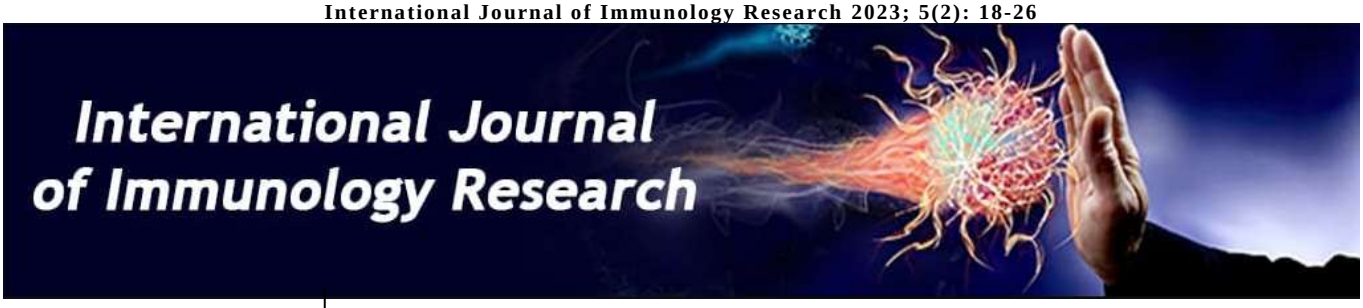

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Vaccination and antibody levels after previous COVID-19 infection in Côte d'Ivoire

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Abstract
Background: COVID-19 immunity can be acquired by vaccination or by natural infection with SARS-CoV-2. The choice of immunisation method depends on a country's resources, the risks involved and the availability of vaccines during the period of high endemicity. Many public health authorities have opted for vaccination protocols that give priority to people at high risk. People who had already been infected may not have been given priority because of existing humoral immunity.
Objective: We compared residual antibody levels between previous COVID-19 infection and COVID-19 vaccination in medical staff.
Methods: A serological assay using a competitive enzyme-linked immunosorbent assay for the quantitative determination of total neutralising anti-S1 SARS-CoV-2, IgG and IgM antibodies on a disposable device with the Chorus TRIO was performed on healthcare staff from the three university teaching hospitals in Abidjan-Côte d'Ivoire. Our population was categorised into 2 and then 4 groups, and antibody levels were compared between the different groups using the Kruskal-Wallis test.
Results: A total of 275 people were recruited with a mean age of 40.1 years. Within each type of immunisation, there was a significant difference between the medians of IgG and neutralising antibodies of vaccinated subjects compared with non-vaccinated. However, among those with a history of infection, there was a difference only in IgG. Neutralising antibody levels were comparable to subjects with no previous infection. IgG and neutralising antibody levels were higher in people with hybrid immunity compared with those with only one mode of immunisation. Hybrid immunity was comparable to immunity acquired by infection in terms of IgG and comparable to vaccination in terms of neutralising antibodies.
Conclusion: Vaccination appears to result in better production of neutralising antibodies, whereas infection results in better production of IgG. Protection against COVID-19 appears to be better with hybrid immunity.

Keywords: Antibody, COVID-19, SARS-CoV-2, Humoral response, Vaccination, Côte d'Ivoire

Introduction

Adaptive immune responses play an essential role in viral clearance and protection against reinfection, and SARS-CoV-2 is no exception [1]. Adaptive immune responses can be acquired actively or passively, either spontaneously through infection or artificially through vaccination.

However, the response to the SARS-CoV-2 pandemic relied heavily on the development, testing and deployment of vaccines, despite the theory of the acquisition of protective herd immunity post-infection, with all the risks. In a short space of time, several different vaccine platforms have been developed.

Since September 2020, in response to the COVID-19 pandemic, the World Health Organisation (WHO) and the US Food and Drug Administration (FDA) have chosen to make COVID-19 vaccines available [2]. However, in Africa, given the relative resistance to infections, and particularly to COVID-19 [3], the vast majority of black African populations south of the Sahara have not embraced the vaccine.

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Instead, they have preferred to take the risk of contracting the disease, thereby acquiring natural protective immunity.

In Côte d'Ivoire, healthcare workers, who are at the forefront of the health response to this pandemic, have not been left out of this debate, despite the fact that they have been one of the targets of the various vaccination programmes, because they are considered to be people at risk who could be contaminated but also, and above all, who could be factors in the spread of the disease around them. As a result, some people contracted the disease while others did not, and some people were vaccinated while others were not.

After several years of circulation of this virus in the population, and given the considerable drop in its infectivity nowadays, it seems necessary to measure and compare the levels of antibodies acquired in the serum of vaccinated agents and those with a previous infection with COVID-19.

Since antibodies are the key components of protective immunity against SARS-CoV-2, their durability is of major interest in understanding protective immunity against SARS-CoV-2 in previously infected, vaccinated or hybrid immunity individuals [4].

The nature, stability and durability of antibody responses in patients with COVID-19 are known to be the subject of many conflicting studies. Some studies report stable and persistent immunity [5], while others show rapidly waning immunity, or a late onset with low antibody levels, and/or a complete absence of long-lasting antibodies [6, 7].

The aim of the present study was therefore to measure and compare the level of IgM and IgG isotype antibodies in serum, in particular the levels of neutralising antibodies, after a period of immunisation, in order to elucidate the best means of protection conferred by one or other means of immunisation against COVID-19 in the Ivorian context.

Methodology

We conducted a descriptive and analytical cross-sectional study over a 3-month period (April to June 2022). It involved random sampling of all health workers regularly assigned to the three university hospital centres (CHU) in Abidjan-Côte d'Ivoire, taking all posts and departments together. We therefore included 275 healthcare workers who were present at their posts at the time of our survey visits and who agreed to take part in the study after giving written informed consent. They were of both sexes and over 18 years of age. They were all subjected to a questionnaire based on survey forms for the collection of information on socio-demographic data, immunisation status, i.e. history of SARS-CoV-2 infection and notion of anti-COVID-19 vaccination (dates, type of vaccine, number of doses, etc.). Nasopharyngeal and blood samples were taken from all patients. The nasal swabs were used to carry out antigenic tests for COVID-19 by SD Biosensor's Standard Q in the presence of the healthcare staff surveyed, with a view to excluding staff with an ongoing infection. If the test was negative, the health worker was included in the study. Blood samples were taken in tubes without anticoagulant (dry tubes). Specimens (sera) for the assays were obtained after centrifugation of the samples at 3.500 rpm for 10 minutes. The anti-COVID-19 antibody assay was performed using a competitive enzyme immunoassay (Chorus SARSCoV-2 "Neutralizing" Ab) for the quantitative determination of total neutralising anti-S1 SARS-CoV-2, IgG and IgM antibodies in sera obtained on a disposable device with the Chorus TRIO instrument from DIESSE. In the case of delayed analyses, sera were frozen at minus 20°C. Results were expressed in BAU/ml (Binding Antibody Units) according to WHO recommendations for anti-SARS-CoV-2. We considered a positive result to be above 50.0 BAU/ml and a negative result to be below 50.0 BAU/ml. In the event of ambiguity, the assay was repeated.

Our data were first categorised into 2 groups according to the notion of vaccination and according to the notion of previous infection. Then into 4 groups by dividing vaccinated and unvaccinated subjects according to whether or not they had had a previous infection. These are: uninfected and vaccinated, uninfected and unvaccinated, infected and vaccinated, and infected and unvaccinated.

As our data did not have a normal distribution for the various normality tests, we used the non-parametric Kruskal-Wallis test (Anova) to compare the medians at the 5% significance level.

Microsoft Word and Excel 2011 were used for data entry and table compilation. GraphPad Prism 8 software was used for statistical analysis and drawing up the figures.

Results

In our study population, we noted a female predominance with a sex ratio of 0.68. The majority of our patients were vaccinated (63.64%), compared with 38.55% who had a previous infection with COVID-19 and 18.90% who had a hybrid immunisation. The average time between sampling and the different types of immunisation was 8 months for vaccination and 11 months for COVID-19 infection. In terms of IgM levels, there was no difference between the different rates, whether or not the patient had been vaccinated, or had had a previous infection. However, within each type of immunisation, there was a significant difference between the median IgG levels of vaccinated subjects compared with non-vaccinated subjects ($p<0.0001$) and of those with a history of infection compared with those without ($p<0.0001$). In terms of neutralising antibodies, vaccinated subjects had higher levels than non-vaccinated subjects ($p<0.0001$). On the other hand, the rates in those who had had a previous infection with COVID-19 were comparable to those in those who had not. A comparison of the different levels when categorised into four groups showed that there was no difference in IgM levels between those exposed to a single type of immunisation and those exposed to a hybrid immunisation. However, IgG levels were higher with hybrid immunisation, with a significant difference compared with levels obtained with vaccination ($p = 0.0008$) but no difference with infection. The opposite effect was observed for neutralising antibodies. The rates in vaccinated subjects and subjects with hybrid immunisation were high compared with those with only infection for immunisation. And the latter had levels comparable to those of subjects who had never been exposed to any form of immunisation.

Discussions

Humoral immune responses to SARS-CoV-2 induced at the start of COVID-19 will lead to the formation of a protective immunological memory in two stages. Initial exposure to the natural virus or any other form of presentation triggers an immune response that induces low-affinity antigen-specific B cells [8]. Subsequently, CD4+ follicular helper T cells (hFTCs) and B cells from secondary lymphoid tissues cooperate and facilitate antibody affinity maturation and

isotype switching in a complex manner that generates long-term immune protection [9]. The fight against this COVID-19 pandemic therefore led to a search for the best means of protection. While some advocated acquiring immunity through vaccination, others opted for herd immunity through infection of larger numbers.

In the present study, we were therefore interested in evaluating these two modes of immunisation after a few years of virus circulation in our country. We enrolled 275 healthcare workers, among whom we found a high proportion of vaccinated subjects: 63.64% (175 patients) had already received at least one dose of anti-COVID vaccine, and 106 patients (38.55%) had at least one history of previous infection with COVID-19 (Table 1). These high rates of immunisation acquired both by artificial challenge (Vaccination) and by natural challenge (infection) confer on these staff a kind of collective immunity which could therefore limit the progression of the disease both within them and within the population as a whole.

Women predominated, with a sex ratio of 0.68, although a higher proportion of men (66.96%) than women (61.35%) were vaccinated. The average age was 40.1, making this a relatively young population. And 38.55% of the staff had a history of COVID-19 disease, with 65.09% having been symptomatic at the time of illness and 34.90% showing no signs. The disease had only been diagnosed during systematic screening after contact with an infected patient or colleague. The fact that our population is relatively young, and therefore has a competent immune system, could justify the high proportion of asymptomatic forms of the disease. Indeed, according to some studies, age-related immunosenescence is considered to be the main cause of increased susceptibility to infection, such as the age-related decline in de novo T-cell reactivity [10, 11]. Our results were in line with the estimates of some studies which have found that between 7.9% and 61.0% of people remain asymptomatic even when PCR tests are positive [12], probably due to the evolution of SARS-CoV-2 [13] and increased vaccination coverage [14]. This favourable immunity acquired actively, both spontaneously and artificially, could therefore limit the contamination of these personnel by the SARS-Cov-2 virus, either by patients or between them, in addition to the significant decline in the circulation of the virus during the study period.

Generally speaking, there was no difference between the medians of the different IgM classes, vaccinated or not, whether or not there had been a previous infection with COVID-19 (Figure 1A). This finding could be justified by the fact that the mean time between the different types of immunisation and sampling was 8 months for vaccination and 11 months for a previous infection. Indeed, studies of anti-COVID-19 antibodies show that 16.7% of patients will be seronegative for IgM antibodies after 8-11 weeks [15]. However, as a result of class switching, which occurs on average within 3 months, IgM levels are likely to fall in favour of IgG, leading to a return to baseline levels. Studies also show that IgM-producing memory B cells are present 20 days after immunisation with SARS-CoV-2 proteins for up to 150 days (5 months), before being replaced by IgG isotype antibodies [16].

In terms of IgG, it was noted that within each type of immunisation, depending on whether the individual had been vaccinated or had had a previous infection, exposed individuals had higher levels than unexposed individuals, with significant differences (Figures 1B). However, whatever the type of immunisation, there was a difference from those who had not been exposed. Our results confirm data in the literature showing that antibodies persist for several months after vaccination [17, 18] and infection. On the other hand, there was no difference between the rates of those vaccinated and those with a previous infection. That said, vaccination and infection confer the same types of immunity. This is because the viral proteins in vaccines and SARS-CoV-2 are immunogenic and will trigger immune reactions leading to the production of specific antibodies, even if the conformations of the vaccine proteins are slightly different from those of the natural proteins [19].

With regard to neutralising antibodies, there was a significant difference between the levels of vaccinated patients compared with non-vaccinated patients ($p<0.0001$) even after an average of 8 months post-vaccination. This indicates the persistence of acquired neutralising antibodies beyond 8 months. However, according to the notion of previous infection, there was no difference between the rates of infected and uninfected patients (Figure 1C). That said, after an average of 11 months, the rates in infected subjects returned to normal and were therefore comparable to the rates in subjects with no previous infection. Indeed, according to Wajnberg Ania *et al*, IgG antibody titres are relatively robust for at least 5 months after infection [20], which is associated with a significantly reduced risk of reinfection [21]. IgG antibodies also decline between 5-7 and 34-42 weeks.

According to some studies, these circulating antibody levels may be related to a number of factors, such as the decrease in response, the size of the peak response, antibody subtypes and the relative contribution of short- and long-lived plasma cells [22, 23]. Also, because of recurrent infections, subjects with no previous history of COVID-19 infection may have been in contact with viruses whose acquired antibodies could cross-react with the SARS-CoV-2 antigens used for testing [24]. Because of the different mean timescales, we cannot effectively compare these two modes of immunisation in terms of their persistence and regression over time. Also, the weakness of our cross-sectional study meant that we were unable to follow changes in antibody titres in our respondents over different periods, in order to use logistic regression tests to compare rates. That said, we cannot state with certainty which of the two persists longer. However, on the basis of our results, we can say that the antibodies acquired after vaccination persist for at least 8 months post-immunisation and that those acquired after infection gradually regress to disappear after an average of 11 months post-infection. However, when vaccination takes place in previously infected areas, the antibodies acquired persist even beyond 11 months.

These results obtained for each type of immunisation can also be seen in the categorisation of the staff into four groups. The same was true for IgM. Levels were more or less the same, with no significant difference (Figure 2A). This confirms what we observed within each type of immunisation taken individually.

However, in terms of IgG (Figure 2B), we observed a very low rate in unvaccinated subjects with no previous infection with COVID-19 (unexposed), with significant differences compared with the very high rates obtained after post-vaccinal and post-infectious immunisations ($p<0.0001$). This testifies firstly to the efficacy of these two methods of

immunisation, but also to the persistence of IgG obtained after immunisation. Both are comparable. However, compared with the rates in people with hybrid immunity, there was a significant difference with immunisation by vaccination (P=0.0008). Our results were similar to those of Shenoy Padmanabha *et al*. who reported that rates after even a single dose of vaccine in individuals with prior infection were higher than rates with two doses [25]. However, they differed from those of Ebinger Joseph E. *et al*. who found in their study a similarity between the IgG levels of people previously infected with SARS-CoV-2 after a single dose and people who had never been infected after two doses of vaccine [26]. Compared with levels after previous infection alone, there was no significant difference. That said, although the IgG level after hybrid immunisation was relatively higher than that after infection alone, the absence of any difference means that the immunity conferred by previous infection is comparable to hybrid immunity but higher than that obtained with vaccination alone. Infection appears here to confer superior IgG immunity. However, our observations differed from those of numerous epidemiological studies which have now validated these immunological results, observing that hybrid immunity results in more robust protection against COVID-19 than immunity to a previous infection or immunity induced by a vaccine [27, 28]. These observations are explained in the study by Hamad Ali *et al*. who noted that the high levels of antibodies in previously infected groups most likely represent the sum of antibodies produced after infection, since antibody-producing B cells multiply after each exposure [29]. Also, the fact that the IgG antibody level after a previous infection is higher than after vaccination could be justified by the fact that the viral proteins in vaccines are in slightly different conformations [19] but also that they are presented to the immune system in a different way to that of a real viral infection. This could therefore lead to differences in the kinetics of the antigens and, consequently, of the antibodies produced [30, 31].

In terms of neutralising antibodies, we found a low level in subjects with only a history of infection compared with vaccinated subjects (p=0.0080) and subjects with hybrid immunisation (p=0.0201), but with levels comparable to subjects who had not been subjected to any form of immunisation. Furthermore, the level of neutralising antibodies acquired after vaccination was comparable to that acquired after hybrid immunisation, and both were higher than in subjects who had not received any form of immunisation ($p<0.0001$). This finding suggests that, in terms of neutralising antibodies, those acquired through vaccination and hybrid immunisation are higher than those acquired after infection. Our observations confirm the conclusions of Wei Jia *et al*., who stated in their study that prior infection conferred substantial immunity, with circulating neutralising antibody titres significantly lower than after vaccination [32]. Furthermore, according to Crotty Shane, with hybrid immunity, neutralising antibody titres and the extent of recognition of SARS-CoV-2 variants are significantly higher in previously infected individuals who have received at least one dose of a COVID-19 vaccine [33]. And, according to some studies, although neutralising antibodies are considered the reference for quantifying protection, a drop in neutralising antibody production can be observed in some patients who have recovered from SARS-CoV-2 [34]. Our results were therefore in line with those reported in the literature. Although high titres of neutralising antibodies in circulation can clearly provide protective immunity, high titres are not always found, particularly after infection with SARS-CoV-2 [35, 36, 37].

Analysis of these two findings in the different groups of our population shows that infection is just as immunising as vaccination. However, the neutralising, and therefore protective, antibodies regress over time to reach levels comparable to those of non-immunised patients. Hybrid immunisation also produces more IgG than vaccination. However, in terms of the production and persistence of neutralising antibodies, these two types of immunisation are comparable. It has recently been shown that natural infection confers stronger and longer-lasting protection against reinfection than vaccination [38, 39, 40], and that the combination of the two types of immunity (Hybrid immunity) can provide even stronger protection [40, 41].

In terms of IgG production, infection appears superior to vaccination because there are several proteins on the natural virus. But in terms of neutralising antibodies, the vaccine appears superior because it has been specifically developed for this purpose (Anti-spike). That said, immunisation with both vaccines is the best way of providing better protection. But natural infection would have the advantage of encouraging cross-reactivity to combat infection by new variants. Vaccines, on the other hand, would be more specific to one variant, and therefore less likely to encourage cross-reactivity.

Table 1: Distribution of the general population according to history of COVID-19 infection, clinical symptoms and vaccination according to sex

			Workforce	History of infection		Vaccination		Symptomatic	
				Yes	No	Yes	No	Yes	No
		Total	n %	n %	n %	n %	n %	n %	n %
Enrolled Personnel		275	275 100	106 38,55	169 61,45	175 63,64	100 36,36	69 65,09	37 34,90
Gender	Female		163 59,27	79 74,53	84 49,7	100 61,35	63 38,65	52 75,36	15 40,54
p	Male		112 40,73	27 25,47	85 50,3	75 66,96	37 33,04	17 24,64	22 59,46
				<0,0001		0,342			

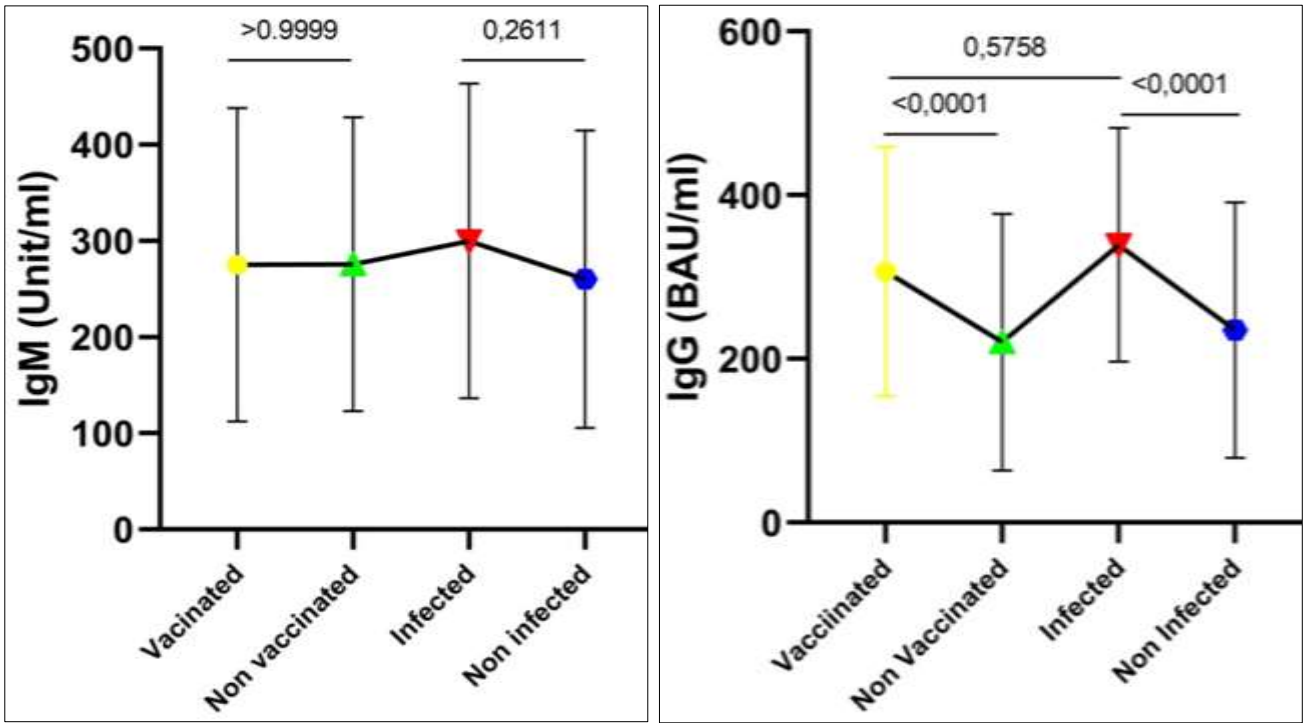
Table 2: Global comparison of medians for antibodies and cytokines according to vaccination and history of infection

			COVID-19 history		No history of COVID-19		
			Vaccinated	Unvaccinated	Vaccinated	Unvaccinated	P
Overall		275	52 (18,9%)	54 (19,64%)	123 (44,73%)	46 (16,73)	
	IgM		4,985	5,195	4;070	4,070	0,2299
	IgG		582,9	482,6	449,9	272,2	<0,0001
	N-Ab		1422	1107	1435	1001	<0,0001

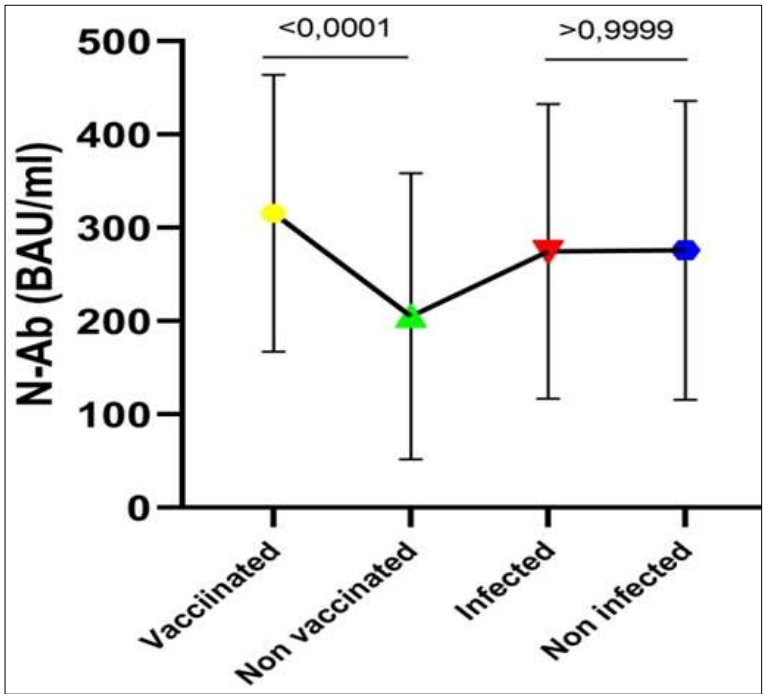
Median	IFN		0,0	0,11	0,54	0,26	<0,0001
	TNF		2,9	0,025	0,01	0,99	<0,0001
	IL-2		0,99	0,32	0,45	0,16	<0,0001
	IL-10		0,96	3,2	4,2	1,5	<0,0001

Table 3: Average time between samples and different types of immunisation

Types of immunisation	Deadlines (Month)	Average	Minimum	Maximum
Vaccination		8	1	26
Infection		11	1	24



A) B)



C)

Fig 1: Comparison of antibodies levels between vaccinated and unvaccinated individuals, and between individuals with and without a history of COVID-19 infection, A) Comparison of IgM antibodies levels between vaccinated and unvaccinated individuals, and between individuals with and without a history of COVID-19 infection, B) Comparison of IgG antibodies levels between vaccinated and unvaccinated individuals, And between individuals with and without a history of COVID-19 infection, C) Comparison of Neutralizing antibodies levels between vaccinated and unvaccinated individuals, and between individuals with and without a history of COVID-19 infection

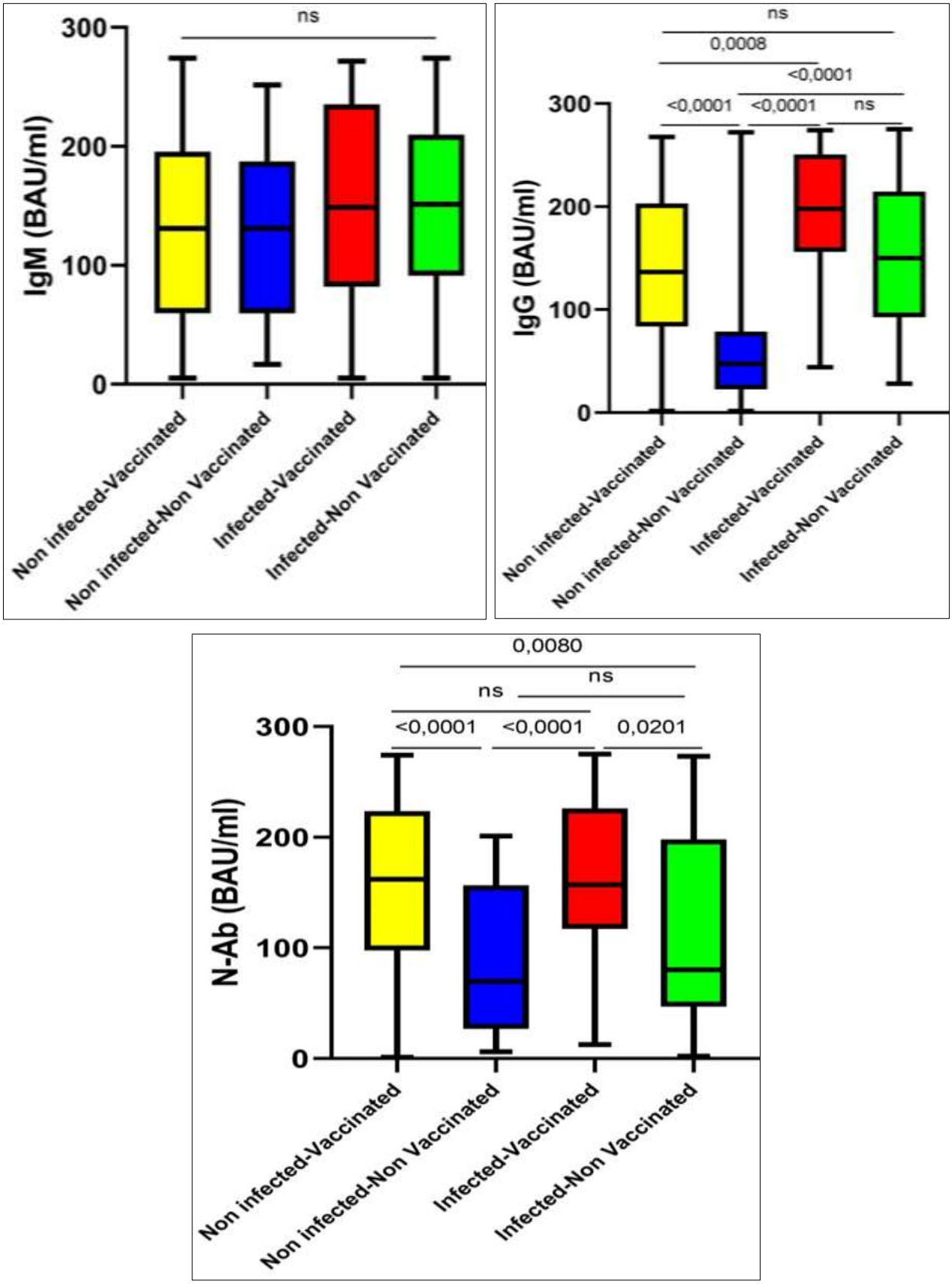


Fig 2: Comparison of antibodies levels between vaccinated and unvaccinated individuals according to history of COVID-19 infection, A) Comparison of IgM antibodies levels between vaccinated and unvaccinated individuals according to history of COVID-19 infection, B) Comparison of IgG antibodies levels between vaccinated and unvaccinated individuals according to history of COVID-19 infection, C) Comparison of Neutralizing antibodies levels between vaccinated and unvaccinated individuals according to history of COVID-19 infection

Conclusion

Our findings from this study confirm that vaccination with prior COVID-19 infection results in a stronger antibody response than vaccination without prior infection. Vaccination also appears to lead to better production of neutralising antibodies, whereas infection leads to better production of IgG antibodies. To save money, our developing countries will be able to recommend a single

dose of vaccine for people who have already been infected, in the event of new epidemics.

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Conflicts of interest: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Ethics Approval: The study was approved by the National Ethics Committee for Life and Health. Reference number: 007-22MSHPCMU/CNESVS-km
The patients/participants provided their written informed consent to participate in this study.

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