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ORIGINAL ARTICLE

Immune response to COVID-19 vaccination in a population with and without a previous SARS-CoV-2 infection

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Abstract

Purpose To evaluate IgG production in a group of vaccinated and unvaccinated subjects previously infected, or not, with SARS-CoV-2.

Methods A total of 316 subjects were enrolled at different times after vaccination and/or infection. IgG against target S1 subunit of the spike protein of SARS-COV-2 was assessed by a chemiluminescent microparticle immunoassay. Participant data was collected using a clinical-epidemiological survey.

Results A total of 56.2% ($n=146$) of our cohort was vaccinated, with 27.5% ($n=36$) reporting a previous infection. Of these, all were IgG positive at the time of the study, regardless of gender, age category, vaccine type, and elapsed time since vaccination. The vaccinated group without a previous infection (72.5%, $n=95$) showed a slightly lower IgG seropositivity and median values, overall, although significantly higher in females and lower with the ChAdOx1 nCoV-19 (AstraZeneca) vaccine. Vaccinated subjects above the age of 65 showed a trend towards higher median IgG values (13,911.0 AU/mL), when previously infected with SARS-CoV-2, but comparatively lower IgG median value (5158.7 AU/mL) in its absence. In all vaccinated groups, IgG antibody production increased at 1–2 weeks, peaking at 4–6 weeks. Afterward, IgG decreased progressively but almost all subjects (97.7%, $n=128$) were seropositive for the remainder of our study. Fully vaccinated individuals with a past infection showed a lower IgG rate of decrease versus their uninfected counterparts (17.9 vs 22.6%, respectively).

Conclusion Our findings suggest a higher effect of vaccination on the production IgG antibodies, as opposed to natural infection. Nonetheless, in general, antibody titers waned rapidly.

Keywords COVID-19 · SARS-CoV-2 · Spike(S) · PCR diagnosis · Immunity · Vaccine

Introduction

On December 2019, a series of acute pneumonia cases of suspected viral etiology surfaced in the Wuhan province of China. The viral agent, later identified as a novel coronavirus, spread quickly, originating a global pandemic. Due to its high similarity to the agent of the severe acute respiratory syndrome (SARS-CoV), on February 2020, the World Health Organization (WHO) designated the new coronavirus SARS-CoV-2, while the resulting disease was named COVID-19 (coronavirus infectious disease 2019) [1].

SARS-CoV-2 is a positive, single-molecule, RNA virus with a single envelope. Its genome contains less than 30,000 nucleotides and codes for approximately twenty-nine identified viral proteins. The 3'-end of SARS-CoV-2 genome encodes 4 major structural proteins: the spike protein (S), the envelope glycoprotein (E), the membrane protein (M),

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and the nucleocapsid protein (N). Proteins S, E, and M form the viral envelope, while the N protein forms the capsid that houses the genomic RNA. Proteins most relevant for infection are the S protein (the viral ligand for cell invasion) and the N protein [2].

Like other coronaviruses, the S protein of SARS-CoV-2 can be cleaved into S1 and S2 subunits by proteases; S1 recognizes and connects to its receptor on the host cell, the angiotensin-converting enzyme-2 (ACE2) [3], and subsequent conformational changes in S2 facilitate the fusion between the viral envelope and the host cell membrane [4].

Epidemiological data has shown that, among all subjects infected with SARS-CoV-2, about 85% develop mild to moderate symptoms, 15% need hospitalization and, of those, 5% develop critical disease, requiring intensive care and organ support therapy [5]. Given that virus transmission rates are high, a progressively higher number of patients eventually seek hospital care, which represents a high burden for national health systems, many of which have nearly collapsed in certain countries severely affected by the pandemic. Severe COVID-19 is rare in children and youngsters. A possible explanation for this is the presence of a higher antibody titer against seasonal coronaviruses, possibly conferring a certain degree of protection against SARS-CoV-2 infection. Higher ACE2 expression might facilitate infection while enabling maintenance of a lesser inflammatory state, by maintaining a functioning ACE2–Angiotensin-(1–7) MAS system. Finally, non-specific protective effects after receiving live vaccines and a more diverse T-cell repertoire in children and young people might contribute to milder presentations. Children with systemic autoimmune or inflammatory conditions might be further protected by overcoming the immune evasion mechanisms of SARS-CoV-2, as well as some treatments might even protect them from the development of cytokine storm syndrome later in the disease course [6].

Severe COVID-19 has been associated with a poor innate immune response followed by a strong inflammatory reaction, leading to a cytokine storm that eventually results in organ failure [7, 8]. Understanding the immune response to SARS-CoV-2 is, thus, of pivotal importance, not only for COVID-19 management but also for the development of new therapeutic and vaccination strategies, aiming to mitigate the current pandemic.

Interest has grown on whether COVID-19 patients develop long-term immunity to SARS-CoV-2. The protection from subsequent infection seems to correlate to both production of specific antibodies directed to the viral spike protein and T cell-dependent immunity [9, 10]. Several studies have demonstrated that the majority of COVID-19 patients become seropositive 10 to 15 days after infection [11], a time period that can be extended in the cases of mild infection. Nevertheless, antibody response against SARS-CoV-2 seems to wane overtime, as reported by different research groups [11–14]. In fact, although severe presenting COVID-19 patients appear to maintain higher neutralizing IgG levels during a longer period, a 40% decrease in neutralizing IgG levels has been observed after only 5 months [11, 12]. A waning antibody response to infection was also observed for SARS and Middle East respiratory syndrome (MERS), with a return to antibody base level in 2 years [15]. In addition, asymptomatic subjects and patients with mild disease develop a weaker antibody response, incompatible with long-term immunity [11, 14]. These findings reinforce the importance of vaccination to prevent infection and possibly achieve herd immunity.

In a global unprecedented effort, only 1 year after the first reported COVID-19 case, several vaccine candidates against SARS-CoV-2 had emerged and been approved by European Union (EU) and other regulators. Vaccine candidates approved by EU regulators by December 2020 used different immunization strategies: mRNA-based vaccines (BTN162b2 – Pfizer/BioNTech and mRNA1273 – Moderna) and adenoviral-vectored vaccines (ChAdOx1 nCoV-19 – University of Oxford/AstraZeneca; Gam-COVID-Vac — Gamaleya Research Institute; and Ad26.COV2.S — Janssen) [16]

Efficacy in preventing symptomatic infection varies between vaccines. Considering the wild type virus, an efficacy of higher than 94% has been reported for BNT162b2 (Pfizer BioNTech) vaccine (2 doses) [17–19], 90% for mRNA1273 (Moderna) (2 doses) [20], 62% for ChAdOx1 nCoV-19 (AstraZeneca) [16, 21, 22], 91% for Gam-Covid-Vac [23], and 67% for Ad26.COV2.S [16]; however, variability occurs among studies. Exposure to variants of concern with higher infection rate and individual differences in generating long-term protective immunity may affect vaccine efficacy. In addition, given that, at the time of this study, not even a full year has passed since the first immunizations occurred the total duration of vaccine protection remains unknown. For all these reasons, follow-up of neutralizing IgG levels in vaccinated individuals is of utmost importance to evaluate vaccine protection and the putative need for subsequent boosts.

This study aimed to assess the kinetics of the humoral immune response to COVID-19 vaccination by evaluating IgG levels in a group of vaccinated and unvaccinated subjects with and without a previous SARS-Cov-2 infection. Antibody assessment was performed at different time points after either vaccination or natural infection. This study was performed in Portugal, a country with one of the higher vaccination rates in the world, as of this date.

Methods

Sample selection

Three hundred sixteen subjects ($n = 316$) who voluntarily sought for a SARS-CoV-2 immunity test (by quantification of IgM and IgG antibodies against the target S1 subunit of the spike protein) were included in this study. Participants were enrolled at Dra. Matilde Sampaio Clinical Analysis Laboratory in Mogadouro (located in Northeast Portugal), between February 2021 and December 2021. Informed consent was obtained from each participant. Each subject was asked to complete an online form concerning their clinical history regarding SARS-CoV-2 infection. This form included history of past infection (if present, date of the diagnosis), history of exposure to a positive case, and vaccination data (type of commercial vaccine, dates of administration). All confidential information was encrypted and could only be accessed by the laboratory clinical director or a delegate (in compliance with the professional duty of secrecy and data protection).

According to Portuguese health authorities and vaccine administration guidelines at the time, a complete vaccination scheme, regardless of vaccine type, consisted of two doses for those previously undiagnosed with COVID-19, and one dose for the previously diagnosed, administered 6 months after diagnosis.

Antibody detection

Analytical tests were carried out using Abbott's ARCHITECT iSystem i1000 equipment, employing a chemiluminescent microparticle immunoassay (CMIA) in serum samples. The ARCHITECT iSystem calculates the medium chemiluminescence calibrator value from 3 replicates of the calibrator (C) and stores the result. The sample results are calculated by dividing the sample (S) by the calibrator.

We quantitatively measured IgG antibodies against the S1 subunit of the virus S protein and IgM antibodies against the N protein using a commercial Abbott kit. According to the manufacturer's instructions, IgG results were deemed positive if ≥ 50.0 AU/mL (arbitrary units by milliliter). For IgM, results were considered positive if the index factor was ≥ 1.1 AU/mL.

Statistical methods

Graphical presentation and statistical analysis were performed using SPSS® Statistics version 26.0 (IBM, Armonk, NY, USA), considering a significance level of 0.05 for all statistical inferences. Counts and proportions (n [%]) were reported for categorical variables and quantitative data was described in median values and corresponding 25th and 75th percentiles (med [interquartile range]), as all were non-normally distributed. Proportions were compared using the chi-square test or Fisher's exact test, whenever appropriate. Z-test adjusted p-values (Bonferroni method) was used to identify categories whose column proportions did not differ significantly from each other at the 0.05 level. Comparison of quantitative data among the categories of the most relevant covariates used was achieved via the Mann–Whitney U or Kruskal–Wallis H tests, whenever suitable. For related samples, the Wilcoxon test was used.

Results

Characterization of the study participants ($n = 316$) is presented in Table 1. The majority are middle-aged individuals with a median age of 45 years (IQR between 36.0 to 59.0). Only a small number of subjects were under the age of 18, which is in line with the age group that goes less frequently to the laboratory for analysis. The study was performed from December 2020 until December 2021. The female gender was predominant in our cohort (69.3%, $n = 219$). Of those who answered the query, 107 (42.8%) reported a previous diagnosis of SARS-CoV-2 while 104 (41.9%) referred a previous exposure to a positive case (Table 1).

At the time of this study, 56.2% ($n = 146$) of our cohort had already been vaccinated, the majority (86.4%, $n = 121$) with BNT162b2, 7.9% ($n = 11$) with ChAdOx1 nCoV-19 (AstraZeneca) and 5.7% ($n = 8$) with mRNA1273 (Moderna).

The interval for the administration of vaccine doses was 21 days for BNT162b2, 28 days for mRNA1273 (Moderna) and 84.5 days for ChAdOx1 nCoV-19.

When compared to their unvaccinated peers, the vaccinated group showed a higher frequency of SARS-CoV-2-specific IgG-positive subjects (97.9% versus 60.5%) as well as a higher median IgG values (7222.6 versus 167.0 AU/ml) (Table 2). We also observed a higher rate of previous infection by SARS-CoV-2 (60.6%, $n = 66$) in unvaccinated subjects, in addition to a higher frequency of past exposure to a positive case (52.9%, $n = 55$), when compared to the vaccinated participants (27.5%, $n = 36$). Most subjects with a positive contact had a positive history of SARS-CoV-2 diagnosis (75.9%, $n = 41$). No significant differences were found between vaccinated and unvaccinated groups considering gender, median age, age categories, IgM seropositivity, or IgM levels (Table 2).

IgG seropositivity for SARS-CoV-2 in vaccinated and unvaccinated groups is shown in Table 3. All vaccinated subjects previously infected with SARS-CoV-2 (100%, $n = 36$) were IgG positive at the time of the study, regardless of gender, age category, vaccine type, and elapsed time since vaccination. In this group, 58.3% ($n = 21$) was

Table 1 Clinical and biological characterization of the sample ($n=316$)

Variable[a]	Total study sample
Gender ($n=316$), *n* (%)	
Female	219 (69.3)
Male	97 (30.7)
Age (years) ($n=316$), med (IQR)	45 (36.0–59.0)
Age categories (years) ($n=316$), *n* (%)	
≤ 18	5 (1.6)
19–40	117 (37.0)
41–64	140 (44.3)
≥ 65	54 (17.1)
SARS-CoV-2 infection ($n=250$), *n* (%)	
Yes	107 (42.8)
No	143 (57.2)
Exposure to a positive case ($n=248$), *n* (%)	
Yes	104 (41.9)
No/unknown	144 (58.1)
COVID-19 vaccination ($n=260$), *n* (%)	
Yes	146 (56.2)
No	114 (43.8)
COVID-19 vaccine type ($n=141$), *n* (%)	
BNT162b2 (Pfizer BioNTech)	121 (86.4)
mRNA1273 (Moderna)	8 (5.7)
ChAdOx1 nCoV-19 (AstraZeneca)	11 (7.9)
Vaccination period (date), min–max	
1st dose ($n=146$)	December 2020–May 2021
2nd dose ($n=114$)	January 2021–December 2021
Analysis period (date) ($n=319$), min–max	February 2021–December 2021

Med (IQR): median (interquartile range), *n (%)*: count (percentage), *min–max*: minimum–maximum

[a]For each variable, the values of n correspond to the total number of answers/results

vaccinated with 1 dose and 41.7% ($n=15$) with 2 doses of the vaccine.

The vaccinated group without a previous SARS-CoV-2 infection showed a slightly lower value of IgG positivity (96.8%, $n=92$), but not significantly different from the former (Table 3 and Fig. 1a). In this group, 88.3% ($n=83$) was vaccinated with 2 doses and 11.7% ($n=11$) with 1 dose. When compared to their male counterparts, women in this group showed both a significantly higher frequency of seropositivity (100% versus 88.9%, respectively), as well as a significantly higher median value of IgG (9275.7 vs 2847.1 AU/mL, $p=0.000$) (Fig. 1b). Also among this group, the ChAdOx1 nCoV-19 (AstraZeneca) vaccine resulted in both a significantly lower IgG seropositivity as well as lower IgG median values than the remaining BNT162b2 (Pfizer BioNTech) and mRNA1273 (Moderna) vaccines (81.8% vs 100% vs 100%, respectively) (Table 3) (480.0 vs 8265.6 AU/mL vs 14,291.5, $p=0.000$) (Fig. 1c). No significant differences in IgG positivity and IgG median values were detected between vaccinated groups (with and without previous SARS-CoV-2 infection), even when accounting for gender, vaccine and age (Table 3 and Fig. 1b–d). Although not statistically significant, vaccinated subjects above the age of 65appeared to display higher median IgG values (13,911.0 AU/mL) than other age groups, when previously infected with SARS-CoV-2. Conversely, this same age group showed comparatively lower IgG median value (5158.7 AU/mL) in the absence of previous infection (Fig. 1d).

In the unvaccinated group, IgG seropositivity and IgG median values were significantly higher in the subjects previously infected with SARS-CoV-2 (81.8% and 427.2 AU/mL, respectively, $n=54$), when compared with the uninfected ones (30.2% and 6.7 AU/mL, respectively, $n=13$) (Table 3 and Fig. 1a). These differences were also observed after accounting for gender and age categories (Table 3, Fig. 1b, d). As expected, the main significant differences on IgG positivity and IgG median values were found between the vaccinated and unvaccinated groups (Table 3 and Fig. 1a, b, and d).

In both vaccinated subgroups (with and without a previous SARS-CoV-2 infection), IgG median values showed an increase from the first 1–2 weeks to the 3rd week, peaking

Table 2 Clinical and biological characterization of the sample with and without COVID-19 vaccination ($n=260$)

Variable**	COVID-19 vaccinated		p*
	Yes	No	
Total sample ($n=260$), n (%)	146 (56.2)	114 (43.8)	0.047
Gender ($n=260$), *n* (%)			
Female	107 (73.3)	75 (65.8)	0.22
Male	39 (26.7)	39 (34.2)	
Age (years) ($n=260$), med (IQR)	43.0 (37.0–59.0)	46.0 (35.0–57.3)	0.901
Age categories (years) ($n=260$), *n* (%)			
≤ 18	1 (0.7)	3 (2.6)	
19–40	58 (39.7)	37 (32.5)	0.178
41–64	62 (42.5)	60 (52.6)	
≥ 65	25 (17.1)	14 (12.3)	
SARS-CoV-2 infection ($n=240$), *n* (%)			
Yes	36 (27.5)	66 (60.6)	0
No	95 (72.5)	43 (39.4)	
Exposure to a positive case ($n=239$), *n* (%)			
Yes	43 (31.9)	55 (52.9)	0.001
No/unknown	92 (68.1)	49 (47.1)	
IgM AU/mL ($n=208$), med (IQR)	0.56 (0.22–1.28)	0.52 (0.08–1.62)	0.286
IgG AU/mL ($n=260$), med (IQR)	7222.6 (3097.0–14,762.4)	167.0 (6.7–639.2)	0
IgM positive (> 1.0 UA/mL) ($n=208$), *n* (%)			
Yes	35 (28.5)	28 (32.9)	0.54
No	88 (71.5)	57 (67.1)	
IgG positive (≥ 50 UA/mL) ($n=257$), *n* (%)			
Yes	143 (97.9)	69 (60.5)	0
No	3 (2.1)	45 (39.5)	

med (IQR): median (interquartile range), *n (%)*: count (percentage)

*Mann–Whitney or chi square test or Fisher's exact test; **For each variable, the values of *n* correspond to the total number of answers/results

at 4–6th week after vaccination (Fig. 2a). Subsequently, IgG values decreased progressively, with a significant decrease being observed past the 10th week of vaccination, when compared to the peak (4–6 weeks, $p=0.016$), for vaccinated subjects without a previous SARS-CoV-2 infection. Although not significant, in general, IgG median values were higher among vaccinated subjects previously infected with SARS-CoV-2 infection when compared to previously uninfected vaccinated subjects. This difference in IgG levels was most notorious in the peak weeks (4–6th week) after vaccination (Fig. 2a). Nevertheless, in both groups, all subjects achieved IgG seropositivity throughout the study (Table 3 and Fig. 2a, b), with the exception of a subset of vaccinated subjects without a previous infection tested in the weeks 1–2 and 7–10 after vaccination, where seropositivity was, respectively, 83.3% and 92.0% (Table 3).

To better understand how IgG levels change over the time after vaccination and/or infection, a second sample (t2) was collected from a subgroup of 30 subjects, 2 to 4 months after the initial collection time (t1) (Table 4). The rate of IgG decrease was lower among fully vaccinated subjects (2 doses administered), in comparison with subjects with only a single dose (median values of 17.9 vs 21.4%, for subjects previously infected; 22.6 vs 27.8% for subjects previously uninfected). Fully vaccinated individuals previously diagnosed *with* COVID-19 also showed a lower IgG rate of decrease than their uninfected counterparts (median values of 17.9 vs 22.6%, respectively). Unvaccinated subjects with a previous COVID-19 diagnosis presented the lowest IgG levels but also a lower IgG decrease (median IgG levels of 363.0 AU/mL at t1 and 271.2 AU/mL at t2; median rate of IgG decrease, 10.8%) compared to vaccinated subjects (Table 4).

Discussion

The currently available COVID-19 vaccines have been reported to be highly effective in preventing symptomatic infection and/or hospitalization in clinical trials [24, 25]. In the real-world scenario, however, efficacy was expected to be lower mostly due to the fast arising of new viral variants [26,

Table 3 Immunization status (IgG seropositivity) of the vaccinated (with and without previous SARS-CoV-2 infection, $n=36$ and $n=95$, respectively) and unvaccinated groups (with and without SARS-CoV-2 infection, $n=66$ and $n=43$, respectively)

Variable**	Vaccinated		Unvaccinated	
	With previous SARS-CoV-2 infection ($n=36$)****	Without previous SARS-CoV-2 infection ($n=95$)***	With SARS-CoV-2 infection ($n=66$)	Without SARS-CoV-2 infection ($n=43$)
	% (n) seropositive	% (n) seropositive	% (n) seropositive	% (n) seropositive
Total sample	100.0 (36)[a]	96.8 (92)[a]	81.8 (54)[b]	30.2 (13)[c]
Gender:				
Female	100.0 (28)[a,b]	100.0 (68)* [a]	81.0 (34)[b]	24.1 (7)[c]
Male	100.0 (8)[a]	88.9 (24)[a]	83.3 (20)[a,b]	42.9 (6)[b]
Age categories (years):				
≤ 18	100.0 (1)[a]	-	100.0 (1)[a]	0.0 (0)[a]
19–40	100.0 (15)[a]	100.0 (37)[a]	83.3 (15)[a]	23.5 (4)[b]
41–64	100.0 (12)[a]	95.2 (40)[a]	83.3 (30)[a]	38.1 (8)[b]
≥65	100.0 (8)[a]	93.8 (15)[a]	72.7 (8)[a]	33.3 (1)[a]
COVID-19 vaccine type:			n/a	
BNT162b2 (Pfizer BioNTech)	100.0 (35)[a]	100.0 (73)[a]		
mRNA1273 (Moderna)	100.0 (1)[a]	100.0 (6)[a]		
ChAdOx1 nCoV-19 (AstraZeneca)	-	81.8 (9)*		
Weeks after vaccination/infection (categories):				n/a
1–2	-	83.3 (5)	-	
3	100.0 (1)[a]	100.0 (4)[a]	-	
4–6	100.0 (5)[a,b]	100.0 (30)[a]	75.0 (3)[a]	
7–10	100.0 (7)[a]	92.0 (23)[a]	100.0 (5)[a]	
> 10	100 (23)[a,b]	100 (26)[a]	78.6 (11)[b]	

Med (IQR) (n): median (interquartile range) (count), *% (n)*: percentage (count), *n/a:* not applicable

*$p<0.05$ inside the variable and the specific COVID-19 group (chi-square test or Fisher's exact test); **For each variable, the values of n correspond to the total number of answers/results; ***88.3% ($n=83$) vaccinated with 2 doses and 11.7% ($n=11$) with 1 dose of the vaccine; ****58.3% ($n=21$) vaccinated with 1 dose and 41.7% ($n=15$) with 2 doses of the vaccine

[a,b,c,d]Each subscript letter (a, b, c, d) denotes a subset of COVID-19 vaccination categories whose column proportions do not differ significantly from each other at the .05 level (*Z*-test, adjust *p*-values (Bonferroni method))

27]. Nevertheless, different studies reported high production of S1 protein-specific IgG's in response to vaccination, although variability in IgG levels does occur across studies [28, 29].

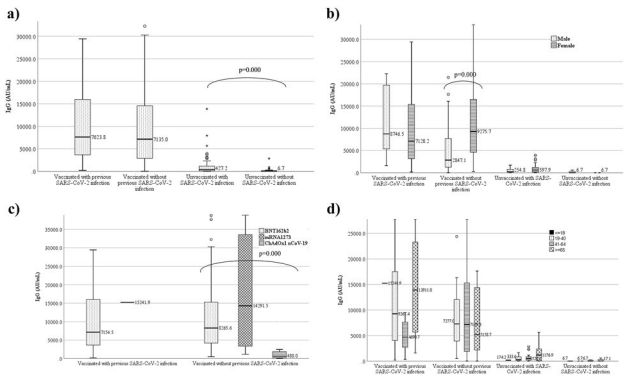

Fig. 1 IgG values (median (interquartile)) to COVID-19 vaccination (with and without a SARS-CoV-2 infection), to SARS-CoV-2 infection in unvaccinated subjects and in naïve subjects (unvaccinated without SARS-CoV-2 infection), according to the total sample (**a**), per gender (**b**), per vaccine type (**c**), and per age category (**d**)

In our study, we analyzed the production of IgG antibodies against the viral spike protein S1 developed after vaccination by two mRNA-based vaccines (BTN162b2, Pfizer BioNTech, and mRNA1273, Moderna) and one viral vector vaccine (ChAdOx1 nCoV-19). Antibody levels were evaluated in both vaccinated and unvaccinated individuals, as well as with and without a previous positive history of SARS-CoV-2 infection. The present study adds to the expanding literature regarding the immunological

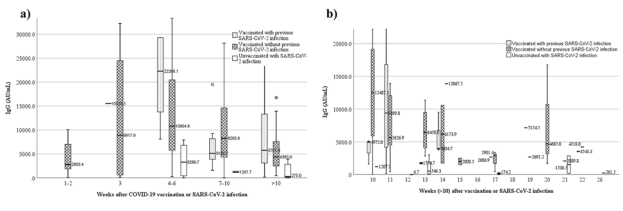

Fig. 2 IgG values (median (interquartile)) along all the weeks (**a**), or for the weeks starting in the 10th week (**b**) after the last dose of COVID-19 vaccine in vaccinated subjects (with and without previous SARS-Cov-2 infection) and after diagnosis in unvaccinated subjects with a SARS-CoV-2 infection

Table 4 IgG decrease (in %) per month in vaccinated groups (with and without previous SARS-CoV-2 infection) with 1 and 2 doses of the vaccine, and in the unvaccinated group with SARS-CoV-2 infection

Status	IgG, med (IQR)		Number weeks from last vaccination dose or infection diagnosis until time 1, med (IQR)	Number months between t1 and t2, med	% IgG decrease/month, med (IQR)*
	Time 1	Time 2			
Vaccinated (1 dose) with previous SARS-CoV-2 infection ($n=5$)	4972.0 (266.2–10,187.3)	390.2 (156.8–2615.4)	11.0 (9.8–11.0)	2.5	21.4 (14.2–28.0)
Vaccinated (2 doses) with previous SARS-CoV-2 infection ($n=4$)	9396.0 (3522.4–24,339.7)	2519.2 (1003.7–3090.9)	9.1 (5.0–12.9)	4.1	17.9 (11.4–23.6)
Vaccinated (1 dose) without previous SARS-CoV-2 infection ($n=1$)	44,976.3	7416.0	4.1	3.0	27.8
Vaccinated (2 doses) without previous SARS-CoV-2 infection ($n=16$)	12,947.4 (7269.2–17,416.0)	1779.2 (866.8–3003.3)	5.9 (4.8–10.2)	3.5	22.6 (18.3–33.0)
Unvaccinated with SARS-CoV-2 infection ($n=4$)	363.0 (274.6–3411.1)	271.2 (200.1–2215.8)	15.5 (8.6–30.2)	3.8	10.8 (7.9–16.8)

Med (IQR) median (interquartile range), *n* count, % percentage

*Median value obtained using the percentage of IgG decrease for each person for the calculation

impact of infection versus vaccination, as well as help to assess the need for the administration of additional booster doses.

In line with other literature reports, we confirmed an increase in IgG antibody production upon 1–2 weeks after vaccination that peaked after 4–6 weeks of vaccination [13, 16, 22, 30]. Afterward, IgG values decreased progressively, although most vaccinated subjects remained seropositive. Only a low number of SARS-CoV-2-specific IgG-negative vaccinated subjects ($n=3$) were detected, which corresponded to subjects without a previous SARS-CoV-2 infection. In the case of one of those subjects, blood samples were collected 1–2 weeks post-vaccination, likely too early for IgG seroconversion. Regarding the other two seronegative vaccinated subjects, despite having been tested 7–10 weeks after vaccination, they solely received a single vaccine dose, which could account for the weak IgG seroconversion (Table 3).

Decreased, or even absent, rates of vaccine-induced seroconversion have been found among immunocompromised individuals, including those who have undergone organ transplantations, or who are receiving immunosuppressive therapy in general, as well as those suffering from some hematological cancers [31–33]. Nonetheless, in our study, weaker seroconversion seemed to be associated with lower compliance with the established vaccination plan (one dose administration instead of two).

Surprisingly, we found virus-specific IgG seropositivity in 30.2% of unvaccinated subjects not previously diagnosed with COVID-19 (Table 3), which may have been a consequence of previously undetected asymptomatic infections.

In line with other studies, our findings suggest a higher effect of vaccination on the production of SARS-CoV-2-specific IgG antibodies, as opposed to natural infection (Table 3 and Fig. 1a–c), [29, 34–40]. In our study, only humoral immunity was addressed and the status of T-cell immunity was not evaluated. Nonetheless, the results seem to indicate that vaccination is of major importance to acquiring significant protection from subsequent infection.

It is interesting to notice that, in subjects not previously exposed to SARS-CoV-2, vaccine-induced IgG levels were significantly higher in females, as compared to males (Fig. 1b). Our observations are consistent with the review by Zimmermann and Curtis [34], suggesting that females tend to develop a higher humoral response to vaccination. In accordance, a recent report on the humoral response to COVID-19 vaccination also demonstrated higher levels of SARS-CoV-2 IgG response in women [35].

Increasing age has been associated with decreased likelihood of seroconversion via disease and vaccination [36–38], with a significantly lower peak of anti-S and neutralizing antibody titers [37, 39]. This was not confirmed by our study, although a trend towards lower IgG levels was observed in the previously uninfected vaccinated subjects over the age of 65. Our results are in line with the study by Wei et al. [40]. In this report, no differences were found in seroconversion across age groups after 2 doses of BTN162b2 vaccine, while differences were noticeable after a single dose of ChAdOx1 nCoV-19, with elderly subjects displaying lower IgG levels. In opposition, and to our surprise, we found that IgG levels in this former age group (over 65 years old) tended to be higher than other groups, when considering vaccinated subjects

with a previous SARS-CoV-2 infection. A possible explanation may be that infection could have impacted the immune system differently in this group, which is in line with our previous study about immune response to natural infections [11]. In the latter, which was conducted with SARS-CoV-2-exposed individuals, the eldest and the youngest groups developed a higher antibody titer to core N protein.

In previous studies, the efficacy rate of ChAdOx1 nCoV-19 (AstraZeneca) vaccine has been shown to be lower than the mRNA-based vaccines [40]. Similarly, our cohort showed a lower protective humoral response (seropositivity and IgG levels) after vaccination with the ChAdOx1 nCoV-19 (AstraZeneca) vaccine, in comparison with the BNT162b2 (Pfizer BioNTech) and mRNA1273 Moderna vaccines (Table 3 and Fig. 1c). This finding suggests a higher efficacy of mRNA-based vaccines, as compared to a viral-vectored vaccine. Nevertheless, in our study, 2 of the 11 subjects vaccinated with ChAdOx1 nCoV-19 (AstraZeneca) did not complete the vaccination plan, which may have affected viral protection.

Although our cohort developed a potent IgG response to COVID-19 vaccination, in general, antibody titers reach half of the initial value after 2.5 months (a decrease of about 20% per month) (Table 4), corroborating other reports [28, 41]. We found that vaccinated subjects with a previous COVID-19 diagnosis showed a lower IgG decrease rate, despite not statistically significant, than previously uninfected subjects (Table 4).

Conclusion

The unprecedented global investment in vaccine development, mass production, and distribution during the COVID-19 pandemic bore serious expectations when it came to the prevention of symptomatic infection and/or hospitalization. Despite these efforts, as we know, vaccine efficacy has been particularly threatened due to the fast emergence of new viral variants.

In our study, we found that IgG antibody levels increased upon 1–2 weeks after vaccination and peaked after 4–6 weeks. Afterward, its values decreased progressively although most subjects remained seropositive throughout the study period (until around 5.5 months after vaccination). In addition, in the absence of a previous SARS-CoV-2 infection, we found that females showed significantly higher vaccine-induced IgG levels, when compared to males. When compared with the other age groups, vaccinated subjects above the age of 65 showed a trend towards higher median IgG values (13,911.0 AU/mL) if previously infected with SARS-CoV-2, but comparatively lower IgG median value (5158.7 AU/mL) in its absence Additionally, a lower immune response (seropositivity and IgG levels) was observed through vaccination with the ChAdOx1 nCoV-19 (AstraZeneca) vaccine, in comparison with the BNT162b2 (Pfizer BioNTech) and mRNA1273 (Moderna) vaccines, which in turn points to a higher efficacy of mRNA-based vaccines, as opposed to viral-vectored vaccines. However, it is important to note that the observed behavior with the ChAdOx1 nCoV-19 (AstraZeneca) vaccine may have been impacted by some cases of non-adherence to the complete vaccination plan.

In conclusion, our findings suggest a stronger effect of vaccination on the production of SARS-CoV-2-specific IgG antibodies, as opposed to natural infection. Nonetheless, antibody titers waned rapidly, in a rate of corresponding to a decrease of approximately 20% per month. Our study is, thus, consistent with a future need for additional vaccination boosters in an effort to help control both infection rates, as well as overall morbimortality during the present pandemic.

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Author contribution MD, CA, AS, and ID contributed to writing of the manuscript. CA also performed the statistical analysis. MD performed the analytical tests and conducted the clinical-epidemiological survey. MD, CA, AS, and ID collaborated on the manuscript revision. All authors read and approved the final manuscript.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Informed written consent was obtained from all participants before the collection of any data regarding the study.

Consent for publication All authors accept the terms and conditions of the editorial for publication.

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References

1. World Health Organization (2020) WHO director-general's remarks at the media briefing on 2019-nCoV on 11 Feb 2020
2. Artika IM, Dewantari AK, Wiyatno A (2020) Molecular biology of coronaviruses: current knowledge. Heliyon 6(8):e04743. https://doi.org/10.1016/j.heliyon.2020.e04743

3. Wang N et al (2020) Subunit vaccines against emerging pathogenic human coronaviruses. Front Microbiol 11:298. https://doi.org/10.3389/fmicb.2020.00298
4. Du L et al (2009) The spike protein of SARS-CoV–a target for vaccine and therapeutic development. Nat Rev Microbiol 7(3):226–236. https://doi.org/10.1038/nrmicro2090
5. World Health Organization (2020) Origin of SARS-CoV-2, 26 March 2020, Geneva
6. Felsenstein S, Hedrich CM (2020) COVID-19 in children and young people. The Lancet Rheumatology 2(9):e514–e516. https://doi.org/10.1016/S2665-9913(20)30212-5
7. Carsetti R et al (2020) Different innate and adaptive immune responses to SARS-CoV-2 infection of asymptomatic, mild, and severe cases. Front Immunol 11:610300. https://doi.org/10.3389/fimmu.2020.610300
8. Boechat JL et al (2021) The immune response to SARS-CoV-2 and COVID-19 immunopathology - current perspectives. Pulmonology 27(5):423–437. https://doi.org/10.1016/j.pulmoe.2021.03.008
9. Grifoni A et al (2020) Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 Disease and unexposed individuals. Cell 181(7):1489–1501 e15. https://doi.org/10.1016/j.cell.2020.05.015
10. Toor SM et al (2021) T-cell responses and therapies against SARS-CoV-2 infection. Immunology 162(1):30–43. https://doi.org/10.1111/imm.13262
11. Duro M et al (2021) Pre-vaccination immune response to COVID-19 in a population in Northeast Portugal. Ir J Med Sci 1–8. https://doi.org/10.1007/s11845-021-02799-6
12. Choe PG et al (2021) Waning antibody responses in asymptomatic and symptomatic SARS-CoV-2 infection. Emerg Infect Dis 27(1). https://doi.org/10.3201/eid2701.203515
13. Kellam P, Barclay W (2020) The dynamics of humoral immune responses following SARS-CoV-2 infection and the potential for reinfection. J Gen Virol 101(8):791–797. https://doi.org/10.1099/jgv.0.001439
14. Post N et al (2020) Antibody response to SARS-CoV-2 infection in humans: a systematic review. PLoS ONE 15(12):e0244126. https://doi.org/10.1371/journal.pone.0244126
15. Mo H et al (2006) Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance. Respirology 11(1):49–53. https://doi.org/10.1111/j.1440-1843.2006.00783.x
16. Sadarangani M, Marchant A, Kollmann TR (2021) Immunological mechanisms of vaccine-induced protection against COVID-19 in humans. Nat Rev Immunol 21(8):475–484. https://doi.org/10.1038/s41577-021-00578-z
17. Barda N, Dagan N, Balicer RD (2021) BNT162b2 mRNA COVID-19 vaccine in a nationwide mass vaccination setting. Reply N Engl J Med 384(20):1970. https://doi.org/10.1056/NEJMc2104281
18. Dagan N et al (2021) BNT162b2 mRNA COVID-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 384(15):1412–1423. https://doi.org/10.1056/NEJMoa2101765
19. Roest S, Hoek RAS, Manintveld OC (2021) BNT162b2 mRNA COVID-19 vaccine in a nationwide mass vaccination setting. N Engl J Med 384(20):1968–1970. https://doi.org/10.1056/NEJMc2104281
20. Thompson MG et al (2021) Interim estimates of vaccine effectiveness of BNT162b2 and mRNA-1273 COVID-19 vaccines in preventing SARS-CoV-2 infection among health care personnel, first responders, and other essential and frontline workers - eight U.S. locations, December 2020-March 2021. MMWR Morb Mortal Wkly Rep 70(13):495–500. https://doi.org/10.15585/mmwr.mm7013e3
21. Broseta JJ et al (2021) Humoral and cellular responses to mRNA-1273 and BNT162b2 SARS-CoV-2 vaccines administered to hemodialysis patients. Am J Kidney Dis 78(4):571–581. https://doi.org/10.1053/j.ajkd.2021.06.002
22. Thompson MG et al (2021) Effectiveness of COVID-19 vaccines in ambulatory and inpatient care settings. N Engl J Med 385(15):1355–1371. https://doi.org/10.1056/NEJMoa2110362
23. Logunov DY et al (2021) Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. Lancet 397(10275):671–681. https://doi.org/10.1016/S0140-6736(21)00234-8
24. Polack FP et al (2020) Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine. N Engl J Med 383(27):2603–2615. https://doi.org/10.1056/NEJMoa2034577
25. Wang H et al (2020) Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2. Cell 182(3):713–721 e9. https://doi.org/10.1016/j.cell.2020.06.008
26. Jalkanen P et al (2021) COVID-19 mRNA vaccine induced antibody responses against three SARS-CoV-2 variants. Nat Commun 12(1):3991. https://doi.org/10.1038/s41467-021-24285-4
27. Li XN et al (2021) Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study. Emerg Microbes Infect 10(1):1751–1759. https://doi.org/10.1080/22221751.2021.1969291
28. Bongiovanni M et al (2021) Evaluation of the immune response to COVID-19 vaccine mRNA BNT162b2 and correlation with previous COVID-19 infection. J Clin Virol 143:104962. https://doi.org/10.1016/j.jcv.2021.104962
29. Psichogiou M et al (2021) Comparative immunogenicity of BNT162b2 mRNA vaccine with natural SARS-CoV-2 infection. Vaccines (Basel) 9(9). https://doi.org/10.3390/vaccines9091017
30. Tut G et al (2021) Profile of humoral and cellular immune responses to single doses of BNT162b2 or ChAdOx1 nCoV-19 vaccines in residents and staff within residential care homes (VIVALDI): an observational study. The Lancet Healthy Longevity 2(9):e544–e553
31. Thakkar A et al (2021) Seroconversion rates following COVID-19 vaccination amongst patients with cancer. Cancer Cell 39(8):1081–1090.e2. https://doi.org/10.1016/j.ccell.2021.06.002
32. Thakkar A et al (2021) Seroconversion rates following COVID-19 vaccination amongst patients with malignant disease-the impact of diagnosis and cancer-directed therapies. https://doi.org/10.1101/2021.05.07.21256824
33. Negahdaripour M et al (2021) Administration of COVID-19 vaccines in immunocompromisedpatients. Int Immunol 108021
34. Zimmermann P, Curtis N (2019) Factors that influence the immune response to vaccination. Clin Microbiol Rev 32(2). https://doi.org/10.1128/CMR.00084-18
35. Tretyn A et al (1952) (2021) Differences in the concentration of anti-sARS-CoV-2 IgG antibodies post-COVID-19 recovery or post-vaccination. Cells 10(8), 1952:1–16. https://doi.org/10.3390/cells10081952
36. Wei J et al (2021) Anti-spike antibody response to natural SARS-CoV-2 infection in the general population. Nat Commun 12(1):1–12
37. Jabal KA et al (2021) Impact of age, ethnicity, sex and prior infection status on immunogenicity following a single dose of the BNT162b2 mRNA COVID-19 vaccine: real-world evidence from healthcare workers, Israel, December 2020 to January 2021. Eurosurveillance 26(6):2100096. https://doi.org/10.2807/1560-7917
38. Bauer G et al (2021) The challenge of avidity determination in SARS-CoV-2 serology. J Med Virol 93(5):3092–3104
39. Levin EG et al (2021) Waning immune humoral response to BNT162b2 covid-19 vaccine over 6 months. N Eng J Med
40. Wei J et al (2021) Antibody responses to SARS-CoV-2 vaccines in 45,965 adults from the general population of the United Kingdom. Nat Microbiol 6(9):1140–1149
41. Lo Sasso B et al (2021) Evaluation of Anti-SARS-Cov-2 S-RBD IgG Antibodies after COVID-19 mRNA BNT162b2 Vaccine. Diagnostics (Basel) 11(7). https://doi.org/10.3390/diagnostics11071135