



## Letters to the Editor

**Limited protection against SARS-CoV-2 infection and virus transmission after mRNA vaccination**

Dear Editor,

Stang and coworkers have recently reported in the Journal that “RT-PCR test results as gold standard for assessing and controlling infectiousness fail”[1]. The authors analyzed two scenarios and used Ct25 and Ct30 as thresholds for infectiousness and conclude that more than the half of their patient cohort was unlikely to have been infectious, i.e. the group of patients tested positive at Ct values >25 or >30.

While we agree that Ct values are insufficient to determine the infectiousness we would like to add that also for the cohort of vaccinated individuals the Ct values are rather misleading. As shown in an early released study vaccinated people can not only become infected with SARS-CoV-2 but the virus can successfully be isolated by cell culture approaches, at least for a shorter time slot as in unvaccinated people[2]. Thereby, the viral loads as determined by the Ct values do not significantly differ between vaccinated and unvaccinated cohorts. While the group from Berlin analyzed an outbreak with the alpha variant, we made similar observations, also including the wild type strain.

In their recent report, Liu and coworkers demonstrated neutralization of newly emerged SARS-CoV-2 variants after properly completed BNT162b2 vaccination[3] and referred to a previous publication, which already showed neutralizing of the B.1.1.7 variant[4].

The first case series resulted from screening examination of medical staff at a local maximum health care provider including a 54 year old male with mild common cold symptoms twelve weeks after second vaccination with BNT162b2. Analysis with the SARS-CoV-2 two target PCR assay (Altona Diagnostics, Hamburg, Germany) revealed CT-values of 23 for the E- and S-gene, while the positive control showed a CT-value of 28. This patient was infected with the SARS-CoV-2  $\alpha$ -variant determined by different probe-based melting curve assays according to the manufacturer (VirSniP SARS-CoV-2 Spike, TIB-MolBiol, Berlin, Germany), which detected the mutations delHV69/70, N501Y, and P681H. In addition two asymptomatic healthcare staff members were tested SARS-CoV-2 positive (CT-values 36/36 and 30/30; E-/S-gene respectively) after proven contact with a COVID-19 patient, despite being completely vaccinated with BNT162b2. The fourth patient was infected 8 weeks after the second vaccination dose and suffered from serious common cold symptoms lasting one week, while being RT-PCR positive (CT-values between 30 and 33) for three weeks.

Regarding humoral immunity, Anchini and colleagues[5] convincingly showed that after BNT162b2 vaccination previously uninfected individuals had a significantly lower neutralizing antibody titer after administration of a second vaccine dose compared to previously infected individuals after a single dose, despite remark-

able antibody titres specifically binding SARS-CoV-2 spike protein. This finding as well as the fact of an aged immune system[6]; [7] must be taken into account when real-world effectiveness of Covid-19 vaccines is discussed.

In this context we investigated a nursing home outbreak in which 12 patients tested positive for SARS-CoV-2 with CT-values between 24 and 37, although all received the second vaccine dose >80 days ago and although staff and visitors were tested negative by rapid antigen tests.

Most striking was that - until the end of April 2021 - 119 cases with confirmed positive PCR results >14 days after second vaccination were reported including one case of fatal SARS-CoV-2 pneumonia. In 37 cases CT-values were <30 with a negative correlation of SARS-CoV-2-mRNA load and days post vaccination. Moreover, in several cases subsequent infections were confirmed by contact tracing (details to be published separately) suggesting that actual SARS-CoV-2 vaccines do not lead to sterile immunity. Considering that all these observations have been made in the relatively small area of Cologne and its surroundings without any claim on completeness this is absolute relevant for further strategies, especially as the findings suggest an insufficient immunity, lack of protection against colonization or infection, and a residual risk for transmission despite SARS-CoV-2 vaccination. For this reason, easing of pandemic-related restrictions or exemptions from hygiene measures based on the vaccination status seems doubtful.

Although determination of SARS-CoV-2 vaccination success, at least as antibody titre (arbitrary units / ml), is not part of the actual vaccination campaign, a vaccine-induced immune response in up to 92% cases must be assumed[8]. Nevertheless, studies on overall neutralizing capacity do not take into account effects like specific T-cell release, heterogeneous antibody populations, or the occurrence of non-spike mutations influencing viral replication and immune response, which was also discussed by Liu et al.[3]. Another effect that should also be considered is the general non-lasting triggering of the innate immunity by contact with foreign RNA[9–11].

This may explain why vaccination based neutralizing effects in some cases seem to be less sustained than assumed. In this context the “green passport”, which is still considered in Europe and Israel, is just a vaccination certificate not more, not less. The individual risk for severe/life threatening COVID-19 may be significantly reduced although fatal courses remain possible, but it must be taken into account that any vaccinated individual may become an, at least short term, spreader of the virus.

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Accepted 25 June 2021

Available online 29 June 2021

<https://doi.org/10.1016/j.jinf.2021.06.023>

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## Detection of a SARS-CoV-2 P.1.1 variant lacking N501Y in a vaccinated health care worker in Italy



Dear Editor,

We read with interest the recently published manuscript of Dimeglio et al., exploring the SARS-CoV-2 immune response and vaccination of healthcare workers post-infection.<sup>1</sup> and here we report a case of SARS-CoV-2 infection with a P.1.1 variant lacking the Y501 mutation in a vaccinated individual in Italy.

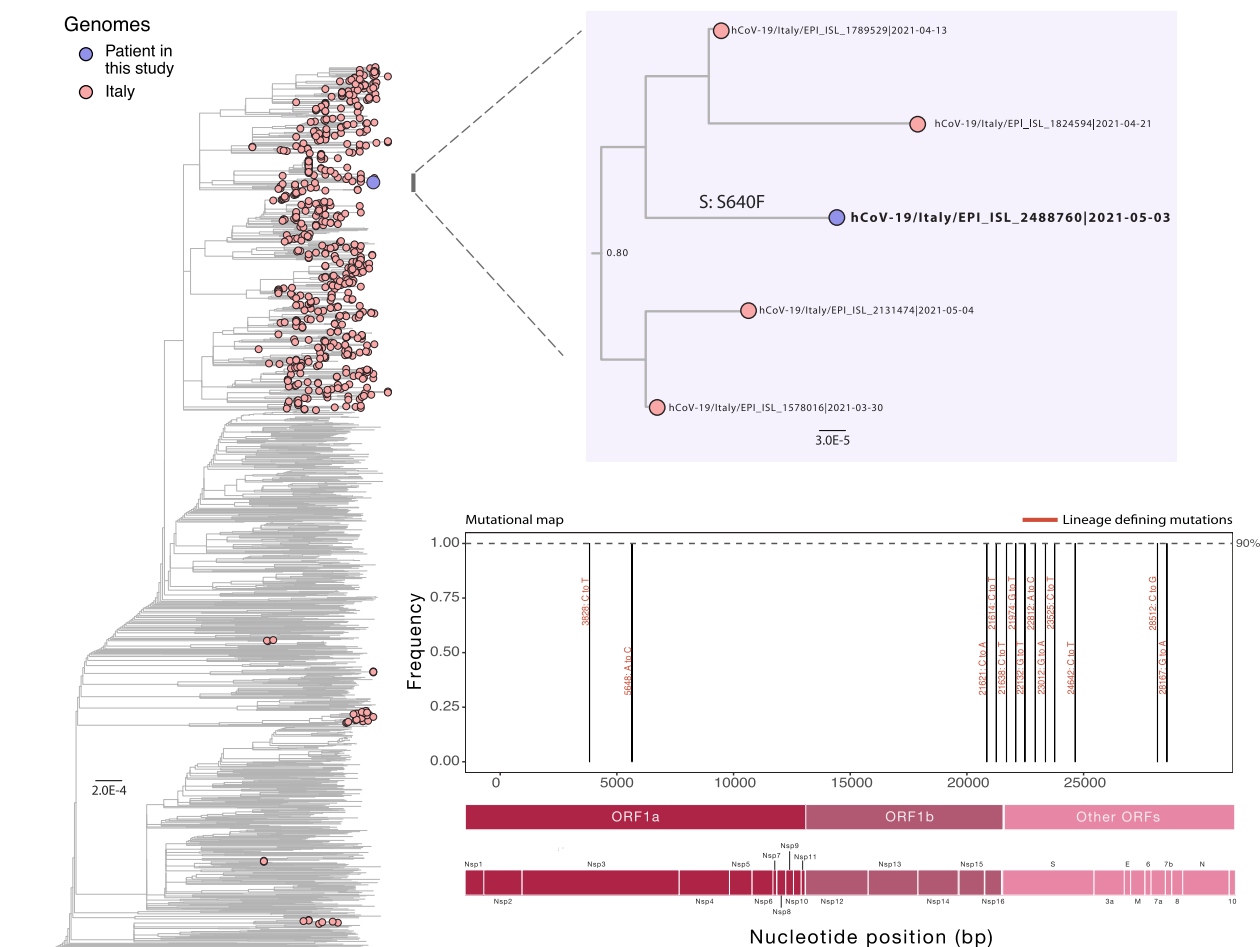
COVID-19 vaccines are very effective in preventing infections, hospitalizations and deaths. However, several cases of SARS-CoV-2 infections have been reported in vaccinated individuals (called “vaccine breakthrough cases”) inoculated with one or both doses of vaccine.<sup>2</sup> This indicates that a small percentage of fully vaccinated subjects can be infected when exposed to the virus. Recently, in the UK, South Africa, Brazil and most recently in India,<sup>3–5</sup> SARS-CoV-2 variants of concern (VOC) have been identified. These new variants harbor mutations in the spike protein, and particularly in the receptor binding domain (RBD). These VOC are important because they show that a number of viral mutations are emerging with a potential impact on infectivity, immune escape and vaccine effectiveness.

In the University Campus Biomedico Hospital (UCBM – Rome, Italy), vaccination of health care workers began on December, 2020 soon after followed by active monitoring of potential breakthrough cases. For this reason, all health care workers, regardless of their symptomatic status, were tested weekly by molecular assay on nasopharyngeal swabs.

Here, we report the first case of SARS-CoV-2 P.1.1 infection lacking N501 mutation in a fully vaccinated (Pfizer) 22-year-old female nurse, working in the COVID-Center of the UCBM.

In early-January 2021, the nurse received the first vaccine dose followed by the second shot three weeks after. Two weeks after second dose of vaccine, a first Quantitative IgG anti-spike chemiluminescent assay tested positive with 2362 BAU/mL. Quantitative IgG anti-spike assay was repeated after 45 and 90 days confirming positive results with 1029 BAU/mL and 432 BAU/mL, respectively. Three months after the second dose the patient started presenting mild symptoms (headache and fever), compatible with a viral infection. At this time, a rapid SARS-CoV-2 N protein Chemiluminescent assay was performed on nasopharyngeal swab, revealing a positive result (> 5000 pg/mL). The same swab was then tested for the detection of SARS-CoV-2 RNA by multiplex real-time PCR Allplex™ SARS-CoV-2 assay (Seegene Inc, Seoul, Korea). Cycle threshold values (Cts) of N, E and RdRp/S targets were 17, 18, and 18, respectively. Whole genome sequencing was then conducted on the same swab by MySeq II Illumina. Consensus sequences were generated by de novo assembling using Genome Detective (<https://www.genomedetective.com/>).<sup>6</sup> A total of 1,323,357 mapped reads were obtained, resulting in a sequencing mean depth > 1,000X and a coverage of > 99.8%. Sequences were aligned using MAFFT<sup>7</sup> and submitted to IQ-TREE 2 for maximum likelihood (ML) phylogenetic analysis.<sup>8</sup>

Lineages assessment, conducted using Phylogenetic Assignment of Named Global Outbreak Lineages tool (available at <https://github.com/hCoV-2019/pangolin>), revealed the new strain belonged to the P.1.1 lineage. Phylogenetic inference by combining our new isolate (EPI\_ISL\_2,488,760) with a representative dataset available on GISAID (<https://www.gisaid.org/>) up to May 31th, 2021 demonstrated that the newly obtained genome belongs to P.1.1 lineage and clustered significantly with SARS-CoV-2 P.1.1 strains isolated in Italy between March and May 2021 (Fig. 1a, b) (Bootstrap=0.80, SH-aLTR=0.80). Further, we analyzed the mutational profile of the newly generated strain to determine its lineage-defining mutations. The identified lineage harbored all the



**Fig. 1.** Genomic detection of the SARS-CoV-2 P.1 variant of concern in a vaccine breakthrough case in Italy. (a) Maximum likelihood (ML) phylogenetic tree including the newly isolate obtained in this study plus  $n=1631$  SARS-CoV-2 strains belonging to the P.1 lineage collected up to May 28th, 2021. (b) Representation of the zoom of the Italian P.1 clade. Branch support (Bootstrap = 0.80, SH-aLTR = 0.80) is shown at key node. c) Variant map of the P.1 lineage-defining-mutations was mapped against the SARS-CoV-2 genome structure. Lineage-defining mutation are highlighted in red (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

P.1.1 lineage specific mutations (Fig. 1c), with the exception of the N501Y, a specific mutation that seems to be linked with an increased transmissibility.<sup>9</sup> We also identified another Spike mutation, namely S640F, which currently is growing in many samples worldwide<sup>10</sup> (Fig. 1c).

As observed through the course of the pandemic, these mutations highlight the ability of SARS-CoV-2 to generate new viral strains. In particular, these newly identified mutations likely represent a new SARS-CoV-2 variant probably able to escape vaccine protection, although to a certain extent, as indicated by the lack of severe symptoms. The lack of the N501Y may be linked to high transmissibility and immune evasion as indicated also for the new path followed by the delta new variant (B.1.617.2). Probably, the vaccine immune pressure could select less prevalent strains leading to their emergence.

At present, it is not clear how widely this variant is currently present in Italy and worldwide. Nonetheless, our case report, clearly indicate the importance of genetic sequencing and analysis to promptly identify and characterize variants as soon as possible both in vaccinated individuals and in the general population.

#### Ethical statement

This research was approved by the University of Campus Biomedico Ethics Review Committee (Approval number 8.1(21).21 OSS).

#### Funding

MG is supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ.

#### Declaration of Competing Interest

The authors declare no competing interests.

#### Acknowledgments

We thank personnel from Health Surveillance System from the University of Campus Biomedico that helped with samples, sources and epidemiological data collection. We also would like to thank all the authors who have kindly deposited and shared genome data on GISAID. A table with genome sequence acknowledgments can be found in Supplementary Table 1.

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.jinf.2021.06.026](https://doi.org/10.1016/j.jinf.2021.06.026).

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Accepted 30 June 2021

Available online 6 July 2021

<https://doi.org/10.1016/j.jinf.2021.06.026>

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## Importance of sample dilution in the evaluation of the antibody response after SARS-CoV-2 vaccination



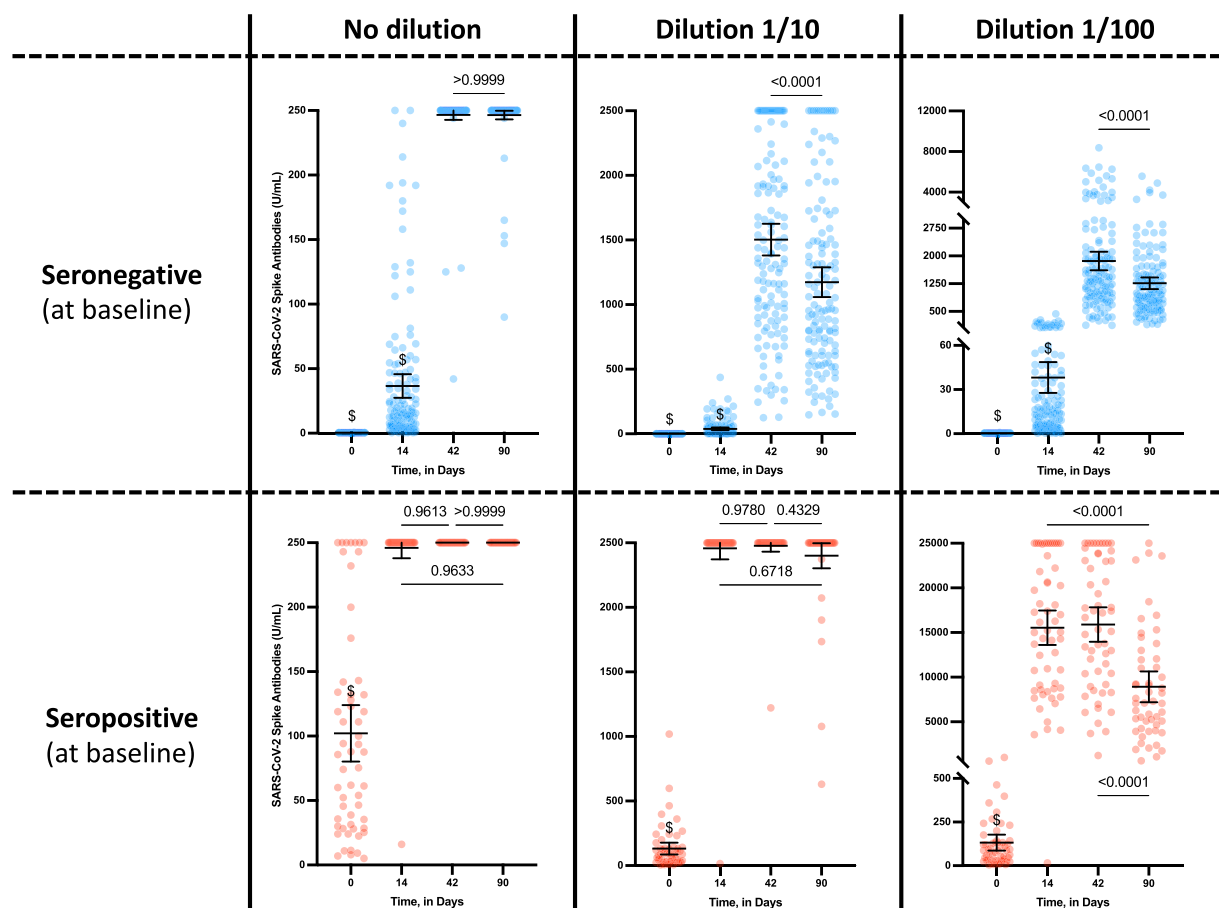
Dear Editor,

We read with great interest the recently published article of Tré-Hardy et al. reporting on the time-related changes in the serological response of healthcare workers having received the mRNA-1273 vaccine.<sup>1</sup> Among 205 individuals, 161 (78.5%) were initially seronegative at baseline while 44 (21.5%) already developed antibodies directed against SARS-CoV-2. The antibody response was assessed 2 weeks after the first vaccine injection (T1), 2 weeks after the second vaccine injection (T2) and 3 months after the first injection (T3). The quantitative analysis of the anti-SARS-CoV-2 IgG antibodies directed against the subunits (S1) and (S2) of the virus spike protein was carried out using the LIAISON® SARS-CoV-2 IgG kit (DiaSorin®, Saluggia, Italy). Almost all samples at T1 and all samples at T2 and T3 in the seropositive cohort were above the maximum quantification value of the assay kit, i.e. >400 AU/mL on neat samples. In the discussion, the authors reported that, in previously seropositive subjects ( $n = 44$ ), no drop in antibody between T2 and T3 was observed.

In order to share our experience on that important topic, we would like to present the results we obtained 3 months post-vaccination in the CRO-VAX HCP study (EudraCT registration number: 2020-006,149-21), an ongoing multicenter study in healthcare workers having received BNT162b2, another mRNA vaccine (Pfizer-BioNTech, Mainz, Germany).<sup>2</sup> Among the 200 individuals who were followed up to 3 months, 58 (29%) were seropositive and 142 (71%) were seronegative at baseline.<sup>3</sup> Antibodies against the SARS-CoV-2 receptor binding domain of the S1 subunit of the spike protein (anti-S; Elecsys® anti-SARS-CoV-2 spike quantitative ECLIA, Cobas 801, Roche Diagnostics®, Machelen, Belgium) were measured. As the Roche system permits samples dilution to increase the range of measurement, we diluted our samples 10 or 100 times when signal was out of range according to the manufacturer recommendations. Similar timepoints as Tré-Hardy et al., i.e. baseline, 14 days, 42 days and 3 months, were collected in our cohort and analyzed.

Using neat or 10-fold diluted samples, we did not observe an antibody drop in seropositive individuals between T1, T2 and T3 (p





**Fig. 1.** Evolution of SARS-CoV-2 spike antibodies (U/mL) in previously seronegative (blue) and seropositive individuals (red) according to the time since administration of the first vaccine dose. Means (95% confidence intervals) are shown. Three different representations according to the dilution titers applied are shown (no dilution: up to 250 U/mL; 10-fold dilution: up to 2500 U/mL; 100-fold dilution: up to 25,000 U/mL). All dilutions were automatically performed by the analyzer. Results < 0.4 U/mL (limit of quantification) were rounded to 0.4. \$ = statistically different from all other groups (i.e.  $p < 0.0001$ ).

**Table 1**

Evolution of SARS-CoV-2 spike antibodies (U/mL) in previously seronegative and seropositive subjects according to the dilution factor. Means (95% confidence intervals) are shown.

		Seronegative	Seropositive	p value
<b>No dilution</b> (up to 250 U/mL)	Before first dose	0.40 (0.39–0.41)	102.2 (80.3–124)	<0.0001
	14 days	36.6 (27.4–45.7)	246 (238–254)	<0.0001
	42 days	247 (243–251)	250 (250–250)	0.9826
	90 days	246 (243–245)	250 (250–250)	0.9753
<b>Dilution 1/10</b> (up to 2500 U/mL)	Before first dose	0.40 (0.39–0.41)	131.8 (86.1–178)	<0.0001
	14 days	38.2 (27.7–48.6)	2457 (2371–2543)	<0.0001
	42 days	1503 (1380–1625)	2400 (2303–2496)	<0.0001
	90 days	1173 (1057–1288)	2477 (2431–2523)	<0.0001
<b>Dilution 1/100</b> (up to 25,000 U/mL)	Before first dose	0.40 (0.39–0.41)	132 (86.1–178)	<0.0001
	14 days	38.2 (27.7–48.6)	15,540 (13,606–17,473)	<0.0001
	42 days	1863 (1613–2113)	15,856 (13,968–17,824)	<0.0001
	90 days	1262 (1104–1420)	8919 (7201–10,637)	<0.0001

>0.05, Fig. 1), a finding which is similar to that of Tré Hardy et al.<sup>1</sup> However, a highly significant drop in antibody titers was observed at 3 months if a 100-fold dilution was performed ( $p < 0.001$ ; from 16,935 U/mL to 8919 U/mL; a decrease of 47.3%) (Fig. 1). Such dilution factor permits to increase the range of measurement until 25,000 U/mL on the Roche assay. Considering seronegative individuals, a highly significant antibody drop was also shown when a 10- or 100-fold dilution was applied ( $p < 0.001$ , Table 1). The application of a 10 or 100-fold dilution (depending on the sample) with our kit permits to show an important difference between the previously seronegative and seropositive subjects (1863 U/mL versus 15,856 U/mL at day 42 and 1262 U/mL versus 8919 U/mL at

3 months), a difference which is not observed when neat samples are used (Table 1). Analytical kits that do not allow a wide range of measurement may thus hide a difference of serological response between previously seronegative and seropositive subjects and does not permit to appreciate the drop in antibody titers in both groups (Table 1).

Currently, data about the long-term kinetics of antibodies in vaccinees are scarce. Two studies found an time-dependent antibody decline with the mRNA-1273 vaccine in only 33 and 34 participants while Tré-Hardy et al. followed more than 200 subjects.<sup>4,5</sup> Nevertheless, in previous investigations, sample dilutions were applied to allow a better discrimination between previously

seronegative and seropositive subjects.<sup>6–9</sup> Compared to the antibody response observed in past-COVID-19 patients, where none or few samples needed to be diluted,<sup>2,9,10</sup> the antibody response in vaccinees is significantly higher and will certainly require dilutions to obtain the real quantitative value with some assays (i.e. not rounded to the upper limit of measurement).

In conclusion, we agree with Tré-Hardy et al. that a persistent antibody response was observed following the administration of the mRNA vaccine, as observed elsewhere using various assays.<sup>1–4,7</sup> However, the absence of “antibody drop” between T1, T2 and T3 observed in their cohort of previously seropositive could depend on the analytical kit used and the application of a dilution factor in case of signal saturation if such procedure is permitted and documented by the manufacturer. The results of the CRO-VAX HCP study showed that the use of undiluted or diluted samples led to different conclusions regarding the antibody kinetics. The fact that the signal is not saturated with the Roche assay also permit to derived more precise pharmacokinetic models which could latter better predict the probable persistence of antibodies.<sup>3</sup> Such data are important, especially since the question about a third dose has been raised.

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Accepted 1 July 2021  
Available online 4 July 2021

<https://doi.org/10.1016/j.jinf.2021.07.001>

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## N-acetylcysteine for the treatment of COVID-19 among hospitalized patients



Dear Editor,

Despite numerous clinical studies conducted over the past year, we have to admit that today we still have a very limited list of effective drugs for the treatment of Novel coronavirus disease 2019 (COVID-19). Dexamethasone improves survival in hospitalized patients requiring supplemental oxygen or respiratory support<sup>1</sup>, and in the presence of systemic inflammation, tocilizumab may provide additional benefits<sup>2</sup>. Thus, there remains an unmet need for therapeutic interventions that prevent disease progression and improve prognosis in patients with COVID-19.

Therefore, we read with great interest a recent article by Hasan et al. who reported data from a small retrospective cases series of COVID-19 patients that received an IL-17A inhibitor (secukinumab)<sup>3</sup>. The authors demonstrated that this type of therapy, aimed at a new target, could reduce the severity of the cytokine storm and ultimately improve clinical outcomes in patients with severe COVID-19 pneumonia. The search for other targets of COVID-19 therapy is of undoubted interest.

Bourgonje et al. described the potential role of hydrogen sulfide (H<sub>2</sub>S) as a fundamental host defense factor against SARS-CoV-2 infection<sup>4</sup>. Low serum levels of hydrogen sulfide (H<sub>2</sub>S) in patients with COVID-19 pneumonia have been shown to be negatively associated with inflammatory biomarkers such as IL-6 and C-reactive protein (CRP), and also associated with a poor prognosis<sup>5</sup>. Endogenous H<sub>2</sub>S production can be increased therapeutically by administering N-acetylcysteine (NAC), which can be seen as a potential treatment strategy for COVID-19 patients<sup>4</sup>. NAC may also replenish intracellular reduced glutathione (GSH) pools by providing L-cysteine, a precursor for GSH synthesis<sup>6</sup>. Moreover, NAC has shown the ability to restore the intracellular redox imbalance in vitro experiments<sup>7</sup>.

To date, the published data on the efficacy of NAC therapy in COVID-19 are very scarce, and their results are rather controversial<sup>8–10</sup>. Herein we describe the response to NAC therapy in a cohort of hospitalized patients with COVID-19 pneumonia.

This case-control study was conducted in the Pulmonology department of a university-affiliated hospital (Sechenov University) between April 12, 2020, and June 20, 2020. The study was approved by the Medical Ethical Committee (protocol number 08–20/1), and written informed consent was obtained from all patients.

**Table 1**

Main characteristics of patients at baseline and on day 10 of the treatment, and clinical outcomes.

Parameters	NAC group(n = 24)	Control group(n = 22)	P value
Age, years	66 (52; 71)	57 (46; 58)	0.08
BMI, kg/m <sup>2</sup>	31.2 (28.5; 32.3)	28.8 (26.4; 31.2)	0.07
Male, n (%)	16 (66.6%)	13 (59.0%)	0.76
Smokers and ex-smokers, n (%)	11 (45.8%)	12 (54.5%)	0.77
Time from symptoms onset, days	7.5 (6; 9)	7 (6; 8)	0.37
Comorbidities			
Cardiovascular diseases, n (%)	18 (75.0%)	16 (72.7%)	0.56
Chronic lung disease, n (%)	5 (20.8%)	3 (13.6%)	0.44
Diabetes mellitus, n (%)	7 (29.2%)	4 (18.8%)	0.37
Chronic kidney disease, n (%)	3 (12.5%)	1 (4.5)	0.37
<b>At baseline</b>			
Body temperature, °C*	37.7 (37.1; 38)	37.4 (37.3; 37.5)	0.08
Respiratory rate, breaths per min	24 (24; 24)	24 (22; 25)	0.11
Heart rate, beats per min	89 (85; 100)	88 (82; 100)	0.48
SpO <sub>2</sub> /FiO <sub>2</sub>	251 (247; 266)	252 (248; 272)	0.93
NEWS <sub>2</sub> scale, points	7 (4; 7)	7 (2; 7)	0.32
White blood cells, × 10 <sup>9</sup> /L	5.9 (3.6; 7.5)	6.3 (5.1; 8.3)	0.54
Platelets, × 10 <sup>9</sup> /L	221 (127; 280)	189 (177; 242)	0.72
Lymphocytes, × 10 <sup>9</sup> /L	0.8 (0.7; 0.8)	0.8 (0.7; 0.9)	0.66
CRP, mg/L	81 (57; 96)	54 (28; 91.5)	0.08
Fibrinogen, g/L	5.6 (4.8; 6.1)	5.1 (4.4; 5.7)	0.07
D-dimer, ng/mL	0.8 (0.6; 0.9)	0.6 (0.2; 0.7)	0.07
CT,% of lung involvement	46 (45; 50)	39 (35; 52)	0.06
<b>Day 10</b>			
Body temperature, °C*	36.5 (36.4; 36.5)	36.6 (36.4; 36.7)	0.07
Respiratory rate, breaths per min	18.5 (18; 20)	18 (18; 18)	0.06
Heart rate, beats per min	80 (75; 84)	76 (74; 78)	0.08
SpO <sub>2</sub> /FiO <sub>2</sub>	459 (399; 476)	401 (331; 451)	0.03
NEWS <sub>2</sub> scale, points	5 (2; 6)	3 (2; 5)	0.04
White blood cells, × 10 <sup>9</sup> /L	8.4 (5.2; 9.5)	6.7 (6.1; 9.9)	0.72
Platelets, × 10 <sup>9</sup> /L	310.5 (239; 353)	264 (191; 375)	0.16
Lymphocytes, × 10 <sup>9</sup> /L	1.4 (1.4; 1.8)	1.5 (1.1; 1.8)	0.86
CRP, mg/L	5 (2; 8)	9 (4; 16)	0.04
Fibrinogen, g/L	1.96 (1.3; 2)	2.1 (1.3; 4)	0.72
D-dimer, ng/mL	0.7 (0.4; 1.6)	0.7 (0.4; 1.4)	0.84
CT,% of lung involvement	35 (28; 35)	35 (30; 45)	0.17
<b>Outcomes</b>			
Transfer to ICU, n (%)	1 (4.2%)	4 (18.2%)	0.18
Intubation and IMV, n (%)	1 (4.2%)	4 (18.2%)	0.18
Non-invasive ventilation, n (%)	3 (12.5%)	6 (27.3%)	0.28
28-day mortality, n (%)	1 (4.2%)	3 (13.6%)	0.34
Length of hospitalization, days	11 (10; 12)	13 (11; 16)	0.01

\* The highest temperature during the day was recorded. Continuous variables are presented as median value [interquartile range (IQR)]. Categorical variables are presented as number and percentage (%). Abbreviations. BMI, body mass index; FiO<sub>2</sub>, inspired oxygen fraction; CRP, C-reactive protein; CT, computed tomography; ICU, intensive care unit; IMV, invasive mechanical ventilation.

We prospectively enrolled patients over 40 years old with additional high-risk criteria ( $\geq 65$  years, cardiovascular comorbidities, diabetes, and BMI  $\geq 30$  kg/m<sup>2</sup>), with SARS-CoV-2 infection confirmed by real-time polymerase chain reaction, and radiological findings compatible with severe COVID-19 pneumonia. The exclusion criteria were as follows: need for immediate endotracheal intubation, chronic respiratory diseases, unstable hemodynamics, and pregnancy. According to the local protocol, all patients received hydroxychloroquine, azithromycin, corticosteroids, prophylactic low-molecular-weight heparin, and tocilizumab if indicated. The experimental group comprised patients who received NAC at a daily dose of 1200–1800 mg intravenously; the comparator arm was drawn from patients who did not receive NAC. All control patients had the same enrollment criteria described for the NAC group, and the measured parameters were collected prospectively on the same data chart, according to a standardized treatment procedure. Controls were matched to patients in the experimental group by age (within  $\pm 5$  years), SpO<sub>2</sub>/FiO<sub>2</sub> ratio (within  $\pm 20$ ), and NEWS<sub>2</sub> score (within  $\pm 1$  point).

The primary objective was to assess the effect of NAC on arterial oxygen saturation to inspired oxygen fraction ratio (SpO<sub>2</sub>/FiO<sub>2</sub>) on Day 10. Clinical and laboratory data were recorded at admission and on day 10. We also analyzed the length of hospitalization

and outcome of the disease, such as transfer to intensive care unit (ICU), need for non-invasive and invasive mechanical ventilation, and 28-day mortality.

A total of 24 consecutive patients were treated with NAC, and 22 patients were included in the control group. The baseline demographic, clinical and laboratory characteristics at baseline did not differ significantly between the groups (Table 1). The time between the symptom onset and NAC administration was 7.2 <sup>6–9</sup> days. On day 1 of a study, 3 and 4 patients in NAC group and control groups, received noninvasive ventilation, and 16 and 13 patients, respectively, required oxygen supplementation via reservoir oxygen face mask.

On Day 10, NAC therapy led to significant improvement in SpO<sub>2</sub>/FiO<sub>2</sub> compared to the controls (Table 1). Furthermore, NAC administration markedly decreased the values of CRP and NEWS<sub>2</sub> scale in comparison to the control group (Table 1). Duration of hospitalization was also significantly shorter in the NAC group ( $p=0.01$ ). All other clinical outcomes (transfer to ICU, need for non-invasive or invasive mechanical ventilation, and 28-day mortality) did not differ between the groups (Table 1). There were no cases of adverse events leading to NAC discontinuation.

Several studies also examined the efficacy of NAC in hospitalized patients with COVID-19. Ibrahim et al. have demonstrated that

in respirator-dependent patients intravenous NAC elicited clinical improvement and reduced CRP and ferritin<sup>8</sup>. In another study by Alamdari et al., the administration of NAC in combination with high doses of methylene blue and vitamin C as a last therapeutic option resulted in a significant clinical response and recovery in four out of five critically ill patients with COVID-19<sup>9</sup>. At the same time, de Alencar et al. have shown that NAC administration in high doses did not affect the evolution of severe COVID-19<sup>10</sup>. However, in this study, patients were not receiving systemic steroids. Thus, the role of NAC in COVID-19 is still controversial. Identifying a population that would likely benefit from NAC is the key question of the treatment of COVID-19. Presently several registered randomized control trials are evaluating the dose, efficacy, and safety of NAC therapy in COVID-19 (NCT04455243, NCT04374461, NCT04419025, NCT04458298).

Our study has several limitations. The case-control design cannot exclude a bias in the analysis of outcomes, and statistical analysis and interpretation of our study results are further limited by the small sample size.

Overall, our study demonstrated that NAC therapy provided a significant improvement in oxygenation parameters and reduction in CRP, NEWS2 scale, and length of hospitalization in hospitalized patients with COVID-19. These results need to be confirmed with further randomized prospective trials in a larger cohort.

#### Declaration of Competing Interest

The authors have no competing interest to declare.

#### Acknowledgments

The authors are grateful to Dr. Tatiana Gorbacheva and Dr. Inna Medvedeva for their help with the clinical management of our patients (University Clinical Hospital №4, Sechenov Moscow Medical University).

#### Funding

No funding was received for this work.

#### Ethical approval

The study was approved by the Medical Ethical Committee of Sechenov Moscow Medical University (protocol number 08–20/1).

#### CRediT authorship contribution statement

**Sergey Avdeev:** Conceptualization, Methodology, Validation, Resources, Data curation, Writing - original draft, Writing - review & editing, Supervision, Project administration. **Viliya Gaynitdinova:** Conceptualization, Investigation, Methodology, Validation, Data curation, Writing - original draft, Writing - review & editing. **Zamira Merzhoeva:** Investigation, Validation, Data curation, Writing - review & editing. **Zelimkhan Berikhanov:** Investigation, Validation, Data curation, Writing - review & editing.

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Accepted 5 July 2021

Available online 10 July 2021

<https://doi.org/10.1016/j.jinf.2021.07.003>

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#### Accuracy of rapid point-of-care antibody test in patients with suspected or confirmed COVID-19



Dear Editor,

We read with interest the article about use of lateral flow antigen detection tests to triage patients presenting to hospital to detect asymptomatic COVID-19<sup>1</sup>. In low-income countries, where most of the population is unvaccinated, and patients often present late, point-of-care rapid antibody tests could also contribute to diagnosis of COVID-19. Although a systematic review found that their overall sensitivity was low at 66% (95% CI 49.3–79.3)<sup>2</sup>, most of the studies were small and under-powered. Most published data is based on stored blood samples from hospitalised patients, with no indication of the duration of illness prior to sampling<sup>2–4</sup>. We report the real-world diagnostic accuracy of a rapid point-of-care antibody test compared to PCR and/or standard laboratory-based antibody tests, in patients with a history of symptoms consistent with COVID-19<sup>5</sup>.

Adult participants were recruited at Watford General Hospital, in two groups: (1) patients who had confirmed COVID-19 on PCR (from 5th March to 18th June 2020) and (2) hospital staff with history of clinically suspected COVID-19 (based on reported symptoms) who had a standard venous antibody test (from 1st May to 22nd July 2020), at least 7 days after onset of symptoms. There



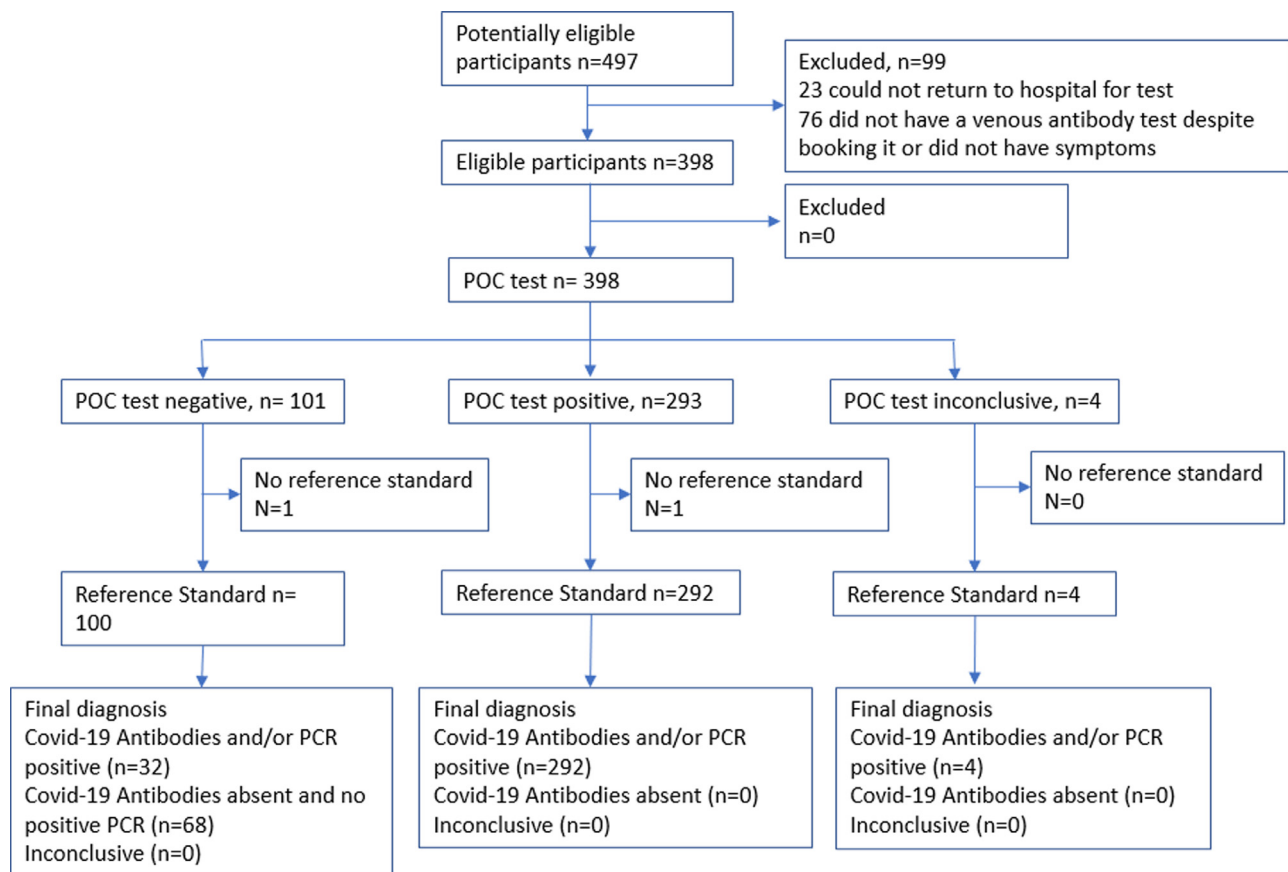


Fig. 1. Flowchart for analysis compared to composite reference standard.

was some overlap as many participants had both a PCR and a venous antibody test.

The index test was the rapid point-of-care (POC) IgM / IgG colloidal gold lateral flow immunoassay (LFIA) manufactured by Livzon (Zhuhai, Guangdong, China) which detects antibodies directed against the spike protein of SARS-CoV-2 from a drop of capillary blood. The test was read by two independent observers after 15 min.

We used a composite reference and evaluated the components of this individually. The first component was SARS-CoV-2 RT-PCR (using the available molecular technology during the study time: PHE laboratories, GeneXpert® system Xpert, Xpress SARS-CoV-2 and Source bioscience laboratory). The second reference test was the Elecsys Anti-SARS-CoV-2 assay, an ECLIA (Electro Chemi Luminescent Immuno Assay) manufactured by Roche Diagnostics GmbH which uses a recombinant protein representing the nucleocapsid (N) protein of SARS-CoV-2.

We recruited 398 participants (173 in the first group and 225 in the second— see Fig. 1); 52.8% had never been seen in hospital, whereas the others had been assessed in A&E and 23.6% were admitted. Regarding the reference test, 130 participants had only a PCR test, 124 had only a reference antibody test, and 144 had both. The median interval between the positive PCR test in group 1 and the POC antibody test was 60 days (IQR 35–91). Of 268 participants who had a reference venous blood test for antibodies to SARS-CoV-2, the test was positive in 190 (70.9%). The median interval between the POC test and the reference antibody test was 3 days (IQR –10 to 24 days). The median interval between onset of symptoms and the POC antibody test was 74 days (IQR 50–96, range 7–173 days). Only 11 participants had the POC test taken 7–14 days after onset of symptoms (of whom only 2 <10 days), 11

had it taken at 11–21 days, 19 at 22–34 days, and the remaining 312 at 35 days or more.

Of the 218 participants with a positive PCR test for COVID-19, the POC test was positive in 197 cases (sensitivity = 90.4%, 95% CI: 85.7% to 93.9%). Compared to the reference venous antibody test, the POC test had a sensitivity of 92.0% (95% CI 87.2–95.5) and specificity of 98.7% (95% CI 93.1–100). Our sensitivity analysis, excluding cases where the interval between the POC test and the venous antibody test was greater than 28 days, resulted in a sensitivity of 137 / 148 = 92.6% (95% CI 87.1%–96.2%) and specificity of 58/59 = 98.3% (95% CI 90.9%–100.0%).

Compared to the composite reference, the POC had an overall sensitivity of 90.1% (292/328, 95% CI 86.3–93.1) and specificity of 100% (68/68, 95% CI: 94.7% to 100% - see Table 1). In the subgroup of participants with presumed milder illness (who were never seen in hospital), the sensitivity was 84.4% (124/147, 95% CI 77.5% to 89.8%) whereas in patients admitted to hospital, the sensitivity was 97.8% (89/92, 95% CI 92.3% to 99.7%). In the subgroup where the POC test was conducted at least 20 days after onset of symptoms, sensitivity was 91.1% (288/316, 95% CI 87.4% to 94.0%). Numbers were too small to calculate diagnostic accuracy in the subgroups of patients in whom the POC test was taken at 7–14 days, 15–21 and 22–34 days after onset of symptoms.

As this was a “real-world” evaluation, some data were missing. In some cases there was a lengthy interval between symptoms and the point-of-care test, and between the point-of-care test and the reference venous antibody test. This was because the reference antibody test was only available from May 2020 and some participants first experienced symptoms in February and early March 2020. Another limitation is that the tests were only evaluated in one centre and from one batch. There have been reports of varia-

**Table 1**Accuracy of the POC antibody test compared to the composite reference standard (PCR and reference antibody,  $n = 396^*$ ).

Point-of-Care antibody test result		Composite reference		Sensitivity (95% CI)	Specificity (95% CI)	ROC	PPV	NPV
		Pos	Neg					
POC IgG	Positive	261	0	261/322 = 81.1% (76.3% to 85.2%)	68/68 = 100% (94.7% to 100%)	0.91 (0.88 to 0.93)	100% (98.6% to 100%)	52.7% (43.7% to 61.6%)
	Negative	61	68					
POC IgM	Positive	242	0	242/318 = 76.1% (71% to 80.7%)	68/68=100% (94.7% to 100%)	0.88 (0.86 to 0.90)	100% (98.5% to 100%)	47.2% (38.9% to 55.7%)
	Negative	76	68					
POC IgG and IgM Combined	Positive	292	0	292/324 = 90.1% (86.3% to 93.1%)	68/68=100% (94.7% to 100%)	0.95 (0.93 to 0.97)	100% (98.7% to 100%)	68% (57.9% to 77.0%)
	Negative	32	68					

\* POC result was inconclusive in 6 for the IgG, 10 for the IgM and 4 overall, excluded from this analysis.

tion in sensitivity between batches of rapid antibody tests<sup>6</sup> so good quality control will be essential.

In conclusion, the Livzon point-of-care antibody test had comparable sensitivity and specificity to the reference antibody test, so could be used to support decision-making about patients presenting with more than 10 days of symptoms of COVID-19. It may be more accurate than rapid antigen tests and PCR for patients with onset of symptoms at least 10 days previously, so it could be particularly useful in settings where access to PCR is limited, patients are unable to access a PCR within their first 10 days of illness, and most have not been vaccinated. The combination of PCR plus IgG and IgM testing in suitable patients has already been suggested to improve diagnostic accuracy<sup>7</sup>.

### Declaration of Competing Interest

All authors have completed the ICMJE uniform disclosure form at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf). Prof Wilkinson reports grants, personal fees and other from AstraZeneca, grants and personal fees from Synairgen, grants and personal fees from MyMHealth, grants from GSK, grants from Bergenbio and grants from UC, outside the submitted work. Prof Griffiths reports grants from Jannsen-Cilag, grants from AZ, grants from Novartis, grants from Astex, grants from Roche, grants from Heartflow, personal fees from CellDex, grants from BMS, grants from BionTech, outside the submitted work. All other authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

### Acknowledgements

We would like to thank Wendy O'Brien and Clare McDermott at the School of Primary Care and Catherine Simpson at Southampton Clinical Trials Unit at the University of Southampton who supported the trial management for this study. Rosie Bourke and Alex Newland Smith arranged follow up for the first group to have the POC tests. The POC tests were funded by The Medical Director's cardiology charity and by individuals donating to a "Just Giving" page. We received no commercial funding or free tests from the manufacturer. West Hertfordshire Hospitals NHS Trust supported clinical staff time. MLW's salary was funded by the National Institute of Health Research (NIHR), under grant CL-2016-26-005. Southampton CTU staff were supported by NIHR CTU Support Funding. The funders had no role in this research.

### Ethical approvals

This study was approved by the Faculty of Medicine Research Ethics Committee at the University of Southampton (reference

56480) and by the Wales Research Ethics Committee 4 (Wrexham, IRAS 283264, REC 20/WA/0148).

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Accepted 11 July 2021  
Available online 14 July 2021

<https://doi.org/10.1016/j.jinf.2021.07.006>

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## Is diffusion of SARS-CoV-2 variants of concern associated with different symptoms?



Dear Editor,

Although the spread of coronavirus disease 2019 (COVID-19) seems to be slowing down in many countries as consequence of widespread vaccination, the gradual accumulation of non-synonymous mutations within the genome of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has lead to appearance of variants of concern (VOCs), characterized by greater infectivity and/or potential of immune evasion especially from anti-SARS-CoV-2 neutralizing antibodies elicited after COVID-19 vaccination.<sup>1</sup> In a recent report, Fantini et al. have proposed an interesting health approach for anticipating COVID-19 outbreaks, based on an index of transmissibility (T-index), calculated from parameters of coronavirus binding to host cells.<sup>2</sup> Although this strategy seems indeed promising, previous evidence has been garnered that easier and more accessible tools such infodemiology, which relies on investigating the volume of Web searches for specific COVID-19 symptoms, may be effective in anticipating local COVID-19 epidemiological trends<sup>3,4</sup> provided that symptoms caused by the emerging VOCs remain constant over time.

With the aim of establishing whether COVID-19 symptoms may have changed over time after introduction of new SARS-CoV-2 VOCs, we conducted an electronic search in Google Trends (Google Inc. Mountain View, CA, US) for the most common self-reported symptoms in patients with SARS-CoV-2 infection, using the Italian search terms “tosse” (cough), “raffreddore” (cold), “mal di testa” (headache) and “febbre” (fever).<sup>5</sup> The country option was set to “Italy”, and the search period ranged between March 1, 2020 to present time (July 7, 2021). The data were downloaded as weekly Google Trends Score (GTS) for all these keywords, thus mirroring the cumulative volume of Google searches recorded for each specific term during the previous week. The GTS was then normalized (i.e., expressed as “ratio”) for the number of COVID-19 diagnoses recorded in Italy during the same week, as officially reported by the Italian National Institute of Health (Istituto Superiore di Sanità; ISS). The correlation between the GTS of the four symptoms was carried out with Spearman’s test. The statistical analysis was performed with analyze-it (analyze-it Software Ltd, Leeds, UK). The study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation. This analysis was based on electronic searches in the unrestricted, publicly available national repositories, and thereby no informed consent or Ethical Committee approvals were required.

The results of this analysis are shown in Fig. 1. As concerns the local diffusion of VOCs in Italy according to ISS data, variants bearing the D614G mutation have replaced the prototype Wuhan strain between April and May 2020, the alpha (B.1.1.7) variant has become largely prevalent (over 80% of COVID-19 cases) between February and March 2021, whilst the prevalence of the delta variant (B.1.617.2) has displayed a dramatic increase in May/June 2021. Irrespective of this evolving epidemiological trend, the normalized GTS of the four symptoms has followed a virtually overlap-

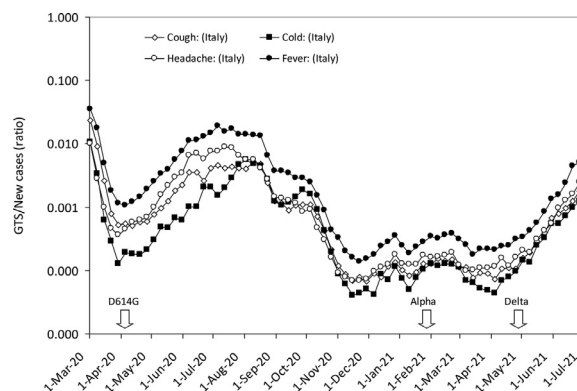


Fig. 1. Google searches for the most common symptoms of coronavirus disease 2019 (COVID-19) between March 1, 2020 and July 7, 2021. Results are expressed as weekly Google trends score (GTS) normalized for the number of new COVID-19 cases recorded during the same week.

ping trend over time, without exhibiting substantial differences (Fig. 1). The absence of a time-dependent variation in the prevalence of COVID-19 symptoms is reflected by highly significant Spearman’s correlations among all normalized GTS for all symptoms (all  $r \geq 0.94$  and  $p < 0.001$ ), as reported in Table 1.

The results of our analysis of normalized GTS for the most common searched COVID-19 symptoms recorded over time in Italy reveals that the progressive introduction of new strains and VOCs in the country was not seemingly associated with significant changes in the primary clinical picture, at least as reflected by the volume of Web searches and Internet interrogations for the most common self-reported COVID-19 symptoms. Therefore, the use of an infodemiology-based approach remains seemingly valuable for monitoring viral epidemiology and anticipating possible outbreaks.

### Funding

None declared.

### Declaration of Competing Interest

The authors have no relevant competing interest to disclose in relation to this work.

### Acknowledgments

The authors are thankful to the entire staff of the Pederzoli Hospital of Peschiera del Garda (Verona, Italy) for accepting to participate to this study.

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Table 1

Spearman’s correlation between volume of Google searches for the most common symptoms of coronavirus disease 2019 (COVID-19) between March 1, 2020 and July 7, 2021. Results are expressed as weekly Google trends score (GTS) normalized for the number of new COVID-19 cases recorded during the same week.

Symptoms	Cold	Headache	Fever
Cough	0.96 (95% CI, 0.94–0.98; $p < 0.001$ )	0.97 (0.95–0.98; $p < 0.001$ )	0.99 (0.99–0.99; $p < 0.001$ )
Cold	–	0.94 (0.90–0.98; $p < 0.001$ )	0.96 (0.93–0.97; $p < 0.001$ )
Headache	–	–	0.98 (0.97–0.99; $p < 0.001$ )

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Accepted 12 July 2021

Available online 15 July 2021

<https://doi.org/10.1016/j.jinf.2021.07.008>

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## Post-SARS-CoV-2 vaccination specific antibody decrease : Let's get the half-full glass perspective



Dear Editor,

We read with great interest the papers recently published in the Journal of Infection tackling the issue of post-vaccination antibody levels to SARS-CoV-2 spike protein.<sup>1,2</sup> Our attention was caught by the report from Favresse and Douxfils<sup>1</sup> showing that high dilution of the tested samples provided a more accurate appreciation of post-vaccination antibody levels. The same authors just published a related study where anti-SARS-CoV-2 serology was followed-up for up to 3 months.<sup>3</sup> Because they found a significant decrease in day 56 and day 90 antibody levels, they conveyed this notion in the title of their publication. This certainly catchy label can however, in this touchy context, be interpreted as bad news. Here we would like to re-interpret these data in a more positive way by emphasizing the high antibody titers detected in this study.

Indeed, numerous reports to date have used the Roche Elecsys® assay (Anti-SARS-CoV-2 S, Roche GMBH) and can be compared. Of note, the manufacturers' recommendations to use 12 or 20 µL of undiluted serum (depending on the analysis instrument) yield an upper positive threshold of 250 U/mL. This already shows an about 300-fold increase compared to the 0.8 U/mL detection threshold. This range allowed for instance to positively compare post-vaccination anti-SARS-CoV-2 antibody levels between allogeneic hematopoietic stem cells recipients and healthcare workers, some reaching this upper threshold after just one injection of BNT612b2 (Pfizer BioNTech, Mainz, Germany).<sup>4</sup> Other studies have however previously indicated that anti-spike antibody levels

could be high above this upper threshold of 250 U/mL. Indeed, in a comparative study of antibody responses of convalescents, vaccinated healthcare personnel and control samples, Suhandynata et al.<sup>5</sup> used a 1:10 dilution and thus raised the upper threshold to 2 500 U/mL. These authors reported a median value above 2500 U/mL for 100% of vaccinated individuals after a booster shot (range 1 009 -> 2 500), thus much above the 250 U/mL threshold of the undiluted serum assay. Similar results were reported by Mueller in assay comparisons,<sup>6</sup> with levels increasing in the course of a 5-week follow-up of vaccinated individuals.

Longer follow-up studies so far have mostly reported sustained antibody levels to the spike protein of SARS-CoV-2. In previously seropositive vaccinated healthcare workers, Tré-Hardy et al.<sup>2</sup> described stable levels over 400 U/mL at 3 months. A comparable result was observed in convalescent patients by Gerhards et al.<sup>7</sup> showing sustained levels up to 1 000 U/mL with little variation over 3 months. What Favresse and Douxfils<sup>1,2</sup> tell us is that antibody levels in the range of 25 ,000 or above are reached after vaccination, i.e. a 30 ,000-fold increase compared to the negativity threshold < 0.8 U/mL. Close examination of their results discloses that, at three months, these titers still exceeded most previously reported upper thresholds. They ranged between 500 and 25 ,000 U/mL in individuals seropositive before the two doses of vaccines administered and between 500 and ~5000 U/mL in subjects seronegative before vaccination. The respective means at day 90 were around 10 ,000 and 1 250 U/mL respectively, thus 40 times and 5 times above the 250 maximal positive threshold of the standard assay and 4 and 3 logs above the detection level. The authors also report wisely on estimated times of possible seronegativation of respectively 1 184 and 554 days for these two groups of patients, pending no other antigenic stimulation has occurred.

The observation by Favresse et al.<sup>2</sup> is typically that of the normal kinetics of a strong post-vaccination humoral response. From a fundamental point of view, it has long been demonstrated that immune responses rely on consecutive cycles of clonal proliferation followed by clonal contraction leaving a progressively increasing pool of memory cells.<sup>8</sup> The latter are then liable to provide a quicker and more important anamnestic or secondary response.

The higher and more sustained response of previously seropositive individuals in the study by Tré-Hardy et al.<sup>2</sup> indicates that the vaccination indeed amplified an already settled immune response. These results can be compared to the smaller study of Doria Rose et al.<sup>9</sup> with different vaccine and assays, yet following patients for 6 months. In this cohort of 35 subjects, individual antibody kinetics clearly showed the booster effect of the second dose of vaccine. Indeed, an initial increase of antibody levels at day 15 was followed by a decrease of the primary immune response by day 29, just before the second injection, especially in neutralizing antibodies. Antibody titers then shot up.

Tré-Hardy et al.<sup>2</sup> pointedly mention that cellular responses of the T-cell compartment were not measured in their study but are likely to follow the same kinetics after infection and/or vaccination. Since cellular responses are even more efficient at eradicating viral infections than humoral responses, the results of this team are in fact quite encouraging.

It should also be mentioned that seropositive individuals are liable to even increase their protection level as long as the virus is still circulating. Indeed, a recent study by Turner et al.<sup>10</sup> has shown that at least 12 weeks after a boost injection of BNT612b2, S protein-binding germinal center B cells were still identified in draining axillary lymph nodes. These results also offer optimism that humoral responses to vaccination will be long-lasting. Close follow-up should thus be continued to assess the ongoing kinetics of immune responses to SARS-CoV-2 in the now well-immunized vaccinated population.



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Accepted 25 July 2021

Available online 29 July 2021

<https://doi.org/10.1016/j.jinf.2021.07.027>

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## SARS-CoV-2 viral dynamics in infections with Alpha and Beta variants of concern in the French community

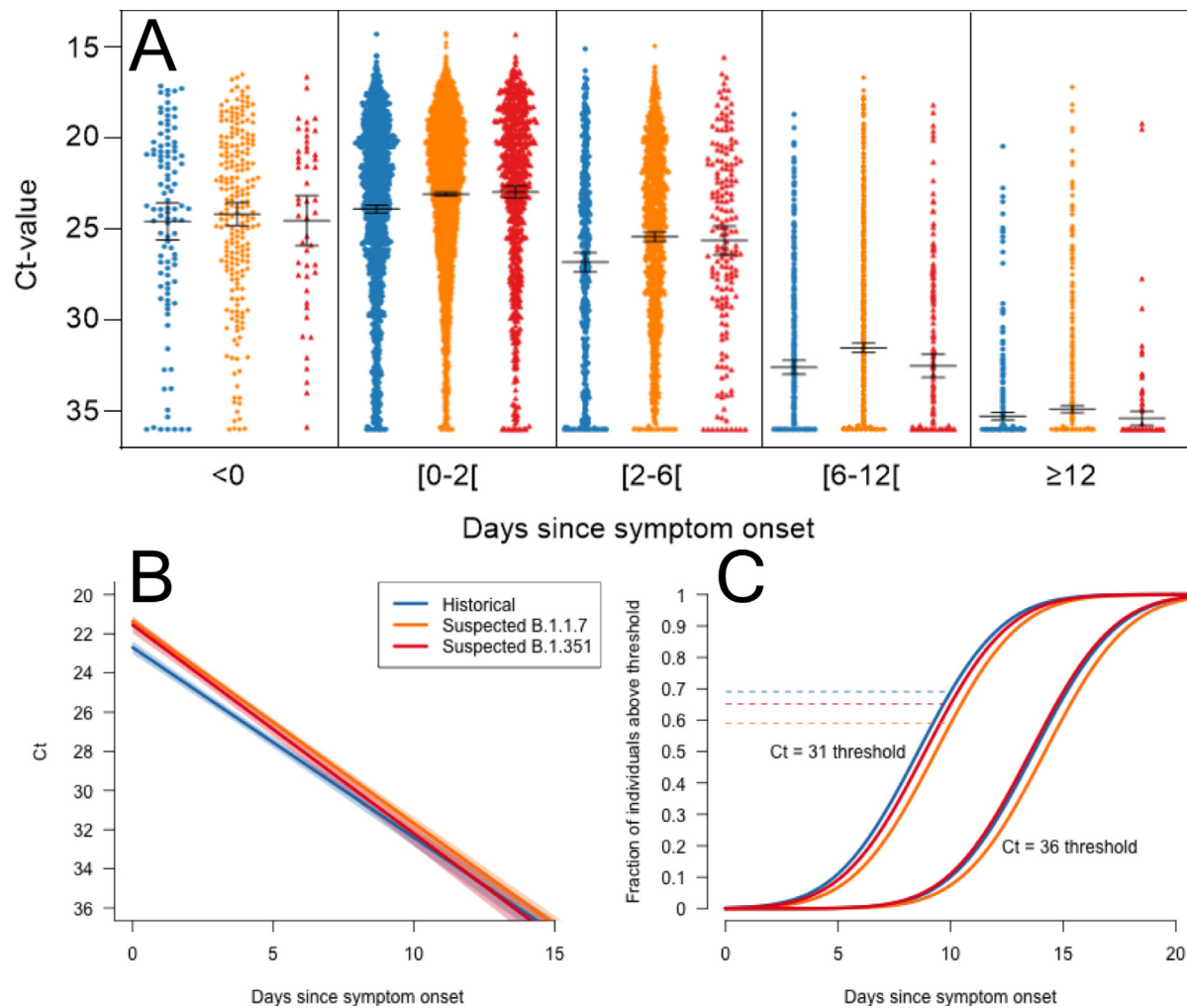


Dear Editor,

We read with interest the article by Tang et al. on the multiple introductions of variant B.1.351 into the UK.<sup>1</sup> As SARS-CoV-2 variants of concern rapidly spread internationally, it is important to know if increased transmissibility<sup>2–4</sup> and virulence<sup>5</sup> is associated with higher viral load to understand pathogenesis and

**Table 1**  
Overview of the test data: number of tests, and percentages of each sex, age category, time from symptom onset category, and number of tests per individual for historical strains, B.1.351 and B.1.1.7 variants.

Strain	N	Sex		Age category							Time from symptom onset (days)							Number of tests			
		Female	Male	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	≥ 90	< 0	[0,2[	[2, 6[	[6, 12[	≥ 12	1	2	≥ 3
Historical	3272	54.3	45.7	1.65	9.32	19.5	21.2	20.2	15.8	7.21	3.51	1.41	0.183	3.18	55.9	13.4	14.3	13.2	52.7	30	17.4
B.1.351	1366	54.9	45.1	1.61	11.6	20	23.2	20.7	13.9	5.56	2.49	0.586	0.366	3.59	59.2	12.6	14.6	10.1	54.4	32.2	13.4
B.1.1.7	11,496	55.3	44.7	1.85	11.4	19.6	21.5	20.6	14.6	6.6	3.01	0.6	0.27	1.87	66.8	12	11.6	7.79	67.5	24.5	7.97



**Fig. 1.** Ct value as a function of time since symptom onset for historical variants, suspected B.1.351 and suspected B.1.1.7. A. Ct value as a function of time since symptom onset categories in the data. B. Prediction of the linear model, with confidence intervals as shaded regions. C. Distribution of the time from symptom onset to thresholds  $Ct = 31$  and  $Ct = 36$  for each variant, as predicted by the linear model. This is the cumulative distribution function of the normal distribution whose mean is the predicted mean time to each threshold for each variant, and standard deviation the standard deviation of the random effect representing the inter-individual variability ( $sd = 2.9$ ). The dashed horizontal lines show the fraction of individuals no longer shedding infectious virus 10 days after symptom onset, the recommended duration of self-isolation in April 2021 in France.

adapt if necessary the duration of isolation of infected individuals. When comparing viral load across variants, controlling for time since symptoms is key, since individuals infected by a variant with faster epidemiological growth will on average be captured earlier in infection<sup>6</sup>. Studies controlling for time since symptoms, and with longitudinal follow-up of individuals are lacking. We used data from 871,604 PCR tests conducted by a large private clinical laboratory in the community in the Ile-de-France region of France from 1st January to 24th March 2021 (17% of all tests in this period). We compared the within-host dynamics of viral load in symptomatic individuals infected by suspected variants of concern B.1.1.7 (Alpha, VOC-202012/01 or 501Y.V1, first detected in England in September 2021) and B.1.351 (Beta, 501Y.V2) to previous strains.

Starting from all tests, negative and positive ( $N = 871,604$ ), we retained 16,134 tests conducted on 12,858 symptomatic individuals for the main analysis (Supplementary Fig. 1). Most individuals had one test ( $N = 10,225$ ), 2121 individuals had two tests and the rest ( $N = 512$  individuals) had three or more tests (Table 1). We conducted the main analysis on the Ct value combining the two Ct values obtained with two primers targeting the RNA-dependent RNA polymerase (RdRp) gene. Variants were detected using two PCRs targeted at the spike deletion 69–70 and at the spike substitution N501Y (IDTM SARS-CoV-2/UK/SA Variant Triplex). Symptom

onset dates were self-reported. We used a censored mixed-effects linear regression<sup>7</sup> describing Ct value as a function of time since symptom onset (continuous variable), variant (historical strains, suspected B.1.1.7, suspected B.1.351), age category (10 categories, 0–9, 10–19, ..., 80–89, 90+ years old), and interactions between time and variant, and time and age (excluding the non-significant interaction between variant and age,  $p = 0.31$ ).

The predicted viral load at symptom onset was inferred to be 22.7 Ct on average (95% confidence interval, CI, [22.4–23.0]) for historical strains (Supplementary Table 1). The inter-individual standard deviation in viral load at symptom onset was 2.9, meaning that 95% of symptomatic individuals had a viral load at symptom onset between 28.4 and 17.0. The viral load declined on average at a rate of +0.97 Ct per day [0.93 – 1.0]. The viral load at symptom onset was higher in B.1.1.7 and B.1.351 variants than in historical variants, with a Ct value  $-1.33$  [ $-1.59, -1.07$ ] and  $-1.15$  [ $-1.57, -0.697$ ] lower than historical strains for B.1.1.7 and B.1.351 respectively ( $p < 10^{-16}$ ). The viral load of the two variants declined slightly faster than that of the historical strains with an additional decline rate of +0.06 [0.015; 0.10] and +0.095 [0.018; 0.16] per day for B.1.1.7 and B.1.351, respectively ( $p = 0.0004$ ). The duration of shedding was longer for individuals infected by variants: the mean time to a Ct of 31, the limit above which the individ-

ual is no longer infectious, was 8.6, 9.3 and 8.9 days for historical strains, B.1.1.7 and B.1.351 (Fig. 1B). The mean time to a Ct of 36, the limit above which the virus is fully cleared, was 13.7, 14.2 and 13.6 days for historical strains, B.1.1.7 and B.1.351. There is substantial inter-individual variability around these mean values (Fig. 1C).

We based the main analysis on the Ct value of the PCR targeting the RdRp gene, but results were very consistent when analyzing the Ct value of the nucleocapsid gene (N), with an even stronger effect of the variants on viral load at symptom onset (effect sizes  $-1.81 [-2.32; -1.32]$  and  $-2.11 [-2.38; -1.81]$  for B.1.1.7 and B.1.351) (Supplementary Table 2, Supplementary Fig. 2). We also ran the same analysis on the more complete dataset where the time from symptom onset was not necessarily known (41,489 tests representing 33,391 individuals), and considered the time since first positive test instead of the self-reported time since symptom onset. Results were very consistent with those of the main analysis (Supplementary Table 3), and in addition, individuals declaring symptoms at all their tests had a larger viral load than asymptomatic individuals ( $-0.95$ , CI  $[-1.1, -0.83]$ ).

Both B.1.1.7 and B.1.351 variants conferred a higher viral load at symptom onset at all ages. The main strengths of our study are the control for time since symptoms, which improves the comparison between historical strains and variants (removing potential confounders due to different epidemiological dynamics of each variant<sup>6</sup>), and the large number of tests for both B.1.1.7 and B.1.351 variants. A limitation is that variant assignment is based on PCR screening; the suspected B.1.1.7 and B.1.351 were not confirmed by whole genome sequencing. In Ile-de-France, whole-genome sequencing of a random subset of 609 cases on March 2nd 2021 indicated that the vast majority of viruses with substitution N501Y and spike deletion 69–70 are B.1.1.7.<sup>8</sup> The prevalence of B.1.351 in France was 6.5%, and the prevalence of P.1 (first detected in Japan in travelers from Brazil) was 0.3%,<sup>8</sup> implying 96% of the suspected B.1.351 are true B.1.351 if only clades B.1.351 and P.1 carried N501Y without del69-70. The evidence presented here from thousands of PCR tests broadly agrees with results from densely sampled trajectories of seven individuals infected with B.1.1.7, showing that individuals infected with B.1.1.7 had a  $-1.2$  Ct higher viral load than those infected with the historical strains, very similar to our estimate of  $-1.33$ .<sup>9</sup> Higher viral load could partly explain the greater pathogenicity of B.1.1.7. It could also explain the selective advantage of B.1.1.7 through a transmission advantage.<sup>10</sup> Individuals infected by suspected B.1.1.7 and B.1.351 excreted virus (Ct < 31) for only slightly longer than those infected by historical strains. Larger datasets with systematic PCR tests and capturing the pre-symptomatic phase will improve our understanding of infection by SARS-CoV-2 variants of concern and potential epidemiological consequences.

## Declaration of Competing Interest

JG has worked as consultant for ROCHE Company.

## CRediT authorship contribution statement

Design: FD, FB, with feedback from all authors Data collection: GC, MB, JA, JMG Data cleaning: FD, FB Analyses: FB Wrote the letter: FB with feedback from all authors.

## Acknowledgments

FB was funded by a Momentum grant from the centre National de la Recherche Scientifique. FD was funded by grant ANR-19-CE45-0009-01 from Agence Nationale de la Recherche.

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Accepted 24 July 2021  
Available online 28 July 2021

<https://doi.org/10.1016/j.jinf.2021.07.031>

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## Immune responses and reactogenicity after ChAdOx1 in individuals with past SARS-CoV-2 infection and those without



Dear Editor,

We have read the article by E. Sansone, et al.<sup>1</sup> with great interest. The authors found the significant protective effect of BNT162b2 vaccination against SARS-CoV-2 infection and symptom development after SARS-CoV-2 infection among healthcare workers (HCWs). However, individuals with past natural infection with SARS-CoV-2 and those without may have different immune responses to COVID-19 vaccination. Some studies have quickly compared the immune responses to the mRNA-based vaccines between individuals with past infection with SARS-CoV-2 and those without<sup>2–5</sup>; but their kinetics in the adenovirus-vector-based vaccines remain unknown. In this study, we evaluated the immunogenicity and reactogenicity of the first dose of the adenovirus-vector-based ChAdOx1 nCoV-19 vaccine in HCWs with or without past infection with SARS-CoV-2.

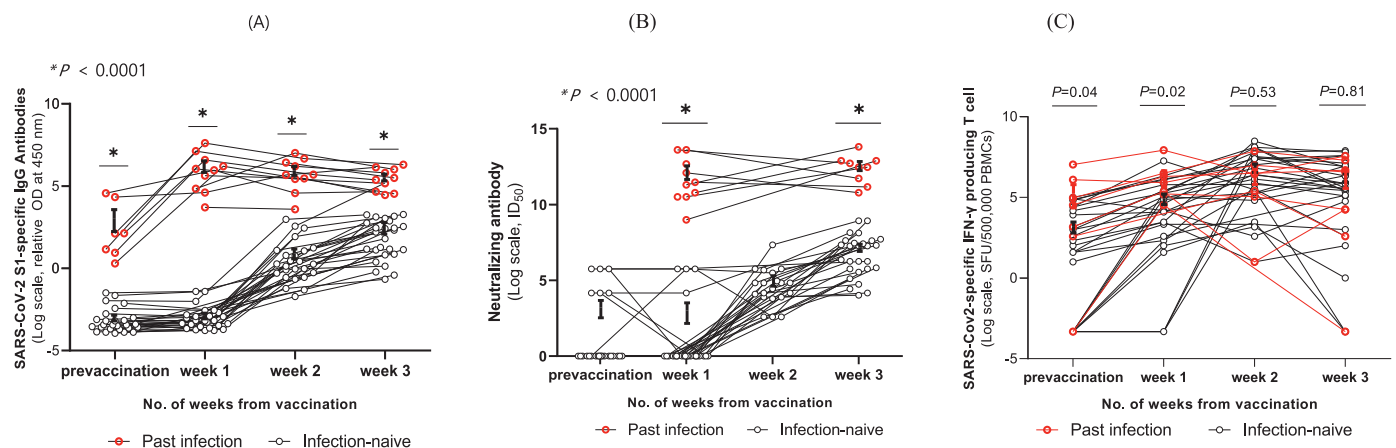
This study enrolled HCWs who received the 1st dose of ChAdOx1 vaccine between March 5 and March 26, 2021, at Asan Medical Center, a 2700-bed tertiary care teaching hospital in Seoul, South Korea. The study was reviewed and approved by the Institutional Review Board of Asan Medical Center (IRB No. 2021-0170). Adverse reactions were evaluated through self-reported questionnaires (Supplementary material). SARS-CoV-2-S1 specific IgG antibody, neutralizing antibody, and T cell responses were measured by ELISA, microneutralization assay by using SARS-CoV-2 ( $\beta$ CoV/Korea/KCDC/2020 NCCP43326), and ELISPOT assay using SARS-CoV-2 spike-overlapping peptides (Miltenyi Biotec, Bergisch Gladbach, Germany), respectively.

A total of 38 HCWs were enrolled in this study. Of them, 11 (29%) had past infection with SARS-CoV-2 confirmed by real-time reverse transcriptase-polymerase chain reaction with nasopharyngeal samples, and the rest of the participants ( $n = 27$ ; 71%) were infection-naïve. The baseline characteristics of the participants are shown in Supplemental Table 1. There was no significant difference in the age and sex distribution between those with past infection and infection-naïve individuals ( $P = 0.52$  and  $P = 0.48$ , respectively).

The baseline SARS-CoV-2-S1 specific IgG titers were positive in all participants with past infection and negative in all infection-naïve participants. The mean values of SARS-CoV-2-specific IgG antibody titer of participants with past infection were significantly higher than those of infection-naïve participants at baseline (8.29 vs. 0.12) and at week 1 (74.98 vs. 0.14), week 2 (61.73 vs. 1.88), and week 3 (46.34 vs. 5.56) after vaccination (all  $P < 0.0001$ ) (Fig. 1A). The neutralizing antibody titer was significantly higher in the past infection group compared with the infection-naïve group at week 1 and week 3 as well (mean  $\pm$  SEM,  $4644 \pm 1416$  vs.  $7.2 \pm 3.6$  and  $6108 \pm 1255$  vs.  $144.6 \pm 24.1$ , respectively, both  $P < 0.0001$ ) (Fig. 1B). IFN-gamma-producing T cell responses were higher in the past infection group at baseline and 1 week after vaccination ( $P = 0.04$  and  $0.02$ , respectively), but were comparable at 2 ( $P = 0.53$ ) and 3 weeks ( $P = 0.81$ ) after vaccination (Fig. 1C).

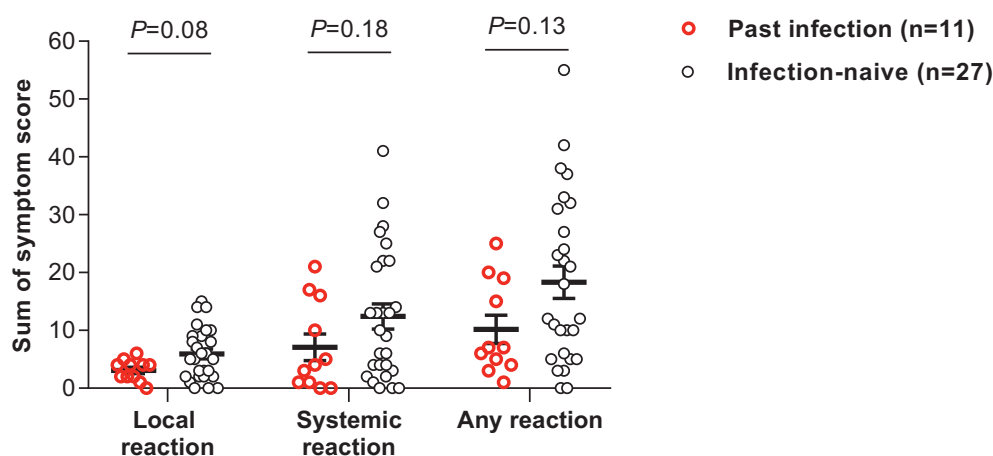
Reactogenicity in terms of adverse reactions during the 7-day reporting period after vaccination was analyzed in the two groups. Local and systemic reactogenicity was similar between individuals with past SARS-CoV-2 infection and those without (Fig. 2). The severities of adverse events in the two groups according to each symptom after vaccination are shown in Supplemental Table 2 and Supplemental Figs. 1 and 2.

Consistent with the previous studies on the immunogenicity of the BNT162b2 vaccine between those with past infection with SARS-CoV-2 and those without<sup>2–4</sup>, we found that the immunogenicity of the ChAdOx1 nCoV-19 vaccine was stronger and more rapid in those with past infection compared with those without. Our study has two unique findings. First, our detailed kinetic data on the immunogenicity of the ChAdOx1 nCoV-19 vaccine revealed that the antibody response peaked at 1 week after vaccination in individuals with past infection, while the antibody response gradually increased until 3 weeks after vaccination in infection-naïve individuals. Moreover, whereas the SARS-CoV-2-specific T cell responses were higher in the past infection group at baseline and 1 week after vaccination but were comparable at 2 and 3 weeks after vaccination. In contrast, a previous study on the BNT162b2 vaccine showed that the SARS-CoV-2-specific T cell responses at 21–



**Fig. 1.** Detailed kinetics of immune responses and reactogenicity after a single dose of ChAdOx1 nCoV-19 vaccine in infection-naïve individuals and those with past SARS-CoV-2 infection (A) Humoral immune response measured by SARS-CoV-2 S1-specific IgG antibodies. (B) Neutralizing antibody measured by microneutralization assay. (C) Cell-mediated immune response measured by IFN-gamma-producing T cells from isolated PBMCs. Error bars denote the standard error of the mean values. Mann-Whitney U test was used for the statistical analysis between past infection and infection-naïve.





**Fig. 2.** Reactogenicity during the 7-day period after the 1st dose of the ChAdOx1 nCoV-19 vaccine in infection-naïve individuals and those with past infection with SARS-CoV-2. Horizontal bars denote the mean values. Error bars denote the standard error of the mean values. Mann-Whitney U test was used for the statistical analysis between past infection and infection-naïve.

25 days after vaccination were higher in individuals with past infection than in infection-naïve individuals.<sup>3</sup> Such difference in the kinetics of T cell response and antibody responses after different types of COVID-19 vaccines provides further insight into the immune response after COVID-19 vaccination according to the history of past natural infection with SARS-CoV-2. For example, the rapid increase of virus-specific T cell response by vaccination in the past infection group likely reflects the presence of memory T cells induced by previous natural infection with SARS-CoV-2. However, in contrast to the wide range of viral epitope stimulation by natural SARS-CoV-2 infection, the ChAdOx1 vaccine only provides spike-derived epitopes; therefore, it may not be able to fully boost the pre-existing virus-specific memory T cells. Moreover, the strong pre-existing T cell response may restrict the efficient supply of antigens, thus resulting in a curtailed expansion of memory T cells. Second, whereas the antibody responses were significantly higher in the past infection group, the reactogenicity after ChAdOx1 vaccine was similar between individuals with past SARS-CoV-2 infection and those without. These findings are in contrast with a previous study on mRNA-based COVID-19 vaccines in which the frequency of systemic reactions was higher in those with previous natural infection than in those without.<sup>4</sup> The stronger immediate reactogenicity in the young population after the ChAdOx1 vaccine than after the BNT162b2 vaccine<sup>6</sup> might partially explain the differences between our data and those from mRNA-based vaccines. Alternatively, the unknown immunologic mechanism that is associated with less adverse reactions after the second dose of the ChAdOx1 vaccine than those after its first dose<sup>6</sup> might explain our observation.

In conclusion, those with past SARS-CoV-2 infection had similar reactogenicity and stronger antibody responses compared with infection-naïve individuals after receiving the ChAdOx1 vaccine. T cell responses were not significantly different between the two groups after 2 weeks after vaccination.

#### Declaration of Competing Interest

None.

#### Funding

This study was supported by a grant (no. [HW20C2062](#)) from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, South Korea and by the National Research

Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. [2021M3A9H5079531](#)).

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jinf.2021.07.032](https://doi.org/10.1016/j.jinf.2021.07.032).

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Accepted 24 July 2021

Available online xxx

<https://doi.org/10.1016/j.jinf.2021.07.032>

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## Decreased neutralization of the Eta SARS-CoV-2 variant by sera of previously infected and uninfected vaccinated individuals

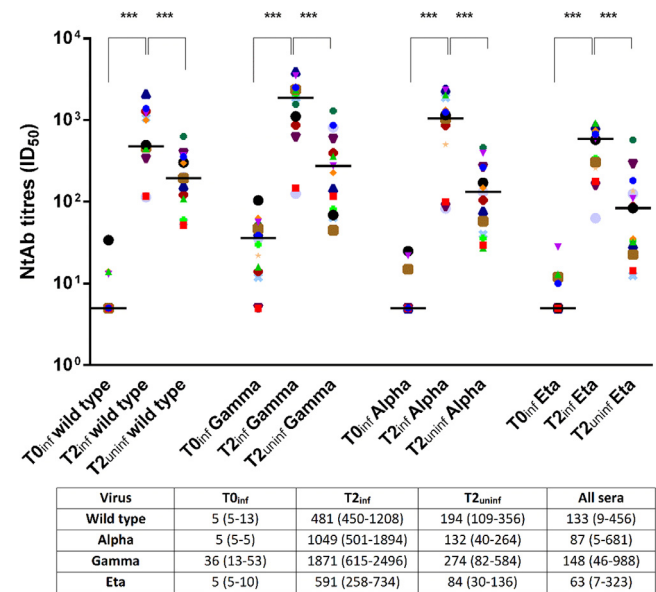


Dear Editor,

In this Journal, the emergence of novel variants with potential to escape vaccine-induced immunity has received commentary.<sup>1</sup>

The emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) variants of concern (VOC) and variants of interest (VOI) are challenging the management of the evolving pandemic across countries. The VOI labelled as Eta (WHO Classification),<sup>2</sup> combines relevant spike mutations detected in several VOC, such as the same 3 deletions of the Alpha lineage (69del, 70del, 144del), the E484K mutation found in the Gamma and Beta lineages as well as in some Alpha isolates and the ubiquitous D614G. In addition, three mutations (A67V, Q677H and F888L) are unique to Eta variant and it is currently unknown whether they favor escape from natural or vaccine induced immunity to the wild type lineage (B.1), as shown for other variants.<sup>3</sup> To test this hypothesis, we measured the serum neutralizing antibody (NtAb) response to Eta variant, as well as to other viral variants, in a cohort of health care workers (HCWs) including both previously infected ( $n=15$ ) and uninfected individuals ( $n=15$ ) vaccinated with two doses of the BNT162b2 COVID-19 mRNA vaccine. The study was approved by the Ethics Committee of the University of Milan (protocol n. 23/21) and conducted in compliance with Good Clinical Practice guidelines and the Declaration of Helsinki. The previously infected group was tested at baseline ( $T0_{inf}$ ) and 17±6 days after receiving the second vaccine dose ( $T2_{inf}$ ); the uninfected HCWs were tested 18±4 days after the second dose vaccination ( $T2_{uninf}$ ). The infected group had median age [IQR] of 38 (31–52) years, included 8 females and was infected during the first wave of the pandemic. The uninfected group had a median age of 38 (29–59) years with 11 females.

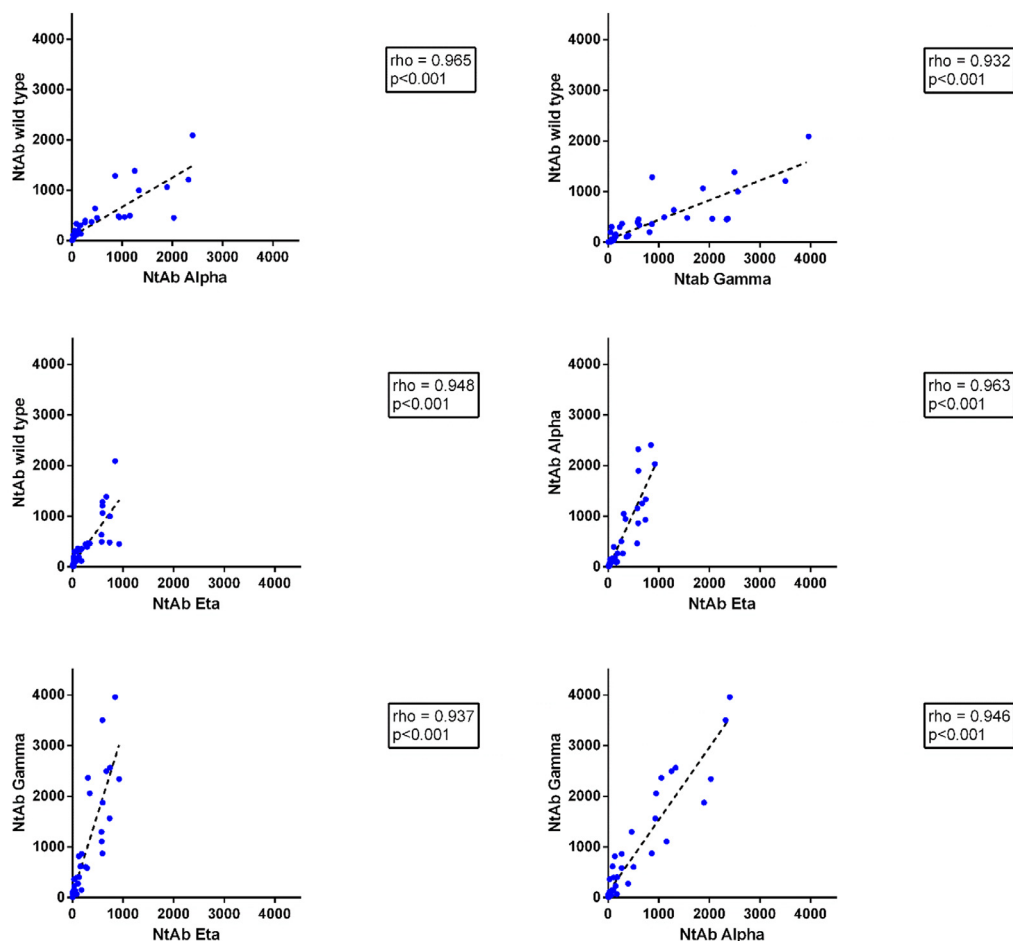
NtAb titers were determined by a microneutralization live virus assay performed in VERO E6 cells using the quantification of cell viability as readout system, as previously described.<sup>4</sup> NtAb titers were expressed as median (IQR) and were defined as the reciprocal



**Fig. 1.** Neutralizing antibody (NtAb) titres to four SARS-CoV-2 variants (wild type, Gamma, Alpha and Eta) in 15 previously infected subjects at baseline ( $T0_{inf}$ ) and after two doses of vaccine ( $T2_{inf}$ ) and in 15 uninfected subjects after two doses of vaccine ( $T2_{uninf}$ ). Asterisks indicate significance levels: \*\*\*,  $p < 0.001$ . Median (IQR) titres of neutralizing antibody are reported below.

value of the sample dilution that showed a 50% protection of virus-induced cytopathic effect ( $ID_{50}$ ). Sera with  $ID_{50}$  titres  $\geq 10$  were defined as SARS-CoV-2 neutralizing, while sera with  $ID_{50} < 10$  were defined as negative and scored as 5 for statistical analysis. Fifteen, 14 and 11 individuals at  $T0_{inf}$ ,  $T2_{inf}$  and  $T2_{uninf}$ , respectively, had also a quantitative anti-spike protein Ab determination, performed by the SARS-CoV-2 IgG II Quant assay (Abbott). The viral isolates used in the microneutralization live virus assay were sequenced by NGS and the full-length SARS-CoV-2 genome was submitted to GISAID (<http://gisaid.org/>) to assign the right variant (Accession numbers: EPI\_ISL\_2,472,896, EPI\_ISL\_1,085,167, EPI\_ISL\_2,472,918 and EPI\_ISL\_2,472,916 for the wild type, Alpha, Gamma and Eta variants, respectively). Statistical analyses were performed using IBM SPSS Statistics, version 20. The non-parametric Friedman test and Wilcoxon Signed Rank Sum test was used to analyze changes in paired data. The non-parametric Mann-Whitney test was used to compare unpaired data. Spearman analysis was used to measure the correlation between NtAb titres against the different variants.

In previously infected HCWs, NtAb titres to all viral variants significantly increased after vaccination (mean  $T2_{inf}/T0_{inf}$  ratio  $119 \pm 66$ ;  $p < 0.001$ ). Notably, 2 to 12 subjects, depending on the reference virus, were negative at  $T0_{inf}$  but all of them seroconverted following vaccination. As expected, the NtAb titer after vaccination was higher in the previously infected compared with the uninfected group (mean  $T2_{inf}/T2_{uninf}$  ratio  $6 \pm 2$ ;  $p < 0.001$  (Fig. 1). Overall, median NtAb titres to the Eta variant (63 [7–323]  $ID_{50}$ ) correlated well with those to the wild type (133 [9–456]), Gamma (148 [46–988]) and Alpha (87 [5–681]) ( $p < 0.001$  for all comparisons) and high correlation was indeed observed between NtAb titres to any pair of virus variants (Fig. 2). Of note, NtAb titres to Eta variant were significantly lower with respect to those obtained for each variant ( $p < 0.001$ ). Anti-spike protein antibodies, as measured by enzyme immunoassay, were highly correlated with NtAb titres to B.1 ( $\rho = 0.934$ ), P.1 ( $\rho = 0.914$ ), B.1.1.7 ( $\rho = 0.913$ ) and B.1.525 ( $\rho = 0.918$ ) viruses ( $p < 0.001$  for all comparisons). Also, a significant increase was observed when comparing the anti-spike Ab median titres at  $T2_{inf}$  and at  $T0_{inf}$  (27,763 [18,282–46,108] vs. 1.7 [0.5–4.4];  $p = 0.001$ ).



**Fig. 2.** Spearman correlation between NtAb titres to each pair of the SARS-CoV-2 variants used in the study. Data were cumulated for all sera tested at T0<sub>inf</sub>, T2<sub>inf</sub> and T2<sub>uninf</sub>.

Overall, in our small cohort of previously infected or uninfected vaccinated-HCWs it appears that cross-neutralization among different viral variants remains substantial, following natural or artificial immunization with the wild type lineage. However, neutralization of Eta variant is significantly reduced with respect to other variants. Indeed, NtAb titres could be ranked with the definite order Gamma>wild type=Alpha>Eta. In vitro correlates of protection against the Eta variant has been investigated in uninfected vaccinated individuals only in two different works delivering inconsistent results. Indeed, Liu et al.<sup>5</sup> observed a modest reduction, while Zani et al.<sup>6</sup> reported an increase in Eta variant NtAb titres with respect to the wild type variant. Of note, NtAb studies published so far have used different combination of strategies (e.g., live virus vs. pseudoparticles), viral variants, cell lines and readouts, in the absence of standardized methods and reference viral strains and neutralizing sera.<sup>7–10</sup> For example, the full-length sequencing of the isolates used in the assay should be always reported and submitted to public repositories. Most importantly, while NtAb studies certainly provide a solid basis to infer cross-protection among vaccines and virus variants, the in vivo correlates of in vitro data remain to be established and must be defined through accurate and continuous monitoring of vaccine induced reduction of morbidity and mortality in the context of molecular surveillance of SARS-CoV-2 lineages.

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Accepted 1 August 2021

Available online 8 August 2021

<https://doi.org/10.1016/j.jinf.2021.08.005>

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## Predominance of delta variant among the COVID-19 vaccinated and unvaccinated individuals, India, May 2021



Dear Sir,

In this Journal, Sansone and colleagues measured effectiveness of BNT162b2 vaccine against the B.1.1.7 variant of SARS-CoV-2 among healthcare workers.<sup>1</sup> India experienced a severe second wave of SARS-CoV-2 infections during the months of April and May 2021. COVID-19 vaccination with BBV152 vaccine (Covaxin; Bharat Biotech) and ChAdOx1 nCoV-19 (Covishield, Serum Institute of India) was started in the country in January 2021, targeting healthcare workers in the first phase and later expanded to include adult population groups.<sup>2</sup> Breakthrough infections following vaccination have been reported in India.<sup>3,4</sup> Breakthrough infections could be due to emergence of newer mutant strains capable of escaping the host immune response.<sup>5</sup> During March 2021, sequencing of more than 10,000 RT-PCR positive samples indicated circulation of viruses of B.1.1.7 (Alpha/UK variant), B.1.351 (beta/South African), P.1 (Gamma/Brazilian) lineage and Kappa/Delta Indian variants (B.1.617).<sup>6</sup> During the course of second wave in India, Delta B.1.617.2 variant emerged as the major sub-lineage among variants that also included B.1.617.1, B.1.617.3 and B.1.1.7.<sup>3</sup> Chennai was one of the worst affected cities in the second wave of COVID-19 in India, with nearly 6000 cases reported daily during the first three weeks of May 2021, despite a high seroprevalence of around 45% estimated during October–November 2020.<sup>7</sup> Chennai has reported more than 520,000 COVID-19 cumulative cases and 7793 deaths since the beginning of the pandemic and vaccinated around 2 million people with at least one dose of COVID-19 vaccine.<sup>8</sup> In this context, we described the prevalence of variants of concern

(VOCs) among vaccinated and unvaccinated COVID-19 positive individuals in Chennai.

Newly diagnosed COVID-19 patients are triaged in screening centers established by the Greater Chennai Corporation. We selected three of the ten such triaging centers for the study with one center each from the northern, central and southern parts of Chennai to ensure representativeness. We consecutively enrolled consenting COVID-19 positive individuals visiting these centers who had taken at least one dose of COVID-19 vaccine 14 days prior to confirmation of the diagnosis. We also recruited unvaccinated COVID-19 cases attending the triage centers. We collected demographic details, clinical history, comorbidities, previous COVID-19 history and date of vaccination. Nasal and oro-pharyngeal (N/OP) swabs and blood samples were collected from the study participants. We tested the N/OP swab samples for the detection of E and RdRP gene using Real-time RT-PCR and only those with Ct < 30 were included for preparation of RNA libraries Illumina Covidseq protocol (Illumina Inc, USA).<sup>9</sup> Amplified and purified libraries were quantified using KAPA Library Quantification Kit (Kapa Biosystems, Roche Diagnostics Corporation, USA) and loaded on NextSeq 500/550 system after normalization. Bcl files generated were analysed after conversion to fastq using CLC genomics workbench version 11.0 (CLC, QIAGEN, Germany). Reference-based mapping was performed to retrieve the sequence of the SARS-CoV-2 and a phylogenetic tree was generated using MEGA software version 7. Blood samples were tested for SARS-CoV-2 IgG antibodies against S1-RBD (Siemens, Munich, Germany).

The participants were followed up telephonically after four weeks to collect information about their symptoms, hospitalisation and treatment details, and clinical outcome. Patients with SpO<sub>2</sub> < 94%, dyspnoea and requiring supplemental oxygen during hospitalization were considered as having moderate/severe illness and remaining as mild illness. Categorical variables were expressed as proportions and continuous variables as median and inter-quartile range (IQR). The study was approved by the Institutional Ethics Committee of ICMR-National Institute of Epidemiology, Chennai.

Of the 3790 COVID-19 cases who visited the triage centers between May 3 and May 7, 2021, 373 reported receiving at least one dose of vaccine 14 days prior to their COVID-19 diagnosis and the remaining 3417 were unvaccinated. We enrolled 354 (94.9%) of the 373 vaccinated (241 had taken one dose, (partially vaccinated) and 113 had taken two doses of COVID-19 vaccine (fully vaccinated)) and 185 (5.4%) of the 3417 unvaccinated individuals in the study. The median age of the individuals who were unvaccinated, received 1 and 2 doses were 47 years (IQR; 33–57), 53 years (IQR; 46–60) and 54 years (IQR: 42–64), respectively (Table 1). Most study participants were male and the proportion having comorbidities was not different in the three groups.

We could retrieve genomic sequences from 414 of the 539 samples. Median RT-PCR cycle threshold values were similar in the unvaccinated (22.4, IQR; 11.9–26.3) partially (22.5, IQR; 19.4–26.9) and fully (23.1, IQR; 18.3–26.4) vaccinated groups. B.1.617.2 (Delta variant) was the predominant VOC: 72.4% (134/185) in unvaccinated, 68.1% (164/241) in partially and 74.3% (84/113) in fully vaccinated groups (Table 1). AY.1 (Delta plus variant) was isolated in five study participants. Of the five patients with AY.1 infection, one required hospitalization for oxygen support and rest had mild disease. The proportion of other VOCs was low (Table 1). A neighbor joining tree was generated using Tamura-3-parameter model and a boot strap of 1000 replication cycle (Fig. 1). Phylogenetic tree revealed the presence four distinct sub-clusters in the delta variant, similar to observed seen in the larger dataset (data unpublished).

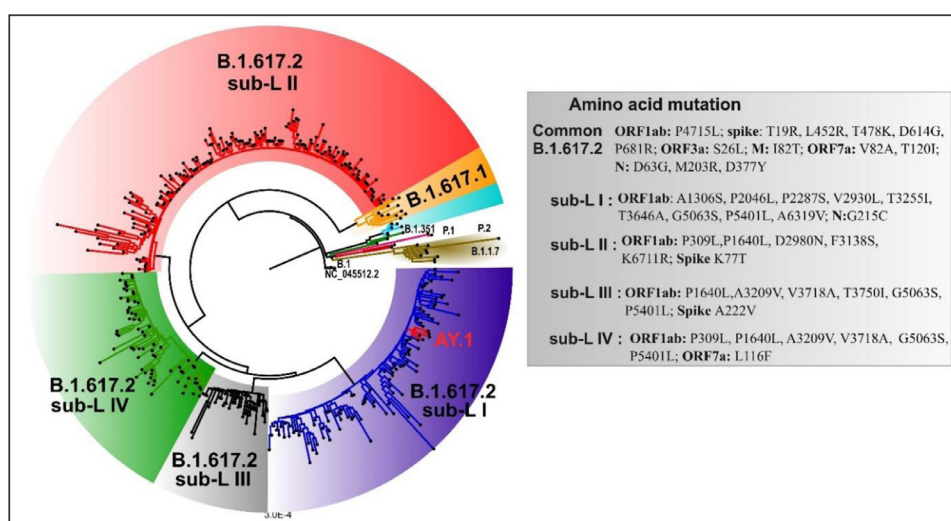
Among the fully vaccinated, majority 85% ( $n = 96$ ) had IgG antibody against SARS-CoV-2 S1-RBD whereas 63.9% ( $n = 154$ ) partially vaccinated and 14.6% ( $n = 27$ ) in the unvaccinated group



**Table 1.**

Demographic characteristics, VOCs prevalence and clinical outcome in the COVID-19 vaccinated and Unvaccinated.

Characteristics	Vaccinated for both doses (N = 113) n (% of total)	Vaccinated for one dose (N = 241) n (% of total)	Unvaccinated (N = 185) n (% of total)
<b>Age (Years)</b>			
Median (Interquartile range)	54 (42–64)	53 (46–60)	47 (33–57)
<b>Gender</b>			
Male	66 (58.4)	149 (61.8)	109 (58.9)
Female	44 (38.9)	87 (36.1)	74 (40.0)
Other	3 (2.7)	5 (2.1)	2 (1.1)
<b>Comorbidities</b>			
Yes	50 (44.6)	110 (46.0)	71 (39.0)
No	62 (55.4)	129 (54.0)	111 (61.0)
missing	1	2	3
<b>Type of Vaccine</b>			
Covaxin	31 (27.4)	80 (33.2)	-
Covishield	80 (70.8)	160 (66.4)	-
Do not Know	2 (1.8)	1 (0.4)	-
<b>Variants of concern</b>			
B.1.617.2	84 (74.3)	164 (68.1)	134 (72.4)
B.1.617.1	1 (0.9)	6 (2.5)	4 (2.2)
AY.1	1 (0.9)	2 (0.8)	2 (1.1)
B.1.1.7	1 (0.9)	2 (0.8)	5 (2.7)
B.1.351	0 (0.0)	2 (0.8)	3 (1.6)
B.1.351.3	0 (0.0)	0 (0.0)	1 (0.5)
B.1.1	0 (0.0)	0 (0.0)	1 (0.5)
B.1.530	0 (0.0)	0 (0.0)	1 (0.5)
Could not be retrieved	26 (23.0)	65 (27.0)	34 (18.4)
<b>Presence of IgG at the time of sample collection</b>			
Yes	96 (85.0)	154 (63.9)	27 (14.6)
No	9 (8.0)	64 (26.6)	118 (63.8)
Not done	8 (7.0)	23 (9.5)	40 (21.6)
<b>Symptoms during the course of illness</b>	<b>N = 104</b>	<b>N = 224</b>	<b>N = 176</b>
Yes	92 (88.5)	212 (94.6)	166 (94.3)
No	12 (11.5)	12 (5.4)	10 (5.7)
<b>Severity of illness</b>	<b>N = 104</b>	<b>N = 224</b>	<b>N = 176</b>
Moderate/Severe illness	7 (6.7)*	46 (20.5)	34 (19.3)
Mild illness	97 (93.3)	178 (79.5)	142 (80.7)
<b>Clinical outcome</b>	<b>N = 104</b>	<b>N = 224</b>	<b>N = 176</b>
Alive	104 (100.0)	221 (98.7)	169 (96.0)
Died	0 (0.0)**	3 (1.3)***	7 (4.0)

\*  $p = 0.003$  for the proportions with severe disease among fully vaccinated and unvaccinated individuals.\*\*  $p$  value (1-tail) = 0.018 for the proportions of deaths among fully vaccinated and unvaccinated individuals.\*\*\*  $p$  value (1-tail) = 0.046 for the proportions of deaths among partially vaccinated and unvaccinated individuals.

**Fig. 1.** A neighbor-joining tree was generated using a Tamura 3-parameter model with gamma distribution and a bootstrap replication of 1000 cycles. Common mutations observed in the B.1.617.2 lineage are mentioned in the figure. Further additional mutations were observed in B.1.617.2 cluster based on which four sub clusters were designated. The sub-clusters are highlighted in different colors: sub-cluster I: blue color; sub-cluster II: red; sub-cluster III: grey; sub-cluster IV: green color. The additional lineages that were found are marked on the nodes: B.1.351: green; red: P.1 and brown: P.2 and B.1.617.1 is highlighted in orange color. NC\_045512.2 (Wuhan Hu-1) is the start of the root. The figure is edited in Figtree v1.4.4 and Inkscape (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

were seropositive. We followed up 504 (93.5%) of the 539 participants and found that majority were symptomatic (88.5% in the fully vaccinated, 94.6% in the partially vaccinated and 94.3% in the unvaccinated). The proportion of patients with moderate/severe illness was significantly lower in the fully vaccinated group (7/104, 6.7%) than in the unvaccinated (34/176, 19.3%) group ( $p = 0.003$ ). No deaths were reported in the fully vaccinated group, whereas 3 partially vaccinated group and seven unvaccinated COVID-19 patients died. The proportion of COVID-19 deaths was significantly lower in the partially vaccinated (1.3%,  $p$  value (1-tail) = 0.046) and fully vaccinated (0%,  $p$  value (1-tail) = 0.018) than the unvaccinated (4.0%).

The study findings indicate that the prevalence of B.1.617.2 was not different between the vaccinated and unvaccinated groups. Delta variant was the dominant circulating strain and one of the primary drivers for the second wave of SARS-CoV-2 in India.<sup>10</sup> Studies have documented reduction in neutralization titres among Covishield and Covaxin recipients after infection with delta variant.<sup>11,12</sup> This might be the reason for the breakthrough infections observed in the fully vaccinated individuals. However, the proportion of patients progressing to severe illness and mortality was lower in the vaccinated group.

Our study has certain limitations. We recruited majority of the vaccinated individuals visiting the triaging center but only 5% of the unvaccinated individuals could be recruited due to logistics challenges. We could not follow up around 5% of the study participants.

B.1.617.2 has the potential to infect both the vaccinated and unvaccinated individuals. However, the progression of illness seems to be prevented by vaccination. Therefore, non-pharmaceutical interventions must continue to slow down the transmission. Additionally, the pace and scale of vaccination has to be increased to mitigate the further waves of the pandemic. Systematic genomic surveillance must be carried out to monitor the emergence of newer variants and assess their capacity to evade infection/vaccine induced immunity.

## Declaration of Competing Interest

None

## Acknowledgements

We sincerely thank Dr Priya Abraham, Director, ICMR-NIV, Pune for encouragement and Ms Manisha Dudhmal, Mr. Yash Joshi, for their support in Genomic sequencing and analysis of sequences. We also thank Augustine D, Punitha, Karunakaran, C.Kanagasivam, P.Tamilselvi, R.Sivakumar, Namratha S Prabhu, MP Sarath Kumar, Arya Vinod, R.Sivakumar, Arun Prasath EB, S Sarath Kumar for data collection and laboratory processing of samples. We acknowledge the support from Greater Chennai Corporation health officials in field operations.

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Accepted 1 August 2021  
Available online 6 August 2021

<https://doi.org/10.1016/j.jinf.2021.08.006>

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# Antibody response in individuals infected with SARS-CoV-2 early after the first dose of the BNT162b2 mRNA vaccine

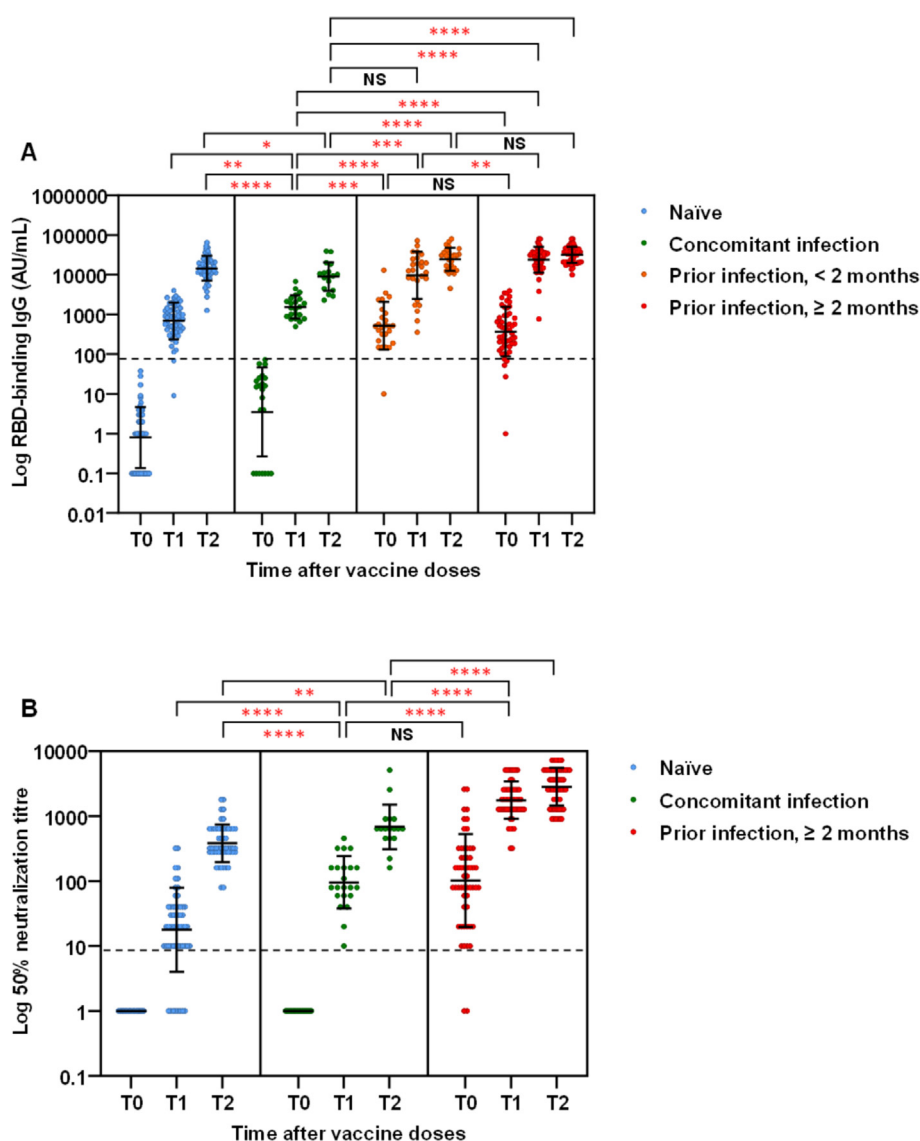


Dear Editor,

We read with interest the study recently published by Tré-Hardy et al. which analyzed the antibody response to the mRNA-1273 vaccine in health care workers (HCWs) according to their serological status before vaccination.<sup>1</sup> In agreement with previous reports and with our study on HCWs vaccinated with the BNT162b2 mRNA vaccine,<sup>2–4</sup> they showed that a single vaccine dose acts as booster in individuals with previous SARS-CoV-2 infection and rapidly induces high antibody titres, even higher than those achieved after two doses in naïve individuals.<sup>1</sup> Follow-up

evaluation at 3 months showed a drop of antibody levels in some vaccinees who were seronegative at baseline, but not in those who were seropositive.<sup>1</sup> These findings support the recommendation of a single vaccine dose for individuals with prior SARS-CoV-2 infection, while suggesting the need of an additional dose in poor responders.

Conceivably, SARS-CoV-2 infection following the first vaccine dose might also act as a booster. However, information about the levels of protective antibodies in these individuals are lacking and there are no indications about the appropriateness of a second dose of vaccine in individuals who were infected with SARS-CoV-2 after having received the first dose. Here, we investigated the dynamics of antibody response to SARS-CoV-2 in HCWs who were infected within 14 days after the first dose of BNT162b2 mRNA vaccine in comparison with the response to vaccination in naïve HCWs and in those with prior infection.



**Fig. 1.** shows serum anti-SARS-CoV-2 RBD IgG and neutralizing antibody titres in the different study groups.

Group A was tested at the time of the first dose of BNT162b2 mRNA vaccine (T0), at about 38 days after the first vaccine dose (T1) and 2,3 weeks after the second vaccine dose (T2); groups B, C and D were tested on the days of the first (T0) and second (T1, i.e. at 21 days after the first dose) vaccine doses and 2,3 weeks after the second dose (T2). **(A)** Anti-SARS-CoV-2 RBD IgG titers were measured by quantitative CMIA and reported as in arbitrary units (AU)/mL; **(B)** SARS-CoV-2 neutralizing antibody titers were measured by microneutralization assays with live virus and reported as IC<sub>50</sub> (50% neutralization titre). The dashed lines indicate the cutoff level of positive antibodies (AU/mL ≥ 50) and neutralizing concentrations (IC<sub>50</sub> > 10). Each coloured dot represents raw values of one serum sample; solid lines indicate geometric means and standard deviation. \*  $p < 0.5$ , \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$  (Mann-Whitney test). Statistical analysis was done using GraphPad Prism 9.1.2.

**Table 1**

Baseline characteristics and response to the BNT162b2 mRNA vaccine in health care workers with (groups A–C) or without (group D) SARS-CoV-2 infection.

	Group A Infection 1–14 days after 1 vaccine dose (n = 22)	Group B Infection ≥ 2 months before vaccination (n = 55)	Group C Infection < 2 months before vaccination (n = 26)	Group D Naïve (n = 55)
<b>Baseline characteristics</b>				
Males, n. (%)	4 (12)	8 (15)	12 (46)	10 (18)
Females, n. (%)	18 (82)	47 (85)	14 (54)	45 (82)
Age at vaccination, median years (IQR)	42 (28–53)	46 (31–53)	43 (31–50)	47 (34–53)
<b>SARS-CoV-2 infection</b>				
Asymptomatic, n. (%)	3 (14)	6 (11)	6 (23)	NA
Mild symptoms, n. (%)	19 (86)	46 (84)	19 (73)	NA
Hospitalization, n. (%)	0 (0)	3 (5)	1 (4)	NA
<b>BNT162b2 vaccination</b>				
Days between infection and dose 1, median (IQR)	– 8 (4–11)	273 (68–291)	46 (42–48)	NA
Days between doses 1 and 2, median (IQR)	75 (72–76)	21 (21–21)	21 (21–21)	21 (21–21)
AE after dose 1, no. (%)	14 (64)	53 (96)	21 (81)	48 (87)
AE after dose 2, no. (%)	16 (73)	50 (91)	24 (92)	50 (91)
<b>Anti-S RBD IgG</b>				
Total positive, T0 (%)	0	52 (95)	21 (81)	0 (0)
Total positive, T1 (%)	22 (100)	55 (100)	26 (100)	54 (98)
Total positive, T2 (%)	22 (100)	55 (100)	26 (100)	55 (100)
<b>Anti-S RBD IgG titre</b>				
T0, GMT (95% CI)	4 (1–11)	371 (250–553)	521 (298–909)	0.8 (0.5–1.0)
T1, GMT (95% CI)	1553 (1151–2097)	23,974 (19,531–29,428)	9687 (5568–16,853)	690 (517–921)
T2, GMT (95% CI)	8997 (5864–13,802)	32,056 (28,088–36,583)	24,476 (18,644–32,131)	14,492 (11,919–17,621)
<b>NT antibodies</b>				
Total positive, T0 (%)	0 (0)	53 (96)	ND	0 (0)
Total positive, T1 (%)	22 (100)	55 (100)	ND	47 (85)
Total positive, T2 (%)	22 (100)	55 (100)	ND	55 (100)
<b>NT antibody titre</b>				
T0, GMT (95% CI)	1 (1–1)	102 (65–160)	ND	1 (1–1)
T1, GMT (95% CI)	96 (64–145)	1769 (1482–2111)	ND	18 (12–27)
T2, GMT (95% CI)	682 (455–1023)	2832 (2369–3384)	ND	382 (318–458)

NA: not applicable; ND: not done; AE: one or more adverse events following vaccine doses; NT antibodies: neutralizing antibodies; T0: day of first vaccine dose; T1: day of second vaccine dose (day 21 after first vaccine dose) in group B, C, D and day 38 after first vaccine dose in group A; T2: 2,3 weeks after second vaccine dose; GMT: geometric mean titre; 95% CI: 95% confidence interval.

In our prospective cohort study, which included 1958 HCWs vaccinated with the BNT162b2 mRNA vaccine between January 1 and March 30, 2021, 22 HCWs were infected with SARS-CoV-2 ≤ 14 days after the first vaccine dose and had the second dose postponed > 2 months. The anti-SARS-CoV-2 antibody response in this group of HCWs (group A: concomitant infection) was compared with that observed in other groups: i.e., HCWs who got infected from March 2020 to November 2020 and were vaccinated in January 2021 (group B: prior infection, ≥ 2 months, n = 55); HCWs who got infected in December 2020 and had vaccination postponed > 1 month (group C: prior infection, < 2 months, n = 26), and naïve HCWs, who were regularly vaccinated in January 2021 (group D: naïve, n = 55). Group A received the second vaccine dose a median of 75 days after dose 1; groups B, C, and D received the second dose 21 days after the first dose (Table 1).

Median age was similar among groups; group C included a higher percentage of males; group A reported less frequently adverse events to vaccination than the other groups (Table 1). All HCWs in groups A, B and C had asymptomatic infection or mild symptoms, with the exception of one in group C who required hospitalization. In group A, SARS-CoV-2 infection was diagnosed a median of 8 days after the first vaccine dose (Table 1).

All study subjects were tested for anti-SARS-CoV-2 spike receptor-binding domain (RBD) IgG antibodies and neutralizing antibodies, as previously reported.<sup>6</sup> Testing was performed upon administration of the first (T0) and the second (T1) vaccine doses, and 2 to 3 weeks after the second dose (T2). For group A, T1 was set on day 38 after the first vaccine dose.

In group A, geometric mean titre (GMT) of RBD-binding IgG antibodies, measured after recovery and at median 38 days (IQR 37–

38) after the first vaccine dose, was about 15-fold and 6-fold lower than that observed 21 days after the first dose in groups B and C ( $p < 0.0001$ ). Conversely, it was 3-fold higher than the peak antibody titer measured after natural infection, i.e., at T0 in group C HCWs in whom antibodies were measured 46 days (IQR 42–48) after diagnosis ( $p < 0.001$ ), and 2-fold higher than in naïve group D HCWs 21 days after the first vaccine dose ( $p < 0.01$ ) (Table 1 and Fig. 1A). Following two vaccine doses, GMT of RBD-binding IgG in group A was similar to GMT in naïve HCWs after two vaccine doses, but significantly lower than in fully vaccinated group B and C HCWs with prior SARS-CoV-2 infection (Table 1 and Fig. 1A). Accordingly, in group A, neutralizing antibody GMT after the first vaccine dose was similar to that observed after natural infection, significantly higher than in naïve HCWs after the first vaccine dose, but lower than the neutralizing antibody titer observed in HCWs with prior infection who received 1 vaccine dose and in fully vaccinated HCWs (Fig. 1B). In addition, after the first vaccine dose, neutralizing antibodies were detected in all group A and B HCWs and in 85% of naïve HCWs (Table 1). A second vaccine dose induced significantly higher neutralizing antibody titers in group A than in naïve HCWs, but significantly lower than in HCWs with prior infection (Fig. 1B).

In conclusion, this study demonstrated that the titers of SARS-CoV-2 RBD-binding IgG and neutralizing antibodies induced by vaccination with BNT162b2 were significantly higher in HCWs infected with SARS-CoV-2 ≤ 14 days after the first vaccine dose than in naïve subjects, but significantly lower than in HCWs infected before vaccination. In addition, the relatively high levels of RBD-binding IgG and neutralizing antibodies in HCWs infected after vaccination were similar to those achieved after natural infection.



This level of immunity probably confers protection against symptomatic SARS-CoV-2 infection and disease, according with data from the literature which showed that the levels of neutralizing antibodies detected in convalescent serum prevent severe infection.<sup>5</sup> However, as the minimum level of antibodies associated with protection has not been defined,<sup>6</sup> a cautionary approach is preferable. Thus, while recommending a single dose for individuals who were infected months before vaccination, the same approach might not be appropriate for those who are diagnosed with the infection soon after the first dose of vaccine, especially in the context of the emergence and spread of variants of concern which escape antibody neutralization.<sup>7</sup> In our study, the strategy to postpone the second dose of two months in this group of HCWs allowed to rapidly achieve an optimal antibody response. This is crucial for elderly and immunosuppressed individuals (not included in our study population), since they mount significantly lower antibody responses than younger and healthy adults and are at risk of breakthrough infections.<sup>8</sup>

### Declaration of Competing Interest

The authors have no relevant competing interest to disclose in relation to this work.

### Funding

This work was funded by the Italian Ministry of Health under “Fondi Ricerca Corrente”- L1P1 and under “Progetto COVID Ricerca Finalizzata 2020 12371675” to IRCCS Sacro Cuore Don Calabria Hospital, and by the European Union’s Horizon 2020 Research and Innovation Programme, under grant agreement no. 874735 (VEO).

### Ethical approval

The study protocol received ethical clearance by the local Ethics Committee (Comitato Etico per la Sperimentazione Clinica delle Province di Verona e Rovigo) on January 13th, 2021 (study protocol n. 17985). All participants gave their written informed consent to participate in this study.

### Acknowledgments

The authors thank the HCWs who participated in this study.

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Accepted 3 August 2021  
Available online 8 August 2021

<https://doi.org/10.1016/j.jinf.2021.08.008>

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