Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

Low neutralizing antibody titers after asymptomatic or non-severe SARS-CoV-2 infection over a 6-month assessment period

Dear Editor:

In their recent publication, Hanrath and co-workers describe protection against SARS-CoV-2 re-infection conferred by prior SARS-CoV-2 infection, as assessed by PCR over 6 months in health-care workers (HCW) with a prior history of COVID-19.[1]

Neutralizing antibodies against SARS-CoV-2 are a key determinant of immunity following infection [2,3]. Data in convalescent patients who experienced moderate to severe disease suggest that this immune response may last for months, and generally correlates with disease severity [4–6]. However, serological responses in patients with asymptomatic or mild SARS-CoV-2 infections are less well defined [7].

We characterized serological responses to SARS-CoV-2 in asymptomatic or non-critical SARS-CoV-2 infections over a 6-month period. Specifically, we monitored the magnitude and kinetics of serological responses in 335 HCW found to be seropositive for SARS-CoV-2 using a Luminex-based technology targeting the trimeric spike protein [8]. This cohort was composed of 198 (59%) seropositive HCW randomly selected throughout our institution[9] without a prior SARS-CoV-2 polymerase chain reaction positive test (PCR+) and 137 (41%) seropositive HCW recruited on the basis of a prior SARS-CoV-2 PCR+ test. SARS-CoV-2-specific antibody response was measured at baseline (end of May 2020), 3- and 6 months thereafter using the luminex-based quantitative anti-spike trimer protein assay to determine quantitative antibody responses, and a cell-free neutralization assay based on the competitive inhibition of trimeric SARS-CoV-2 spike protein binding to the angiotensin-converting enzyme 2 (ACE2) receptor to determine neutralizing capacity of the antibodies developed [8,10]. Importantly, in this neutralizing activity assay a value of 50 percent is predictive of robust neutralization capacity in cell assays [10]. At each time-point, serological assessments were paired with a survey to assess symptoms, risk of exposures or PCR documentation of repeat SARS-CoV-2 infection. Of note, the 6-month period (June to December 2020) coincided with the tail-end of the first COVID-19 wave in Switzerland (at baseline), a very low SARS-CoV-2 infection period (3-month visit) and the beginning of a second COVID-19 wave (6-month visit).

Median range of anti-spike trimer immunoglobulin G (IgG) titers (expressed as mean fluorescence intensity relative to control) decreased from 35 (baseline) to 20 (3-month) and 22 (6-month) (Linear mixed model for the IgG titers across time: $p < 0.001$). However, median range of neutralizing activity (expressed as inverse of the serum dilution required for 50 percent inhibition) was 40, 44 and 53 at 0-, 3- and 6-months, respectively (Fig. 1a). Compared with baseline, the mean neutralization activity was 1.34 and 1.62 times higher at 3- and 6-months, respectively (Linear mixed model for the neutralizing activity across time: $p < 0.001$).

Anti-spike IgG titers and neutralizing activity were stratified according to the severity of the clinical manifestations using the NIH COVID-19 classification. Among 334 participants with available neutralizing activity at baseline, 105 (31%) had asymptomatic infection, 139 (42%) had mild infection and 90 (27%) had moderate to severe infection that did not require ICU admission. At baseline, median range of neutralizing activity was significantly lower for the 105 asymptomatic vs. 229 symptomatic participants (median 8 vs. 63, respectively; $p < 0.001$ Mann-Whitney-U). Thus, the neutralizing activity of sera from asymptomatic participants was very weak.[10] Specifically, the median neutralizing activity of asymptomatic participants was significantly lower compared to those with mild infection (median 8 vs. 59; $p < 0.001$) and those with moderate-severe infection (median 8 vs. 71; $p < 0.001$). Albeit not statistically significant, the median neutralizing activity was higher in HCW with moderate-severe infection than in those with mild infections, suggesting an association between levels/titers of neutralizing antibodies and COVID-19 clinical severity (median 59 vs. 71; $p = 0.056$), adjusting for multiple testing (Benjamini-Hochberg) (Fig. 1b). Neutralizing activity was sustained over time within each severity category, with the same trend being present at 3- and 6-months.

The survey assessed the rate of re-infection in participants post-baseline. No participant reported a SARS-CoV-2 PCR+ test at 3-months. In contrast 9 (3%) participants reported PCR positivity at 6-months. Out of 9 re-infected participants, the initial SARS-CoV-2 infection was asymptomatic in 5, mild in 3 and moderate/severe in one participants respectively. These participants had a significant rise in neutralizing activity at the last time point (median 5, 13 and 180 at baseline, 3-months and 6-months, respectively; $p < 0.001$), strongly supporting the likelihood of re-infection. Albeit not formally excluded in the absence of a comparison of SARS-CoV-2 sequence data, the possibility of persistent viral shedding is unlikely considering the time lapse and temporal association of symptomatic disease.

While our results support the finding of Hanrath et al. that prior infection with SARS-CoV-2 can protect against re-infection, we observe that re-infection risk varies according to initial infection severity.[1] Consistent with recent data, we find that antibody titers can be influenced by infection severity. [4–6] However, in this cohort of seropositive HCWs, participants with asymptomatic and mild SARS-CoV-2 infections had relatively lower antibody titers and neutralizing activity and they experienced higher rates of re-infection. We acknowledge that the subset of re-infected participants is small (possibly due to the timing of the survey) and results are only hypothesis generating. Nevertheless our findings strengthen the recommendation to boost by vaccination persons

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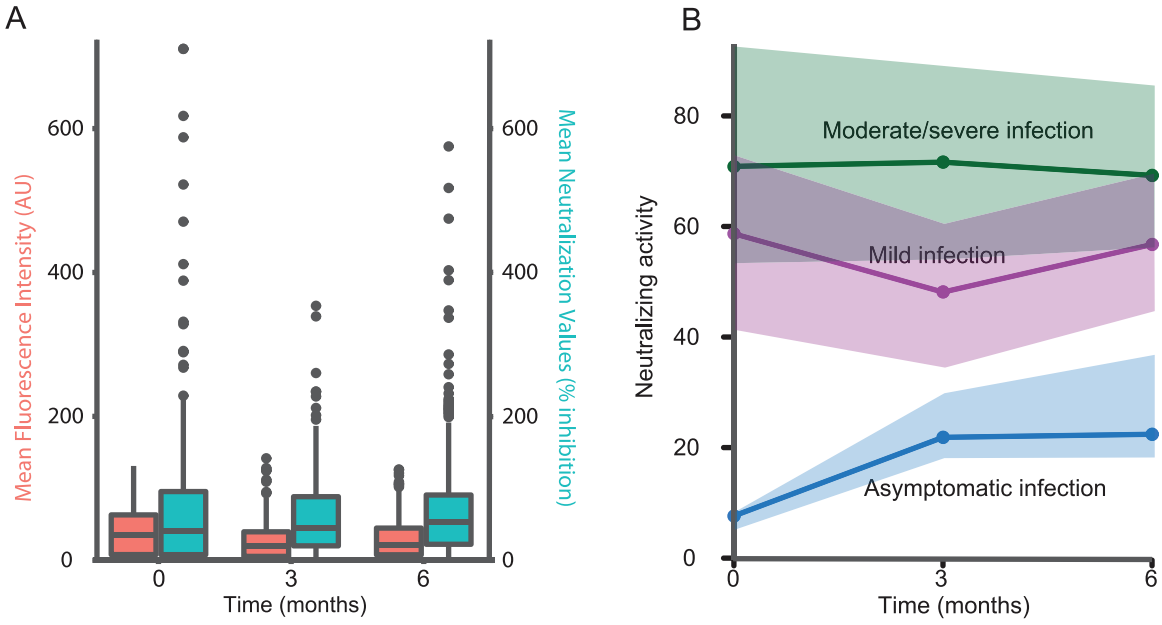

Fig. 1. Evolution of Anti-S IgG titers and neutralization capacity in HCWs across time (A): HCWs assayed for Anti-S protein (red) expressed in mean fluorescence intensity and mean neutralization values (green) expressed in percent of inhibition at 0, 3 and 6 months: 335/334, 302/289 and 294/294 HCWs Evolution of median neutralization activity in HCWs according to disease severity (B). A total of 334 SARS-CoV-2 seropositive HCWs had recorded neutralizing activity at baseline with 289 and 294 presenting for follow-up visits at 3- and 6-months, respectively. Participants were segregated according to disease severity at baseline, as per NIH classification (asymptomatic – blue, mild [myalgia, anosmia, cough, ageusia, fever, sore throat, diarrhea, common cold but without shortness of breath] – purple, moderate to severe [shortness of breath with or without hospitalization] – green). Units of neutralizing activity are expressed as inverse of the serum dilution required for 50 percent inhibition. Distribution according to disease severity at 0/3/6 months: Asymptomatic 105/91/88 HCWs, Mild 139/125/120 HCWs, moderate-severe 90/73/86 HCWs.

who are seropositive following natural infection with SARS-CoV-2, particularly those who experienced asymptomatic or mild infections in light of the low overall neutralizing activity observed over time in this subgroup.

The study was approved by the local institutional review board (Research Ethics Committee of the Canton de Vaud - CER-VD, Switzerland, Req-2020–00990).

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All authors have completed the Unified Competing Interest form (available on request from the corresponding author) and declare: no support from any organization for the submitted work [or describe if any]; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years [or describe if any], no other relationships or activities that could appear to have influenced the submitted work [or describe if any].

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C. Lazor-Blanchet
Occupational health unit, Service of Hospital Preventive Medicine, Lausanne University Hospital, Lausanne, Switzerland

P. Zygoura
Frontier Science Foundation-Hellas, Athens, Greece

U. Dafni
Frontier Science Foundation-Hellas, Athens, Greece
National and Kapodistrian University of Athens, Athens, Greece

F. Lamoth
Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland
Institute of Microbiology, Lausanne University Hospital and University, Lausanne, Switzerland

Z. Tsourti
Frontier Science Foundation-Hellas, Athens, Greece

M.A. Lobritz
Roche Pharma Research and Early Development, Roche Innovation Center Basel, Basel, Switzerland

J. Regina
Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

B. Grandbastien
Service of Hospital Preventive Medicine, Lausanne University Hospital, University of Lausanne, Lausanne, Switzerland

C. Fenwick, G. Pantaleo
Immunology Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

T. Calandra, S. Meylan*
Infectious Diseases Service, Department of Medicine, Lausanne University Hospital and University of Lausanne, Lausanne, Switzerland

*Corresponding author.
E-mail address: Sylvain.meylan@chuv.ch (S. Meylan)

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An off-season outbreak of human metapneumovirus infections after ending of a COVID-19 lockdown

Dear Editor,

We read with great interest the article by Lumley et al.[1] who described changes in pediatric respiratory infections at a British hospital after ending of a national COVID-19 lockdown. They showed off-season increased infection rates with Respiratory Syncytial Virus (RSV), rhinovirus and adenovirus, whereas influenza virus infection rates remained low. Here, we report that in addition, also a change in human metapneumovirus (HMPV) epidemiology has been observed in our hospital. Normally, the peak incidence of HMPV infections is found in late winter and early spring in the northern hemisphere.[2] In our hospital, this pattern has always been very stable with a peak incidence in March. Among 239 patients hospitalized with HMPV, we have previously observed only one infection in June.[3] In contrast, in 2021, we observed an off-season outbreak of HMPV in June and July among both adults and children. The outbreak occurred just after national lockdown measures due to coronavirus disease 2019 (COVID-19) had been lifted. To characterize the outbreak further, clinical characteristics were studied of the patients and phylogenetic analysis of the viral samples was performed in order to investigate the relationship of the viruses.

Between June and July 2021, 28 patients were hospitalized with an HMPV infection in the Zuyderland Medical Center, which is a large teaching hospital situated in Heerlen in the south of The Netherlands. After exclusion of two patients who refused informed consent and three patients who were unable to communicate, 23 patients were analyzed. None of the patients had known relationships with each other. Twelve patients were children ranging from 0 to 4 years and the 11 adult patients were between 20 and 90 years old. None of the patients were immunocompromised. The most prominent comorbidity in adults was chronic obstructive pulmonary disease (COPD) in 5 (46%) of the patients. In general, the clinical disease severity at presentation and the course of disease was not different from that observed in other cohorts.[3] Temperature in children and adults was 38.4 ± 1.1 °C and 37.9 ± 0.8 °C, respectively, heart rate 146 ± 17 and 100 ± 22 min^{-1} and respiratory rate 24 ± 12 and 23 ± 7 min^{-1}. Antibiotics were administered to 3 (25%) children and 9 (82%) adults. No patient was admitted to the intensive care unit. Mean length of hospital stay was 4.0 ± 4.0 days for children and 4.4 ± 2.9 days for adults. One adult patient died after hospital dismissal but within 30 days after admission. This patient had severe comorbidity (end-stage neuroendocrine tumor and ileus).

For the purpose of this study, surplus samples initially tested for routine clinical care were obtained. For sequence analysis 14 samples were selected, in which HMPV could be detected by routine diagnostic qRT-PCR assays at a cycle threshold less than 27. For all samples, full length F sequences, and for 8 of these samples that of the attachment protein (G), were obtained with sanger sequencing as described previously.[4] Phylogenetic analysis of complete F gene nucleotide sequences was performed using the MEGA 10 software with the best fit DNA model determined by the MEGA software with 1000 bootstraps. This phylogenetic analysis demonstrated that all viruses clustered in two smaller clusters within the A2.2.2 lineage (Fig. 1). All eight viruses, for which sequences were obtained for the G gene, had a 111 nt duplication in that gene.

Taken together, this study described an off-season outbreak of infections with HMPV in both adults and children caused by viruses belonging to the HMPV A2.2.2 lineage. Because all 8 viruses for which sequences of the G gene could be obtained showed a 111-nt duplication in the G gene, it may be considered likely that this variant has been responsible for most infections during the outbreak. This variant was first described in 2016 and has gradually become the dominating strain worldwide but is not the only circulating variant.[5] Some authors initially postulated that this strain may be more virulent;[6] however, in time, HMPV incidence has not changed significantly.[3] The cause of the unusual summer outbreak of HMPV infections is likely related to reopening of the society after a severe COVID-19 lockdown, thus enabling the spread of HMPV. During the first COVID-19 wave in 2020, both SARS-CoV-2 and HMPV had circulated independently, which suggested that there was no competition between the viruses at the time.[3] During subsequent lockdowns HMPV incidence was very low, like that of RSV.[7]. Together these data suggest that public health measures were probably more important drivers for the shift in HMPV incidence than viral interference. Whether the currently identified clusters of patients, all hospitalized with the same HMPV genotype, were limited to local transmission only or spread to the region, country or continent remains to be determined. However, because the cluster was further divided into two smaller clusters within the same A2.2.2 lineage and because the patients in our study did not have known relationships with each other, the HMPV outbreak probably did not occur locally only. Of note, whereas the incidence of HMPV in this cohort and the previously reported incidence of RSV in the study by Lumley et al.[1] and others[8,9] both peaked after ending of the COVID-19 lockdown, the incidence of influenza viruses was not affected at that time.[1, 9] Hence, different factors drive seasonal variation among different viruses and this concurrent outbreak of HMPV and RSV suggests that outbreaks of these viruses may be less depending on weather conditions.

Declaration of Competing Interest

None

Ethical statement

The study protocol was approved by the board of the METC-Z (medical-ethics board of Zuyderland Medical Center; approval number: METCZ20190153).

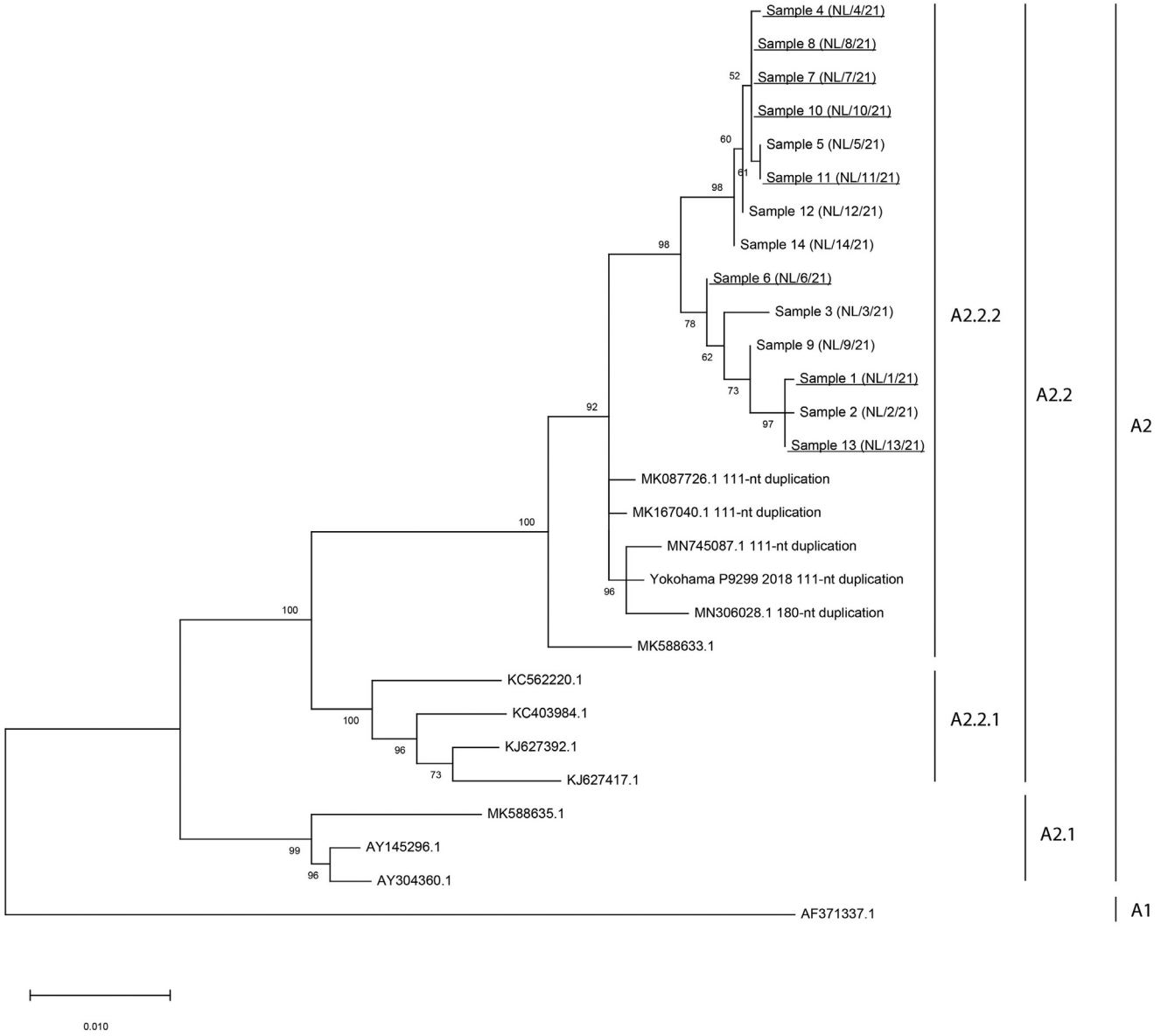

Fig. 1. Maximum likelihood phylogenetic tree reconstructed from HMPV complete F gene sequences. The tree was reconstructed using the GTR $+G + I$ substitution model with 1000 bootstraps. Fourteen complete F gene sequences (thirteen from GenBank, indicated with accession numbers, and one from[10]) were included for the tree topology. Viruses sequenced in this study that contain a 111-nucleotide duplication in the G gene that was confirmed by Sanger sequencing of the G gene are underlined.

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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.2022.01.042.

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CMHJ Kivit
Department of Pulmonology, Zuyderland Hospital, Henri Dunantstraat 5, Heerlen 6419, PC, Netherlands

K Groen
Department of Viroscience, Erasmus MC, University Medical Center Rotterdam the Netherlands, Department of Infectious Diseases, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

M Jongbloed
Department of Pulmonology, Zuyderland Hospital, Henri Dunantstraat 5, Heerlen 6419, PC, Netherlands
Faculty of Health, Medicine and Life Sciences, Maastricht University, Universiteitssingel 60, Maastricht 6229, ER, Netherlands

CFM Linssen
Department of Medical Microbiology, Zuyderland Hospital, Henri Dunantstraat 5, Heerlen 6419, PC, Netherlands

A van Loo
Department of Paediatrics, Zuyderland Hospital, Henri Dunantstraat 5, Heerlen 6419, PC, Netherlands

ECM van Gorp, S van Nieuwkoop, BG van den Hoogen
Department of Viroscience, Erasmus MC, University Medical Center Rotterdam the Netherlands, Department of Infectious Diseases, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands

MD de Kruif*
Department of Pulmonology, Zuyderland Hospital, Henri Dunantstraat 5, Heerlen 6419, PC, Netherlands

*Corresponding author.
E-mail address: m.dekruif@zuyderland.nl (M.d. Kruif)

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COVID-19 vaccines efficacy in preventing or limiting SARS-CoV-2 infections

Dear Editor,

We read with interest the recent article of Hsu and colleagues,[1] who concluded that vaccination against coronavirus disease 2019 (COVID-19) is highly effective to lower the risk of developing severe and/or life-threatening illness, but does not seem very efficient for averting the likelihood of becoming infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and, therefore, for preventing or limiting virus spread.

In order to provide further insights on this pivotal healthcare aspect, we used data from the nationwide COVID-19 vaccination campaign to assess to which extent COVID-19 vaccines may be effective for preventing newly diagnosed SARS-CoV-2 infections in the general Italian population. The information for this analysis was retrieved by accessing the report published on weekly basis by the Italian National Institute of Health (Istituto Superiore di Sanità, ISS; Last available update, January 4, 2022), and which contains official data on COVID-19 vaccinations and newly diagnosed cases of SARS-CoV-2 infection.[2] The odds ratio (OR) with 95% confidence interval (95%CI) of SARS-CoV-2 infection in different cohorts was calculated with MetaXL, software Version 5.3 (EpiGear International Pty Ltd., Sunrise Beach, Australia). The study was carried out in accordance with Helsinki Declaration, under terms of relevant local legislation. The research was based on public ISS data, so that Ethical Committee approval was unnecessary.

The total number of people who completed a primary COVID-19 vaccination cycle within 120 days, by over 120 days, as well as of those who received a recent COVID-19 vaccine booster dose was 26.3, 13.6 and 5.7 million people at the time of this analysis (i.e., January 4, 2022), while 6.9 million people were still unvaccinated. Overall, the rate of SARS-CoV-2 infections per 10,000 was 248.0 (170,551/6,876,688) in unvaccinated people, decreasing to 100.9 (265,724/26,348,254), 73.4 (99,757/13,585,896) and 35.8 (20,375/5,694,939) in those who completed the primary COVID-19 vaccination by over 120 days, within 120 days and in those who received the booster dose, respectively. Compared to unvaccinated people these figures translated into a cumulatively lower risk of SARS-CoV-2 infection of 68% (OR, 0.32; 95%CI, 0.19–0.56) in the entire cohort of COVID-19 vaccine recipients, with such risk reduction being the highest in those who received the vaccine booster dose (OR, 0.14; 95%CI, 0.14–0.14), followed by those who completed the primary vaccination within 120 days (OR, 0.29; 95%CI, 0.29–0.29) and, finally, by those who had completed the primary vaccination cycle by over 120 days (OR, 0.40; 95%CI, 0.40–0.40) (Fig. 1).

The results of our analysis on available data of the ongoing nationwide Italian COVID-19 vaccination campaign suggest that although the viral load may be basically similar between unvaccinated subjects with primary SARS-CoV-2 infection and vaccinated people with breakthrough infections, the overall risk of being infected by SARS-CoV-2 is nearly 70% lower in vaccine recipients, further decreasing to nearly 90% in those who received a vaccine booster dose after completing the primary vaccination cycle. According to our analysis, the risk that any vaccinated individual may become a virus spreader, as emphasized by Hsu et al.,[1] was hence found to be many times lower compared to unvaccinated people. This would lead us to conclude that COVID-19 vaccination should be further encouraged and supported, especially in countries with low vaccination rate, since this seems a quite reasonable and effective strategy for preventing or limiting SARS-CoV-2 circulation, thus ultimately reducing the medical, social and economic burden of SARS-CoV-2, as well as for mitigating the risk that new and highly mutated variants (like Omicron B.1.1.529) will emerge.[3]

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; COVID-19, Coronavirus Disease 2019.

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Declaration of Competing Interest

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

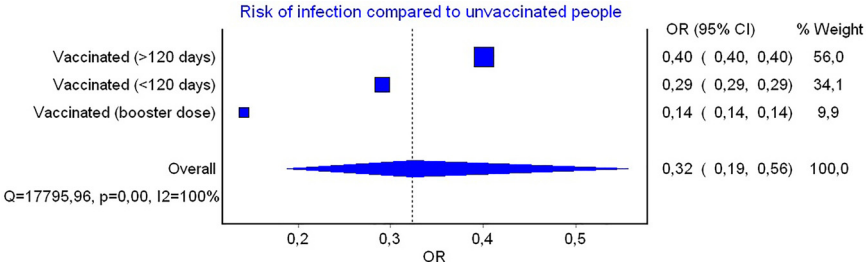

Fig. 1. Impact of coronavirus disease (COVID-19) vaccination in preventing SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infections in the general Italian population. Risk of infection compared to unvaccinated people.
OR, odds ratio; 95%CI, 95% confidence interval.

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Camilla Mattiuzzi
Service of Clinical Governance, Provincial Agency for Social and Sanitary Services, Trento, Italy

Giuseppe Lippi*
Section of Clinical Biochemistry and School of Medicine, Department of Neurosciences, Biomedicine and Movement Sciences, University of Verona, Verona 37134, Italy

*Corresponding author.
E-mail address: giuseppe.lippi@univr.it (G. Lippi)

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Safety and efficacy of the COVID-19 vaccine in children and/or adolescents:A meta-analysis

Dear Editor,

Since 2019, the COVID-19 epidemic has raged worldwide, with children and adolescents accounting for a quarter of the world's population being greatly threatened. The safety and effectiveness of the COVID-19 vaccine for children have been examined. At present, clinical trials of COVID-19 vaccines for children are gradually increasing. The most common ones are attenuated and inactivated vaccines.The efficacy and safety of many randomized clinical trials (RCTs) and observational trials of the COVID-19 vaccine in children have been published through a systematic search of common databases between 2019, and November 8, 2021.

We read with great interest the report in this Journal by Chappell et al. who found that SARS-CoV-2 infections have occurred in immunocompromised children and young people with no increased risk of severe disease.[1] Even without an increased risk of contracting COVID-19 in immunocompromised children and young adults. However, a meta-analysis of the safety and efficacy of COVID-19 vaccines in children and/or adolescents is still warranted. An extensive literature search was performed in PubMed, Web of Science, EMBASE, Elsevier ScienceDirect, and Cochrane Library to find all compliant articles published from January 1, 2020, to November 8, 2021. The following keywords were used on the search strategy: "COVID-19″, "2019-nCoV", "SARS-CoV-2″, "2019 novel coronavirus", "coronavirus disease 2019″, "severe acute respiratory syndrome coronavirus 2″, "children", "child", "adolescent", and "teenager". The reference lists, cited in the included studies and reviews, were eligible as exploratory targets to identify extensive articles. The inclusion criteria included[1]: adult COVID-19 children/adolescents confirmed by reverse transcriptase-polymerase chain reaction (rt-PCR)[2]; peer-reviewed original articles in English[3]; individual study populations being at least fifteen cases[4]; the key available data tabulated data or effect (95% confidence interval (CI)), must be clearly stated. Case reports, repeated articles, review papers, and preprints were eliminated.

Single-group rates and corresponding 95% CIs were used to assess the association between children/adolescents and the COVID-19 vaccine in a whole random-effects meta-analysis model. The model includes effectiveness rates, adverse effects rates, and injection site pain rates in the COVID-19 group. The I^2 statistic was used to quantify the heterogeneity of the effects among the included studies. A sensitivity analysis was perform to determine the robustness of the results. The "META" package of the *R* software (version 4.1.1) was applied. A significant association was not recognized until the two-tailed $P < 0.05$.

A total of 9 articles involving 264,674 patients were identified, including 7 RCTs and 2 observational studies. Table 1 describes the detailed characteristics of the effectiveness and safety studies.[2–10] Seven studies have shown that the overall effectiveness of the COVID-19 vaccine is 96.09% (95% confidence interval [CI]:93.35–98.90, $p < 0.01$) (Fig. 1A), of the attenuated vaccine is 95.05% (95% [CI]:90.21–100.16, $p < 0.01$) (Fig. 1A), and the inactivated vaccine 97.32% (95% [CI];95.17–99.52, $p > 0.01$) (Fig.1A). Safety is important for children. In 5 studies, we found that the adverse reaction was 0.59 (95% [CI]; 0.45–0.73, $p < 0.01$) (Fig.1B). The adverse reaction of the attenuated vaccine was 0.78 (95% [CI]: 0.62–0.94, $p < 0.01$) (Fig.1B), the adverse reaction of the inactivated vaccine was 0.47 (95% [CI]: 0.19–0.75, $p < 0.01$) (Fig.1B) The adverse reactions of inactivated vaccines are significantly less than that of attenuated vaccines. We also counted the pain at the injection site. In 7 studies, the pain at the injection site was 0.58 (95% [CI]: 0.43–0.72, $p < 0.01$) (Fig.1C), the injection pain of the attenuated vaccine was 0.78 (95% [CI]:0.60–0.95, $p < 0.01$) (Fig. 1C), the

Table 1
The basic information of the included literature.

Study	Population	Study type	Country	Intervention	All person	controls	Vaccine efficacy(95%CI)	Injection-site pain	Adverse reactions
Walter et al.[2]	5–11years	Randomized controlled trial	United States	BNT162b2 mRNA	2268	750	90.7% (95% CI, 67.7–98.3)	1093	/
Frenck et al.[3]	12–15years	Randomized controlled trial	United States	BNT162b2 mRNA	2260	1129	100% (95% CI, 78.1 to 100)	939	/
Olson et al.[4]	12–18years	Randomized controlled trial	United States	Pfizer-BioNTech mRNA.2doses	464	285	93% (95% CI = 83–97%)	/	/
Hause et al.[5]	12–17years	observational study	United States	BNT162b2 mRNA	66,550	/	/	41,927	46,585
Freedman et al.[6]	12–15years	observational study	Israel	BNT162b2 mRNA	187,707	/	91.5% (95% CI 88.2–93.9%	/	/
Ali et al.[7]	12–17years	Randomized controlled trial	United States	mRNA-1273	3732	1243	98.8(95%CI= 97.0 to 99.7)	2290	2140
Han et al.[8]	3–17 years	Randomized controlled trial	China	CoronaVac	333	114	96●8% [95%CI= 93●1–98●8]	35	59
Zhu et al.[9]	6–17years	Randomized controlled trial	China	Recombinant Adenovirus Type-5-Vectored Coronavirus	150	50	98.0% (95%CI= 93.0–99.5)	50	82
Xia et al.[10]	3–17years	Randomized controlled trial	China	BBIBP-CorV	810	90	100%	53	229

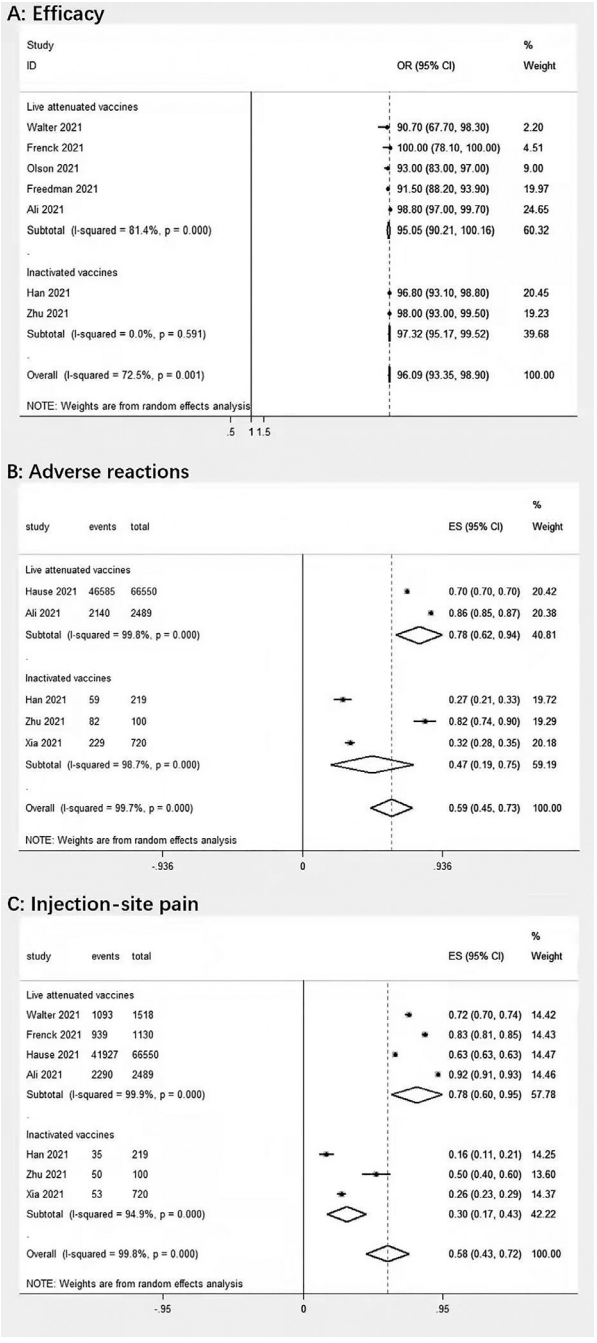

Fig. 1. Forest plot of Vaccine efficacy (95%CI), njection-site pain, and adverse reactions.

injection pain of the inactivated vaccine was 0.30(95% [CI]: 0.17–0.43, $p < 0.01$)(Fig. 1C), the injection of inactivated vaccine is less painful and more friendly to children and adolescents.

In conclusion, our research shows that the current COVID-19 vaccine for children is effective and safe, and is more effective than the adult COVID-19 vaccine. There is a gap between the effectiveness of attenuated and inactivated vaccines, and the effectiveness of inactivated vaccines is the highest. We found that the inactivated vaccine is less painful at the injection site and is more friendly to children and adolescents. More carefully designed further studies based on risk factor adjusted estimates are necessary to confirm our findings.

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Data sharing statement

All the data and materials mentioned in the manuscript are available.

Ethics approval and consent to participate

This study was approved by the ethics committee of Affiliated Hospital of Hangzhou Normal University. This study was carried out according to the Declaration of Helsinki.

Declaration of Competing Interest

The authors declare no competing interests.

Acknowledgment

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Wencheng Xu[1]
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, China

Jiake Tang[1], Chen Chen[1], Chunyi Wang, Wen Wen
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, China
Hangzhou Normal University, Hangzhou 311121, China

Yongran Cheng
School of Public Health, Hangzhou Medical College, Hangzhou 311300, China

Mengyun Zhou
Department of Molecular & Cellular Physiology, Shinshu University School of Medicine, 3900803, Japan

Qi Wu, Xingwei Zhang
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, China

Zhanhui Feng*
Department of Neurology, Affiliated Hospital of Guizhou Medical University, Guiyang, China

Mingwei Wang*
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, China

*Corresponding authors.
E-mail addresses: h9450203@126.com (Z. Feng), wmw990556@163.com (M. Wang)

[1] These authors contributed equally to this work.
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Exploring the recovery curves for long-term post-COVID functional limitations on daily living activities: The LONG-COVID-EXP-CM multicenter study

Dear Editor,

Evidence supports that almost 60% of COVID-19 survivors will experience post-COVID symptoms during the first months after infection.[1] These symptoms lead to a decrease in health-related quality of life and function.[2] One full-text[3] and three letters to the editor[4–6] published in *Journal of Infection* have evaluated the presence of functional limitations as post-COVID sequalae in individuals who had survived to COVID-19. Most of studies investigating post-COVID functional limitations are cross-sectional since they assessed related-disability just at one follow-up period. Understanding the longitudinal evolution of post-COVID functional limitations might have implications for optimizing patient care and public health outcomes. We present here two approaches for potentially analyzing the longitudinal recovery curves of post-COVID functional limitations in a sample of previously hospitalized COVID-19 survivors: (1) mosaic plots of the prevalence of functional limitations during the first year after hospitalization; and, (2) a bar plot of the evolution of functional limitations, fitted with an exponential decay model to help in its longitudinal interpretation.

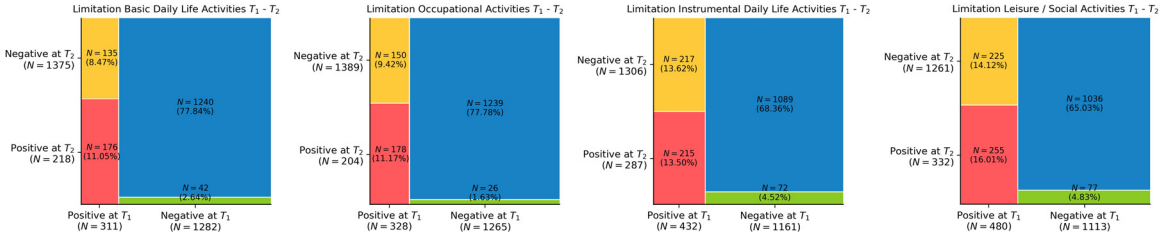

Fig. 1. Mosaic plots of self-reported limitations with daily living activities at T1 (8.4 months after hospital discharge) vs T2 (13.2 months after hospital discharge).

The LONG-COVID-EXP-CM is a multicenter cohort study including individuals with a diagnosis of SARS-CoV-2 (ICD-10 code) by RT-PCR technique and radiological findings hospitalized during the first wave of the pandemic (from March 10 to May 31, 2020) in five urban hospitals of Madrid (Spain). From all patients hospitalized during the first wave, a sample of 400 individuals from each hospital was randomly selected. The Ethics Committees of all hospitals approved the study (HCSC20/495E, HSO25112020, HUFA 20/126, HUIL/092–20, HUF/EC1517). Informed consent was obtained from all participants.

Patients were scheduled for a telephone interview conducted by trained healthcare professionals at two follow-up periods with a 5-month period in between to evaluate the functional status of the patient. Participants were asked for self-perceived limitations in occupational, leisure/social activities, instrumental, and basic daily living activities as we previously described the relevance of specifically asking for different activities.[5] They were asked for determining their functional status at the moment of the interview (post-COVID) in comparison with their previous status before hospitalization. Clinical features (i.e., age, gender, height, weight, medical comorbidities) and hospitalization data (e.g., COVID-19 symptoms at hospital admission, days at the hospital, and intensive care unit admission) were collected from hospital medical records.

Mosaic plots were created with Python's library statsmodels 0.11.1 while Matplotlib 3.3.4 was used for the bar plots. The exponential curves were fitted to the data according to the formula $y = Ke^{ct}$, where y represents the modeled prevalence of the functional limitation (occupational, leisure/social activities, instrumental, and basic) at a time t (in months), and K and c are the parameters of the model.

From 2000 patients randomly selected and invited to participate, a total of 1593 (80.9%) completed both assessments. Patients were assessed at T1 (mean: 8.4, range 6–10) and T2 (mean: 13.2, range 11–15) months after hospital discharge. Between 20 and 30% of participants reported limitations during at least one daily living activity. **Fig. 1** shows mosaic plots of self-perceived limitations in occupational, leisure/social activities, instrumental, and basic daily living activities comparing T1 to T2. Looking at **Fig. 1**, self-perceived limitations in daily living activities decreased during the following year after the infection (occupational activities from 20.9% at T1 to 12.8% at T2; leisure/social activities from 30.1% at T1 to 20.8% at T2; instrumental activities from 27.1% at T1 to 18.1% at T2; and basic activities from 19.9% at T1 to 13.7% at T2). In **Fig. 2**, vertical bars represent the percentage of patients reporting limitations at daily living activities at any time (opacity approximately indicates the sample size at a particular time). The mean values used for the development of the mosaic plots have been marked with asterisks in the graphs. Finally, fitted exponential curves were added to visualize the prevalence trend.

To the best of our knowledge, this is the first analysis showing the recovery curves of post-COVID functional limitations in previously hospitalized COVID-19 survivors. The mosaic plots showed that a large number of patients developed "de novo" functional limitations after the infection. Despite this, more individuals recovered their functional status during daily living activities than those developing functional limitation, explaining the decrease prevalence trend observed. This decrease was, however, not as pronounced as expected suggesting that functional limitations during daily living activities will be long-lasting post-COVID sequelae. Although previous studies have investigated health-related quality of life in COVID-19 survivors, we differentiated the type of daily living activity perceived as limited, a distinction that is not commonly conducted in former post-COVID literature. The exponential recovery curves identified suggest that limitations during basic daily living activities showed the less pronounced decrease tendency and could be present up to five years after infection. Identification of risk factors associated to these functional limitations would help for early identification and monitorization of patients at a high risk of developing functional limitations as post-COVID sequelae. In fact, the number of COVID-19 associated onset symptoms at hospital admission (high symptom load), intensive care unit admission and female sex have been identified as risk factors associated with functional limitations.[5]

Although this is the first-time using mosaic plots and tendency analysis for analysis the recovery curves of post-COVID functional status with a large and multicenter design, potential weaknesses should be also admitted. First, only hospitalized individuals aged 60-years old were included. Second, we collected self-reported functional limitations on daily living activities. The use of validated questionnaires, e.g., EuroQol-5D, assessing health-related quality of life could help to characterizing functional status of COVID-19 survivors. Finally, we did not collect objective data of COVID-19 severity and measures of lung damage, although current literature suggests that these factors are not related to post-COVID sequelae.

In conclusion, our tendency analysis revealed that post-COVID functional limitations during daily living activities tend to slowly recover during the following five years after SARS-CoV-2 infection in previously hospitalized COVID-19 survivors.

Consent to participate

Participants provided informed consent before collecting data.

Consent for publication

No personal info of any patient is provided in the text.

Role of the funding source

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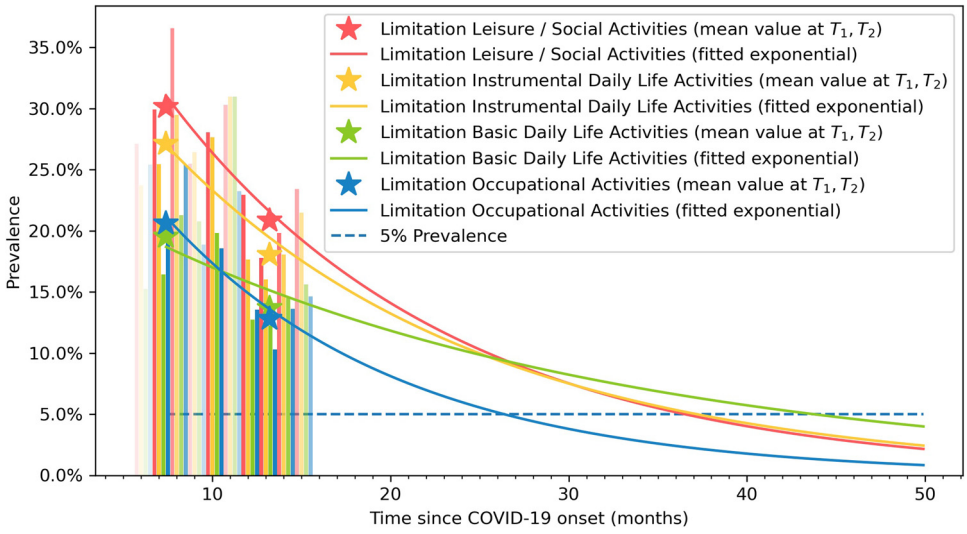

Fig. 2. Recovery curve of self-reported post-COVID limitations with leisure/social (in red), instrumental (in yellow), basic (in green) and occupational (in blue) daily living activities. Opacity indicates the sample size at that follow-up time. Asterisks represent the mean values taken at T1 and T2 follow-up periods.

its content. The authors were responsible for the decision to submit the manuscript for publication, and the sponsor did not participate in this decision.

Author contributions

All authors contributed to the study concept and design. CFdlP, JMG and OPV conducted literature review and did the statistical analysis. All authors recruited participants and collected data. OPV supervised the study. All authors contributed to interpretation of data. All authors contributed to drafting the paper. All authors revised the text for intellectual content and have read and approved the final version of the manuscript.

Declaration of Competing Interest

No conflict of interest is declared by any of the authors

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César Fernández-de-las-Peñas*
Department of Physical Therapy, Occupational Therapy, Physical Medicine and Rehabilitation, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos (URJC), Avenida de Atenas s/n, Alcorcón, Madrid 28922, Spain

José D. Martín-Guerrero
Department of Electronic Engineering, Intelligent Data Analysis Laboratory, ETSE (Engineering School), Universitat de València (UV), Valencia, Spain

Ignacio Cancela-Cilleruelo, Paloma Moro-López-Menchero, Jorge Rodríguez-Jiménez
Department of Physical Therapy, Occupational Therapy, Physical Medicine and Rehabilitation, Facultad de Ciencias de la Salud, Universidad Rey Juan Carlos (URJC), Avenida de Atenas s/n, Alcorcón, Madrid 28922, Spain

Esperanza Navarro-Pardo
Department of Developmental and Educational Psychology, Universitat de València (UV), València, Spain

Oscar J. Pellicer-Valero
Department of Electronic Engineering, Intelligent Data Analysis Laboratory, ETSE (Engineering School), Universitat de València (UV), Valencia, Spain

*Corresponding author.
E-mail address: cesar.fernandez@urjc.es (C. Fernández-de-las-Peñas)

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Post-COVID-19 fatigue among COVID-19 in patients discharged from hospital: A meta-analysis

Dear Editor,

In this Journal, Xiaoyu Fang et al. reported post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19, suggesting that we should pay more attention to patients' symptoms after discharge.[1] We had a valuable op-

Table 1
The basic information of the included literature. Total: number of patient included in the study; Any one of them: Any of fatigue,anxiety,Dyspnea,sleep difficulties,hair loss,smell disorder ,decreased appetite,joint pain etc.; N: no data.

Author	Year	Total	Any one of them(n)	Fatigue (n)	Anxiety (n)	Dyspnea (n)
Xiaoyu Fang[1]	2021	1233	630	400	141	44
Qiutang Xiong[2]	2021	538	267	152	35	140
Chaolin Huang[3]	2020	1655	1265	1038	367	N
Angelo Carfì[5]	2020	143	125	76	N	62
Eve Garrigues[6]	2020	120	N	66	N	50
Marwa Tolba[7]	2020	287	256	209	109	81

portunity to carefully read this interesting manuscript and additional published studies.

We found that a number of published studies explored the symptoms of patients with COVID-19 after discharge. Xiong et al. found that most discharged patients had symptoms of fatigue.[2] Chaolin Huang reported 6-month consequences of COVID-19 in patients discharged from the hospital; 76% of patients (1265 of 1655) reported at least one symptom at follow-up.[3]

It was found that physical, cognitive, and psychological impairments persisted for multiple years in many cases.[4] As COVID-19 research progresses, it has become increasingly apparent that a high proportion of patients experience persistent symptoms, such as fatigue. A unified taxonomy for fatigue in neurological disorders to define fatigue objectively, which, in our opinion, can be used as a template for post-COVID-19 fatigue. We define post-COVID-19 fatigue as the decrease in physical and/or mental performance that results from the changes in central, psychological, and/or peripheral factors due to COVID-19, such as fatigue, anxiety, dyspnea, sleep difficulties, hair loss, smell disorder, decreased appetite, joint pain, and so forth.[4]

PubMed, Web of Science, Embase, and Cochrane Library databases were extensively searched for all compliant studies published from January 1, 2020, to December 25, 2021. The search strategy used the following keywords: “COVID-19,” “2019-nCoV,” “SARS-CoV-2,” “2019 novel coronavirus,” “coronavirus disease 2019,” “severe acute respiratory syndrome coronavirus 2,” “Post-COVID-19,” “Fatigue,” and “persistent symptoms.” The reference lists of included studies and relevant reviews were searched for additional studies. The inclusion criteria were as follows: (1) adult patients with COVID-19 confirmed by reverse transcriptase–polymerase chain reaction; (2) peer-reviewed original studies in English; (3) individual study populations with at least 15 cases; and (4) key available data of the included studies, four-table data, or effect [95% confidence interval (CI)] clearly stated. Case reports, repeated articles, review papers, and preprints were eliminated. After searching PubMed and other websites, six eligible studies encompassing 3976 patients with COVID-19 were included in our meta-analysis. Six studies reported persistent symptoms of patients with COVID-19 discharged from the hospital. The general information of included studies is summarized in Table 1.[1,2,3,5,6,7] We focused on several of the most common symptoms after discharge, such as fatigue, anxiety, and dyspnea.

The results of six studies listed in Fig. 1 showed fatigue in 51% of patients (95% CI, 0.35–0.66; $P < 0.01$). The anxiety rate in four other was 19% (95% CI, 0.10–0.28; $P < 0.01$), and the dyspnea rate in five studies was 28% (95% CI, 0.12–0.45) during the follow-up ($P < 0.01$). It indicated that, out of every 100 patients, 51 experienced fatigue for any reason after discharge, 19 felt anxiety after discharge, and 28 had difficulty breathing after discharge. This suggested that these symptoms might indeed be the sequelae of recovery for COVID-19 survivors.

The reasons for fatigue may be as follows. The central factors contributing to COVID-19 fatigue include neurotransmitter levels,

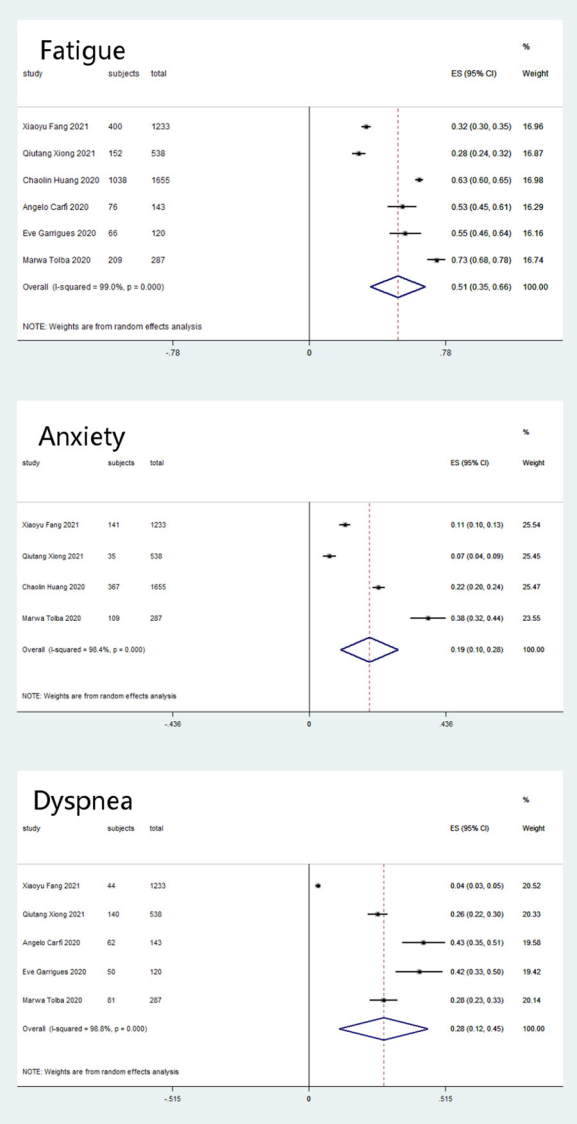

Fig. 1. Forest plot of fatigue,anxiety and dyspnea rates of among COVID-19 in patients discharged from hospital. ES: fatigue,anxiety and dyspnea rates.

inflammation, and so forth. Some negative psychological factors included stress, anxiety, depression, and anger. When these are taken together, it is presumed that they may be a significant contributor to fatigue. Also, some studies suggest that COVID-19 may directly impact skeletal muscle, hence contributing to fatigue.[3]

We found that most patients after discharge from the hospital had at least one of the aforementioned related symptoms. After acute infection, COVID-19 survivors mainly suffered from fatigue or muscle weakness, and anxiety or dyspnea. Similar to Rudroff and

colleagues[4], we should regard fatigue as a decline in physical or mental performance rather than just focusing on one of them. Further, the increase in age is related to the increase in the severity of the disease course, and the severity of the disease is related to patients' symptoms after discharge.[8] New variants of coronavirus may have a different profile of fatigue, which warrants further investigation.[9] Fatigue, along with other symptoms, may also affect the ability to function and work. Our aim is to advise medical staff to better understand the long-term prognosis of patients with COVID-19 after discharge so as to prevent various complications, including physical and mental fatigue. Many comorbidities such as cardiovascular disease, hypertension, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, and cancer, present with fatigue as a common symptom, The health status of these patients with underlying diseases after discharge from the hospital deserves our attention because it is difficult for us to identify the causes of fatigue. Therefore, after the acute infection is relieved, we should pay attention to fatigue among patients after discharge from the hospital.

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Declaration of Competing Interest

The authors declare no conflict of interest

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Guohua Ji, MS[1], Chen Chen, MB[1]
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou Normal University, Hangzhou, 310015, China

Mengyun Zhou, MD[1]
Department of Molecular & Cellular Physiology, Shinshu University School of Medicine,3900803, Japan

Wen Wen, MB, Chunyi Wang, MB, Jiake Tang, MB
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou Normal University, Hangzhou, 310015, China

Yongran Cheng, MS
School of Public Health, Hangzhou Medical College, Hangzhou, 311300, China

Qi Wu, MS, Xingwei Zhang, MD, PhD, Mingwei Wang, MD, PhD*
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou Normal University, Hangzhou, 310015, China

Zhanhui Feng, MD, PhD*
Department of Neurology, Affiliated Hospital of Guizhou Medical University, Guiyang, China

*Corresponding authors at: Hangzhou Normal University, Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, China.
E-mail address: wmw990556@163.com (M. Wang)

[1] Guohua Ji, Chen Chen and Mengyun Zhou contributed equally to this work.

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Impact of previous SARS-CoV-2 infection on the rate of mortality in dengue. A preliminary report from Pakistan

Dear Editor,

An interesting article recently published in this journal offers an insightful overview of the COVID-19 and alarming dengue outbreak in Pakistan [1]. To this aim, an elegant parallel is also made with dengue mortality in patients previously exposed to SARS-CoV-2 infection.

The outbreak of SARS-CoV-2 spread rapidly across the globe resulting in unprecedented public health problems worldwide. As of 23 November 2021, over 258 million cases and over 5 million deaths have been reported around the world. Similarly, in Pakistan, the toll of confirmed COVID-19 cases reached 12,82,510 including 28,668 deaths since 26 February 2020 [2].

On the other hand, the incidence of dengue has grown dramatically around the world in recent decades. It is estimated that 390 million dengue infections and 40,000 related deaths occurred

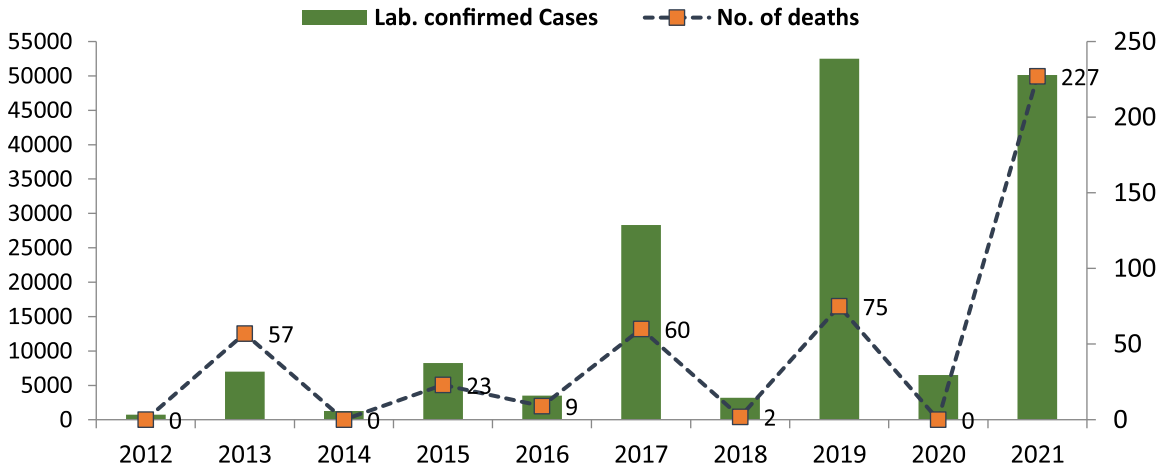

Fig. 1. Dengue reported case counts and deaths from 2012 to 2021.

per year with 70% of the world's dengue burden being in Asia. Out of 390 million dengue cases, only 24% manifest clinical symptoms, and the other 76% remain asymptomatic. The total number of dengue cases reported to WHO increased over 10 fold from 0.5 million cases in 2000 to over 5.2 million in 2019 [3]. In 2021, 1 316 518 dengue cases have been reported, the majority of the cases are from Brazil, India, Vietnam, Peru Philippines, Pakistan, Bangladesh, Nepal and Colombia [4].

Unfortunately, dengue is endemic in Pakistan since 1994, when the first laboratory-confirmed dengue outbreak occurred in Karachi city of Sindh province. From 1994 to 2009 localized dengue outbreaks with 8549 cases including 215 deaths were reported in Karachi, Pakistan. During the last decade 2010–2020, yearly outbreak of dengue has more frequent and expanded to every corner of the country with 144,855 cases including 625 deaths. In Pakistan the largest ever dengue outbreak was reported in 2019, infecting over 52,485 individuals with 75 related deaths [5].

During the current year, sporadic dengue cases were detected since February 2021. However, this dengue outbreak gets intensified during the post moon soon season from September to November 2021. As of 23 November 2021, a total of 2,36,773 suspected and 50,120 confirmed dengue cases including 227 deaths have been reported in Pakistan. 50% (25,000/50,120) of the total cases were reported from Punjab province. Province-wide distribution of dengue cases showed that over 85% of total cases reported by each province are from the highly populous cities such as Lahore, Karachi, Peshawar, Quetta, Islamabad, and Muzaffarabad, which are considered as the hotspot for dengue outbreaks for the last many years. It is speculated that the actual number of dengue cases and deaths might be high than reported as most dengue cases remain asymptomatic and become a source of infection for the community. According to the current data, the rate of mortality due to dengue is very high as compared to previous dengue outbreaks. It is already reported that the dengue infection provides the protective immunity against the same serotype and increases the severity of the infection if infected by the other serotype due to the antibodies dependent enhancement (ADE). We closely observed and reviewed the data and recent lab results of dengue virus serotypes circulating in Pakistan. Previous and current serotyping data showed that the dengue virus serotype-2 (DENV-2) is circulating as a dominant strain in Pakistan since 2017 [5,6,7]. During the current dengue outbreak over 500 samples covering almost all regions of the country were screened for the identification of dengue serotypes at the department of Virology, National Institute of Health Islamabad and DENV-2 was detected from all positive samples (NIH unpublished data 2021). In the presence of a single serotype from the last five years, the unprecedented rise (33%, 75/227) in dengue-related deaths in 2021 as compared to 2019 showed that the previous COVID-19 infection increases the risk of severe dengue and dengue-related deaths due to the ADE, Fig. 1. We thoroughly reviewed the hospital medical records of deceased dengue patients and dengue NS1 positive lab reports were available for all 201 patients. However, 71% (162/227) of patients who died due to dengue had a history of pre-exposure to SARS-CoV-2 infection. COVID-19 PCR and IgG positive reports were found in the hospital records for 59 patients; however, only PCR positive reports were available for 103 patients. We are unable to trace the COVID-19 PCR or serology results for the other 65 patients who died due to dengue virus infection. Out of total deceased dengue patients, 63% (143/227) were male and the other 37% (84/227) were female patients. Most deceased dengue patients belonged to the 35–75 year of age group. The cross-reactivity of COVID-19 and dengue antibodies has already been reported [8]. In our previous study, we have already reported the co-infection of dengue and COVID-19 with unfavorable outcomes. A recently published study in the journal of clinical infectious diseases also reported that the previous dengue infection increases the risk of severe COVID-19 due to the cross-reactivity of non-neutralizing antibodies [9]. If the previous infection of dengue increases the risk of severe COVID-19 and the previous exposure to covid-19 ultimately increases the risk of severe dengue as indicated by the results of the present study. Both COVID-19 and dengue are significant public health problems, especially in dengue-endemic countries. The present study explored the preliminary observational evidence that the previous COVID-19 infection might increase the risk of mortality due to dengue virus infection. The Bidirectional impact of ADE in COVID-19 versus dengue is a growing concern. This phenomenon is enormously important not only for the understanding of viral pathogenesis but also for developing antiviral strategies such as vaccines for COVID-19 and dengue. Rigorous research work on deep cellular and immunological aspects from dengue-endemic countries is needed to further elucidate the effect of antibodies cross-reactivity in patients having pre or post exposure to COVID-19 and dengue

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Muhammad Suleman Rana*
Muhammad Usman
Muhammad Masroor Alam
Aamer Ikram
Muhammad Salman
Massab Umair
National Institute of Health, Islamabad, Pakistan
*Corresponding author.
E-mail address: ranavirologist@gmail.com (M.S. Rana)

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Persistence of Long-COVID symptoms in a heterogenous prospective cohort

Dear Editor,

We read with interest the article by Fang and colleagues,[1] showing the results of a multicenter prospective cohort study related to COVID-19 sequelae one year after acute illness, as well as potential risk factors.

We present our data collected from May 11, 2020 to September 24, 2021 at the "Luigi Sacco" University Hospital, Milan, where the ARCOVID (Ambulatorio Rivalutazione COVID) outpatients' clinic began to follow "long haulers", periodically controlling the persistence of physical and psychological symptoms, in order to assess their duration and the predictive factors associated with their resolution. Patients aged >18 years with confirmed COVID-19 (by PCR/antibody detection) were either referred by the physicians who had taken care of them in the acute phase or came voluntarily. After signing written informed consent they were enrolled in the AntiCROWN longitudinal study of anti-S1/S2 IgG response and clinical follow-up, approved by the "Comitato Etico Interaziendale Area 1", n. 2020/ST/158. Throughout the first visit patients received a standardized clinical examination, serological sample to detect anti-S1/S2 IgG levels, 6-minute-walk test in case of dyspnea, and, if necessary, blood work and then sometimes were referred to other specialists. Moreover, they were asked about ongoing symptoms. The follow-up continued using questionnaires sent to each patient every 3 months.

Descriptive statistics included absolute frequencies and percentages for categorical variables and medians with interquartile range [IQR] for continuous variables. Patients were grouped according to the WHO classification of severity of SARS-CoV-2 infection.[2]

Demographic and clinical characteristics of subjects in the four groups were compared using the χ^2 test or Fisher's exact test where appropriate for categorical variables and the nonparametric Kruskal-Wallis test for continuous variables. With regard to the outcome of symptom resolution in the acute phase of infection, a survival analysis was conducted using Kaplan-Meier curves and univariable and multivariable Cox regression models to identify factors associated with symptom disappearance. R software v.3.6.2 was used for statistical analysis. A p-value <0.05 was considered statistically significant.

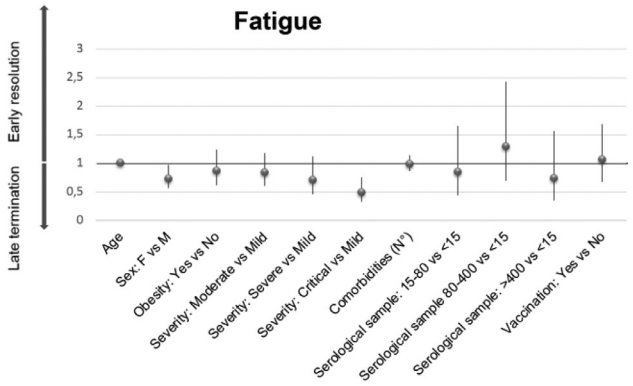
We enrolled 1168 patients, 49,7% females and 50,3% males, median age 57 years: 41% were mild, 26% moderate, 11% severe and 22% critical based on WHO COVID-19 disease severity classification. Of our patients 58,3% had been discharged from an hospital after acute COVID-19. Overweight patients were 59%, the median BMI being 25.47. Regarding comorbidities 37.8% were cardiovascular disease, 26,1% metabolic disorders, 11.4% pulmonary disease, 10,2% diabetes, 6,2% immunological diseases, 4,3% renal diseases, 3,7% autoimmune diseases, 2,7% cancer, 1,7% liver disease. Table 1 shows the distribution of the main Long-COVID symptoms among the different WHO severity groups.

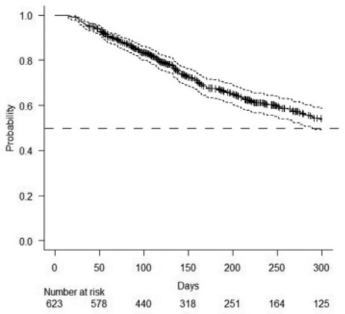
Fig. 1 shows the decay curves and estimated median time to resolution for the main symptoms with the multivariate analysis of predictive factors of persistence. Ageusia and anosmia displayed similar curves, steeper in the first 100 days, with a flattening of the slope thereafter and a median time to resolution of 155 and 200 days, respectively, 46% and 48% of still reporting persistent symptoms at 300 days. Baseline antibody production was associated with protracted ageusia.

Palpitations are disproportionate accelerations of the heartbeat during exercise or at rest that resolve slowly. Their estimated median time to resolution (95% CI) was 425 days (282–489). The estimated median time to resolution of anxiety and panic attacks was 391 days (313-NA). The median time to resolution of headache was estimated to be 379 days (264–505). For amnesia and insomnia the slow decay rate currently makes it impossible to foresee a median time of resolution. After one year less than 20% of our patients have resolved memory problems, and only about 15% have resolved insomnia. After median 35 days beyond the onset of COVID-19 phase of the disease 17.3% of the population reported telogen effluvium, the only truly post-COVID symptom, which had a median time to resolution of 299 days.

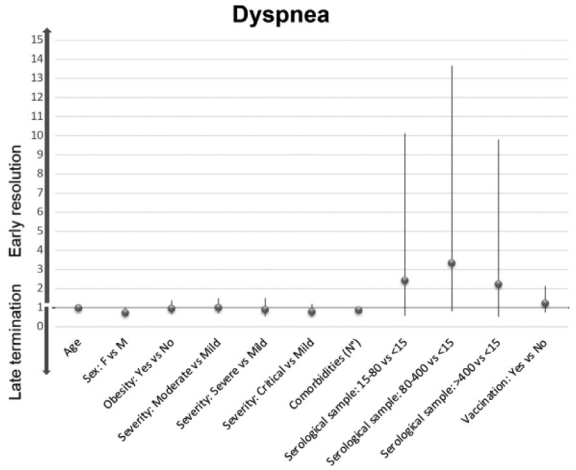
Researchers have initially approached Long-COVID by phone calls performed 60 days after discharge, which revealed the persistence of at least one symptom in 66–100% of subjects, according to disease severity,[3,4] with implications of job loss and mental health impact,[5] A review of such short-term evaluations lead to a comprehensive description of the frequency of each Long-COVID symptom.[6] Subsequently, clinical cohorts provided medical visits, physical examination and questionnaire-based follow-up. Huang et al., on a cohort of 1655 patients discharged from hospital reported the persistence at six months of fatigue in 63%, sleep difficulties in 26% and anxiety/depression in 23%. The severity of sequelae correlated to the severity of the acute phase.[7] Liu et al. described in

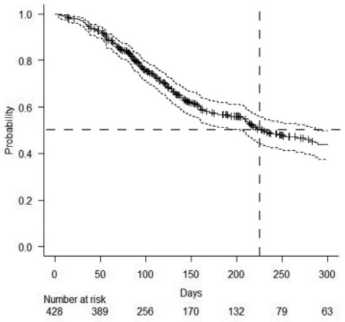
Abbreviations: COVID, COronaVIrus Disease; SARS-CoV-2, Severe Adult Respiratory Syndrome - CoronaVirus 2.



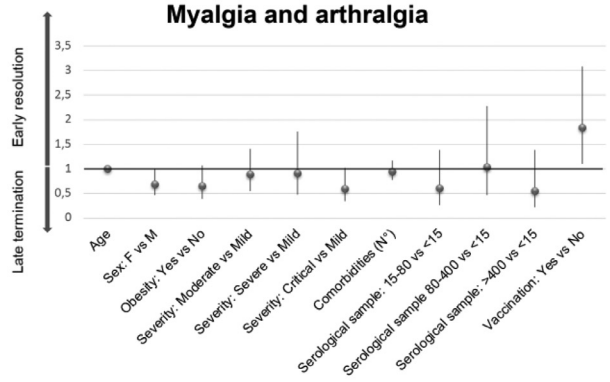


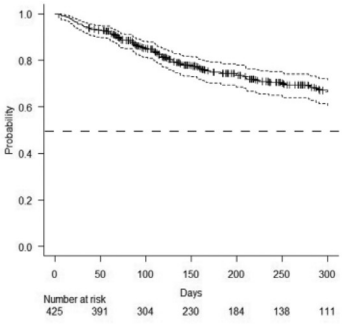
Median time to resolution (95% CI): 383 days (289-512)
Percentage of patients with symptoms after 300 days: 54%



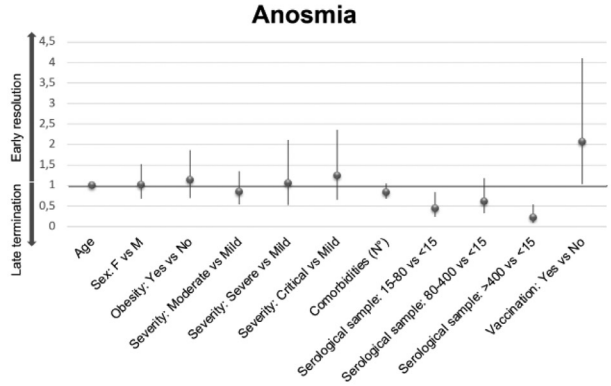


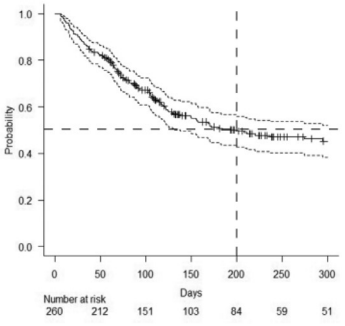
Median time to resolution (95% CI): 229 days (203-289)
Percentage of patients with symptoms after 300 days: 43%





Median time to resolution (95% CI): 512 days (402-NA)
Percentage of patients with symptoms after 300 days: 66%





Median time to resolution (95% CI): 200 days (138-319)
Percentage of patients with symptoms after 300 days: 48%

Fig. 1. Decay curves and median decay rate of the main Long-COVID symptoms and multivariate analysis of associated risk factors.
Serological sample: anti-spike IgG (AU/mL).

Table 1
Long-COVID symptoms' prevalence according to the WHO severity score.

Symptoms, n (%)	WHO COVID-19 disease severity score					*p*-value
	Overall	Mild	Moderate	Severe	Critical	
Dyspnea,	428 (36.6)	118 (24.9)	132 (43.7)	57 (43.5)	121 (46.4)	**<0.001**
Telogen effluvium	117 (10.0)	50 (10.5)	34 (11.3)	14 (10.7)	19 (7.3)	0,407
Fatigue	624 (53.4)	224 (47.3)	181 (59.9)	79 (60.3)	140 (53.6)	**0,002**
Myalgia and arthralgia	426 (36.5)	161 (34.0)	112 (37.1)	50 (38.2)	103 (39.5)	0,474
Palpitations	147 (12.6)	67 (14.1)	46 (15.2)	15 (11.5)	19 (7.3)	**0,02**
Anosmia	261 (22.3)	141 (29.7)	63 (20.9)	24 (18.3)	33 (12.6)	**<0.001**
Ageusia	246 (21.1)	130 (27.4)	55 (18.2)	25 (19.1)	36 (13.8)	**<0.001**
Amnesia	168 (14.4)	62 (13.1)	54 (17.9)	14 (10.7)	38 (14.6)	0,164
Headache	113 (9.7)	57 (12.0)	31 (10.3)	7 (5.3)	18 (6.9)	**0,041**
Anxiety and panic attack	121 (10.4)	52 (11.0)	36 (11.9)	12 (9.2)	21 (8.0)	0,442
Insomnia	123 (10.5)	46 (9.7)	34 (11.3)	10 (7.6)	33 (12.6)	0,405

594 patients discharged from Tongji Hospital, Wuhan, the persistence of at least one symptom in 28.4% at 12 months. Obstructive, restrictive, and mixed pulmonary function impairment persisted in 1.9%, 4.7%, 0.2% of the patients. Electrocardiogram abnormalities, including arrhythmia, ST-T change and conduction block remained in 242 patients (49.8%).[8] Our study' peculiarity is that the population is wider, including all grades of the WHO severity scale, and the observation period is longer than 12 months. Biases are the fact that symptoms were reported as present/absent, without severity scales. Specialists have gradually gathered around the project and we hope that more insight can be given in the future. Clinical visits detected sometimes a fluctuating course of the symptoms, leading to depression. The cause of fatigue has not been clarified yet, but it has been compared to post-infectious fatigue. A discrepancy between dyspnea, leading to a significant reduction in exercise endurance, and persistent lung damage is not uncommon. Anosmia and ageusia are so peculiar that pathophysiology is still unclear. Symptoms often evolve into altered smell and taste, which worsens the patients' quality of life. The mechanism underlying the onset of COVID-related amnesia is still debated as the virus has shown some neurotropic and vasculotropic affinity, as well as the ability to stimulate the production of neurotoxic cytokines.[9] What happens after COVID-19 disease cannot be summarily referred to a simple convalescence period in which symptoms gradually but linearly decrease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2022.01.024.

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Chiara Mariani, Fabio Borgonovo, Amedeo F Capetti*
1st Division of Infectious Diseases, 'Luigi Sacco' University Hospital, Milan, Italy

Letizia Oreni
Clinic of Infectious Diseases, DIBIC University of Milan, Italy

Maria Vittoria Cossu, Martina Pellicciotta, Luciana Armiento, Simona Bocchio, Gianfranco Dedivitiis
1st Division of Infectious Diseases, 'Luigi Sacco' University Hospital, Milan, Italy

Angelica Lupo, Massimo Galli
Clinic of Infectious Diseases, DIBIC University of Milan, Italy

Giuliano Rizzardini
1st Division of Infectious Diseases, 'Luigi Sacco' University Hospital, Milan, Italy
School of Medicine, University of the Witwatersrand, Johannesburg, South Africa

*Corresponding author.
E-mail address: amedeo.capetti@unimi.it (A.F. Capetti)

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Host-cell recognition through Cs-GRP78 is enhanced in the new Omicron variant of SARS-CoV-2, *in silico* structural point of view

Dear Editor,

New SARS-CoV-2 variants started to evolve and spread worldwide in late 2020 and until today. The new variants bear many modifications (insertion, deletion, and mutations) in the spike protein. Some lie in the receptor-binding domain (RBD), which mediates viral entry to the host cell utilizing different host-cell agents. The new variant Omicron (B.1.1.529) shed scientific concern as its spike bear many mutations (A67V, Δ69–70, T95I, G142D/Δ143–145, Δ211/L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F) that alter vaccination strategy to reduce the infection spread. Angiotensin-converting enzyme 2 (ACE2) was reported as the principal entry agent for SARS-CoV-2, but not the only gate. Different host-cell receptors are defined to mediate SARS-CoV-2 recognition and entry (1). In this Journal, we previously published a predicted SARS-CoV-2 spike-host cell surface receptor, glucose-regulated protein 78 (GRP78), binding site (2), which has been confirmed experimentally by Carlos et al. lately (3). The receptor-binding domain region of the spike protein can be recognized by GRP78 substrate-binding domain β (SBDβ), where the C480-C488 was the predicted binding motif of the spike. Mutation at D484 (to Q, K, D, G, V, and A) was detected in delta and Omicron variants, among other mutations which impacted the binding of Cs-GRP78 to the viral spike protein as reported by our group previously (Beta and Gamma variants) (4).

In this report, we simulate the cell-surface GRP78 (Cs-GRP78) recognition to the spike of Omicron variant of SARS-CoV-2 after performing 100 ns molecular dynamics simulation (MDS) to the wildtype (WT) & Omicron spikes and the GRP78 structure (PDB ID: 5E84, Chain A). PyMOL v2.2.2 was utilized to perform the mutations in the RBD of the spike before the minimization and MDS performed by Nanoscale molecular dynamics software (NAMD) 2.13 software using CHARMM 36 force field and TIP3P water model (5, 6). The MDS calculations and analysis were performed on the King Abdullah University of Science and Technology (KAUST) supercomputing facility, SHAHEEN (project no. k1482), and a local workstation. The input files for MDS were generated using the CHARMM-GUI webserver (7). Temperature, pressure, and salt concentration were set to be 310 K, 1 atm, and 0.154 M NaCl as the physiological conditions. Before the simulation, the system was minimized for 20,000 steps in a constant number of atoms, constant volume, constant temperature (NVT) ensemble using a conjugate gradient algorithm. The system was then equilibrated in an NPT ensemble for one nanosecond period before the 100 ns production run.

Additionally, the binding of GRP78 to the spikes was predicted using HADDOCK 2.4 webserver (8). We docked GRP78 with both WT and mutant SARS-CoV-2 spike RBD. GRP78 and SARS-CoV-2 spike RBD's active sites were selected to be T428, V429, V432, T434, F451, S452, V457 & I489, and C480-C488, respectively. Other options of HADDOCK were kept as default during the docking. The carbohydrate moieties (NAG) attached to the proteins were held in the structure during the simulations. After docking, the best-scored complexes were used to predict the binding energies using PRODIGY of the WeNMR portals (9).

RBD dynamics of the WT spike versus the Omicron variant

Fig. 1 shows the MDS analysis of the WT spike (blue curve) and the Omicron variant (orange curve) spike. The root-mean-square deviation (RMSD) in Å (A), the radius of gyration (RoG) in Å (B), surface accessible surface area (SASA) in Å^2 (C), and the number of H-bonds (D) is plotted against the simulation time in ns. Additionally, the per-residue root-mean-square fluctuations are plotted for the two spike RBDs (Fig. 1E). The two systems are equilibrated at around 3.4 Å (WT) and 2.3 Å (Omicron) during the first 50 ns as reflected from the RMSD curves. Additionally, the two systems are stable during the simulation period as reflected from the RoG, SASA, and the total number of H-bonds values (17.5 Å, 11,200 Å^2, and 225, for the WT RBD and 18 Å, 11,000 Å^2, and 240, for the Omicron RBD, respectively).

On the other hand, the RMSF (see Fig. 1E) of the WT RBD (blue curve) show a significantly elevated level of fluctuations in the region 470–490 of the protein (yellow cartoon) and from which the GRP78 recognition site (C480-C488) in the WT RBD is at least two-fold more flexible than that of the Omicron RBD. This indicates the stabilization of the Omicron variant of the spike RBD at the GRP78 binding site exerted by the RDB mutants.

The binding affinity of GRP78 to WT spike versus the Omicron variant

After MDS, the trajectories are subjected to clustering using TTclust software (10). Two different clusters are found in GRP78 and Omicron spike RBD trajectories, while three are found in the WT spike RBD. A representation member from each cluster is used in the protein-protein docking of HADDOCK. Fig. 2A shows the average HADDOCK score values (columns) and their corresponding binding affinity values (points) calculated using PRODIGY software for the WT RBD-GRP78 (blue) and the Omicron RBD-GRP78 (green) complexes. The average binding affinity of the Omicron RBD to GRP78 is lower (-9.68 ± 0.63 kcal/mol) compared to the binding affinity of the WT RBD to GRP78 (-8.83 ± 0.60 kcal/mol). This reflects the higher probability of the association between the Omicron spike and the host-cell surface receptor GRP78 than the WT spike. A result we reported before in alpha and beta variants of SARS-CoV-2 as well (4).

Table 1 summarizes the established interactions upon docking the two GRP78 representative structures into the three WT RBD conformations and the two Omicron RBD conformations. The average number of the formed interactions is increased in the case of Omicron RBD variants compared to the WT RBDs. On average, eight hydrophobic contacts and 8 H-bonds are formed in the case of Omicron RBD docking against GRP78. Those numbers are 4.67 (hydrophobic contacts) and 9.33 (H-bonds) for the docking of GRP78 against WT RBD variants. The mutant E484A has an impact on the recognition of the C480-C488 region of the spike as it raises the average hydrophobicity index (Kyte & Doolittle) (see Fig. 2B) of the peptide to be closer to the value of the Pep42 cyclic peptide that was confirmed before to bind Cs-GRP78 over cancer cells (11). In addition, the E484A mutant increased the number of hydrophobic contacts formed between the spike and the GRP78 SBD β, as reflected in Table 1 (bold residues).

Conclusively, in this letter, we shed light on the modified affinity of the spike RBD of the new variant Omicron against the host cell-surface GRP78. This recognition could be targeted by peptides, antibodies, or phytochemicals (12).

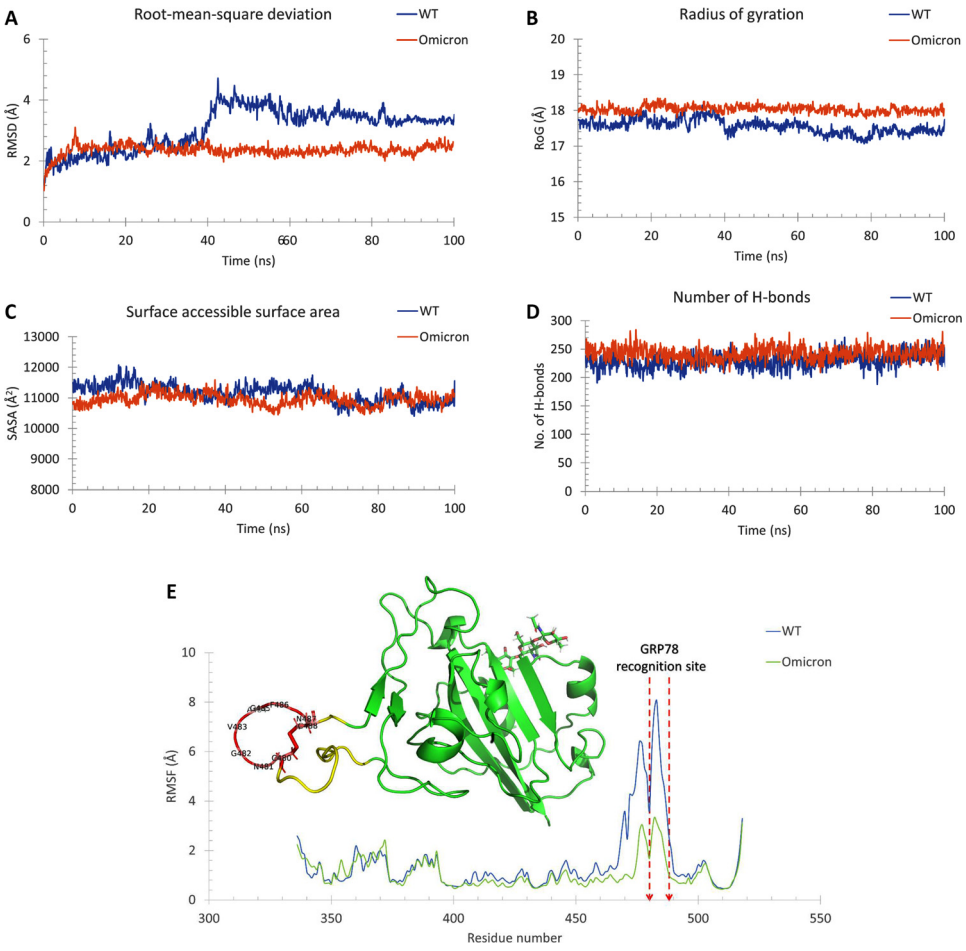

Fig. 1.. The molecular dynamics simulation analysis of the WT RBD spike (blue curves) and the Omicron RBD variant (orange curves). (A) the root-mean-square deviation versus the simulation time. (B) the radius of gyration versus the simulation time. (C) the surface accessible surface area versus the simulation time. (D) the total number of H-bonds versus the simulation time. (E) the per-residue root-mean-square fluctuations among Omicron RBD representative structure taken at 43.1 ns. GRP78 binding site of the spike is labeled and depicted in the red cartoon, while the region of high deviation from the WT is shown in the yellow cartoon.

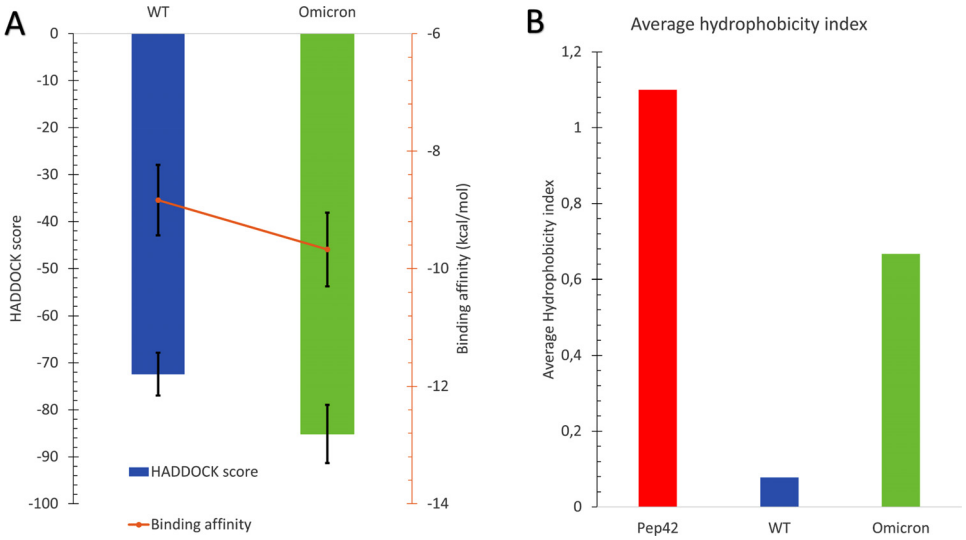

Fig. 2.. The average binding affinity (in kcal/mol) calculated by PRODIGY (line) and the average HADDOCK scores (columns) for the WT RBD-GRP78 complexes (blue) and the Omicron RBD-GRP78 complexes (green) calculated from the representative cluster members of each protein after the MDS trajectory analysis.

Table 1.
The interactions established upon docking the GRP78 into WT RBD and Omicron RBD of SARS-CoV-2 spike. Red residues are the amino acids involved in salt bridge formation, while blue residues form π-cation interactions upon docking.

CLUSTER NUMBER		NUMBER OF HYDROGEN BONDS	AMINO ACIDS IN GRP78	AMINO ACIDS IN RBD	NUMBER OF HYDROPHOBIC INTERACTIONS	AMINO ACIDS IN GRP78	AMINO ACIDS IN RBD
WT RBD	C1_grp1	11 1	V245(2), D348, S349, D350(2), **D350,** K435, N440, Q449(2), and S452	T345, R346(4), K444, N450, N481, V483(2) R509, and **R509**	2	E347 and F451	K444 and F486
	C1_grp2	11	E427, T428, V429, S452(2), G454, P487, R488(2), and G489(2)	P479, C480, N481, G485, N487, C488, Q493(4), and S494	5	F451, V453, V457(2), and P485	Y449, P479, and F486(3)
	C2_grp1	8	T428, G430, S452(2), T456(2), T458, and R488	Q474, N481(2), **E484(2),** G485, N487, and Y505	5	I426, V429, F451, I459, and V490	T478, F486(3), and Y489
	C2_grp2	7 1	T428, T434, K435, **K435,** S452(3), and Q492	N481(4), **E484(2), E484,** and G485	6	T428, V429, T434, F451, and V457(2)	N481, **E484(2),** F486(2), and Y489
	C3_grp1	9	V429, T434, K447, S448(2), I450, S452(2), and G454	G446, Y449(2), T478, N481(2), V483, **E484**, and G485	3	I450, V457, and I459	V483 and F486(2)
	C3_grp2	10 1	T428, T434, **K435,** V442, T445(2), K446, Q449, S452(2), and Q492	Y449, T478, P479, N481, V483, **E484, E484,** G485, Q498(2), and T500	7 1	I426, T434, V442, K447, **K447,** F451, V457, and I459	Y449, **Y449, E484,** F486(4), and T500
Omicron RBD	C1_grp1	10	T428, V429, G430, Q449(2), S452(2), Q492(2), and T514	Y449, N481, G482, V483, Y489(2), F490, and R493(3)	12	I426(2), T434, L436, Q449, I450, F451(2), V457, I459, and V490(2)	**A484(3)**, F486(6), Y489, and F490(2)
	C1_grp2	6 1	E121, **E121,** V429, S452(2), T458, and D525	N487(2), C488, R493, **R498**, T500, and Y501	7	V429(2), V432(2), T434, F451, and V453	I472(2), V483, **A484**, F486(2), and Y489
	C2_grp1	7	V429, Q449, S452(4), and G454	K478, C480, N481(3), V483, and C488	6	I426, V429, T434, V453(2), and V457	K478(2), **A484(2),** F486, and Y489
	C2_grp2	9	E347(2), V429, K435, I450, S452(2), T458, and Q492	K444, V445, E471(4), V483, F490, and S494	7	E347, T428, V432(2), T434, I459, and P467	K444, Y449, V483, and F490(4)

Declaration of Competing Interest

All the authors declare no competing interest in this work.

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Abdo A Elfiky*
Ibrahim M Ibrahim
Biophysics Department, Faculty of Science, Cairo University, Giza, Egypt
*Corresponding author.
E-mail addresses: dr_abdo@cu.edu.eg, abdo@sci.cu.edu.eg (A.A. Elfiky)

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Intra-host SARS-CoV-2 single-nucleotide variants emerged during the early stage of COVID-19 pandemic forecast population fixing mutations

Dear Editor,

We read with great interest the recent study by J Zhu et al [1]. reported that SARS-CoV-2 in saliva from infected patients presented viral load dynamics. With error-prone polymerase and recombination events occurring during the replication, the SARS-CoV-2 has been continuously spreading across populations. The highly diversity of SARS-CoV-2 genome leads to worries of emerging strains with high transmissibility or those escaping from immunity induced by infection or vaccination. Intra-host single-nucleotide variant variants (iSNVs) that appear during the course of infection might provide valuable information on distinguishing mixed founder viruses, their potential of escaping immune response, as well as potential clues for drug designing [2–4]. A sub-lineage of SARS-CoV-2 was first defined by T4959 mutation that appeared as an iSNV in two individuals in Massachusetts [5]. Additionally, studies of intra-host diversity have revealed that the most iSNVs are either lost, or occasionally fixed as population dominant mutations [6]. Collectively, these observations suggest that close monitoring of iSNVs would help predict dominant mutations and gain time in besieging and addressing variants of concern.

Here, taking advantage of iSNVs profiles from specimens collected from January 2020 to March 2020, the early stage of the pandemic, we investigated the use of iSNVs in predicting mutations that can be fixed at the population level. Sixty-three confirmed COVID-19 patients from domestic cases and oversea importation cases admitted to Huashan Hospital or Shanghai Public Health Clinical Center from January to March 2020 were included in this study. Viral RNA was isolated from patients' sputum or nasopharyngeal swabs and amplicon sequencings were performed on Illumina Nova-seq Platform (Illumina, USA). This study was approved by the ethical committee of Huashan Hospital.

Raw sequencing data were filtered and mapped to the reference genome (Accession number: NC_045512.1). Bowtie2 (v 2.3.3.1) was used for mapping reads and candidate SNPs were identified using SAMtools (v 1.9). The number of mapping reads, mapping ratio, sequence coverage, and depth were generated to evaluate the quality of specimens. The iSNVs sites were determined as described earlier [2,3]; briefly, first, criteria of Phred Quality Score (base quality and mapping quality) $\geq$ 20 and $\geq$ 200x depth were met. Second, [1] minor allele frequency $\geq$5%, [2] at least ten reads to support the minor allele, and finally, [3] strand bias of the minor allele and reads with major allele $<$ 10-folds. Additionally, iSNVs positioned in the 20 bp upstream and downstream of the primers-targeted SARS-CoV-2 genome region was removed. The annotations were made using the reference genome available at NCBI (NC_045512.1). The data related to fixed SARS-CoV-2 mutation sites deposited by November 8th, 2021 were downloaded from National Genomics Data Center [7].

Overall, 836 iSNV sites were identified among specimens obtained from 61 patients. As of November 8th, 2021, 829 out of 836 (99.16%) iSNVs in our samples were repeatedly found as fixed single-nucleotide polymorphisms (SNPs) in the sequences deposited from laboratories across the world in National Genomics Data Center platform. Among these sites, 29 iSNVs gradually increased in frequency and eventually became consensus variants worldwide, with at least 1‰ proportion (5,202) in 5201,737 strains, suggesting they are advantageous within small subsets of population (Table 1). Other variants either 'reverted' in subsequent infections or did not transmit as effectively during onward transmission. Four sites were considered as lineage-specific fixed mutation with existence in more than 1% strains. These four iSNVs, 10,029 (ORF1ab: T3255I), 11,418(ORF1ab: V3718A), 26,149 (ORF3a: S253P) and 28,932 (N: A220V) were non-synonymous regarding change in amino acids (Fig. 1a and 1b). Surprisingly, iSNV site 10,029 accounted for approximately 20.19% (1050,005/5201,737), especially in Delta and Omicron variant. The iSNVs 11,418 and 28,932 accounted for approximately 2.82% (146,468/5201,737) and 2.35% (122,070/5201,737), respectively. The iSNV 22,992 (S: S477N) was later identified as a marked mutation of the Iota and Omicron Variant. (Fig. 1c)

Among 29 iSNVs, 18 (62.07%) iSNVs led to non-synonymous substitutions, 8 (27.59%) led to synonymous substitutions, whereas the remaining 3 iSNVs were located in non-coding regions; the high proportion of non-synonymous substitutions indicated en-

hanced viral replication after cross-species transmission. The iSNVs to be fixation for C>U/G>A transitions (10.75%, 23/214) is stark significantly higher than the other substitutions (0.96%, 6/622) ($P < 0.001$, OR=11.14) (Fig. 1d).

Based on the emergence and time-line of these fixed SNPs, we inferred that among the 29 iSNVs, 16 iSNVs occurred in our samples far before fixing consensus SNPs emerged. Time-line wise, the other 13 iSNVs were observed close to the emergence of fixed mutations, but before the progression to fixation (Table 1). Apart from the lineage-specific mutations, 4 iSNVs (21,789, 22,088, 22,992, and 24,334) were located at the region encoding the spike protein, which may help in immune escape, especially iSNV 22,992 (S: S477N) [8,9]

Among publicly available sequenced database that had been deposited 18 months into the COVID-19 pandemic, we identified several intra-host variants that were eventually fixed, and their proportion increased in population. Among these, substitutions in the receptor binding domain (RBD) attracted our attention as they may affect receptor binding or neutralization by antibodies, although most iSNVs identified in this study may have been lost during transmission because of the narrow bottleneck.

Although previous studies reported occasional fixed mutations from iSNVs [6], in this study, we observed that a large proportion of iSNVs could be found in the several dominant lineages in the samples obtained during early stage of COVID-19 epidemic.

During origin of iSNVs, interferon-induced expression of restriction factors belonging to APOBEC family exclusively deaminate an adenine or cytosine on the viral RNA, initiating C-U/G-A transitions, which facilitates evading degradation [10]. The large proportion of C-U/G-A in these iSNVs may be linked with APOBECs driven under innate immune pressure. As most of the fixed mutations are APOBEC related, APOBEC RNA editing may drive SARS-CoV-2 adaptation to the human host. In conclusion, close monitoring of variants conferring immune-escape ability via iSNVs would aid in variants surveillance and forecasting immune-escape variants that may emerge in the future.

Author contribution

WZ, CQ, ZZ and JWA designed the study. WQQ and TL collected the samples and clinical data. YZ and NJ analyzed clinical and sequencing data. YZ completed figures and tables. YZ and CQ drafted the article. YZ and YMZ, JW, HZ performed RNA extraction and amplification. All the authors contributed to this article and reviewed the final version.

Availability of data and materials

All data are available from the corresponding author.

Declarations of Competing Interest

All authors report no potential conflict of interest.

Acknowledgments

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Table 1
Prospective dominant fixed mutations indicated by iSNV.

Position	Region	Substitution alteration	Annotation of alteration	Amino Acid alteration	Accumulated mutation rate by 2021/11/08	Timeline of SNP
iSNVs existed before fixed mutation emerge						
5392	ORF1ab	C>T	synonymous	/	0.13%	Emerge from 2020/03, ascend from 2020/08
6027	ORF1ab	C>A	nonsynonymous	P1921Q	0.13%	Emerge from 2020/03, ascend from 2020/07
7767	ORF1ab	T>C	nonsynonymous	I2501T	0.40%	Emerge from 2020/03, ascend from 2020/08
8084	ORF1ab	G>A	nonsynonymous	E2607K	0.10%	Emerge from 2020/08, ascend from 2021/03
11,418	ORF1ab	T>C	nonsynonymous	V3718A	2.82%	Emerge from 2020/05, ascend from 2021/06
12,053	ORF1ab	C>T	nonsynonymous	L3930F	0.16%	Emerge from 2020/03, ascend from 2021/01
18,395	ORF1ab	C>T	nonsynonymous	A6044V	0.15%	Emerge from 2020/03, ascend from 2021/07
21,306	ORF1ab	C>T	synonymous	/	0.39%	Emerge from 2020/03, ascend from 2021/02
21,789	S	C>T	nonsynonymous	T76I	0.13%	Emerge from 2020/03, ascend from 2021/03
22,088	S	C>T	nonsynonymous	L176F	0.11%	Emerge from 2020/02, ascend from 2021/02
24,334	S	C>T	synonymous	/	0.35%	Emerge from 2020/03, ascend from 2020/09
25,703	ORF3a	C>T	nonsynonymous	P104L	0.20%	Emerge from 2020/03, ascend from 2021/02
25,710	ORF3a	C>T	synonymous	/	0.63%	Emerge from 2020/02, ascend from 2020/08
26,149	ORF3a	T>C	nonsynonymous	S253P	1.20%	Emerge from 2020/03, ascend from 2021/04
28,932	N	C>T	nonsynonymous	A220V	2.35%	Emerge from 2020/02, ascend from 2020/08
29,750	3′UTR	C>T	non-coding	/	0.18%	Emerge from 2020/03, ascend from 2020/09
iSNVs existed before epidemic of fixed mutations						
2485	ORF1ab	C>T	synonymous	/	0.14%	Emerge from 2020/01, ascend from 2020/11
4878	ORF1ab	C>T	nonsynonymous	T1538I	0.14%	Emerge from 2020/03, ascend from 2020/05
9430	ORF1ab	C>T	synonymous	/	0.25%	Emerge from 2020/03, ascend from 2020/12
9693	ORF1ab	C>T	nonsynonymous	A3143V	0.21%	Emerge from 2020/01, ascend from 2021/01
10,029	ORF1ab	C>T	nonsynonymous	T3255I	20.19%	Emerge from 2020/01, ascend from 2021/07
11,521	ORF1ab	G>T	nonsynonymous	M3752I	0.17%	Emerge from 2020/01, ascend from 2021/01
14,708	ORF1ab	C>T	nonsynonymous	A4815V	0.23%	Emerge from 2020/03, ascend from 2020/06
14,724	ORF1ab	C>T	synonymous	/	0.23%	Emerge from 2019/12, ascend from 2020/03
16,092	ORF1ab	C>T	synonymous	/	0.10%	Emerge from 2020/03, ascend from 2020/12
22,992	S	G>A	nonsynonymous	S477N	0.96%	Emerge from 2020/01, ascend from 2020/07
27,999	ORF8	C>T	nonsynonymous	P36S	0.15%	Emerge from 2020/01, ascend from 2021/07
29,743	3′UTR	C>T	non-coding	/	0.12%	Emerge from 2020/01, ascend from 2020/09
29,779	3′UTR	G>T	non-coding	/	0.22%	Emerge from 2020/01, ascend from 2020/05

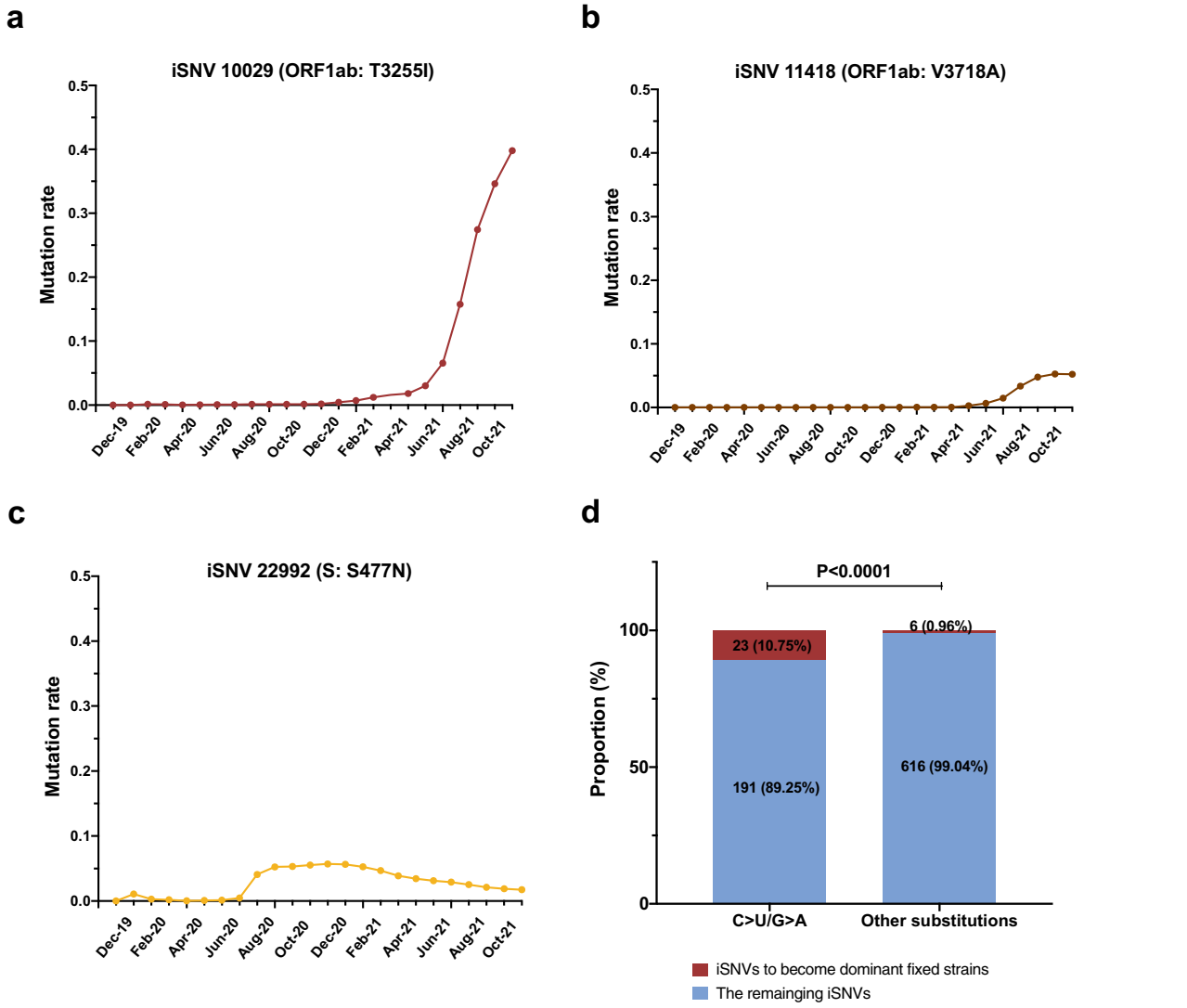

Fig. 1. The dynamics mutation rate of iSNV 10029, 11428 and 22992 since December, 2019. (a) The dynamics fixed mutation rate of iSNV 10029 since December, 2021. The fixed mutation emerged from 2020/01 and ascend dramatically from 2021/07.(b) The dynamics fixed mutation rate of iSNV 11418 since December, 2021. The fixed mutation emerged from 2020/05 and ascend dramatically from 2021/06.(c) The dynamics fixed mutation rate of iSNV 22992 since December, 2021. The fixed mutation emerged from 2020/01 and ascend quickly from 2020/07.(d) The proportion of dominant fixed mutations in substitution C>U/G>A and the other five substitutions.

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Yi Zhang[#]
Department of Infectious Diseases, National Clinical Research Center for Aging and Medicine, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Huashan Hospital, Fudan University, Shanghai, China

Ning Jiang[#]
Department of Infectious Diseases, National Clinical Research Center for Aging and Medicine, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Huashan Hospital, Fudan University, Shanghai, China
State Key Laboratory of Genetic Engineering and Institute of Biostatistics, School of Life Sciences, Fudan University, Shanghai, China

Weiqiang Qi[#], Tao Li[#]
Shanghai Public Health Clinical Center, Shanghai, China

Yumeng Zhang, Haocheng Zhang, Jing Wu
Department of Infectious Diseases, National Clinical Research Center for Aging and Medicine, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Huashan Hospital, Fudan University, Shanghai, China

Zhaoqin Zhu[*]
Shanghai Public Health Clinical Center, Shanghai, China

Jingwen Ai[*]
Department of Infectious Diseases, National Clinical Research Center for Aging and Medicine, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Huashan Hospital, Fudan University, Shanghai, China

Chao Qiu[*]
Department of Infectious Diseases, National Clinical Research Center for Aging and Medicine, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Huashan Hospital, Fudan University, Shanghai, China
Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai, China

Wenhong Zhang[*]
Department of Infectious Diseases, National Clinical Research Center for Aging and Medicine, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, Huashan Hospital, Fudan University, Shanghai, China
Institutes of Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai, China
State Key Laboratory of Genetic Engineering and Institute of Biostatistics, School of Life Sciences, Fudan University, Shanghai, China
Key Laboratory of Medical Molecular Virology (MOE/MOH), Shanghai Medical College, Fudan University, Shanghai, China

[*]Corresponding authors.
E-mail addresses: zhaoqinzhu@163.com (Z. Zhu), jingwenai1990@126.com (J. Ai), qiuchao@fudan.edu.cn (C. Qiu), zhangwenhong@fudan.edu.cn (W. Zhang)

[#] These authors contributed equally (Y Zhang, N Jiang, W Qi and T Li)

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Persistent post COVID-19 symptoms and functional status after 12–14 weeks of recovery, Tamil Nadu, India, 2021

Dear Editor,

Most of the infected patients completely recovered after COVID-19 infection. However, a substantial proportion of patients who have been infected with SARS-CoV-2 continue to have symptoms long past the time that they recovered from the initial phases of COVID-19 disease. Clinicians worldwide called these long-term effects of COVID-19 as "Long-Haul COVID-19".[1] The knowledge about long COVID-19 is evolving day by day. Multiple articles published in the Journal of Infection discussed the persistent symptoms, quality of life, and functional status post recovery.[2–4] Long COVID symptoms were reported in 12 countries and none were from LMIC.[5] The proportion of the Indian Population who have been experiencing the symptoms following the recovery is unknown. Understanding the burden of post COVID-19 symptoms is vital in planning the health systems for essential Post COVID care. We rapidly assessed the burden of persistent post COVID-19 symptoms and functional status after 12–14 weeks among those recovered from COVID-19 in Chennai, Tamil Nadu, India.

We obtained the line list of COVID-19 positive cases between February 25 and March 09, 2021, from the COVID-19 surveillance unit of Chennai, India. During the period, RT-PCR was the only method of testing and having any of the comorbidity or requiring oxygen therapy were the criteria for hospitalization. We defined persistent Post COVID-19 symptoms as clinical symptoms that develop during or after an infection consistent with COVID-19, persistent for more than 12 weeks and are not attributable to alternative clinical diagnoses.[1] The assessment was done during June 11–20, 2021. A team of doctors tele-consulted all these COVID-19 case-patients aged >18 years during the 12–14 post recovery period of COVID-19. The team collected data on persistent symptoms and ruled out the possible differential diagnosis based on the reported symptoms. They also evaluated the functional status of activities of daily living using post COVID-19 functional scale (PCFS).[6] The PCFS was evaluated between the time of the interview and the pre-COVID-19 diagnosis. The team graded the persistent breathlessness reported by the participants using modified Medical Research Council (mMRC) dyspnea scale.[7] Mean, Standard deviation (SD), and proportions were calculated as appropriate. We estimated odds ratio (OR) with 95% confidence interval (95% CI) for the association between hospitalization, persistent symptoms and functional limitations. We computed the adjusted OR (aOR) with 95% CI using multiple logistic regression after adjusting for age, gender, having any comorbidity, and hospitalization. P value <0.05 was considered as statistically significant.

We contacted all the 1241 case-patients who were found positive for COVID-19 between February 25, 2021, and March 09, 2021. Of the 1241, 1001 (81%) responded. The mean age (SD) of the case-patients was 46.9 (16.1) years and 596 (60%) were females, 341 (34%) had at least one of the comorbidities. Eight-hundred and fifty-two (85%) of the case-patients experienced symptoms during active phase of COVID-19, 482 (48%) were hospitalised. All the 482 who were hospitalised either had a comorbidity or required

Table 1
Predictors of persistent post COVID-19 symptoms, Tamil Nadu, India, 2021.

Exposures	# Reported persistent symptom ($n=249$)	%	# Reported no persistent symptom ($n=752$)	%	OR	95% CI	aOR*	95% CI*
Aged >45 years	143	57	402	53	1.8	0.9–1.6		
Male gender	147	59	449	60	1	0.7–1.3		
Having at least one comorbidity	98	39	243	32	1.4	1.0–1.8	1.2	0.8–1.6
Hospitalised for severe COVID-19 during active phase of infection	157	63	325	43	2.2	1.7–3.0	2.2	1.6–3.1
	# Severe limitation in ADL ($n=27$)	%	Nil to negligible limitation in ADL ($n=974$)	%	OR	95% CI	aOR*	95% CI*
Aged >45 years	19	70	526	54	2	0.9–4.6		
Male gender	18	67	578	59	1.4	0.6–3.0		
Having at least one comorbidity	17	63	324	33	3.4	1.5–7.5	2.7	1.2–6.8
Hospitalised for severe COVID-19 during active phase of infection	20	74	462	47	3.2	1.3–7.6	2.4	1.0–6.0

*Adjusted for all the exposures.

oxygen therapy. Among the hospitalised, 137 (28%) required oxygen support. Almost one in four (24%) reported at least one of the symptoms as persistent (Fig. 1). Among the 249 who reported symptoms post recovery, 136 (55%) were males. Persistent symptoms were higher among the age group 45–59 years (40%), followed by 30–44 years (24%). Four percent of those who remained asymptomatic during the active phase of infection also reported symptoms. Weight loss (40%), Hair loss (29%), Fatigue/Tiredness (26%), myalgia (10%), and sleeplessness (9%) were the most common reported symptoms (Fig. 1). Of the 1001 case-patients, sixteen reported persistent breathlessness and none had dyspnea more than grade 3 of mMRC dyspnea scale.

Based on PCFS scale, 868 (87%) participants did not report any functional limitations in the activities of daily living, and they have been carrying out with the same intensity when compared to period of pre-COVID diagnosis. Around 106 (11%) reported negligible differences in the day-to-day activities. Despite symptoms they were performing all day-to-day activities without assistance. Twenty-four (2%) reported that their usual activities reduced due to symptoms and anxiety and 3 (0.3%) participants reported that their functional status affected drastically and required constant support for the activities of daily living when compared to period of pre-COVID diagnosis.

On bivariate analysis, having at least one comorbidity and hospitalised for severe COVID-19 were significantly associated with persistent post COVID-19 symptoms (Table 1). On multivariate analysis, hospitalised for severe COVID-19 was independently associated with persistent post COVID-19 symptoms (aOR= 2.2, 95% CI= 1.6–3.1) (Table 1). Severe limitations of the functional status were significantly higher among those with comorbidity than those who did not [aOR= 2.7, 95% CI= 1.2–6.8].

The prevalence of at least one persistent symptom in our study was lower than the studies from high income countries.[5,8] Weakness, general malaise, fatigue, brain fog, and breathlessness were the most commonly reported long COVID-19 symptoms in high income countries.[5] On the other hand, weight loss, hair loss, and excessive tiredness were the most common reported symptoms in our setting. Globally, almost one in four reported brain fog and breathlessness post COVID-19 compared to 2% and 6% respectively in Chennai. Our study also found that hospitalization for severe COVID-19 was the predictor for long COVID-19 which is consistent with the findings from other studies.[9]

Although our study provides the estimates of burden of long COVID-19 and functional status post recovery, it suffers from a few limitations. We collected follow up data among hospitalized individuals for a two week period. The non-response was 20% which might have lead on under or over estimate of the prevalence. Also, we could not stratify the case-patients as ICU and non-ICU admissions. Due to overwhelmed health system during the pandemic, the ICU services were provided in the non-ICU wards also; hence, the team faced difficulties in eliciting accurate hospitalization history. Thus we analyzed the whole data among hospitalized case-patients. Lastly, the cross-sectional study design did not permit us to establish the temporality between the exposure and outcome. Longitudinal follow-ups, prospective cohort study or establishing registry in clinical settings is critical to understand the post COVID-19 sequel in the long term.

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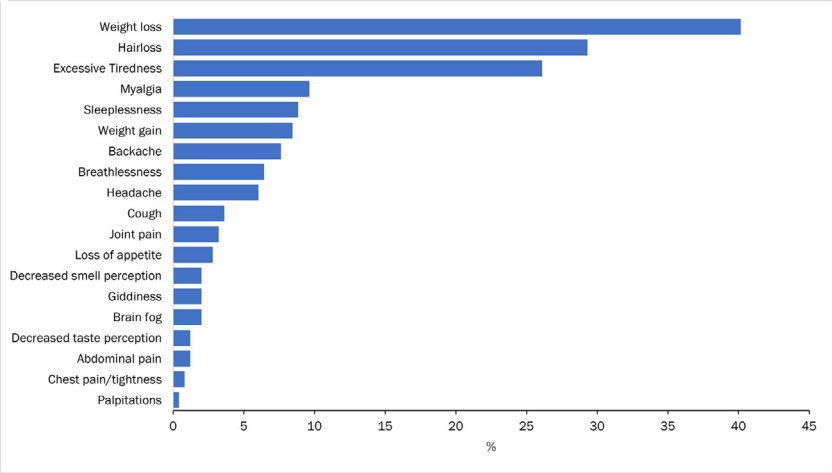

Fig. 1. Persistent post COVID-19 symptom reported by those recovered after 12–14 weeks, Chennai, Tamil Nadu, India, 2021 ($n = 249$).

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Polani Rubeshkumar
ICMR-National Institute of Epidemiology, R 127 TNHB, Ayappakkam, Chennai, Tamil Nadu 600 077, India

Alby John, Manish Narnaware, Jagadeesan M, Fermi Vidya
Greater Chennai Corporation, Government of Tamil Nadu, Chennai, Tamil Nadu, India

Rajeshkumar Gurunathan, Parasuraman Ganeshkumar*
ICMR-National Institute of Epidemiology, R 127 TNHB, Ayappakkam, Chennai, Tamil Nadu 600 077, India

Gagandeep Singh Bedi
Greater Chennai Corporation, Government of Tamil Nadu, Chennai, Tamil Nadu, India

Prabhdeep Kaur
ICMR-National Institute of Epidemiology, R 127 TNHB, Ayappakkam, Chennai, Tamil Nadu 600 077, India

*Corresponding author.
E-mail address: ganeshkumar@nieicmr.org.in (P. Ganeshkumar)

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