	0.6164	0.4303	-
COVID-19–negative individuals					
Anti-S1 IgG positivity	**2021**	189/42.9%	45/62.5%	299/87.9%	NA
Nr/%,		(38.2-47.7)	(50.3-73.6)	(84.0-91.2)	
(95% CI)	**2022**	205/76.2%	25/92.6%	326/93.1%	132/98.5%
		(70.7-81.2)	(75.7-99.1)	(89.9-95.5)	(94.7-99.8)
P-value	-	<0.0001	0.0035	0.0197	-
	2021	0.84	1.78	4.84	NA
Anti-S1 IgG level		0.61-1.02	1.14-2.72	4.40-5.57	
Median IR,	**2022**	3.98	7.31	6.85	7.91
(95% CI)		3.25-4.70	4.93-7.66	6.56 -7.05	7.55-8.36
P-value	-	<0.0001	<0.0001	<0.0001	-
Individuals aged ≤60 yrs					
Anti-S1 IgG positivity	**2021**	468/59.2%	73/78.5%	290/93.2%	NA
Nr/%,		(55.7-62.7)	(68.8-86.3)	(89.8-95.7)	
(95% CI)	**2022**	367/79.1%	53/98.1%	664/96.1%	155/98.1%
		(75.1-82.7)	(90.0-99.9)	(94.4-97.4)	(94.6-99.6)
P-value		<0.0001	0.001	0.0471	
Anti-S1 IgG level	**2021**	1.69	4.36	7.18	NA
Median IR,		(1.48-1.92)	(2.62-6.48)	(6.78-7.5)	
(95% CI)	**2022**	4.4	7.71	7.00	7.51
		(3.89-4.80)	(6.83-8.12)	(6.86-7.2)	(7.21-7.79)
P-value	-	<0.0001	<0.0001	0.3016	-
Individuals aged ≥61 years					
Anti-S1 IgG positivity	**2021**	127/70.9%	33/78.6%	240/90.2%	NA
Nr/%,		(63.7-77.4)	(63.2-89.7)	(86.0-93.5)	
(95% CI)	**2022**	63/80.8%	13/92.9%	160/91.4%	197/99.5%
		(70.3-88.4)	(66.2-99.8)	(86.2-95.1)	(97.2-99.9)
P-value	-	0.1026	0.2541	0.6779	-
Anti-S1 IgG level	**2021**	3.00	5.52	5.11	NA
Median IR,		(2.48-4.00)	(3.23-7.24)	(4.48-5.59)	
(95% CI)	**2022**	4.12	6.46	7.50	7.93
		(3.56-5.03)	(3.75-7.83)	(7.1-7.81)	(7.61-8.2)
P-value	-	0.0064	0.3498	<0.0001	-

CI = confidence interval; Ig = immunoglobulin; IR = index ratio; NA = not available; S = spike.

increased almost uniformly from the unvaccinated group to those with one vaccine dose, reaching the highest values in double-vaccinated participants (Table 2). This dynamic pattern of seropositivity and antibody levels differed in the 2022 cohort, where the maximum average values were already reached in individuals who had received only a single vaccine dose. Their antibody levels did not differ from those with two vaccine doses in the same year (Figure 1a). Among individuals in the 2022 cohort who had received a third booster vaccine dose, the anti-S1 antibody response was found to be higher than those with two vaccine doses (P = 0.002 and P <0.0001 for seropositivity rate and antibody levels, respectively) but not significantly than those who received only one vaccine dose (Table 2).

Relationships between previous COVID-19 infection- and vaccination-induced immune response

Among all individuals studied, the reported previous symptomatic COVID-19 infection rate was 57.5% in the 2022 cohort compared with 49.1% in 2021 (P <0.0001). In unvaccinated individuals, despite the rates of previous COVID-19 infections being very similar in the 2021

Table 3
Anti-N IgG SARS-CoV-2 seropositivity rates and antibody levels in Albanian individuals studied in August 2021-2022 by COVID-19 infection and age, stratified by vaccination status.

Parameters studied	Years	Not vaccinated	One vaccine dose	Two vaccine doses	Three vaccine doses
All individuals					
Anti-N IgG positivity (Nr, %, 95% CI)	**2021**	428/44.4% (41.2-47.6)	50/37.9% (29.6-46.8)	179/33.3% (29.3-37.5)	NA
	2022	349/58.9% (54.8-62.9)	43/62.3% (49.8-73.7)	540/61.9% (58.6-65.1)	209/57.7% (52.4-62.8)
P-value	-	<0.0001	<0.0001	<0.0001	.
Anti N-IgG level (median IR, 95% CI)	**2021**	0.87 (0.80-0.99)	0.75 (0.60-0.94)	0.49 (0.41-0.58)	NA
	2022	1.48 (1.30-1.57)	1.75 (1.18-2.62)	1.80 (1.60-1.90)	1.47 (1.20-1.60)
P-value	-	<0.001	<0.0001	<0.0001	-
COVID-19–positive individuals					
Anti-N IgG positivity (Nr, %, 95% CI)	**2021**	304/58.5% (54.1-62.9)	36/59.0% (45.7-71.4)	130/60.5% (53.6-67.1)	NA
	2022	203 / 66.6% (61.0-71.9)	25 / 59.2% (43.0-74.0)	355 / 68.8% (64.6-72.8)	145 / 65.9% (59.2-72.1)
P-value	-	0.0211	0.9839	0.0864	-
Anti-N IgG level (median IR, 95% CI)	**2021**	1.34 (1.21-1.56)	1.45 (0.96-2.66)	1.40 (1.20-1.67)	NA
	2022	1.6 (1.50-1.93)	2.27 (1.03-3.05)	2.04 (1.87-2.30)	1.78 (1.51-2.20)
P-value	-	0.1721	0.01375	<0.0001	-
COVID-19–negative individuals					
Anti-N IgG positivity (Nr, %, 95% CI)	**2021**	122/27.8% (23.7-32.2)	14/19.7% (11.2-30.8)	49/15.3% (11.5-19.7)	NA
	2022	133/49.4% (40.6-58.2)	18/66.6% (46.0-83.4)	181/51.7% (46.3-57.0)	61/45.5% (36.5-54.7)
P-value	-	<0.0001	<0.0001	<0.0001	-
Anti-N IgG level (median IR, 95% CI)	**2021**	0.50 (0.40-0.56)	0.25 (0.14-0.50)	0.29 (0.25-0.33)	NA
	2022	1.10 (0.78-1.30)	1.56 (0.94-2.16)	1.23 (1.00-1.57)	0.90 (0.62-1.33)
P-value	-	<0.0001	<0.0001	<0.0001	-
Individuals aged ≤60 years					
Anti-N IgG positivity (Nr, %, 95% CI)	**2021**	316/40.3% (36.8-43.8)	30/33% (23.5-43.6)	99/34.4% (29.9-40.2)	NA
	2022	267/57.5% (52.9-62.0)	34/63.0% (48.8-75.7)	415/60.0% (56.2-63.7)	91/57.6% (49.5-65.4)
P-value	-	<0.0001	0.0005	<0.0001	-
Anti-N IgG level (median IR, 95% CI)	**2021**	0.80 (0.73-0.87)	0.60 (0.40-0.89)	0.54 (0.44-0.76)	NA
	2022	1.40 (1.17-1.53)	1.72 (1.16-2.60)	1.68 (1.51-1.87)	1.46 (1.09-1.60)
P-value	-	<0.0001	<0.0001	<0.0001	-
Individuals aged ≥61 years					
Anti-N IgG positivity (Nr, %, 95% CI)	**2021**	112/62.6% (55.0-69.6)	21/51.2% (35.1-61.1)	81/32.5% (26.7-38.7)	NA
	2022	62/79.5% (68.9-87.8)	8/57.1% (28.8-82.3)	119/68.0% (60.5-74.8)	113/57.1% (49.9-64.1)
P-value	-	0.0078	0.7052	<0.0001	-
Anti-N IgG level (median IR, 95% CI)	**2021**	1.86 (1.35-2.20)	1.0 (0.60-2.51	0.42 (0.33-0.51)	NA
	2022	2.16 (1.80-2.93)	1.51 (0.68-3.05)	2.09 (1.78-2.53)	1.47 (1.11-2.02)
P-value	-	0.0042	0.0588	<0.0001	-

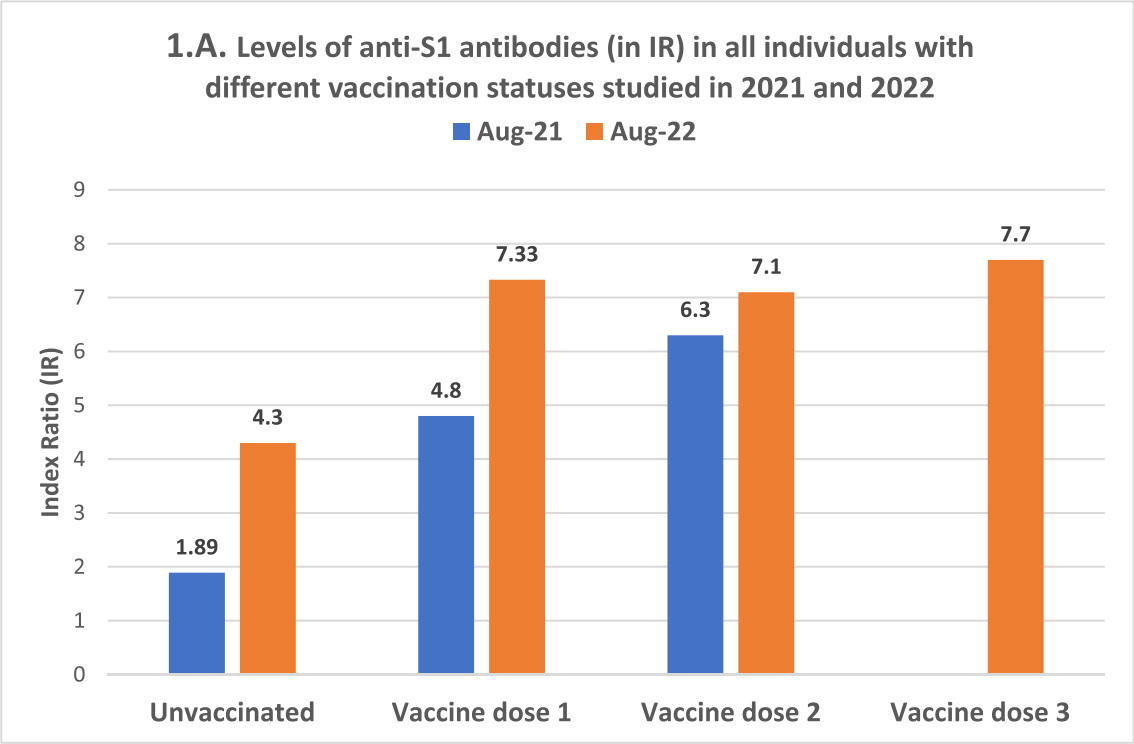
CI = confidence interval; Ig = immunoglobulin; IR = index ratio; NA = not available; N = nucleoprotein.

and 2022 cohorts (Table 1), there was a significant increase in anti-S1 and anti-N antibodies in 2022 compared with 2021 (P <0.0001). This increase was considerably more pronounced (three-fold increase) among individuals who were COVID-19–negative than those with COVID-19 (Table 2).

In August 2021, the increase in anti-S1 seropositivity and antibody levels among vaccinated individuals who were COVID-19–negative was proportional to the number of vaccine doses, similar to the dynamic observed in the general 2021 cohort (Table 2). However, this dynamic differed in the same year's COVID-19–positive group, where we observed that anti-S1 seropositivity and antibody levels, likely in all the 2022 cohort, already reached their maximum average level in individuals with only one vaccine dose (Figure 1b). No significant difference existed between them and those who received two vaccine doses (Table 2).

Individuals who were COVID-19–positive of the 2022 cohort who had received the two doses of the vaccine presented a higher anti-S1 response than the same year individuals who were COVID-19–negative with two vaccine doses (P = 0.01 and P = 0.002, respectively, for seropositivity and antibody level) (Table 2).

Among the individuals who were COVID-19–negative of the 2022 cohort who received a third vaccine dose, the anti-S1 antibody response was higher than in those who received two vaccine doses (P = 0.01 and P <0.0001 for seropositivity and level of antibodies, respectively). However, in individuals who were COVID-19–positive, the corresponding values presented a minor increase in the seropositivity rate (P = 0.034)



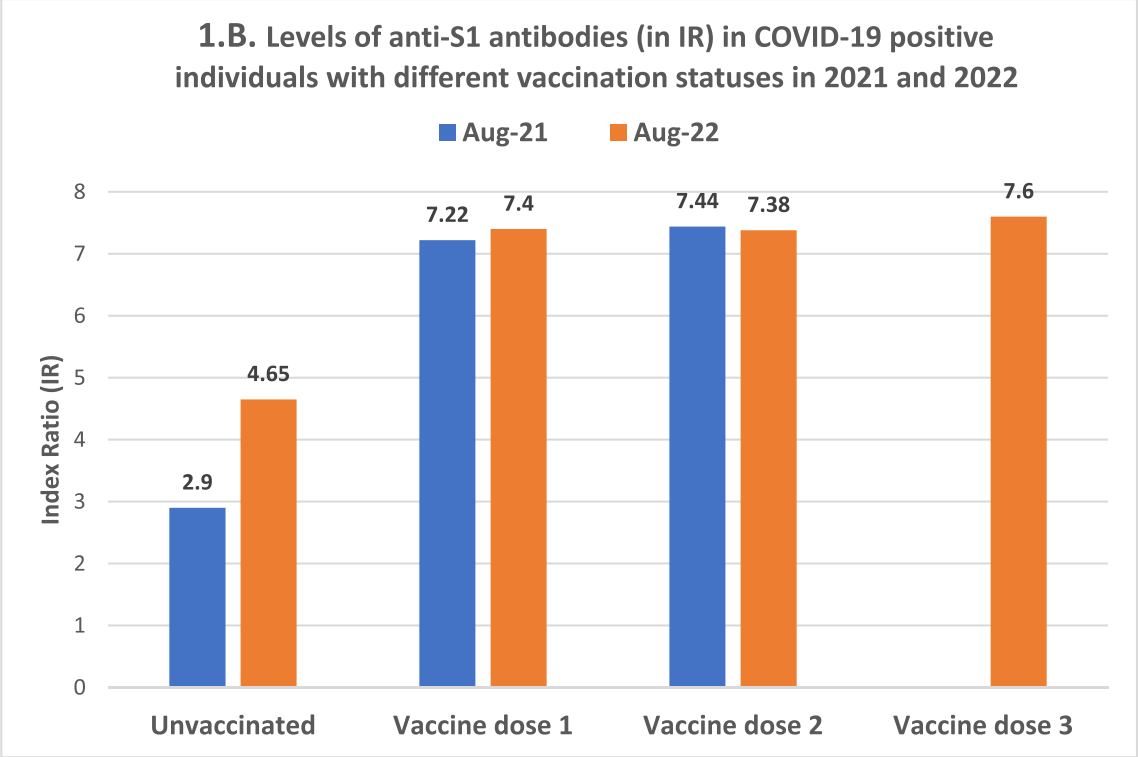


Figure 1. Levels of anti-S1 antibodies (in index ratio values) in all individuals with different vaccination statuses (1a) and in only individuals with reported past COVID-19 infection (1b) in the 2021 and 2022 cohorts.

and were nonsignificant for the antibody levels ($P = 0.177$). Similarly, no differences were observed between the COVID-19–positive and -negative groups that received the third vaccine dose (Table 2).

Relationship of age and anti-SARS-CoV-2 antibody response among groups of individuals with different vaccination statuses.

In August 2021, among unvaccinated individuals, those aged ≥61 years exhibited significantly higher anti-S1 seropositivity rates and antibody levels than those aged ≤60 years, with differences of 11.7% and 77.8%, respectively ($P = 0.0006$ and $P < 0.0001$, Table 2). However, this disparity was not observed in the 2022 cohort. Similarly, anti-N seropositivity and antibody levels were notably higher in the unvaccinated ≥61 years age group than those aged ≤60 years in both studied years ($P < 0.0001$, Table 3). In August 2022, the average level of anti-S1 antibodies in the ≥61 age group who had received two vaccine doses was

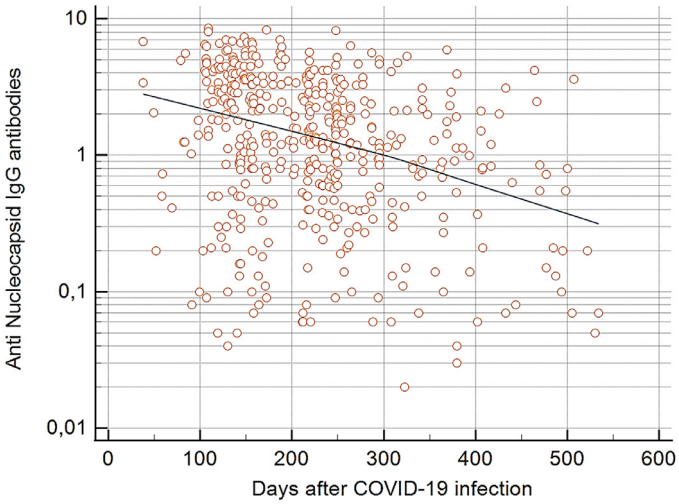


Figure 2. Correlation between the level of N SARS-CoV-2 IgG antibodies (in index ratio values) and time interval after the infection among the unvaccinated individuals with past COVID-19 infection (vaccine− COVID-19+) in August 2021 (Spearman rho = −0.286; P <0.0001; n = 474). Ig, immunoglobulin.

46.8% higher than in the same age group in 2021 (P <0.0001), whereas seropositivity rates remained similar. Conversely, in the ≤60 age group, seropositivity and antibody levels were very similar in 2022 compared with 2021 (Table 2).

In the 2022 cohort, the ≤60 years age group reached its peak average anti-S1 antibody rates and levels with a single vaccine dose, whereas the ≥61 years group reached its highest level only after the second vaccine dose. Notably, among the ≥61 years age group in 2021, no difference in anti-S1 antibody levels was detected between individuals receiving one or two vaccine doses (P = 0.3917) (Table 2). In August 2022, in the ≤60 years age group who received a third vaccine dose, the increase in anti-S1 seroprevalence compared with the two-dose vaccine group was not statistically significant (P = 0.084). However, the median antibody level exceeded the significance threshold compared with the two-dose vaccine group (P = 0.002) but not compared with the single-dose group (Table 2). Conversely, in the ≥61 years age group, the increase in seroprevalence rate between the second and third doses was highly statistically significant (P = 0.00011). However, the rise in antibody levels did not reach the significance threshold in this group (P = 0.105).

Anti-nucleoprotein seropositivity rates and antibody levels vs vaccination statuses by year

The dynamics of anti-N antibodies over time differed from that of anti-S1 antibodies. In 2021, anti-N seropositivity rates and mean antibody levels were lower in individuals who received two doses of the vaccine than those who were not vaccinated (P <0.0001 and P <0.0001, respectively). However, in 2022, these levels remained unchanged among the groups of individuals with different vaccine doses (Table 3). In both years, the correlation analyses of anti-N antibody levels and the time interval (in days) since the previous COVID-19 infection or the vaccination date revealed a significant negative correlation, as demonstrated in August 2021 with anti-N IgG antibodies (P <0.0001) (Figure 2).

Discussion

In this study, we present the results of our study on the anti-S1 and anti-N humoral immune responses in different groups within the Albanian population, categorized by varying vaccination statuses. The study involved two independent samples from the general population, with a 1-year interval between August 2021 and August 2022. Up until August 2021, the Albanian population experienced three COVID-19 waves: the first two were caused by the original Wuhan virus variants and the third was caused by the Alpha variant. In September 2021, the Delta variant triggered a fourth wave. This was followed by two consecutive waves from the beginning of 2022 until August 2022, driven by the Omicron variants, which have largely subsided in Albania at the time of this writing.

Among the unvaccinated participants of the 2022 cohort, the rate of previous symptomatic infection was 51.4%, with asymptomatic infections occurring in 42.3% of those older than 20 years [14]. Despite these findings, anti-S1 seropositivity was 80.1% among unvaccinated individuals of the August 2022 cohort. However, among all vaccinated individuals, comprising approximately 70% of the sample population, seropositivity ranged from 95% to 99%. These data underscore the critical importance of vaccination in achieving the population's collective immunity threshold [15–17] and suggest that it may have played a significant role in controlling COVID-19 transmission in Albania. This is evident in the absence of epidemic waves in the country since September 2022 [18].

The rates of previous symptomatic COVID-19 infections were similar among unvaccinated individuals in both cohorts. However, from 2021 to 2022, a significant increase in seropositivity and antibody levels was observed among unvaccinated individuals who were COVID-19–negative compared with unvaccinated individuals who were COVID-19–positive. This finding seems to be attributed to the accumulation of non-reported asymptomatic (or quasi-symptomatic) COVID-19 infections during the last year of the pandemic [19,20]. The elevated levels of anti-S1 and anti-N seropositivity observed among unvaccinated individuals aged 61 years and above in 2021, in comparison to the ≤60 years age group, imply that older Albanians experienced heightened exposure to the SARS-CoV-2 virus during the initial phases of the pandemic. This discovery contrasts with findings from a United States study, which indicated that individuals aged 65 years and above had lower prevalence of infection-induced and hybrid immunity than younger demographics [21]. Nevertheless, by August 2022 in Albania, both age groups had experienced a plateau in virus exposure and antibody response.

In the 2022 cohort of vaccinated individuals, there was no significant difference in seropositivity and levels of anti-S1 antibodies between those who received one vaccine dose and those who completed the full vaccination schedule. Also, as we have reported in another study, the anti-S1 antibody response was uniformly elevated across all individuals, regardless of vaccine type or dosage received [22]. However, this pattern was not observed in the 2021 cohort. This distinction likely stems from the higher prevalence of individuals with previous COVID-19 infections among the vaccinated group in August 2022 than the previous year. The presence of hybrid immunity, combining natural infection with vaccination, likely contributed to the robust immune response observed among individuals who tested positive for COVID-19 and received only one vaccine dose in 2021. They exhibited similar seropositivity rates and antibody levels as the 2022 sample. Although the effect of hybrid immunity in enhancing immune response has been documented in numerous studies [23–27], to the best of our knowledge, no other report has demonstrated this effect across two distinct samples of the general population over a 1-year interval, where significant differences in previous COVID-19 infection rates were observed.

The impact of previous COVID-19 infection on the immune response is evident in the ≥61 years age group who had received two vaccine doses. Although the anti-S1 seropositivity rate in this age group was comparable to the same group in 2021, the levels of anti-S1 antibodies were significantly higher than the previous year. The higher rate of previous COVID-19 infections in this age group is further supported by the elevated anti-N seropositivity and antibody levels observed in 2022 compared with 2021. In contrast, the ≤60 years age group of the 2022 cohort who received only a single vaccine dose exhibited similar anti-S1 seroprevalence and antibody levels as those who received two or three

vaccine doses. However, individuals aged ≥61 years reached these levels only after receiving two vaccine doses. Moreover, in the ≥61 years age group, individuals who received a third vaccine dose demonstrated a significant increase in seropositivity but not in antibody levels. These findings suggest that individuals aged ≥61 years may have limitations in generating a robust humoral immune response [28,29].

Interestingly, the time elapsed from the previous COVID-19 infection or the last vaccine dose did not play a significant role in the amplitude of the anti-S1 immune response. The immune response remained robust despite the longer intervals between infection and vaccination in 2022 compared with 2021. This finding supports other studies reporting that protection from only vaccination can decrease over time; however, vaccination after infection can maintain significantly higher antibody titer levels for a prolonged period [30,31].

The presence of anti-N antibodies indicates a natural SARS-CoV-2 infection [32]. However, in the 2021 and 2022 cohorts, anti-N antibodies demonstrated lower sensitivity than anti-S1 antibodies. This finding is clearly shown among the unvaccinated individuals, in whom the anti-N seropositivity rates were in the 2021 and 2022 cohorts, respectively, 17.0% and 21.2% lower than the corresponding anti-S1 seropositivity rates. The levels of anti-N antibodies also decreased over time, which is also reported by other authors [33,34]. This effect in our study has been found in the 2021 cohort but not 1 year later, possibly due to the considerably longer time interval since the previous COVID-19 infection in the 2022 cohort and the effect of hybrid immunity.

There were limitations of our study, particularly, our inability to track a complete cohort over time. Due to the low vaccination rates among individuals under 16 years, we could not investigate antibody responses according to vaccine doses in this age group. Our study measured the humoral immune response using anti-S1 and anti-N antibodies rather than virus-neutralizing antibodies. In addition, data on previous clinical COVID-19 infections and vaccination status relied on self-reported information. Furthermore, we did not collect data on post-vaccination COVID-19 infections.

To the best of our knowledge, no other reports, especially in Eastern Europe, have illustrated the role of hybrid immunity in generating anti-S1 and anti-N immune responses concerning vaccination status and vaccine doses within the general population studied prospectively across two different cohorts over the last year of the COVID-19 pandemic. The population-based seroepidemiological data presented here corroborate previous studies, which have indicated that individuals with previous COVID-19 infection may achieve a robust immune response with just one vaccine dose, comparable to those who received two vaccine doses [35,36]. This finding is particularly relevant for Albania's August 2022 epidemiological situation, when an anti-S1 antibody immune response in more than 90% of the population and a high prevalence rate of hybrid immunity are detected. To achieve optimal protective levels of anti-S1 antibodies in individuals over 60 years and those with immune deficiencies, a second and, perhaps, a third booster dose is necessary to obtain a sufficiently protective level of immunity. Our findings may contribute to a better understanding of the dynamics of humoral immune responses against SARS-CoV-2 and improve vaccination strategies for COVID-19 control in the Albanian population. Our study's specific anti-SARS-CoV-2 antibody immune response patterns should be evaluated in other countries when adequate population-based age-specific immunization data becomes available.

Declarations of competing interest

The authors have no competing interests to declare.

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Ethical considerations

The study was approved by decision Nr. 33, May 7, 2020, of the Academy of Sciences of Albania Ethics Committee. Study participants provided written informed consent.

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Author contributions

Conceptualization, G.S., A.Y., F.C., and MJP; methodology, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., E.L. and JDP; software, G.S., F.C. and A.Y.; validation, G.S., F.C., A.Y., and M.K.P.; formal analysis, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., E.L., JDP and MJP; investigation, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., E.L., and JDP; resources, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., E.L. and JDP; data curation, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., E.L., and JDP; writing—original draft preparation, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., and MJP; writing—review and editing, G.S., A.Y., F.C., M.K.P., and MJP; visualization, G.S., A.Y., F.C., I.S.Q., M.K.P., E.S., E.L., and MJP; supervision, G.S., A.Y., F.C., I.S.Q., M.K.P., and MJP; project administration, G.S. and A.Y.; funding acquisition, G.S. and A.Y. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data presented in this study are available on request from the corresponding author.

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