# Reactogenicity, safety and antibody response, after one and two doses of mRNA-1273 in seronegative and seropositive healthcare workers

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Letters to the Editor

## Remdesivir treatment and transient bradycardia in patients with coronavirus diseases 2019 (COVID-19)

*Dear Editor,*

As described recently in this journal, natural coronavirus diseases 2019 (COVID-19) is associated with cardiac arrhythmias[1]; cardiovascular complications were risk factors for severe COVID-19 and poor outcome[1] as well as increased CD4/CD8 ratio, fever, LDH >250 U/l, d-dimer >1000 ng/ml.[2,3]

Remdesivir, a viral RNA-dependent RNA-polymerase inhibitor, was authorized for severe COVID-19 patients without mechanical ventilation[4] but still little is known about its safety except for reports about hepatic disorders and skin reactions.[5] More recently, concern arose about RDV-related cardiac adverse events, especially bradycardia.[6–9] The purpose of this study is to describe bradycardia incidence in a group of directly-observed COVID-19 patients and its possible association with RDV.

We retrospectively evaluated all the patients consecutively admitted to our ward with COVID-19 confirmed diagnosis from September 14th to December 14th 2020. We excluded ( i) patients coming from other wards and/or hospitals, (ii) patients that did not receive a complete course of RDV during their stay in our ward, (iii) patients with life expectancy <48 h at admission. The study population was divided into 2 groups: patients who received RDV (cases) and patients who did not receive RDV (controls).

We collected data about demographic and clinical characteristics, laboratory tests, treatments and outcome using an electronic case report form. Heart rate (HR) was measured at least 3 to 6 times daily according to patients' clinical conditions. RDV was administered as follows: 200 mg as loading dose on Day 1 and 100 mg on Day 2 to 5.

Transient bradycardia was defined as HR <60 bpm in two consecutive measurements or HR <50 bpm in one measurement. To be considered "positive for bradycardia", cases had not presented other bradycardia episodes before or after RDV administration period. Positive outcome was defined as clinical healing and/or discharge independently of the virological status.

We compared bradycardia incidence between cases and controls and we evaluated risk factors for bradycardia by univariate and multivariate logistic regression. Moreover, as a *post hoc* analysis, we performed a univariate and multivariate logistic regression analysis about risk factors for mortality.

Frequencies and medians were compared using chi-squared test and Mann–Whitney *U* test, respectively. Statistical analysis was made using SPSS vers. 23.

We enrolled 141 patients, 62 cases and 79 controls. Table 1 shows patients' characteristics at admission, treatments and outcomes. Cases and controls are homogeneous at admission except for body temperature >38 °C. Transient bradycardia was observed in 29/62 (46.8%) cases and 22/79 (27.8%) controls ($p = 0.023$). Univariate and multivariate logistic regression analysis confirmed the association between RDV treatment and bradycardia (OR 2.153, 95%CI 1.052–4.405, $p = 0.036$) (Table 2). All patients were asymptomatic for bradycardia, and we did not observe any cardiac event nor electrocardiographic clinically significant alterations in both groups.

Mortality was higher in the control group (22.8% vs 9.7%, $p = 0.045$). To better understand this finding, we performed a univariate and multivariate logistic regression for the assessment of risk factors for mortality. Although RDV still represented a protective factor at the univariate analysis, this finding was not confirmed at the multivariate model (OR 0.3, 95%CI 0.086–1.049, $p = 0.059$) (Table 2). Age and elevated C-RP, instead, were risk factors for mortality, while female sex and a longer time from disease's onset to admission as protective ones (OR 0.827, 95%CI 0.701–0.976, $p = 0.025$). In particular, patients with negative outcome were admitted to the hospital after a median of 4 days (interquartile range [IQR] 2–6.25 days) from the onset of the symptoms, while patients with a positive outcome after 6 days (IQR 4–8 days) with $p = 0.047$.

Our study described transient bradycardia as a very common finding in patients administered with RDV (incidence about 47%). With this study, we confirmed the previously published data by our group, in which 60% of patients treated with RDV had transient bradycardia.[8] Some other authors also described this association in case reports and small reviews.[6,7] How RDV could provoke bradycardia is still unknown. A possible explanation comes from the similarity between a nucleotide triphosphate metabolite of RDV and adenosine triphosphate (ATP).[10] ATP has a negative chronotropic and dromotropic activity by an adenosine-mediated pathway; this mechanism was hypothesised to be also used by RDV's metabolite.[9,10]

We found an elevated incidence of bradycardia. On the other hand, Touafchia and colleagues in a recently published extensive article reported only 94 cases of bradycardia out of 2603 RDV side effects reports.[9] Moreover, we did not observe any severe adverse event while in the above-mentioned study, 80% were serious, and 17% were fatal.[9] These substantial differences may lie in the design of the studies. While we directly observed every enrolled patient, Touafchia and colleagues evaluated "just" a big amount of reports of possible RDV associated adverse events. Theoretically, the indirect observation might be considered as a sort of selection bias that could lead to underestimating the incidence of bradycardia. At the same time, the small sample size of our study was probably responsible for the lack of severe adverse events.

In the first analysis, we found RDV administration significantly associated with positive outcome. This finding made mandatory an additional *post hoc* analysis that did not confirmed RDV association with reduced mortality, while the threshold of significance

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**Table 1**
Cases' and controls' characteristics at admission, treatments and outcomes.

| | *Study population n = 141* | *Cases n = 62* | *Controls n = 79* | *P* |
|---|---|---|---|---|
| Female sex *n (%)* | 66 (46.8) | 32 (51.6) | 34 (43) | >0.1 |
| Age *years, median (IQR)* | 69 (56–80) | 69 (59–80.75) | 67 (53.5–80) | >0.1 |
| CCI *median (IQR)* | 4 (1–5) | 4 (2–5) | 3 (1–6) | >0.1 |
| CVD *n (%)* | 84 (59.6) | 40 (64.5) | 44 (55.7) | >0.1 |
| Beta-blockers *n (%)* | 46 (32.6) | 19 (30.6) | 27 (34.2) | >0.1 |
| Days onset-admission*median (IQR)* | 6 (4–7) | 6 (4.25–7) | 7 (3–8.5) | >0.1 |
| T ≥ 38°C *n (%)* | 23 (16.3) | 16 (25.8) | 7 (8.9) | **0.011** |
| $PaO_2/FiO_2$ <300 *n (%)* | 60 (42.6) | 31 (50) | 29 (36.7) | >0.1 |
| C-RP >5 *mg/dl n (%)* | 94 (66.7) | 46 (74.2) | 48 (60.8) | >0.1 |
| C-RP *mg/dl median (IQR)* | 7.8 (3–12.36) | 8.93 (5.01–12.92) | 6.76 (2.3–11.17) | 0.077 |
| Lymphocytes <800/$mm^3$ *n (%)* | 56 (39.7) | 26 (41.9) | 30 (38) | >0.1 |
| Lymphocytes *n/$mm^3$ median (IQR)* | 920 (650–1310) | 890 (595–1185) | 970 (700–1340) | >0.1 |
| IL-6[a] *pg/ml median (IQR)* | 29.9 (9.05–78.75) | 30.25 (13.4–51.025) | 28.8 (7.6–86.725) | >0.1 |
| d-dimer >1000 ng/ml *n (%)* | 59 (41.8) | 21 (33.9) | 38 (48.1) | >0.1 |
| d-dimer *ng/ml median (IQR)* | 789 (473–1399.5) | 720.5 (480–1155) | 964 (472–1595) | >0.1 |
| CD4/CD8[a] *median (IQR)* | 1.9 (1.2–2.65) | 2.1 (1.3–3.1) | 1.6 (0.975–2.3) | 0.069 |
| Bradycardia *n (%)* | 51 (36.2) | 29 (46.8) | 22 (27.8) | **0.023** |
| Steroids *n (%)* | 129 (91.5) | 62 (100) | 67 (84.8) | >0.1 |
| LMWH *n (%)* | 139 (98.6) | 62 (100) | 77 (97.5) | >0.1 |
| Exitus *n (%)* | 24 (17) | 6 (9.7) | 18 (22.8) | **0.045** |

Abbreviations: CCI, Charlson comorbidity index; CVD, cardiovascular diseases; Days onset-admis, days from disease onset to hospital admission; *T*, temperature; C-RP, C-reactive protein; IL-6, interleukin 6; LMWH, low molecular weight heparin. Notes: a, *n* = 83, 49 patients and 34 controls.

**Table 2**
Univariate analysis and multivariate logistic regression assessing risk factors for transient bradycardia and for mortality.

| | *Univariate analysis* | | *Multivariate analysis* | |
|---|---|---|---|---|
| *Transient Bradycardia* | OR (95% CI) | *p* | OR (95% CI) | *p* |
| Female sex | 1.62 (0.807–3.256) | 0.175 | | |
| **Age** | **1.025 (1.000–1.051)** | **0.048** | 1.016 (0.986–1.047) | 0.291 |
| **CVD** | **2.1 (1.011–4.362)** | **0.047** | 1.628 (0.643–4.125) | 0.304 |
| Beta-blockers | 1.385 (0.671–2.86) | 0.378 | 0.935 (0.389–2.244) | 0.88 |
| T ≥38°C | 1.075 (0.421–2.742) | 0.88 | | |
| Lymphocytes | 1 (0.999–1) | 0.402 | | |
| C-RP | 1.027 (0.982–1.074) | 0.246 | | |
| d-dimer | 1 (1-1) | 0.934 | | |
| **RDV therapy** | **2.277 (1.13–4.588)** | **0.021** | **2.153 (1.052–4.405)** | **0.036** |
| $PaO_2/FiO_2$ <300 | 1.161 (0.577–2.337) | 0.676 | | |
| ***Mortality*** | | | | |
| **Female sex** | 0.405 (0.156–1.049) | 0.063 | **0.267 (0.072–0.997)** | **0.049** |
| **Age** | **1.138 (1.072–1.208)** | **<0.001** | **1.138 (1.046–1.238)** | **0.003** |
| CCI | **1.63 (1.281–2.074)** | **<0.001** | 0.977 (0.636–1.499) | 0.914 |
| **Days onset-admission** | **0.799 (0.679–0.94)** | **0.007** | **0.759 (0.603–0.956)** | **0.019** |
| $PaO_2/FiO_2$ <300 | 1.523 (0.62–3.738) | 0.359 | | |
| Pneumonia | 1.029 (0.273–3.875) | 0.966 | | |
| T ≥38°C | 1.447 (0.479–4.373) | 0.655 | | |
| Lymphocytes | 0.999 (0.998–1) | 0.256 | | |
| **C-RP** | **1.082 (1.024–1.143)** | **0.005** | **1.11 (1.022–1.206)** | **0.013** |
| IL-6[a] | 1 (0.999–1.002) | 0.58 | | |
| d-dimer | 1.0001 (1–1.0003) | 0.055 | 1 (1-1) | 0.78 |
| CD4/CD8 | 0.62 (0.326–1.179) | 0.145 | | |
| LMWH administration | 0.198 (0.012–3.286) | 0.259 | | |
| Steroids administration | 5.806 (0.332–101.45) | 0.228 | | |

Abbreviations: OR, odds ratio; CI, confidential interval; CVD, cardiovascular diseases, T, body temperature; C-RP, C-reactive protein; RDV, remdesivir; CCI, Charlson comorbidity index; IL-6, interleukin 6; LMWH, low molecular weight heparin.

was reached by other factors such as C-RP, age, female sex. These data were consistent with the literature.[4] Since patients receiving RDV had a greater clinical improvement and a faster time to recovery, they did not have benefits in terms of mortality.[4]

This study had some limitations, especially the limited sample size and the retrospective design. However, it was performed in a real-life setting and provided directly-observed data that could possibly improve the clinical management of COVID-19 patients.

In conclusion, RDV is associated with a quite high incidence of transient bradycardia. Clinicians should be aware of this frequent adverse event in order to provide appropriate care to COVID-19 patients. A serial electrocardiographic control during RDV administration could be suggested to avoid severe cardiologic adverse events.

## Ethics

Patients provided their consent for data analysis and submission

## Authors' contribution

CP, AG, LM, PB and MADP conceived the study; CP, LRS, SE, FV, ADE, PP, LA, ES and FB collected data; CP drafted the manuscript; CP, LRS, AG, LM, PB, DF, SE, FV, ADE, PP, LA, ES and FB revised the manuscript; CP, PB, DF and MADP supervised the study. All Authors approved the final version of the manuscript to be submitted.

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## Prior COVID-19 protects against reinfection, even in the absence of detectable antibodies

*Dear Editor,*

Several studies, including from our own centres, have shown the strong protection from reinfection conferred by previous SARS-CoV-2 infection[1–5] However these studies did not address whether prior infection is protective in the absence of a detectable humoral immune response. Patients with primary or secondary antibody deficiency syndrome and reduced or absent B cells can recover from COVID-19[6,7]. Although there have been few mechanistic studies, preliminary data show that such individuals generate striking T-cell immune responses against SARS-CoV-2 peptide pools[8]. SARS-CoV-2 specific T cell immune responses but not neutralising antibodies are associated with reduced disease severity suggesting the immune system may have considerable redundancy or compensation following COVID-19[9]. It is plausible that mucosal immunity, memory B-cells, or other classes of antibody may also play a significant role in protection, although direct evidence is lacking[10].

We examined datasets from four UK laboratories and identified a subset of patients with proven SARS-CoV-2 infection, defined as laboratory detection of RNA, in the first wave of the pandemic between March and May 2020, but with negative serology results in June and July. SARS-CoV-2 RNA test results (PCR or other nucleic acid amplification technology) between August 2020 and January 2021 were reviewed to identify patients with likely reinfection in the second wave of the UK pandemic. Repeat positive results within 90 days were discounted. A comparator group of patients with no evidence of infection in the first wave – *i.e.* negative serology with either a negative or no RNA assay performed - was used to calculate the relative risk of infection in those with and without prior infection. A second comparator group was also examined, who were RNA-positive and antibody-positive in the first wave. A significant proportion of the patients were healthcare workers, who were offered serology as part of a national policy. We terminated the study at the end of January, as we judged that the national vaccination rollout might interfere with the reliability of results thereafter.

The results are summarised in Table 1. We identified 224 RNA-positive antibody-negative patients in the first wave, with two laboratory-confirmed reinfections in the second wave (0.89%), compared to 2054 second-wave infections in the 47139 patients with previous negative serology and either no RNA result, or a negative RNA result (4.36%.) This implies a significantly reduced risk of reinfection (relative risk 0.20, 95% CI 0.05 to 0.81) in those with prior SARS-CoV-2 infection but without detectable antibod-

**Table 1**
Numbers of patients and SARS-CoV-2 (re)infections identified in the participating laboratory datasets.

| | | SWLP | NWLP | NCLE | KCH | Total | Significance; 95% confidence intervals |
|---|---|---|---|---|---|---|---|
| Test group: Confirmed infection, serology negative in first wave | RNA-positive, Antibody negative in first wave | 98 | 75 | 28 | 23 | 224 | |
| | Reinfected in second wave | 1 | 1 | 0 | 0 | 2 | |
| | Proportion reinfected | 1.02% | 1.33% | 0% | 0% | 0.89% | |
| Comparator Group 1: No laboratory evidence of infection in first wave | RNA negative or not tested; Antibody negative in first wave | 23289 | 6389 | 10138 | 7323 | 47139 | |
| | Infected in second wave | 639 | 562 | 443 | 410 | 2054 | |
| | Proportion infected | 2.74% | 8.80% | 4.37% | 5.60% | 4.36% | |
| | Relative risk | 0.37 | 0.15 | 0 | 0 | 0.20 | $p = 0.02$; CI = 0.05 to 0.81 |
| Comparator Group 2: Confirmed infection, serology positive in first wave | RNA positive, Antibody positive in first wave | 852 | 311 | 380 | 544 | 2087 | |
| | Reinfected in second wave | 5 | 8 | 0 | 5 | 18 | |
| | Proportion reinfected | 0.59% | 2.57% | 0% | 0.92% | 0.86% | |
| | Relative risk | 1.74 | 0.52 | * | 0 | 1.04 | $p = 0.96$; CI = 0.24 to 4.43 |

SWLP: South West London Pathology
NWLP: North West London Pathology
NCLE: Newcastle-upon-Tyne Laboratories
KCH: King's College Hospital Laboratory
* Relative risk undefined (0/0)

ies, compared to those with no previous evidence of infection. We also found 2087 RNA-positive antibody positive patients in the first wave, with 18 reinfections (0.86%) – this was similar to the proportion in the RNA-positive antibody-negative patients (relative risk 1.04).

Our results indicate that antibodies (as detected by routine laboratory assays) are not essential for protection against reinfection. To our knowledge this is a novel observation, though it is supported by a recent report that immunity to SARS-CoV-2 in patients without antibodies can occur if there is a significant T cell immune response[8]. IgG memory B cells against SARS-CoV-2 increase and exhibit greater affinity maturation over time despite a decline in serum antibody titres[11,12]. This is consistent with the known development of the immune response: the loss of antibody may reflect not so much waning immunity but rather standard contraction of immune responses following SARS-CoV-2 infection, with development of antigen specific memory B cells. In addition, mucosal IgA or IgG may explain some of the protective effect we have observed. Furthermore, given the long incubation and slow onset of severe disease in SARS-CoV-2 infection, it is biologically plausible that the 2-3 day response time of antigen specific memory T- or B-cells is sufficient to protect against reinfection independently of circulating antibody, as is seen with Hepatitis B vaccination[13]

The principal limitation of this study is that it is a retrospective pragmatic review of pooled clinical laboratory datasets. As such, SARS-CoV-2 RNA testing in many individuals will have been event-driven, rather than routine screening, and some cases of SARS-CoV-2 infection may have been missed, for example if asymptomatic. Furthermore, the criteria for seropositivity were set by the assay manufacturers; it is possible that some patients had specific antibody below the limit of assay detection, that nonetheless contributed to protection. Due to the deployment of different assays across laboratories, we were unable to examine the relationship between antibody index and risk of reinfection. The comparator group, of necessity, may have included some patients who also had antibody-negative infection in the first wave but who did not have an RNA assay performed. However, any such missed cases would have tended to reduce the apparent difference between the two groups, which increases confidence in our findings. We cannot exclude the possibility that positive test results may have influenced individual behavior, potentially increasing the risk of (re)infection or making seropositive individuals reluctant to come forward for further testing if they developed COVID-19 symptoms. However a recent Danish study showed no difference in protection from reinfection in health care workers tested regularly for SARS-CoV-2 infection, compared to other population groups[4]. Finally, given the evolving epidemiology of the SARS-CoV-2 Pandemic, and the continual emergence of new strains, we can only say with confidence that our results apply to the situation in the UK up to the end of January 2021.

In conclusion, our results add to the emerging evidence that detectable serum antibody may be an incomplete marker of protection against reinfection. This could have implications for public health and policy-making, for example if using seroprevalence data to assess population immunity, or if serum antibody levels were to be taken as official evidence of immunity – a minority of truly immune patients have no detectable antibody and could be disadvantaged as a result. Our findings highlight the need for further studies of immune correlates of protection from infection with SARS-CoV-2, which may in turn enhance development of effective vaccines and treatments. Serum antibody, whilst convenient to measure, is but a small window on the complex world of the human immune system.

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## One-year durability of anti-spike IgG to SARS-CoV-2: Preliminary data from the anticrown prospective observational study one year durability of COVID-19 anti-spike IgG

*Dear Editor,*

We read with interest the article by Ross J Harris and colleagues,[1] showing assay-dependent durability of antibodies to SARS-CoV-2 after 6 months of symptomatic and asymptomatic COVID-19 as assessed by five different immunoassays with a prediction of durability at one year. We present our preliminary data on DiaSorin anti S1/S2 IgG production after one year from disease onset or detection of SARS-CoV-2 infection. The AntiCROWN study is being run in an hospital-based outpatient clinic open to all people who have serological or PCR evidence of SARS-Cov-2 infection. On May 13, 2020, the Infectious Diseases Department of Luigi Sacco Hospital, Milan, Italy, started an outpatient clinic for the follow-up of COVID-19 patients, diagnosed by a positive nasopharyngeal swab or a combination of clinical and epidemiological criteria and a positive serological test. Only patients whose onset was dated between February 20 and April 30, 2020 are included in

**Table 1**
Antibody response and new COVID-19 events, total 368/503 patients with results at one year.

| Baseline value (AU*/mL) | < 15 | 15–50 | >50 |
|---|---|---|---|
| N (%) | 29 (7.9) | 65 (17.7) | 274 (74.4) |
| one died of mesothelioma | | | |
| Female sex, n (%) | 9 (31) | 41 (63.1) | 126 (46) |
| Median age (range) | 48.5 (18-92) | 46.5 (15–83) | 59 (4–87) |
| Immune depression/ immune suppression, n (%) | 1 (3.4) | 9 (14.3) | 43 (15.9) |
| WHO severity scale represented (m: mild; M: moderate; S: severe; C: critical, n, %) | 20 m (68.9); 7 M (24.2); 1 S (3.4); 1 C (3.4) | 49 m (77.8); 11 M (14.3); 2 S (3.2); 3 C (4.8) | 93 m (34.1); 73 M (26.3); 46 S (16.7); 62 C (23) |
| Lost response (<15 AU/mL) | | 4 (6.1% of evaluable natural course) | 0 |
| §RR vs >50 for losing reponse [95% †CI] | | 37.5 [2.0–688.0] *p = 0.0146* | |
| Lost response (<3.8 AU/mL) | | 0 | 0 |
| Maintained natural response | | 49 | 241 |
| Lost AU/mL, median [‡IQR], variance; | | -4.4 [13; +1-2.5], 1125.2 | -25 [-56; +7], 12184.2 |
| P for lost AU/mL vs >50 | | *p = 0.0295* | |
| Vaccinated, n (% achieved >400 AU/mL) | 4 (75), all baseline >10 AU/mL, 1 with recall dose | 15 (100), 3 with recall dose | 57 (100), 5 with recall dose |
| Acquired response later | 3 | | |
| Repeated clinical COVID-19, n (%=) | 4 (13.8) one admitted for pneumonia | 1 (1.5) | 1 (0.4) |
| RR vs >50 for repeating clinical COVID [95%CI] | 37.7 [4.4–326.9] *p = 0.001* | 4.2 [0.3–66.5] *p = 0.3067* | |

*AU = Arbitrary Units
†CI = Confidence Interval
‡IQR = InterQuartile Range
§RR = Relative Risk

this analysis (the so called “first wave”). The LIAISON® SARS-CoV-2 S1/S2 IgG solution (DiaSorin, Saluggia, Italy) used to quantify the antibody response shows a positive agreement of 94.4% (88.8%–97.2%) with *in vitro* neutralising antibody titre.[2] The response was tested at the first outpatient visit (T1) set at week 12 ± 3 weeks from symptoms onset or diagnosis in asymptomatic subjects, at T2 (20 ± 3 weeks), T3 (32 ± 3 weeks) and T4 (52 ± 3 weeks). Since December, 2020, we were imposed a ceiling cut-off of 400 AU/mL. According to the WHO classification for COVID-19 severity patients were divided into mild, moderate, severe and critical.[3] We calculated the relative risk of falling < 15 Arbitrary Units (AU)/mL with 95% confidence interval and statistical significance according to Altman and the significance of decay or increase over time through the Mann-Whitney and Wilcoxon log-rank test. The study was approved by the “Comitato Etico Interaziendale Area 1″. All patients signed a written informed consent. The full ARCOVID cohort counts 1048 outpatients, of whom 503 from the ‘first wave’. We present preliminary data of 368 patients who had a one-year control of serum anti-S1/S2 IgG levels (11 to 14 months, median 12.5 months). Our patients belonged to all severity classes according to the WHO definition. However, since response better correlated with baseline antibody production, we used this criterion to analyse data presented in Table 1, stratifying by <15 (positive cut-off value), 15–50 (arbitrary cut-off for low antibody production) and >50 AU/mL. The mean age was 58.9 years (range 4–92 years) and 174 (41.8%) were females. Immune suppression or immune depression (HIV infection, cancer, immune diseases and autoimmunity, steroids, anti-cancer chemotherapy, monoclonal anti-B lymphocyte drugs) were present in patients. Our data suggest that loss of anti-S1/S2 IgG response at one year may be a rare event, occurring only in subjects who produce less than 50 AU/mL within the initial 4 months since disease onset. Of note, 12 patients had unexpected increase of antibody production in the absence of vaccination (+ 40 AU/mL and at least double compared to baseline), which suggests renewed exposure to the virus without developing symptoms. Stratifying for baseline antibody production did not show significant differences in such phenomenon. Moreover, only 6 patients had a new clinical COVID-19 event, four having IgG levels below 15 AU/mL. Events were mild and only one patient, who had recently been receiving monoclonal anti-B lymphocyte suppressive therapy for lymphoma, developed moderate pneumonia and was admitted to hospital, showing rapid clinical improvement during a 5-day stay. This patient subsequently responded minimally to recall vaccination with the BNT162b2 vaccine (15.1 AU/mL), whereas all the remaining 75 patients who were vaccinated showed an increase in antibody production over the ceiling cut-off, 67 (88.2%) having received a single vaccine shot. Fig. 1 allows overall visual understanding of the antibody production over time. In conclusion, our observation suggests that antiS1/S2 antibodies are fairly stable over one year. The few clinical events seem to occur almost only in those patients who had never responded and loss of protection in those who showed poor initial antibody response. This confirms similar observations reported in a shorter time frame by Lumley et al.[4] Our aim now is to continue the observation until two years and widen the population with the “second wave”, which, by November, will bring our one-year observation to almost 1.100 patients, as well as to follow the response to single-dose vaccination over time in COVID-19 patients. If data are confirmed, we feel that COVID-19 provides long-lasting immunity to symptomatic subjects as well as to a proportion of asymptomatic subjects, although boosting immunity with a single dose, irrespective of the time lapsed from the clinical event, may improve the intensity[5] and possibly the duration of such response. Similar observations may help inform health policy decisions,[6] although their main limitation remains the fact they are necessarily performed in a setting influenced by the WHO advices[7] and further restricted by local authorities’ measures. Indeed, nobody knows what this means in a world free of masks and social distancing.

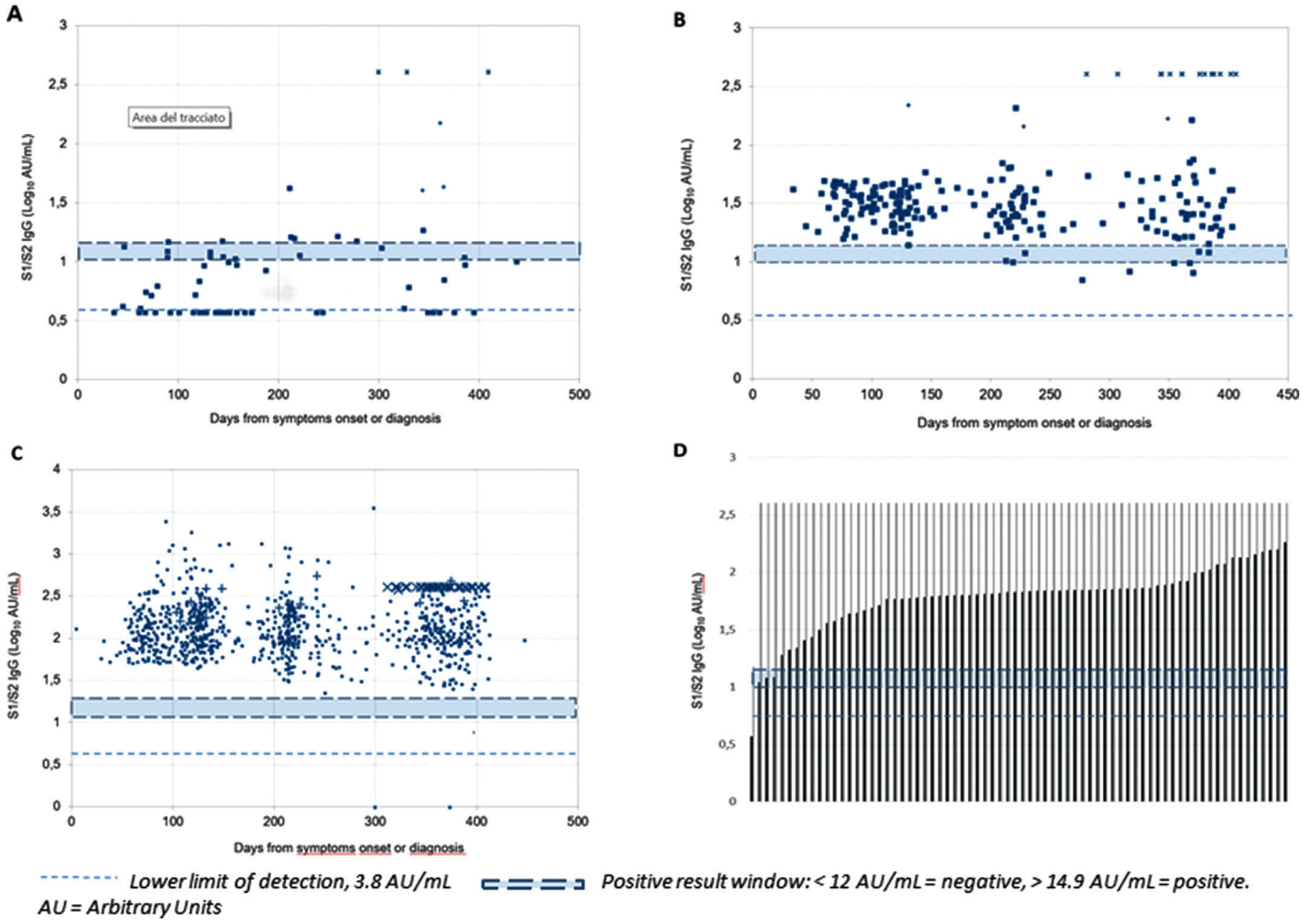


**Fig. 1.** One Year follow-up of anti-S1/S2 antibody levels in subjects: A, with baselibe lavels <15 AU/mL; B, with baseline levels 15 to 50 AU/mL; C, with baseline levels >50 AU/mL. D: responses in vaccinated subjects (Results limited by ceiling cut-off effect at 400 AU/mL)).

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## The performance of the SARS-CoV-2 RT-PCR test as a tool for detecting SARS-CoV-2 infection in the population

*Dear Editor,*

Worldwide, detection and monitoring of SARS CoV-2 infection continues to be based on results of the real-time reverse-transcription polymerase chain reaction (RT-PCR) test. A recent scoping review in this journal reported that assessment of the diagnostic accuracy of the RT-PCR test for SARS-CoV-2 has been less than perfect [1]. We analysed real-world data from a large laboratory in the city of Münster (population 313,000), Germany, derived from a single fully automated high throughput RT-PCR platform (cobas SARS-CoV-2 RT-PCR system, Roche Diagnostics) utilizing the same two gene targets for the entire study period (weeks 10-49, 2020). This laboratory performed about 80% of all SARS-CoV-2 RT-PCR tests in the Münster region during this time. We explored changes in the percentage of positive RT-PCR tests (positive rate) over time. In addition, we assessed the influence of covariates such as age, sex, calendar time, and symptoms at the time of first RT-PCR test on the distribution of cycle threshold (Ct) values.

Nearly all swab specimens were tested within 24 hours of collection. The tests and their interpretation were carried out in accordance with the Roche cobas SARS-CoV-2 emergency use authorization (EUA) protocol, the specific targets of the test being the open reading frame (ORF) 1ab and the pan-Sarbecovirus E genes. The limit of detection, defined as the concentration of analyte that will be detected in 95% of replicate tests was 0.007 median tissue culture infectious doses (TCID50) per ml for target 1 and 0.004 TCID50/ml for target 2, corresponding to Ct values of approximately 33 and 36, respectively (cobas® SARS-CoV-2 package insert, version 1.0).

RT-PCR tests that had not crossed the positivity threshold after the 40th cycle were reported as “negative”. The Ct value is inversely proportional to the initial amount of target nucleic acid and is thus a relative indicator of the concentration of viral particles in the clinical specimen. An increase in Ct value of three points indicates that the initial amount of viral particles was smaller by a factor of about ten.

We categorized our population-based Ct values according to the recommendations of the UK Office for National Statistics (ONS) COVID-19 household survey as $< 25$ and $\geq 25$ [2]. Since there has been some discussion regarding this Ct-threshold [3-5], we performed a second categorization using a cutoff of $< 30$ versus $\geq 30$. For a small subset of 58 people, sufficient clinical information was available to allow classification as symptomatic or asymptomatic.

Of 162,457 tested individuals, 4,164 (2.6%) had a positive RT-PCR test. The positive rate was lower among children aged 0-9 years (2.2%) and among adults aged 70 or more (1.6%), compared to the intermediate group aged 10-69 years (2.8%). The positive rate was strongly linked to the national SARS-CoV-2 test strategy. During the first and third phase of national testing, predominantly symptomatic people were tested. During these phases, the positive rates were higher than during the intermittent second phase corresponding to the summer season, when predominantly asymptomatic individuals were tested. The positive rate during the third phase was considerably higher than during the first phase. During the peak of testing asymptomatic individuals, only 0.4% tested positive with a mean Ct value of 28.8. Higher mean Ct values were observed among children aged 0-9 years (28.6) and adults above 70 years (27.0). Only 40.6% of positive tests showed Ct values below the threshold of 25, indicating a likelihood of the person being infectious (**Table 1**). In the small group of individuals for whom clinical information was available, symptomatic subjects had a markedly lower mean Ct value of 25.5 compared to asymptomatic subjects, who showed a mean Ct value of 29.6 (**Figure 1**).

Most positive tests in our sample showed Ct values of 25 or higher, indicating a low viral load. Ct values were on average lower in symptomatic than in asymptomatic individuals. Our results are similar to the observations made in the ONS Survey with consistently low positive rates (0.06%) during the summer months,

**Table 1**
Characteristics of people who underwent PCR testing in the region of Münster, North Rhine-Westphalia, Germany, March 26 - December 6, 2020

| | Number of tests[1] | Positive tests | | Mean Ct value among positive tests[2] | | Percentage of positive tests with Ct values[2] | |
|---|---|---|---|---|---|---|---|
| | N | N | % | Mean | SD | < 25 | <30 |
| All | 162,457 | 4164 | 2.6 | 26.5 | 5.2 | 40.6 | 69.6 |
| Men | 70,043 | 1981 | 2.8 | 26.4 | 5.3 | 42.0 | 69.6 |
| Women | 92,113 | 2165 | 2.4 | 26.6 | 5.1 | 39.4 | 69.5 |
| Unknown | 301 | 18 | 6.0 | 27.4 | 5.2 | 38.9 | 66.7 |
| Swab site | | | | | | | |
| Nose & throat | 8637 | 222 | 2.6 | 25.9 | 5.4 | 43.0 | 72.9 |
| Throat | 7059 | 151 | 2.1 | 26.2 | 4.5 | 41.7 | 77.2 |
| Unspecified/other | 146,761 | 3791 | 2.6 | 26.6 | 5.2 | 40.4 | 69.1 |
| Age group | | | | | | | |
| 0-9 | 9978 | 222 | 2.2 | 28.6 | 4.7 | 21.1 | 56.5 |
| 10-19 | 15,200 | 536 | 3.5 | 26.8 | 4.9 | 38.2 | 71.4 |
| 20-29 | 21,613 | 745 | 3.5 | 26.4 | 5.1 | 41.6 | 69.4 |
| 30-39 | 21,830 | 572 | 2.6 | 26.3 | 5.1 | 42.7 | 72.3 |
| 40-49 | 21,373 | 600 | 2.8 | 26.3 | 5.4 | 43.8 | 69.1 |
| 50-59 | 25,367 | 665 | 2.6 | 26.0 | 5.3 | 44.4 | 72.9 |
| 60-69 | 17,460 | 351 | 2.0 | 26.0 | 5.1 | 46.0 | 73.5 |
| 70-79 | 12,155 | 214 | 1.8 | 27.1 | 5.2 | 35.3 | 65.8 |
| 80-89 | 13,196 | 185 | 1.4 | 26.8 | 5.2 | 37.4 | 64.5 |
| 90-99 | 3699 | 55 | 1.5 | 27.0 | 5.4 | 37.0 | 63.0 |
| 100+ | 29 | 1 | | | | | |
| unknown | 557 | 18 | 3.2 | 31.3 | 4.9 | 11.8 | 29.4 |
| Calendar week | | | | | | | |
| 10-19 | 12,985 | 305 | 2.4 | 28.7 | 5.1 | 22.1 | 46.8 |
| 20-44 | 132,488 | 2418 | 1.8 | 26.5 | 5.2 | 40.5 | 69.6 |
| 45-49 | 16,984 | 1441 | 8.5 | 26.4 | 5.1 | 41.8 | 70.7 |
| Specific phases of the pandemic[3] | | | | | | | |
| Peak 1st wave | 2190 | 36 | 1.6 | 27.8 | 5.4 | 26.5 | 55.9 |
| Traveler return | 16,874 | 68 | 0.4 | 28.8 | 5.5 | 26.9 | 55.2 |
| Peak 2nd wave | 4022 | 367 | 9.1 | 26.6 | 5.1 | 39.5 | 69.8 |

Legend table: SD = standard deviation
[1] only persons with tests that were clearly either positive or negative were included
[2] among 4164 people tested positive, the Ct value was available for 3810 people (91.5%); Ct values were not retrievable for positive tests during the calendar weeks 12-13 and 16-25 in 2020
[3] Peak of 1st wave in weeks 12-13 (16.-29.3.2020); proxy weeks 13-14; unselective testing in weeks 33-34 (peak of tests for traveler return); peak of 2nd wave in weeks 50-51 (7.-20.12.2020), proxy weeks 48-49

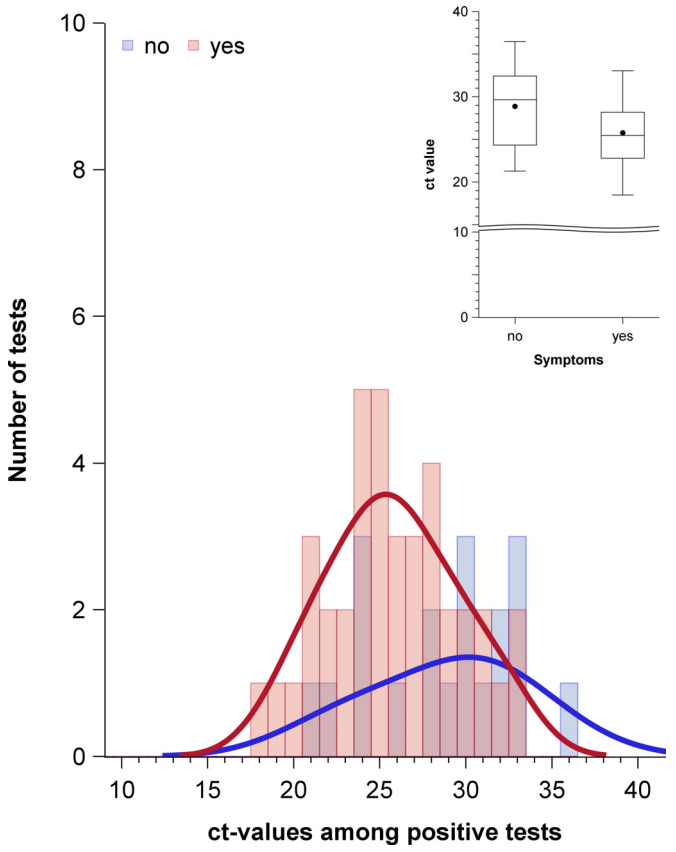


**Figure 1.** Ct value distribution among symptomatic and asymptomatic individuals´with positive tests in the region of Münster, North Rhine-Westphalia, Germany, 2020
Legend: "no" means "no symptoms", "yes" means "symptoms"; dots in the box plot indicate mean values and horizontal lines in the boxes indicate median values. Asymptomatic individuals : n=19, median 29.6, mean 28.8, SD 4.3; symptomatic individuals: n=39 median 25.5, mean 25.8, SD 3.7

followed by a rise to more than 1% by the end of October 2020. A substantial proportion (45%-68%) of test positive individuals in the UK did not report symptoms at the time of their positive PCR test [6].

In light of our findings that more than half of individuals with positive PCR test results are unlikely to have been infectious, RT-PCR test positivity should not be taken as an accurate measure of infectious SARS-CoV-2 incidence. Our results confirm the findings of others that the routine use of "positive" RT-PCR test results as the gold standard for assessing and controlling infectiousness fails to reflect the fact "that 50-75% of the time an individual is PCR positive, they are likely to be post-infectious" [7].

Asymptomatic individuals with positive RT-PCR test results have higher Ct values and a lower probability of being infectious than symptomatic individuals with positive results. Although Ct values have been shown to be inversely associated with viral load and infectivity, there is no international standardization across laboratories, rendering problematic the interpretation of RT-PCR tests when used as a tool for mass screening.

## Declaration of Competing Interest

Paul Cullen has received speaker's fees from Roche Diagnostics. None of the other authors declares any conflict of interest.

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## Rate and risk factors for breakthrough SARS-CoV-2 infection after vaccination

*Dear Editor,*

We read with interest the recent article by Sansone et al. regarding the effectiveness of BNT162b2 vaccine against the B.1.1.7 variant of SARS-CoV-2 among healthcare workers in Brescia, Italy.[1] Recently available data from other groups also confirms very high levels of effectiveness of the Pfizer-BNT-162b2 vaccine in the real-world settings.[2-6] Despite such high efficacy and effectiveness, there are anecdotal reports of breakthrough infection among vaccine recipients. Clinical characteristics and risk factors for SARS-CoV-2 infection after a full recommended course of vaccination is not known. We report the rate and risk factors associated with infection among US Veterans who received a recommended course of vaccination. The Veterans Health Administration (VA) is the largest provider of integrated health services in the United States. The VA provides care to over 9 million enrolled Veterans at 170 VA medical centers and 1074 outpatient sites.[7]

### Methods

*Creation of the study dataset*

We identified all Veterans who received two doses of the Pfizer-BNT-162b2 or Moderna-mRNA-1273 vaccine between December 15, 2020 and March 31, 2021 from the national VA COVID-19 Shared Data Resource. We excluded those with a positive SARS-CoV-2 PCR on a nasopharyngeal swab within 14 days of receiving the first vaccine dose. From the remaining persons, we retained those who had at least one SARS-CoV-2 PCR test performed on a nasopharyngeal swab ≥7 days after the second dose vaccine dose. Cases were those with confirmed SARS-CoV-2 infection and controls were those who remained uninfected with at least one confirmed negative test for SARS-CoV-2 ≥ 7 days after their second vaccine dose.

### Results

Among a total of 258,716 fully vaccinated persons, we identified 410 persons with breakthrough infection and 14,465 controls. Median age (IQR) was 73 (68,78) years for the infected group and 72 (66,76) for the uninfected group ($P = 0.0002$). There were more Whites in the infected group (76.6% vs. 69.1%; $P = 0.01$)) compared with the uninfected group. Prevalence of comorbidities was similar in the two groups except anemia, which was more common in the infected group.

Overall infection rate ≥7 days after the second vaccine dose was 0.66 (95% CI 0.60,0.72) per 1000 person-days of follow up. (Table 1) The rates were not statistically significantly different by age group, sex, or the type of vaccine administered. Rate was lower among Black compared with Whites (0.49 [95% CI 0.37,0.60] vs 0.73 [95% CI 0.65,0.81] per 1000 person-years; $P = 0.002$) and among those with no comorbidities (0.44 [95% CI 0.25,0.62]) compared with those with 1–3 comorbidities (0.68 [95% CI 0.59,0.76]; $P = 0.05$) and those with 4 or more comorbidities (0.69 [95% CI 0.57,0.81]; $P = 0.05$).

In a Cox proportional hazards model, factors associated with SARS-CoV-2 infection included increasing age (HR 1.11; 95% CI 1.01,1.23), Black race (HR 0.65; 95% CI 0.50,0.85), and presence of anemia (HR 1.37; 95% CI 1.09,1.73). (Table 2) Increasing number of comorbidities was not associated with a higher risk of infection while other factors demonstrated similar hazards ratios.

**Table 1**
Infection rate per 1000 person-days ≥7 days after second vaccine dose, by subgroups.

| | N | Rate (95% CI) | P-Value |
|---|---|---|---|
| Infection rate, overall | 410 | 0.66 (0.60,0.72) | N/A |
| By age | | | |
| <=40 | 6 | 0.41 (0.08,0.73) | comparator |
| >40 – 60 | 41 | 0.54 (0.37,0.70) | 0.53 |
| >60 – 70 | 96 | 0.60 (0.48,0.72) | 0.36 |
| >70 | 267 | 0.72 (0.637,0.81) | 0.16 |
| By race | | | |
| White | 314 | 0.73 (0.65,0.81) | comparator |
| Black | 70 | 0.49 (0.37,0.60) | 0.002 |
| Other/Unknown | 26 | 0.54 (0.33,0.74) | 0.13 |
| By sex | | | |
| Female | 24 | 0.69 (0.42,0.97) | comparator |
| Male | 386 | 0.66 (0.59,0.72) | 0.81 |
| By comorbidities | | | |
| None | 22 | 0.44 (0.25,0.62) | comparator |
| 1–3 | 257 | 0.68 (0.59,0.76) | 0.05 |
| 4 or more | 131 | 0.69 (0.57,0.80) | 0.05 |
| By vaccine type | | | |
| Pfizer | 266 | 0.69 (0.60,0.77) | comparator |
| Moderna | 144 | 0.62 (0.52,0.72) | 0.32 |

**Table 2**
Factors associated with SARS-CoV-2 infection after vaccination (infection ≥7 days after second vaccine dose; Cox proportional hazards model).

| | Hazards ratio (95% CI) | P-value |
|---|---|---|
| Age (per 10 years increase) | 1.11 (1.01,1.23) | 0.04 |
| Race (comparator: White) | | |
| Black | 0.65 (0.5,0.85) | 0.001 |
| Other/unknown | 0.75 (0.5,1.13) | 0.17 |
| Body mass index >30 (comparator: <30) | 0.91 (0.73,1.12) | 0.36 |
| Comorbidities | | |
| Diabetes | 1.1 (0.9,1.35) | 0.36 |
| Coronary artery disease | 0.97 (0.79,1.2) | 0.78 |
| Chronic kidney disease | 1.11 (0.88,1.39) | 0.38 |
| Chronic lung disease (COPD) | 0.88 (0.72,1.08) | 0.21 |
| Anemia (Hb<13 for men; <12 for women) | 1.37 (1.09,1.73) | 0.01 |
| Cancer diagnosis | 0.86 (0.7,1.05) | 0.14 |
| Human immunodeficiency virus infection | 0.38 (0.1,1.54) | 0.18 |
| Vaccine type (comparator: Pfizer) | | |
| Moderna | 0.82 (0.67,1.01) | 0.06 |

### Discussion

To our knowledge, this is the first study to describe the rate and risk factors for SARS-CoV-2 breakthrough infection in persons who have been fully vaccinated. We found a low rate of infection among those who were fully vaccinated and age, race and anemia to be associated with confirmed infection.

We found relatively few factors associated with infection after vaccination. Increasing age increased the risk, as did presence of anemia at baseline. Increasing age is a well-recognized risk factor for SARS-CoV-2 infection and is also associated with more severe disease and poorer clinical outcomes. Therefore, it is not surprising that it would also be associated with infection after vaccination. Multiple comorbid conditions are also associated with a higher risk and increased severity of infection. The reason for the association of anemia with infection after vaccination while no association was demonstrable other comorbidities is unclear. While we used the standard World Health Organization definition of anemia (i.e. hemoglobin <13 g/dL for men and <12 g/dL for women), this may be too permissive. We did not assess the association of the degree of anemia with the risk of infection. Whether this association is limited to more severe anemia, which may worsen oxygenation, is not known.

Surprisingly, Black race was associated with a lower risk of infection. The reason for this is entirely unclear. It is possible that the

Blacks who were vaccinated were younger and healthier and at a lower risk of infection at the outset. It is equally possible that they were older and less healthy and due to those reasons they were less mobile and therefore less likely to be exposed to persons with confirmed infection. Further studies are warranted to confirm this finding and to understand the reasons for this finding.

Our study has several strengths. We studied a national population with diverse geographical and demographic characteristics who receive care within a single integrated healthcare network. Vaccines, SARS-CoV-2 testing, and clinical care are provided free or cost or with minimal expense to qualified Veterans. The VA created a national database of SARS-CoV-2 infected Veterans using validated definitions and algorithms which is regularly updated and provides a rich resource for clinical and observational studies. Despite these strengths, several limitations need to be noted. Veterans are predominantly male. We did not assess the actual exposure to confirmed cases. We also did not assess the clinical severity of disease and outcomes in our study population, which will be the subject of a subsequent study.

In conclusion, the rate of infection among persons who have been fully vaccinated is low but not insignificant. Increasing age and presence of anemia increase the risk, while Black race is associated with a lower risk. An awareness campaign, particularly targeted to those at risk is needed to mitigate the risk.

## Authorship statement

Dr. Butt had complete access to data at all times and accepts the responsibility of the integrity of this article.

## Disclosures

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## Genomic survey of SARS-CoV-2 vaccine breakthrough infections in healthcare workers from Kerala, India

*Dear Editor,*

Tré-Hardy et al. in this journal recently discussed the immunogenicity of mRNA-1273 in healthcare workers.[1] Vaccines based on different strategies are being deployed across the globe to curb the recurring waves of COVID-19. Of these, inactivated SARS-CoV-2 virus-based BBV152/COVAXIN and adenoviral vector-based AZD1222/Covishield (ChAdOx1 nCoV-19) are widely used in India.[2,3] Breakthrough infections in fully vaccinated individuals have been documented in many countries including India.[4] The emergence of SARS-CoV-2 variants threatens the continued efficacy of these vaccines with increasing reports on reduced efficacy against different SARS-CoV-2 variants of concern (VoC).[5]

We surmise that genomic surveillance is useful to understand and monitor evolving SARS-CoV-2 variants. In this study, we describe the genomic characterization of vaccine breakthrough infections following vaccination in 6 healthcare workers (P1–P6) from Kerala, India. All 6 patients were fully vaccinated with two standard doses of the AZD1222/Covishield vaccine.

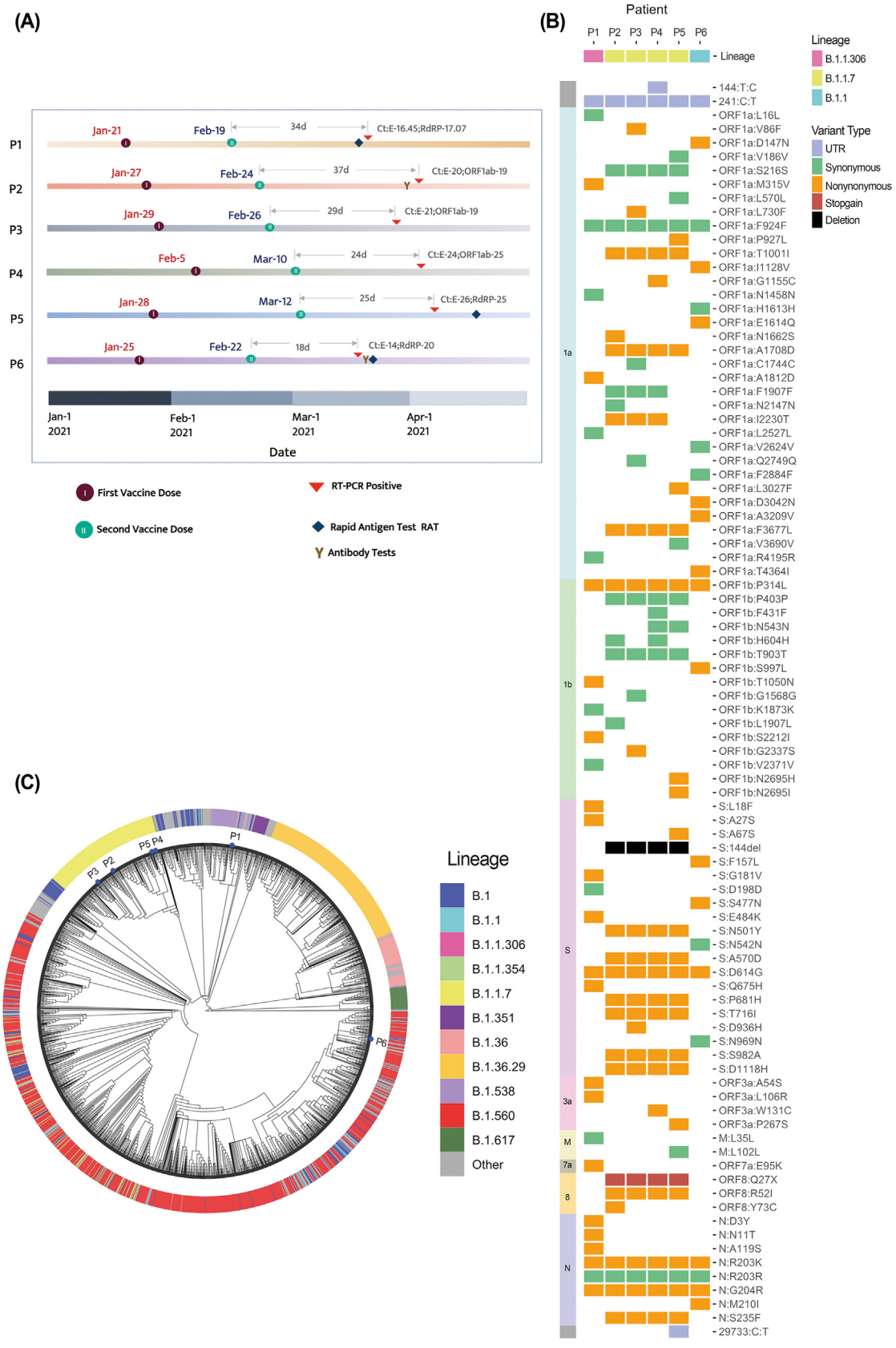


**Fig. 1.** (A) History and timelines of infection for the 6 patients and intervals between the second dose of vaccine and date of RT-PCR testing (B) Presence of variants in the genome isolates of the 6 cases (P1–P6). (C) Phylogenetic context of the 6 genome isolates with 2630 additional SARS-CoV-2 genomes from the state of Kerala.

P1, a 25-year-old female, was administered the first and second dose of vaccine on January 21, and February 19, 2021, respectively. P1 was found to be positive for SARS-CoV-2 antigen on March 23 with influenza-like illness (ILI) symptoms and tested RT-PCR positive for COVID-19 on March 25.

P2, a 50-year-old male, received the two doses on January 27 and February 24 respectively. He developed fever, malaise, anosmia and headache on March 31 and tested RT-PCR positive on April 2. P2 was found to be SARS-CoV-2 IgG positive (12.94) and SARS-CoV-2 IgM negative on March 31.

P3, a 53-year-old female, received the two doses on January 29 and February 26 respectively. She developed rhinitis on March 23 and tested RT-PCR positive on March 27.

P4, a 25-year-old female, received the two doses on February 5 and 10 March respectively. She developed fever, loose stools, abdominal pain, dry cough, myalgia, rhinitis and anosmia on March 27 and tested RT-PCR positive on April 3.

P5, a 32-year-old male, received the two doses on January 28 and March 12 respectively. He tested RT-PCR positive on April 6 and developed mild nasal congestion and headache. P5 tested antigen negative after 10 days.

P6, a 33-year-old female, received the two doses on January 25 and February 22 respectively. She developed loss of smell, loose stools and rhinitis and tested RT-PCR positive on March 12. P6 tested antigen negative after 5 days. Neutralizing antibody titres for P6 were above 320 (S/Co value −14.9) on March 16.

The prognosis of the breakthrough infections in all cases shows the effective protection of the vaccine in preventing severe COVID-19. Fig. 1A summarizes the history and timeline of infection for the 6 patients.

RNA extracted from nasopharyngeal swab samples were collected as part of routine COVID-19 testing after informed consent as per the institutional ethical committee guidelines (IHEC–CSIR-IGIB/IHEC/2020–21/01) for individuals who tested positive following two doses of the AZD1222 vaccine. Antigen assay (Standard Q Covid-19 Ag Kit, SD Biosensor) was carried out in five out of six patients (Supplementary Table 1). Genomes were sequenced on NovaSeq 6000 platform following the COVIDSeq protocol[6] with read length of 100 2́ base pairs. Sequences were assembled using the NC_045512.2 reference genome. Variants were called using VarScan. Phylogenetic clustering for the isolates was done using Nextstrain with additional SARS-CoV-2 genomes isolated from Kerala. Lineages were assigned using pangolin (v2.3.9).[7]

Genomes for the 6 isolates were assembled at a mean genome coverage of 7476.27X. 4 samples (P2-P5) had the spike variant N501Y, while P1 and P6 had spike variants E484K and S477N respectively. N501Y, E484K and S477N are key mutations in the receptor-binding domain of spike protein with substantial evidence reported in the context of immune evasion.[8–10]

Genomic variants present in all 6 isolates are summarized in Fig. 1B.

Isolates P1 and P6 belonged to PANGO lineage B.1.1.306 and B.1.1 respectively. P2-P5 belonged to the lineage B.1.1.7 (VOC 202012/01), defined by 6 key spike variants including N501Y. Phylogenetic context of P1–P6 with 2630 genome sequences from Kerala is summarised in Fig. 1C. P1–P5 clustered closely with other genomes from their respective lineages. Isolate P6 clustered near genomes belonging to the lineage B.1.560 which was the most prevalent lineage ($N = 1130$) in additional genomes included in the analysis.

All 6 patients in the study were vaccinated at an interdose interval range of 4–6 weeks and COVID-19 symptoms were observed in all at least 15 days post second dose. Considering the efficacy of AZD1222 against symptomatic COVID-19 following two standard doses is 63%,[3] a small percentage of fully-vaccinated people may still get infected, however, it is important to note that none of the 6 patients presented with severe illness or required hospitalization. Characterization of clinically important SARS-CoV-2 variants in vaccinated individuals confers possible exploration of selection of viral escape mutants following immunization. Genome sequencing revealed that 4 patients in this study were infected by the B.1.1.7 variant of SARS-CoV-2. N501Y, a key mutation in the B.1.1.7 lineage has been reported to escape neutralization by some monoclonal antibodies (mAbs), and a small decrease in neutralization activity in patients vaccinated with Moderna (mRNA-1273) or Pfizer–BioNTech (BNT162b2).[10] B.1.1.7 has also been shown to lower neutralising antibody titres against AZD1222 as compared to non-B.1.1.7 variants.[5] Both E484K and S477N, found in P1 and P6 respectively, are reported to escape neutralization by a range of mAbs. E484K is also associated with a decrease in neutralizing activity of convalescent and post-vaccination (BNT162b2) sera.[8–10] While it remains unclear if these breakthrough infections are related to vaccine efficacy, immune evasion, or other factors, the study highlights the importance of continued genomic surveillance for tracking emergent SARS-CoV-2 variants.

## Declaration of Competing Interest

The authors report no potential conflicts of interest.

## Data for reference

Genome sequences for the viral isolates P1-P6 have been deposited to GISAID (Accession IDs: EPI_ISL_2000674, EPI_ISL_2000675, EPI_ISL_2000676, EPI_ISL_2000677, EPI_ISL_2000678, and EPI_ISL_20006749 respectively).

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## Seroprevalence of SARS-CoV-2 antibodies in a national hospital and affiliated facility after the second epidemic wave of Japan

*Dear Editor,*

### Introduction

Healthcare workers (HCWs) are at high risk for coronavirus disease (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1,2] However, relative to the general population, there was no increase in the infection risk

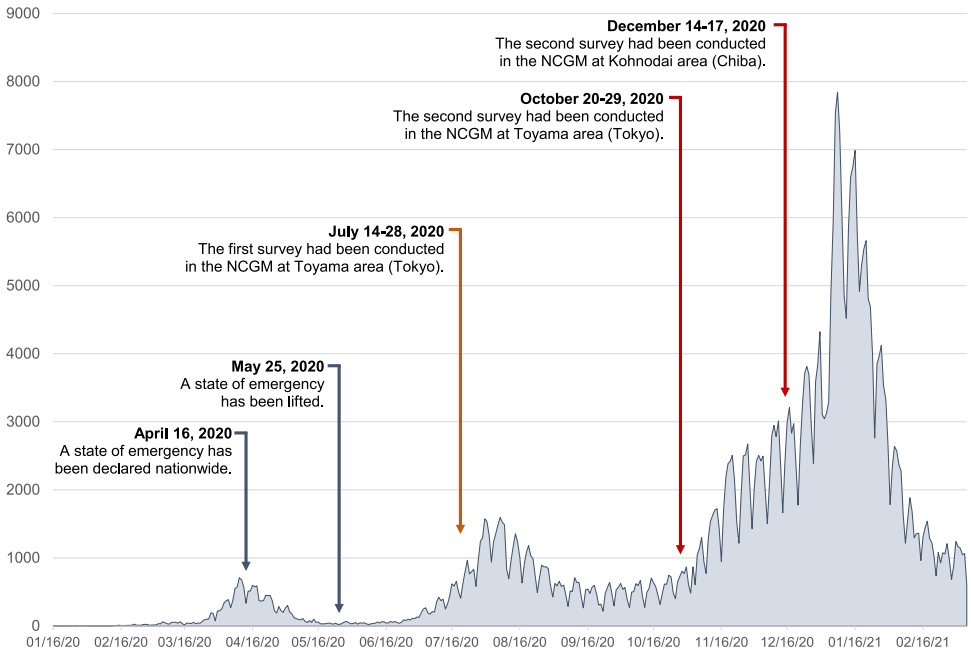


**Fig. 1.** Change in the number of patients diagnosed with COVID-19 in Japan.

among HCWs in hospitals with adequate control measures against the infection.[3] Studies on the source of infection among HCWs showed a stronger association with community factors than occupational factors,[4–6] suggesting the importance of infection prevention outside the hospital. Although Japan recorded a relatively high number of COVID-19 cases in Asia, data on SARS-CoV-2 infection and its source among HCWs are limited.

The National Center for Global Health and Medicine (NCGM) has played a leading role in patient care and COVID-19 research since the early phase of the epidemic in Japan. Additionally, the staff were involved in screening for returnees from Wuhan, infection control on the Diamond Princess cruise ship, and running a fever clinic and local polymerase chain reaction (PCR) testing center.[7] To estimate the cumulative SARS-CoV-2 infection rate over time, we designed a repeat seroprevalence study among the NCGM staff. Previously, we reported a very low seroprevalence of SARS-CoV-2 IgG antibody (0.16%) as of July 2020, after the first COVID-19 wave in Japan.[8] Here, we report the seroprevalence and its related factors in a follow-up survey after the second, larger wave (Fig. 1).

## Methods

We invited all NCGM staff (Toyama and Kohnodai areas) and asked participants to complete a questionnaire and donate venous blood in October (Toyama) and December (Kohnodai) 2020. We collected data on demographics, occupational factors, close contact with patients with COVID-19, symptoms indicative of COVID-19, PCR testing results, use of public transportation, and adherence to infection prevention practices (IPPs). We qualitatively measured IgG (Abbott ARCHITECT®) and total antibodies (Roche Elecsys®) against the SARS-CoV-2 nucleocapsid protein, according to the manufacturers' instructions at an in-house (Toyama) or external laboratory (Kohnodai). We performed a confirmatory analysis of seropositive samples on either test with the EUROIMMUN anti-S IgG immunoassay. If it was positive, neutralizing antibody titers were measured using the live virus (Supplemental Text). Written informed consent was obtained from each participant. This study was approved by the ethics committee of NCGM.

Seropositivity was defined as positivity of either test (sensitivity priority). Seroprevalence with 95% confidence intervals (CI) were calculated using the exact binomial technique. We performed Poisson regression with a robust variance estimator to assess the association between exposure variables and seropositivity. Participants who had both tests positive were classified as being seropositive (specificity priority).

## Results

Of 2,893 staff invited, 2,563 (88.6%) participated. The major occupations included nurses (36%), doctors (16%), allied healthcare professionals (14%), and administrative staff (11%). Nearly half of the participants (47.6%) had been engaged in COVID-19-related work (Table 1). The adherence to the recommended IPPs was quite high (e.g., cough etiquette [99.8%], washing or sanitizing hands [99.3%], and wearing a mask [98.8%]) (Fig. S1).

Eighteen staff had one positive test (10 on Abbott and 13 on Roche), giving a seroprevalence of 0.70% (95% CI: 0.42–1.11). None of them belonged to the same department. Using the second definition (two positive tests), only 5 were seropositive (seroprevalence: 0.20%, 95% CI 0.06–0.45). Of the seropositive staff, 8 (44%) were positive on the EUROIMMUN assay, but none had a neutralizing antibody.

A history of loss of taste and smell and PCR testing were associated with an increased seropositivity rate. Close contact with patients with COVID-19 at home and in the community (family members, cohabitants, acquaintances, or friends), but not in the hospital (coworker or patients), was associated with seropositivity. The seropositivity rate was not high among those working in the COVID-19 ward or engaged in COVID-19-related work (Table 1).

## Discussion

After the second COVID-19 wave in Japan, the seroprevalence rate among the NCGM staff remained low (0.70%), which was even lower than those of the general population in Tokyo during the same period (1.94%, recalculated according to the definition used in this study).[9]

**Table 1.**
Seroprevalence of SARS-CoV-2 antibodies by participants' characteristics.

| Characteristics[a] | Total participants, No. | Participants with seropositive | | Prevalence Ratio (95% CI) |
|---|---|---|---|---|
| | | No. | % (95% CI) | |
| **Total, No.** | 2563 | 18 | 0.70 (0.42–1.11) | |
| **Location of workplace** | | | | |
| Tokyo | 2054 | 16 | 0.79 (0.45–1.26) | 1.0 [reference] |
| Chiba | 509 | 2 | 0.39 (0.05–1.41) | 0.50 (0.12-2.19) |
| **Sex** | | | | |
| Male | 779 | 7 | 0.94 (0.38–1.93) | 1.0 [reference] |
| Female | 1784 | 11 | 0.64 (0.32–1.14) | 0.68 (0.26-1.75) |
| **Age range, year** | | | | |
| <30 | 797 | 8 | 1.05 (0.46–2.07) | 1.0 [reference] |
| 30-39 | 633 | 1 | 0.17 (0.00–0.93) | 0.16 (0.02–1.27) |
| 40-49 | 596 | 3 | 0.52 (0.11–1.50) | 0.49 (0.13–1.84) |
| ≥50 | 537 | 6 | 1.13 (0.42–2.44) | 1.07 (0.37–3.07) |
| **Job category** | | | | |
| Doctors | 410 | 2 | 0.49 (0.06–1.75) | 1.0 [reference] |
| Nurses | 921 | 8 | 0.87 (0.38–1.70) | 1.78 (0.38–8.35) |
| Allied healthcare professionals | 362 | 3 | 0.83 (0.17–2.40) | 1.70 (0.29–10.11) |
| Administrative staff | 284 | 1 | 0.35 (0.01–1.95) | 0.72 (0.07–7.93) |
| Others | 492 | 4 | 0.81 (0.22–2.07) | 1.67 (0.31–9.06) |
| **Department** | | | | |
| Non-medical departments | 551 | 4 | 0.73 (0.20–1.85) | 1.0 [reference] |
| The other medical departments | 1619 | 11 | 0.68 (0.34–1.21) | 0.91 (0.36–2.31) |
| COVID-19-related departments | 299 | 3 | 1.00 (0.21–2.90) | 0.92 (0.23–3.66) |
| **The risk of SARS-CoV-2 infection at work**[b] | | | | |
| Low | 1184 | 12 | 1.01 (0.52–1.76) | 1.0 [reference] |
| Moderate | 690 | 3 | 0.43 (0.09–1.27) | 0.43 (0.12–1.52) |
| High | 595 | 3 | 0.50 (0.10–1.47) | 0.50 (0.14–1.76) |
| **Engagement in COVID-19-related work** | | | | |
| Screening of returnees of the charter flight from Wuhan | 119 | 0 | 0.00 (0.00–3.05) | NA |
| Infection control on the cruise ship | 48 | 0 | 0.00 (0.00–7.40) | NA |
| COVID-19 testing center, fever consultation clinic | 178 | 0 | 0.00 (0.00–2.05) | NA |
| Care facility for COVID-19 patients with mild symptom | 34 | 0 | 0.00 (0.00–10.28) | NA |
| Works done within 1 m of COVID-19 patient | 798 | 4 | 0.50 (0.14–1.28) | 0.60 (0.20–1.81) |
| Works done at 1 m or more of COVID-19 patient | 491 | 3 | 0.61 (0.13–1.78) | 0.81 (0.23–2.77) |
| SARS-CoV-2 laboratory testing | 147 | 1 | 0.68 (0.02–3.73) | 0.93 (0.12–6.94) |
| Handling SARS-CoV-2 other than testing | 194 | 2 | 1.03 (0.13–3.67) | 1.47 (0.34–6.33) |
| Cleaning, laundry, sterilization, waste disposal | 315 | 2 | 0.63 (0.08–2.27) | 0.85 (0.20–3.70) |
| Fever screening of outpatient and visitors | 198 | 0 | 0.00 (0.00–1.85) | NA |
| Others | 96 | 1 | 1.04 (0.03–5.67) | 1.45 (0.20–10.8) |
| Any of the above | 1176 | 6 | 0.51 (0.19–1.11) | 0.55 (0.21–1.46) |
| **Symptom indicative of COVID-19** | | | | |
| Common cold-like symptom lasting 4 days or longer | 327 | 4 | 1.22 (0.33–3.10) | 1.85 (0.61–5.60) |
| High fever | 117 | 2 | 1.71 (0.21–6.04) | 2.49 (0.58–10.70) |
| Severe fatigue | 197 | 2 | 1.02 (0.12–3.62) | 1.43 (0.33–6.17) |
| Dyspnea | 64 | 1 | 1.56 (0.04–8.40) | 2.19 (0.30–16.22) |
| Loss of sense of taste or smell | 25 | 3 | 12.0 (2.55–31.22) | 19.4 (5.98–38.4)[c] |
| **History of previous PCR testing** | | | | |
| No | 2075 | 12 | 0.58 (0.30–1.01) | 1.0 [reference] |
| Yes | 393 | 6 | 1.53 (0.56–3.29) | 2.64 (2.05–3.39)[c] |
| **Close contact with COVID-19 cases** | | | | |
| Contact in the hospital[d] | 94 | 1 | 1.06 (0.03–5.79) | 0.67 (0.09–5.00) |
| Contact at home and in the community[e] | 5 | 2 | 40.0 (5.27–85.34) | 61.6 (18.9–200.4)[c] |
| **Use of public transportation** | | | | |
| <1 times/wk | 888 | 9 | 1.01 (0.46–1.92) | 1.0 [reference] |
| ≥1 times/wk | 1563 | 9 | 0.58 (0.26–1.09) | 0.57 (0.23–1.43) |

Abbreviations: COVID-19, coronavirus disease 2019; NA, not applicable; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

[a] The number of missing data for each characteristics was as follows: job category (94), department (94), the risk of SARS-CoV-2 infection at work (94), engagement in COVID-19-related work (94), symptom indicative of COVID-19 (111), history of previous PCR testing (95), close contact with COID-19 cases (95), and use of public transportation (112).

[b] Categorized as follows: low (those who were not engaged in COVID-19-related work), moderate (those who were engaged in COVID-19-related work without heavy exposure to the virus), and high (those who were heavily exposed to SARS-CoV-2).

[c] $P < 0.05$ (2-tailed).

[d] Close contact with COVID-19-positive patients or coworkers.

[e] Close contact with COVID-19-positive family members, cohabitants, friends, or acquaintances.

We found no evidence of clustering of seropositive staff in the center and no significant association between occupational factors and seropositivity. These data refute an increased risk of inpatient-to-HCW and HCW-to-HCW transmission in hospitals well prepared for COVID-19. NCGM has introduced and strengthened multiple infection control measures since the early phase of the epidemic, including the provision of personal protective equipment, universal masking, hand washing, and routine checking of staff's body temperature, and PCR testing in case of suspected infection.[8] These results support the effectiveness of these measures against infection associated with occupational exposure.

Regarding non-occupational factors, close contact with patients with COVID-19 at home and in the community was associated with increased seropositivity. Given few seropositive staff who had close

contact in these settings ($n = 2$, 11% of seropositive staff), it is reasonable to assume that the primary route of infection might be unrecognized contact with asymptomatic cases in the community. The NCGM is located in an epicenter of the second wave; therefore, the infection control division sends e-mails to all staff weekly to enhance their awareness of preventive behaviors,[8] leading to high adherence to the recommended IPPs by the staff (Fig. S1). With the correlation between the infection rate of HCWs and the cumulative community incidence,[4,10] there is need for more emphasis on the prevention of community-acquired infection in preventing nosocomial infection.

This study provides more evidence on the contribution of comprehensive control measures targeting both occupational and community risk of SARS-CoV-2 infection in the protection of HCWs from infection during the epidemic.

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## Role of the funder/sponsor

The funder did not play any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

## Declaration of Competing Interest

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## Mucormycosis—A serious threat in the COVID-19 pandemic?

*Dear Editor,*

We read with great interest the work by Lansbury et al.[1], who perform systematic review and meta-analysis concerning on co-infection in people with COVID-19. At the end of 2019, a new type of coronavirus appeared in China - SARS-CoV-2, which, rapidly spreading around the World, has become a huge challenge for the health care system[2]. The classic picture of COVID-19 disease may vary in severity, from very mild/asymptomatic to life-threatening pneumonia accompanied by bacterial or fungal co-infections[3,4]. There have been reports of the development of severe opportunistic infections such as Gram negative bacteria, *Staphylococcus aureus*, oropharyngeal candidiasis, *Pneumocystis jiroveci* pneumonia (PCP), pulmonary aspergillosis, bloodstream candida infections in patients undergoing COVID-19. Opportunistic infections are especially common in patients who, apart from the current COVID-19 disease, also have other comorbidities such as diabetes or COPD. An additional factor contributing to exposure to co-infections is treatment with mechanical ventilation, antibiotic therapy, monoclonal antibodies and the use of corticosteroids. Especially corticosteroids are commonly used to treat serious form of COVID-19 disease and reduce the damage caused by the own body's immune system during SARS-CoV-2 infection. Unfortunately, corticosteroids are also immunosuppressive and increase blood sugar levels in both diabetic and non-diabetic patients. Both of these effects are now believed to contribute to mucormycosis[5]. Recently, the Indian Council of Medical Research (ICMR) recommended that doctors and medical facilities should pay special attention to signs of mucormycosis such as sinus pain, nasal obstruction on one side of the face, one-sided headache, swelling or numbness, toothache, and loosening of the teeth. Mucormycosis usually leads to discoloration or reddening of the nose, blurred or double vision, chest pain, coughing up blood and difficulty breathing which is an additional very heavy burden for COVID-19 patients. The International Diabetes Federation has determined that India has a very high incidence rate of type 2 diabetes (8.9% adults, 77 million patients)[6]. According to the World Health Organization, 2% of all deaths in India are due to diabetes, diabetes itself poses a risk of a very severe course of COVID-19 and is associated with higher in-hospital mortality[7]. Diabetes, being closely related to mucormycosis infection and a much higher risk of SARS-CoV-2 infection, may have tragic consequences for the local community.

In India, where apart from a high percentage of diagnosed diabetes, there are still many people who do not receive health care and do not undergo diagnostics. Mucormycosis with COVID-19 infection can be a very serious problem for them. Hospitals that are overloaded and no longer have places to spread further promote the spread of mycoses, the widespread use of steroids and broad-spectrum antibiotics to combat COVID-19 may lead to the development or significant exacerbation of pre-existing fungal diseases. A very important issue is the high incidence of mucormycosis in India, which is about 0.14 cases per 1000 inhabitants (about 80 times more often than in developed countries) - so secondary invasions with a huge primary occurrence can have dramatic effects[8]. Studies have also shown an increased incidence with a fairly severe course of mycormycosis, in patients with a history of COVID-19, the most common infection was the sinuses (100%), intraorbital dilation was observed in 43.47% of cases, while intracranial dilation was seen only in 8.69%. Diabetes mellitus occurred in over 91% of cases and was not controlled in over 52% of cases. All patients have used steroids in the past while being treated with the COVID-19[9].

Healthcare professionals should pay special attention to the possibility of invasive secondary fungal infections in patients with COVID-19 infection. Moreover, the use of therapeutic measures should be carefully monitored to achieve a therapeutic effect with the lowest possible dose in the shortest possible time, in line with the gold standard of treatment in order to minimize reduction of the patient's immunity, also the mucormycosis infection itself in the course of COVID-19 and after it should be further investigated.

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## Genomic evidence of SARS-CoV-2 reinfection case with the emerging B.1.2 variant in Brazil

*Dear Editor,*

We read with interest the recently published manuscript of Santos et al., about evidence of reinfection and enhanced severity in Brazilian healthcare worker[1] and here we report the first confirmed case of SARS-CoV-2 reinfection of a 29-year-old male, medical doctor, from Minas Gerais state, Southeast Brazil.

The duration of acquired immunity conferred by infection with SARS-CoV-2 is still poorly understood and recently released data suggest that having COVID-19 may not protect against getting infected again with some of the new variants, evoking the nightmare of a never-ending pandemic.

Since the report of the first confirmed case of COVID-19 on 26 February 2020 in São Paulo (SP) state, Brazil, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has affected more than 12 million people and to date, has caused approximately 300 thousand deaths in Brazil. [2] Infection with SARS-CoV-2 leads to detectable, short-lasting, IgG responses[3,4] likely to provide protection to reinfection. Nonetheless, susceptibility of previously infected individuals to reinfection, due to the circulation of different SARS-CoV-2 variants and lineages,[5–7] is now starting to be considered a growing concern.

Here, we present the first confirmed case of SARS-CoV-2 reinfection in Minas Gerais state, presenting two distinct COVID-19 illnesses from genetically distinct SARS-CoV-2 variants, including the emerging B.1.2 lineage in Brazil. Identifying cases of SARS-CoV-2 reinfection is essential to better understand the course of the COVID-19 pandemic, to monitor the evolution of population herd-immunity, and to guide strategies for vaccine development.

A 29-year-old male, medical doctor, resident in Sabará, Minas Gerais state, southeast Brazil, with no comorbidities, presented two clinical episodes of SARS-CoV-2 infection separated by a 225-day interval (**Fig. 1 panel A**).

In the first episode on May 21st, 2020 the patient presented fever, myalgia, cough, sore throat, and diarrhea for approximatively 10 days (**Fig. 1 panel A**). Two months after testing positive by RT-PCR in the first episode, an IgG test against S1 protein by chemiluminescence, was performed and showed a positive result (index value: 5.07 on 07/08/2020), followed by a negative IgG assay on Mid-December 2020.

The patient's symptoms returned on January 4th, 2021, after returning from holiday from Rio de Janeiro, when a second nasopharyngeal swab (on January 06th, 2021) (**Fig. 1 panel A**) was obtained and presented a positive result for SARS-CoV-2 infection by re-al-time RT-PCR testing.

Viral RNA was extracted from nasopharyngeal swabs and tested for SARS-CoV-2 using the protocol established by the Center for Disease Control and Prevention that targets the Nucleocapsid gene (CDC, Atlanta).[8] On both occasions, results of RT-PCR tests targeting 2 genes (N1 and N2) were positive for SARS-CoV-2. Antibody testing (IgG) after the first and the second episode was performed by chemiluminescence (Alinity$^{TM}$, Abbott).

Cycle threshold values (Cts) of N1, and N2 targets were 15.7, and 18.9 in the first episode and 17.6, and 19.6 in the second episode. In early February 2021, a second positive IgG assay was also detected (index value: 7,58) (**Fig. 1 panel A**).

Genome sequencing was then conducted by PGM Ion Torrent (Life Technologies, USA) and a total of 1.486.791 mapped reads for sample A and 1.228.341 reads for sample B were obtained, resulting in a sequencing mean depth >1000 for both samples and a coverage > 99%.

The distinct viral origin of the two infections was evaluated by combining our new isolates (EPI_ISL_1182550 and EPI_ISL_1182549) with $n = 3852$ representative full-length viral genomes available on GISAID (https://www.gisaid.org/) up to March 23rd, 2021, with which phylogenetic inference was performed. Low-quality genomes (> 10% of ambiguous positions) were excluded. Sequences were aligned using MAFFT[9] and submitted to IQ-TREE for maximum likelihood (ML) phylogenetic analysis.[10] The statistical robust-ness of individual nodes was determined using the SH- aLTR test.

Sequence data and phylogenetic analysis, indicated that the two COVID-19 episodes, were indeed caused by different SARS-CoV-2 lineages, confirming reinfection. In the first episode, the lineage B.1.1.28 was detected and genomic sequence analysis identified $n = 7$ mutations ORF1ab: P4715L and M6078I; Spike: D614G and V1176F; Nucleocapsid: R203K and G204R; ORF14: G50N. In the second infection, the B.1.2 lineage was detected for the first time in Brazil (**Fig. 1 panel B**) which showed $n = 15$ mutation ORF1ab (**Fig. 1 panel C**): T265I, M2606I, L3352F, P4075S, A4489V, P4715L, N6054D, T6938I, R7014C and T265I; Spike: D614G; ORF3a: Q57H and G172V; ORF8: S24L; Nucleocapsid: P67S.

In conclusion, our case report describes the first individual in Minas Gerais state to have symptomatic reinfection with SARS-CoV-2 with no increases in symptom severity from the first to the second episode. Our study reports the first detection of the B.1.2 lineage in Brazil, which is mainly circulating in North America, reinforcing how the high connectivity of countries can mediate the introduction of new viral strains. Considering the recent concern of the rapid rise (starting from late January 2020) of the B.1.2 infections carrying a substitution affecting amino acid position 677 of the Spike protein,[7] our findings reinforce the need for active monitoring of travelers, to follow the real-time spread of new SARS-CoV-2 variants with possible implications for public health policies and immunization strategies.

### Ethical approval

This research was approved by the Ethics Review Committee of the Federal University of Minas Gerais (CEP/CAAE: 32912820.6.1001.5149 approval number). The availability of these samples for research purposes during outbreaks of national concern is allowed to the terms of the 510/2016 Resolution of the National Ethical Committee for Research – Brazilian Ministry of Health (CONEP - Comissão Nacional de Ética em Pesquisa, Ministério da Saúde), that authorize, without the necessity of an informed consent, the use of clinical samples collected in the Brazilian Central Public Health Laboratories to accelerate knowledge building and contribute to surveillance and outbreak response.

### Data availability

Newly generated SARS-CoV-2 sequences have been deposited in GISAID under accession numbers EPI_ISL_1182550 and EPI_ISL_1182549.

### Declaration of Competing Interest

The authors declare no conflict of interest.

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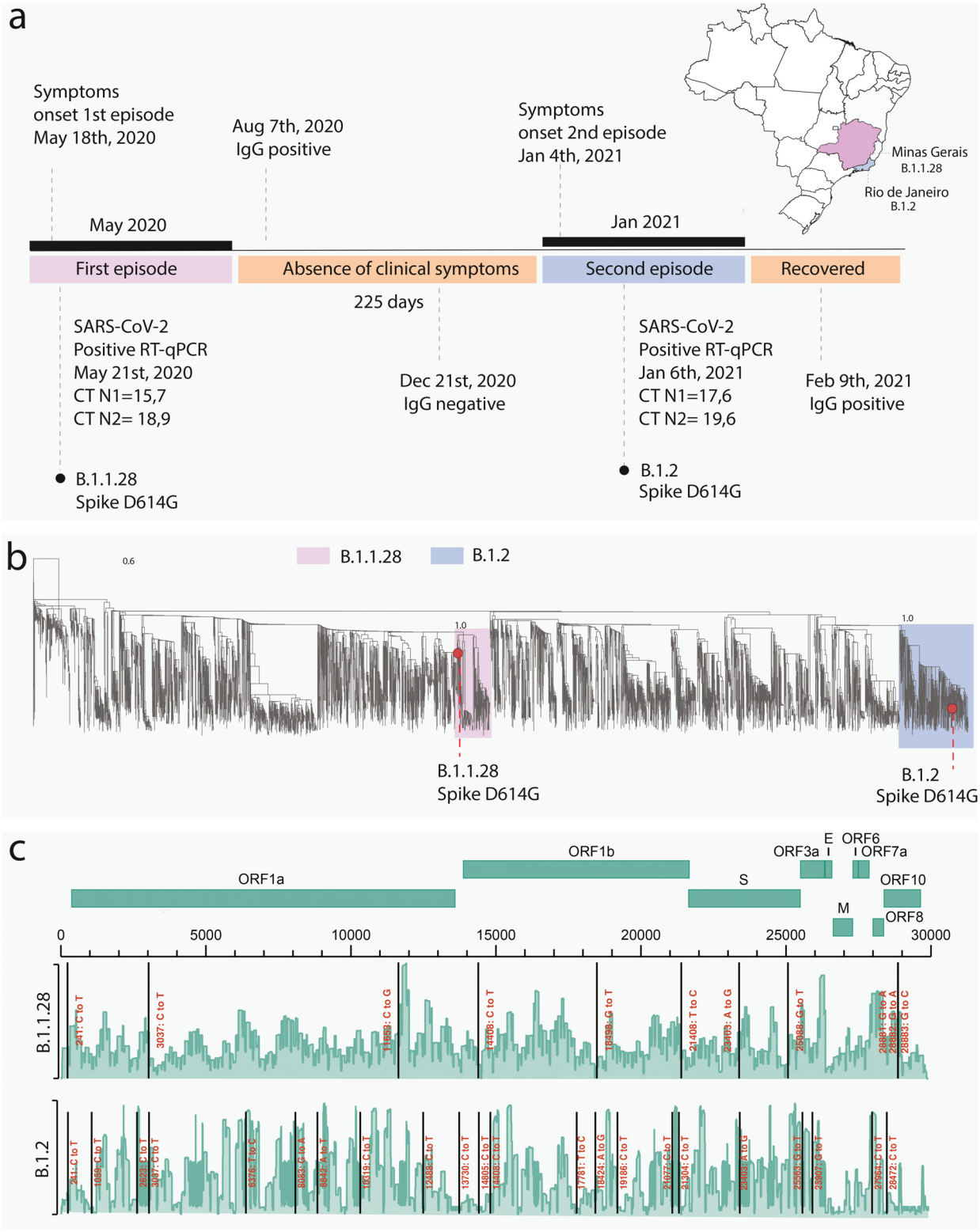


**Fig. 1.** Genomic characterization of a COVID-19 reinfection case in Minas Gerais state, Southeast Brazil. (A) Timeline of symptom onset, molecular diagnosis, and sequencing of specimens; (B) ML tree including the newly SARS-CoV-2 genomes (EPI_ISL_1182550 and EPI_ISL_1182549) recovered from a 29-year-old male resident in Sabará, Minas Gerais state, Southeast Brazil, with $n = 3852$ representative full-length viral genomes available on GISAID (https://www.gisaid.org/) up to March 23rd, 2021. New genomes are highlighted with red circles. Branch support (SH-aLTR >0.8) is shown at key nodes; (C) Variant mapping of specimens recovered from the first and the second episode (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

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## Are antigenic tests useful for detecting SARS-CoV-2 infections in patients accessing to emergency departments? Results from a North-West Italy hospital

*Dear Editor,*

In the article “Clinical application of a rapid antigen test for the detection of SARS-CoV-2 infection in symptomatic and asymptomatic patients evaluated in the emergency department: A preliminary report.”,[1] Turcato et al. presented a study on the use of rapid antigenic tests (Ag-RDTs) instead of the usual real time reverse transcription polymerase chain reaction (RT-PCR) assay to de-

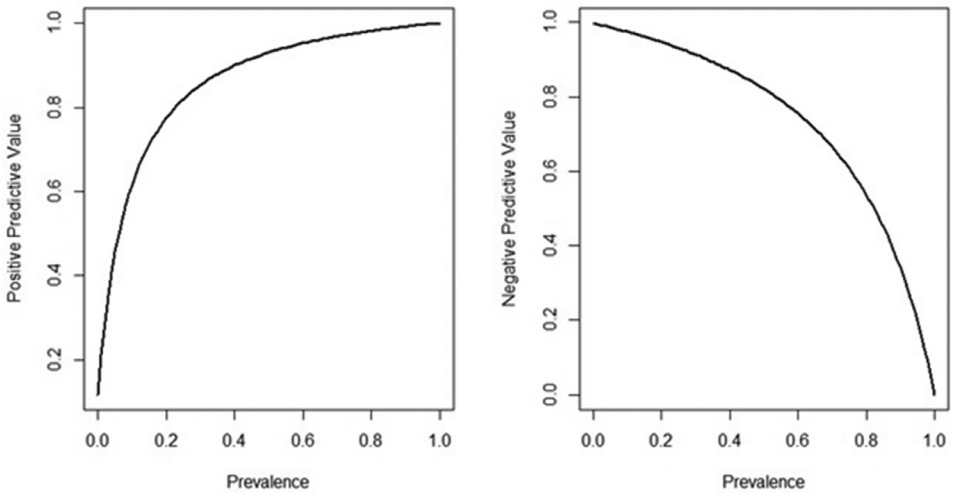


**Fig. 1.** Positive and negative predictive value estimates in relation to prevalence, using the sensitivity and specificity of the test found in our population (sensitivity = 0.800; specificity = 0.939).

tect the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in the context of Emergency Departments (ED). They observed a general good sensitivity and specificity, lower in the subgroup of asymptomatic patients. Their conclusion is in favour of the use of Ag-RDTs in EDs as an additional tool to address the challenge of containing the SARS-CoV-2 pandemic.

We agree with the authors that the development of reliable but cheaper and faster point-of-care diagnostic tests was expected to be useful either for population-screening or as first aid tests in the emergency room.[2,3] Data on the sensitivity and specificity of currently available Ag-RDTs derive from studies that vary in design, setting, population and type of specimen, thus strongly limiting the comparability and ability to make general inferences. Sensitivity appears to be highly variable, ranging from 29 to 94% compared to the RT-PCR test, but specificity is consistently high (>97%).[4–7] Ag-RDTs were found to perform better in patients with high viral loads (Ct values ≤25 or >106 genomic virus copies/mL)[5,7,8] which usually happens in the pre-symptomatic (0.5–3 days before symptom onset) and early symptomatic phases of the illness (within the first week from symptom onset) but limited data are available about other possible individual modifiers of the accuracy of the assay. A recent Cochrane review highlighted that patients' characteristics were not available or poorly detailed in many studies, with only three out of 22 studies coming from an ED setting.[8]

Between October 26th and November 10th 2020, 455 patients accessed the ED of San Luigi Gonzaga University Hospital in Orbassano (Turin, Italy) and 324 underwent both RT-PCR and Ag-RDT testing. This period corresponds to the first two weeks of the second pandemic wave, with a weekly incidence of SARS-CoV-2 infection in the Region of about 500 confirmed cases/100,000 inhabitants. Data were obtained as part of an observational study described elsewhere[9] and a detailed presentation of methods is available in supplementary material.

The prevalence of SARS-CoV-2 infection in this cohort was 65% measured using RT-PCR as a gold standard. Supplementary Table 1 reports test results: 275 (84%) patients showed concordant results (168 positive and 107 negative), while 49 (15%) showed discordant results (42 patients had a positive RT-PCR and a negative Ag-RDT and 7 vice versa). Cohen's Kappa Statistics ($k = 0.68$ – 95% CI 0.61–0.77) highlighted substantial agreement. Specificity and sensitivity of Ag-RDT were 0.939 (95% CI: 0.895–0.983) and 0.800 (95% CI: 0.746–0.854), respectively, taking RT-PCR as the reference. Overall, the Ag-RDT positive predictive value was 0.960 (95% CI 0.931–0.989), and the negative predictive value was 0.718 (95% CI: 0.646–0.790). The variation of positive and negative predictive values due to difference in prevalence can be observed in Supplementary Table 2 and Fig. 1. Positive predictive value could vary from 0.12, when the prevalence of the disease is 0.01, to 0.77 when the prevalence is 0.20. The negative predictive value could vary from about 1, considering a low prevalence (0.01) to 0.95, considering a higher prevalence (0.20).

No difference in patients' characteristics between true positive and false negative tests was observed (Supplementary Table 3). On the contrary, false negative patients were significantly younger and they were tested significantly later after symptoms onset compared with true negative patients (Table 1). Moreover, fever (64.3% vs 19.6%, $p < 0.0001$) and cough (42.9% vs 15.0%, $p = 0.0003$) were significantly more frequent in false than true negatives, while chronic obstructive pulmonary disease was more frequent in true than false negatives, with a borderline significance (16.5% vs 4.8%, $p = 0.06$). Few true negative patients had bilateral pneumonia ($n = 10$, 9.4%), that was highly present in false negative patients ($n = 25$, 61.0%, $p$-value for difference $< 0.0001$) and multivariable analysis confirm these results, suggesting that wrong group allocation for negative patients occurred more frequently in patients with fever, cough, and pneumonia, while it was less likely in patients with COPD.

The infection prevalence and the clinical context where the test is used affect the effectiveness of the test itself[10]: the ideal test in a crowded ED context should help in identifying asymptomatic patients arriving to the ED for reasons other than COVID-19, who are concurrently found COVID-19 positive.

Our results suggest that a negative Ag-RDT test should not exclude COVID-19 in patients that clinically have symptoms that are strongly suggestive of COVID-19. Ag-RDTs alone had a low negative predictive value (we cannot trust a negative result of the test), thus they need to be evaluated in association with clinical judgement. A high level of suspicion should be maintained in patients with fever, cough or pneumonia notwithstanding a negative Ag-RDT. Since the predictive value is strictly related to the prevalence of disease, and then to the pre-test odds, Ag-RDTs are not really useful in settings where the prevalence of disease is high or in patients with high pre-test odds. On the contrary, in periods with low prevalence of the disease or in patients with a low pre-test odds (asymptomatic) or with symptoms probably related to a known COPD, Ag-RDTs can be used alone and we can trust a negative result.

In conclusion, our results confirm the limits of antigenic tests as first line screening tests in settings with high prevalence of disease

**Table 1**
Ag-RDT negative patients: comparison of patients' characteristics between true negative and false negative patients. Wilcoxon sum rank test (quantitative variables) and chi-square or Fisher's exact test (qualitative variables) are used and multivariable logistic model (including significant variables) to evaluate the association between being a false negative and patients' characteristics.

| | True negative ($n = 107$)*Mean (SD), medianor Frequency (%)* | False negative ($n = 42$)*Mean (SD), medianor Frequency (%)* | *P*-values | OR(95% CI) |
|---|---|---|---|---|
| Age, years | 68.4 (18.6), med: 74.4 | 63.1 (16.3), med: 64.4 | 0.03 | *For 1 year increase* 1.00 (0.96 – 1.03) |
| Days from symptoms onset | 3.9 (6.7), med: 2 | 6.3 (4.7), med: 6 | 0.0003 | *For 1 day increase* 1.06 (0.97 – 1.16) |
| NEWS at arrival | 2.1 (3.0), med: 1 | 2.4 (2.5), med: 2 | 0.14 | |
| *Symptoms* | | | | |
| Fever | 21 (19.6%) | 27 (64.3%) | <0.0001 | 4.31 (1.30 – 14.28) |
| Cough | 16 (15.0%) | 18 (42.9%) | 0.0003 | 5.72 (1.63 – 20.07) |
| Dyspnoea | 33 (30.8%) | 16 (38.1%) | 0.40 | |
| Respiratory failure | 16 (15.1%) | 8 (19.1%) | 0.56 | |
| Gastrointestinal symptoms | 29 (27.1%) | 7 (16.7%) | 0.18 | |
| Anosmia | 0 (0.0%) | 2 (4.8%) | 0.07* | |
| Ageusia | 6 (5.6%) | 3 (7.1%) | 0.72 | |
| Asthenia | 15 (14.0%) | 11 (26.2%) | 0.08 | |
| *Comorbidities* | | | | |
| Obesity | 5 (6.2%) | 1 (2.8%) | 0.66* | |
| Hypertension | 43 (41.7%) | 13 (31.0%) | 0.22 | |
| Diabetes | 17 (16.5%) | 5 (11.9%) | 0.48 | |
| Heart disease | 26 (25.2%) | 7 (16.7%) | 0.26 | |
| COPD | 17 (16.5%) | 2 (4.8%) | 0.06* | 0.12 (0.01 – 1.29) |
| Cancer | 18 (17.5%) | 4 (9.5%) | 0.31* | |
| immunosuppression | 8 (7.8%) | 1 (2.4%) | 0.45* | |
| neurological disease | 14 (13.7%) | 4 (9.5%) | 0.59* | |
| *Pneumonia* | | | | |
| No | 87 (82.1%) | 12 (29.3%) | <0.0001 | Reference |
| Monolateral | 9 (8.5%) | 4 (9.8%) | | 4.12 (0.59 – 28.60) |
| Bilateral | 10 (9.4%) | 25 (61.0%) | | 14.89(4.14 – 53.52) |

* Fisher's exact test.

or in patients with high pre-test odds, where a negative test is not informative (i.e. in ED in a pandemic period). This suggests that in these situations the antigenic test should be integrated in a clinical algorithm.

## Declaration of Competing Interest

All authors declare no conflict of interests.


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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.05.012.

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## Clinical efficacy of nitric oxide nasal spray (NONS) for the treatment of mild COVID-19 infection

*Dear Editor,*

### Summary

Baek et al.[1] investigated the duration of COVID-19 virus shedding in infected patients and demonstrated that even in patients demonstrating prolonged viral clearance, the virus was no longer viable after 15 days post onset of symptoms. Our study aimed to measure whether nitric oxide nasal spray (NONS) could accelerate the reduction in SARS-CoV-2 RNA load versus control with a saline spray. Our study recruited 80 participants who were divided into a NONS treatment or a placebo arm to test the efficacy of NONS as a treatment for mild COVID-19 infection.

### Introduction

The coronavirus (COVID-19) pandemic has had a profound impact on the world, resulting in a worldwide death toll of over 2.6 million and global cases in excess of 119 million as at March 2021.[2] These figures demonstrate the necessity of rapidly developing new and effective ways in which to control and treat the virus in support of the emergency use of already-available COVID-19 vaccines.[3]

There are currently no evidence-based treatments for mild COVID-19 infection. This double-blind phase IIb clinical trial used a placebo control to evaluate the efficacy of nitric oxide in the treatment of mild, symptomatic COVID-19 infection in the form of a self-administered nasal spray. Nitric oxide (NO) is a free radical gas molecule involved in innate immunity, as well as wound healing, vasodilation, neurotransmission, and angiogenesis.[4] Although produced physiologically, NO has been shown to exhibit a number of antimicrobial actions at therapeutic dosage regimens both *in vitro* and *in vivo*.[5–7]

### Materials and methods

This trial was carried out at Ashford and St. Peter's Hospitals NHS Foundation Trust (ASPHFT). 80 adults (18–70 years) who were isolated with mild COVID-19 infection confirmed by laboratory SARS-CoV-2 RT-PCR nasal and throat swab within the 48 h of randomisation were eligible for recruitment. Participants were randomised 1:1 to receive NONS ($n=40$) placebo ($n=40$). The nasal sprays were self-administered 5–6 times daily (two sprays per nostril/dose, 120–140 $\mu$L of solution/spray) for 9 days.

Treatment with NONS or placebo commenced on day 1. Participants took self-sampled nasal and throat swabs on days 1 (at baseline, before initiating treatments), 2, 4, and 6 in the mornings, prior to treatment. Quantitative RT-PCR was carried out at Berkshire Surrey Pathology Services Virology laboratory to determine SARS-CoV-2 RNA levels. SARS-CoV-2 sequencing for variants was performed at Public Health England Colindale. Daily self-reporting questionnaires on symptoms, compliance, and treatment tolerance were completed by patients and follow-up continues for a total of 18 days.

### Results

Patients in both trial groups started on NONS or placebo at least 4 days after the onset of symptoms and were well balanced in terms of risk factors (Table 1). 34 (85%) of the NONS group and the placebo group were determined to be lineage B.1.1.7 (VOC202012/01) and the remainder were not determined to be a variant of concern. There were no serious adverse events in patients within either trial group. NONS versus placebo started on at least day 4 of symptom onset was independently associated with an accelerated decrease in log(10) SARS-CoV-2 RNA concentration of −1.21 (95% CI, −2.07 to −0.35; $P=0.01$) and −1.21 (95% CI, −2.19 to −0.24; $P=0.02$) on days 2 and 4 respectively (Fig. 1). Mean SARS-CoV-2 RNA concentration was lower on NONS by a factor of 16.2 at days 2 and 4. A rapid reduction (95%) in the SARS-CoV-2 viral load was observed within 24 hours, with a 99% reduction observed within 72 hours with NONS treatments.

The mean SARS-CoV-2 RNA concentration at day 6 was lowered to −3.32 on NONS, with a treatment difference of −0.98 (95% CI, −2.04 to 0.08; $P=0.069$). The mean treatment difference using an area under curve estimate from baseline through day 6 was −5.22 with a 95% CI, −9.14 to −1.31; $P=0.001$), where the mean change was −10.17 for the NONS group and −4.95 for the placebo group.

40 subjects (15 NONS and 25 placebo subjects) completed and returned the trial assessment questionnaire. A total of 46.7% (7 of 15) of NONS respondents reported feeling better versus 8% (2 of 25) of placebo respondents on treatment. NONS subjects typically reported being better by day 2-4 on treatment, whereas the placebo subjects typically did not report feeling better until after day 5.

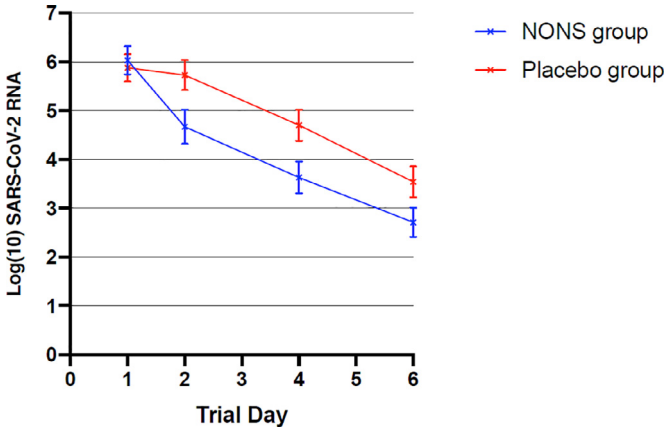


**Fig. 1.** Mean Log(10) SARS-CoV-2 RNA at days 1 to 6
Shown is the difference in the change from baseline in SARS-CoV-2 RNA between the active (NONS) group and the placebo (saline) group from day 1 to day 6. The I bars represent standard error.

**Table 1**
Characteristics of the patients at baseline.

| | NONS group ($n = 40$) | Placebo group ($n = 40$) | p-value |
|---|---|---|---|
| Age (mean, sd) | 44 (12.1) | 43.9 (12.6) | 0.966 |
| Sex | | | |
| Male | 16 (40.0%) | 13 (32.5%) | 0.488 |
| Female | 24 (60.0%) | 27 (67.5%) | |
| Ethnicity | | | 0.692 |
| White | 34 (85.0%) | 37 (92.5%) | |
| Black African + Caribbean | 1 (2.5%) | 0 (0%) | |
| South Asian | 2 (5.0%) | 3 (7.5%) | |
| Mixed | 2 (5.0%) | 0 (0%) | |
| Other | 1 (2.5%) | 0(0%) | |
| BMI | | | 0.034* |
| BMI ≥ 30 | 12 (30.0%) | 7 (17.5%) | |
| BMI < 30 | 19 (47.5%) | 30 (75.0%) | |
| No data | 9 (22.5%) | 3 (7.5%) | |
| Comorbidities | | | |
| Any comorbidity | 6 (15.0%) | 4 (10.0%) | 0.502 |
| Chronic lung disease | 2 (5.0%) | 0 (0%) | 0.155 |
| Chronic liver disease | 0 (0%) | 0 (0%) | |
| Chronic heart disease | 0 (0%) | 0 (0%) | |
| Diabetes | 3 (7.5%) | 2 (5.0%) | 0.646 |
| Hypertension | 2 (5.0%) | 3 (7.5%) | 0.646 |
| Presenting symptoms | | | |
| Dry cough | 22 (55.0%) | 27 (67.5%) | 0.496 |
| Fever | 7 (17.5%) | 16 (40.0%) | 0.081 |
| Loss of sense of smell | 9 (22.5%) | 5 (12.5%) | 0.323 |
| None of the above | 6 (15.0%) | 8 (17.5%) | 0.743 |
| No data | 5 (12.5%) | 3 (7.5%) | |
| SARS-CoV-2 variant | | | |
| B.1.1.7 | 34 (85.0%) | 34 (85.0%) | |
| Not known variant | 6 (15.0%) | 6 (15.0%) | |

## Discussion

Treatment with NONS in this trial was found to be effective and safe in reducing the viral load in patients with mild, symptomatic COVID-19 infection. Patients with recent disease onset were enrolled in the trial to evaluate the effect of early intervention with NONS on SARS-CoV-2 RNA load. Patients in the NONS treatment arm demonstrated viral loads, as determined from PCR testing of nose and throat swab sampling, that were lower at days 2 and 4 by a factor of 16.2 than those on placebo, and symptom resolution was also found to be faster on NONS treatment than on placebo in this study.

Lower SARS-CoV-2 RNA loads in patients with NONS may be beneficial in the prevention of SARS-CoV-2 transmission. It has been described that higher viral loads in patients with SARS-CoV-2 earlier than SARS-CoV may have contributed to greater difficulties in reducing the onward transmission.[8] Furthermore, it has been observed that the risk of symptomatic COVID-19 was associated with the SARS-CoV-2 RNA levels of contacts and incubation time was shortened in a dose-dependent manner.[9]

Accelerated SARS-CoV-2 clearance with NONS may reduce symptom duration, decrease infectivity period, reduce hospital admissions, and lower disease severity. Consequently, this study could be used as supporting evidence for emergency use of NONS for patients with mild COVID-19 infection.

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## Genetic characteristics of a New HIV-1 subtype B/C intersubtype circulating recombinant form (CRF118_BC) identified in Yunnan, China

*Dear Editor,*

Recent correspondence in this Journal has highlighted that HIV-1 frequent intersubtype recombinants lead to its rapid evolution, extraordinary genetic variability and vast genetic diversity.[1] By the end of April 2021, 117 circulating recombinant form (CRFs) and a large number of unique recombinant forms (URFs) have been very well documented worldwide.[2] In China, CRF07_BC and CRF08_BC are the first prevalent intersubtype recombinants consisting of subtype B and C among intravenous drug users (IDUs) in Yunnan province,[3] which located in the areas on the China-Myanmar border, and was regarded as the "hotspots" for new HIV-1 recombination occurrence.[4,5] Over the past three decades, numerous newly CRF_BC recombinants have been identified in Yunnan, such as CRF57_BC, CRF62_BC, CRF64_BC, CRF86_BC, CRF88_BC, and our recently reported CRF110_BC.[6] These findings demonstrated that the high frequency of multiple intersubtype recombinant events between B and C were substantial and ongoing in here. In this study, we characterized a newly emerging HIV-1 CRF118_BC comprising subtype B and C in Yunnan, and analyzed its evolutionary history.

Three HIV-1 positive plasma specimens were collected from Baoshan prefecture (YN23II and YN245F) and Kunming city (YN287_168) in Yunnan province. The remaining one strain DH33 (KF250409) obtained from a previous report in Dehong prefecture, exhibited a high degree of genetic similarity with the three strains in this study based on Blast analysis. This study was approved by the Yunnan Provincial Hospital of Infectious Diseases Ethics Committee. All participants supplied written informed consent for specimen collection and subsequent analyses.

The HIV-1 near full-length genome (NFLG) sequences from the three subjects were successfully amplified and sequenced. And the three sequences obtained have been deposited in GenBank under accession numbers MZ063027 to MZ063029. The NFLG sequences from the three isolates were 8864, 8907, and 8834 bp in size for strains YN23II, YN245F, and YN287_168, respectively, spanning from gag gene to part of 3 long terminal repeat (LTR) corresponding to the location 738–9718 of HXB2 strain.

Combined with the strains D33 (KF250409) reported previously, the maximum likelihood tree of HIV-1 NFLGs exhibited that the four sequences formed a distinct monophyletic branch with a bootstrap value of 100%, distantly related to all known HIV-1 CRFs (Fig. 1A). Further, the recombination structures were determined based on RIP, jpHMM, and BootScan analyses. The results showed that the viruses belong to BC recombinant, with twelve breakpoints delimiting six short subtype B fragments inserted into gag, pol, vpu, env-rev overlap, and nef, respectively, in the subtype C backbone (Fig. 1B).

The twelve recombinant breakpoints were positioned at 1234 nt, 1820 nt, 2285 nt, 2840 nt, 3799 nt, 4217 nt, 4540 nt, 5000 nt, 6037 nt, 6200 nt, 8494 nt and 8869 nt corresponding to the HXB2 coordinate according to informative sites analysis. These recombination breakpoints were shared among all four strains. To characterize the recombinant structure, genomic map was performed by the Map-Draw Tool available at the Los Alamos HIV sequence database. The results depicted thirteen mosaic fragments of the viruses: six subtype B fragments and seven subtype C fragments (Fig. 2A). Next, neighbor-joining phylogenetic tree analyses for the thirteen mosaic fragments further confirmed the breakpoints of the four NFLG sequences as follows: I (738–1234 nt) subtype C, II (1235–1820 nt) subtype B, III (1821–2285 nt) subtype C, IV (2286–2840 nt) subtype B, V (2841–3799 nt) subtype C, VI (3800–4217 nt) subtype B, VII (4218–4540 nt) subtype C, VIII (4541–5000 nt) subtype B, IX (5001–6037 nt) subtype C, X (6038–6200) subtype B, XI (6201–8494 nt) subtype C, XII (8495–8869) subtype B, and i (8870–9718) subtype C (Fig. 2A). Taken together, these results mentioned above allow defining a new HIV-1 CRF, which was named CRF118_BC.

To better understand the time of emergence of CRF118_BC, we performed bayesian molecular clock analyses using combined subtype B regions (Regions II+IV+VI+VIII+X+XII) and combined subtype C regions (Regions *I*+III+*V*+VII+IX+XI+*i*) to estimate the time to tMRCA. As shown in Fig. 2B, the estimated tMRCAs for the concatenated subtype B regions and the concatenated subtype C regions were 1998.3 [95% highest probability density (HPD): 1996.8, 2002.8] and 1996.7 95% HPD: (1994.5, 1999.2), respectively. Hence, the tMRCA estimates of the subtype B and subtype C regions were consistent, revealing that CRF118_BC originated around 1996–1998.

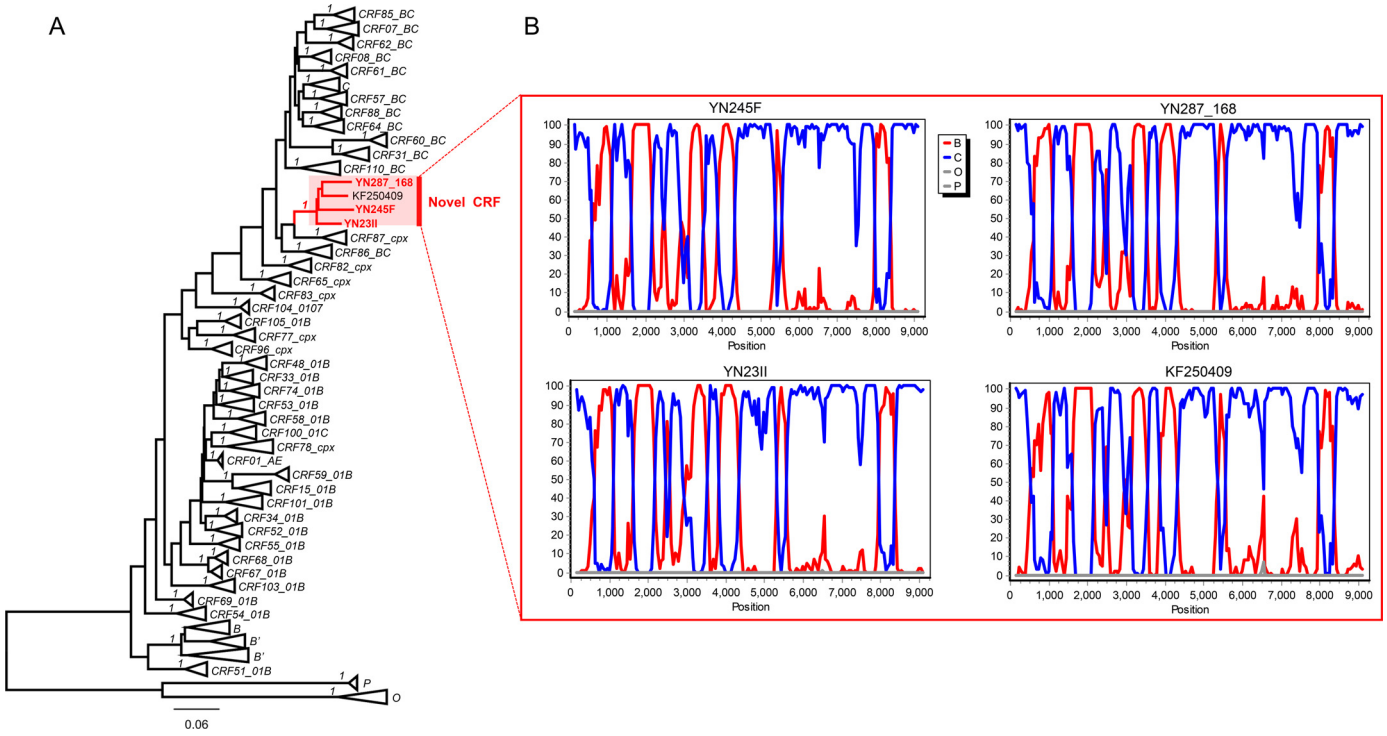


**Fig. 1.** Phylogenetic and recombinant analyses based on near full-length HIV-1 genome sequences. (A) The representing different HIV-1 CRFs reference sequences were used to construct the maximum likelihood phylogenetic tree. The sequences of CRF118_BC (YN23II, YN245F, YN287_168 and KF250409) are marked in branch. The stability of the nodes was assessed by bootstrap analysis with 1000 replications. Reference strains of established and informative HIV-1 genotypes involving subtype B of Thai origin, subtype C, 13 known CRFs comprising of B and C, were included in the analysis. (B) Bootscan analysis was conducted using a window size of 300 bp and a step size of 50 bp along with reference strains of subtype B, C and representative HIV-1 O and P subtypes.

In the early 1990s, HIV-1 subtypes B and C were first imported into Yunnan from Thailand and India, respectively. Subsequently, CRF07_BC and CRF08_BC generated by recombination of B and C were identified among IDUs, and then the two recombinants began to spread rapidly from high-risk group to the general population.[7,8] Currently, 11 CRF_BC, and multiple B/C unique recombinant forms has become the most predominantly recombination types in the southwest of China, and specifically west Yunnan near the Myanmar border, as the epicenter of the HIV/AIDS.[6,9] In the current study, after our recently identified CRF110_BC, another one novel HIV-1 CRF_BC designated CRF118_BC containing a subtype C backbone with six B segments inserted was first characterized in Yunnan, China. Compared with the 13 CRF_BC recombinants reported in the world, CRF118_BC shows the most complex mosaic patterns involving twelve recombinant breakpoints and six B segments inserted (Fig. S1) and exhibits markedly different from previously documented 13 known CRF_BC recombinants in its distinct backbone, inserted fragment size, and breakpoints, suggesting CRF118_BC did not originated from the offspring recombination between CRF07_BC/CRF08_BC and B/ C.

In conclusion, we describe a complex new recombinant HIV-1 CRF consisting of subtype B and C named CRF118_BC, which has a subtype C backbone with six subtype B segments inserted into gag, pol, vpu, env-rev overlap, and nef, respectively. And CRF118_BC were estimated to have originated around the year 1996–1998. Our results further highlighted that the evolving intersubtype recombinants of HIV-1 pose a major obstacle to the development of an effective vaccine against HIV-1, diagnostic assays, viral load measurements, and antiretroviral treatments. Therefore, it is necessary to strengthen the prevention and control measures for HIV-1 infection in Yunnan.

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## Declaration of Competing Interest

The authors declare no competing financial interests.

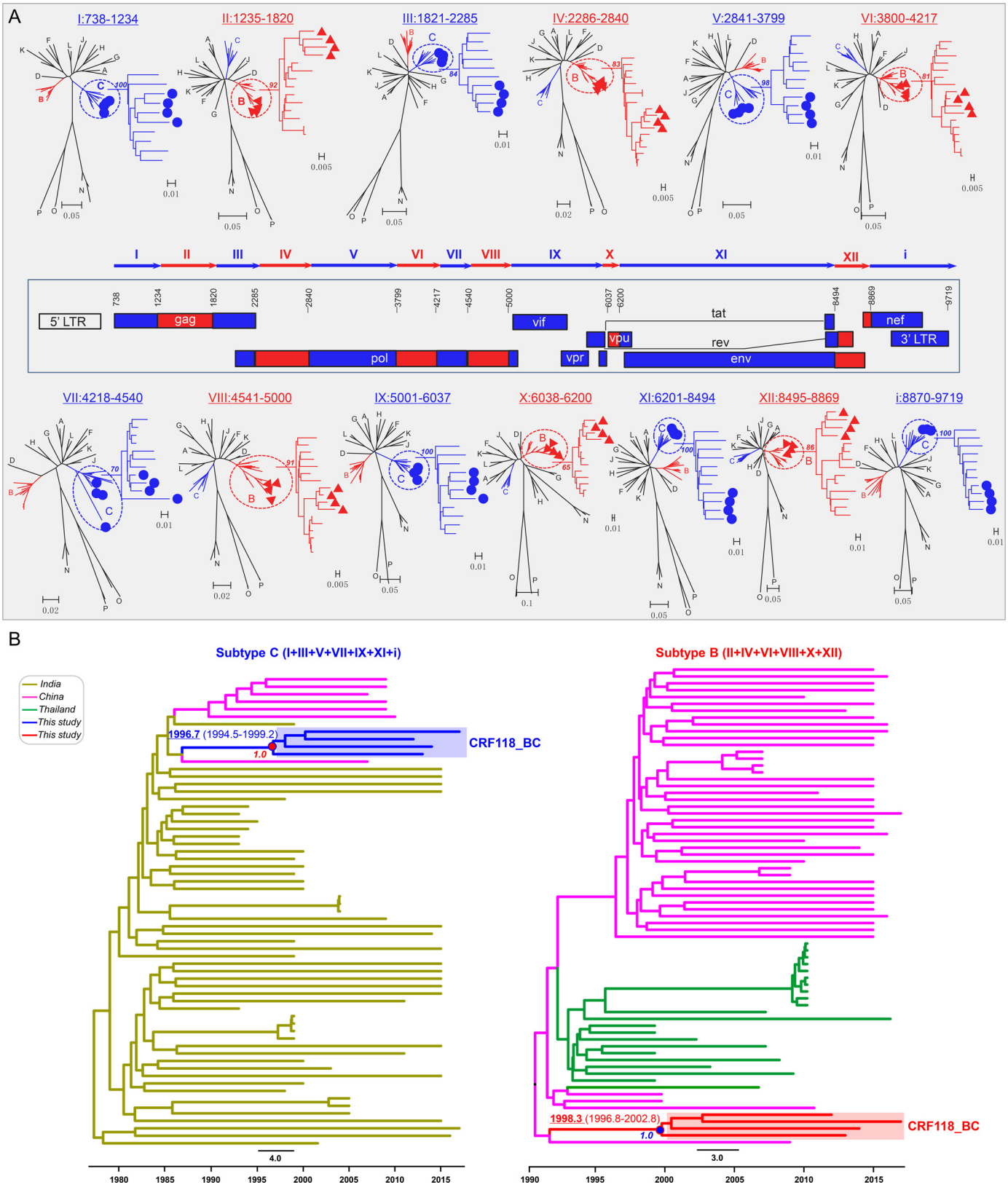


**Fig. 2.** The analyses of HIV-1 recombination breakpoint, subregion trees, and maximum clade credibility (MCC) trees. (A) Genomic structure of CRF118_BC. The mosaic map was generated using the Recombinant HIV-1 Drawing Tool (https://www.hiv.lanl.gov/content/sequence/DRAW_CRF/recom_mapper.html). The phylogenetic trees of the five mosaic fragments identified by bootscan analysis were constructed using the neighbor-joining method based on the K-2 model in MEGA. The reliability of tree branches was evaluated by 1000 bootstrap replicates, and bootstraps of the strains from this study only are shown. To discern the genotypes of HIV-1 recombinant fragment, HIV-1 genotypes of group M involving subtypes A, B, B'(Thai origin), C, D, F, G, H, K, J, L, N, O, and P were also included in the analysis. (B) MCC trees for combined subtype B segments (Regions II+IV+VI+VIII+*X*+XII, 2557 bp) and subtype C segments (Regions *I*+III+*V*+VII+IX+XI+*i*, 6426 bp) are shown. Timescale is shown at the bottom of the tree. The mean tMRCA and 95% highest probability density (HPD) for the key nodes are indicated. CRF118_BC strains from this study are highlighted in red (subtype B) and blue (subtype C).

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.05.007.

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## Potential future implications of the COVID-19 pandemic on Norovirus infections in England

*Dear Editor,*

I read with interest the recent article by Eigner et al., in the Journal of infection, highlighting the reduction in Norovirus (NoV) infections reported in Germany since the introduction of COVID-19 containment measures in 2020[1]. In the UK, there has been a reduction in both NoV infections and outbreaks reported to Public Health England (PHE) since the introduction of Nonpharmaceutical Interventions (NPIs) by the UK government to contain the COVID-19 pandemic in March 2020[2]. The GII.4 NoV strain was detected in the mid-20th century and has undergone evolution in the form of 7 new variants since the 1990s which have been associated with global epidemics[3]. These have appeared on average every 2–3 years, but a new variant has not been detected since 'The Sydney strain' in 2012[3]. There are concerns about the potential for an undetected new strain shift, as a result of the reduced surveillance over the last year[4].

PHE surveillance data on NoV is collected via four main sources[2]. The Second-Generation Surveillance System (SGSS) records all positive norovirus tests reported to labs in England[2]. The Hospital Norovirus Outbreak Reporting System (HNORS) includes online reporting of both confirmed and suspected hospital outbreaks of norovirus across England[2]. HPZone is used by Health Protection Teams (HPTs) to record suspected and confirmed enteric virus (EV) outbreaks notified to them[2]. The Enteric Virus Unit monitors genotype and characterisation of NoV nationally[2]. Data is collected across the 'Norovirus season' which runs from week 27 (July) in year 1 to week 26 (June) in year 2, in order to capture the winter peak of activity[2].

PHE surveillance data since week 12 of the 2019/2020 season through to the 2020/2021 season have shown markedly reduced levels of norovirus positive tests and EV outbreaks reported compared with the 5-season average[2]. The cumulative total of EV reports in the 2020/2021 season to week 7 is 89% lower than the 5-season average[2]. It is hard to argue that the NPIs introduced to contain COVID-19 have not had a significant impact on the transmission of NoV. However, this must be interpretated with caution. The underreporting of enteric virus cases is an issue that will only have worsened during the pandemic, due to factors such as reduced access to NHS services and the impact on testing capacity[4].

Douglas et al., discussed the reduction in the referral of NoV-positive samples in the UK for characterisation and genotyping during the pandemic in the Journal of hospital infection[4]. They suggest that such a significant reduction could result in key indicators for NoV strain replacement events being missed[4]. With UK COVID containment measures set to ease, the authors warn of the ongoing risk to healthcare services due to the potential occurrence of a new NoV strain in a population with low levels of immunity[4]. There have been reports of increasing numbers of NoV outbreaks across China since the easing of COVID-19 restrictions there. This highlights the reality of the risk of significant NoV outbreaks occurring to a greater degree once containment measures are lifted.

It is clear to see that, despite factors leading to underreporting of surveillance data, there has been a substantial impact on NoV infections due to COVID-19 containment measures implemented both in the UK and globally. What is not clear is what the implications of this may be for the future. There remains the possibility for the emergence of new NoV strains with epidemic potential as measures are lifted in the short term. Whether the significant societal and behavioural changes that have occurred during the pandemic will lead to a reduction in transmission of infectious diseases, such as influenza and norovirus, remains to be seen.

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**Conflict of interest statement**

No conflict of interest to declare.

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## Asymptomatic SARS-CoV-2-infected children attending hospital with non-COVID-19 diagnoses, March 2020-February 2021

*Dear Editor,*

We read with interest the article by de Paul et al.[1], which highlighted gastrointestinal manifestations of SARS-CoV-2 infection in children. Most children infected with SARS-CoV-2 exhibit COVID-19 symptoms, but about 20–30% may be truly asymptomatic[2], who may then pose an undiagnosed infection hazard to other hospital staff and patients - especially as children are not yet eligible for COVID-19 vaccination.

This may become a seasonal problem, as we have seen with other respiratory viruses, such as respiratory syncytial virus (RSV)[3] and influenza[4]. Indeed, children with asymptomatic SARS-CoV-2 infection can show higher nasopharyngeal viral loads than hospitalised adults with severe disease[5], and can shed virus for up to 3 weeks[2].

Throughout the COVID-19 pandemic in the UK during 2020, asymptomatic SARS-CoV-2 infections in children were difficult to assess directly, as only symptomatic children were tested during the first wave of the pandemic, and only if they required hospitalisation. Most community SARS-CoV-2 testing was stopped after 12 March 2020[6] for both adults and children.

From June 2020, UK national guidance mandated that all new hospital admissions undergo screening for COVID-19[7]. This universal screening policy allowed us to monitor SARS-CoV-2 infection rates in children who were both symptomatically and asymptomatically infected with SARS-CoV-2, with some of the latter group being admitted for other medical problems.

Our Children's Hospital serves a paediatric population of 233,796 throughout Leicester, Leicestershire and Rutland[8], seeing over 60,000 children in the children's Emergency Department (ED), annually. We performed a 1-year retrospective surveillance audit to determine the incidence of asymptomatic paediatric SARS-CoV-2 infections admissions.

**Inclusion criteria**: all under-18-year olds who had been seen and swabbed (nasopharyngeal) in ED or their destination ward, within 72 h of admission, who tested SARS-CoV-2 PCR positive, during 1 March 2020 to 21 February 2021.

**Exclusion criteria**: all swabs taken during this same study period, by other teams or by other referring hospitals, including any repeat positive swabs from the same patient and/or positive tests from samples taken from beyond their first 72 h of admission (based on the average incubation period of 5–6 days for SARS-CoV-2 infection)[9].

Using hospital electronic patient records, children with symptomatic SARS-CoV-2 infection had their symptoms classified as COVID-19-compatible or not, according to the World Health Organization symptom list of COVID-19 symptoms[10].

An 'unclear' COVID-19 status was assigned when a patient presented with at least one COVID-19-compatible symptom but who also had a concurrent illness with overlapping symptom patterns, e.g. a child admitted with fever and abdominal pain, who had surgically proven appendicitis, but who was also found to be SARS-CoV-2 positive.

Out of a total of 11,793 nasopharyngeal swabs, 202 (1.71%) were SARS-CoV-2 PCR positive. Of these, swabs from 80 patients met our inclusion criteria for laboratory-confirmed SARS-CoV-2 infection. Of these 80 cases, 68 were swabbed in ED (85%) and 11 (13.75%) by their destination inpatient ward and 1 (1.25%) by the mortuary following an out-of-hospital cardiac arrest admitted via ED.

**Table 1** shows the trends of SARS-CoV-2 infections amongst the 1427 new paediatric admissions during the audit period, with 5.61% (80/1427) being infected with the virus.

The majority of these children were of preschool (53/80, 66.25%), then secondary school (21/80, 26.25%), then primary school (6/80, 7.5%) age. The highest proportions of new SARS-CoV-2 cases occurred in April 2020 (14/46, 30.43%) and January 2021 (14/103, 13.59%), immediately following the government implementation of school closures (23 March 2020 and 5 January 2021.) (**Fig. 1**)

In terms of clinical presentation, of the 80 SARS-CoV-2-infected cases, 52/80 (65%) had COVID-19-compatible symptoms, 16/80 (20%) were asymptomatic and in 12/80 (15%) it was unclear. The 20% asymptomatic infections reported here in this Leicester, UK cohort is similar to the 22% (20/91) figure reported in a South Korean paediatric cohort by Han et al. 2021[2].

Of the 52 children displaying COVID-19 symptoms; 23/52 (44.23%) had fever only, 13/52 (25%) had fever and a respiratory symptom, 11/52 (21.15%) had respiratory symptoms only, 2/52 (3.85%) presented with febrile seizures, 2/52 (3.85%) with headaches alone and 1/52 (1.92%) with skin discoloration of their extremities.

Stratifying by school stage, the symptomatic and asymptomatic infections were, respectively: preschool: 38/80 (47.5%), 8/80 (10%);

**Table 1**
Trends of SARS-CoV-2 infections in new paediatric admissions during March 2020 to February 2021, Leicester Children's Hospital, Leicester, UK.

| Month | Total number of monthly admissions | SARS-CoV-2 positive cases | | Symptomatic SARS-CoV-2 infections | | Asymptomatic SARS-CoV-2 infections | | Unclear group SARS-CoV-2 infections | |
|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | n | % |
| Mar-20 | 132 | 3 | 2.27% | 2 | 1.52% | 1 | 0.76% | 0 | 0 |
| **Apr-20** | **46** | **14** | **30.43%** | **12** | **26.09%** | **0** | **0** | **2** | **4.35%** |
| May-20 | 82 | 1 | 1.22% | 1 | 1.22% | 0 | 0 | 0 | 0 |
| Jun-20 | 111 | 2 | 1.80% | 1 | 0.90% | 1 | 0.90% | 0 | 0 |
| Jul-20 | 96 | 3 | 3.13% | 2 | 2.08% | 1 | 1.04% | 0 | 0 |
| Aug-20 | 104 | 1 | 0.96% | 1 | 0.96% | 0 | 0 | 0 | 0 |
| Sep-20 | 147 | 7 | 4.76% | 4 | 2.72% | 3 | 2.04% | 0 | 0 |
| Oct-20 | 125 | 2 | 1.60% | 1 | 0.80% | 1 | 0.80% | 0 | 0 |
| Nov-20 | 187 | 14 | 7.49% | 7 | 3.74% | 4 | 2.14% | 3 | 1.60% |
| Dec-20 | 177 | 11 | 6.21% | 9 | 5.08% | 1 | 0.56% | 1 | 0.56% |
| **Jan-21** | **103** | **14** | **13.59%** | **8** | **7.77%** | **2** | **1.94%** | **4** | **3.88%** |
| Feb-21 | 117 | 8 | 6.84% | 4 | 3.42% | 2 | 1.71% | 2 | 1.71% |
| **Total** | **1427** | **80** | **5.61%** | **52** | **3.64%** | **16** | **1.12%** | **12** | **0.84%** |

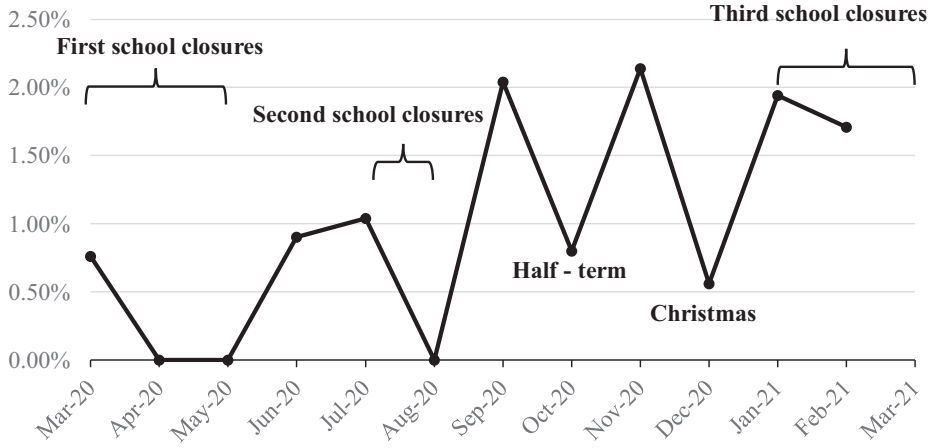


**Fig. 1.** Percentage of monthly asymptomatic SARS-CoV-2 infections admitted during March 2020 to February 2021, to Leicester Children's Hospital, Leicester, UK. First school closures: national lockdown 23 of March to 31 of May 2020; Second school closures: local lockdown 4 of July 2020 to 31 August 2020; Third school closures: national lockdown 5th January to 7th March 2021.

primary school: 2/80 (2.5%), 2/80 (2.5%); secondary school: 12/80 (15%), 6/80 (7.5%).

The overall percentage of new paediatric admissions with asymptomatic SARS-CoV-2 infection remained at or below 1.04%, until September 2020 when it rose to 2.04%, then further increased to 2.14% in November 2020. In addition, whilst schools remained open throughout the 2020 autumn term, it was noticeable that dips in asymptomatic SARS-CoV-2 case numbers occurred in October 2020 (0.80%) and December 2020 (0.56%), which coincided with the school half-term and Christmas holidays (Fig. 1). This may have been due to an overall reduction in social contacts between children during these school breaks.

Conversely, and compatible with this explanation, during the 5 months that the schools were open (June, September-December), there was a 1.65-fold increase (1.29% vs. 0.78%) in the mean percentage of new asymptomatic paediatric SARS-CoV-2 infections admitted, compared to the 7 months when the schools were closed (March-May, July-August, January-February). Thus, the trend of asymptomatic paediatric SARS-COV-2 infections appears to follow the timing of the school terms.

Asymptomatic SARS-CoV-2 infections presenting to hospital for other, non-COVID-19-related medical reasons may pose a nosocomial transmission risk to other patients and staff, as has been seen with other seasonal respiratory viruses[3,4]. Even where bedside rapid diagnostic tests are available, these can still take 30–60 min to complete, and patient waiting areas can still allow some degree of close-contact mixing, particularly with active young children. This risk may increase during school term times, as the percentage of asymptomatic paediatric SARS-CoV-2 infections being seen in hospital rises.

We therefore urge paediatric ED and outpatient teams to be particularly vigilant for potentially asymptomatic SARS-CoV-2-infected children during school terms, particularly as SARS-CoV-2/COVID-19 becomes more endemic and seasonal, and whilst children are still not eligible for COVID-19 vaccinations.

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## Rapid antigen test for SARS-CoV-2 and primary health care

*Dear Editor,*

We have carefully read the article published by Buliete et al. in your prestigious journal.[1] This is, in our opinion, an excellent article about the usefulness of rapid antigen detection tests (RADTs) in the diagnosis of SARS-CoV-2 infection. Its strengths are that it is a real-life, primary care study, its careful design and the large and calculated sample size, congratulations. However, there are some issues that we believe should be highlighted and others that should be nuanced based on their results, especially with regard to policy implications.

Firstly, we believe that the high specificity found in both, symptomatic and asymptomatic patients, close to 100%, has not been sufficiently highlighted. This near absence of false positives, as the authors comment, has been noted in other published articles. This finding is consistent with two recently published papers by our research group in two different contexts: population screening[2] and an outbreak in a nursing home.[3] As the authors conclude, this means that a positive test is a source of infection, but in both symptomatic and asymptomatic patients, so confirmatory tests are unnecessary. Based on the internal validity provided by the manufacturer, other authors recommend confirmatory testing in screening cases because of the expected high false positive rate.[4] It is well known that if the expected prevalence is higher than 1 - Specificity the positive predictive value will be very low and even all positives could be false positives.[5] However, if the prevalence is close to 100% the positive predictive value will be very high even with pre-test probabilities below 5%, which is the WHO recommended limit for the use of RADTs.[6]

With regard to nuance, we were surprised that the authors praise the reliability of the negative results in symptomatic subjects and question those of asymptomatic subjects with similar results and with confidence intervals that overlap widely. In both cases we believe that a negative test does not rule out the presence of infection. Even in those cases where the reason for the request for testing is unknown, the pre-test probability is high, 7.8%,[1] and therefore a clear scenario of maintaining caution, the same in the case of close contacts, the quarantine situation should be maintained for the stipulated time regardless of the result of the test not only for antigen, but even for PCR.[7,8] On the contrary, in a low pre-test probability scenario of less than 5%, as may be the case in population-based screening, the negative predictive value is very high and the presence of infection can be reasonably ruled out.[2]

In any case, we would like to congratulate the COVID-19 Primary Care Research Group for its interesting work and just remind that diagnostic tests are not to be read but must be interpreted in their context.

### Conflict of interest

The authors declare that they have no conflict of interest.

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## Impact of COVID-19 vaccination program on seroprevalence in blood donors in England, 2021

*Dear Editor,*

We read with interest in this journal the letter of Tré-Hardy et al.[1] which contrasts serological responses following mRNA vaccination in individuals with and without prior infection; good responses were seen in all study participants. England introduced a mass vaccination programme against COVID-19 on 8th December 2020 primarily based on age, starting with those over 80 years of age, along with health and social care workers.[2] Since the beginning of the programme to 7th March 2021 over 19 million individuals in England have been vaccinated with at least one dose of vaccine: either Pfizer BioNTech (from 8th December) or AstraZeneca (from 4th January).[3] We describe the impact of vaccination rollout on antibody prevalence in blood donors in England.

As part of COVID-19 infection monitoring, Public Health England, in collaboration with the National Health Service Blood and Transplant Service has arranged regular collections of plasma from English blood donors to be sent for COVID serology testing; results are reported weekly.[4] Approximately 250 samples per week are collected from each of seven NHS regions. We present seropositivity estimates from 23rd November 2020 onward, which covers the period of vaccine rollout and the peak of England's B.1.1.7-variant dominated epidemic wave.

The vaccination status of donors is not available but parallel testing using a nucleoprotein (Roche N) and a spike (Roche S) assay allows us to monitor trends in natural infection transmission and vaccine-induced seropositivity. Nucleoprotein assays (Roche N) only detect antibodies post natural infection, whereas spike assays (Roche S) detect both post natural infection and vaccine-induced antibodies. Antibody responses to both targets reflect infection/vaccination occurring 2–3 weeks previously given the time taken to generate a SARS-CoV2 antibody response.[5] We have shown strong agreement between serological responses using these two assays following natural infection that was sustained 6 months post infection.[6]

Seropositivity estimates are calculated on a 4-week rolling basis and are population weighted by NHS region, age group and sex. Estimates are not adjusted for assay sensitivity and specificity, which are estimated to be in excess of 97% and 99.8% respectively.[7, 8] Additionally, estimates are compared against vaccine uptake, which is calculated using the National Immunisation Management System (NIMS), a new national vaccine register to facilitate management of the vaccination programme in England.

7720 samples were available during the most recent 4-week period 22nd February-21st March 2021, of which 3224/7720 were Roche S positive and 1111/7717 were Roche N positive. Overall population weighted seropositivity amongst blood donors was 46.4% (95% CI 45.4% - 47.5%) using the Roche S assay. This compares with all-England seropositivity of 54.7%3 (95% CrI 49.3% - 60.5%) from the UK Office of National Statistics (ONS) Infection Survey for the period 18th February – 14th March, based on a single spike target based assay.[9] Roche N seropositivity was considerably lower at 14.5% (95% CI 13.7% - 15.4%).

Based on Roche S assay results, seroprevalence has been clearly increasing across all age groups from survey weeks 7th December 2020 – 3rd January 2021 (Fig. 1). For the most recent 4-week period, the population weighted seroprevalence was highest in the age 70–84 group at 93.5% (95% CI 90.9% - 95.4%). In parallel, the Roche N assay, a marker for natural infection, showed not only the lowest seroprevalence in the age 70–84 group for the same period at 4.7% (95% CI 3.1% - 7.1%), but this also stabilised over successive four week intervals; for example over the period 1st-31st January 2021 seropositivity was 5.2% (95% CI 3.1% - 8.5%). Seropositivity based on Roche N was highest in the youngest donor cohort and continues to increase, suggesting transmission was ongoing.

Cumulative first dose vaccine uptake was 91.6% to the week ending 21st February, which roughly corresponds with the most recent 4-week period given 2–3 weeks for antibody response (Fig. 2). The increase in S positive N negative outcomes accelerated from survey weeks 11th January – 7th February 2021 following a rise in uptake. Note that age 70+ uptake in Fig. 2 is weighted by the 70+ donor age distribution, which tails off with age.

The vaccine uptake of 8.7% to the week ending 7th February in those 18–59y is lower than S positive N negative seroprevalence in younger blood donors, suggesting that health and social care workers are over-represented in the latter group.

Since vaccine rollout commenced Roche S seropositivity has increasingly risen above Roche N seropositivity and clearly shows trends in vaccine-induced antibodies, especially within the 70–84 age group who were amongst the first to be targeted for vaccination. Second dose coverage is less an 1% amongst the oldest donor age group, hence we observe a robust antibody response following a single vaccine dose. Meanwhile Roche N seropositivity in this age group has remained stable, suggestive of vaccine impact. This adds to a growing body of evidence suggestive of vaccine impact in the UK population.[10]

### Ethics

PHE has legal permission, provided by Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002, to process patient information for national surveillance of communicable diseases. Specific ethical approval was not required for this surveillance work.

### Author's contributions

HW, SE, IH, SR, KB and GA wrote the manuscript, with input from MR. GA, KB and MR contributed to conceptualization, funding acquisition and project administration. HW performed statistical analysis. EC, AL, CT, CC collated vaccine uptake statistics. EL, IH,

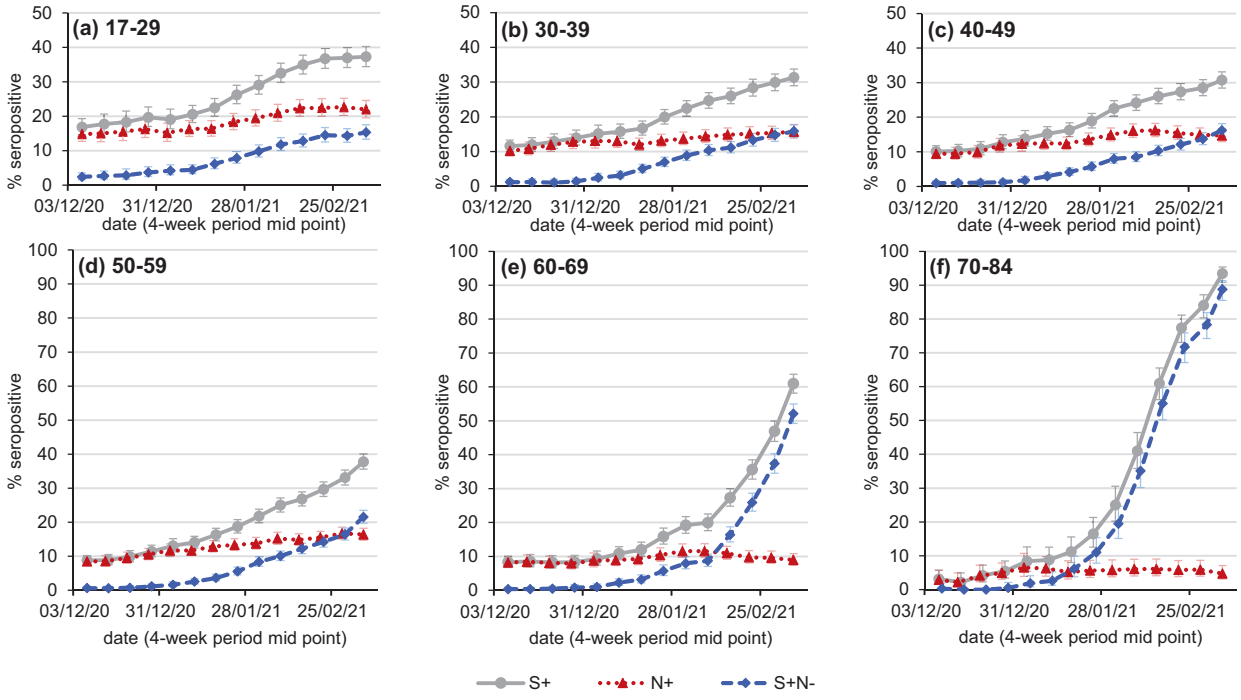


**Fig. 1.** SARS-CoV-2 antibody seropositivity based on the Roche S assay (S+, grey solid lines), the Roche N assay (N+, red dotted lines) in English blood donors by age group, weighted by NHS region and sex, rolling four weekly average from the 4 week period 25/11/2020 - 20/12/2020 to the 4 week period 22/02/2021 – 21/03/2021. Also shown is the percentage Roche S seropositive, Roche N seronegative (S+N-, blue dashed lines).

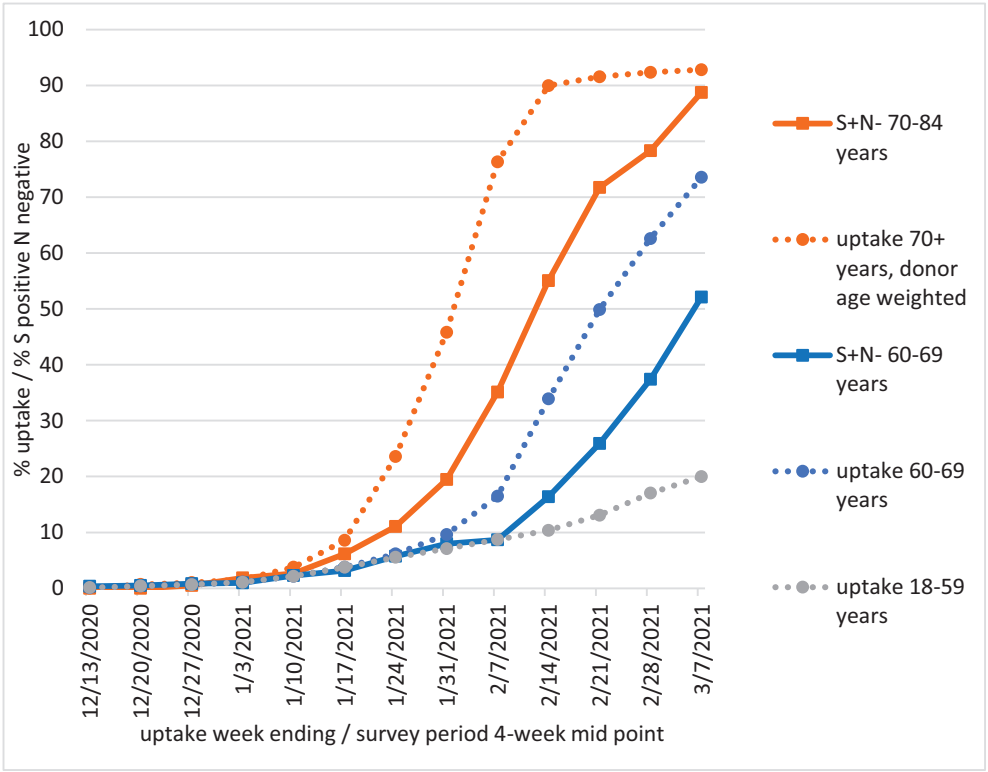


**Fig. 2.** Cumulative dose 1 COVID-19 vaccine uptake by age group (age on 31/03/2021). Roche S positive, N negative is plotted for the 70–84 and 60–69 age groups to demonstrate the lag in antibody response. Age 70+ uptake is weighted by the 70–84 donor age distribution.

SR, JH curated and managed serology data. CR, AO, TB performed the testing. All authors read and approved the submission.

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## Declaration of Competing Interest

EL reports the Public Health England Vaccine Evaluation Unit performs contract research on behalf of GSK, Sanofi and Pfizer which is outside the submitted work.

HW, SE, IH, SR, KB, GA, EC, AL, CT, CC, IH, SR, JH, CR, AO, TB, MR report no conflicts of interest.

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## Long-term post-COVID symptoms and associated risk factors in previously hospitalized patients: A multicenter study

*Dear Editor,*

The word is in front of a second pandemic associated with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), i.e., post-COVID sequelae and “long-haulers”. A preprint meta-analysis has found that 80% of COVID-19 survivors exhibit at least one post-COVID symptom after infection.[1] However, most of the studies included in this meta-analysis had follow-up periods <3 months, sample sizes < 300 participants, and were conducted at a single center.[1] In a letter to the editor in *Journal of Infection,* Garrigues et al. found that fatigue, dyspnea, and loss of memory were the most prevalent post-COVID symptoms 3 months after hospital discharge.[2] More recently, Moreno-Perez et al. observed that 59% of hospitalized and 37% of non-hospitalized patients exhibited post-COVID symptoms 3 months after the infection.[3] Here we report a multicenter study assessing post-COVID symptoms and associated risk factors seven months after hospital discharge.

This multicenter observational study included patients hospitalized with a positive diagnosis of SARS-CoV-2 by RT-PCR technique and radiological findings during the first wave of the pandemic (March 10th to May 31st, 2020) in four public hospitals in Madrid (Spain). From all hospitalized patients, a randomized sample of 300 patients from each hospital was selected. The study was approved by all the Local Ethics Committees (URJC0907202015920, HCSC20/495E, HUFA 20/126, HUF/EC1517, HUIL/092–20). Informed consent was obtained from participants before collecting data.

Patients were scheduled for a telephone interview by trained researchers. Clinical (i.e., age, gender, height, weight, pre-existing comorbidities) and hospitalization (e.g., symptoms at hospital admission, days at hospital, intensive care unit [ICU] admission) data were collected from hospital medical records. Participants were systematically asked about a list of post-COVID symptoms (dyspnea, fatigue, anosmia, ageusia, hair loss, chest pain, palpitations, diarrhea, skin rashes, brain fog, memory loss, cough) but they were

**Table 1**
Demographic and clinical data of the sample ($n = 1142$).

| | |
|---|---|
| **Age, mean (SD), years** | 61 (17) |
| **Gender, male/female (%)** | 601 (52.5%) / 541 (47.5%) |
| **Weight, mean (SD), kg.** | 70 (15) |
| **Height, mean (SD), cm.** | 166 (10) |
| **Body Mass Index, mean (SD), kg/cm$^2$** | 25.4 (3.0) |
| **Smoking status, n (%)** | |
| Active | 96 (8.5%) |
| None or Former | 1046 (91.5%) |
| **Main Symptoms at hospital admission, n (%)** | |
| Fever | 812 (71.1%) |
| myalgia | 380 (33.2%) |
| dyspnea | 380 (33.2%) |
| Cough | 315 (27.6%) |
| Headache | 209 (18.3%) |
| Gastrointestinal Disorders-Diarrhoea | 140 (12.2%) |
| Anosmia | 108 (9.5%) |
| Ageusia | 99 (8.7%) |
| Throat Pain | 61 (5.4%) |
| **Medical co-morbidities** | |
| Hypertension | 291 (25.5%) |
| Diabetes | 145 (12.7%) |
| Chronic Heart Disease - Cardiovascular Disease | 144 (12.6%) |
| Rheumatological Disease | 61 (5.5%) |
| Asma | 55 (4.8%) |
| Obesity | 54 (4.7%) |
| Chronic Obstructive Pulmonary Disease | 51 (4.4%) |
| Stroke | 29 (2.5%) |
| Other (Cancer, Kidney Disease) | 105 (9.1%) |
| **Stay at the hospital, mean (SD), days** | 14 (12) |
| **Intensive Care Unit (ICU) admission** | |
| Yes/No, n (%) | 80 (7%) / 1062 (93%) |
| Stay at ICU, mean (SD), days | 15 (13) |
| **Number of persistent post-COVID symptoms, n (%)** | |
| None | 212 (18.6%) |
| 1 or 2 | 505 (44.2%) |
| 3 or more | 425 (37.2%) |
| **Persistent post-COVID symptoms, n (%)** | |
| Fatigue | 695 (60.8%) |
| loss hair | 305 (26.3%) |
| dyspnea | 268 (23.5%) |
| Loss memory | 217 (19.0%) |
| Skin Rashes | 117 (10.2%) |
| Brain fog | 110 (9.6%) |
| Attention Disorders | 93 (8.1%) |
| Gastrointestinal Disorders-Diarrhoea | 82 (7.2%) |
| Chest Pain | 80 (7.0%) |
| Tachycardia-Palpitations | 77 (6.7%) |
| Ocular/Vision Disorders | 52 (4.5%) |
| Ageusia | 38 (3.3%) |
| Anosmia | 34 (3%) |
| Cough | 24 (2.1%) |

free to report any symptom that they considered relevant. More than one symptom could be reported by the same participant.

Descriptive data are presented as mean (standard deviation, SD) or percentages as appropriate. Chi-square or Mann-Whitney tests were used to compare the post-COVID symptoms by gender or ICU or not admission. Multivariate Poisson regression prediction and risk models were constructed to identify those clinical and hospitalization variables associated with the number of persistent post-COVID symptoms. Adjusted incident rate ratios (IRR) with 95% confidence intervals (95%CI) were calculated.

From 1200 patients randomly selected and invited to participate, 13 refused, 10 were not contacted, and 35 had deceased after hospital discharge. A total of 1142 (48% women, mean age: 61, SD: 17 years) were included. The most prevalent symptoms at hospital admission were fever (71.1%), myalgia (33.2%), and dyspnea (33.2%). Four hundred and eighty-two (42.2%) had no comorbidities, 406 (35.5%) had one comorbidity, 174 (15.3%) had two, and the remaining 80 (7%) had at least three comorbidities (**Table 1**).

Participants were assessed a mean of 7.0 months (SD 0.6) after hospital discharge. Only 212 (18.6%) were completely free of any post-COVID symptom, 238 (20.8%) had one symptom, 267 (23.4%) had two symptoms, and 425 (37.2%) had 3 or more. The mean number of post-COVID symptoms was 2.5 (SD 1.2). Women (mean: 2.5, SD: 1.5) had significantly (IRR1.37, 95%CI 1.26–1.49, $P < 0.002$) higher number of post-COVID symptoms than men (mean 1.8, SD: 1.4). Patients requiring ICU admission (mean: 2.5; SD; 1.5) also showed greater (IRR1.20, 95%CI 1.03–1.38, $P = 0.016$) number of post-COVID symptoms than those not requiring ICU admission (mean: 2.0, SD: 1.5). The most frequent symptoms were fatigue (60.8%), hair loss (26.3%), and dyspnea (23.5%). Women experienced fatigue (OR1.75, 95%CI 1.37–2.24; $P < 0.001$), hair loss (OR4.34, 95%CI 3.2–5.79; $P < 0.001$), and dyspnea (OR1.70, 95%CI 1.29–2.24; $P < 0.001$) more frequently than men (**Fig. 1**).

The regression model revealed that female (IRR1.37, 95%CI 1.25–1.49, $P < 0.001$), number of days at hospital (IRR1.005, 95%CI 1.002–1.009, $P = 0.002$), number of medical comorbidities (IRR1.11,

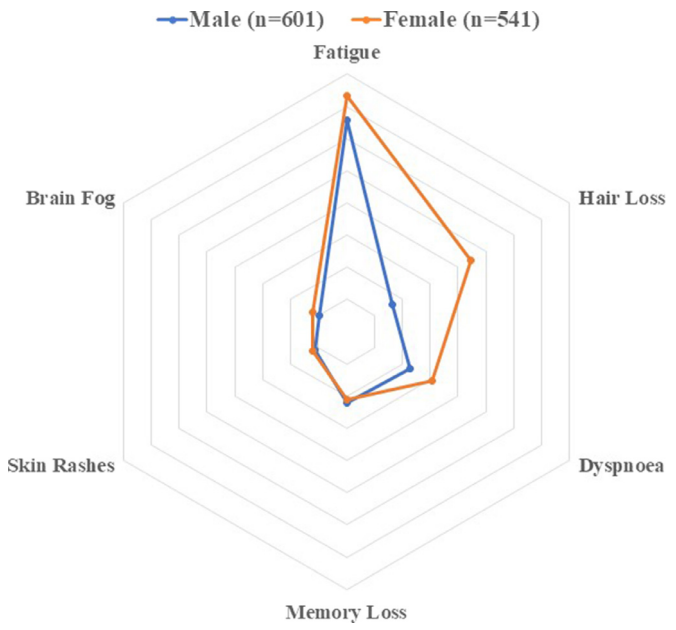


**Fig. 1.** Distribution of the most prevalent post-COVID symptoms (fatigue, hair loss, dyspnea, memory loss, skin rashes, and brain fog) in male and female patients.

95%CI 1.05–1.16, $P < 0.001$) and number of acute COVID-19 symptoms at hospital admission (IRR1.24, 95%CI 1.17–1.31, $P < 0.001$) were significantly associated with the number of long-term post-COVID symptoms.

This multicenter study found that 80% of hospitalized COVID-19 survivors exhibited at least one post-COVID symptom seven months after hospital discharge. Fatigue, hair loss, and dyspnea were the most prevalent symptoms. Female gender, number of days at hospital, previous comorbidities, and number of symptoms at hospital admission were associated with a higher number of long-term post-COVID symptoms.

Our prevalence rates of fatigue (60.8%), hair loss (26.3%), and dyspnea (23.5%). as post-COVID sequelae agree with pooled prevalence data reported by Lopez-Leon et al.[1] Although most studies investigating post-COVID symptoms have included follow-up periods < 3 months,[1] a small number of single-center studies have included follow-ups > 6 months.[4–7] Our study increases evidence to the current literature with a large, multicenter design evaluating long-term post-COVID symptoms. Based on the available evidence, the term persistent post-COVID is supported, since symptoms are present more than six months after infection.[8]

It seems that the post-COVID-19 symptom burden will be comparable to the long-term burden of severe acute respiratory syndrome (SARS), where subjects present with symptoms one year after infection.[9] In fact, unlike other acute respiratory syndromes, COVID-19 survivors also exhibit multiple non-respiratory symptoms, e.g., tachycardia, ageusia, anosmia, brain fog, memory loss and gastrointestinal problems, several months after infection. Biological (e.g., cytokine storm) and emotional (e.g., posttraumatic stress, uncertainty on prognosis, social alarm) factors surrounding COVID-19 are suggested to be responsible of this plethora of post-COVID symptoms. This heterogeneity in post-COVID symptoms supports that they will certainly need a multidisciplinary treatment.

Identification of risk factors associated with persistent COVID-19 sequelae will facilitate diagnosis and counselling strategies for these patients. We identified that female gender, longer stay at hospital, higher number of comorbidities, and higher number of symptoms at hospital admission were risk factors associated with a higher number of post-COVID symptoms seven months after discharge. These results agree with potential risk factors previously identified in other single-center studies[1].

Our study has some weaknesses. First, only hospitalized patients were included. Second, the number of patients requiring ICU admission was small. Third, we did not collect objective measures of COVID-19 disease, e.g., inflammatory biomarkers, blood oxygen saturation.

## Author contributions

All authors contributed to the study concept and design. CFdlP, DMP, VGM, and VHB conducted literature review and did the statistical analysis. VGM, MVA, CG, CMEM, MLC, JAAN, LJMT, TSV, JTM, MGCD, and SPC recruited participants. JRJ, MPC, AIdlLR, SFN, LLF, ROS, MGM, SAQ and JLAB collected data. LAN supervised the study. All authors contributed to interpretation of data. CFdlP, DPC, VGM, MLC and LA contributed to drafting the paper. All authors revised the text for intellectual content and have read and approved the final version of the manuscript.

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## Similar prevalence of long-term post-COVID symptoms in patients with asthma: A case-control study

*Dear Editor,*

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disproportionately impacts people with some pre-existing medical comorbidities, e.g., diabetes, hypertension or cardiovascular conditions. For instance, hypertensive patients exhibit higher mortality risk than normotensive patients with SARS-CoV-2 infection.[1] Asthma is another medical comorbidity which could influence the course of COVID-19. Interestingly, asthma seems to be a "protective factor", since the risk of presenting severe COVID-19 in people with asthma is small;[2]although a recent meta-analysis concluded that pre-existing asthma was a predictor of intubation particularly just in young and obese COVID-19 patients.[3]

Current evidence supports the presence of long-COVID, that is, individuals who have recovered from COVID-19 but exhibit symptoms after the acute phase far longer than it would be expected.[4]In a letter to the editor in *Journal of Infection,* Garrigues et al.analysed the presence of post-COVID symptoms in hospitalized patients and found that the most prevalent persistent symptoms were fatigue, dyspnoea, and loss of memory.[5]Very recently, Moreno-Perez et al. observed that 59% of hospitalized and 37% of non-hospitalized patients exhibited post-COVID symptoms 3 months after the infection.[6] In a posterior letter to the editor in *Journal of Infection*, Garcia-Pachon et al. described a series of patients with asthma showing low prevalence of symptoms 3 months after infection.[7]However, this study did not include a comparison control group including COVID-19 patients without asthma. We present the first case-control study comparing the differences in post-COVID symptoms between hospitalized patients with and without asthma.

From all patients admitted to Hospital Universitario Infanta Leonor-Virgen de la Torreand Hospital Universidad Fundación Alcorcon (Madrid, Spain) with a diagnosis of SARS-CoV-2 by RT-PCR technique during the first wave of the pandemic (March 10th to May 31st, 2020), a randomized sample of 400 patients from each hospital was selected. From those selected, patients with asthma prior to hospitalization were included as cases. Additionally, age-

**Table 1.**
Demographic, hospitalisation data, and post-COVID symptoms of COVID-19 patients with and without pre-existing asthma.

| | Asthmatic ($n = 61$) | Non-asthmatic ($n = 122$) |
|---|---|---|
| **Age, mean (SD), years** | 55 (17) | 55 (16.5) |
| **Gender, male/female (%)** | 15 (24.6%) / 46 (75.4%) | 30 (24.6%) / 92 (75.4%) |
| **Weight, mean (SD), kg.*** | 79.5 (23) | 77.0 (15.5) |
| **Height, mean (SD), cm.** | 164 (11) | 163 (9) |
| **Body Mass Index, mean (SD), kg/cm$^2$*** | 29.8 (9.5) | 29.0 (4.5) |
| **Smoking status, n (%)** | | |
| Active | 4 (6.6%) | 9 (7.3%) |
| None or Former | 57 (93.4%) | 112 (92.7%) |
| **Medical co-morbidities** | | |
| Asthma Treatment | 61 (100%) | 0 (0.0%) |
| Hypertension | 13 (18.9%) | 28 (22.9%) |
| Cardiovascular Disease | 4 (6.5%) | 10 (8.2%) |
| Diabetes* | 1 (1.6%) | 10 (8.2%) |
| Obesity | 2 (3.3%) | 7 (5.7%) |
| Rheumatological Disease | 1 (1.6%) | 4 (3.3%) |
| Chronic Obstructive Pulmonary Disease | 3 (4.9%) | 5 (4.1%) |
| Migraine | 3 (4.9%) | 3 (2.4%) |
| Other (Cancer, Kidney Disease) | 9 (14.7%) | 23 (18.8%) |
| **Symptoms at hospital admission, n (%)** | | |
| Fever | 38 (62.3%) | 90 (73.7%) |
| Dyspnoea* | 28 (45.9%) | 35 (28.6%) |
| Myalgia* | 32 (52.4%) | 42 (34.4%) |
| Cough | 21 (34.4%) | 35 (28.6%) |
| Headache | 12 (19.7%) | 27 (22.1%) |
| Diarrhoea | 11 (18.0%) | 24 (19.7%) |
| Anosmia | 6 (9.8%) | 13 (10.6%) |
| Ageusia | 4 (6.6%) | 8 (6.6%) |
| Throat Pain | 6 (9.8%) | 10 (8.2%) |
| **Stay at the hospital, mean (SD), days** | 14.5 (14.4) | 12.8 (10.1) |
| **Intensive Care Unit (ICU) admission** | | |
| Yes/No, n (%) | 7 (11.5%) / 54 (88.5%) | 5 (4.1%) / 117 (95.9%) |
| Stay at ICU, mean (SD), days | 18.3 (21.1) | 9.4 (8.4) |
| **Number of post-COVID symptoms, n (%)** | | |
| None | 8 (13.2%) | 25 (20.4%) |
| 1 or 2 | 23 (37.7%) | 45 (36.9%) |
| 3 or more | 30 (49.1%) | 52 (42.7%) |
| **Post-COVID symptoms, n (%)** | | |
| Dyspnoea on exertion | 46 (75.4%) | 71 (58.2%) |
| Fatigue | 40 (65.6%) | 75 (61.5%) |
| Dyspnoea rest | 21 (34.4%) | 34 (27.8%) |
| Memory Loss | 11 (18.0%) | 20 (16.4%) |
| Skin Rashes | 8 (13.1%) | 13 (10.6%) |
| Concentration loss | 8 (13.1%) | 14 (11.4%) |
| Cognitive Blunting - Brain fog | 6 (9.9%) | 10 (8.2%) |
| Gastrointestinal Disorders - Diarrhoea | 2 (3.3%) | 5 (4.1%) |
| Tachycardia-Palpitations | 6 (9.8%) | 8 (6.5%) |
| Ocular/Vision Disorders | 4 (6.6%) | 9 (7.3%) |
| Ageusia/Hypogeusia | 1 (1.6%) | 3 (2.5%) |
| Anosmia/Hyposmia | 4 (6.6%) | 2 (1.6%) |
| Throat Pain | 2 (3.3%) | 3 (2.5%) |
| **HADS-D (0–21), mean (SD)*** | 6.1 (5.6) | 5.4 (4.9) |
| Depressive Symptoms (HADS-$D \geq 10$ points), n (%) | 17 (27.9%) | 27 (22.1%) |
| **HADS-A (0–21), mean (SD)*** | 5.5 (5.5) | 5.3 (4.8) |
| Anxiety Symptoms (HADS-$A \geq 12$ points), n (%) | 9 (14.75%) | 14 (11.5%) |
| **PSQI (0–21), mean (SD)*** | 8.8 (4.6) | 7.5 (4.3) |
| Poor Sleep Quality (PSQI $\geq$8 points), n (%)* | 33 (54.1%) | 51 (41.8%) |

HADS: Hospital Anxiety and Depression Scale (A: Anxiety; D: Depression); PSQI: Pittsburgh Sleep Quality Index; SD: Standard Deviation.

* Significant differences between asthmatic and non-asthmatic patients ($P<0.01$).

and sex- matched hospitalized COVID-19 patients without pre-existing asthma were recruited as controls. Asthma was classified according the 2019 Global Initiative for Asthma (GINA) guidelines (www.ginasthma.org/). The study was approved by both local Ethics Committees (HUIL/092–20, HUF/EC1517). Participants provided informed consent before collecting data.

Clinical and hospitalization data were collected from hospital records. Participants were scheduled for a telephonic interview by trained healthcare professionals around 7.5 months (SD 0.5) after hospital discharge. Patients were asked to report the presence of symptoms after hospitalization, and if these symptoms persisted at the time of the study. Participants were systematically asked about a predefined list of post-COVID symptoms including fatigue,dyspnoea at rest, dyspnoea on exertion, chest pain, headache, anosmia, ageusia, cough, palpitations,diarrhoea, cognitive blunting/brain fog, or memory loss, but they were free to report any further symptom that they considered relevant.

The Hospital Anxiety and Depression Scale (HADS) and the Pittsburgh Sleep Quality Index (PSQI) were used to assess anxiety/depression symptoms and sleep quality, respectively, as both can be adequately administered by telephone.[8]We considered cut-off scores considered on the Spanish populationfor determin-

ing the presence of anxiety (HADS-$A \geq 12/21$ points) and depressive (HADS-$D \geq 10/21$ points) symptoms and poor sleep quality (PSQI≥8/21 points).[9]

The statistical analysis was conducted with STATA 16.1 (StataCorp. 2019, USA). The McNemar and paired Student t-tests were applied to compare proportions and means between groups. Multivariable conditional logistic regression models were constructed to identify variables associated to the presence of pre-existing asthma. Adjusted odd ratios (OR) or Incident Rate Ratios (IRR) with their 95% confidence intervals (95%CI) were calculated.

From 800 randomized COVID-19 patients hospitalized during the first wave of the pandemic, 61 patients with asthma and 122 age- and sex-matched patients without asthma were recruited. A greater proportion of patients with asthma experienced dyspnoea and myalgia as onset symptoms at hospital admission ($P<0.05$, Table 1). Higher number of patients with asthma also presented diabetes as comorbid condition when compared with those without asthma ($P=0.045$).

From the total sample, just 34 (18.6%) were completely free of any post-COVID symptom 7 months after hospital discharge. Individuals with pre-existing asthma showed similar (IRR1.07, 95%CI 0.87–1.33, $P=0.476$) number of post-COVID symptoms (mean: 2.4, SD: 1.4) than those without asthma (mean: 2.2, SD: 1.6). The most prevalent post-COVID symptoms weredyspnoea on exertion, fatigue, anddyspnoea at rest (Table 1). In fact, a greater proportion of patients with pre-existing asthma reporteddyspnoea on exertion (OR2.73; 95%CI 1.23–6.08; $P=0.013$) than those without asthma. No differences in the presence of fatigue (OR1.23; 95%CI 0.61–2.49; $P=0.556$),dyspnoea at rest (OR1.38; 95%CI 0.70–2.75; $P=0.347$), depressive symptoms (OR1.39, 95%CI 0.67–2.89), anxiety symptoms (OR1.32, 95%CI 0.55–3.18) or poor sleep quality (OR1.71, 95%CI 0.89–3.27, $P=0.105$) between patients with or without asthma were observed (Table 1).

Identification of the phenotype of patients at a higher risk of death during the acute infection or a higher risk of developing post-COVID symptoms is crucial. In our sample, the presence of long-term post-COVID symptoms was similar between patients with and without pre-existing asthma, suggesting that asthma seems not to be a risk factor for more severe long-term post-COVID symptoms but either was a “protective” factor for that. Our results are contrary to those found by Garcia-Pachon et al. in their letter to the Editor.[7] It should be considered that Garcia-Pachon et al. did not include a “control” group without asthma and also included a shorter follow-up.[7] Additionally, these authors included non-hospitalized patients, which could explain the discrepancies. Fatigue anddyspnoea where the most common post-COVID symptoms in agreement with current literature,[5,6] but the presence of dyspnoea with exertion was more frequent in patients suffering from asthma. Distinction between dyspnoea at rest and on exertion maybe crucial in these patients.

Our study has limitations. First, the prevalence of asthma in our sample was 7.6%, in agreement with a meta-analysis reporting a pooled prevalence of asthma in COVID-19 patients of 7.46% (95%CI 6.25–8.67); [10]however, this sample could be considered small. Second, we conducted the follow-up by telephone. Third, just hospitalized patients were included. Fourth, we did not collect objective measures of COVID-19 disease such as inflammatory biomarkers. Finally, we collected data cross-sectionally; therefore, future longitudinal studies are needed.

## Declaration of Competing Interest

The authors declare no conflict of interest

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## Reactogenicity, safety and antibody response, after one and two doses of mRNA-1273 in seronegative and seropositive healthcare workers

*Dear Editor,*

We read with great interest the prospective SARS-CoV-2 serosurveillance study of Harris et al. in your columns.[1] During 6 months and before the UK vaccination campaign, they followed the antibody response in a cohort of 2246 healthcare workers (HCWs). They relied on 4 commercial kits and an in-house test to track the antibody response to natural SARS-CoV-2 exposure. As expected with kits that targets different proteins and total or specific immunoglobulin sub-groups, they observed, along time, fluctuating seropositivity from one test to another. Nevertheless, they showed that SARS-CoV-2 antibodies do not decline as quickly as predicted by smaller cohorts of patients with shorter follow-up.

With the start of worldwide vaccination campaigns, scattered evidence is emerging from the medical literature to dispute the second injection of mRNA vaccines in individuals previously infected with SARS-CoV-2.[2,3] Knowing that a significant proportion of the population would be seropositive at the time of the first injection, we wanted to investigate the utility of a second dose under both supply and time constraints. Here we report our observations in a cohort of healthcare workers (HCWs) who were administered mRNA1273 at the inception of the national vaccination campaign. Manisty et al., first questioned the administration of the second dose of BNT162b2, another mRNA vaccine, so as to reserve it only for individuals not previously infected.[3] They showed that the antibody response after a first dose in HCWs previously infected ($n = 24$) reached levels 140 times higher than their peak value before vaccination. Krammer et al. observed in previously infected individuals antibody titers 10–45 times higher than in their uninfected counterparts after a first-dose of either BNT162b2 ($n = 88$) or mRNA-1273 ($n = 22$) vaccines[2].

In our prospective study, we compared not only the antibody response (Fig. 1) but also the local and systemic side effects in terms of duration and intensity after the first and second dose of mRNA-1273 (Fig. 2).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) on a LIAISON®XL analyzer previously validated in our laboratory.[4] In order to assess the serological status of the participants ($n = 160$), a first dosage was carried out with a median time (± 95% confidence interval [CI]) of 2 (± 0.29) days before the first injection (T0). Among those, 36 participants were found to be seropositive. Two other samples were taken from all participants 2 weeks after the first injection (T1) (median time [± 95% CI]: 16 [± 0.25] days), and 2 weeks after the second injection (T2) (median time [± 95% CI]: 14 [± 0.21] days). Except for 2 individuals, all participants who were seropositive at T0, saw their antibody levels boosted by the first dose but no additional boosting effect was observed after the second injection. In these two individuals (1.6%), the second injection made it possible to raise their antibody levels from 59.7 and 105 AU/mL to above the maximum detection limit (> 400 AU/mL) at T2. In seronegative participants, the anti-S antibody titers obtained after a single dose were comparable to those obtained in unvaccinated seropositive participants while the second injection was necessary to achieve higher antibody levels approaching those obtained for seropositive individuals (T1). We also explored the frequency of side effects after the first dose in a slightly larger cohort ($n = 206$, mean age, 48.6 (± 11.6) years) including 151 seronegative (71% female) and 55 seropositive participants (69% female), as well as after the second dose in 113 participants (mean age, 49.2 (± 11.3) years) including 89 seronegative participants (69% female) and 24 seropositive participants (58% female). The intensity of local and systemic side effects reported by participants was graded into 4 levels of severity: (very mild, mild, moderate, severe). Common side effects such as articular pain, muscular pain, headache, fatigue, fever, adenopathy and oedema from the first dose appear to be more frequent and severe in previously infected individuals ($P < .05$). Nevertheless, it seems that the second injection generates a greater overall systemic reaction than that observed after the first one, regardless of the initial serological status of the participants. Seven days after the first or the second dose, all observed side effects disappeared in all participants and none were hospitalized. Two weeks after the last injection, a clinical follow-up questionnaire was sent to the 113 participants. Only 41 were returned at the time of redaction. None of the respondents reported thinking they had been infected. Ten of them had to undergo a RT-qPCR and all were negative.

Our results plead, in a supply-limited environment, for reserving the second dose scheme to seronegative individuals prior to vaccination, especially when the serological status is easily accessible, as the additional protective effect of the second dose has yet to be demonstrated in seropositive individuals. The determination of the antibody titers after the initial dose could be used in order to catch-up the very few vaccinees with a weaker response.

In the worrying context of an increase in the spread of mutant viruses around the world[5] and given that most registered vaccine platforms use the two-dose-prime boost approach,[6–8] this strategy would help speed up vaccination campaigns and achieve group immunization goals more rapidly. Even though titers of antibodies against S1 spike protein seem to correlate with viral neutralization studies,[6,9,10] only long-term serosurveillance studies will not only confirm the results of our investigation but will also determine the IgG protection thresholds.

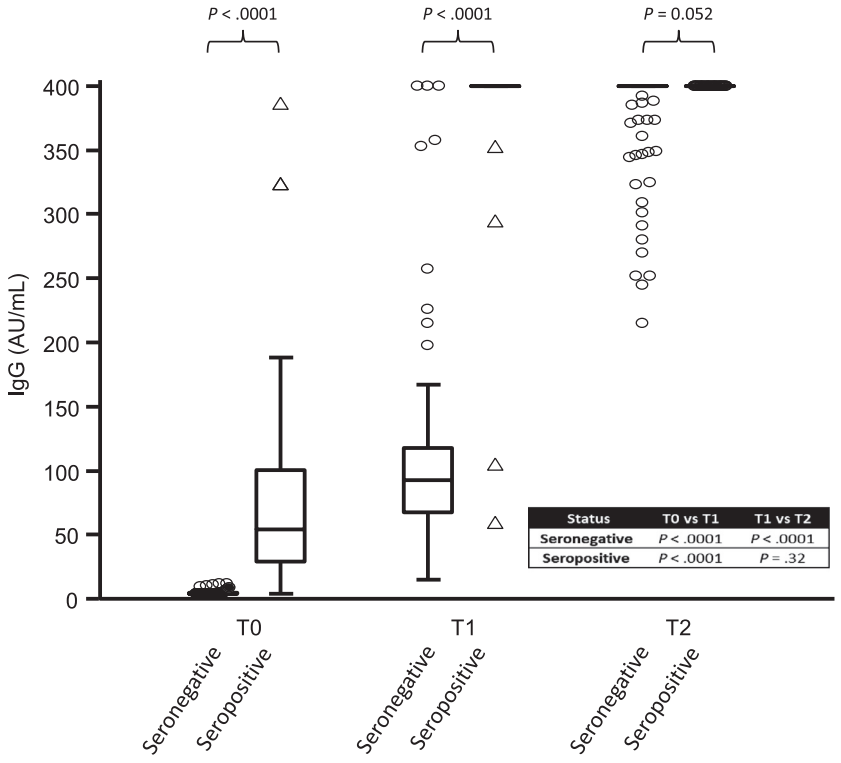


**Fig. 1.** Antibody responses to one and two doses of the mRNA-1273 in seronegative and seropositive individuals.

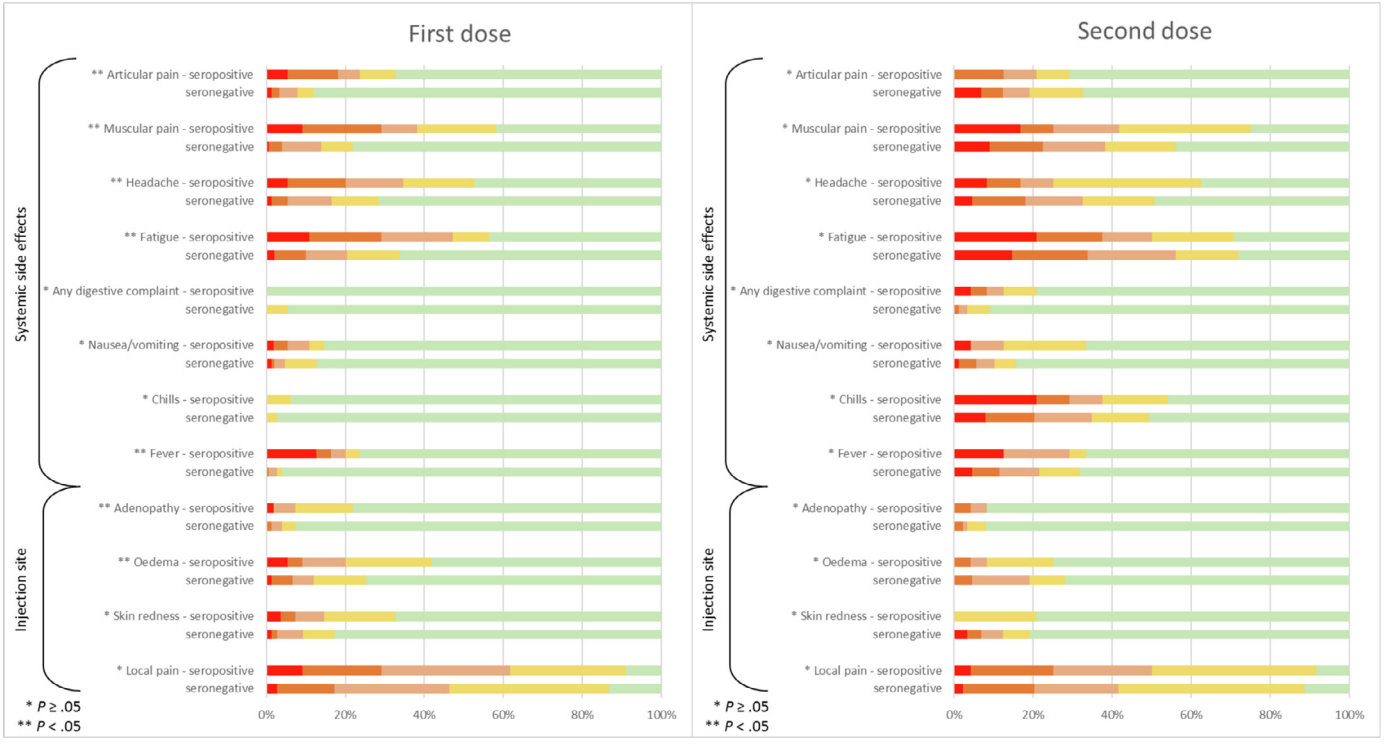


**Fig. 2.** Reactogenicity and side effect profile of the mRNA-1273 in seronegative and seropositive individuals after the first and second doses.

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None declared.

## Ethical approval

This study has been approved by the Ethical Committee of the HIS-IZZ (ethical agreement number: CEHIS/2021-7).

## Authorship

*Study concept and design*: Marie Tré-Hardy, Roberto Cupaiolo, Emmanuelle Papleux, Alain Wilmet, Marc Vekemans, Ingrid Beukinga, Laurent Blairon. *Investigation:* All. *Acquisition, analysis, interpretation of data and visualization:* Marie Tré-Hardy, Roberto Cupaiolo, Laurent Blairon. *Supervision and manuscript-first draft*: Marie Tré-Hardy, Laurent Blairon. *Critical revision of the manuscript*: All.

Fig. 1 shows SARS-CoV-2 IgG antibodies titres directed against the subunits (S1) and (S2) of the virus spike protein before (T0), after the first (T1) and second injection (T2), according to the participant serological status ($n = 160$). The Box-and-Whisker plot represents the 25th and 75th percentiles. Inside the box, the horizontal line indicates the median (the 50th percentile). Discs and triangles respectively represent outside and far out values. A Mann–Whitney *U* test was used to assess the differences in IgG levels between seronegative and seropositive subjects on the one hand and to assess the changes in these levels between T0, T1 and T2 times within each of these groups on the other hand.

Fig. 2 lists the reported side-effects according to their nature and severity during the first ($n = 206$) and second ($n = 113$) dose administration. The gradation was as follow: absence (green), very mild (yellow), mild (light orange), moderate (dark orange), severe (red). A given participant possibly experienced more than one symptom. A Chi-square test was used for the comparison of side effects in seronegative versus seropositive subjects. A *P*-value $< .05$ was considered significant.

## Declaration of Competing Interest

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

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