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Anti-spike IgG against COVID-19 three months after the end of the pandemic in Northeast Portugal

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Abstract: The emergence of new COVID-19 strains and variants and immune escape from vaccines forces reflection on the need to continue vaccinating the entire population. This study intends to monitor and understand the reinfection in recently vaccinated people. The amount of anti-Spike IgG, the number of vaccine doses, and infections/reinfections in 82 volunteers, three months after the declaration of the end of the COVID-19 pandemic, was evaluated. All participants, asymptomatic at the time, presented IgG, including those who had no infection or vaccination (n = 3). Those vaccinated showed high levels of antibodies, even 36 months after the last booster. There was no significant difference in the immunological status with the type of vaccine, age, and sex, although women and older people had higher median IgG values. A significant positive correlation was observed between vaccine doses and IgG (rs = 0.373; $p < 0.001$). Women vaccinated before coming into contact with the virus showed higher antibody levels (16607 vs. 6233; $p = 0.012$). This study suggests women's immune systems are more effective at fighting the virus. It also supports the effectiveness of vaccines on the humoral response. However, the timing of infections is inconsistent with the expected immunity. Therefore, continuation of booster doses is questionable except for immunocompromised patients.

Keywords: COVID-19; reinfection; vaccine efficacy; booster

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Introduction

At the end of 2019, the global healthcare system faced a new pandemic. Following the identification of the causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the urgency for an effective treatment and the development of a vaccine to halt the spread of the infection rose, with its main obstacle being the understanding of the immune response to the virus [1-3]. Studies published in the pre-vaccine era were consensual regarding the ability of the immune system to react to SARS-CoV-2 infection and produce specific IgM and IgG [4], but at the time some data suggested that these antibodies might not be detected in all patients [5,6].

The pharmaceutical industry allowed the quick development of laboratory tests that enabled monitoring of IgM and IgG antibodies in the infected, and later, when vaccines were discovered, in vaccinated individuals, a fundamental step in evaluating the immune response to natural infection and long-term vaccine effectiveness [2,7]. With this monitoring, it was observed that natural infection produced fewer antibodies

than vaccines. The vaccines proved to be highly effective in producing anti-SARS-CoV-2 antibodies, limiting severe disease and the spread of the virus among the population, and being a turning point in the history of this infection [8,9]. These antibodies produced after vaccination could be detected in serum a few days post-infection, reaching their peak around the third week. However, their levels subsequently had a noticeable decrease, to the extent that they could be neutralized. This observation suggested the need for a vaccination plan incorporating multiple boosters [2,7,10,11] and, initially, it was believed that this approach would lead to the eradication of the infection [12]. However, in the ensuing years, it became evident that many individuals experienced infections and reinfections shortly after vaccination, including some who had received four doses and exhibited high IgG antibody levels. This likely resulted from an immunological failure against emerging strains and variants [8,12,13]. Another fundamental problem is determining the protective threshold of the IgG titer. The available data on the subject are confounded by variability [8] due to the lack of standardized laboratory testing, despite the World Health Organization (WHO)'s introduction of international binding antibody units (BAU/ml) in an attempt to standardize results [14,15].

Finally, recognized adverse effects of vaccination [16], which more recently also appear to establish a link between vaccination and clotting problems and an increase in D-dimers [17,18], have raised questions regarding indiscriminate vaccination across the population, besides in healthcare workers and immunocompromised people.

We previously monitored a population from Northeast Portugal in the different stages of the COVID-19 infection. The first stage, before the appearance of the vaccine (2020/2021), revealed that IgG displayed within 1/2 weeks, reaching a peak in the third week, with a significant decrease after the sixth week, translating into a loss of immunity at the 21st-week post-diagnosis [4].

A publication, in 2023, after what was considered complete vaccination in Portugal [19], showed that vaccination promoted a greater effect on IgG production, as opposed to natural infection; however, despite the lower number of antibodies in the unvaccinated, their rate of decline appeared to be lower [20].

This study aimed to evaluate the IgG anti-spike antibody status, three months after the WHO declared the end of the pandemic, to understand its persistence over time and the relationship between infection, reinfection, and concomitant vaccination and boosters with IgG concentration, facts that are still poorly understood.

Materials and Methods

During August 2023, three months after the declaration of the end of the COVID-19 pandemic, eighty-two users of the Nordeste Lab volunteered to quantify the serum IgG antibody against the target S1 subunit of the spike protein, aiming to verify the state of immunity to the SARS-CoV-2 virus. Participants were clearly informed of the objective of the work, and all signed a consent form for the present study and publication of results. They also agreed to fill out a small questionnaire regarding their status of SARS-CoV-2 infection, reinfection, and vaccination, namely: a) data and type of the last confirmed vaccine dose; b) timing of infection in relation to the vaccination process (before/after); and c) the self-reported number of infections, reinfections, and previous vaccine doses. No other medical records were available, given that the work took place in an outpatient analysis laboratory. Moreover, none of the participants exhibited any symptoms suggestive of infection at the time of blood collection or since the last confirmed infection, which served as an inclusion criterion for this study. All personal information and serum samples provided were encrypted and could only be accessed by the laboratory's clinical director or a delegate in compliance with the professional duty of confidentiality and current legislation for data protection.

Analytical tests were performed using the ARCHITECT iSystem i1000 equipment (Abbott), 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 using a commercial Abbott kit. The same kit was used for all the samples. The analytical measurement interval is stated as 21 to 40,000 arbitrary units per milliliter (AU/ml). According to the manufacturer's instructions, results were deemed positive if > 50.0 AU/ml.

Data analysis was performed with IBM® SPSS® Statistics 28.0 (IBM Corp. 2023, Armonk, NY). Descriptive and inferential analyses were conducted to study whether a difference in IgG titer was detected per group. Quantitative data was expressed as median values, as it was non-normally distributed. Counts and percentages were reported for categorical variables. Comparison of median IgG between groups regarding gender, age groups, COVID-19 situation, vaccine type, and vaccine doses were performed with Mann-Whitney (2 groups) or Kruskal-Wallis tests (> 2 groups). The relationships between age groups (years), number of vaccine doses, and IgG were assessed using the Spearman's correlation coefficient ($r_{spearman}$). Whenever statistical tests have been applied, the level of significance considered was $\alpha = 0.05$.

Results

Among the 82 volunteers, there was an equitable distribution by sex and age groups (≤ 60 years and > 60 years). The characterization of the sample is summarized in Table 1.

Table 1. Characterization of the sample regarding sociodemographic and clinical variables and comparison of median IgG values for those groups.

Variable[#]	Sample *n* (%)	Median IgG (AU/ml)	*p**
Sample (*n* = 82)			
Female	47 (57.3%)	13176	0.334
Male	35 (42.7%)	7250	
Age, years (*n* = 82)			
≤ 60 years (min = 21 years)	43 (52.4%)	6866	0.195
> 60 years (max = 86 years)	39 (47.6%)	12424	
Infection (*n* = 82)			
No	34 (41.5%)	9448	0.745
Yes	48 (58.5%)	8367	
Vaccination/Infection (*n* = 82)			
No vaccination, no infection	1 (1.2%)	172	0.143
No vaccination, infection	2 (2.4%)	2407	
Vaccination, no infection	33 (40.2%)	9448	
Vaccination, infection	46 (56.1%)	8651	
Vaccine type (*n* = 79)			
mRNA-based (Pfizer or Moderna)	68 (86.1%)	9620	0.105
Adenoviral-vectored (AstraZeneca or Janssen)	7 (9.8%)	4489	
Mix	1 (1.3%)	6542	
Unknown	3 (3.8%)	12424	
Last dose of vaccine in (*n* = 74)			
2021	32 (43.2%)	8585	0.611
2022	38 (51.4%)	12803	
2023	4 (5.4%)	12110	
Vaccination, doses (*n* = 80)			
1	5 (6.3%)	2465	**< 0.001**
2	25 (31.5%)	3741	
3	32 (40.0%)	8939	
4	18 (22.5%)	24779	
Months (m) after vaccination (*n* = 74)			
0-12 m	26 (35.1%)	7947	0.260
13-24 m	28 (37.8%)	13500	
25-36 m	20 (27.0%)	8619.5	

n (%): count (percentage); AU/ml: arbitrary units per milliliter. [#]For each variable, the values of *n* correspond to the total number of answers/results. *Bold *p*-values correspond to significant differences in the variables' median values, all calculated using non-parametric tests.

Three of the sample participants were not vaccinated. Of those vaccinated, 79 (96%) used the BTN162b2 mRNA vaccine – Pfizer/BioNTech or Moderna –, and the last vaccine dose was given at a minimum of 9 and a maximum of 31 months. All participants had positive IgG levels (> 50 AU/ml), including those who had no infection or vaccination and those who had the last dose of vaccine 32 months before. The IgG peak was seen in those who had their last vaccine booster between 13 and 24 months, although the median IgG level did not differ ($p = 0.260$) from the period before (0-12 m) or after (25-36 m) (Table 1). There was no significant difference in IgG median value concerning sex, age group (Table 1), as well as by type of vaccine and whether volunteers had been infected or not (Fig. 1). However, the data of this sample shows that women [female vs. male: 13176 vs. 7520; $p = 0.334$] and older people [> 60 vs. ≤ 60 years old: 12424 vs. 6866; $p = 0.195$] presented higher median IgG values. Vaccines based on mRNA (Pfizer or Moderna) showed greater efficacy in the production of IgG antibodies when compared to those vectored by adenovirus (AstraZeneca or Janssen) [9620 vs. 4489; $p = 0.105$]. A significant positive correlation was observed between COVID-19 IgG antibodies and the number of vaccine doses administered, compared to the unvaccinated group ($n = 80$; rs = 0.373; $p < 0.001$) (Fig. 2).

When asked about the infection, 34 individuals (41.5%) said they had never been infected, while 18 (22%) had an infection before vaccination and 29 (35.4%) after starting the vaccines (Table 2).

Of the infected, those who had previous vaccination had a higher median IgG value [13233 vs. 8433 ($p = 0.165$)], although not statistically significant. When adjusting for sex (Table 3), there were no significant differences if the infection occurred before vaccination; however, in the opposite case, where the vaccine was first administered before contact with the virus, the female group had a significantly higher median IgG value (17607 vs. 6233; $p = 0.012$).

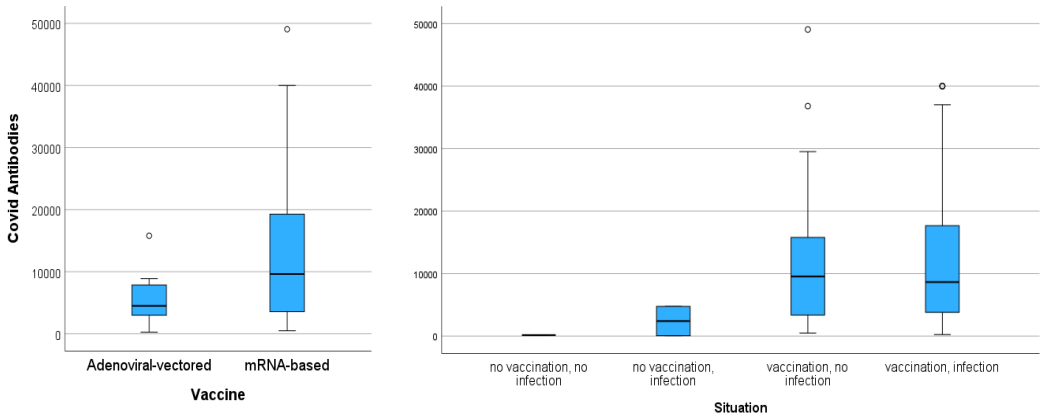

Figure 1. COVID IgG antibody concentration (AU/ml) following administration of different vaccine types and in different situations (vaccination and/or infection).

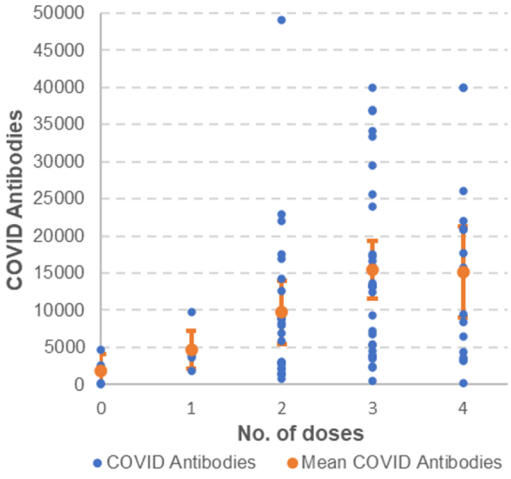

Figure 2. Relationship between COVID IgG antibody levels and the number of inoculated vaccine doses (blue dots show COVID IgG antibody concentrations, and orange dots and bars represent mean COVID IgG antibodies and their 95% confidence level error bars).

Table 2. IgG values according to infection status, before or after vaccination.

	Infection before vaccination		
	No	**Yes**	***p***
n (%)	64 (78%)	18 (22%)	
median (P25-P75)	9448 (3395.3-17660.3)	6827 (3395.3-13853)	0.364
mean (st.dev)	13110.8 (11765.4)	9789.4 (9729.2)	
min-max	57-49061	1853-40000	
	Infection after vaccination		
	No	**Yes**	
n (%)	53 (64.6%)	29 (35.4%)	
median (P25-P75)	8433 (2964.5-15784.5)	13233 (3839.5-22083.5)	0.165
mean (st.dev)	11090.1 (10635.2)	14742.3 (12469)	
min-max	57-49061	255-40000	

n (%): count (percentage); AU/ml: arbitrary units per milliliter. #For each variable, the values of *n* correspond to the total number of answers/results. *Bold *p*-values correspond to significant differences in the variables' median values, all calculated using non-parametric tests.

Table 3. Comparison of IgG values according to infection status before and after vaccination and by sex.

	Female			Male		
	Infection before vaccination					
	No	**Yes**	***p****	**No**	**Yes**	***p****
n (%)	38 (59.4%)	9 (50%)	0.386	26 (40.6%)	9 (50%)	0.810
median	13752.5	4419		7058	8301	
(P25-P75)	(3152.8-21253.8)	(3277.5-14530)		(3617.3-14583.8)	(2929.5-13591.5)	
mean (st.dev)	14420.7 (12046.4)	10548.3 (12073.4)		11196.2 (11297.9)	9030.4 (7354.3)	
min-max	172-40000	1853-40000		57-49061	2162-23983	
	Infection after vaccination					
	No	**Yes**	***p****	**No**	**Yes**	***p****
n (%)	26 (49.1%)	8 (72.4%)	**0.012**	27 (50.9%)	21 (27.6%)	0.166
median	6233[b]	17607[a]		8938	4182	
(P25-P75)	(2538.5-15389)	(6027-28204)		(3372-17487)	(3709.5-7154)	
mean (st.dev)	9955.7 (10088)	18289.3 (12832.6)		12182.4 (11217.7)	5431.3 (3818.4)	
min-max	172-40000	751-40000		57-49061	255-13233	
p**	0.113	0.125		0.729	0.370	

St.dev: standard deviation; [a,b]: different letters stand for significant differences in IgG median value, according to the Mann-Whitney test. *Bold *p*-values correspond to significant differences in the variables' median values comparing No vs. Yes Infection; ** comparing Before vs. After vaccination.

Discussion

The number of participants who revealed that they had not been infected – 34 (41.5%) – is, in our opinion, very high, given that the infection spread across almost the entire population. It is known that the combination of infection and vaccination has a synergistic effect on the acquisition of antibodies against SARS-CoV-2 [21]. Here, as there was no difference in IgG antibody levels in this group compared to that of vaccinated individuals who also became infected, we are led to believe that there was some prejudice in accepting the infection.

Most publications conclude that sex and age are not significant factors for the acquisition of antibodies [22], although some studies report more reactivity in young people and men [23]. We, who have already studied this population twice (before and after the emergence of vaccines) [4,20], once again found a higher level of antibodies in the elderly and in women, even though we are aware that the population studied was small and women predominated in this study. It has also been proven that the serological immune response lasts longer in naturally infected versus non-infected patients and that the combination of infection and vaccination has a synergistic effect [8,9]. Although the immune system of the elderly is less reactive than that of young people, the fact that they require greater adherence to the vaccination plan, with more doses of vaccine, have been infected more times, and possibly experienced more intense clinical manifestations leads us to believe that this group benefits from these synergistic effects, resulting in a higher concentration of antibodies. When it comes to women, it is now being proven that they are more immunoreactive than men. Their estrogens can activate cells involved in antiviral reactions, and masculine testosterone can inhibit inflammation [24]. Many studies have identified the *TLR7* gene, located on the X chromosome, as one of the players involved. The X chromosome strengthens a woman's immune system through differences in the activation and regulation of natural killer (NK) cells against viral agents [25-30]. Overall, published results show that cellular immunity from SARS-CoV-2 infection provides a significantly greater boost to the neutralizing antibody response compared to two doses of vaccine alone, indicating an enhancement in both potency and breadth of the antibody response in hybrid immunity [31]. This is consistent with our results (Table 3), especially in the case of women, who generally exhibit higher antibody levels, but notably higher when vaccination precedes contact with the virus, which suggests that vaccination before contact with the virus represents not only prevention of the much more serious primary infection, but also a particular improvement in antibody production.

The effectiveness of vaccines in the production of IgG was verified here, but on the other hand it is clear that no vaccine can completely prevent the transmission of the virus, since a significant proportion of our vaccinated patients suffered COVID-19 infections after vaccination [32,33]. The hypothesis that infection in vaccinated people occurs after a loss of immunity is contradictory [2,10,34,35] with the timing of the infections, as they all occurred shortly after the vaccine and with high levels of antibodies. It is now more credible that these failures result from the emergence and immune escape of new variants of the virus [36-38].

For this reason, booster injection for a population infected with or vaccinated against COVID-19 is questionable, except for immunocompromised patients, given that natural infection confers protection because it also involves cellular immunity, with function being more important than quantity [39].

Believing in immune protection by antibodies, the fundamental problem lies in determining the protective threshold of the IgG titer. The available data on this subject are confounded by the variability of non-standardized laboratory tests [8]. The level of immunological response also varies according to the type

of vaccine [14,15]. The WHO promoted the introduction of binding antibody units per milliliter (BAU/ml) to standardize results [15,40]. Nevertheless, even selecting the highest limit (> 2,000 BAU/ml) from our literature search [35] and converting our results according to WHO guidelines (1 BAU/ml corresponds to 0.142 Abbott AU/ml), the booster injection would only be deemed necessary for a single unvaccinated, uninfected person who had a median value of 172 AU/ml, which corresponds to 1211 BAU/ml.

Regarding limitations, this follow-up study presents both observed data and data collected by participants through self-report, which is prone to bias. Moreover, the study had a smaller sample size, when compared to our previous studies, which was a consequence of both pandemic fatigue, which led to a decreased willingness to engage in research related to the novel coronavirus, and our eligibility criteria, which limited participation to asymptomatic patients only. Furthermore, the study was not conducted in a hospital setting and, therefore, participants' clinical histories were not accessible. However, it can be reasonably assumed that the population in question would only attend the hospital in the event of a severe condition. Likewise, SARS-CoV-2 persists in the population with low virulence and has the potential to trigger an immune response, which could influence the data, a fundamental aspect of any work that deals with virus, the complexity of the immune system in all its multiple responses and variables.

In this third study, we confirmed once again that the human immune system can develop protective antibodies against SARS-CoV-2, whether through contact or infection, and we evaluated the effectiveness of vaccines in generating IgG, particularly with a booster plan. IgG levels increase significantly with the number of doses administered. Overall, our results demonstrate that SARS-CoV-2 infection before or after vaccination provides a significantly greater boost to the neutralizing antibody response. Regarding the timing of vaccination, there seems to be a consensus that it is beneficial before actual contact with the virus, as it prevents primary infection, which is normally more serious.

The fact that only women show a significant positive difference in antibody concentrations, when vaccinated before contact with the virus, reminds us of the need for future, more in-depth research into hormonal differences and the role of the double X chromosome.

Despite the generation of high levels of antibodies and their persistence for many months after vaccination, the population continues to suffer infections and reinfections, as demonstrated here. Hence, immune failure is likely due to newly emerging variants, rather than to a decline in antibodies.

Considering the side effects of vaccination and the costs associated with this plan, we are forced to reflect on the need for booster doses for the entire population, besides high-risk groups and healthcare professionals.

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Author Contributions

All authors contributed to the study's conception and design. ID participated in the study's conceptualization, formal analysis and investigation, original draft preparation, draft review, and editing. MCM was involved in the methodology, formal analysis and investigation, original draft preparation, draft review, and editing. CC contributed to formal analysis and investigation, original draft preparation, and draft review and editing. MD participated in the study's conceptualization, methodology, formal analysis and investigation, original draft preparation, draft review and editing, and supervision. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no competing interests.

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