ACE I/D polymorphism and epidemiological findings for COVID-19: One year after the pandemic outbreak in Europe

Ristić, Smiljana; Pavlić, Sanja Dević; Nadalin, Sergej; Čizmarević, Nada Starčević

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Letter to the Editor

A stark difference in the profiles of defective viral transcripts between SARS-CoV-2 and SARS-CoV

Dear Editor,

Recently, Fantini et al. reported that mutations in the N-terminal (NTD) and receptor binding (RBD) domains of the SARS-CoV-2 spike protein act synergistically to optimize virus infection[1]. However, genomic variants beyond the coding region of spike protein is poorly understood, especially the large structural variants within a single or between closely related coronaviruses.

SARS-CoV and SARS-CoV-2 are two closely related β coronaviruses that caused the global pandemics of SARS and COVID-19 in 2003[2] and 2019[3], respectively. Despite similarities in their receptor, tropism, and clinical manifestations, the two viruses demonstrate a drastic difference in their transmissibility, which remains largely unexplained. Defective viral transcripts are known to attenuate the replication of a parental virus by competing translational machinery in host cells[4].

To determine whether the two viruses produce a differential profile of defective transcripts, we performed direct RNA sequencing (dRNA-seq) of the transcripts derived from the Vero E6 cells infected with SARS-CoV, SARS-CoV-2 or MERS-CoV for 24 h as we did previously[5,6]. We chose this sequencing technology for its potential to generate ultra-long read[7]. Notably, dRNA-seq generates reads from 3′ to 5′ end and depends on the presence of poly(A) tail at 3′ end, meaning that all reads produced with dRNA-seq will carry a poly(A) tail. We generated approximately 2.88, 0.86 and 2.04 million reads for the SARS-CoV, SARS-CoV-2 and MERS-CoV samples, respectively, with N50 sizes of approximately 1.9, 2.5 and 2.2 k nucleotides (nts), respectively (Table S1, Fig. S1). Approximately 87.6%, 84.6% and 59.8% of the total reads were viral reads in the samples infected with SARS-CoV, SARS-CoV-2 and MERS-CoV, respectively. We focused mainly on the full-length reads that carried both a leader at the 5′ end and a poly(A) tail at the 3′ end (Fig. 1A). These reads represented 12.2%, 26.3% and 11.2% of the total viral reads of SARS-CoV and SARS-CoV-2, respectively. Mapping of these reads allowed us to unambiguously determine the global structure of both DVGs and dsgRNAs, because the reads without a leader may be subject to the uncertainty in terms of the absence of an unknown portion at the 5′ end.

As expected, the coverages of the full-length reads demonstrated a precise demarcation that coincided precisely with the predicted ORF boundaries for all structural proteins except for the ORFs 6 and 10 (Fig. 1B). We were surprised to find that although few reads that contained a leader were mapped to the start of ORF 6, approximately 6000 reads were precisely mapped to the internal part of ORF M (Fig. S2), indicating that the ORF 6 sgRNAs do not begin from their own start codon, but from the transcriptional regulatory sequence (TRS) within the ORF M, which is consistent with the previous findings, in which the core sequence was identified but the global structure of ORF 6-specific sgRNAs was not clear[8,9].

We were able to recover two distinct categories of defective viral transcripts that contain both a 5′ leader and a 3′ poly(A) tail. The first category carries an extended 5′ and 3′ end sequence separated by a large deletion, which is referred to as defective viral genome (DVG). The second category consists of defective subgenomic RNAs (dsgRNAs) that lack various portions of the 3′ co-terminal end (Fig. 1A). Strikingly, we found that full-length profiling of viral transcripts revealed a stark difference in the profiles of sgRNAs between SARS-CoV and SARS-CoV-2. Despite the high abundance of dsgRNAs produced by SARS-CoV and MERS-CoV, few dsgRNAs were detected in SARS-CoV-2 (Fig. 2A-G, Figs. S3 and S4). dsgRNAs were observed for all structural proteins with various abundances in the case of SARS-CoV. For example, dsgRNAs accounted for as many as 60% of all full-length reads mapped to the ORF S that bear the longest full-length sgRNA, and dsgRNAs accounted for approximately 25% of all full-length reads mapped to the ORF N that bear the shortest full-length sgRNA (Fig. 2A-G). Apparently, the longer the ORF, the greater the abundance of dsgRNA, which suggests poor processivity of the RNA-dependent RNA polymerase (RdRp) of SARS-CoV in the generation of long transcripts. Sequencing of the antisense RNAs of SARS-CoV-2 revealed that the abundance of antisense RNAs was approximately 1000 times lower than that of sense RNAs, thus indicating the great efficiency of sgRNA synthesis.

In addition to the dsgRNAs, our full-length transcript profiling revealed the presence of DVGs in both SARS-CoV and SARS-CoV-2 (Fig. 2H-K, Fig. S4). The 5′ UTR extended into the ORF 1ab up to the ORF of nonstructural protein (nsp) 3 in DVGs. These DVGs lacked most coding regions, including those encoding the remaining nsps, and those encoding various ORFs of structural proteins at their 3′ ends (Fig. 1A). One important finding was that the two viruses demonstrated a significant difference in their 3′ ends. Specifically, nearly a half of the junction site between the 5′ and 3′ parts of DVGs began precisely at the beginning of the ORF N in the case of SARS-CoV and MERS-CoV (Fig. 2I, K, and Fig. S4B), whereas such a bias was absent in the DVGs produced by SARS-CoV-2 (Fig. 2H and J). These results indicate a differential DVG-generation mechanism between SARS-CoV-2 and other coronaviruses. Sequencing data also showed that overall SARS-CoV-2 transcripts had substantially longer poly(A) tails than SARS-CoV transcripts (Fig. S5A), and this was also the case in terms of DVGs (Fig. S5B) and dsgRNAs (Fig. S5C). Functional relevance of the shorten poly(A) tail warrants further investigation.

In summary, our dRNA-seq results indicate that SARS-CoV-2 has evolved a unique capability to generate full-length sgRNAs but has lost the ability to retain the full-length ORF of N in its DVGs, which may have implications for its transmissivity. The ex-

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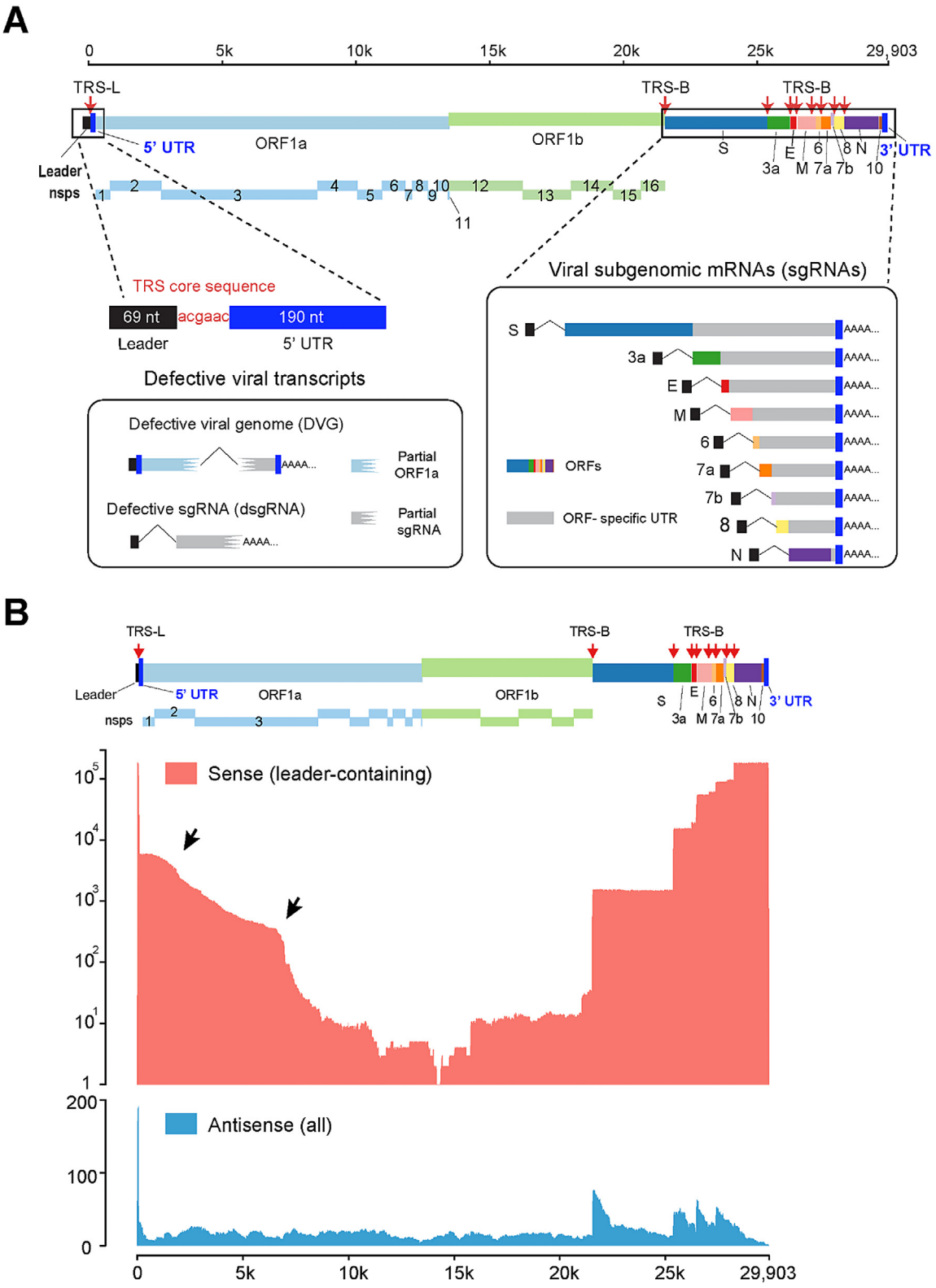

Fig. 1. Full-length dRNA-seq read coverage reveals precise boundaries of sgRNAs. (A) Schematic representation of genomic and subgenomic organizations of SARS-CoV-2 RNAs and their defective formats. Full-length genomic, subgenomic RNAs and UTRs are depicted in scale and are differentially color coded. Name of ORF and non-structural protein (nsp) are indicated. Positions of TRS-B and TRS-L inferred from the presence of core TRS are indicated with red arrow. A magnified view of 5′ end (leader and UTR) and subgenomic RNAs are shown below. Also shown on the left bottom are two types of defective viral transcript, including DVG and dsgRNA. Color codes for ORF, nsp and structural proteins are used throughout. (B) Shown on the top is the coverage of full-length reads carrying a leader and a poly(A) tail derived from dRNA-seq of sense-strand RNA. Note the precise punctuation between the read coverage and existing ORFs except ORF 6. Also note the two imprecise coverage drops within the ORF of nsp1–3 (indicated with arrowhead). Shown on the bottom is the coverage of all antisense reads derived from dRNA-seq with a poly(A) tailing step. Note the precise punctuation between the read coverage and the existing ORFs and a sharp jump in the antisense leader. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

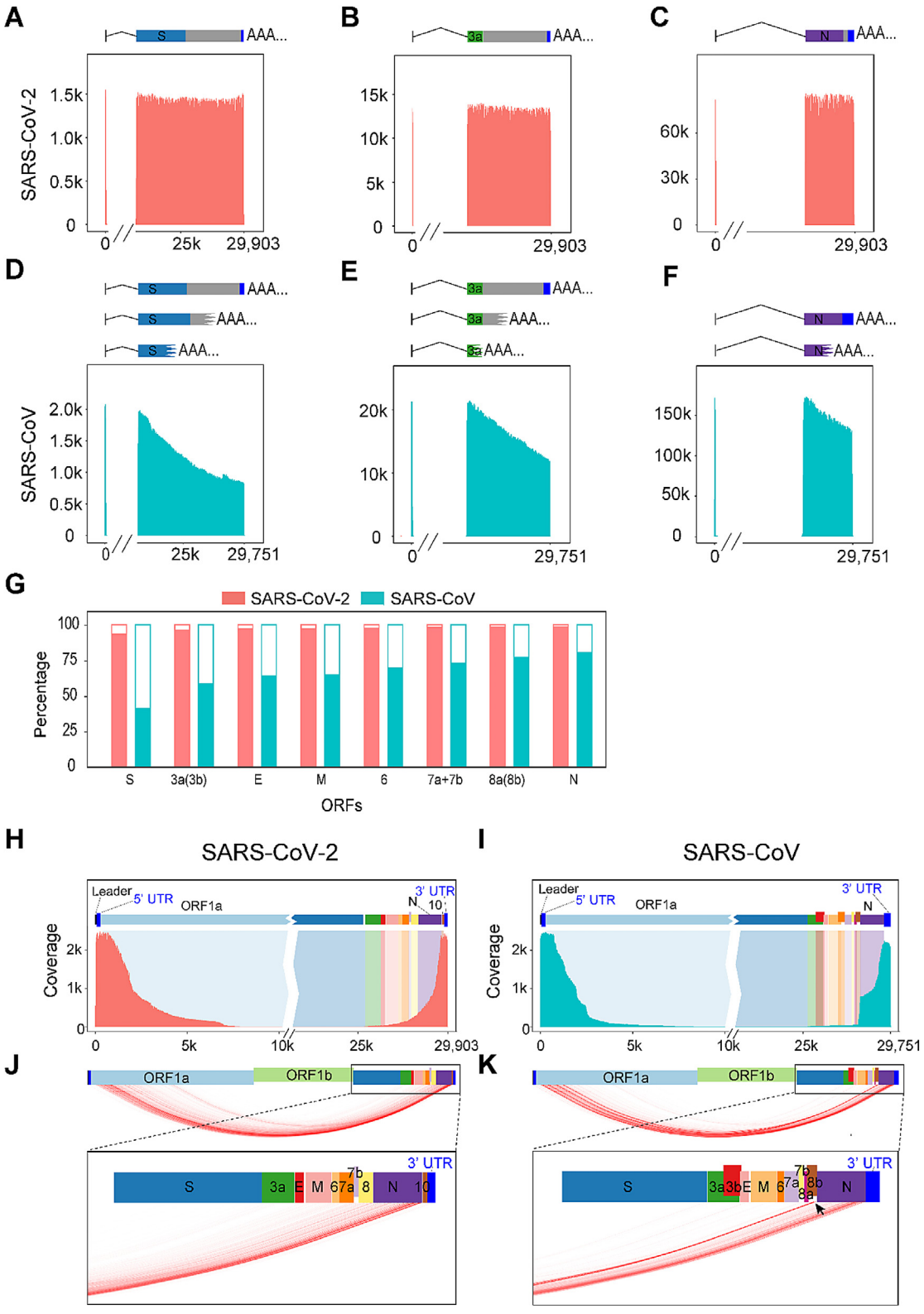

Fig. 2. A stark difference in the profiles of defective viral transcripts between SARS-CoV-2 and SARS-CoV. (A-G) Abundant defective sgRNAs in SARS-CoV but not in SARS-CoV-2. (A-C) Coverage of ORF-specific reads from SARS-CoV-2 for S (A), 3a (B) and N (C), respectively. Diagrams showing the full-length sgRNA are depicted on the top. (D-F) Coverage of ORF-specific reads from SARS-CoV for S (D), 3a (E) and N (F), respectively. Diagrams showing the both the full-length and defective sgRNA are depicted on the top. (G) Quantification of full-length and defective sgRNAs for each ORF in SARS-CoV and SARS-CoV-2. (H-K) Selective retention of full-length ORF N in the DVGs of SARS-CoV. (H-I) Shown are the coverages of DVGs that contain approximately 10k nts of sequences at the 5′ end and various parts at the 3′ end in SARS-CoV-2 (H) and SARS-CoV (I). (J) and (K) 5′ and 3′ junctions of the DVGs in (H) and (I) respectively. Curved lines represent the 5′ and 3′ locations of the junctions in SARS-CoV-2 (J) and SARS-CoV (K).

tremely low abundance of antisense strand of SARS-CoV-2 genome makes these RNAs an ideal target for development of inhibitory agents.

Ethics

Ethical approval was not required for this service evaluation and audit of practice.

Declaration of Competing Interest

No conflicts of interests declared by an author.

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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.2021.06.020.

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Dongying Xie[1]
Department of Biology, Hong Kong Baptist University, Hong Kong, China

Hin Chu[1], Dong Yang[1]
Department of Microbiology, the University of Hong Kong, Hong Kong, China

Qiutao Ding
Department of Biology, Hong Kong Baptist University, Hong Kong, China

Gefei Huang
Department of Biology, Hong Kong Baptist University, Hong Kong, China
State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

Luo Chen
Department of Biology, Hong Kong Baptist University, Hong Kong, China

Zongwei Cai*
State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China
Department of Chemistry, Hong Kong Baptist University, Hong Kong, China

Jiandong Huang*
School of Biomedical Sciences, the University of Hong Kong, China
Institute of Synthetic Biology, Shenzhen Institute of Advanced Technology (SIAT), Chinese Academy of Sciences (CAS), Shenzhen 518055, China

Zhongying Zhao*
Department of Biology, Hong Kong Baptist University, Hong Kong, China
State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China

*Corresponding authors.
E-mail addresses: zwcai@hkbu.edu.hk (Z. Cai), jdhuang@hku.hk (J. Huang), zyzhao@hkbu.edu.hk (Z. Zhao)

[1] These authors contributed equally to this work.

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Waning antibodies in SARS-CoV-2 naïve vaccinees: Results of a three-month interim analysis of ongoing immunogenicity and efficacy surveillance of the mRNA-1273 vaccine in healthcare workers

Dear Editor,

We read with interest the study recently published by Capetti and colleagues showing one-year durability of anti-spike IgG after natural exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] Although the antibody kinetics in symptomatic and asymptomatic patients is known,[1,2] we still ignore how it evolve beyond 6 months in vaccinees and if and how the initial serological status of vaccinees might influence it.

To date, antibody kinetics data after vaccination remain fragmented. The study by Doria-Rose et al., showed persistence of antibodies 6 months after the second dose of mRNA-1273 vaccine in 33 participants included in the phase 1 follow-up of the Moderna study without knowing their initial serological status before the vaccination.[3] Likewise, interim results from a phase 3 trial of the mRNA-1273 vaccine indicated 94.5% efficacy in preventing coronavirus disease 2019 (Covid-19).[4] Since efficacy trials have focused on individuals without prior exposure to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), little is known about the immune responses induced by mRNA-1273 in participants who have suffered from Covid-19. Finally, this large-scale, phase 3 study conducted by the firm Moderna was carried out from July 27 to October 23, 2020, away from the worrying spread of new SARS-CoV-2 variants.[5–7]

In our independent study, we compared the antibody response 2 weeks after the first injection (T1) (median time [± 95% CI]: 16 [± 2.26] days), 2 weeks after the second injection (T2) (median time [± 95% CI]: 14 [± 1.83] days) and 3 months after the first injection (T3) (median time [± 95% CI]: 86 [± 4.59] days) from 205 healthcare workers (HCWs) stratified according to their initial serological status. 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) previously validated in our laboratory[8] and also used by Capetti et al.[1] Effectiveness of the mRNA-1273 vaccine was also assessed through a medical questionnaire. Participants were asked to declare any results of RT-qPCR tests regardless of the reason behind, even in asymptomatic situations, and any eventual Covid-19 infection after vaccination (including severity of symptoms). To better apprehend the observed efficacy, a comparison of the level of antibodies directed against the nucleocapsid was carried out on part of the cohort of seronegative participants at T0 and T3 with the Platelia® SARS-CoV-2 Total Ab test (Bio-Rad®, Marnes-la-Coquette, France) detecting total antibodies (IgM, IgA and IgG) (n = 86/161). Since only these antibodies are produced during a natural infection, their detection allows us to identify the participants who have been infected by SARS-CoV-2 since their vaccination.

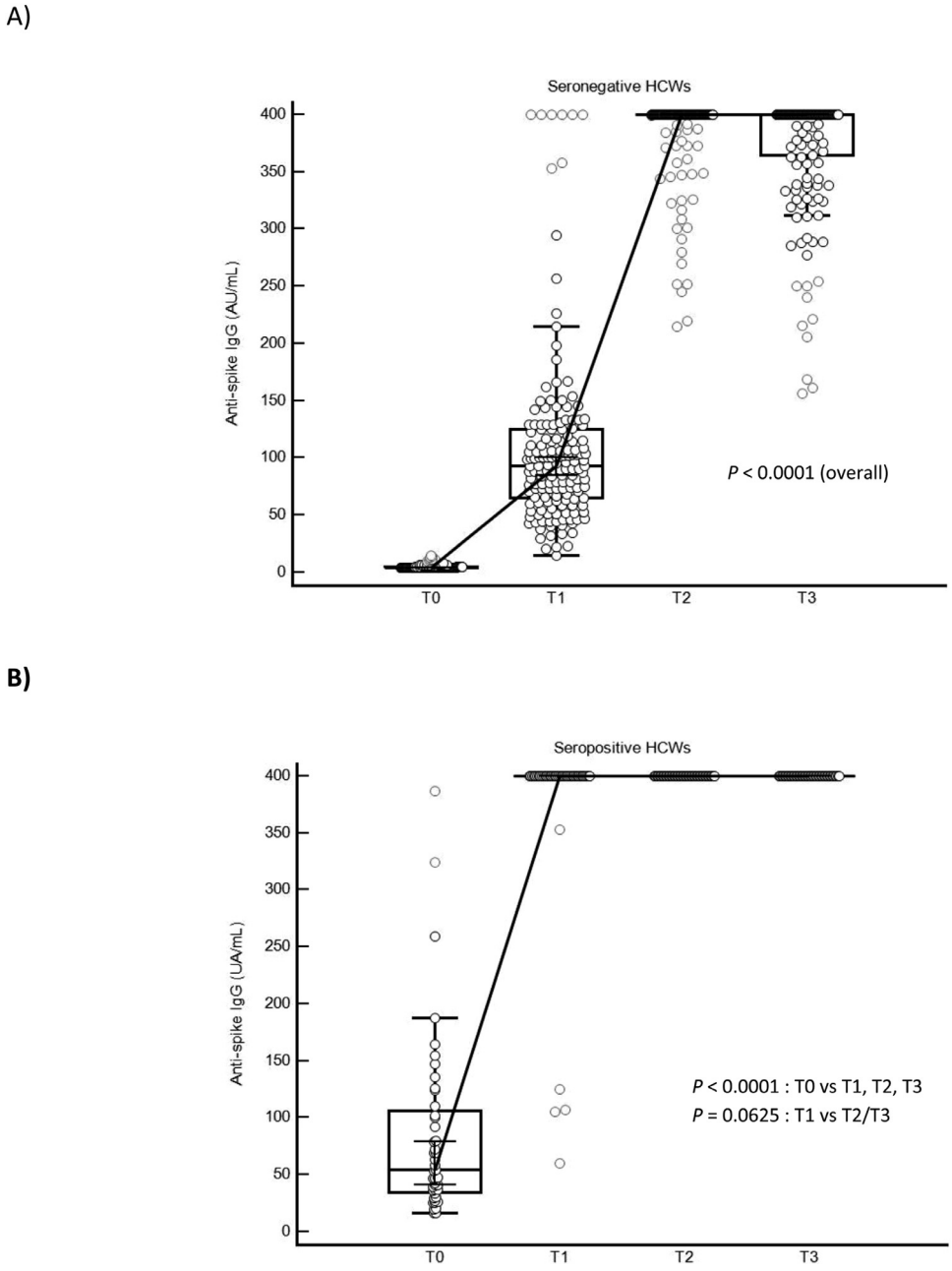

Fig. 1. Antibody responses in seronegative (A) and seropositive (B) HCWs after mRNA-1273 vaccination.
It shows the titers of SARS-CoV-2 IgG antibodies directed against the subunits (S1) and (S2) of the virus spike protein before (T0), 2 weeks after the first injection (T1), 2 weeks after the second injection (T2) and 3 months after the first infection (T3) according to the participant serological status (n = 205). The Box-and-Whisker plot represents the 25th and 75th percentiles. Inside the box, the horizontal line indicates the median (the 50th percentile). Discs in light grey represent far out values. A Wilcoxon test was used to assess the changes in IgG levels between T0, T1, T2 and T3 times within seronegative (n = 161) and seropositive subjects (n = 44). A P-value < 0.05 was considered significant.

In the initially seronegative participants ($n = 161$), we observed a persistence of anti-S-antibody levels 3 months after vaccination with nevertheless a decrease in the antibody levels observed between T2 and T3 in 48 participants (Fig. 1A). Conversely, an increase in antibody levels was observed in 15 seronegative HCWs. Interestingly, in seropositive people ($n = 44$), no drop in antibody was observed between T2 and T3. The measured levels are all above the maximum quantification value (> 400 AU/mL). Moreover, the administration of a second dose of vaccine in participants initially seropositive made it possible to catch-up the very few vaccinees ($n = 5$) with a weaker response at T1 by reaching the maximum level of antibodies at T2 (Fig. 1B).

Analysis of the clinical follow-up questionnaires revealed that none of the respondents reported thinking they had been infected ($n = 167$). Thirty-six of them had to undergo a RT-qPCR and all were negative. Finally, among the seronegative ones, only 2 participants developed antibodies directed against the nucleocapsid at T3 while all were negative at T0 ($n = 86$). Based on these results and given that none of the participants developed symptoms, the mRNA-1273 vaccine is effective at preventing Covid-19 illness. However, additional long-term serosurveillance studies based on larger cohorts will be necessary to confirm these observations. Monitoring of anti-nucleocapsid antibodies remains a complementary aid in detecting infections which are sometimes asymptomatic in vaccinated persons known to be initially seronegative.

Faced with an unprecedented global vaccine deployment, a close follow-up of vaccinees remains crucial to confirm both safety and long-lasting immune protection. In this study, we evaluated the immune response of the participants but also the effectiveness of the mRNA-1273 vaccine in a context different from the previous phase 1 and 3 studies by Moderna, under a higher virological pressure, and by categorizing the participants into 2 cohorts according to their serological status at initiation. Three months after vaccination, we confirm a very high efficacy and a persistence of anti-spike antibodies. However, the decrease observed in some seronegative participants argues for an additional dose of vaccine in the upcoming months for this specific subgroup.

Ethical approval

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

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Conflicts of Competing Interest

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

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Marie Tré-Hardy*
Department of Laboratory Medicine, Iris Hospitals South, rue Jean Paquot 63, Brussels 1050, Belgium
Faculty of Medicine, Université libre de bruxelles, Brussels, Belgium
Department of Pharmacy, Namur Research Institute for Life Sciences, University of Namur, Belgium

Roberto Cupaiolo, Alain Wilmet, Ingrid Beukinga, Laurent Blairon
Department of Laboratory Medicine, Iris Hospitals South, rue Jean Paquot 63, Brussels 1050, Belgium

*Corresponding author at: Department of Laboratory Medicine, Iris Hospitals South, rue Jean Paquot 63, Brussels 1050, Belgium.
E-mail address: mtrehardy@his-izz.be (M. Tré-Hardy)

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One year later: SARS-CoV-2 immune response and vaccination of healthcare workers post-infection

Dear Editor,

A recent study in this journal reported that prior SARS-CoV-2 infection is protective even in the absence of a detectable humoral immune response.[1] Our prospective longitudinal study was designed to measure the changes in the binding and neutralizing antibody titers in seropositive healthcare workers (HCWs) over a year and the changes in individuals who had and had not been vaccinated. We also assessed the incidence of positive SARS-CoV-2 RNA tests among HCWs who were seropositive for SARS-CoV-2 antibodies and in those who were seronegative.

From June 10, 2020 to July 10, 2020, 1 to 2 months after the end of lockdown in France, 8758 HCWs were screened for total serum antibodies by enzyme linked immunosorbent assay (ELISA) kit supplied by Wantai (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, China). All the 276 ELISA-positive personnel identified by the first serological screening were re-tested twice (November 30 to December 9 and March 30 to 15 April) to determine the evolution of SARS-CoV-2 neutralizing antibodies.[2] This study was approved by Toulouse University Hospital Ethics Committee

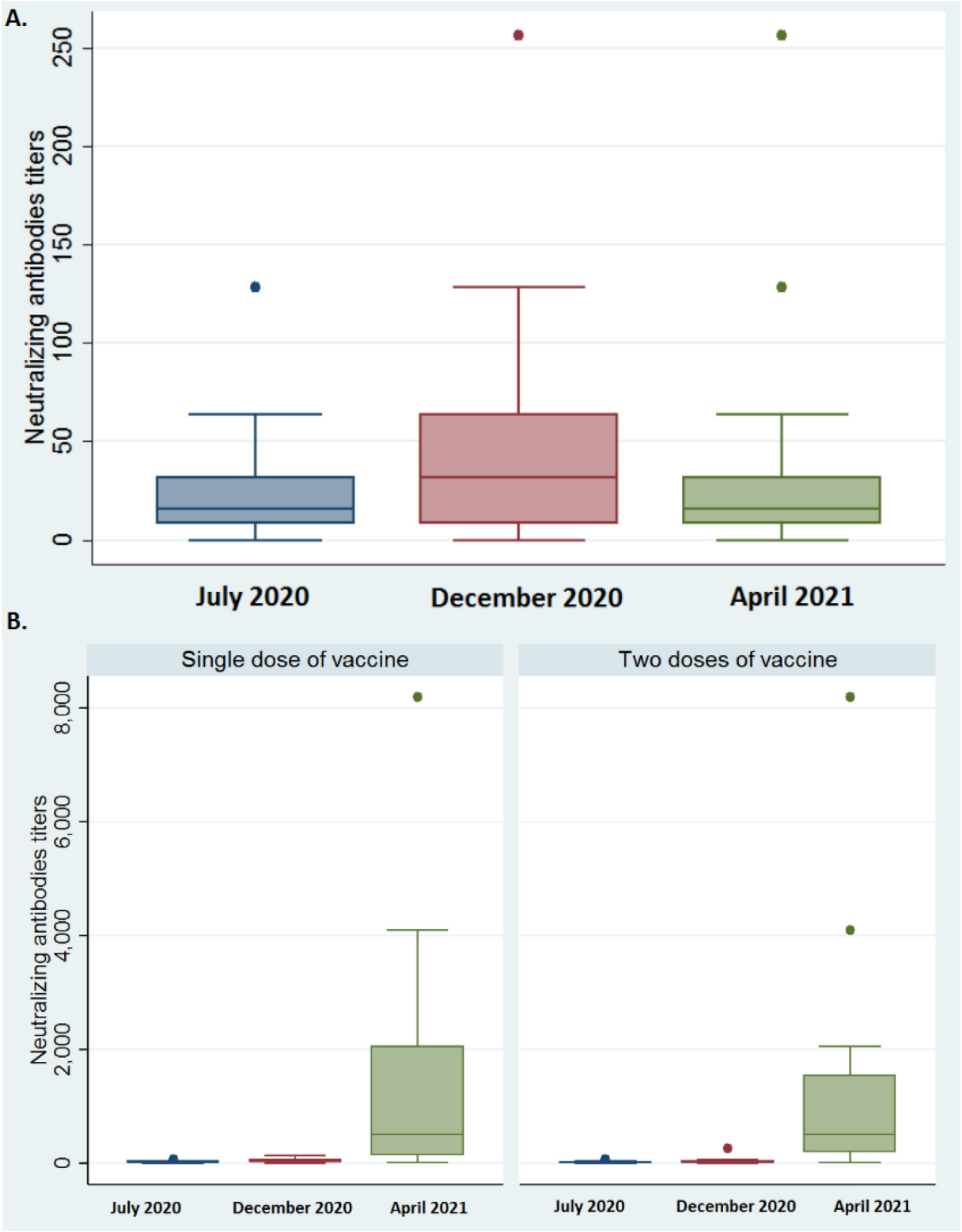

Fig. 1. Distributions of neutralizing antibody titers in July 2020, December 2020 and April 2021:
1A: among unvaccinated HCWs
1B: one month post vaccination, among vaccinated HCWs given either one or two doses of vaccine.

(COVIDBIOTOUL RC31/20/0162, CPP number: 20.05.09, CNIL number: 2020-A01292-37).

We monitored the ELISA total antibody values and the neutralizing antibody titers of 194 HCWs, 70.3% of the 276 who were serologically positive in July 2020, until April 2021. Only 40 (20.6%) were vaccinated: 17 (42.5%) with two doses of BNT162b2, 16 (40%) with one dose of ChAdOx1 nCoV-19 and 7 (17.5%) with one dose of BNT162b2. The correlation between the Wantai total antibody values and the neutralizing antibodies titers in April 2021 was 76.1% for unvaccinated HCWs and 72.7% for vaccinated HCWs. The

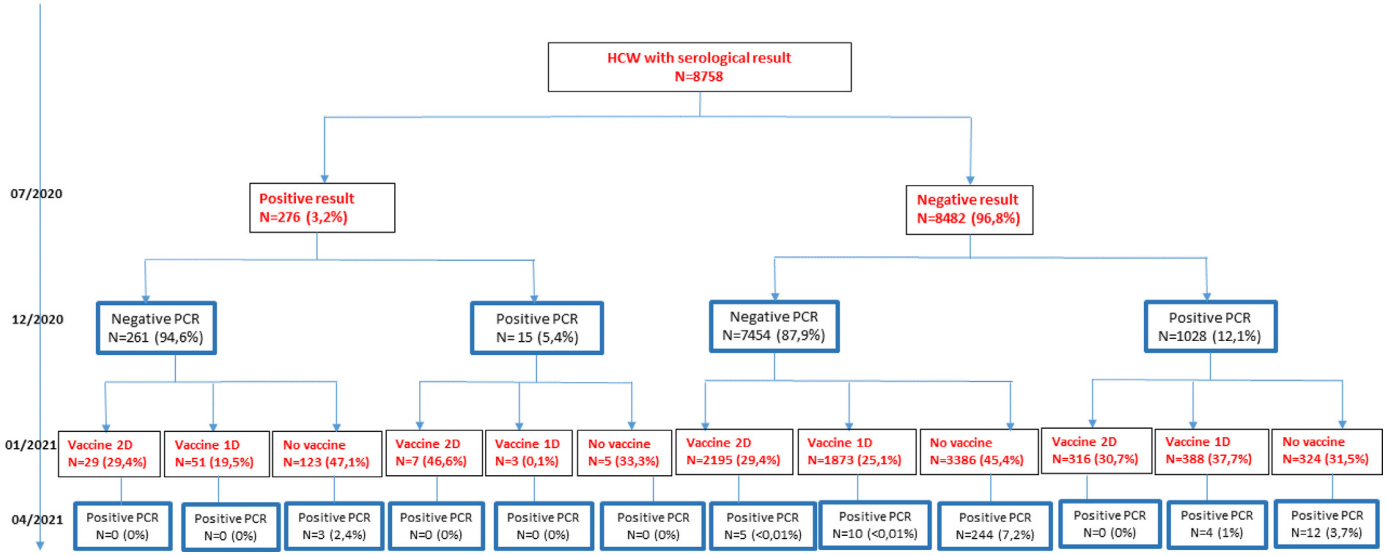

Fig. 2. Study flowchart.

December 2020 neutralizing antibody titers (median 32, IQR 8-64) were statistically higher than the April 2021 titers (median 16, IQR 8–32, $p = 0.02$, Wilcoxon signed rank test, Fig. 1A). The distributions of neutralizing antibody titers in April 2021 and July 2020 were not statistically different (median 16, IQR [8–32] for both; $p = 0.95$; Wilcoxon signed rank test, Fig. 1A). The neutralizing antibody titers were significantly higher in April 2021 after both one dose (median 512, IQR [128–2048]) and two doses (median 512, IQR [192–1536]) than in December 2020 (1 dose median 32, IQR [16–64]; $p < 0.01$, two doses median 16, IQR [8–32]; $p < 0.01$; Wilcoxon signed rank test, Fig. 1B). The neutralizing antibody titers of HCWs who received one or two doses of vaccine did not differ ($p = 0.95$, Wilcoxon rank test). All 40 vaccinated HCWs who were infected before July 2020 had higher neutralizing antibody titers in April 2021 than in July 2020. The neutralizing antibody titers for April 2021 were also much higher than those for July 2020 (median 16, IQR [16–32], Fig. 1B), regardless of the number of doses given ($p < 0.01$; Wilcoxon signed rank tests). The reinfection rate of HCWs first infected before July 2020, median follow-up of 275 days (IQR: 265–281), was 18/276 (6.5%), significantly lower than the first infection rate over the same period (1272/8482; 15%; $p < 0.01$; Chi2 test) (Fig. 2). While 6.5% of unvaccinated HCWs became re-infected within 10 to 13 months of their first SARS-CoV-2 infection, 15% of HCWs who had never been previously infected and were unvaccinated became infected. Thus 56.1% of HCWs were protected against re-infection about one year after their first infection, without being vaccinated.

These findings are consistent with the data for neutralizing antibody titers. The distributions in April 2021 and July 2020 were identical: 91.4% of these titers were the same or increased 1 to 3 months after the first infection. However, the neutralizing antibody concentration peaked approximately 9 months after the first infection. The titers for April 2021 were lower than those of December 2020. A previous study on the same sample showed that a high neutralizing antibodies titer protected up to 84.8% HCWs from re-infection for 9 months after their first infection.[3] We infer that protection against re-infection peaks at around 9 months after the first SARS-CoV-2 infection, although most of the HCWs remained protected against re-infection a year post-infection, even without vaccination. A recent study on rhesus macaques experimentally infected with SARS-CoV-2 showed that neutralizing antibodies that protected against reinfection developed within 35 days.[4] This result, together with those of ourselves and others, indicates that a SARS-CoV-2 infection induces a protective humoral response that can be correlated with the serum neutralizing activity. Further studies are now needed to determine the factors contributing to post-infection protection.

Our results also indicate that vaccination boosts the immune response of infected HCWs. The most striking finding is that there was no re-infection in HCWs vaccinated 9–12 months after their initial SARS-CoV-2 infection. This contrasts favourably with the rates for infected but unvaccinated HCWs. This is undoubtedly due to the very high neutralizing antibody concentrations found in those given one or two doses of vaccines. Maximal protection against SARS-CoV-2 reinfection seems to occur in people who have been infected and vaccinated, although we have no neutralizing antibody titers for HCWs vaccinated but not previously infected. The present results are consistent with those obtained for a small sample; they showed that the neutralizing antibody titers of infected people are lower than those who have been infected and vaccinated.[5] However, we found no difference in re-infections or distributions of neutralizing antibodies between HCWs infected before July 2020 who received a single dose of vaccine and those of people with the same infection profile who received two doses. This supports the recent recommendation that people who have already been infected with SARS-CoV-2 should be given a single dose of vaccine,[6] even if infection is "old" (over 9 months).

In conclusion, we find that binding and neutralizing antibodies persist for up to one year post-infection. Our data also suggest that vaccinating individuals who have already been infected induces a high level of protection, much higher than that following infection alone. This is particularly important for HCWs, who remain more exposed to SARS-CoV-2 than most of the general population.

Declaration of Competing Interest

The authors declare no competing interests.

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Chloé Dimeglio*
IFB, Laboratoire de Virologie, CHU Toulouse, Hôpital Purpan, 330 av de Grande Bretagne, Toulouse 31300 France
INSERM UMR 1291 – CNRS UMR 5051, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), Toulouse 31300, France

Fabrice Herin
Occupational Diseases Department, Toulouse-Purpan University Hospital, Toulouse 31000, France
Centre for Epidemiology and Research in Population Health Unit (CERPOP), UMR1295, Unité Mixte INSERM - Université Toulouse III Paul Sabatier, Toulouse 31000, France

Marcel Miedougé, Isabelle Da-Silva, Marion Porcheron
IFB, Laboratoire de Virologie, CHU Toulouse, Hôpital Purpan, 330 av de Grande Bretagne, Toulouse 31300 France

Guillaume Martin-Blondel
INSERM UMR 1291 – CNRS UMR 5051, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), Toulouse 31300, France
Infectious and Tropical Diseases Department, Toulouse University Hospital, Toulouse 31300, France

Jean-Marc Soulat
Occupational Diseases Department, Toulouse-Purpan University Hospital, Toulouse 31000, France
Centre for Epidemiology and Research in Population Health Unit (CERPOP), UMR1295, Unité Mixte INSERM - Université Toulouse III Paul Sabatier, Toulouse 31000, France

Jacques Izopet
IFB, Laboratoire de Virologie, CHU Toulouse, Hôpital Purpan, 330 av de Grande Bretagne, Toulouse 31300 France
INSERM UMR 1291 – CNRS UMR 5051, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), Toulouse 31300, France

*Corresponding author at: IFB, Laboratoire de Virologie, CHU Toulouse, Hôpital Purpan, 330 av de Grande Bretagne, Toulouse 31300 France.
E-mail address: dimeglio.c@chu-toulouse.fr (C. Dimeglio)

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The surge in Covid related mucormycosis

Dear Editor,

Mucormycosis(MM), a sequelae of clinical event post COVID-19 infection, is an uncommon opportunistic infection caused by a filamentous fungus (class: *Zygomycetes* and order: Mucorales) with a high degree of morbidity & mortality. [1,2] The point to ponder therefore is, whether SARSCoV2 is the major culprit which compromises the immune system of the host and thereby make the host more vulnerable to this secondary opportunistic infection, thus accounting for higher incidence of MM during second wave in India? Earlier published literature including several retrospective case series analyses have reported vulnerability of immune-compromised patients with pre-existing comorbidities e.g. diabetic ketoacidosis(DKA) treated with systemic glucocorticoids, Zn supplement, and longer ICU stay with O2 support towards mucormycosis.[3–7] However, These observations are not backed by sufficient scientific evidence to account for the proportionately higher Covid-associated MM in the second wave.

There are several clinical forms of MM infection reported till date including pulmonary, gastrointestinal, cutaneous, and rhinocerebral.[8] MM has a typical clinical presentation characterized by rapid progression of tissue necrosis due to sequential invasion and thrombosis of blood vessels. Rhino-cerebro-orbital mucormycosis, the major form in this pandemic is diagnosed through CT paranasal sinus and MRI brain.[9,10]

It is now known that the surge in second wave is related, at least in part, to the new variants of concern in the SARSCOV-2 virus making it more transmissible and difficult to treat.[11,12] It was well established that the virus gains entry into cells using the ACE-2 receptors.[13] A greater rate of endocytosis will be facilitated if the virus has additional “routes” of entry. Ibrahim et al. and others have reported that the GRP 78 could act as an alternative and additional route for the virus to gain entry into the host cell.[14,15] The genome of the prevalent SARSCoV2 variant (B.1.1.7 & B.6.117) in India is believed to be the cause of the increased infection.[16,17] In-silico studies have shown stable interaction between RBD domain of spike protein (C480-488,) with that of GRP 78 predicting its role in endocytosis.[18,19] It is to be noted that MM also have the same port of entry i.e GRP78 into the nasal and paranasal sinus mucosa through its coat protein CotH3.[20] Two hypotheses can be formulated for over expression of GRP78 one due to High glucose and iron content found during DKA and second dexamethasone induced GRP78 expression and thus may facilitate the invasion of MM into target cells for further proliferation.[21-23] There is still a less explored reason for GRP78 over expression namely endoplasmic reticulum(ER) stress. In perfectly healthy condition, the protein folding ability of endoplasmic reticulum matches with the body’s protein synthesis ability. However, in stress condition e.g. virus infection cells accumulates excessively high number of unfolded viral structural proteins in ER leading to over expression of GRP78 at cell surface making the cells vulnerable to fungal pathogens e.g. MM.[24,25] The GRP 78 binding being common to both the new variants as well as MM, could explain both the higher transmissibility of SARSCoV2 and surge in COVID-19 associated MM in the second wave.

We propose, therefore, that this is the right time to conduct a stringent medical audit of MM cases with a detailed questionnaire and medical records to identify risk predictors for future plans of action. Assessment of GRP78 expression in target cells or in circulation during hospital discharge, If not in all cases, at least in high-risk individuals like diabetics supplemented with steroid and had a history of long ICU admission, can be recommended. Additionally, such individuals could be considered for low-dose anti-fungal prophylaxis to decrease the morbidity and mortality due to MM.

Declaration of Competing Interest

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Somesh Chandra*
Rakesh Rawal
Department of Life Sciences, Gujarat University, Ahmedabad, Gujarat, India
*Corresponding author.
E-mail addresses: somesh@sterlinghospitals.com (S. Chandra), rakeshrawal@gujaratuniversity.ac.in (R. Rawal)

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Will achieving herd immunity be a road to success to end the COVID-19 pandemic?

Dear Editor,

Our previous work estimated the minimum, i.e. 'critical', level of population immunity acquired via vaccination or natural infection (P_{crit}) to stop the spread of Coronavirus Disease 2019 (COVID-19) among 32 selected study populations.[1] Currently, over 1 billion COVID-19 vaccine doses are administered in 208 territories. Early insights from countries with high vaccine uptake offer the hope that mass vaccination can bring an end to the pandemic, though this does not necessarily mean a complete virus eradication, which is likely to persist to become endemic and seasonal in most populations.[2]

The crucial question of what is the minimal vaccine coverage needed for different countries to achieve SARS-COV-2 herd immunity (i.e. that required to block exponential virus spread in a population) is an important one, when COVID-19 vaccine supplies are limited and unreliable, and different vaccines have different efficacies. With evidence demonstrating natural immunity effectiveness (i.e. immunity acquired after natural SARS-COV-2 infection), we can factor this into the minimum vaccine coverage required for any given population. Much of the COVID-19 vaccine and incidence data can only be estimated from publicly available and various websites, but these can be combined to provide useful estimates of the required herd immunity level - and therefore the COVID-19 vaccine coverage still required - for different countries.

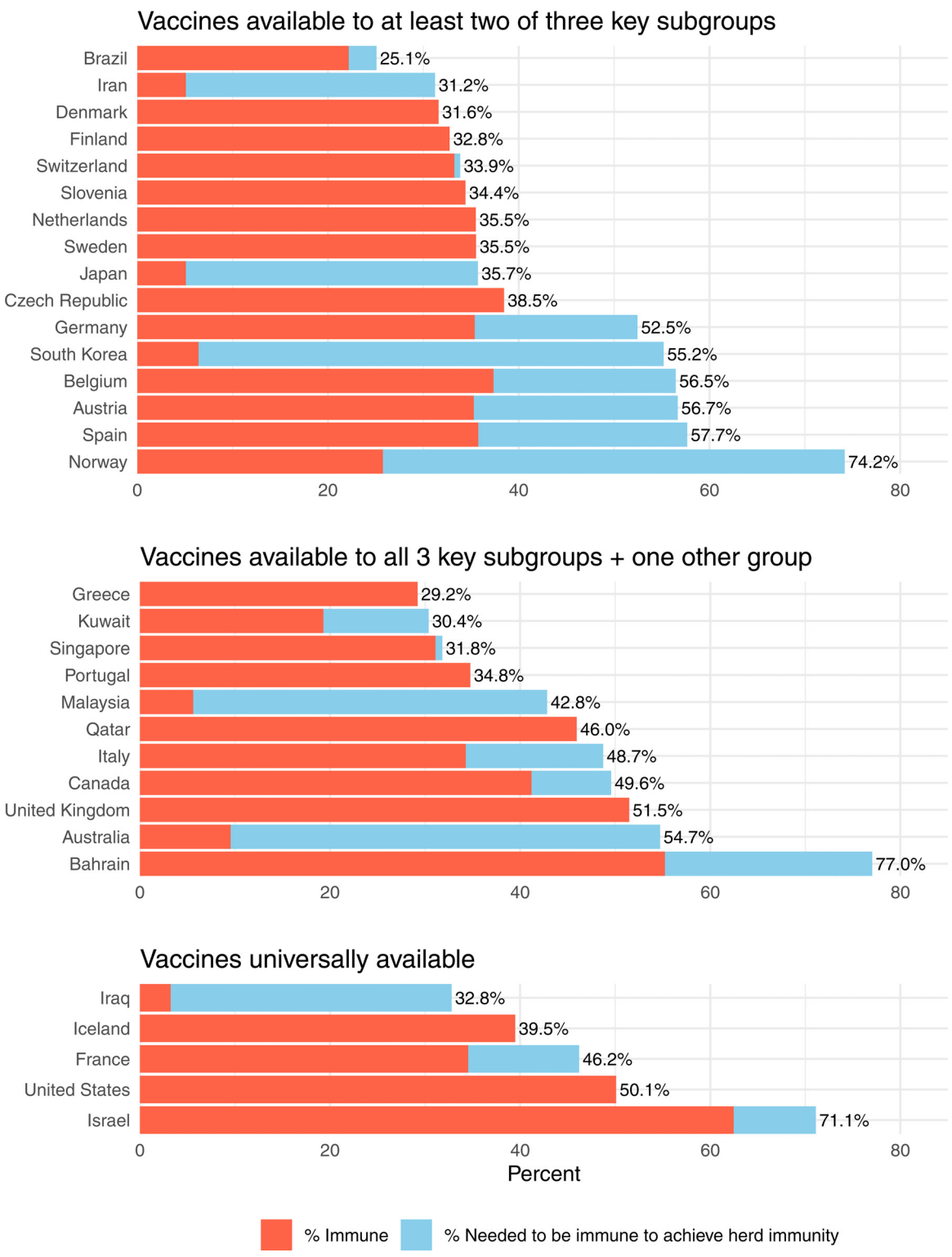

Fig. 1. Proportion of population already immune (P_{im}) (red) and the additional proportion still required to achieve herd immunity (P) (blue) in the 32 study populations stratified by vaccine availability for various key priority groups. With the most recent data for the numbers of vaccine doses given and naturally occurring COVID-19 cases, as reported from each country's population on 26th May 2021,[1] assumed estimates for VE_1, VE_2, and P_{ni} to be 70%[2], 88%[3] and 80%[4,5], respectively, P_{im} can be estimated. Percentages to the right of each bar represent the minimum proportion of the total population required to recover from COVID-19 to confer immunity with vaccine availability (P_{crit}). (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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Table 1
Characteristics of vaccine deployment in the 32 selected study populations.

Study country	Total population	R_0*	Cumulative % of population reported as infected†	First vaccination rollout	Days since first rollout (up to 26/5/2021)	Number of priority groups‡	% of population receiving 1 dose of the vaccine§	%% of population receiving 2 doses of the vaccine
Australia	25,499,881	2.21	0.12	22/02/2021	93	4[a]	9.5	1.9
Austria	9,006,400	2.31	7.13	27/12/2020	150	3	35.3	15.0
Bahrain	1,701,583	4.36	13.31	25/12/2020	152	4	55.2	43.8
Belgium	11,589,616	2.30	9.08	28/12/2020	149	3	37.4	16.2
Brazil	212,559,409	1.33	7.66	21/01/2021	125	3	22.2	10.0
Canada	37,742,157	1.98	3.64	14/12/2020	163	4[b]	41.2	4.6
Czech Republic	10,708,982	1.60	15.50	27/12/2020	150	3	38.5	12.3
Denmark	5,792,203	1.32	4.78	27/12/2020	150	3	31.6	21.0
Finland	5,540,718	1.39	1.66	27/12/2020	150	3	32.8	7.9
France	67,564,251	1.86	8.41	27/12/2020	150	5	34.5	15.3
Germany	83,783,945	2.10	4.38	27/12/2020	150	3	35.4	15.6
Greece	10,423,056	1.24	3.79	27/12/2020	150	4	29.2	18.0
Iceland	341,250	1.47	1.92	29/12/2020	148	5	39.5	23.6
Iran	83,992,953	1.45	3.41	9/02/2021	106	3	5.1	0.5
Iraq	40,222,503	1.49	2.94	2/03/2021	85	5	3.2	–[9]
Israel	8,655,541	3.46	9.70	19/12/2020	158	5	62.5	59.2
Italy	60,461,828	1.95	6.95	27/12/2020	150	4[c]	34.3	18.1
Japan	126,476,458	1.56	0.58	17/02/2021	98	2	5.1	2.4
Kuwait	4,270,563	1.44	7.10	24/12/2020	153	4	19.3	0.9
Malaysia	32,365,998	1.75	1.65	24/02/2021	91	4	5.6	3.1
Netherlands	17,134,873	1.42	9.70	8/01/2021	138	3	35.5	14.3
Norway	5,421,242	3.87	2.28	27/12/2020	150	3	25.8	16.6
Portugal	10,196,707	1.53	8.30	27/12/2020	150	4	34.8	16.1
Qatar	2,881,060	1.26	7.51	23/12/2020	154	4	46.0	35.5
Singapore	5,850,343	1.47	1.06	8/1/2021	138	4	31.1	27.6
Slovenia	2,078,932	1.18	12.14	27/12/2020	150	3	34.4	17.5
South Korea	51,269,183	2.23	0.27	26/02/2021	89	3	6.4	3.9
Spain	46,754,783	2.36	7.82	27/12/2020	150	3	35.8	18.4
Sweden	10,099,270	1.45	10.57	27/12/2020	150	3	35.5	12.2
Switzerland	8,654,618	1.51	7.99	23/12/2020	154	3	33.3	18.7
United Kingdom	67,886,004	1.66	6.61	8/12/2020	169	4[d]	51.5	35.4
United States	331,002,647	1.72	10.03	14/12/2020	163	5	50.1	39.8

* We first estimate $R_o$with the exponential growth method[1] using COVID-19 case series from 21st January 2020 to 31st July 2020 (**Fig. 1**) coupled with estimates of the serial interval[2] (mean = 4.7 days, standard deviation = 2.9 days). Each country's exponential phase was defined as the period from onset (the first day of a consecutive 3-day period with at least 3 cases) to the peak (maximum cases) of the first wave. The first wave was defined as the period from onset to the day when the number of cases decreased by more than 50% of the maximum up to that day for at least 3 consecutive days or did not exceed the maximum for 7 consecutive days.

† Information updated on 26/5/2021.

‡ Three priority groups were key workers, clinically vulnerable people and the elderly.
2, 3: vaccines available for 2 and 3 of the above priority groups, respectively.
4: vaccines available for all of three priority groups plus partial additional availability for various other subgroups or age groups.
5: universal availability, when vaccine is available to everyone ≥16 or ≥18 (depends on the lowest age permitted by the vaccine brand currently).

§ Information updated on 26/5/2021, except for Iceland and Malaysia (updated 25/5/2021), Iran and Singapore (updated 24/5/2021), Netherlands (updated 23/5/2021), Iraq (updated 11/5/2021), and Kuwait (updated 18/4/2021).

[a] Indigenous people aged 50 or above were eligible as a priority group under the current phase of vaccinations. (Reference: https://www.health.gov.au/initiatives-and-programs/covid-19-vaccines/phase-1b#aboriginal-and-torres-strait-islander-people).

[b] In Canada, some provinces including Saskatchewan, Alberta, New Brunswick and Ontario added pregnancy to the vaccine priority groups. (Reference: https://www.cbc.ca/news/canada/montreal/pregnant-women-not-prioritized-covid-19-vaccine-1.5999304).

[c] Students in the final year of high school in Lazio, Italy were prioritized to receive the COVID-19 vaccine. (Reference: https://www.salutelazio.it/vaccinazione-maturandi).

[d] Adults experiencing homelessness in Scotland were one of the eligible priority groups for COVID-19 vaccination.(Reference: https://www.nhsinform.scot/COVID-19-vaccine/invitations-and-appointments/who-will-be-offered-the-coronavirus-vaccine).

[e] Iraq had no data for 2 doses.

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We revisit the calculation of P_{crit} and estimate the current immune proportion, P_{im}, with the following formulae:

$$P_{crit} = 1 - \frac{1}{R_o} \quad (1)$$

$$P_{im} = P_{v1} \times VE_1 + P_{v2} \times VE_2 + P_{cc} \times P_{ni} \quad (2)$$

where R_o is the basic reproductive number, P_{v1} and P_{v2} are the proportions of the population vaccinated with one and two doses, respectively, VE_1 and VE_2 are the overall real-world population effectiveness of the vaccine for one and two doses, respectively, P_{cc} is the proportion of confirmed cases, and P_{ni} is the proportion of the population who have naturally-induced immunity against symptomatic SARS-COV-2 infection. From Eqs. (1) and (2), we define P as the proportion still required to gain immunity for the country to achieve herd immunity: $P = P_{crit} - P_{im}$. A country with $P > 0$ indicates that its population had achieved herd immunity. All analyses were performed in R (version 4.1.0; R Foundation for Statistical Computing) (**Table 1**).

The estimates of R_o varied by country, ranging from 1.18 to 4.36, resulting in the corresponding P_{crit} estimates ranging from 15.3% to 77.1%. Our lower end estimate of herd immunity threshold was consistent with those of 10 to 20% from a recent study[3]. The Czech Republic had the highest proportion of reported cases (15.5%) followed by Bahrain (13.3%) and Slovenia (12.1%). Although vaccine rollout was delayed in most Asian countries compared to Europe and North America, COVID-19 vaccines were currently available in

our 32 study populations. According to their eligibility for vaccination within the national guidance of each country[4], they were further classified with different levels of vaccine availability into three subpopulations. In 16 countries, vaccines were available for at least two priority subgroups (key workers, clinically vulnerable, elderly), in 11 countries vaccines were available to all three aforementioned priority groups and extra availability for selected broader subgroups (e.g. indigenous peoples, pregnant women) or age groups (e.g. ≥18, ≥30) in some countries and in 5 countries vaccines were universally available. Countries with universal vaccine availability such as Israel and the United States had higher P_{im} values (62.5% and 50.1%, respectively). Surprisingly, countries in Asia such as Malaysia, Japan, and South Korea, regardless of level of vaccine availability, reported low single-digit P_{im} values (5.6%, 5.1%, and 6.4%, respectively). Of the 32 study countries, 11 had achieved herd immunity, 6 others required P to be between 0.01% and 8.6% to reach the herd protection level, and the rest required proportions ranging from 11.1 to 48.8%. (**Table 1, Fig. 1, and Supp Fig. 1**)

Our study suggested that the majority of the study populations had lower proportions that were immune compared to Israel, the exemplar in reducing the infection rate after successful vaccine deployment.[5] This might be partly attributable to the inequitable distribution of vaccines globally, which may be shaping different government policies on vaccination, but also cultural and socioeconomic barriers leading to vaccine refusal and hesitancy, particularly amongst Asian and African populations. Thus, to improve COVID-19 vaccination coverage and raise the levels of population immunity, sufficient vaccine supplies need to be more reliable,[6] with improved, culturally sensitive, and appropriate communication to encourage their uptake. This will partly depend on whether we can successfully identify determinants of vaccine hesitancy and refusal amongst various populations.[7]

The exact proportion in any population that is required to achieve herd immunity to stop the spread of the virus will vary, depending on the virus variant circulating, as well as the natural degree of mixing in that population - which also depends on population density and mobility and so on. In addition, the duration of protection conferred by natural and vaccine-induced immunity is not well-established, and different vaccines may confer differing durations and degrees of humoral (B-cell) and cell-mediated (T-cell) immunity.[8,9] It is also not known how long and effective the immunity conferred by mixed vaccine regimens and third dose boosters will be in different populations - including those of different ethnicities. Finally, children are still not routinely vaccinated as most COVID-19 vaccines are not yet licensed for this subgroup, particularly primary school children, which means they will mostly remain a susceptible population where any degree of herd immunity will be uncertain. Therefore, the precise level of population immunity required, as estimated by the equation of herd immunity, to 'end' the pandemic in each country and globally is difficult to determine.

From a practical viewpoint, estimates of P_{crit} will be considered to be transient and herd immunity is likely to be a spectrum instead of a specific threshold that determines if and when the entire pandemic is over.[10] The current pandemic might end gradually with an increasing proportion of immune individuals. Also, since all COVID-19 vaccines seem to protect against severe disease and death, and against most viral variants, universal vaccination is still the key message. As this will take time, maintaining social distancing, universal mask-wearing, and improved ventilation indoors to control the virus spread, are all still important as the vaccine coverage in different countries improves.

Declaration of Competing Interest

None

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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.2021.06.007.

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Kin On KWOK*
JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China
Stanley Ho Centre for Emerging Infectious Diseases, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China
Shenzhen Research Institute of the Chinese University of Hong Kong, Shenzhen, China

Edward B MCNEIL, Margaret Ting Fong TSOI, Vivian Wan In WEI, Samuel Yeung Shan WONG
JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China

Julian Wei Tze TANG*
Respiratory Sciences, University of Leicester, Leicester, United Kingdom
Clinical Microbiology, Leicester Royal Infirmary, Leicester, UK

*Corresponding author at: JC School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong Special Administrative Region, China.Corresponding author.
E-mail addresses: kkokwok@cuhk.edu.hk (K.O. KWOK), jwtang49@hotmail.com (J.W.T. TANG)

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An observed association between conjunctivitis and severity of COVID-19

Dear Editor,

We read with interest the article by Zhong et al.[1]

Conjunctivitis, also called "Pink eye", is a disease of bacterial or viral etiology that has been associated with Coronavirus infection in humans and animals.[2,3]

Some aspects of this disease have not yet been fully elucidated, such as its prevalence, which is quite variable across countries,[4] and association with mortality.

Regarding the association between conjunctivitis and mortality in COVID patients, the data are scarce. We performed a meta-analysis, which showed a higher prevalence of conjunctivitis in COVID19 patients with severe disease, which was defined as a composite of severe pneumonia, mortality, ARDS, use of mechanical ventilation or intensive care unit recovery[2]; therefore, analysis of the impact of conjunctivitis versus mortality alone was lacking. Thus, in this observational study we want to analyze the prevalence of conjunctivitis and its association with mortality in Italian patients hospitalized in medical wards for COVID-19 disease. Furthermore, we analyzed the frequency of admission to intensive care units (ICU).

Two hundred and eighteen consecutive non-selected patients with acute COVID-19 infection and medical conditions requiring hospitalization were recruited.

This observational cohort study was performed at Sapienza University of Rome (Italy) in wards devoted to COVID-19 care. We included in the study adult (≥18 years) patients with laboratory-confirmed COVID-19 and acute respiratory syndrome coronavirus-2 (SARS-CoV2)-related pneumonia consecutively hospitalized from February 2020 to January 2021. COVID-19 was diagnosed on the basis of WHO interim guidance.

A COVID-19 case was defined as a person with laboratory confirmation of COVID-19 infection, irrespective of clinical signs and symptoms. Oropharyngeal and nasopharyngeal swabs for laboratory diagnosis of COVID-19 were performed in duplicate: SARS CoV2 E and S gene were detected by a reverse transcriptase polymerase chain reaction (RT-PCR) (RealStar SARS-CoV2 RT-PCR, Altona Diagnostics).

Conjunctivitis was diagnosed at admission and was defined in the presence of conjunctival hyperemia, chemosis, epiphora, or increased secretions.[5] Patients with Continuous Positive Airway Pressure (CPAP) conjunctivitis were excluded from the study.

Demographic and clinical characteristics were collected after receiving informed consent.

Routine analysis included serum albumin and high sensitivity C-reactive protein (hs-CRP).

Ethical approval for this study was obtained from Ethics Committee of Azienda Ospedaliera Universitaria Policlinico Umberto I (ID Prot.109/2020).

Continuous variables are reported as mean ± SD, and categorical variables are reported as *n* (%). Statistical analyses were performed using SPSS 18.0 software for Windows (SPSS, Chicago, IL, USA). Between group differences were analyzed by T-test. Differences between percentages were assessed by the $\chi 2$ test. A p value of <0.05 was considered statistically significant. The cumulative incidence of death was estimated using a Kaplan–Meier product–limit estimator. Survival curves were formally compared using the log-rank and Breslow tests. A p value lower than 0.05 was considered statistically significant.

Two hundred and eighteen subjects were recruited in the study. The hospitalization period was 17 ± 10 days. The prevalence of conjunctivitis was 13% (29/218 patients). Clinical characteristics of the patients with and without conjunctivitis are reported in the Table 1. Compared to patients without conjunctivitis, patients with conjunctivitis had similar characteristics (Table 1).

During the follow-up 34% subjects with (10/29) and 13% (24/189) without conjunctivitis died (Table 1). Furthermore, 24% (7/29) of patients with and 7% (13/189) without conjunctivitis needed ICU treatment (Table 1).

A Kaplan–Meier with log-rank test analysis showed that, compared to the patients without conjunctivitis those with conjunctivitis had a lower survival (log-rank test: $p = 0.02$; Breslow test: $p < 0.001$) (Fig. 1 Panel A) and a higher frequency of admission to ICU (log-rank test $p = 0.02$; Breslow test: $p < 0.001$) (Fig. 1 Panel B).

In a population of consecutive Italian patients suffering from COVID-19 hospitalized in medical wards we found a conjunctivitis prevalence of 13%. Differently from studies performed in COVID-19 Asian subjects, that reported an extremely wide prevalence (0–30%)[6] of conjunctivitis, our finding is consistent with that of 10% previously reported in Italian population.[7]

The novelty of the present study is in reporting a new and intriguing clinical relationship between conjunctivitis and the severity of COVID-19. Thus, we show that COVID-19 patients with conjunctivitis, hospitalized in medicine wards, have a higher rate of ICU treatment and lower survival, suggesting that conjunctivitis is warning sign of poor outcome.

An open issue is if conjunctivitis is a sign related to the route of entry of the virus in the human body or, more interestingly, occurs during a later phase of infection as suggested by previous studies showing that many conjunctivitis are detected after the onset of COVID.[4,8] If so, conjunctivitis could represent a sign of a systemic disease and a warning sign of poor outcome consequent to the systemic inflammation. This hypothesis may be supported by the multisystem inflammatory syndrome (Kawasaki disease) in children with COVID-19, where conjunctivitis has been described as a sign of a storm of cytokines and inflammatory molecules[9] independently from COVID-19 infection. Other examples of conjunctivitis as a manifestation of systemic disease are mucous membrane pemphigoid, vasculitis, Stevens-Johnson syndrome, and Graves disease.[10]

This study has limitations. The sample size was low and the study was conducted in a single Italian center. Future studies with a larger population are necessary to establish the real weight of conjunctivitis in terms of prevalence and whether this clinical manifestation can represent an early marker of COVID-19 poor outcome.

In conclusion, the results of this study suggest that conjunctivitis could be a clinical manifestation associated with a poor outcome as mortality or ICU hospitalization in patients with COVID-19.

Abbreviations: COVID- 19, Coronavirus disease 2019; ARDS, acute respiratory distress syndrome; ICU, intensive care units.

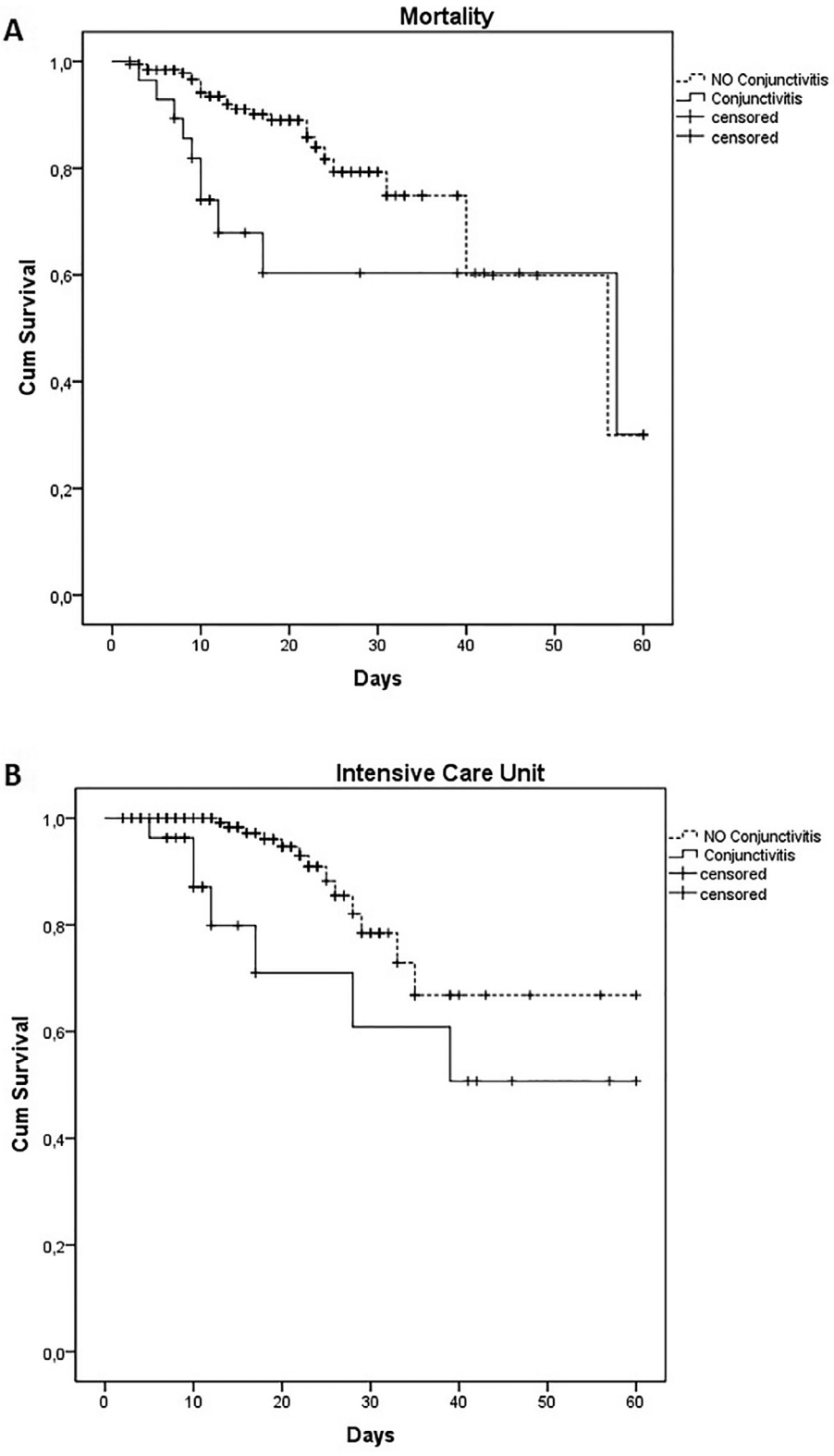

Fig. 1. Kaplan–Meier curves estimate of death (Panel A) and ICU admission (Panel B) in COVID-19 patients. Continuous line: patients with conjunctivitis. Dashed line: patients without conjunctivitis.

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Declaration of Competing Interest

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Table 1
Clinical characteristics of patients with and without conjunctivitis.

	COVID-19 without conjunctivitis	COVID-19 with conjunctivitis	*P*
N.	189	29	–
Age, years	61±16	62±15	0.746
Male/Female	107/82	15/14	0.275
Days of hospitalitazion	17±9	18±16	0.692
Obesity (no/yes)	159/30	22/7	0.269
Smoke (no/yes)	175/14	28/1	0.615
COPD (no/yes)	162/27	26/3	0.329
Diabetes (no/yes)	155/34	28/1	0.051
Hypertension (no/yes)	100/89	18/11	0.356
Atrial Fibrillation (no/yes)	175/14	28/1	0.432
Dementia (no/yes)	171/18	25/4	0.477
Neoplasia (no/yes)	171/18	25/4	0.477
Hematological neoplasm (no/yes)	181/7	26/3	0.113
Hb (g/dL)	13.6 ± 1.8	12.9 ± 1.7	0.061
WBC (x10^6/L)	6180±3082	7380±4430	0.070
PLT (× 10^9/L)	210±84	196±75	0.393
Albumin (g/dL)	3.7 ± 0.5	3.7 ± 0.6	0.924
LDH (mU/ml)	319±146	319±124	0.991
P/F ratio	372±114	346±105	0.306
SpO2 (%)	95±7	95±4	0.841
Follow-up			
ICU (no/yes)	176/13	22/7	0.003
Death (no/yes)	165/24	19/10	0.003

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Lorenzo Loffredo*

Department of Clinical, Internal Medicine, Anesthesiology and Cardiovascular Sciences, Sapienza University of Rome, Viale del Policlinico 155, Rome 00161, Italy

Alessandra Oliva[1]
Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy

Aurora Paraninfi
Department of Clinical, Internal Medicine, Anesthesiology and Cardiovascular Sciences, Sapienza University of Rome, Viale del Policlinico 155, Rome 00161, Italy

Giancarlo Ceccarelli
Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy

Federica Orlando, Paolo Ciacci
Department of Clinical, Internal Medicine, Anesthesiology and Cardiovascular Sciences, Sapienza University of Rome, Viale del Policlinico 155, Rome 00161, Italy

Fernanda Pacella[1], Elena Pacella[1]
Department of Sense Organs, Faculty of Medicine and Dentistry, Sapienza University of Rome, Italy

Silvia Di Bari, Valeria Filippi, Alessia Cruciata, Claudio Maria Mastroianni
Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy

Francesco Violi
Department of Clinical, Internal Medicine, Anesthesiology and Cardiovascular Sciences, Sapienza University of Rome, Viale del Policlinico 155, Rome 00161, Italy

*Corresponding author.
E-mail addresses: lorenzo.loffredo@uniroma1.it, lorenzo.loffredo@alice.it (L. Loffredo)

[1] These authors contributed equally to this work.
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ACE I/D polymorphism and epidemiological findings for COVID-19: One year after the pandemic outbreak in Europe

Dear Editor,

We have read with great interest several recent articles on the insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (*ACE*) gene and its potential relevance to the risk of a SARS-CoV-2 infection and the severity of the consequent coronavirus disease 2019 (COVID-19).[1–6] These epidemiological studies, which analyzed either European populations[1, 5, 6] or global populations that included Europe,[2–4] have reported conflicting results (Table 1). Variability in results may arise, due to various factors, including differences in the ethnicities/countries included in the analysis, which might reflect differences in genetic background; differences in other biological, environmental, and social risk parameters; and differences in the prevalence of the *ACE* I/D polymorphism. Indeed, it is well documented that the frequency of the *ACE*-D allele varies according to the ethnic/geographic origin of the study cohort. The prevalence of the *ACE*-D allele increases from Eastern to Western countries, worldwide. The prevalence in Asian populations (approximately 25–40%) is lower than the prevalence in Caucasian (generally approximately 40–60%) and African (60%) populations. Therefore, we focused our interest on studies that analyzed the European region, which also provided conflicting results, despite the similarities among these populations, in terms of ancestry, other risk covariates for COVID-19, and strategies for controlling the pandemic.

Several factors could potentially explain the variable results, including the study design (i.e., the source and method of data collection), the approach to data analysis (e.g., adjustments for potential confounders), or the timing of the analysis. In addition, previous studies analyzed data during the first wave of the pandemic, when most European countries were under total lockdown. The opening of borders at the end of June 2020 led to an increase in social contacts and virus transmission, which caused the second COVID-19 wave in the early autumn of 2020. The epidemiological situation changed markedly during the second wave of the pandemic. Countries that had largely avoided the pandemic during the first wave, such as the Czech Republic, or countries with a favorable epidemiological situation, such as those in Southeast Europe, were affected by the second wave.

In this study, we conducted a replication analysis in the European population to investigate the impact of the *ACE* I/D polymorphism on the prevalence and mortality of COVID-19 after the second wave of the pandemic. Data were collected on February 1, 2021, one year after the World Health Organization declared the outbreak as a Public Health Emergency of International Concern and at a time when the populations of European countries had not yet been immunized by vaccinations.

Thirty-four European countries were included in the analysis: Albania, Austria, Belarus, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, the Czech Republic, Croatia, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Moldova, Montenegro, The Netherlands, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey, the United Kingdom, and Ukraine. We performed a multiple regression analysis, after adjusting for possible confounders, including the number of diagnostic tests, the onset of the epidemic (days) in each country, and the Human Development Index (HDI). We retrieved data on the prevalence (number of cases/10^6 inhabitants), mortality (number of deaths/10^6 inhabitants), the number of diagnostic tests per 10^6 persons, and the time elapsed since the onset of the epidemic (days since January 1, 2020), in each country, from the Worldometer website (https://www.worldometers.info/coronavirus/#countries).[7] The HDI reflects three main dimensions of human development: life expectancy at birth, education, and gross national income per capita. For each country, these data were obtained from the United Nations Human Development Reports website (http://hdr.undp.org/en/content/latest-human-development-index-ranking).[8] Data on the distribution of *ACE* genotypes were collected from recent studies.[3, 5, 6]

The multiple regression analysis revealed no significant associations between the *ACE* I/D polymorphism and the log-transformed prevalence of COVID-19 (DD genotype: partial $r = 0.161$, $P = 0.386$; ID genotype: partial $r = -0.375$, $P = 0.841$; II genotype: partial $r = -0.129$, $P = 0.490$). Moreover, the *ACE* I/D polymorphism was not associated with the log-transformed mortality (DD genotype: partial $r = 0.191$, $P = 0.302$; ID genotype: partial $r = 0.343$, $P = 0.855$; II genotype: partial $r = -0.218$, $P = 0.238$).

The lack of associations between the *ACE* I/D polymorphisms and COVID-19 prevalence or mortality could arise from the fact that there was a significant change in the age structure of patients during the pandemic. During the second pandemic wave, the number of younger patients increased. Importantly, in European populations, the prevalence of the *ACE*-D allele was found to be age-dependent; thus, higher frequencies of the *ACE*-D allele were detected among older individuals,[9] who were most affected by COVID-19 infections during first pandemic wave.

Population-level studies have some inherent limitations, due to the ecological study design. Therefore, future studies are needed, based on the different clinical strata of COVID-19 manifestations

Table 1
Epidemiological studies on associations between *ACE* I/D polymorphisms and COVID-19 prevalence/mortality.

Author (reference)	Geographic region	Date assessed	*ACE* I/D allele/genotype	Association with COVID-19 prevalence and/or mortality
Delanghe (1)	Europe (25 countries)	20 March 2020	D allele	negative association
Delanghe (2)	European (26 countries), North African and Middle Eastern countries	1 April 2020	D allele	negative association
Yamamoto (3)	European (19 countries), Middle Eastern, South Asian, and East Asian countries	23 May 2020	II genotype	negative association
Aung (4)	Worldwide countries (9 European)	8 June 2020	DD genotype II genotype	no association negative association
Cenanovic (5)	Europe (18 countries)	10 July 2020	D allele	no association
Bellone (6)	Europe (24 countries)	5 August 2020	DD genotype II genotype	positive association negative association

(i.e., asymptomatic, mild, severe and fatal), to clarify the impact of the *ACE* I/D polymorphism on SARS-CoV-2 infections. Moreover, the frequencies of the *ACE*-D allele and *ACE*-DD genotype are associated with many different diseases and/or conditions,[10] including some that might increase the risk of COVID-19 mortality, such as diabetes and hypertension. Therefore, it might be relevant, in future studies, to include those diseases and/or conditions as potential confounders.

Declarations of Competing Interest

The authors declare no conflict of interest.

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Smiljana Ristić, Sanja Dević Pavlić
Department of Medical Biology and Genetics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

Sergej Nadalin
Department of Psychiatry, General Hospital "Dr. Josip Benčević", Slavonski Brod, Croatia

Nada Starčević Čizmarević
Department of Medical Biology and Genetics, Faculty of Medicine, University of Rijeka, Rijeka, Croatia

*Corresponding author at: Department of Medical Biology and Genetics, Faculty of Medicine, University of Rijeka, 51000 Rijeka, Croatia.
E-mail addresses: smiljana.ristic@uniri.hr (S. Ristić), sanja.devic@uniri.hr (S.D. Pavlić), sergej.nadalin@uniri.hr (S. Nadalin), nadasc@uniri.hr (N.S. Čizmarević)

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Lower respiratory tract and plasma SARS-CoV-2 RNA load in critically ill adult COVID-19 patients: Relationship with biomarkers of disease severity

Dear Editor,

We read with interest the work published by Lui and colleagues in the Journal of Infection[1], in which the dynamics of SARS-CoV-2 RNA at different body sites was investigated in a rather small cohort including 5 patients with critical/severe COVID-19. The authors supported the assumption that viral loads in lower respiratory tract (LRT) better reflected clinical progression in severe disease than those in upper respiratory tract (URT) samples. To further address this issue, we conducted an observational study (approved by the Ethics Committee of Hospital Clínico Universitario INCLIVA in May,2020) aimed at characterizing the kinetics of SARS-CoV-2 RNA load in the LRT and plasma (viral RNAemia) and assessing how these relate to the inflammatory state and mortality of critically ill COVID-19 patients. Seventy-three consecutive patients (51 males and 22 females; median age, 65 years; range, 21 to 80 years) were recruited during ICU stay (median,18 days; range, 2–67 days), between October 2020 and February 2021 (Supplementary Table 1). Patients were admitted to ICU at a median of 9 days (range, 2–25) after onset of symptoms. Sixty-four patients underwent mechanical ventilation, from whom 165 tracheal aspirates (TA) were collected (median of 2 specimens/patient; range, 1–11). A total of 340 plasma specimens (median, 4 samples/patient; range, 1–16) were available from the 73 patients. SARS-CoV-2 RNA quantitation in TA and plasma was carried out by the Abbott Real*Ti*me SARS-CoV-2 assay Abbott Molecular (Des Plaines, IL, USA) (See Supplementary Material). SARS-CoV-2 viral loads (in copies/ml) were estimated using the AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Vircell SA, Granada, Spain). The analytical sensitivity of the RT-PCR assay in TA and plasma specimens was 100 copies/ml (95%) for both matrices.

SARS-CoV-2 RNA (median, 6.5 $\log_{10}$ copies/ml; range, 3.03–10.6 $\log_{10}$) was detected in 109 TA from 56 patients (91.8%). Viral load remained relatively stable across the first two weeks from symptoms onset and began to decrease afterwards (Fig. 1A). No patient tested positive for SARS-CoV-2 RNA in TA beyond day 42. As reported for URT[2,3], neither remdesivir nor tocilizumab administration appeared to have a major impact on the dynamics of SARS-CoV-2 RNA load in TA (Supplementary Table 2).

SARS-CoV-2 RNAemia (median, 3.03 $\log_{10}$ copies/ml; range, 1.69 to 5.27 $\log_{10}$) was detected in 37 plasma specimens from 26 patients (35.6%). Median time to first detection of viral RNA in plasma was 10 days after symptoms onset (range, 3–32 days) Viral RNA cleared faster in plasma than in TA (Fig. 1B). Previous studies using a droplet-based digital PCR, which seemingly outperforms conventional RT-PCR assays in terms of sensitivity, reported higher rates of viral RNAemia detection in ICU patients (77% in[4] and 88% in[5]) than found in the current study. This discrepancy could also be related to different timing of sample collection across studies.

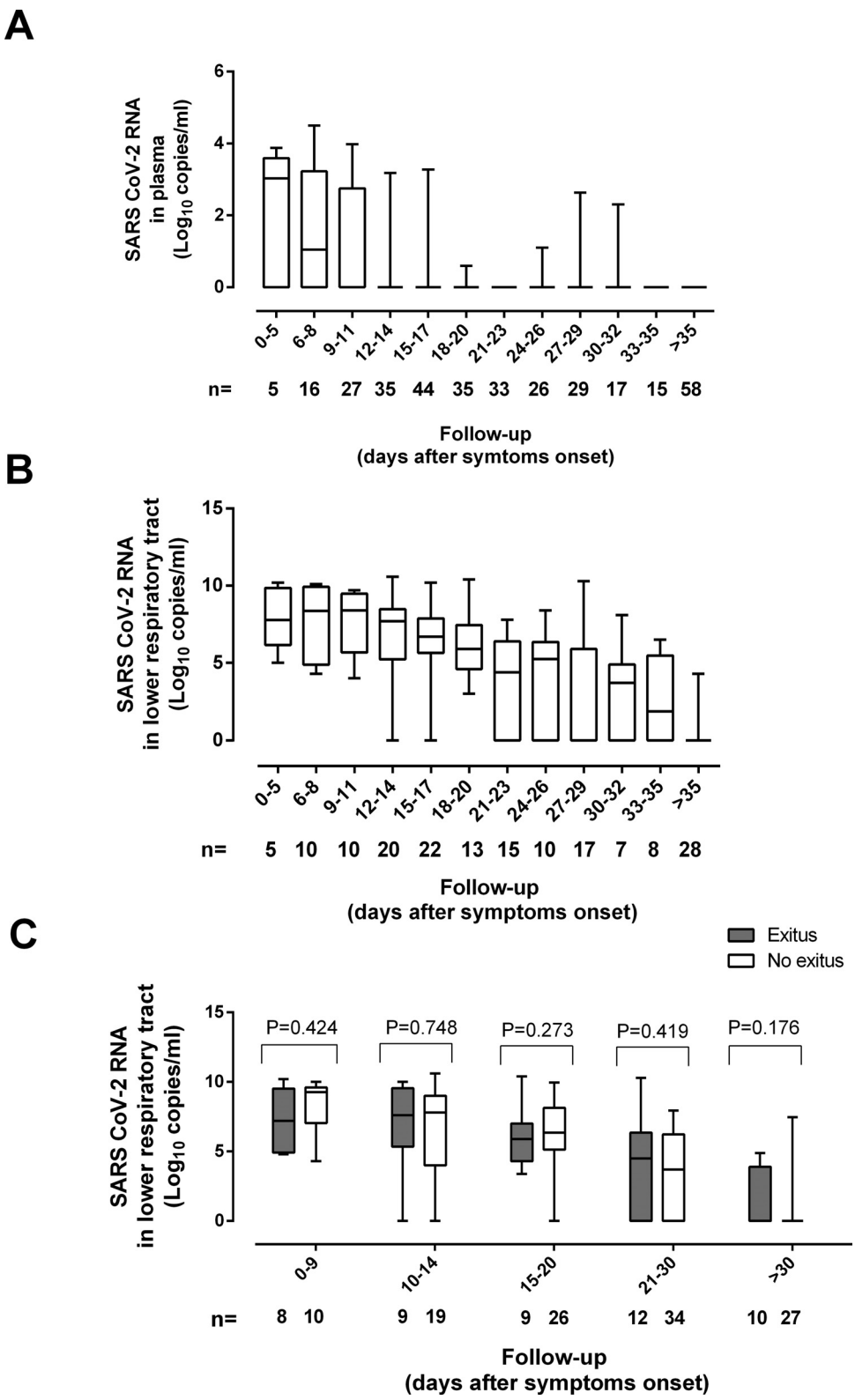

Fig. 1. Kinetics of SARS-CoV-RNA load in tracheal aspirates (A) and plasma (B) of critically ill patients undergoing invasive ventilation. Panel C shows the kinetics of SARS-CoV-2 RNA load in the lower tracheal aspirates in patients who either died or

A moderate yet significant correlation was found between SARS-CoV-2 RNA levels in TA and in paired plasma specimens (rho, 0.41; $p < 0.001$). SARS-CoV-2 RNA load in TA was significantly higher ($p < 0.001$) in presence than absence of concomitant viral RNAemia (median, 9.5 $\log_{10}$ copies/ml; range, 4.3 to 10.4 $\log_{10}$ copies/ml vs. median, 6.2 $\log_{10}$ copies/ml; range, 3.0–10.6 $\log_{10}$ copies/ml), this suggesting that LRT may be a substantial source of SARS-CoV-2 RNA.

Plasma levels of ferritin, lactose dehydrogenase (LDH), but not interleukin-6 (IL-6), C-reactive protein (CRP), or D-Dimer (D-D),

Table 1
Qualitative detection of SARS-CoV-2 RNA in the lower respiratory tract or plasma or SARS-CoV-2 N protein in plasma and blood levels of biomarkers of COVID-19 severity.

Qualitative result of a given virological parameter		no. of paired specimens	Parameter (Median range)		*p* value
SARS-CoV-2 RNA load in tracheal aspirates	Pos	23	IL-6 in pg/ml.	111.4 (4–3,548)	0.84
	Neg	2		142 (22–262)	
	Pos	82	Ferritin in ng/ml.	805.5 (69–6,440)	0.01
	Neg	34		421.5 (46–2,659)	
	Pos	101	D-D in ng/ml.	1,730 (270–29,940)	0.87
	Neg	49		1,790 (270–16,160)	
	Pos	105	LDH in UI/l.	687 (93–2,132)	0.001
	Neg	51		520 (214–1,395)	
	Pos	107	CRP in mg/l.	35 (1–746)	0.62
	Neg	54		32.85 (1–606.7)	
	Pos	74	Lymphocytes in cell/µl	0.96 (0.02–3.73)	<0.001
	Neg	42		1.40 (0.44–2.43)	
SARS-CoV-2 RNAemia	Pos	9	IL-6 in pg/ml.	111.4 (10.8–1363.)	0.92
	Neg	40		141.8 (4–3,548)	
	Pos	31	Ferritin in ng/ml.	1,176 (147–6,440)	<0.001
	Neg	211		590 (42–5,847)	
	Pos	36	D-D in ng/ml.	1,535 (320–9,170)	0.17
	Neg	274		1740 (270–60,000)	
	Pos	36	LDH in UI/l.	765.5 (329–1,720)	0.002
	Neg	281		637 (58–2,132)	
	Pos	36	CRP in mg/l.	47.95 (1.2–459)	0.38
	Neg	299		30.7 (1–746)	
	Pos	26	Lymphocytes in cell/µl	0.72 (0.02–3.13)	<0.001
	Neg	209		1.13 (0.17–3.73)	

CRP, C-reactive protein; D-D, Dimer-D; IL-6, interleukin-6; LDH, lactose dehydrogenase.

were significantly higher when SARS-CoV-2 RNA was detected in paired TA or plasma specimens (Table 1), yet SARS-CoV-2 RNA loads in these specimens correlated modestly (Rho < 0.31) with plasma levels of ferritin and LDH (Supplementary Fig 1). Lymphocyte counts were significantly lower in the presence of SARS-CoV-2 RNA in TA and plasma (Table 1). Nevertheless, the level of correlation (inverse) between SARS-CoV-2 RNA load in TA and plasma and lymphocyte counts was modest ((rho, -0.43; $p < 0.01$ and rho, -0.25, $p < 0.01$, respectively). A significant association between SARS-CoV-2 RNAemia detection and blood levels of IL-6, interleukin-10, CRP, ferritin, D-D and LDH was previously reported[4,6]. In these studies, a single time point specimen per patient collected at ICU admission was considered for the analyses, as opposed to the serial specimens used herein.

Dynamics of SARS-CoV-2 RNA load (initial, peak and trajectory) in TA following ICU admission were comparable across patients who either died or survived (Fig. 1C). Moreover, neither initial nor peak viral load in TA was associated with increased mortality (OR, 0.81; 95% CI, 0.68–2.24; $p = 0.68$, and OR, 0.39; 95% CI, 0.22–1.82; $p = 0.39$, respectively) (Supplementary Table 3). Other studies, in contrast, pointed to an association between protracted SARS-CoV-2 RNA clearance in LRT and/or simple presence of SARS-CoV-2 RNA in LRT and increased risk of mortality[7–9]. In these studies, a wide variety of LRT specimens were used, and no data proving a dose-dependent relationship between SARS-CoV-2 RNA load in LRT and mortality were provided.

We found a trend towards an association between qualitative detection of SARS-CoV-2 RNA in plasma and increased mortality in adjusted multivariate logistic regression models (OR, 2.82, 95% CI, 0.94–8.47), and failed to demonstrate such a trend for initial or peak viral loads (supplementary Table 3). SARS-CoV-2 RNAemia has been previously associated with poor clinical outcome in series including only ICU patients, in which patients who died displayed higher viral RNA loads in plasma collected at ICU admission than those who survived[4].

The main limitation of the current study is its relatively small sample size. Analysis of sequential specimens from patients could be considered a strength of the research.

The current study provides a further insight into the pathogenesis of SARS-CoV-2 infection in ICU patients. In our view, our data fit better with a pathogenetic model, in which SARS-CoV-2 replication in the LRT or its presence in blood at a certain point over the course of ICU stay might not be a major driver of systemic inflammation, lymphopenia, lung dysfunction, multisystemic organ failure and death. This does not invalidate the importance of virus replication rate in the URT in the early stage after infection in determining the clinical course of COVID-19[10]. Further studies are needed to resolve this issue.

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Author Contributions

BA, EA, IT, RG-R, RC, JC and JR: Methodology and validation of data. NC, JF and MLB: Medical care of ICU patients. DN: Conceptualization, supervision, writing the original draft. All authors reviewed the original draft.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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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.2021.05.036.

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Beatriz Olea
Eliseo Albert
Ignacio Torres
Roberto Gozalbo-Rovira
Nieves Carbonell
José Ferreres
Sandrine Poujois
Rosa Costa
Javier Colomina
Jesús Rodríguez
María Luisa Blasco
David Navarro*
School of Medicine Valencia, Valencia Spain
*Corresponding author.
E-mail address: david.navarro@uv.es (D. Navarro)

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Previous COVID-19 infection, but not Long-COVID, is associated with increased adverse events following BNT162b2/Pfizer vaccination

Dear Editor,

We read with interest the study recently published by Tré-Hardy et al., who reported that Adverse Events (AEs) after the first dose of mRNA-1273/Moderna vaccine were greater in those previously infected with COVID-19[1]. Their findings are consistent with other studies that suggest mRNA vaccines may cause more AEs in those with a history SARS-CoV-2 infection [2-4]. These results warrant further investigation into the effects of prior COVID-19 history on vaccine reactions, particularly whether time between previous infection and vaccination administration, or the presence of 'Long-COVID' [5], can predict AEs. This information is important, as it could identify individuals more likely to experience side effects to COVID-19 vaccines. Furthermore, there are implications regarding vaccine hesitancy, which is partially driven by fear of AEs [6]. As part of an observational study of COVID-19 outcomes in healthcare workers in North-East England, we evaluated AEs following first doses of BNT162b2/Pfizer vaccine, with reference to previous COVID-19 and Long-COVID.

Healthcare workers completed an electronic survey, which captured self-reported COVID-19 symptoms, PCR/antibody results, and AEs following first doses. The FDA Toxicity Grading Scale [7] was modified, allowing participants to self-report AEs for severity (mild/moderate/severe/very severe), duration (≤24 h/>24 h) and onset (≤24 h/>24 h); lymphadenopathy was also included. A composite score for symptom nature and severity was calculated, to provide an overall estimate of AE-related morbidity. Individual and composite AE scores were compared between those with and without a prior history of COVID-19, as indicated by self-reported prior positive antibody and/or PCR result. Long-COVID was defined as symptoms persisting for >2 months prior to vaccination. Effects of age, gender and time between past infection to vaccination were also considered.

Respondents who permitted laboratory results to be accessed (SARS-CoV-2 PCR/antibody), formed a subgroup for a 'sensitivity analysis'. Statistical analysis was conducted using JASPv0.14.1.0. Composite scores were compared using 2-way ANCOVA. Multivariable logistic regressions were used, to identify the relationship between COVID-19 status and moderate/severe symptoms in each category, and the Bonferroni correction applied to the resulting significance/confidence intervals. The study was approved by Cambridge East Research Ethics Committee.

Of 974 healthcare workers (aged 19–72-years) responding to the survey and providing complete data for analysis, 265 (27%) participants (84% female, mean-age 48.9) reported a prior positive PCR and/or antibody result, and 709 (80% female, mean-age 47.0) had no COVID-19 history. Within the previous COVID-19 group (symptoms median 8.9 months before vaccination), 30 (83% female, mean-age 48.8) complained of Long-COVID (median duration 9.3 months, range 2.8–10.4).

Fig. 1A shows frequencies of each symptom by COVID-19 status. The proportion of participants reporting at least one moderate-to-severe symptom was higher in the previous COVID-19 group (56% v 47%, OR=1.5 [95%CI, 1.1–2.0], p=.009). Symptom onset was mostly within 24 h (75%) with no onset >48 h. Number and total duration of reported symptoms was greater in women (1.24 (1.67) v 0.84 (1.46) symptoms, d = 0.25 [0.09–0.42], p=.002; 2.10 (2.99) v 1.39 (2.54) symptom-days, d = 0.22 [0.09–0.42], p=.001) and significantly decreased with age (symptoms: r_s=−0.25, p<.001; symptom-days: r_s=−0.24, p<.001). After controlling for age and sex, higher symptom number (1.61 (2.26) v 0.89 (2.02) symptoms, d = 0.34 [0.20–0.49], p<.001) and severity (2.7 (6.65) v 1.5 (2.21) symptom-days, d = 0.41 [0.27–0.55], p<.001) were significantly associated with reporting previous COVID-19.

Logistic regressions (Table 1) controlling for age and sex showed five systemic symptoms were significantly associated with previous COVID-19 status: fever, fatigue, myalgia, arthralgia and lymphadenopathy. Arthralgia was regularly co-reported with myalgia

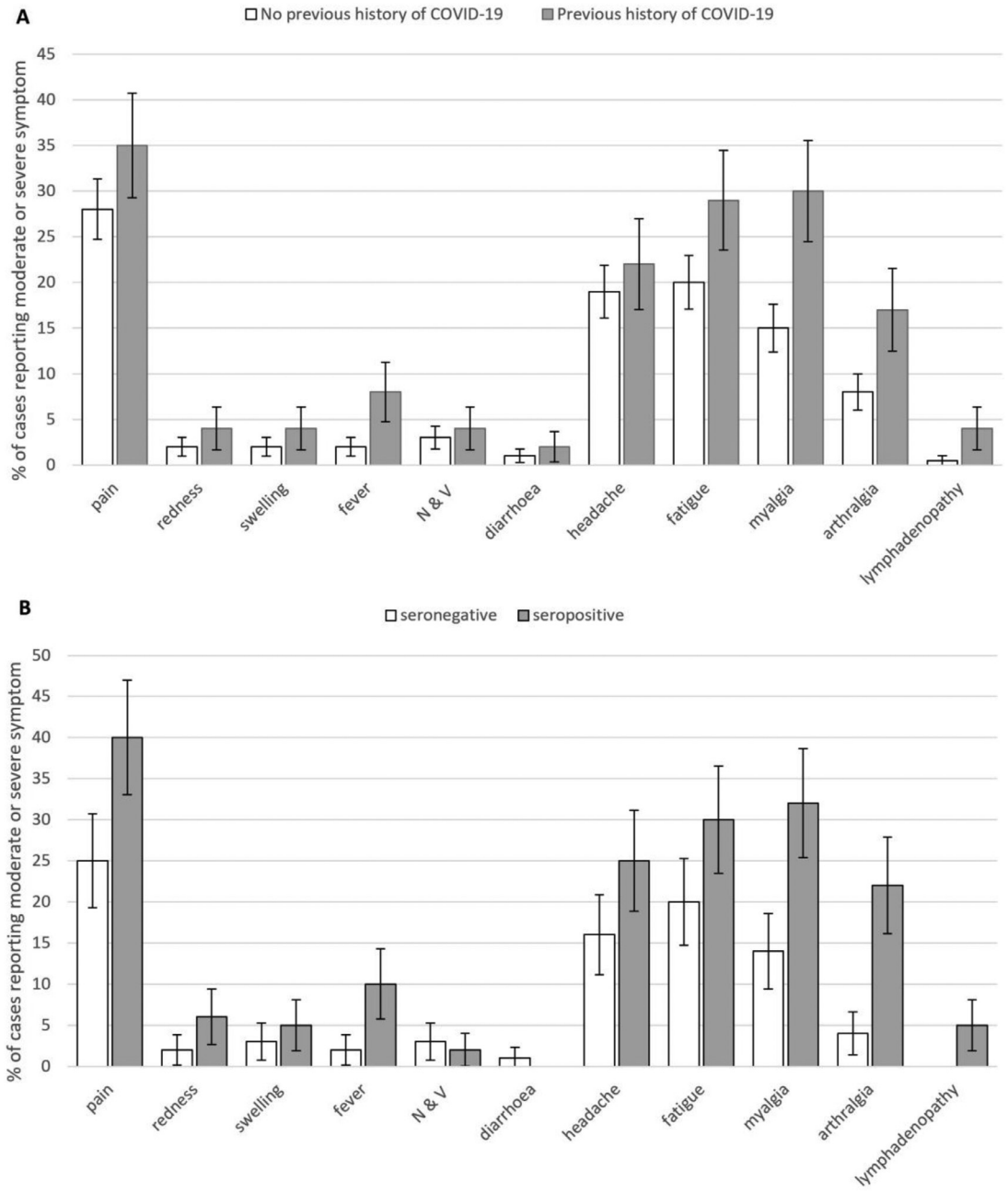

Fig. 1. *Moderate and Severe Symptoms by COVID-19 Status*: Percentage of cases reporting moderate or severe symptoms (95% CI) in those with and without a history of COVID-19 (the former including Long-COVID). N & V: nausea and vomiting. Upper panel (A): entire cohort; lower panel (B): sensitivity analysis subset.

(87 cases), but rarely alone, and was not independently associated (OR 1.4 [95%CI 0.86–2.37], p=.49) with COVID-19 exposure once myalgia was controlled for. Neither local nor gastrointestinal symptoms were significantly associated with previous COVID-19 history.

Symptom number and duration was not significantly higher in those with Long-COVID after accounting for gender and age effects. No individual symptom was significantly associated with this condition. Importantly, among those with prior COVID-19, there was no significant relationship between illness-vaccine time interval and either composite score (r_s=0.09, p=.44 for symptoms; r_s=0.10, p=.42 for symptom–days), nor any difference in mean time interval based on presence of any of the symptoms (all p>.05).

For the 'sensitivity analysis', PCR/antibody results were verified for 412 participants. Of this subgroup, 228 (55%) were PCR/antibody negative (80% female, mean-(SD)-age 47.0 [11.1]) and 184 (45%) were PCR or antibody positive (91% female, mean-(SD)-age 47.3 [11.5]). Nine (5%) complained of Long-COVID (range 2.8–10.4 months). The pattern of results was broadly replicated in this subgroup analysis (Fig. 1B), with more previous-COVID-19 individuals reporting at least one moderate symptom (63% v 43%, OR=2.2 [1.2–4.0], p=.006) and previous-COVID-19 being associated with higher symptom number (1.81 (3.09) v 0.85 (4.12) symptoms, d = 0.25 [0.05–0.44] p=.012) and severity (3.0 (8.3) v 1.5 (5.6) symptom days d = 0.2 [95% CI 0.02–0.41], p=.0350). Only myalgia and arthralgia remain as significant outcomes once multiple comparisons were controlled for though pattern of outcomes remains similar.

This study of healthcare workers demonstrated that prior COVID-19, but not Long-COVID, was associated with increased risk

Table 1
Results of Logistic Regression Analyses: Logistic regressions showing those symptoms significantly predicted by previous history of COVID-19 after controlling for differences in age and gender, and with p values and confidence intervals corrected (Bonferroni) for multiple comparisons.

	Whole cohort		Sensitivity Analysis Subset	
	Odds Ratio (95% C.I.)	p	Odds Ratio (95% C.I.)	p
Fever	2.87 (1.10 – 7.51)	.044	5.68 (0.69 – 46.65)	.32
Fatigue	1.78 (1.12 – 2.84)	.011	2.17 (0.85– 5.54)	.31
Myalgia	2.34 (1.44 – 3.88)	<0.001	3.18 (1.16 – 8.69)	.02
Arthralgia	2.25 (1.23 – 4.12)	.004	7.06 (2.05 – 36.91)	.01
Lymphadenopathy	5.18 (1.19 – 22.63)	.033	****	****
Local Pain	1.55 (0.99 – 2.40)	.09	2.28 (0.96 – 5.43)	.11
Local Redness	2.93 (0.84 – 10.20)	.24	3.92 (0.43 – 35.79)	>0.99
Local Swelling	2.0 (0.64 – 6.27)	.14	2.1 (0.29 – 15.33)	>0.99
n & v	1.47 (0.48 – 4.42)	>0.99	0.72 (0.05 – 8.81)	>0.99
diarrhea	2.35 (0.30 – 18.25)	>0.99	****	****
Headache	1.31 (0.80 – 2.15)	>0.99	1.78 (0.65 – 4.83)	>0.99

**** No model could be calculated due to absence of cases in this cohort. In all cases age and gender were included in the null model as nuisance variables. Adjusted P values and adjusted confidence intervals corrected (Bonferroni) for 11 outcomes in each case.

of AEs following BNT162b2/Pfizer vaccination, although there was no relationship with duration since COVID-19 illness. Women and younger individuals were also more likely to report AEs. Our study adds to other reports supporting the wider understanding of AEs following COVID-19 vaccination [1-4]. Importantly, given hesitancy surrounding recently developed COVID-19 vaccines [6], our findings may help inform those with previous COVID-19 of increased susceptibility to certain AEs. Our study also adds weight to the question of whether a second dose of mRNA vaccine is necessary in those with previous COVID-19, assuming effective immunity is established after the first dose [1,2,8,9]. This is relevant, given that Tre-Hardy's and other studies have reported worse AEs following second doses of vaccine [1,3].

Our study has several limitations. Firstly, some non-responder bias[10] is likely, with 27% of participants reporting previous COVID-19. Secondly, AE information was gathered via self-reported questionnaires, and hence was subjective. Thirdly, PCR/antibody results were self-reported. We addressed this via a sensitivity analysis on a subset with laboratory data available, which mostly confirmed the findings. Finally, numbers of participants with Long-COVID were relatively small for comparison.

Author contributions

DRC/CK/RKR conceived the study and DRC is chief investigator of CHOIS. RKR acted as site principal investigator. DRC/RKR/CW contributed to the study protocol, design, and data collection. JR/RKR/DRC did the statistical analysis. RKR/JR/DRC prepared the manuscript. All authors critically reviewed and approved the final version.

Declarations of Competing Interest

No conflicts of interest.

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Rachael Kathleen Raw, BSc, MBBS, PhD*
Newcastle University, United Kingdom
James Cook Universiry Hospital, United Kingdom

Clive Anthony Kelly, MBBS, MRCP, FRCP, MD
James Cook Universiry Hospital, United Kingdom

Jon Rees, BSc, MBBS
University of Sunderland

Caroline Wroe, MBChB, MRCP, PhD, David Robert Chadwick, MB BChir, MRCP, PhD
James Cook Universiry Hospital, United Kingdom

*Corresponding author.
E-mail address: rachael.raw3@nhs.net (R.K. Raw)

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Follow-up study of pulmonary function among COVID-19 survivors 1 year after recovery

Dear Editor,

Introduction

Coronavirus disease 2019 (COVID-19) is a novel systemic disease that affects multiple organs, with the lungs being most affected.[1-3] Previous studies have demonstrated that carbon monoxide diffusing capacity (DLCO) is impaired in patients who had recovered from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection at the time of discharge.[4] However, long-term pulmonary function in survivors is poorly understood. Here, we assessed pulmonary function in survivors who had recovered from SARS-CoV-2 infection 1 year previously.

Methods

In this cohort study conducted from March 16 to March 28, 2021, we followed up a total of 119 survivors with SARS-CoV-2 infection who had been hospitalized during January 24–March 18, 2020 in Huanggang, Hubei Province, China. Study inclusion criteria included a previous COVID-19 diagnosis (positive PCR result for SARS-CoV-2) and the willingness and ability to provide informed consent. Baseline demographics, smoking status, body mass index and comorbidities were extracted from the electronic medical record. The severity of the disease was defined according to the World Health Organization COVID-19 guidelines. Severe COVID-19 refers to fever or suspected respiratory infection, plus one of the following: respiratory rate $>$ 30 breaths/min; severe respiratory distress; or SpO2 $\leq$ 93% on room air.

Lung function tests were performed by technicians in the lung function laboratory using the Master Screen Body (Jaeger, Germany). The procedure followed was in accordance with American Thoracic Society/European Respiratory Society guidelines.

This study was approved by the Hunan Provincial People's Hospital Ethics Commission. All participants provided their written or verbal consent to participate.

Results

A total of 119 survivors participated in this study (asymptomatic, n = 9; non-severe, n = 82; severe, n = 28) (Table 1). The median patient age was 52.97 (±12.17) years; 49 survivors (41%) were men and 70 (59%) were women. Twenty-four survivors (20%) had at least one chronic comorbidity, 10 (8%) with hypertension and 11 (9%) with diabetes; only 2 (2%) patients were reported as having chronic obstructive pulmonary disease. There were no statistically significant differences in age, sex, body mass index, and smoking status among the three groups.

Anomalies were found for the percent predicted DLCO (n = 47, 39%), DLCO/alveolar volume (n = 10, 8%), percent predicted total lung capacity (TLC; n = 50, 42%), percent predicted residual volume (n = 50, 42%), percent predicted forced expiratory volume in 1 second (FEV1; n = 11, 9%), maximal mid-expiratory flow (MMEF) 75/25 (n = 41, 34%), percent predicted forced vital capacity (FVC; n = 11, 9%), and FEV1/FVC (n = 6, 5%).

As shown in Table 1, there was no statistically significant difference in damaged diffusing capacity among groups with different disease severity, with 11% in the asymptomatic group, 38% in the non-severe group, and 54% in the severe group, respectively (P = 0.605). However, the gradual decline in lung diffusion capacity among survivors was consistent with varying degrees of severity. There was no significant difference in other measures (TLC, RV/TLC, FVC, FEV1, FEV1/FVC, and MMEF 75/25) among COVID-19 survivors with different disease severity.

Discussion

Previous studies have shown that survivors of COVID-19 may have lung damage.[4-7] In follow-up studies lasting 3–6 months among rehabilitating COVID-19 severe/critical patients, DLCO damage was the most common abnormality, accounting for 56%–82% of cases, followed by TLC deficiencies.[6-8] They found significant differences in diffusing capacity damage among groups with different disease severity.

Alessia et al. found that 10 of 13 patients with COVID-19 pneumonia were damaged at the time of discharge.[9] After 6 weeks, lung function was improved but a certain degree of restrictive changes remained.[9] In this cohort study, lung functional impairment are highly prevalent in survivors with COVID-19 1 year after discharge. Forty-seven (39%) survivors had impaired diffusing capacity during the 1-year follow-up, with no significant difference between the severe and non-severe groups. This may indicate that pulmonary function damage from COVID-19 can improve over time.

DLCO abnormalities occurred in 39% of survivors, indicating damaged intra-alveolar diffusion pathways. Autopsy in patients who died from SARS-CoV-2 infection showed diffuse alveolar injury, accompanied by thrombosed small vessels with remarkable associated hemorrhage.[10] Changes in lung pathology can explain the diffusing capacity damage to a certain extent. Moreover, a proportion of patients with COVID-19 developed acute respiratory distress syndrome (ARDS). Pulmonary fibrosis can develop as a result of chronic inflammation of the lungs owing to ARDS. Pulmonary fibrosis associated with ARDS in COVID-19 patients may damage alveolar-capillary units, causing loss of alveolar units and impaired gas exchange.

Patients with severe or critical COVID-19 may need to use ventilators in the intensive care unit for several weeks. The breathing muscles are affected, which weakens the ability to breathe. Pulmonary rehabilitation involves suggestions for physical exercise and management of symptoms and is important to help survivors fully recover.

Our study had several limitations. First, the lack of baseline pulmonary function data before the illness onset made it difficult to conduct comparisons with post-illness results. Moreover, we only carried out 1 year of follow-up; the long-term dynamic changes of pulmonary function after SARS-CoV-2 infection need further study.

In summary, in this cohort study, we found that lung functional impairment are highly prevalent in survivors with COVID-19 1 year after discharge, and persistent lung function impairment was found in about 40% of survivors. Lung damage might be related to pulmonary fibrosis. Further long-term research is needed to understand the mechanisms underlying long-term SARS-CoV-2-related pulmonary function damage.

Author contributions

YZhu, XH, YZeng, and XY generated the research question and analysis plan. XY, HH, CW, ZJ, ZZ, JH, SY, MF, JH, FC were involved

Table 1
Demographics and pulmonary function characteristics of survivors with COVID-19.

Variable	Total (n = 119)	Asymptomatic cases (n = 9)	Non-severe cases (n = 82)	Severe cases (n = 28)	P-value*
Age, median(SD), y	52.97±12.17	46.44±10.48	52.66±12.39	56.00±11.40	0.111
Gender					
Male, no, (%)	49 (41%)	3 (33%)	35 (43%)	11 (39%)	0.841
Female, no, (%)	70 (59%)	6 (67%)	47 (57%)	17 (61%)	
Cigarette smoking					
Never-smoker	86 (72%)	6 (67%)	58 (%)	22 (%)	0.737
Current smoker	15 (13%)	1 (11%)	10 (%)	4 (%)	
Former smoker	18 (15%)	2 (22%)	14 (%)	2 (%)	
BMI $kg \bullet m^{-2}$	25.07±3.22	24.48±3.09	24.98±3.21	25.51±3.26	0.638
Comorbidities	24 (20%)	0	13 (16%)	11 (39%)	0.008
Hypertension	10 (8%)	0	6 (7%)	4 (14%)	0.331
Diabetes	11 (9%)	0	4 (5%)	7 (25%)	0.005
Cardiovascular diseases	2 (2%)	0	0	2 (7%)	0.037
Malignant tumor	1 (1%)	0	1 (1%)	0	0.797
COPD	2 (2%)	0	1 (1%)	1 (4%)	0.649
Liver disease	1 (1%)	0	1 (1%)	0	0.797
Chronic kidney disease	1 (1%)	0	1 (1%)	0	0.797
Spirometry					
FVC% pred	97.7 ± 13.76	98.82±12.36	97.93±13.72	96.68±14.70	0.890
FVC <80% pred	11 (9%)	0	7 (9%)	4 (14%)	0.404
FEV1% pred	98.22±14.25	98.11±13.84	98.12±14.19	98.54±14.10	0.991
FEV1 <80% pred	11 (9%)	0	8 (10%)	3 (11%)	0.602
FEV1/FVC%	80.56±7.82	81.26±4.30	80.36±7.95	80.90±8.46	0.917
FEV1/FVC <70%	6 (5%)	0	5 (6%)	1 (4%)	0.672
MMEF75/25	77.60±26.06	80.24±16.59	76.70±26.89	79.38±26.64	0.855
MMEF75/25 <65%	41 (34%)	2 (22%)	30 (37%)	9 (32%)	0.661
Diffusion capacity					
DLCO% pred	81.27±13.06	84.38±5.94	81.94±12.56	78.34±15.74	0.347
DLCO <80% pred	47 (39%)	1 (11%)	31 (38%)	15 (54%)	0.605
DLCO/VA% pred	103.74±16.86	106.21±10.84	103.66±16.94	103.18±18.56	0.895
DLCO/VA <80% pred	10 (8%)	0	7 (9%)	3 (11%)	0.600
Lung volume					
TLC% pred	81.52±9.41	80.70±7.47	82.41±9.90	79.16±8.25	0.281
TLC <80% pred	50 (42%)	4 (44%)	34 (41%)	12 (43%)	0.980
RV% pred	70.67±17.61	61.49±11.93†	73.52±18.38	65.27±14.70†	0.026
RV <65% pred	50 (42%)	6 (67%)	28 (34%)	16 (57%)	0.031
RV/TLC% pred	85.36±20.11	75.30±11.48	87.61±19.59	82.02±22.73	0.132

Data are mean (SD), median (IQR), or n (%), unless otherwise specified.
Comparisons between continuous variables were performed with one-way ANOVA. Chi-squared test and Fisher's exact test were applied to categorical variables as appropriate.
*Difference among all types.
†P<0.05 versus non-severe cases;.
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; FVC, forced vital capacity; pred, predict; FEV1, forced expiratory volume in the first second; MMEF, maximum mid-expiratory flow; DLCO, diffusing capacity of the lung for carbon monoxide; DLCO/VA: DLCO corrected for alveolar volume; TLC, total lung volume; RV, residual volume.

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Declaration of Competing Interest

No conflicts of interests declared by an author.

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Xiquan Yan
Department of Emergency Medicine, Hunan Provincial Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's

Hospital/The First Affiliated Hospital, Hunan Normal University, Changsha, Hunan, China
School of Life Sciences, Hunan Normal University, Changsha, Hunan, China

Huxiang Huang, Changfeng Wang
Department of Respiratory and Critical Care Medicine, Huanggang Central Hospital, Huanggang, Hubei, China

Zhaoxia Jin
Department of Cardiology, Huanggang Central Hospital, Huanggang, Hubei, China

Zhongwei Zhang
Department of Emergency Medicine, Hunan Provincial Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's Hospital/The First Affiliated Hospital, Hunan Normal University, Changsha, Hunan, China

Jiangming He, Siqing Yin
Department of Public Health, Huangzhou General Hospital, Huanggang, Hubei, China

Maiying Fan
Department of Emergency Medicine, Hunan Provincial Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's Hospital/The First Affiliated Hospital, Hunan Normal University, Changsha, Hunan, China

Juanshu Huang
Department of Respiratory and Critical Care Medicine, The Third Hospital of Changsha, Changsha, Hunan, China

Fang Chen
Department of Emergency Medicine, Hunan Provincial Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's Hospital/The First Affiliated Hospital, Hunan Normal University, Changsha, Hunan, China

Yong Zeng*
Huanggang Central Hospital, Huanggang, Hubei, China

Xiaotong Han*
Department of Emergency Medicine, Hunan Provincial Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's Hospital/The First Affiliated Hospital, Hunan Normal University, Changsha, Hunan, China

Yimin Zhu*
Department of Emergency Medicine, Hunan Provincial Institute of Emergency Medicine, Hunan Provincial Key Laboratory of Emergency and Critical Care Metabonomics, Hunan Provincial People's Hospital/The First Affiliated Hospital, Hunan Normal University, Changsha, Hunan, China
School of Life Sciences, Hunan Normal University, Changsha, Hunan, China

*Corresponding authors.
E-mail addresses: zengyong8000@163.com (Y. Zeng), hanxiaotong2021@163.com (X. Han), zhuyimincs@163.com (Y. Zhu)

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Mouth care matters – A HAP prevention strategy

Dear Editor,

Globally, Morbidity and Mortality due to hospital-acquired pneumonia (HAP) is considerable, as Lim and colleagues found in their systematic review of *Acinetobacter baumannii* pneumonia prevalence.[1] Results of the 2016 Point Prevalence Survey (PPS) (ESPAUR) indicate that HAP is the most prevalent healthcare-associated infection in England (29.2%); 74% of these were not associated with mechanical ventilation.[2] The term non-ventilated hospital-acquired pneumonia (NV-HAP) describes a pneumonia in patients admitted to hospital, who have not received mechanical ventilation.[3] Giuliano et al., (2018) estimated the burden of NV-HAP, demonstrating an association with increased total hospital charges, longer length of stay and greater likelihood of death.[4] Quinn et al., (2013) stated that preventing even 100 cases of NV-HAP may save up to $4 million, ~900 hospital days, and the lives of ~30 patients.[5] Strategies to prevent NV-HAP include frequent mouth care, increasing mobilisation, changing patients' bed position and appropriate management of dysphagia.[3] Here, we report observations on the impact of a mouth care education intervention, entitled '*mouth care matters*', on the prevalence of HAP at a major UK tertiary referral hospital.

University Hospitals Birmingham NHS Foundation Trust (UHB) is one of the largest Trusts in the UK, treating over 2.2 million patients per year. UHB utilised the expertise of a dental nurse to improve mouth care in patients. The dental nurse delivered basic mouth care education at the bedside to nurses and healthcare assistants via presentations with practical demonstrations. The education package included information on: the relationship between oral health and general health; risk assessments of the oral cavity; practical demonstration of new specialist equipment to overcome any barriers to providing mouth care, especially for patients with aspiration risk; delivering standard mouth, dry mouth treatment ulcer and denture care. A set of protocols for '*mouth care matters*' were developed and carried out at initial assessment, last time at night and one other time during the day.[6] Mouth assessment included: assessment of the lips, tongue, teeth, gums, cheeks palate and dentures; level of support for patients and aspiration risk. A risk level was calculated and a treatment plan formed.[6] The dental nurse undertook a short evaluation with assessment questions at the end of the education session to confirm competency. The training lasted one month. All mouth care was initially taught with a basic tooth brush and paste, with mouth moisturisers. Specialist mouth care equipment was available but only when there were challenges that the basic equipment failed to meet.

'*Mouth care matters*' was undertaken on four wards between April-October 2019; two respiratory wards, a neurosurgery ward (each consisting of 36 beds), and a geriatric medicine ward (27 beds). Two additional general medicine wards (36 beds each), were chosen as controls where no interventions were undertaken. Prior to implementation of the programme, a baseline audit and staff questionnaire was performed on each study ward to explore the current delivery of mouth care. These were reprised 6 months after the interventions to review progress. A PPS for NV-HAP was undertaken on one day, a month before the intervention and 6 months after the intervention. Definitions for NV-HAP were based on the European Centre for disease prevention and Control definitions for lower respiratory tract infections.[7] A Poisson regression model on the number of HAP's, offset by the number of bed days, was used to check if the intervention affected the number of NV-HAP cases.[8] The explanatory variables in this multivariate model were a factor representing whether the PPS occurred pre or post intervention, a

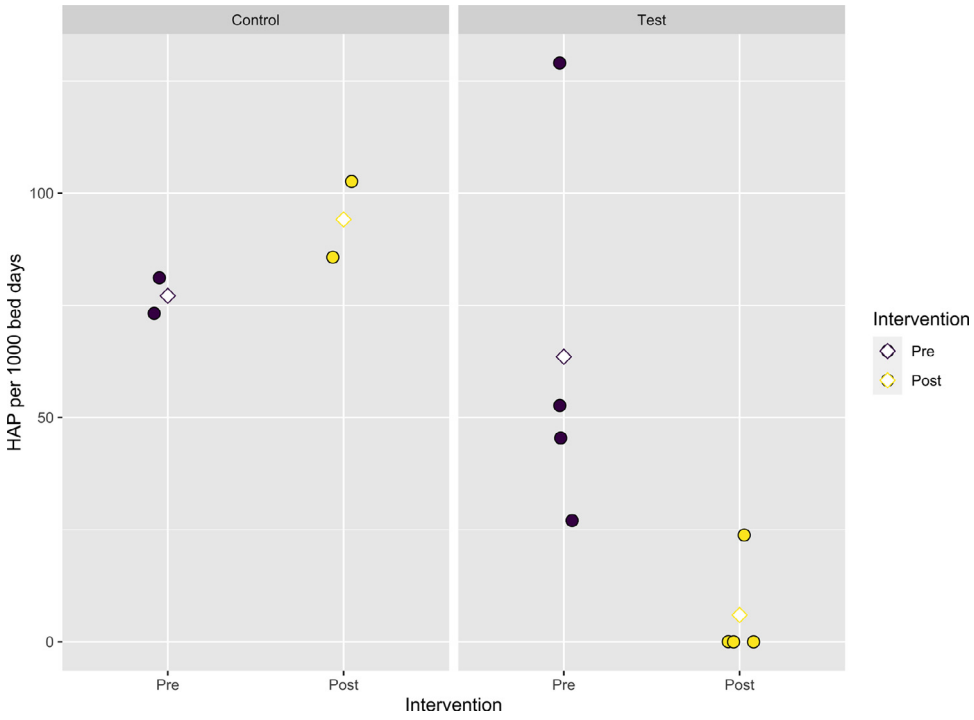

Fig. 1. The number of HAPs per 1000 bed days pre and post the 'mouth care matters' interventions on all the wards in the study.
Key: the white diamonds represent the mean values, circles represent the wards, blue circles represent pre intervention, yellow circles post intervention.

factor representing which 'arm' the ward was in (control or test) and an interaction between the two. The model was used to calculate pairwise contrasts for each level of factor/interaction, with p values corrected for multiple contrasts.

The model suggests that HAP did not significantly change on the wards where no intervention was undertaken comparing before and after roll out ($p = 0.710$). On the wards where the intervention was undertaken, there was a nine-fold reduction in HAP after the implementation, $p = 0.0383$. Comparing the pre intervention HAP levels between the two arms, there was no significant difference, ($p = 0.637$). Assessing the post intervention HAP levels between the two arms, the mean HAP per 1000 bed days was approximately 14-fold higher in the control arm compared to the test arm, $p = 0.0136$ (Fig. 1). There was also a 37% increase in the number of patients receiving twice-daily mouth care after mouth care matters on the intervention wards.

Here we have shown a package of comprehensive mouth care reduces the number of HAPs. It is not surprising mouth care matters was associated with a reduction in HAP, as oral hygiene has been well documented as an intervention for HAP. A systematic review incorporating 28 RCTs identified oral health care was associated with a reduction in HAP.[9] Azarpazhooh and Leake (2006) explained why oral care reduced HAP.[10] They examined the aetiology of oral health and pneumonia, stating that microorganisms in saliva/dental plaque were risk factors for HAP, detailing how poor oral hygiene leads to these organisms causing HAP.[10] A significant limitation of studies looking at NV-HAP is that the data could potentially be biased in terms of evaluation of the results.[3] The methodology identifying patients with NV-HAP is not standardised, so whether the effects reported in our study are as pronounced as suggested warrants further investigation. Potential limitations to the current study also include seasonality.[3] On one ward, the baseline PPS was completed during the influenza season, which could have contributed to an increased rate of NV-HAP, as influenza is a risk factor for HAP.[3] A further limitation is that it is a single centre study, and so may not be reproducible in different healthcare settings. In conclusion, NV-HAP places a significant burden on healthcare, according to the two most recent national prevalence studies is the most common HCAI and more can be done to prevent this serious infection.[2] Here, we have illustrated that a basic care intervention such as mouth care can reduce NV-HAP. Larger, controlled multicentre studies are required to validate this approach for the prevention of NV-HAP in the secondary and tertiary care setting.

Authors contributions

All authors have contributed to the manuscript.

Patient and public involvement

None, paper is of interest to patients and public in light of current pandemic. An observational study.

Transparency declaration

The author affirms the manuscript is an honest accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Mark I. Garvey*
University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Edgbaston, B15 2WB Birmingham, United Kingdom
Institute of Microbiology and Infection, The University of Birmingham, Edgbaston, Birmingham, United Kingdom

Martyn A.C. Wilkinson, Helen Woodall, Caroline Smith
University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Edgbaston, B15 2WB Birmingham, United Kingdom

Kerry Holden
Gloucestershire NHS Foundation Trust, Gloucester, United Kingdom

Jenna Cartin
University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Edgbaston, B15 2WB Birmingham, United Kingdom

Davinder Dosanjh
University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Edgbaston, B15 2WB Birmingham, United Kingdom
Institute of Inflammation and Ageing, University of Birmingham, Edgbaston, Birmingham, United Kingdom

Elisabeth Holden
University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, Edgbaston, B15 2WB Birmingham, United Kingdom

Martin Kiernan
University of West London, Richard Wells Research Centre, London, United Kingdom

*Corresponding author at: University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Hospital Birmingham, B15 2WB Edgbaston, Birmingham United Kingdom.
E-mail addresses: mark.garvey@uhb.nhs.uk, m.i.garvey@bham.ac.uk (M.I. Garvey)

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Rapid lateral-flow immunochromatographic tests to assess anti N/S IgG seropositivity after BNT162b2 vaccine: A cross-sectional study

Dear Editor,

As reported recently in this Journal, antibodies against SARS-CoV-2 can be detected as early as 7–14 days after natural infection and the antibody titre could persist for more than 6 months.[1] Theimmune response is elicited against several viral epitopes, yet the nucleocapsid (N) protein and the spike (S) protein (with its subunits S1 – containing the receptor binding domain – and S2 – which mediates viral fusion and entry) were those selected to develop diagnostic methods. Anti-S antibodies have been found to correlate with in vitro neutralization activity.[2] Consequently, the S protein was selected as the target for the development of SARS-CoV-2 vaccines.[3]

Rapid lateral-flow immunochromatographic tests (RLITs) can detect IgG and/or IgM antibodies against SARS-CoV-2 proteins N and/or S in capillary blood, serum, and plasma: this point of care method has already been successfully used in population studies.[4–7]

Another application of RLITs could be in the qualitative determination of antibody response after SARS-CoV-2 vaccination. In Italy, the immunization campaign started on 27th December 2020 with the priority given to health care workers (HCWs) vaccinated with BNT162b2 vaccine**.** BNT162b2 has been demonstrated to elicit a robust anti S antibody response in up to 95% of individuals after the second shot.[3] Although the detection of antibodies in peripheral blood samples is the gold standard, it is expensive and needs expert personnel. These barriers could be overcome by RLITs, but studies assessing the performance of RLITs after BNT162b2 vaccine are lacking and whether they could adequately detect this response is unknown.

The aim of our study was to estimate the qualitative antibody response elicited by BNT162b2 vaccine using different RLITs in a sample of vaccinated HCWs at Luigi Sacco Hospital, Milan, Italy.

In this cross-sectional study, we estimated the antibody response to SARS-CoV-2 antigens (N and S proteins) using three different RLITs in a group of vaccinated HCWs. RLITs were performed between 25th January and 16th February 2021, 7 (±3) days after the second BNT162b2 dose. All the hospital staff was invited to participate on a voluntary basis, and everyone gave written informed consent. A questionnaire was filled to assess gender, age, and previous self-reported SARS-CoV-2 exposure (defined as having had a previous positive nasopharyngeal swab and/or a positive IgG serology). The anti-N protein COVID-19 IgG/IgM rapid test (PRIMA Lab SA, Balerna, Switzerland) and the anti-N and anti-S COVID-19 IgG/IgM rapid test cassette (Zhejiang Orient Gene Biotech Co., InnoLiving, Zhejiang, China) were performed on a single capillary blood sample. The anti-N and anti-S COVID-19Speed IgG/IgM test (BioSpeedia SAS – Institut Pasteur, Paris, France) was later available and simultaneously performed only on a subsample of HCWs. RLITs were read by two investigators (LP and FC). Only the IgG band was considered for the present analysis. RLITs IgG results were categorized as positive, negative or indeterminate (if the IgG band was incomplete). All subjects underwent a concomi-

tant anti-S serological examination on peripheral blood assessed by means of Chemiluminescent immunoassay (CLIA) (LIAISON SARS-CoV-2 trimericS IgG DiaSorin, Saluggia, Vercelli, Italy). An anti S titre ≥ 33.8 Binding Arbitrary Units (BAU)/mL on peripheral blood was considered as positive.[8]

To estimate vaccine response, assuming a response rate ≥95% with a 95% confidence interval and a precision of at least 5%, a minimum of 73 subjects was needed. The study was approved by University of Milan's Ethical Committee.

Of the 160 HCWs included in the analysis, 110 (68.8%) were female and the median age was 41 years (Table 1). Twenty-six (16%) reported a previous SARS-CoV-2 exposure. All subjects tested positive on anti S peripheral blood with significantly higher titers observed in subjects previously exposed to SARS-CoV-2 when compared to the unexposed ones [6745 BAU/mL (Inter Quartile Range (IQR) 4452–9960) vs 1995 BAU/mL (IQR 1202–3257), respectively; $p<0.001$]. The anti-N and anti-S COVID-19 IgG/IgM rapid test cassette RLIT resulted positive in 26/26 (100%) of exposed and 129/134 (96.3%) of unexposed HCWs.

One-hundred and fifty-five out of 160 and 56/88 subjects tested positive with anti-N and anti-S COVID-19 IgG/IgM rapid test cassette and anti N and anti-S COVID-19Speed IgG/IgM test: assuming CLIA on peripheral blood as the reference, this accounts for a sensitivity of 96.9% [95% Confidence Interval (CI) 92.9%−99%] and 63.6% [95% CI 52.7%−73.6%], respectively.

In our study anti-N and anti-S COVID-19 IgG/IgM rapid test cassette showed a good performance in identifying antibody response (96.9%) after the second dose of BNT162b2 vaccine. Whereas, the anti-N and anti-S COVID-19Speed IgG/IgM test identified only 63.6% of subjects with a positive anti S response after BNT162b2 vaccine.

Only two subjects with no known previous SARS-CoV-2 exposure tested negative with anti-N and anti-S COVID-19 IgG/IgM rapid test cassette. Although both subjects tested positive with CLIA, they showed the lowest antibody titre in the cohort (64 BAU/mL and 253 BAU/mL). In addition, both subjects reported autoimmune disorders: in one case atopic dermatitis treated with janus kinase inhibitor and in the other systemic lupus erythematosus treated with hydroxychloroquine.

The observed higher titers in subjects with a previous SARS-CoV-2 exposure when compared to those unexposed before vaccination was in line with previous observations suggesting that just one single dose of BNT162b2 vaccine could be sufficient to elicit an adequate antibody titre.[9]

Our study presents some limitations. First, not all subjects had an available anti-N titre to systematically ascertain SARS-CoV-2 exposure before vaccination and consequently asymptomatic infections could not be definitely ruled out. Second, the study population is a convenience sample of HCWs not representative of the vaccinated general population. Third, a single serological determination was performed, thus not allowing a longitudinal assessment of test performance overtime. In the end, the absence of vaccine non-responders (with a negative antibody titre) does not allow the assessment of different tests' specificity.

In conclusion, RLITs could be considered for a qualitative assessment of BNT162b2 vaccine antibody response. RLITs could serve as a tool for a rapid point of care evaluation in people at risk of non-response (i.e. those exposed to immunosuppressant agents). In this population a negative result should be further evaluated by means of CLIA.

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Table 1
Characteristics of the study population and different tests' results. The presence of a clearly identifiable and complete IgG band was considered positive, the complete absence was considered negative and a partial/incomplete band was considered as indeterminate.

		Overall	Self-reported SARS-CoV-2 exposure before vaccination		
			NO	YES	p-value
		$n = 160$	$n = 134$ (84%)	$n = 26$ (16%)	
Gender, n (%)	***Females***	110 (68.8)	92 (68.7)	18 (69.2)	0.999
	Males	50 (31.2)	42 (31.3)	8 (30.8)	
Age, median [IQR]		41.00 [32.00, 52.25]	41.00 [33.00, 53.00]	34.00 [28.00, 45.75]	*0.028*
Positive serological test*, n (%)		160 (100.0)	134 (100.0)	26 (100.0)	
Ab Anti-SARS-CoV-2 measured by CLIA (BAU/mL), median [IQR]		2125 [1312, 4250]	1995 [1202, 3257]	6745 [4452, 9960]	*<0.001*
Rapid lateral-flow immunochromatographic tests					
COVID-19 IgG/IgM rapid test (anti-N protein), n (%)	***Negative***	146 (91.2)	132 (98.5)	14 (53.8)	*<0.001*
	Positive	10 (6.2)	1 (0.7)	9 (34.6)	
	Indeterminate	4 (2.5)	1 (0.7)	3 (11.5)	
COVID-19 IgG/IgM rapid test cassette (anti-N and anti-S), n (%)	***Negative***	2 (1.2)	2 (1.5)	0 (0.0)	0.999
	Positive	155 (96.9)	129 (96.3)	26 (100.0)	
	Indeterminate	3 (1.9)	3 (2.2)	0 (0.0)	
COVID-19Speed IgG/IgM test (anti-N and anti-S), n (%) ($n = 88$)	***Negative***	20 (22.7)	17 (22.7)	3 (23.1)	0.374
	Positive	56 (63.6)	46 (61.3)	10 (76.9)	
	Indeterminate	12 (13.6)	12 (16.0)	0 (0.0)	

*Cut-off for positivity > or = 33.8 BAU/mL.
List of abbreviations: S, spike; N, nucleocapsid; IQR, Inter Quartile Range; n, number; CLIA, Chemiluminescent immunoassay; BAU, Binding Arbitrary Units.

Laura Pezzati*
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy
Department of Biomedical and Clinical Sciences, Università di Milano, Milan, Italy

Andrea Giacomelli
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy

Davide Mileto
Laboratory of Clinical Microbiology, Virology and Bioemergencies Diagnostics, ASST Fatebenefratelli-Sacco, Milan, Italy

Federico Conti
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy
Department of Biomedical and Clinical Sciences, Università di Milano, Milan, Italy

Gloria Gagliardi, Alberto Rizzo
Laboratory of Clinical Microbiology, Virology and Bioemergencies Diagnostics, ASST Fatebenefratelli-Sacco, Milan, Italy

Laura Milazzo
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy

Marco Schiuma, Alice Covizzi, Matteo Siano, Cinzia Bassoli, Matteo Passerini, Marco Piscaglia
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy
Department of Biomedical and Clinical Sciences, Università di Milano, Milan, Italy

Alessandro Torre, Letizia Oreni, Giuliano Rizzardini
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy

Massimo Galli
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy
Department of Biomedical and Clinical Sciences, Università di Milano, Milan, Italy

Anna Lisa Ridolfo
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy

Spinello Antinori
Infectious Diseases Department, ASST Fatebenefratelli-Sacco, Milan, Italy
Department of Biomedical and Clinical Sciences, Università di Milano, Milan, Italy

*Corresponding author at: Department of Biomedical and Clinical Sciences, Università di Milano, Milan, Italy.
E-mail address: laura.pezzati@unimi.it (L. Pezzati)

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Persistence of humoral immune response to SARS-CoV-2 up to 7 months post-infection: Cross-sectional study, South India, 2020–21

Dear Editor,

Hanrath and colleagues, reported in this Journal that the prior SARS-CoV-2 infection was associated with protection against symptomatic reinfection.[1] Authors suggested further studies to know the durability of protection. SARS-CoV-2 elicits rapid immune response, with seroconversion occurring in majority cases by 10 days post-symptom onset.[2–5] Information about longevity of antibody mediated immune response in convalescent COVID-19 patients is vital in understanding the duration of immunity. Several published studies have reported rapid waning of antibodies within 3–4 months.[5] Others have reported presence of IgG antibodies up to 3 and 8-months post infection.[6-8] Few studies are available about persistence of humoral immune response from low and middle-income countries. We estimated prevalence of SARS-CoV-2 specific antibody response among COVID-19 patients at multiple time points over a 7-month period post RT-PCR confirmation.

We conducted a cross-sectional study among recovered COVID-19 patients between November 10 and December 15, 2020 across all age groups in Chennai, India. We obtained the line-list of recovered COVID-19 patients from the local civic body and grouped these patients into seven time-points (i.e. 15–30, 31–60, 61–90, 91–120, 121–150, 151–180 and 181–232 days) based on days since their RT-PCR confirmation. We enrolled a minimum of 100 consenting individuals from each of the seven-time groups and interviewed them to collect information on basic demographic details, clinical history, comorbidity and current health status and collected 3–5 ml of venous blood.

The sera were tested for the presence of IgG antibodies against nucleocapsid (NC) (Abbott Park, IL, USA, Sensitivity: 100%, Specificity: 99.6%[9]) and spike (S1-RBD) (Siemens Healthineers India, Mumbai, Sensitivity: 100%, Specificity: 99.9%) proteins using chemiluminescent immunoassays, and neutralizing antibodies (Nabs) using surrogate virus neutralization test (sVNT) (GenScript, Piscataway, USA) (Supplementary material.[8]) The data were analyzed to estimate the proportion IgG positivity during different time-windows (Supplementary material). Institutional ethics committee of ICMR-National Institute of Epidemiology and ICMR-National Institute for Research in Tuberculosis, Chennai approved the study protocol.

We enrolled 755 individuals in the study (minimum 100 participants in each time-group). The mean age of the study participants was 41.8 (SD: 12.5) years, and 58.3% (n = 440) were males. 81 (10.7%) individuals reported that they were asymptomatic, 44 (5.8%) had severe illness (admitted in ICU or required supplemental oxygen while hospitalization) and 630 (83.4%) were classified into mild to moderate illness category. Majority were either isolated in COVID care centres (33.1%) or in their homes (37%) and 194 (25.7%) were directly admitted to a hospital or medical institution. 280 (37.1%) reported a chronic co-morbidity; the most common being diabetes mellitus (n = 176, 23.3%) and hypertension (n = 155, 20.5%) (Table-1).

IgG seropositivity against NC protein 15–30, 31–60, 61–90, 91–120, 121–150, 151–180 and 181–232 days after RT-PCR diagnosis was 83.2% (95%CI: 76.1% - 90.3%), 85.1% (95%CI: 78.2% - 92.1%), 75.7% (95%CI: 67.8% - 83.5%), 71.3% (95%CI: 62.5% - 80.1%), 58.2% (95%CI: 49.0% - 67.4%), 51.4% (95%CI: 41.9% - 61.0%), and 37.1% (95%CI: 28.3% - 45.9%) respectively (Fig-1, Supplementary Table-1). Sero-positivity to S1-RBD was higher compared to that of NC pro-

tein at all time-windows except during the first-time-window of 15–30 days. The proportion of COVID-19 patients sero-positive to NC or S1-RBD declined over time, with respectively 43 (37.1%) and 73 (62.9%) of the 116 patients having antibodies against NC and S1-RBD 180 days , after RT-PCR diagnosis (Supplementary Table-1, Fig-1). More than 90% (range: 91.3%- 96.4%) of the recovered COVID-19 patients had NAbs till 121–150 days. NAbs during the time-window of 151–180 and 181–232 days after RT-PCR diagnosis was 85.7% (95%CI: 79.0% - 92.4%) and 86.2% (95%CI: 79.9% - 92.5%) respectively.

Seropositivity for IgG against NC, S1-RBD and NAbs observed during 15–30-day time period was higher among individuals with severe illness compared to those with a mild/moderate or asymptomatic illness. This pattern was observed during each of the time-window. In particular, IgG seropositivity against NC and S1-RBD protein during the time-window of 151–232 days was 37.5% and 50.0% among individuals with asymptomatic COVID-19 and 43.9% and 63.6% respectively among mild/moderate patients. However, individuals who had a severe illness had higher levels of IgG NC (60%) and S1-RBD (80%) during the same time-window. Similarly, percentage of NAbs among individuals with severe illness (90.0%) was higher compared to who had a mild/moderate (81.3%) or asymptomatic (70.8%). (Supplementary Table-2).

Seropositivity for IgG against NC, S1-RBD and NAbs was not different among males and females during all time-windows (Supplementary table-3). Seropositivity for NAbs was also not different among those with and without comorbidity during all time-windows. Although individuals with comorbidity had higher seropositivity for IgG against NC and S1-RBD during each time-windows, proportion seropositives for these antibodies were not significantly different among those with and without comorbidity (Supplementary table-4).

The decline of anti-NC and anti S1-RBD has an implication on the serosurveys conducted to estimate the proportion of population previously infected with SARS-CoV-2. Most serosurveys use NC or spike assays to estimate seropositivity.[2] Since the pandemic is continuing for more than a year, serosurveys using only one assay would grossly underestimate the seroprevalence. Hence a standard algorithm to use laboratory assays for serosurvey needs to be developed to account for the waning of antibodies.

The IgG anti-NC, anti S1-RBD and neutralizing antibody waned faster among the individuals with no to mild/moderate symptoms than individuals who had severe illness. Antibody response was more pronounced and long-lasting in individual who had severe disease as documented in other studies.[6,10,11] Lower antibody response and relatively faster waning among asymptomatic and individuals with mild/moderate symptoms might be because of strong innate immunity and T cell response in these individuals [2].

Our study has certain limitations. We used cross-sectional design to measure humoral response to SARS-CoV-2 over time whereas cohort design involving longitudinal measurement may be ideal for this purpose. Nevertheless, our cross-sectional estimates provide quick snapshot of durability of immune response among COVID-19 patients. We could not compare antibody response over time by age groups (Supplementary table-5) since majority of our study participants were in working age group than children and older adults. As a secondary objective, we examined host immune response by clinical severity based on self-reported symptoms, this could have led to misclassification specifically between those reporting asymptomatic status versus mild/moderate symptoms. However, we could validate the severity status from hospitalization records for those categorized as having severe illness.

Table 1
Demographic and clinical characteristics of the study participants.

Characteristics	Number of study Participants (% of the total) $N = 755$
Age (in years)	
6 - 18	17 (2.3)
19 - 45	413 (54.7)
46 - 60	293 (38.8)
61 - 82	32 (4.2)
Mean (SD)	41.8 (12.5)
Gender	
Male	440 (58.3)
Female	314 (41.6)
Transgender	1 (0.1)
Severity of illness	
Severe	44 (5.8)
Mild/Moderate	630 (83.4)
Asymptomatic during the entire course of illness	81 (10.7)
Admission status	
Home isolation throughout the entire course of illness	279 (37.0)
Initially was in home isolation, but later hospitalised	14 (1.9)
COVID Care center throughout the entire course of illness	250 (33.1)
Initially was in COVID care center, but later hospitalised	18 (2.4)
Directly admitted to a hospital/medical institution	194 (25.7)
Duration since RT-PCR confirmation	
15–30 days	107 (14.2)
31–60 days	101 (13.4)
61–90 days	115 (15.2)
91–120 days	101 (13.4)
121–150 days	110 (14.6)
151–180 days	105 (13.9)
181–232 days*	116 (15.4)
Symptoms ($n = 674$)	
Fever	504 (74.8)
Muscle aches/Body pain	460 (68.2)
Loss of smell	335 (49.7)
Loss of taste	334 (49.6)
Cough	313 (46.4)
Headache	303 (45.0)
Joint pain	300 (44.5)
Sore throat	272 (40.4)
Fatigue	272 (40.4)
Shortness of Breath	146 (21.7)
Running nose	97 (14.4)
Diarrhea	92 (13.6)
Chills	85 (12.6)
Vomiting	75 (11.1)
Abdominal pain	51 (7.6)
Conjunctivitis	33 (4.9)
Confusion	18 (2.7)
Seizures	1 (0.1)
Presence of Comorbidity ($n = 280$)	
Hypertension	155 (20.5)
Diabetes mellitus	176 (23.3)
Heart diseases	14 (1.9)
Asthma	19 (2.5)
Other diseases (CKD, Liver diseases, malignancies, neurological disorders, rheumatic disorders, etc.)	28 (3.7)

(*includes 16 patients between 210 and 232 days after RT-PCR infection).

In conclusion, findings of our study indicated that IgG antibodies against NC and S1-RBD waned over time but the neutralization function of the antibody remained stable in majority of the COVID-19 infected patients till 7 months of post-infection. These findings suggest a lower possibility of reinfection by the

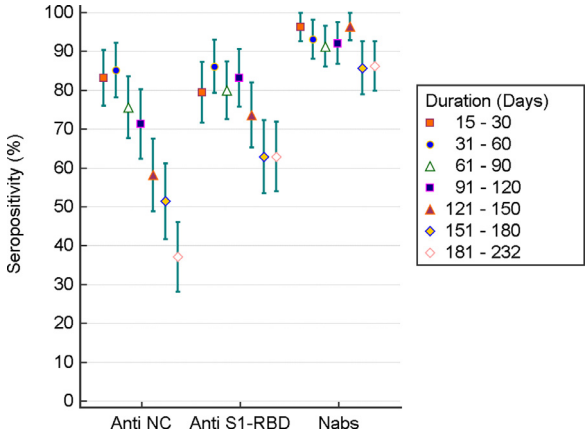

Fig. 1. SARS-CoV-2 specific IgG seropositivity (%) with 95% CIs among recovered COVID-19 patients by duration since infection ($N = 755$).

same viral strain among infected individuals during this time period.

Fig. 1.

Table 1.

Declaration of Competing Interest

NIL

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.jinf.2021.05.026.

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Jeromie Wesley Vivian Thangaraj, Muthusamy Santhosh Kumar, CP Girish Kumar, V Saravana Kumar, Nathella Pavan Kumar, Tarun Bhatnagar, Manickam Ponnaiah, R Sabarinathan, D Sudharani
ICMR National Institute of Epidemiology, Chennai, India

Arul Nancy
ICER-ICMR-NIRT-International Center for Excellence in Research, Chennai, India

M Jagadeesan
Greater Chennai Corporation, Chennai, India

Subash Babu
ICER-ICMR-NIRT-International Center for Excellence in Research, Chennai, India

Manoj Murhekar*,**
ICMR National Institute of Epidemiology, Chennai, India

*Corresponding author. **Chennai COVID immunity study team D. Augustine, Annamma Jose, P.R. Asish, Sauvik Dasgupta, S. Kalaivani, C. Kanagasivam, T. Karunakaran, P. Ashok Kumar, K. Sathish kumar, T. Mahesh, M. Beula margrete, M. Sheeba Mary, C. Prabakaran, Y. Radhakrishnaiah, R. Sivakumar, P. Gnana Soundari, R. Swapna Shinde.
E-mail address: mmurhekar@nieicmr.org.in (M. Murhekar)

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