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Letters to the Editor

## Depression increases the risk of tuberculosis: Preliminary observations

*Dear Editor*,

We read with great interest the systematic review by Alene et al.[1] and the comment on it by Cao et al.,[2] who pooled data of the incidence rate of depression in patients with multidrug-resistant tuberculosis (MDR-TB). As reported, the incidence of depression is high among patients with TB, particularly for individuals with MDR-TB, as an adverse effect of anti-TB drugs and/or a consequence of the host's perturbed inflammatory and neurobiological response[1,3,4]. Both depression and TB are major public health issues with high prevalence and incidence.[4] The association between TB and depression is presumed to be bidirectional.[5] TB increases the risk of depression has been analyzed in several studies, however, the evidence of the association between depression and the subsequent risk of TB is limited.

In this letter, we pooled the data published that depression increased the risk of TB and presented our preliminary observations. First, we conducted a systematic review and meta-analysis to quantify the incidence of TB in patients with depression. After hand-searching and processing, a total of 237,696 enrollees including 67,137 patients with depression and 170,559 control individuals from only two articles was recruited according to predefined selection criteria.[6,7] The overall incidence rate of TB was 1.16-fold [95% Confidence Interval (CI): 1.12–1.20] greater in the depression cohort than that in the control cohort (Fig. 1). Furthermore, a dose-response relationship was shown between depression and the risk of TB.[7] Although depression is a common belief to influence the neuroendocrine response and modify the behavioral function with consequences of the delays in TB diagnosis and treatment, whether depression impacts the immune response against *Mycobacterium tuberculosis (Mtb)* infection remains to be investigated.

Macrophages are served as both the habitats, and the first line of defense against intracellular pathogen, *Mtb*.[8] We then hypothesized that depression might impair macrophage function to fight against *Mtb* infection. To test our hypothesis, we recruited 6 depression patients and 6 age- and sex-matched healthy donors from Shenzhen Kangning Hospital (Shenzhen, China). The participants who had any comorbidities including HIV infection, chronic obstructive pulmonary disease, and diabetes mellitus were excluded. Adherent monocytes enriched from peripheral blood mononuclear cells were differentiated into human monocyte-derived macrophages (hMDMs).[9] We found that depression significantly impaired the bactericidal activity of hMDMs against *Mtb* H37Rv. Specifically, compared to the hMDMs differentiated from healthy donors, intracellular H37Rv colony forming unit (CFU) was significantly increased in the hMDMs from patients with depression (Fig. 2A).

Finally, to further recapitulate the data in mouse macrophages, we established a chronic social defeat stress (SDS)-induced mouse model that reliably exhibits depressive symptoms, as previously described.[10] Mice that underwent SDS procedure were separated into resilient and susceptible groups based on their depressive-like behaviors.[10] Mouse bone marrow-derived macrophages (BMDMs) from control (housed in pairs during SDS procedure with no contact with aggressor mice), susceptible and resilient mice were infected with H37Rv and macrophage bactericidal activity was assessed by intracellular CFU counts. As expected, compared to the BMDMs from control and resilient mice, intracellular H37Rv growth was significantly increased in the BMDMs from susceptible mice (Fig. 2B), suggesting that depression dampens macrophage bactericidal activity against *Mtb* infection.

In summary, we performed a systematic review for the first time to estimate the incidence rate of TB in patients with depression and found that depression posed a high risk for TB with 1.16-fold [95% CI: 1.12–1.20] in the overall pooled incidence rate. More importantly, our *in vitro* data showed that depression significantly impaired the bactericidal activity of macrophages against *Mtb* infection, which might provide evidence partially explaining how depression increased the risk of TB. This letter spotlights interesting and exciting observations that could potentially open up a new area of research to reveal the mechanism underlying how mental disorders such as depression influences the immune response to anti-*Mtb* infection.

### Ethics statement

The present study was approved by the Ethics Committees of the Shenzhen Kangning Hospital (Shenzhen, China). Written informed consent was provided by all study participants. The animal experiments were approved by the Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences Research Committee.

### Funding

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### Declaration of Competing Interest

The authors declare no competing financial interests.

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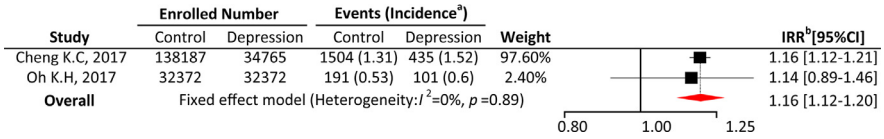


**Fig. 1.** Effect of depression on the incidence of tuberculosis. In this systematic review and meta-analysis, the studies that reported data on TB among depression patients were searched in PubMed and Web of Science, without any language restrictions. The reference list was also hand-searched according to inclusion and exclusion criteria. The data of the incidence of TB in patients with depression was extracted and pooled into a meta-analysis using the R package metafor (version 3.0). Thompsons' $I^2$ was used to assess the heterogeneity.
[a]Incidence: per 1000 person-years.
[b]IRR (incidence rate ratio): depression vs. control cohort (95% CI).

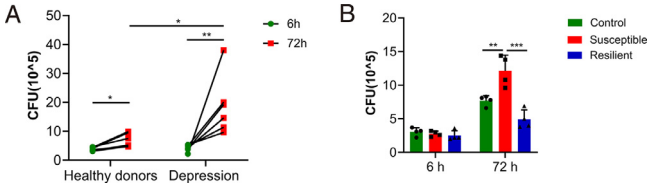


**Fig. 2.** Depression impaired the bactericidal activity of macrophages against *Mtb* infection. (A) Adherent monocytes enriched from PBMCs (from six depression patients and six healthy donors) were differentiated into hMDMs under conditions of stimulation (50 ng/ml M-CSF in culture. hMDMs were infected with H37Rv (MOI=1) for 6 h and then washed three times with prewarmed PBS. After a further 72 h culture, macrophage bactericidal activity was assessed by determination of intracellular *Mtb* CFU. (B) Mouse MDMs from control, susceptible and resilient mice were infected with H37Rv and macrophage bactericidal activity was assessed by intracellular CFU counts as described for panel A. Data represented as mean ± SD, *, $P < 0.05$, **, $P < 0.01$; ***, $P < 0.001$. by Student's two-tailed paired *t*-test (A) and two-way ANOVA with Bonferroni post hoc test (B).

## References


1. Alene K.A., Clements A.C.A., McBryde E.S., Jaramillo E., Lonnroth K., Shaweno D., et al. Mental health disorders, social stressors, and health-related quality of life in patients with multidrug-resistant tuberculosis: a systematic review and meta-analysis. *J Infect* 2018;**77**(5):357–67.
2. Cao Y., Yu C., Wu Y.. Incidence rate of depression as an adverse effect of multidrug-resistant tuberculosis treatment. *J Infect* 2019;**79**(1):61–74.
3. Ambaw F., Mayston R., Hanlon C., Medhin G., Alem A.. Untreated depression and tuberculosis treatment outcomes, quality of life and disability, Ethiopia. *Bull World Health Organ* 2018;**96**(4):243–55.
4. Sweetland A.C., Jaramillo E., Wainberg M.L., Chowdhary N., Oquendo M.A., Medina-Marino A., et al. Tuberculosis: an opportunity to integrate mental health services in primary care in low-resource settings. *Lancet Psychiatry* 2018;**5**(12):952–4.
5. Zhang K., Wang X., Tu J., Rong H., Werz O., Chen X.. The interplay between depression and tuberculosis. *J Leukoc Biol* 2019;**106**(3):749–57.
6. Cheng K.C., Liao K.F., Lin C.L., Lai S.W. Increased risk of pulmonary tuberculosis in patients with depression: a cohort study in Taiwan. *Front Psychiatry* 2017;**8**:235.
7. Oh K.H., Choi H., Kim E.J., Kim H.J., Cho S.I.. Depression and risk of tuberculosis: a nationwide population-based cohort study. *Int J Tuberc Lung Dis* 2017;**21**(7):804–9.
8. Hmama Z., Pena-Diaz S., Joseph S., Av-Gay Y.. Immunoevasion and immunosuppression of the macrophage by Mycobacterium tuberculosis. *Immunol Rev* 2015;**264**(1):220–32.
9. Dai Y., Cai Y., Wang X., Zhu J., Liu X., Liu H., et al. Autoantibody-mediated erythrophagocytosis increases tuberculosis susceptibility in HIV patients. *MBio* 2020;**11**(1):e03246–19.
10. Cheng J., Umschweif G., Leung J., Sagi Y., Greengard P.. $HCN_2$ channels in cholinergic interneurons of nucleus accumbens shell regulate depressive behaviors. *Neuron* 2019;**101**(4):662–72 e665 .



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## Emerging polio hotspots in Pakistan: Challenges and the way forward

*Dear Editor*,

We read with great interest the article entitled "Impact of COVID-19 pandemic on Measles surveillance in Pakistan" by Rana et al. The authors of this article discuss the various infectious diseases that surfaced during the COVID-19 pandemic.[1] Therefore, the current article will spotlight the re-emergence of polio in Pakistan, which is another serious public health concern.

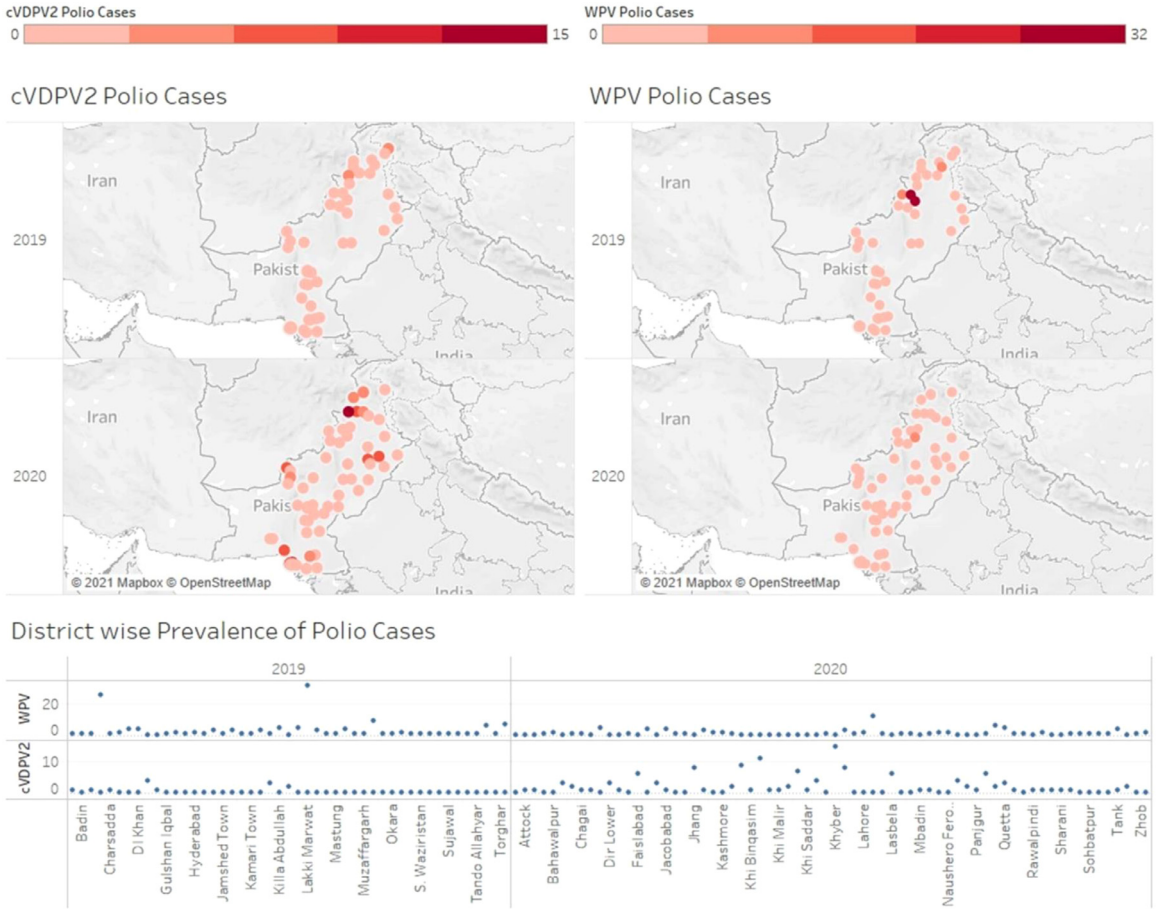


**Fig. 1.** Polio hotspots in Pakistan from in year 2019 to 2020.

It is estimated that after the Global Polio Eradication Initiative (GPEI) unveiling in 1988, the global incidence of polio has been reduced by 99%.[2] The tremendous efforts of GPEI to eliminate polio for the last three decades are now on the verge of downfall owing to the COVID-19 pandemic. Pakistan is the fifth most populated country and has a population of over 200 million with an annual growth rate of 2.04% and is listed as a polio-endemic country.[3,4] The catastrophic impacts of COVID-19 in Pakistan are exacerbated by the disruption of routine immunization against a myriad of vaccine-preventable diseases. In amidst of 2020, all immunization operations in Pakistan were halted owing to the lockdown, depriving 40 million children of the polio vaccine. [5]

Pakistan's Polio program has operated commendably through 2015, reducing 306 Wild Poliovirus (WPV) cases to 54 in 2015, 20 in 2016, 08 in 2017, 12 in 2018, and re-emergence reported in 2019 with 147 new cases. Geospatial analysis revealed that the worst-affected regions in Pakistan from WPV were the slum and squatter territories in Khyber Pakhtunkhwa province (Fig. 1). Between 2015 to 2020, the prevalence of WPV cases was significantly higher in Khyber Pakhtunkhwa (51%), followed by Sindh (23%), Balochistan (17%), and Punjab (9%) (Fig. 2).[4]

Several conspiracy theories have previously been used to sabotage the country's Polio vaccine campaigns, including myths regarding vaccine quality, religious beliefs, and the presence of live viruses in the vaccine, which have resulted in vehement parental refusal to vaccinate their children. Pakistan's initiative to eradicate polio GPEI was endeavoring to overcome these obstacles, but regretfully, a pandemic hit Pakistan, and a lockdown was imposed, thus suspending the country's whole immunization program. A comparative survey of child polio vaccination statistics revealed that the number of daily vaccination visits decreased by 52.8%. Dreadfully, only 92,492 children received vaccinations, compared to 608,832 children who received vaccination shots before the lockdown.[6]

Discontinuation of polio immunization in Pakistan may not affect immediately, but a surge in polio cases might potentially result in the importation of infections to vulnerable countries.[2] Thus, following established World Health Organization (WHO) processes and preventive measures, Pakistan's government and policymakers should actively implement supplementary immunization activities (SIAs) and mop-up immunization strategies in hotspot areas. Additionally, inter-district mobility, particularly between districts with positive environmental samples, should be closely monitored, and susceptible children should be permitted to travel only after receiving immunization. In particular, health practitioners must educate and address vaccine adverse effects with the people in Pakistan to ensure effective polio immunization.

## Declaration of Competing Interest

The authors declare that there is no conflict of interest or financial disclosure about this publication.

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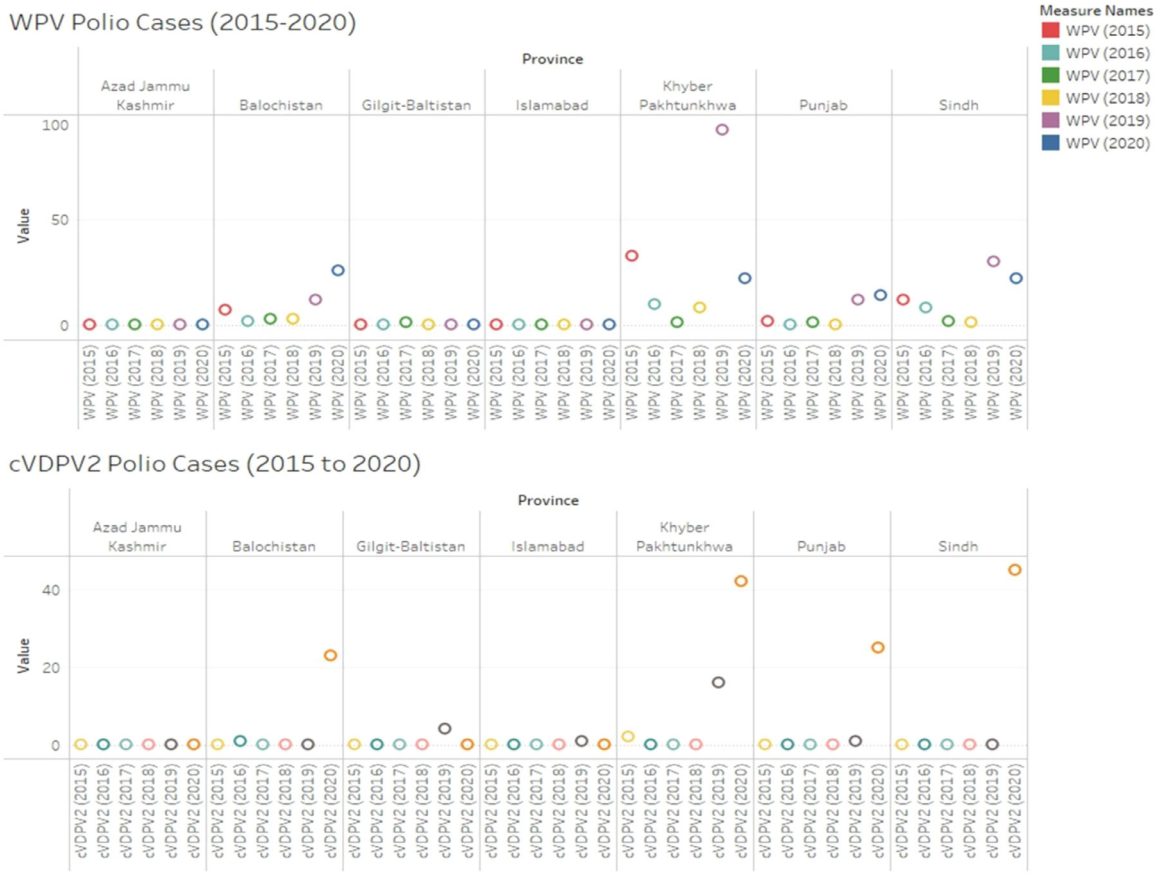


**Fig. 2.** Number of WPV Polio cases reported in different provinces of Pakistan from the year 2015–2020. Comparative analysis showed the highest number of polio cases were seen in 2020 due to halting vaccination program.

## References


1. Rana M.S., Usman M., Alam M.M., et al. Impact of COVID-19 pandemic on Measles surveillance in Pakistan. *J Infect* 2021;**82**:414–51.
2. Global Polio Eradication Initiative (GPEI)- Pakistan. 2021. https://polioeradication.org/where-we-work/pakistan/. Accessed 01-07-2021.
3. Silva N.M., Santos N.C., Martins I.C.. Dengue and Zika viruses: epidemiological history, potential therapies, and promising vaccines. *Trop Med Infect Dis* 2020;**5**:150.
4. Pakistan Polio Eradication Program - Polio Cases in Provinces. 2021. https://www.endpolio.com.pk/polioin-pakistan/polio-cases-in-provinces. Accessed 15-06-2021.
5. Din M., Ali H., Khan M., et al. Impact of COVID-19 on polio vaccination in Pakistan: a concise overview. *Rev Med Virol* 2020;**31**(4):e2190. doi:10.1002/rmv.2190.
6. Chandir S., Siddiqi D.A., Setayesh H., Khan A.J.. Impact of COVID-19 lockdown on routine immunisation in Karachi, Pakistan. *Lancet Global Health* 2020;**8**:e1118–e1e20.



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## Significant increase in hospital admissions for the management of severe dental infection in England 2000–2020

*Dear Editor,*

Severe odontogenic infection is the most serious consequence of dental disease.[1] Maxillo-facial cellulitis is most commonly a result of dental caries where the carious lesion extends into the tooth pulp causing irreversible pulpal inflammation leading to pu-

pal necrosis and subsequent peri-apical abscess. Spread of the infection beyond the immediate periapical area is influenced by both local anatomic considerations such as location of tooth apex to mucosal surfaces, tissue planes, muscle attachments and systemic factors[2,3] such as, poorly controlled diabetes mellitus. Spread of infection along tissue planes often leads to pain, swelling, trismus, dysphagia and loss of airway, together with signs and symptoms of sepsis syndrome. Severe odontogenic infection is an emergency that must be promptly treated by a combined surgical (incision and drainage, tooth extraction, airway protection) as well as medical management for infection (IV antibiotics) and symptoms of sepsis as required. The large majority of localised dental abscesses respond to early local surgical intervention such as, tooth extraction or root canal therapy. In 2008 Thomas et al.[4] reported a case series of life threatening dental infections and described a doubling in such admissions over the period 1998–2006, this report updates and extends the data on hospital admission in England for the period 2000–2020.

Following the method reported by Thomas et al.[4] we accessed routinely captured data on all admissions to NHS hospitals in England between 2000 and 2020. The Hospital Admitted Patient Care Activity (formerly known as Hospital Episode Statistics) data includes information on all patients treated in NHS hospitals regardless of funding source, residential status or treatment centre location. The Hospital Admitted Care Activity records store information on the main operation and currently up to 24 procedures in total for each hospital episode coded using the Office of Population Censuses and Surveys: Classification of Surgical Operations and Procedures, 4th Revision (OPCS4). According to convention the main operation is normally the most resource intensive procedure or intervention performed during the treatment episode. This report focused only on cases where the management of the dental abscess was the main intervention. We downloaded freely available aggregated data from the NHS Digital website[5] .Trends are reported for episodes where the main operation code was “drainage of abscess of alveolus of tooth” (OPCS4.2 code F16.1). Information was available for each year from 2000 to 2020 (1 April to 31 March). We calculated the absolute number of admissions and the rate of admission per hundred thousand head of population in order to account for population change over the study period. Over the last 20 years 36,197 people have been admitted to England’s hospitals for the surgical drainage of dental abscesses (Table 1). The number of people admitted for incision and drainage of the alveolus has risen steadily over the last two decades from 842 in 2000–2001 to 3018 in 2019–2020 (Fig. 1). The raw number of cases is more than three and a half times (3.58) higher in 2019–2020 compared with 2000–2001. The rate per 100,000 head of population has also increased steadily from 1.71/100,000 to 5.36/100,000 over the same time period. The rate was 3.13 times higher in 2020 compared with 2000. In the year 2000–2001 1887 bed days were taken up with the management of dental abscesses, by 2019–2020 this figure had risen to 5629.

**Table 1**
Number and Rate/100,000 pops of Hospital admissions for drainage of dental abscess 2000–2020.

| Year | N | Rate /100,000 |
|---|---|---|
| 2000–01 | 842 | 1.71 |
| 2001–02 | 884 | 1.79 |
| 2002–03 | 963 | 1.94 |
| 2003–04 | 1060 | 2.12 |
| 2004–05 | 1219 | 2.43 |
| 2005–06 | 1431 | 2.83 |
| 2006–07 | 1522 | 2.99 |
| 2007–08 | 1625 | 3.16 |
| 2008–09 | 1799 | 3.47 |
| 2009–10 | 1749 | 3.35 |
| 2010–11 | 1774 | 3.37 |
| 2011–12 | 2086 | 3.93 |
| 2012–13 | 2127 | 3.98 |
| 2013–14 | 2174 | 4.04 |
| 2014–15 | 2281 | 4.20 |
| 2015–16 | 2270 | 4.14 |
| 2016–17 | 2244 | 4.06 |
| 2017–18 | 2558 | 4.60 |
| 2018–19 | 2571 | 4.59 |
| 2019–20 | 3018 | 5.36 |

Although odontogenic infection is one of the most common causes of infection in the maxillofacial area it is however a largely preventable condition. The majority of cases are caused by dental caries which occurs due to excessive sugar consumption and inadequate dental hygiene. Despite its preventable nature dental caries rates in the United Kingdom at the last national survey remain high at 31% and dental infection has been shown to be present in 2% of the population[6]. Dental caries and its complications remains a disease linked to health inequalities with the burden of disease falling on the most deprived populations. Moles[7] subsequently analysed the data initially reported by Thomas et al.[4] and showed that there was a widening social gradient meaning that patients from lower socioeconomic groups were also far more

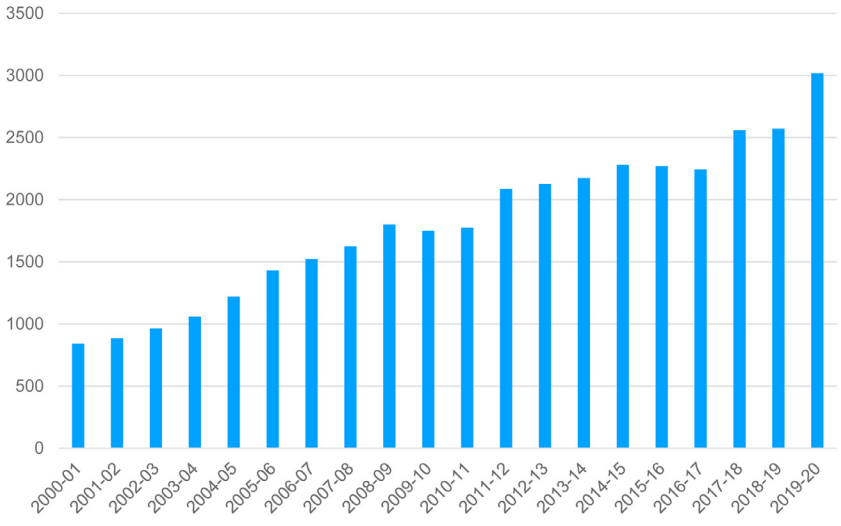


**Fig. 1.** Rising number of hospital admissions for drainage of dental abscesses in England 2000–2020.

likely to experience the most serious of sequelae. The majority (86.3%) of these admissions were classified as emergencies and again the most affluent patients were least likely to be admitted as an emergency. In response Freeman[8] expressed hope that reforms begun in 2006 would lead to "accessible NHS dentistry for all". While it is not possible to attribute causality we note that numbers have continued to rise in the twelve years since these concerns were first raised and show no sign of levelling off or improving. The increase may be due to poor access to dental care in deprived areas, an inability to pay for dental treatment leading to neglect until serious complications arose or other systemic changes in the provision of dental care in England. The identification of this surprising and sustained increase in admissions to hospital for the management of severe odontogenic infections should cause policy makers and commissioners to investigate this phenomenon further, identify risk factors and improve dental health especially in socio-economic groups at higher risk of hospital admission for this potentially life threatening infection.

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and material

Not applicable.

### Authors contributions

All authors have contributed to the manuscript.

### Patient and public involvement

None, paper is of interest to patients and public. An observational study.

### Transparency declaration

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

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### Declaration of Competing Interest

None.

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### References

1. Robertson D.P., Keys W., Rautemaa-Richardson R., Burns R., Smith A.J.. Management of severe acute dental infections. *BMJ* 2015;**350**:h1300. doi:10.1136/bmj.h1300.
2. Seppänen L., Lauhio A., Lindqvist C., Suuronen R., Rautemaa R.. Analysis of systemic and local odontogenic infection complications requiring hospital care. *J Infect* 2008;**57**(2):116–22. doi:10.1016/j.jinf.2008.06.002.
3. Park J., Lee J.Y., Hwang D.S., Kim Y.D., Shin S.H., Kim U.K., et al. A retrospective analysis of risk factors of oromaxillofacial infection in patients presenting to a hospital emergency ward. *Maxillofac Plast Reconstr Surg* 2019;**41**(1):49. doi:10.1186/s40902-019-0238-9.
4. Thomas S.J., Atkinson C., Hughes C., Revington P., Ness A.R.. Is there an epidemic of admissions for surgical treatment of dental abscesses in the UK? *BMJ* 2008;**336**(7655):1219–20. doi:10.1136/bmj.39549.605602.BE.
5. Hospital Admitted Patient Care Activity. NHS Digital. https://digital.nhs.uk/data-and-information/publications/statistical/hospital-admitted-patient-care-activity (last accessed 01/05/2021).
6. White D., Tsakos G., Pitts N., Fuller E., Douglas G.V.A., Murray J.J., et al. Adult dental health survey 2009: common oral health conditions and their impact on the population. *Br Dent J* 2012;**213**:567–72. doi:10.1038/sj.bdj.2012.1088.
7. Moles D.R.. Epidemic of dental abscesses? Dental abscesses have increased most among poorer people. *BMJ* 2008;**336**(7657):1323. doi:10.1136/bmj.39605.430243.3A.
8. Freeman R.. Reforming NHS dentistry. *BMJ* 2008;**336**(7655):1202–3. doi:10.1136/bmj.39546.440822.80.

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## The impact of case and contact characteristics on contact tracing during the West Africa Ebola epidemic

*Dear Editor,*

A recent paper by Etard et al. highlights how clustering of Ebola exposures around a few cases, and overdispersion of contacts per case, leads to super-spreader events and epidemic propagation.[1] Based on this evidence and that seen with SARS-Cov-2,[2] they suggest that targeting epidemic screening and communication control strategies in low resource settings may be beneficial.

Here, we summarise a study we conducted to assess the success of contact tracing in relation to different characteristics of cases and contacts. To our knowledge, the association of a contact's characteristics with their likelihood of being successfully traced during a pandemic has not previously been assessed.

We analysed data collected during the 2014–2016 West African Ebola epidemic to identify case and contact characteristics associated with success of contact-tracing. Data were collected in the Ebola Contact Tracing proof-of-concept Study (ECTS) in Port Loko district, northern Sierra Leone, April-August 2015. The study aimed to design and evaluate the use of a mobile health (mHealth) app relative to paper-based contact-tracing.[3] We found that the app improved data completeness, storage, and accuracy, but that challenges in tracing remained.

**Table 1**
Baseline characteristics of participants recorded on The Ebola Contact Tracing Study App and the distribution of these characteristics between successfully and unsuccessfully traced contacts.

| | Contact tracing | |
|---|---|---|
| | Traced $N = 365$ | Not traced $N = 281$ |
| **Ebola Case Factors** (17 cases: 15 laboratory confirmed; 2 secret burials) | *n* (%)* | *n* (%)* |
| **Age of Ebola case (years), median (IQR)** | 29 (22, 29) | 29 (24, 50) |
| **Sex of Ebola case** | | |
| Male | 142 (38.9%) | 173 (61.6%) |
| Female | 223 (61.1%) | 108 (38.4%) |
| **Status of Ebola case** | | |
| Dead | 213 (58.4%) | 174 (61.9%) |
| Alive | 152 (41.6%) | 107 (38.1%) |
| **Number of contacts per Ebola case, median (IQR)** | 52 (24, 120) | 92 (66, 112) |
| **Ebola contact factors** | | |
| **Age of Ebola contact (years), median (IQR)** | 19 (5, 35) | 24 (9, 36) |
| **Sex of contact** | | |
| Male | 160 (43.8%) | 157 (55.9%) |
| Female | 205 (56.2%) | 124 (44.1%) |
| **Type of contact with Ebola case**** | | |
| Touched the body fluids of the case | 62 (17.0%) | 11 (3.9%) |
| Had direct physical contact with the body of a case | 108 (29.6%) | 132 (47.0%) |
| Touched or shared the linen, clothes, or dishes/utensils of the case | 56 (15.3%) | 21 (7.5%) |
| Slept, ate or spent time in the same household or room as the case | 139 (38.1%) | 51 (18.2%) |
| **Ebola contact location factors** | | |
| **Level of development***** | | |
| Urban | 270 (74.0%) | 34 (12.1%) |
| Rural | 95 (26.0%) | 247 (87.9%) |

* Percentage of those with complete data.
** Each contact was assessed for the highest risk level of contact and only this level was recorded.
*** Based on standardised categories developed for The Ebola Contact Tracing Study (3).

We have subsequently further analysed the app-based data to investigate which case and contact characteristics were associated with successful mHealth contact-tracing. A total of 16 laboratory-confirmed Ebola virus disease (EVD) cases (one of whom was excluded from this analysis as they had no contacts), and two secret burial cases, were registered on the study app. From the 17 included cases, 646 contacts were identified and recorded on the app by the local case investigation team.

Successful contact tracing was defined as a contact being visited by a contact-tracer (CT), and an ECTS app form being completed daily from first contact with a CT throughout the remaining incubation-period. The association of successful tracing with Ebola case characteristics (age, sex and survival) and contact characteristics (age, sex, type of contact with the Ebola case, and urban/rural location) was investigated by estimating adjusted hazard ratios (aHR) using multivariable Cox regression, adjusted for clustering by case. Follow-up time was time between last contact with the Ebola case and first visit from a CT. For all non-traced contacts, follow-up time was set at 21-days (maximum incubation-period). The multivariable model was built by first fitting univariable models, including those variables with $p < 0.05$ in an initial multivariable model, and retaining those independently associated with the outcome in a final multivariable model. Models were compared using the Likelihood Ratio test.

Of the 646 registered contacts, 365 (57%) were traced Table 1. The median age of cases was 29 years for both traced and non-traced groups. Those not traced were more likely to be contacts of male cases ($n = 173$, 61.6%) than those traced ($n = 142$; 38.9%). The median number of contacts per case was higher in the non-traced group (92 contacts vs 52 contacts). Non-traced contacts were older (median 24-years vs 19-years) and more likely to be male ($n = 157$, 55.9% vs $n = 160$, 43.8%) than the traced contacts. In the non-traced group, the most common form of contact was physical contact with the body ($n = 132$, 47.0%) whereas in the traced group the most common form of contact was sharing a room with the case ($n = 139$, 38.1%). A greater proportion of non-traced contacts than traced contacts lived in rural settings ($n = 247$, 87.9% vs $n = 95$, 26.0%).

In the final multivariable Cox model Table 2, three factors were independently associated with contact tracing success. The case being female was positively associated with successful contact tracing (aHR 4.96; CI 1.55–15.90, $p < 0.01$). The contact living in a rural as opposed to urban setting (aHR=0.04; CI 0.01–0.12, $p < 0.01$) and the contact having direct contact with the Ebola-case, their personal items, or bodily fluids (Table 2) were negatively associated with successful tracing.

To our knowledge, this is the first study of its kind to utilise app-based data to examine factors affecting contact-tracing. Introduction of mHealth into the tracing process removes obstacles to tracing, such as large travel distances and poor transportation. However, this study shows additional issues associated with living in a rural environment that affect the likelihood of successful tracing, even once the travel barriers have been removed. Mode of contact with the Ebola case had a complex relationship with success of contact tracing. Those who had the closest contact with the case were twice as likely to be contact traced as those who had not had physical contact, but half as likely as those who had minor physical contact with the Ebola case. We hypothesise that there is an interaction between social factors such as stigma, personal perception of disease-risk, concerns regarding financial loss, and isolation at play.[4]

Based on our findings, in future epidemics in low-resource settings, contact-tracing programs need to be designed with a strategy in mind for reaching those in rural locations, as well as contacts of specific case characteristics including those who are of male sex and the super-spreaders described by Majra et al.[2] This must however, not be to the detriment of those currently being traced.

With the ongoing SARS-CoV2 pandemic, and increasing evidence of epidemics becoming more common, contact-tracing pro-

**Table 2**
Cox-regression shared frailty model showing the effect of Ebola Case and Contact factors on Hazard ratios of successful contact tracing over a 21-day follow-up period adjusted for clustering around cases.

| Ebola case factors | unadjusted hazard ratio | Confidence interval (95%) | *p* value | adjusted hazard ratio | confidence interval (95%) | *p* value |
|---|---|---|---|---|---|---|
| *Age of Ebola case (years)* | | | <0.01 | | | |
| | reference | | | | | |
| 30–60 | 0.57 | 0.44–0.74 | | | | |
| Over 60 | 0.16 | 0.05–0.51 | | | | |
| Missing data | 0.26 | 0.17–0.40 | | | | |
| **Sex of Ebola case** | | | <0.01 | | | <0.01 |
| Male | reference | | | reference | | |
| Female | 1.63 | 1.32–2.01 | | 4.96 | 1.55–15.90 | |
| **Number of contacts per Ebola case**(per 1 contact increase) | 0.99 | 0.99–1.00 | <0.01 | | | |
| *Mortality status of Ebola case* | | | 0.03 | | | |
| Dead | reference | | | | | |
| Alive | 1.26 | 1.02–1.55 | | | | |
| *Ebola contact factors* | | | | | | |
| *Age of Ebola contact (years)* | | | | | | |
| 0–30 | reference | | <0.01 | | | |
| 30–60 | 0.81 | 0.63–1.03 | | | | |
| Over 60 | 0.87 | 0.57–1.32 | | | | |
| *Sex of contact* | | | <0.01 | | | |
| Male | reference | | | | | |
| Female | 1.35 | 1.09–1.65 | | | | |
| *Type of contact with Ebola case*** | | | <0.01 | | | <0.01 |
| Touched the body fluids of the case (blood, vomit, saliva, urine faeces) | 0.81 | 0.60–1.10 | | 0.57 | 0.37–0.88 | |
| Had direct physical contact with the body of a case (alive or dead) | 0.38 | 0.30–0.49 | | 0.44 | 0.30–0.65 | |
| Touched or shared the linen, clothes, or dishes/utensils of the case | 0.58 | 0.42–0.79 | | 0.21 | 0.13–0.32 | |
| Slept, ate or spent time in the same household or room as the case | reference | | | reference | | |
| *Ebola contact location factors* | | | | | | |
| *Level of development* *** | | | <0.01 | | | <0.01 |
| Urban | reference | | | reference | | |
| Rural | 0.17 | 0.14–0.22 | | 0.04 | 0.01–0.12 | |

** Each contact was assessed for the highest risk level of contact and only this level was recorded.
*** Based on standardised categories developed for The Ebola Contact Tracing Study (3).

grammes are going to become more integral to maintaining normality and preventing the economic and societal damage seen when blanket restrictions are enforced. We recommend further qualitative research to understand whether our findings are generalisable, and to develop strategies to reach those who are harder to trace.

## Availability of data and materials

The datasets analysed during the current study are available in the London School of Hygiene & Tropical Medicine data repository https://datacompass.lshtm.ac.uk/1069/

## Ethics approval and consent to participate

The London School of Hygiene & Tropical Medicine Observational/Interventions Research Ethics Committee (reference 8749–01) and the Sierra Leone Ethics and Scientific Review Committee (SLESRC) approved this study. Written informed consent was obtained from eligible Contact Tracing Coordinators and Contact Tracers who consented to take part in the study. Consent was not required from individual Ebola contacts as the smartphone app mirrored the existing paper-based system that was in use for contact tracing throughout the country.

## 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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## References

1. Etard J.F., Touré A., Sow M.S., et al. Heterogeneity of contact patterns with Ebola virus disease cases. *J Infect* 2021.
2. Majra D., Benson J., Pitts J., et al. SARS-CoV-2 (COVID-19) superspreader events. *J Infect* 2021;**82**(1):36–40.
3. Danquah L.O., Hasham N., MacFarlane M., et al. Use of a mobile application for Ebola contact tracing and monitoring in northern Sierra Leone: a proof-of–concept study. *BMC Infect Dis* 2019;**19**(1):810.
4. Saurabh S., Prateek S.. Role of contact tracing in containing the 2014 Ebola outbreak: a review. *Afr Health Sci* 2017;**17**(1):225–36.

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## Anxiety, depression and poor sleep quality as long-term post-COVID sequelae in previously hospitalized patients: A multicenter study

*Dear Editor*,

Evidence supports the presence of a plethora of symptoms after suffering severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The prevalence rate of these post-COVID symptoms ranges from 35 to 60% depending on the symptom and the follow-up period.[1] One letter to the Editor and one full-text in *Journal of Infection* found that fatigue and dyspnea are the most prevalent post-COVID symptoms in both hospitalized[2] and non-hospitalized[3] patients. Post-COVID sequalae include general, respiratory, physical, gastrointestinal, neurological, and mental symptoms. A scoping review observed that anxiety and depression are also prevalent post-COVID symptoms;[4] however, most of published studies have follow-up periods < 3 months, sample sizes <300 participants, and were conducted at single centers.[4] We have recently published in a letter to the Editor in *Journal of Infection,* a multicenter study assessing post-COVID symptoms and their associated risk factors seven months after hospital discharge[5]. This study observed that 80% of hospitalized COVID19 survivors exhibited at least one post-COVID symptom seven months after hospital discharge, being fatigue and dyspnea the most prevalent. No data about the prevalence of mood disorders was provided. Here we present secondary data of that multicenter study analyzing the prevalence of depressive and anxiety levels and sleep quality.

Briefly, this multicenter observational study included 1200 hospitalized patients randomly selected from four public hospitals in Madrid, Spain (300 from each hospital) with a diagnosis of SARS-CoV-2 by RT-PCR technique and radiological findings during the first wave of the pandemic (March 10th to May 31st, 2020). Local Ethics Committees of all hospitals approved the study (HCSC20/495E, HUFA 20/126, HUF/EC1517, HUIL /092–20, URJC0907202015920). Informed consent was obtained from all participants.

**Table 1**
Demographic, clinical and hospitalization data ($n$ = 1142).

| | |
|---|---|
| Age, mean (SD), years | 61 (17) |
| Gender, male/female (%) | 601/541 |
| Weight, mean (SD), kg. | 70 (15) |
| Height, mean (SD), cm. | 166 (10) |
| Medical co-morbidities | |
| Hypertension | 291 (25.5%) |
| Diabetes | 145 (13%) |
| Chronic Heart Disease - Cardiovascular Disease | 144 (13%) |
| Rheumatological Disease | 61 (5.5%) |
| Asma | 55 (5%) |
| Obesity | 54 (5%) |
| Chronic Obstructive Pulmonary Disease | 51 (4.5%) |
| Stroke | 29 (2.5%) |
| Other (cancer, kidney disease) | 105 (9%) |
| Stay at the hospital, mean (SD), days | 14 (12) |
| Intensive Care Unit (ICU) admission | |
| Yes/No, n (%) | 80 (7%) / 1062 (93%) |
| Stay at ICU, mean (SD), days | 15 (13) |
| Anxiety/Depressive Symptom, n (%) | |
| Anxiety Symptoms (HADS-$A \geq$ 12 points) | 185 (16.2%) |
| Depressive Symptoms (HADS-$D \geq$ 10 points) | 225 (19.7%) |
| Sleep Quality, n (%) | 394 (34.5%) |
| Poor Sleep Quality (PSQI $\geq$ 8 points) | |

Patients were scheduled for a telephone interview by trained researchers a mean of 7.0 months (SD 0.6) after hospital discharge. Clinical (i.e., age, gender, height, weight, pre-existing comorbidities) and hospitalization (e.g., symptoms at hospital admission, days at hospital, intensive care unit [ICU] admission) data were collected from hospital medical records. In addition to a predetermined list of post-COVID symptoms previously described[5], depressive/anxiety level and sleep quality were also collected. The Hospital Anxiety and Depression Scale (HADS) was used to assess anxiety (HADS-A, 7 items, 0–21 points) and depressive (HADS-D, 7 items, 0–21 points) symptoms.[6] We considered the cut-off scores recommended for Spanish population (HADS-$A \geq$ 12 points; HADS-$D \geq$ 10 points) suggestive of clinical anxiety and depressive symptoms, respectively[7]. The Pittsburgh Sleep Quality Index (PSQI) was used to assess the sleep quality the previous month by using 19 self-reported questions assessing the usual bed time, usual wake time, number of hours slept, and number of minutes to fall asleep.[8] A total score ranging from 0 to 21 points is calculated, higher scores indicate worse sleep quality, and a score > 8.0 points is indicative of poor sleep quality.[8]

Descriptive data are presented as mean (standard deviation, SD) or percentages as appropriate. Multivariate Poisson regression prediction and risk models were constructed to identify clinical/hospitalization variables associated with the presence of post-COVID anxiety/depressive symptoms and poor sleep quality. Adjusted Odd Ratio (OR) with 95% confidence intervals (95%CI) were calculated.

As previously reported, a total of 1142 (48% women, mean age: 61, SD: 17 years) were included. Data on symptoms at hospital admission, previous co-morbidities, or other post-COVID symptoms have been previously published.[5] **Table 1** summarizes clinical and hospitalization data related to the current analysis. Seven months after hospitalisation, 16.2% patients showed anxiety symptoms ($\geq$ 12 points), 19.7% depressive symptoms ($\geq$ 10 points), and 34.5% poor sleep quality ($\geq$ 8 points). Only 50.4% ($n$ = 575) of the included patients did not report depressive/anxiety symptoms or poor sleep quality at seven months after hospital discharge. Significant positive associations between anxiety and depressive symptoms (r: 0.759, $P$ < 0.001) and between sleep quality with anxiety

(r: 0.239, $P < 0.001$) and depressive (r: 0.340, $P < 0.001$) symptoms observed.

The regression models revealed that female (OR1.88, 95%CI 1.35–2.51, $P < 0.001$), the number of days at the hospital (OR1.02, 95%CI 1.01–1.03, $P = 0.04$), the number of pre-existing medical comorbidities (OR1.23, 95%CI 1.02–1.45, $P = 0.022$) and the number of symptoms at hospital admission (OR1.37, 95%CI 1.13–1.67, $P = 0.001$) were associated with depressive symptoms. Only the number of symptoms at hospital admission (OR1.29, 95%CI 1.04–1.58, $P = 0.015$) was significantly associated with anxiety. Finally, poor sleep quality was associated with female gender (OR2.15, 95%CI 1.65–2.80; $P < 0.001$), number of days at hospital (OR1.02, 95%CI 1.001–1.035, $P < 0.001$), the number of comorbidities (OR1.34, 95%CI 1.15–1.57, $P < 0.001$), and number of symptoms at hospital admission (OR1.31, 95%CI 1.12–1.54, $P < 0.001$).

This multicenter study revealed that almost 50% of hospitalised COVID-19 survivors experienced anxiety or depressive symptoms and/or poor sleep quality seven months after hospital discharge. Specifically, anxiety symptoms were observed in 16.2% of patients, depressive symptoms in 19.7%, and poor sleep quality in 34.5%. Our prevalence rates of post-COVID depressive or anxiety symptoms and poor sleep quality should be considered significant since patients were evaluated seven months after hospital discharge, but were lower than prevalence data previously reported (40–45%) during the acute hospitalisation period.[9] Previous studies investigating post-COVID mood disorders included follow-up < 3 months, smaller samples and individuals recruited from just one center. The current study increases evidence to the literature with a large, multicenter design evaluating long-term post-COVID anxiety/depressive levels and sleep quality.

Early recognition of long-term post-COVID effects and associated risk factors will facilitate diagnosis and multidisciplinary strategies for these patients.[10] We identified that female gender, longer stay at hospital, higher number of comorbidities, and higher number of symptoms at hospital admission were risk factors associated with depressive symptoms and poor sleep quality, but not with anxiety levels. Similar risk factors were identified for the number of post-COVID symptoms (not including mood disorders) in our previous letter[5]. Future studies determining the role of these risk factors in the development of long-term post-COVID symptoms are guaranteed.

Finally, these results should be considered attending study weaknesses. First, only hospitalized patients were included. Second, patients with diagnosed psychiatric diseases were excluded. Third, we did not collect objective measures of COVID-19 disease, e.g., inflammatory biomarkers, blood oxygen saturation.

## Author contributions

All authors contributed to the study concept and design. CFdlP, DMP, VGM, and VHB conducted literature review and did the statistical analysis. VGM, MVA, CG, CMEM, MLC, JAAN, LJMT, TSV, JTM, MGCD, and SPC recruited participants. JRJ, MPC, AIdlLR, SFN, LLF, ROS, MGM, and SAQ collected data. DPC supervised the study. All authors contributed to interpretation of data. CFdlP, DPC, VGM, and MLC 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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## References

1. Fernández-De-Las-Peñas C., Palacios-Ceña D., Gómez-Mayordomo V., Florencio L.L., Cuadrado M.L., Plaza-Manzano G., Navarro-Santana M.. Prevalence of post-COVID-19 symptoms in hospitalized and non-hospitalized COVID-19 survivors: a systematic review and meta-analysis. *Eur J Intern Med* 2021. doi:10.1016/j.ejim.2021.06.009.
2. Garrigues E., Janvier P., Kherabi Y., Le Bot A., Hamon A., Gouze H., et al. Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. *J Infect* 2020;**81**:e4–6.
3. Moreno-Pérez O., Merino E., Leon-Ramirez J.M., Andres M., Ramos J.M., Arenas-Jiménez J., et al. Post-acute COVID-19 syndrome. Incidence and risk factors: a Mediterranean cohort study. *J Infect* 2021;**82**:378–83.
4. Shanbehzadeh S., Tavahomi M., Zanjari N., Ebrahimi-Takamjani I., Amiri-Arimi S.. Physical and mental health complications post-COVID-19: scoping review. *J Psychosom Res* 2021;**147**:110525.
5. Fernández-de-Las-Peñas C., Palacios-Ceña D., Gómez-Mayordomo V., Rodríguez-Jiménez J., Palacios-Ceña M., Velasco-Arribas M., Guijarro C., de-la-Llave-Rincón A.I., Fuensalida-Novo S., Elvira-Martínez C.M., Cuadrado M.L., Arias-Navalón J.A., Florencio L.L., Ortega-Santiago R., Molina-Trigueros L.J., Sebastián-Viana T., Torres-Macho J., Canto-Diez G., Plaza-Canteli S., Cigarán-Méndez M., Ambite-Quesada S., Hernández-Barrera V., Arias-Buría J.L., Arendt-Nielsen L.. Long-term post-COVID symptoms and associated risk factors in previously hospitalized patients: a multicenter study. *J Infect* 2021 May 11:S0163-4453(21)00223-1.
6. Herrmann-Lingen C., Buss U., Snaith R.P.. *Hospital Anxiety and Depression Scale – Deutsche Version (HADS-D)*. Bern: Verlag Hans Huber; 2011.
7. Grupo de Trabajo de la Guía de Práctica Clínica para el Manejo de Pacientes con Trastornos de Ansiedad en Atención Primaria. *Guías de Práctica Clínica en el SNS - UETS N° 2006/10. Madrid: Plan Nacional para el SNS del MSC*. Unidad de Evaluación de Tecnologías Sanitarias, Agencia Laín Entralgo, Comunidad de Madrid; 2008.
8. Carpenter J.S., Andrykowski M.A.. Psychometric evaluation of the pittsburgh sleep quality index. *J Psychosom Res* 1998;**45**:5–13.
9. Deng J., Zhou F., Hou W., et al. The prevalence of depression, anxiety, and sleep disturbances in COVID-19 patients: a meta-analysis. *Ann N Y Acad Sci* 2021;**1486**:90–111.
10. Korompoki E., Gavriatopoulou M., Hicklen R.S., Ntanasis-Stathopoulos I., Kastritis E., Fotiou D., Stamatelopoulos K., Terpos E., Kotanidou A., Hagberg C.A., Dimopoulos M.A., Kontoyiannis D.P.. Epidemiology and organ specific sequelae of post-acute COVID19: a narrative review. *J Infect* 2021;**83**:1–16.

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## Increase in circulation of non-SARS-CoV-2 respiratory viruses following easing of social distancing is associated with increasing hospital attendance

*Dear Editor,*

In this Journal, we recently reported that the appearance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and the accompanying social restrictions were associated with a dramatic reduction in circulation of non-SARS-CoV-2 viruses.[1] We have also previously reported on the resurgence of rhinovirus circulation following the re-opening of schools in the United Kingdom in September 2020.[2] Apprehension now surrounds the effects of complete cessation of social distancing measures in the United Kingdom on the transmission of SARS-CoV-2, and other respiratory viruses. The effect of social distancing on suppressing respiratory viruses are well documented, therefore, as these restrictions ease multiple routes of transmission increase. [3]

At University Hospital Southampton NHS Foundation Trust (UHSFT), UK, we have tested all adult medical patients admitted to hospital using point-of-care multiplex PCR testing (using the QIAstat-Dx Respiratory SARS-CoV-2 panel and the BioFire Respiratory Panel 2.1 plus) for a wide range of respiratory viruses, since the start of the SARS-CoV-2 pandemic.[4] From 20th March 2020 to the 30th June 2021, 17,804 adult patients were tested. There was a near absence of detection of non-SARS-CoV-2 respiratory viruses following the introduction of social distancing measuring during the first wave of the pandemic. In addition to the increases seen in rhinovirus detection after September 2020, detections of parainfluenza viruses and non-SARS-CoV-2 coronaviruses were seen to increase after the 18th April 2021. This corresponds with the opening of non-essential retail and outdoor hospitality in England on the 12th April 2021. There was a further sharp increase following the resumption of indoor socialising and hospitality which started on the 17th May 2021 (Fig. 1). At peak circulation, 13% of admitted adult patients had parainfluenza viruses detected and 5% had non-SARS-CoV-2 coronaviruses detected. These findings are consistent with national surveillance data.[5] Furthermore, Emergency Department attendances for acute respiratory illness increased at UHSFT following schools returning after the Easter holidays on the 8th March 2021. This corresponded with an increase in rhinovirus detection. Thereafter, a further sustained increase in Emergency Department attendances has been seen coinciding with increased parainfluenza and non-SARS-CoV-2 detection (Fig. 2).

Our data is aligned with previous research supporting the impact of social distancing on reducing the circulation of non-SARS-CoV-2 respiratory viruses, and that non-enveloped viruses such as rhinovirus, re-emerge initially as social distancing is eased, followed by other viruses.[1,2] The increase in detection of non-SARS-CoV-2 coronaviruses is in keeping with other reports of increased respiratory virus detections outside of the normal viral epidemiological cycles.[6] These findings might have important implications for the complete relaxing of social distancing measures in the coming months and particularly on the forthcoming circulation of respiratory syncytial virus (RSV) and influenza viruses.

### Declaration of Competing Interest

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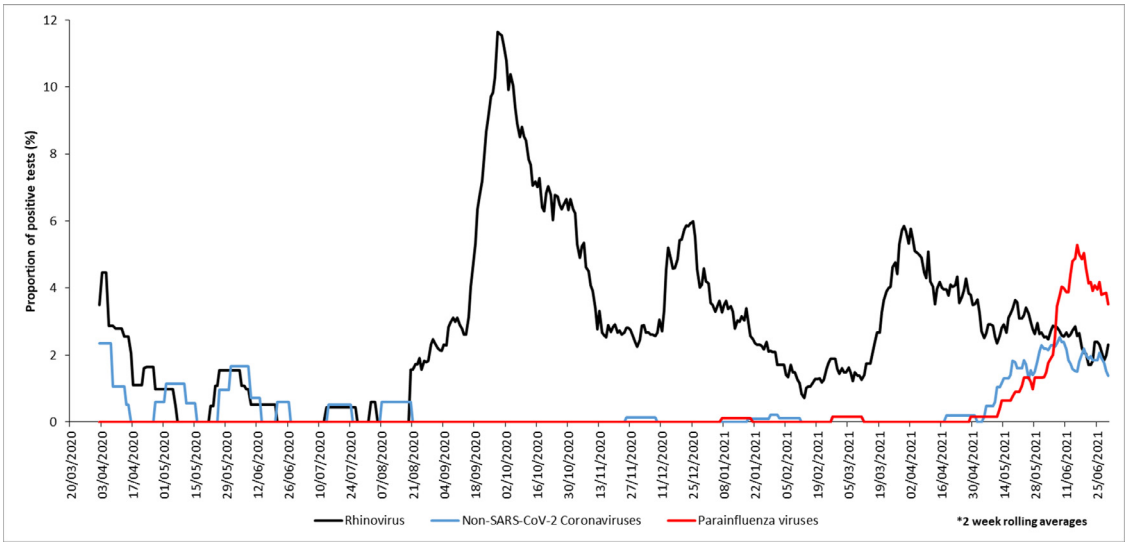


**Fig. 1.** Proportion of tests positive for non-SARS-CoV-2 respiratory viruses over time.

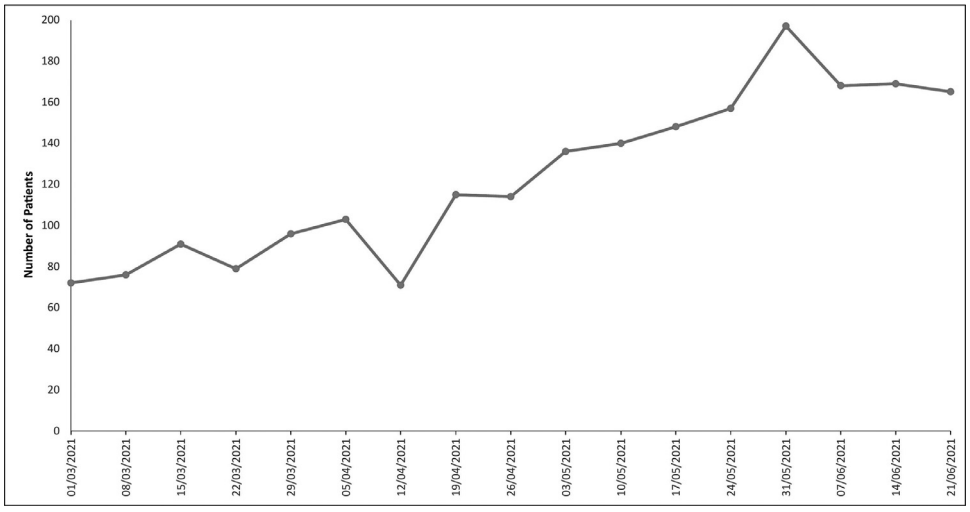


**Fig. 2.** Emergency department attendances with acute respiratory illness, March to June 2021.

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## References

1. Poole S., Brendish N.J., Clark T.W.. SARS-CoV-2 has displaced other seasonal respiratory viruses: results from a prospective cohort study. *J Infect* 2020;**81**(6):966–72.
2. Poole S., Brendish N.J., Tanner A.R., Clark T.W.. Correspondence physical distancing in and the resurgence of rhinovirus. *Lancet Respir Med* 2020;**2600**(20):4–5.
3. Kim M.C., Kweon O.J., Lim Y.K., Choi S.H., Chung J.W., Lee M.K.. Impact of social distancing on the spread of common respiratory viruses during the coronavirus disease outbreak. *PLoS ONE* 2021;**16**(6):e0252963.
4. Brendish N.J., Poole S., Naidu V.V., Mansbridge C.T., Norton N.J., Wheeler H.. Clinical impact of molecular point-of-care testing for suspected COVID-19 in hospital (COV-19POC): a prospective, interventional, non-randomised, controlled study. *Lancet Respir Med* 2020;**8**(12):1192–200.
5. Public Health England. Weekly National Influenza & COVID-19 Report: week 25 report (up to week 24 data). 2021. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/996369/Weekly_Flu_and_COVID-19_report_w25.pdf. Last accessed: 29th June 2021.
6. Foley D.A., Yeoh D.K., Minney-Smith C.A., Martin A.C., Mace A.O., Sikazwe C.T.. The interseasonal resurgence of respiratory syncytial virus in Australian children following the reduction of coronavirus disease 2019–related public health measures. *Clin Infect Dis* 2021.

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## Comparison of self-collected mouth gargle with deep-throat saliva samples for the diagnosis of COVID-19☆,☆☆

*Dear Editor,*

We read with interest the study by Zhu et al.[1] that proved saliva as an acceptable alternative to nasopharyngeal or oropharyngeal swabs for diagnosis and monitoring of SARS-Coronavirus-2 (SARS-CoV-2) patients. While saliva is a well-accepted self-collected sample, mouth gargle is a potential alternative with characteristics more favorable for laboratory handling. Mouth gargle are non-viscous in nature, which minimizes cross-contamination during re-suspension and transfer that poses risk of generating false-positive results. Furthermore, the non-viscous nature also reduces the chance of clogging of liquid handling systems that would result in testing failure. To date, only a few comparative studies analyzed the analytical performance of mouth gargle in the diagnosis of Coronavirus Disease 2019 (COVID-19)[2,3]. We performed a prospective head-to-head comparison on the analytical performance of self-collected mouth gargle and Deep-Throat Saliva (DTS) samples for the detection of SARS-CoV-2 using four different Nucleic Acid Amplification Test (NAAT) platforms performed in three independent laboratories.

We recruited patients admitted to the Prince of Wales Hospital, Hong Kong SAR, China, with active SARS-CoV-2 infection confirmed by the presence of SARS-CoV-2 RNA in their respiratory tract (nasopharyngeal swab, sputum or DTS) using reverse transcription polymerase chain reaction (RT-PCR). Recruitment started from November 23 to December 1, 2020 for patients aged above 10 years who can follow instructions of gargle collection. Disease severity was classified into asymptomatic, mild, moderate, severe, and critical as previously described[4,5]. Informed consents were obtained from all participants. The study was approved by The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee.

Mouth gargle and deep-throat saliva (DTS) samples were collected at least 30 min apart. Mouth gargle samples were collected with sterile bottles pre-filled with 4 mL of 0.9% sterile normal saline according to the illustrations printed on the instruction sheet (Supplementary figure 2). DTS samples were collected according to the instructions produced by the centre for Health Protection, HKSAR (https://www.chp.gov.hk/files/pdf/information_sheet_on_dts_en.pdf). DTS samples were diluted with 3 mL of Phosphate Buffered Saline (PBS) that ended up in about 4 mL similar to the final volume of gargle samples that were undiluted. Samples were mixed and aliquoted into equal portions for head-to-head comparison using four different NAAT platforms by three independent laboratories (Supplementary figure 1): (i) In-house assay quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR), Roche Cobas 6800, and Cepheid GeneXpert by local Public Health Reference Laboratory. (ii) In-house RT-PCR assay by University Laboratory A; and (iii) Roche Cobas 6800 and Cepheid GeneXpert assays by University Laboratory B (Supplementary data).

We recruited 49 patients (30 females) aged 12–81 (median: 61) years, with seven asymptomatic, 20 mild (no pneumonitis), 15 moderate (pneumonitis), and four had severe disease that required oxygen support, and three were critical and required ventilator support. All participants recovered and discharged. Samples were collected between 1 and 19 days (mean 7 ± 4) from symptom onset.

A total of 109 mouth gargle-DTS sample-pairs (mean of 2 ± 1 sample-pairs per patient) with at least one positive result for SARS-Co-V-2 RNA were analyzed. The overall positive rate ranged from 89.9% to 96.3% (Fig. 1) with no significant difference between mouth gargle and DTS samples by all four assays. Six sample-pairs had discrepant results of either having detectable RNA in mouth gargle only or in DTS only, all with Ct values of >30. Analysis of diagnostic yield reflected by 1/Ct showed strong positive correlation between the paired mouth gargle and DTS samples regardless of the assay used, and with Spearman's correlation index ranged from 0.662 to 0.727 (Fig. 2B). Of note, DTS had a significantly higher diagnostic yield than mouth gargle (Fig. 2A).

A total of 26 sample-pairs that had adequate volume were additionally tested for inter-laboratory and inter-assay consistencies. We found a strong inter-laboratory correlation when using Cobas 6800 and GenXpert with both sample types with correlation coefficients of > 0.8 (Fig. 2C). Inter-assay performance comparison by E gene detection using Cobas 6800 and GeneXpert performed in Public Health Reference Laboratory and University Laboratory B again showed no significant difference in diagnostic yield using mouth-gargle and DTS (Fig. 2D). When analyzing the diagnostic yield among various clinical situations, we found that the diagnostic yield of DTS was significantly higher in patients with respiratory symptoms, but there was no significant difference in asymptomatic patients and those who were symptomatic but without respiratory symptoms (Supplementary figure 3)

Our study found that the positive rate for SARS-CoV-2 RNA detection from mouth gargle samples was similar to paired deep-throat saliva (DTS) samples collected from patients with active COVID-19 infection irrespective of the laboratory or assay used.

Mouth gargle had been shown to be a suitable sample for diagnosis of COVID-19 and respiratory pathogens[6,7]. Previous studies

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☆☆ Potential competing interests: none declared

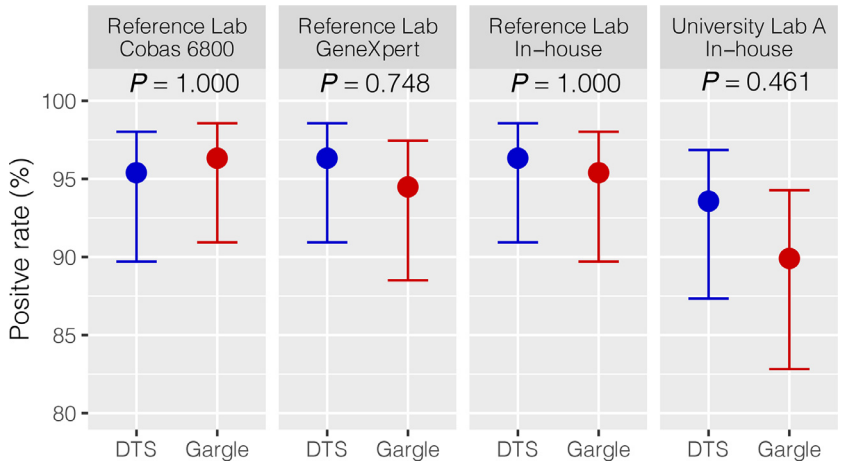


**Fig. 1.** Positive rates of SARS-CoV-2 RNA detection between mouth gargle and saliva samples using four different assays in three different laboratories
Reference Laboratory (Cobas 6800) positive rate for mouth gargle: 96.3% (95% CI 90.0–98.6), and DTS: 95.4% (95% CI 89.7–98.0). Reference Laboratory (GeneXpert) positive rate for mouth gargle: 94.5% (95% CI: 88.5–97.5), and DTS: 96.3% (95% CI: 90.9–98.6). Reference Laboratory (In-house method) positive rate for mouth gargle: 95.4% (95% CI 89.7–98.0), and DTS: 96.3% (95% CI 90.9–98.6). University Laboratory A (In-house method) positive rate of for mouth gargle: 89.9% (95% CI 82.8–94.2), and DTS 93.6 (95% CI 87.3–96.9). DTS – Deep-throat saliva samples; Gargle – mouth gargle samples. Fisher test was used to assess the difference in positive rates between detection assays and/or specimens. The 95% confidence interval (CI) was calculated using *epi*.conf (ctype="prop.single") in epiR.

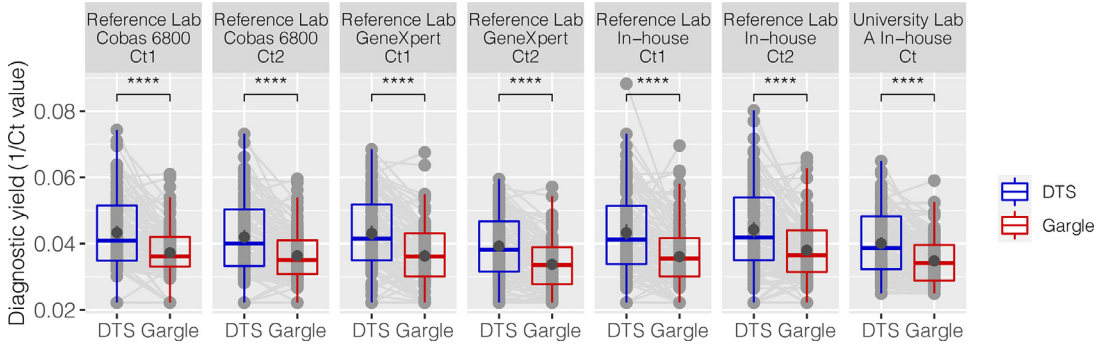


**Fig. 2.** Comparison of diagnostic yield between sample types, assays, and laboratories
A. Comparison of diagnostic yield of the 109 mouth gargle and DTS sample pairs by test method. Midline: median; Box: interquartile range; DTS, Deep-throat saliva; Gargle, mouth gargle. **** $p \leq 0.0001$. Comparisons of viral concentration between detection assays and/or specimens were performed using non-parametric Mann-Whitney Wilcoxon rank-sum test (unpaired) or Wilcoxon signed rank test (paired).
B. Correlation analysis between 109 mouth gargle and DTS sample pairs by test method. Correlation performed by Spearman's correlation index; R2adj, Adjusted R-squared. Spearman's *rho* and linear regression were used to evaluate their associations. A two-sided *p* value of $< 0.05$ was considered statistically significant.
C - Inter-laboratory comparison of Cobas 6800 and GeneXpert of 26 paired samples by Reference Laboratory and University Laboratory B. Correlation performed by Spearman's correlation index; R2adj, Adjusted R-squared. DTS – deep-throat saliva; Gargle – mouth gargle. Spearman's *rho* and linear regression were used to evaluate their associations. A two-sided *p* value of $< 0.05$ was considered statistically significant.
D - Inter-assay comparison of 26 paired samples by analyzing E genes detection by Cobas 6800 and Genexpert. Midline: median; Box: interquartile range; DTS – deep-throat saliva; Gargle – mouth gargle. Comparisons of viral concentration between detection assays and/or specimens were performed using non-parametric Mann-Whitney Wilcoxon rank-sum test (unpaired) or Wilcoxon signed rank test (paired). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)


**Fig. 2.** Continued

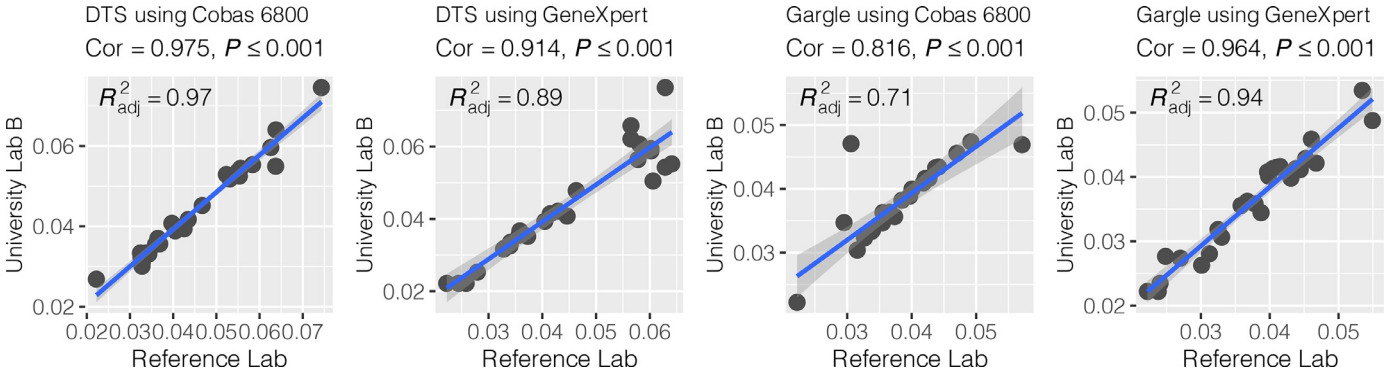


**Fig. 2.** Continued

have shown that gargle samples are comparable, but with slightly higher cycle threshold (Ct) values than those of nasopharyngeal and oropharyngeal swabs[8]. Mouth gargle samples are easy to collect, acceptable to patients[2], and have the advantage over saliva that it is non-viscous and can dilute inhibitors present in the samples.

Our study has the strength of being prospective, inclusion of a wide age range (12 to 81 years), wide spectrum of clinical severity (from asymptomatic to critically ill), and covering all stage of illness (from 1 to 19 days of onset). There are limitations in our study. First, our cohort were all confirmed COVID-19 patients, thus we cannot analyze specificity. Secondly, we did not specify the sequence of mouth gargle and DTS collection; however, we gave explicit instructions to separate the collection of the specimens to be at least 30 min apart, which should negate the collection sequence bias. In conclusion, our findings suggested mouth gargle showed excellent correlation with DTS and can be a choice of self-collected specimen for mass screening of asymptomatic individuals.

## Declaration of Competing Interest

None declared

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.07.012.

## Reference

1. Zhu J., Guo J., Xu Y., Chen X.. Viral dynamics of SARS-CoV-2 in saliva from infected patients. *J Infect* 2020 Sep;**81**(3):e48–e50 Epub 2020 Jun 25. PMID: 32593658; PMCID: PMC7316041. doi:10.1016/j.jinf.2020.06.059.
2. Goldfarb D.M., Tilley P., Al-Rawahi G.N., Srigley J.A., Ford G., Pedersen H., Pabbi A., Hannam-Clark S., Charles M., Dittrick M., Gadkar V.J., Pernica J.M., Hoang L.M.N.. Self-collected saline gargle samples as an alternative to health care worker-collected Nasopharyngeal Swabs for COVID-19 diagnosis in outpatients. *J Clin Microbiol* 2021 Mar 19;**59**(4) e02427-20PMID: 33514627; PMCID: PMC8092743. doi:10.1128/JCM.02427-20.
3. Saito M., Adachi E., Yamayoshi S., Koga M., Iwatsuki-Horimoto K., Kawaoka Y., Yotsuyanagi H.. Gargle lavage as a safe and sensitive alternative to swab samples to diagnose COVID-19: a Case Report in Japan. *Clin Infect Dis* 2020 Jul 28;**71**(15):893–4 PMID: 32241023; PMCID: PMC7184508. doi:10.1093/cid/ciaa377.
4. Wu J., Liu J., Zhao X., Liu C., Wang W., Wang D., Xu W., Zhang C., Yu J., Jiang B., Cao H., Li L.. Clinical characteristics of imported cases of Coronavirus Disease 2019 (COVID-19) in Jiangsu Province: a multicenter descriptive study. *Clin Infect Dis* 2020 Jul 28;**71**(15):706–12 PMID: 32109279; PMCID: PMC7108195. doi:10.1093/cid/ciaa199.
5. Lui G., Ling L., Lai C.K., Tso E.Y., Fung K.S., Chan V., Ho T.H., Luk F., Chen Z., Ng J.K., Chow K.M., Cheng P.K., Chan R.C., Tsang D.N., Gomersall C.D., Hui D.S., Chan P.K.. Viral dynamics of SARS-CoV-2 across a spectrum of disease severity in COVID-19. *J Infect* 2020;**81**(2):318–56 Aug Epub 2020 Apr 18. PMID: 32315724; PMCID: PMC7166038. doi:10.1016/j.jinf.2020.04.014.
6. Saito M., Adachi E., Yamayoshi S., Koga M., Iwatsuki-Horimoto K., Kawaoka Y., Yotsuyanagi H.. Gargle Lavage as a safe and sensitive alternative to swab samples to diagnose COVID-19: a case report in Japan. *Clin Infect Dis* 2020 Jul 28;**71**(15):893–4 PMID: 32241023; PMCID: PMC7184508. doi:10.1093/cid/ciaa377.
7. Bennett S., Davidson R.S., Gunson R.N.. Comparison of gargle samples and throat swab samples for the detection of respiratory pathogens. *J Virol Methods* 2017;**248**:83–6 Oct Epub 2017 Jun 17. PMID: 28633963. doi:10.1016/j.jviromet.2017.06.010.
8. Mittal A., Gupta A., Kumar S., Surjit M., Singh B., Soneja M., et al. Gargle lavage as a viable alternative to swab for detection of SARS-CoV-2. *Indian J Med Res* 2020;**152**(1 & 2):77–81 Jul & Aug PMID: 32820725; PMCID: PMC7853282. doi:10.4103/ijmr.IJMR_2987_20.

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## Low incidence rate of diarrhoea in COVID-19 patients is due to integrin

*Dear Editor,*

Coronavirus disease-19 (COVID-19), which was caused by Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2), has been a global pandemic. The first key step of SARS-CoV-2 entry is attachment and binding to cell receptor angiotensin-converting enzyme 2 (ACE2) by spike protein (S).[1] Integrin family consists of 18 $\alpha$ and 8 $\beta$ subunits, which form tens of transmembrane heterodimers. It has been determined that many integrins associated with other proteins by recognizing RGD or KGD peptide. Previously we proposed an inhibitory role for integrin in the receptor targeting of SARS-CoV-2.[2] Both S protein of SARS-CoV-2 and ACE2 have an RGD in their sequences, with ACE2 possessing a KGD.[2] Based on the integrin-binding motif in S sequence, other researchers hypothesized that integrin could be an alternative receptor for SARS-CoV-2.[3] However, the real function of integrin in SARS-CoV-2 entry is not determined experimentally.

To verify the interaction of integrin with SARS-CoV-2 S and ACE2, we over-expressed these proteins in 293T cells and performed co-immunoprecipitation (co-IP). Integrin $\beta$5 was found to interact with S and ACE2 (Fig. 1A). The association of integrin $\alpha$V with S and ACE2 was also confirmed (Fig. S1). These results indicated that integrin can interact with the S protein of SARS-CoV-2 and ACE2, respectively. Because RGD/KGD residues are classical integrin-binding motifs, we went on to determine the dependence of these motifs in S-integrin and ACE2-integrin interactions. We constructed RGE and RGA mutants of S. Co-IP showed that either RGE or RGA bound with integrin $\beta$5 less (Fig. 1B), suggesting that RGD motif was important for integrin binding. S 405E mutation significantly suppressed integrin $\alpha$V binding (Fig. 1B). The KGD motif in ACE2 was mutated to KGE and KGA. The amount of mutant ACE2 co-immunoprecipitated with integrin $\alpha$V and $\beta$5 was lower than wild type ACE2 (Fig. S2), indicating that KGD residues was involved in ACE2-integrin interaction.

Having confirmed the interaction of integrin with S and ACE2, we proposed that integrin potentially interrupted receptor targeting of SARS-CoV-2 S. To test our hypothesis, we transfected plasmids expressing ACE2 and S into 293T cells in the presence or absence of integrin. GFP-tagged S co-precipitated less ACE2 in the presence of integrin $\alpha$V (Fig. 1C). Co-IP using S, ACE2 and integrin $\beta$5 came to the same result (Fig. 1C). These results demonstrated that integrin could interfere with the binding of ACE2 and SARS-CoV-2 S protein.

The inhibitory role of integrin in S-ACE2 binding suggested that the integrin could perturb the entry of SARS-CoV-2. Taking advantage of pseudovirus system which has been successfully established in our lab, we went further to determine the role of integrin in SARS-CoV-2 entry. This SARS-CoV-2 pseudovirus particle (SARS-CoV-2pp) was packaged by four plasmids expressing firefly luciferase, gag, rev, S protein, respectively. The S is changed to envelope G protein of vesicular stomatitis virus (VSV) for constructing VSVpp. We first tested whether integrin itself supported SARS-CoV-2 entry by transfecting 293T cells with integrin $\alpha$V only. In contrast to 293T cells transfected with human ACE2, 293T cells expressing integrin $\alpha$V produced undetectable luciferase activity signal (Fig. S3). This result indicated that integrin was not a receptor for SARS-CoV-2 pseudovirus. Then we examined the effect of exogeneous integrin on SARS-CoV-2 entry into 293T cells expressing ACE2. Compared to unconditioned group, pretreatment of either SARS-CoV-2pp or cells with exogenous integrin $\alpha$V$\beta$5 reduced luciferase activity (Fig. 1D). However, incubating VSVpp with integrin $\alpha$V$\beta$5 did not affect VSVpp entry (Fig. 1D). Pretreating 293T cells slightly inhibited VSVpp entry. Integrin $\alpha$V$\beta$5 addition did not change cell viability in all these conditions (Fig. 1D).

Gastrointestinal symptoms have been described in COVID-19 patients.[4–7] However, the incidence rate of diarrhoea in COVID-19 patients is low.[8] We evaluated the expression of SARS-CoV-2 receptor ACE2 in intestinal tract by analyzing single-cell sequencing data (GSE125970) containing 14,537 human intestinal cells. 14 different cell clusters were verified in intestinal tissues. ACE2 was found mainly in intestinal epithelial progenitor cells and intestinal epithelial cells. Since integrin plays an inhibitory role in the receptor targeting of SARS-CoV-2, we examined the expression of various integrin subtypes in intestinal tract and found broad distribution of integrin A1, A2, A3, A6, AE, AV, B1, B4, B5, B6 and B8 in all 14 cell clusters (Fig. 2). In this case, the high level of intestinal integrin may be related to rare diarrhoea in COVID-19 patients.

In conclusion, our work found that both $\alpha$V and $\beta$5 subtypes of integrin inhibited S-ACE2 interaction and entry of SARS-CoV-2pp by binding to S and ACE2, which largely relied on RGD/KGD motifs. ACE2 has been proven to mediate SARS-CoV-2 entry into human cells by researches on multiple levels. ACE2 was found highly expressed in digestive system by single-cell RNA sequencing, while diarrhoea was not often reported in patients infected with SARS-CoV-2. This raised a question about the existence of inhibitory factors. The integrin-binding motif in S sequence makes integrin on plasma membrane become a candidate mediator. Our research for the first time experimentally identified the association between integrin and SARS-CoV-2 S. At the same time, co-IP results showed that both two types of integrin interacted with ACE2. These results indicate that integrin could play a role in S-ACE2 binding, which may not be promoting effect but inhibitory function. To verify our hypothesis, we performed co-IP in the presence of integrin and confirmed the inhibitory role of it in S-ACE2 binding. Integrin kills two birds with one stone that neutralizing virus by binding to S, meanwhile, masking the receptor by binding to ACE2. In accordance with co-IP results, less SARS-CoV-2pp entered into 293T cells when the virus and cells were preincubated with recombinant integrin $\alpha$V$\beta$5 protein. This result not only demonstrated the inhibitory effect of integrin on SARS-CoV-2 entry, but also provided a strategy for antivirus research. Combined with our sc-RNA analysis which showed high expression levels of multiple integrin types in intestine, the low incidence of diarrhoea in COVID-19 patients

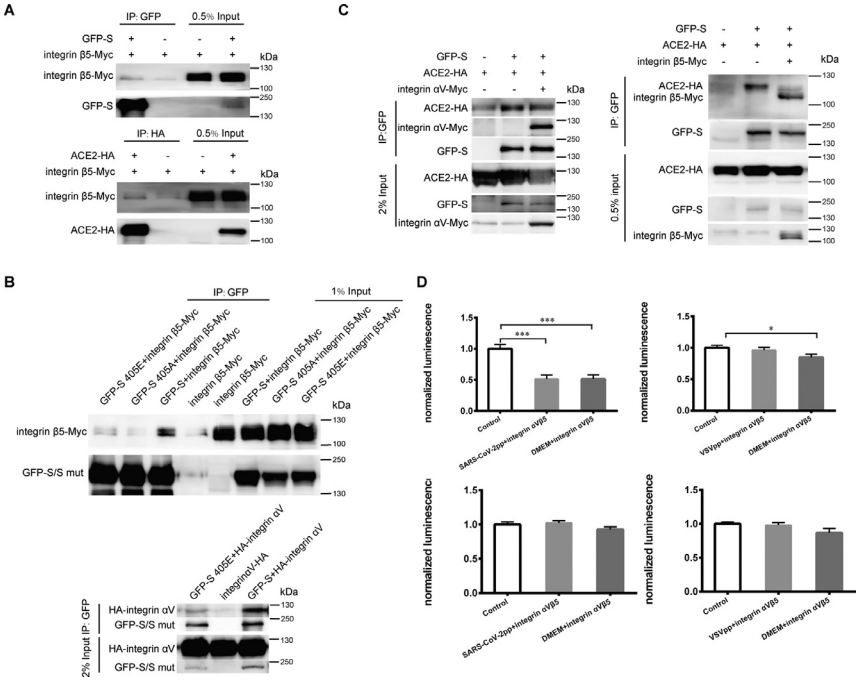


**Fig. 1.** *Integrin inhibited SARS-CoV-2 pseudovirus infection through inhibition of S-ACE2 binding.* (A) Integrin $\beta5$ interacted with SARS-CoV-2 S and ACE2. Top, Myc-tagged integrin $\beta5$ with or without GFP-tagged S was transfected into 293T cells. Cell lysates were immunoprecipitated using anti-GFP antibody. Bottom, integrin $\beta5$-Myc was expressed in 293T cells with or without ACE2-HA. Cell lysates were precipitated with anti-HA antibody. (B) RGD motif in S protein was important for its binding with integrin $\beta5$ and integrin $\alpha V$. Top, Wild type S and two single-amino acid mutants (405E, 405A) were expressed in 293T cells receptively with integrin $\beta5$-Myc. Bottom, wild type S or S 405E mutant was expressed in 293T cells. Lysates was immunoprecipitated by GFP. (C) SARS-CoV-2 S and ACE2 interaction was inhibited by integrin $\alpha V$ and integrin $\beta5$. Left, GFP-tagged full-length S and HA-tagged ACE2 were co-expressed in 293T cells with or without Myc-tagged integrin $\alpha V$. Right, GFP-S and ACE2-HA were transfected with or without integrin $\beta5$-Myc. Cell lysates were immunoprecipitated by GFP antibody. (D) Integrin $\alpha V\beta5$ protein inhibited SARS-CoV-2 pseudovirus infection. 293T cells were transiently transfected with ACE2-expressing plasmid. Left top: SARS-CoV-2 pseudovirus and ACE2-expressing cells were separately treated with recombinant human integrin $\alpha V\beta5$ protein. Then the 293T cells were infected by SARS-CoV-2 pseudovirus for 48 h and pseudovirus entry was indicated with luciferase activity. Left down: Cell viabilities were measured. Right top: ACE2-expressing 293T cells and VSVpp were pretreated with integrin $\alpha V\beta5$ protein. Then the cells were infected with integrin $\alpha V\beta5$ conditioned or unconditioned VSVpp. Luciferase activity was measured after 48 h. Right down: Cell viability under two conditions were not affected.

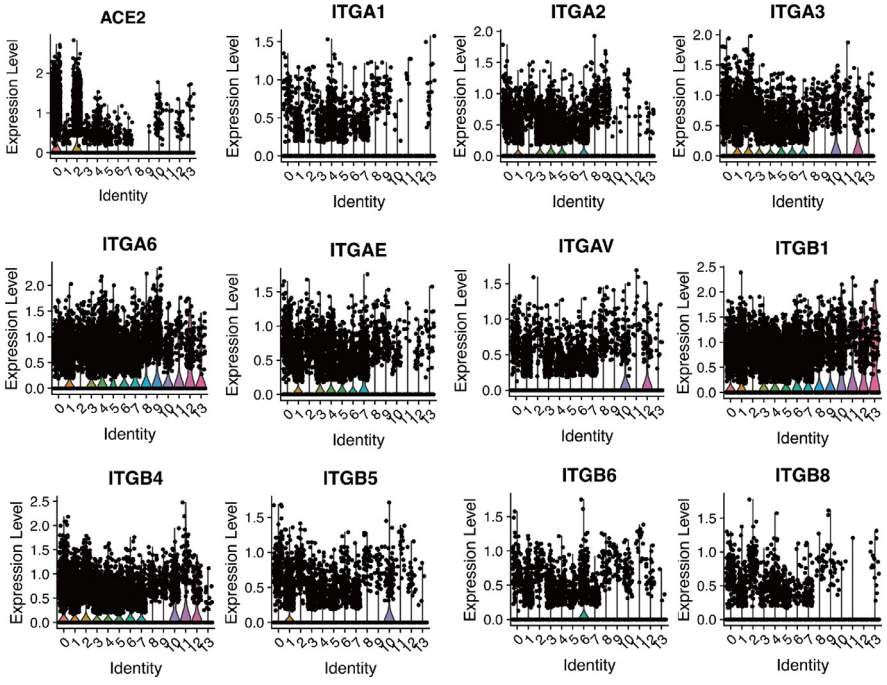


**Fig. 2.** *Single-cell analysis of ACE2 and different integrin subtypes expression in human intestine.* The expression of ACE2, integrin A1 (ITGA1), A2(ITGA2), A3(ITGA3), A6(ITGA6), AE(ITGAE), AV(ITGAV), B1(ITGB1), B4(ITGB4), B5(ITGB5), B6(ITGB6) and B8(ITGB8) in 14 cell populations were presented by violin plots. 0, small intestine enterocyte progenitor cell; 1, large intestine paneth-like cell; 2, small intestine enterocyte; 3, large intestine stem cell; 4, small intestine stem cell; 5, small intestine paneth cell; 6, large intestine enterocyte; 7, small intestine transit amplifying cell; 8, large intestine goblet cell; 9, small intestine goblet cell; 10, large intestine enterocyte progenitor cell; 11, large intestine enteroendocrine cell; 12, large intestine transit amplifying cell; 13, small intestine enteroendocrine cell.

may be partially explained. The function of integrin in virus-host interaction may need re-defined.

## Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.07.007.

## References

1. Zhou P., Yang X.L., Wang X.G., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020;**579**:270–3.
2. Luan J., Lu Y., Gao S., Zhang L.. A potential inhibitory role for integrin in the receptor targeting of SARS-CoV-2. *J Infect* 2020;**81**:318–56.
3. Sigrist C.J., Bridge A., Le Mercier P.. A potential role for integrins in host cell entry by SARS-CoV-2. *Antiviral Res* 2020;**177**:104759.
4. Jin X., Lian J.S., Hu J.H., et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut* 2020;**69**:1002–9.
5. Liang W., Feng Z., Rao S., et al. Diarrhoea may be underestimated: a missing link in 2019 novel coronavirus. *Gut* 2020;**69**:1141–3.
6. Lin L., Jiang X., Zhang Z., et al. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut* 2020;**69**:997–1001.
7. Song Y., Liu P., Shi X.L., et al. SARS-CoV-2 induced diarrhoea as onset symptom in patient with COVID-19. *Gut* 2020;**69**:1143–4.
8. Mao R., Qiu Y., He J.S., et al. Manifestations and prognosis of gastrointestinal and liver involvement in patients with COVID-19: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol* 2020;**5**:667–78.

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## Cytomegalovirus infection in critically ill patients with COVID-19

*To the Editor,*

Previous studies have shown that patients hospitalised with coronavirus disease 2019 (COVID-19) have a high risk of secondary infections; intensive care unit (ICU) admission, prolonged mechanical ventilation, and severe lymphopenia are likely causes. [1] Cytomegalovirus (CMV) has infected the majority of people globally. The reactivation of CMV occurs in 30% of immunocompetent patients with acute respiratory distress syndrome (ARDS) and is associated with increased mortality.[2] CMV reactivation is especially concerning in patients with COVID-19 because ARDS is a common complication of severe COVID-19. However, data on CMV infection in critically ill patients with COVID-19 is scarce. Therefore, we evaluated the frequency and clinical characteristics of CMV cases in COVID-19 patients who required mechanical ventilation.

We retrospectively reviewed consecutive patients with COVID-19 requiring invasive mechanical ventilation for more than one week in our centre between April 2020 and February 2021. We collected data on clinical characteristics and laboratory findings from medical charts. All patients were confirmed as having severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positivity by reverse-transcription polymerase chain reaction (PCR) on nasopharyngeal and throat swabs. We screened for CMV infection using the CMV antigenaemia assay for detecting pp65 antigen in peripheral blood leukocytes. We analysed patients with serial assays for CMV screening during their ICU stay. CMV infection was defined as $\geq$ 1 antigen-positive cell per 50,000 leukocytes and two consecutive positive assays. CMV disease was classified as proven, probable, or possible according to a previous study.[3] The institutional review board approved this study and waived the requirement for informed consent. Data is presented as median and interquartile range. Continuous variables were analysed using the Mann-Whitney *U* test, while categorical variables were analysed using Fisher's exact test. The duration of mechanical ventilation was analysed using the Kaplan–Meier method, and the differences between groups were compared using the log-rank test. The duration of mechanical ventilation in the CMV group was defined as the time from initiation of mechanical ventilation to CMV diagnosis. All *p*-values were two-sided, and a $p$-value $< 0.05$ was considered statistically significant. All statistical analyses were conducted using *R*, version 3.6.3.

A total of twenty-six patients were enroled in our study. All patients had negative CMV antigenaemia screening tests on ICU admission. Of twenty-six patients, 6 patients (23.1%) developed CMV infection during mechanical ventilation (CMV group), while the remaining patients did not develop CMV during mechanical ventilation (non-CMV group). There was no significant difference in the testing interval for CMV antigenaemia assay between the groups (median, 8 days vs. 7 days, $P = 0.76$). Table 1 summarises patient characteristics at ICU admission and clinical course. The median ages were 76.5 (66.25–80) years and 72 (62.5–76) years in the CMV and non-CMV groups, respectively ($P = 0.38$). Lymphocyte count on ICU admission was lower in the CMV group than in the non-CMV group (393 /$\mu$L vs. 525 /$\mu$L; $P = 0.062$). *C*-reactive protein, serum albumin, and *D*-dimer levels were not significantly different between the groups. The CMV group had a significantly longer duration of mechanical ventilation than the non-CMV group (40.5 days vs. 18.0 days, respectively, $P = 0.010$). The CMV group also had a significantly higher incidence of complications from bacterial or fungal infections and mortality rate. Two out of six patients in the CMV group died, whereas none of the patients in the non-CMV group died ($P = 0.046$).

**Table 1**
Characteristics at ICU admission and clinical course according to CMV infection.

| | CMV infection ($n = 6$) | Non-CMV infection ($n = 20$) | p-value |
|---|---|---|---|
| **Characteristics at ICU admission** | | | |
| Age, yr | 76.5 (66.25–80) | 72 (62.5–76) | 0.38 |
| Sex, female, *n* (%) | 3 (50%) | 8 (40%) | >0.99 |
| PaO2 / FiO2 ratio | 173.5 (161.3–180.8) | 188.2 (155.6–246.2) | 0.39 |
| Lymphocyte count, /$\mu$L | 393 (234–476) | 525 (412–723) | 0.062 |
| Neutrophil-to-lymphocyte ratio | 20.2 (13.0–32.2) | 12.0 (7.5–21.7) | 0.083 |
| C-reactive protein, mg/dL | 8.42 (5.45–16.02) | 5.29 (4.26–10.24) | 0.32 |
| Serum albumin, g/dL | 2.75 (2.63–3.10) | 2.70 (2.40–3.23) | 0.78 |
| D-dimer, $\mu$g/mL | 1.49 (1.08–1.95) | 2.13 (1.21–6.79) | 0.24 |
| **Clinical course** | | | |
| Corticosteroid use, *n* (%) | 6 (100%) | 20 (100%) | >0.99 |
| Duration of mechanical ventilation, days | 40.5 (31.0-NA) | 18.0 (13.0–31.0) | 0.010 |
| Bacterial infection, *n* (%) | 5 (83.3%) | 5 (25%) | 0.018 |
| Fungal infection, *n* (%) | 4 (66.7%) | 2 (10%) | 0.013 |
| Death, *n* (%) | 2 (33.3%) | 0 (0%) | 0.046 |

Duration of mechanical ventilation in the CMV group indicates the time from initiation of MV to CMV diagnosis. CMV: cytomegalovirus; NA: not available.

**Table 2**
Clinical summary of six patients with CMV infection.

| Case | Age/Sex | MV duration (days) | Median test interval (days) | Days since the last negative test | pp65 cell count (/$10^5$ WBC) | CMV disease | Outcome |
|---|---|---|---|---|---|---|---|
| 1 | 80/F | 33 | 9 | 14 | 1348 | Proven | Death |
| 2 | 80/M | 31 | 8 | 6 | 16 | Possible | Recovery |
| 3 | 82/M | 38 | 7 | 6 | 2 | – | Recovery |
| 4 | 58/F | 43 | 7 | 8 | 8 | Possible | Recovery |
| 5 | 64/F | 58 | 9 | 3 | 42 | Possible | Recovery |
| 6 | 73/M | 38 | 8 | 5 | 2 | – | Death |

MV duration indicates the time from initiation of MV to CMV diagnosis. CMV: cytomegalovirus; MV: mechanical ventilation; WBC: white blood cell.

Table 2 summarises the six patients who developed CMV infection. Although all patients received ganciclovir therapy for CMV infection, two patients eventually died from refractory respiratory failure. The patient in Case 1 died four days after a positive CMV antigenaemia test result; the patient was diagnosed with CMV pneumonia from post-mortem lung findings. In this patient, the antigenaemia assay was performed four times, with a median test interval of 9 days, except for a two-week gap between the negative third and the positive fourth results (high positive cell count). The patient in Case 6, the only patient who developed neither bacterial nor fungal secondary infection, was considered to have died from ARDS due to refractory COVID-19 pneumonia.

We investigated the frequency and characteristics of CMV infection in critically ill patients with COVID-19 requiring mechanical ventilation for more than one week. In our study, approximately one in four patients developed CMV infection during mechanical ventilation and one patient died from CMV pneumonia. CMV infection was associated with lymphopenia on ICU admission, prolonged mechanical ventilation, and increased mortality. Our results suggest that CMV disease may be underestimated in COVID-19 patients in the ICU setting.

Risk factors for CMV disease or its recurrence include corticosteroid use, prolonged mechanical ventilation, and lymphopenia.[4,5] COVID-19 contains these aspects due to its disease characteristics. Moreover, these are risk factors for disease recurrence or secondary infections in patients with COVID-19.[1,6,7] Further, infection with SARS-CoV-2 induces M1 polarisation of macrophages that promote the reactivation of latent CMV; inflammatory cytokines such as tumour necrosis factor-$\alpha$ may be directly associated with CMV reactivation. Importantly, CMV infection may be associated with accelerated immunosenescence, leading to the attrition of naive T cells.[8] The decreased naive T-cell response may contribute to subsequent uncontrolled cytokine production and worse clinical outcomes. Therefore, physicians should be extremely aware of CMV infection in patients with COVID-19 ARDS compared to that in those with non-COVID-19 ARDS.

CMV reactivation and virus-induced immune dysfunction may be overlooked as a cause of immunomodulation in patients with severe COVID-19.[9] Considering that the lung is a major reservoir for CMV and patients with COVID-19 are at risk for CMV disease,[10] CMV pneumonia may be underestimated in critically ill patients with COVID-19 pneumonia.[11] Untreated infection can lead to rapid deterioration and fatal outcomes. Therefore, routine monitoring for CMV infection may help improve outcomes in COVID-19 patients in the ICU setting.

This study had some limitations. First, this is a preliminary retrospective study with a small sample, precluding definite conclusions. Second, we screened for CMV infection using the CMV antigenaemia assay. This might be inferior to PCR in case of leukopenia. Finally, in patients with COVID-19, the optimal threshold value for CMV infection and the significance of pre-emptive anti-CMV therapy remains unclear. Further research is needed to define the management of secondary CMV infection in critically ill patients with COVID-19 who are at high risk for CMV reactivation.

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## Declaration of Competing Interest

The authors declare no conflict of interest.

## References

[1]. Ripa M., Galli L., Poli A., et al. Secondary infections in patients hospitalized with COVID-19: incidence and predictive factors. *Clin Microbiol Infect* 2021;**27**:451–7.
[2]. Ong D.S.Y., Spitoni C., Klein Klouwenberg P.M.C., et al. Cytomegalovirus reactivation and mortality in patients with acute respiratory distress syndrome. *Intensive Care Med* 2016;**42**:333–41.
[3]. Ljungman P., Boeckh M., Hirsch H.H., et al. Definitions of cytomegalovirus infection and disease in transplant patients for use in clinical trials. *Clin Infect Dis* 2017;**64**:87–91.
[4]. Al-Omari A., Aljamaan F., Alhazzani W., et al. Cytomegalovirus infection in immunocompetent critically ill adults: literature review. *Ann Intensive Care* 2016;**6**:110.
[5]. Gardiner B.J., Nierenberg N.E., Chow J.K., et al. Absolute lymphocyte count: a predictor of recurrent cytomegalovirus disease in solid organ transplant recipients. *Clin Infect Dis* 2018;**67**:1395–402.
[6]. Adachi Y., Shiroyama T., Yamaguchi Y., et al. Predicting recurrence of respiratory failure in critically ill patients with COVID-19: a preliminary study. *J Infect* 2021;**82**:e33–5.
[7]. van Paassen J., Vos J.S., Hoekstra E.M., et al. Corticosteroid use in COVID-19 patients: a systematic review and meta-analysis on clinical outcomes. *Crit Care* 2020;**24**:696.
[8]. Moss P.. The ancient and the new": is there an interaction between cytomegalovirus and SARS-CoV-2 infection? *Immun Ageing* 2020;**17**:14.
[9]. Söderberg-Nauclér C.. Does reactivation of cytomegalovirus contribute to severe COVID-19 disease? *Immun Ageing* 2021;**18**:12.
[10]. Poole E., Juss J.K., Krishna B., et al. Alveolar macrophages isolated directly from human cytomegalovirus (HCMV)-seropositive individuals are sites of HCMV reactivation in vivo. *J Infect Dis* 2015;**211**:1936–42.
[11]. Le Balc'h P., Pinceaux K., Pronier C., et al. Herpes simplex virus and cytomegalovirus reactivations among severe COVID-19 patients. *Crit Care* 2020;**24**:530.



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## Initial viral load and decay kinetics of SARS-CoV-2 lineage B.1.1.7 in the upper respiratory tract of adults and children

*Dear Editor,*

We read with interest the systematic review published by Walsh et al. in the Journal of Infection,[1] focusing on the dynamics of SARS-CoV-2 RNA at the upper respiratory tract (URT). In this context, a novel SARS-CoV-2 variant lineage (B.1.1.7), first detected in the UK at the end of 2020 has transmission advantage over other lineages.[2] Increased transmissibility of the B.1.1.7 variant has been linked to enhanced ACE2 affinity[3] allegedly resulting in higher viral loads in URT, an observation that has been reported in some,[3–6] but not all[7] large series published to date. In addition, longer duration of SARS-CoV-2 RNA shedding in URT has been reported in individuals infected by the B.1.1.7 variant as compared to controls;[8] if proven, this may have important implications regarding isolation policies. The current retrospective, observational study was undertaken to gain further insight into the above issues. It included a convenience sample of 990 individuals (799 aged >18 years; 507 females) testing positive for SARS-CoV-2 RNA in nasopharyngeal specimens (NP) by the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, MS, USA) between June 2020 and April 2021. The study was approved by INCLIVA Research Ethics Committee. A total of 338 subjects (median age, 38 years; range, 1–93 years) were infected by the SARS-CoV-2 lineage B.1.1.7 (179 had COVID-19-Supplementary Table 1). The study included a control group of 652 individuals, 339 presenting with COVID-19 (median age, 40.6 years; range, 0–95 years) infected by other variants, of which 390 were characterized by whole-genome sequencing (Supplementary Table 2). Individuals belonging to the control group were matched with the B.1.1.7 group for sex and age. Among patients with COVID-19, the time from symptoms onset to RT-PCR testing was 5 days (range, 1–10 days) in the B.1.1.7 group and 4 days (range, 1–10 days) in the control group, with no differences between children and adults. As for asymptomatic individuals (140 infected by the B.1.1.7 variant), RT-PCR testing was performed within the first 10 days since diagnosis (for household) or contact with (for non-household) the index case in individuals from both groups. A total of 1152 NP specimens (median 1 specimen/patient; range, 1–3) were included in the analyses described below.

We found that SARS-CoV-2 B1.1.7-infected individuals displayed initial NP viral loads around 1 $\log_{10}$ higher than controls (median, 7.6 $\log_{10}$ copies/ml; range, 3.3–12.1 vs. 6.8 $\log_{10}$ copies/ml; range, 2.4–13.1; $P < 0.001$), a figure that concurs remarkably with that observed by Jones et al.,[4] and overall support previous observations[3–6] reported in studies involving large cohorts, that were nevertheless poorly defined regarding subject age, individual clinical condition at diagnosis, timing of URT specimen collection or all of the above. A subanalysis including only SARS-CoV-2 B1.1.7-infected individuals as confirmed by whole-genome sequencing ($n = 108$) yielded comparable results ($P < 0.001$) (not shown). A novel observation was that the difference in viral load between B.1.1.7 and non-B.1.1.7 infected individuals remained significant ($P < 0.001$) for adults (Fig. 1B), but not for children ($P = 0.41$) (Fig. 1C). When symptomatic patients infected by either the B.1.1.7 or other variants were analyzed separately, initial viral loads were also higher ($P = 0.04$) for adults (Fig. 1D), but again not ($P = 0.16$) for children (Fig. 1E), although a trend towards higher viral loads was noticeable in B.1.1.7 infected subjects. We hypothesize that a more robust early innate immune response to SARS-CoV-2 in children as compared to adults may minimize the apparent replicative advantage of the B.1.1.7 variant over other less transmissible ones.[9] Likewise, Asymptomatic adults infected by the B.1.1.7 variant had higher initial SARS-CoV-2 RNA loads ($P = 0.02$) than controls (Fig. 1F).

It is of interest that SARS-CoV-2 RNA loads measured in the comparison groups were unlikely to be biased by differences in cellularity across NP specimens, as determined by amplification of the $\beta$-glucuronidase housekeeping gene by RT-PCR[10] in 50 randomly selected participants (24 infected by the B.1.1.7 variant and 26 from controls). In fact, median $C_T$ was similar ($P = 0.43$) between NP samples in both groups (not shown).

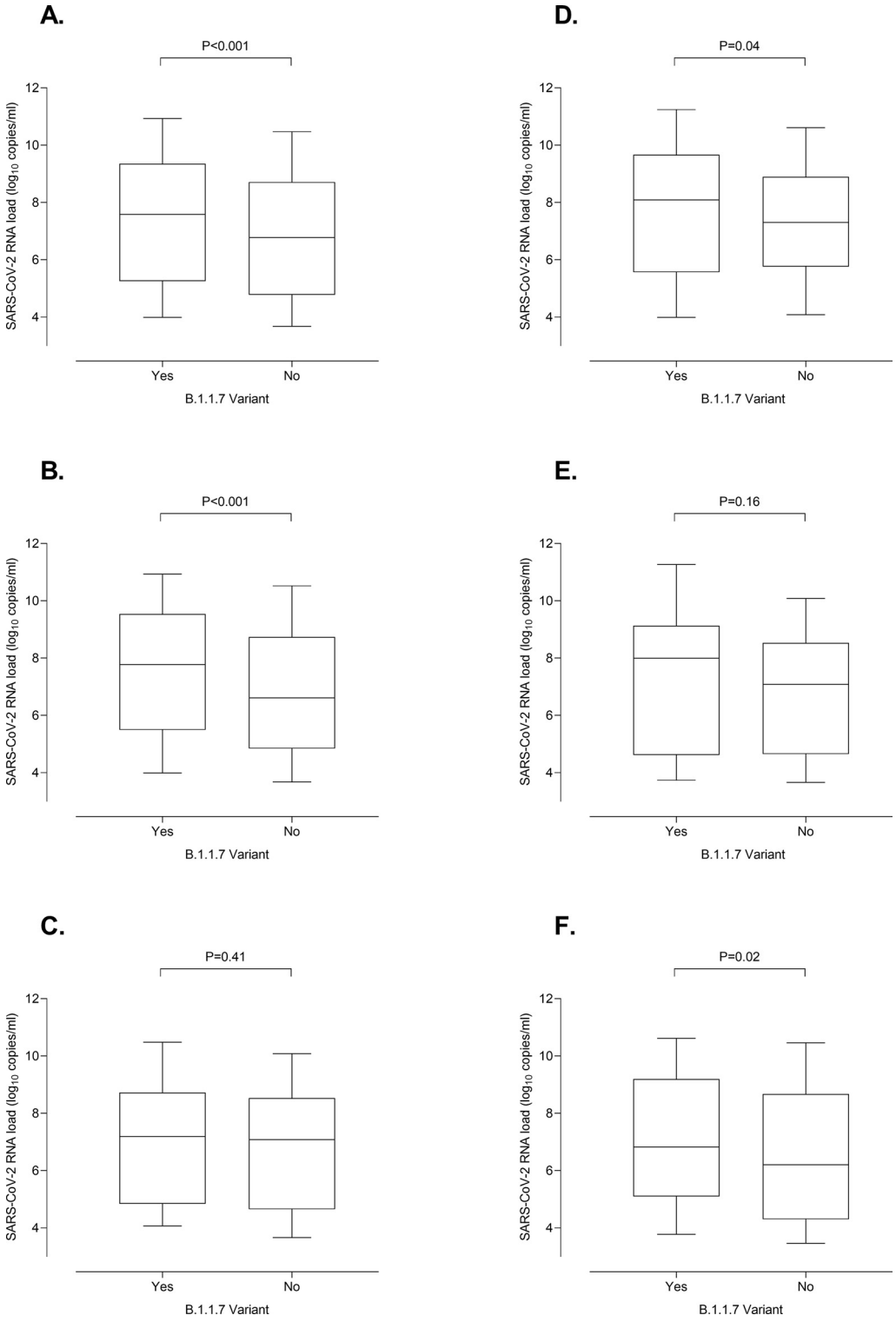


**Fig. 1.** Whisker plots depicting initial SARS-CoV-2 RNA loads in nasopharyngeal specimens from individuals infected by either the B.1.1.7 variant or other variants. Participants were sampled at either primary care centers affiliated to the Clínico-Malvarrosa Health Department, which attends 350,000 inhabitants in Northwest Valencia (Spain), or at its tertiary reference hospital (Hospital Clínico Universitario de Valencia, Spain-HCU-). NP were collected by trained nurses, placed in 3 ml of Universal Transport Medium (UTM, Becton Dickinson, Sparks, MD, USA) and delivered to the Microbiology Service of HCU for testing. Specimens were analyzed by RT-PCR within 24 h of receipt. We used the TaqPath COVID-19 Combo Kit (Thermo Fisher Scientific, MS, USA), which targets SARS-CoV-2 ORF1ab, N and S genes, following RNA extraction carried out by using the Applied Biosystems™ MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kits coupled with Thermo Scientific™ KingFisher Flex automated instrument. The AMPLIRUN® TOTAL SARS-CoV-2 RNA Control (Vircell SA, Granada, Spain) was used as the reference material for estimating SARS-CoV-2 RNA load (in copies/mL, taking RT-PCR $C_T$ for the N gene). The B.1.1.7 lineage was confirmed by whole-genome sequencing in 108 cases, whereas in the remaining 230 it was inferred by S-gene target failure (SGTF) in the RT-PCR as within the timeframe of specimen collection (mid-February-April 2021) more than 95% of SGTFs detected in the Clínico-Malvarrosa Health Department belonged to that lineage (not shown). Control individuals were sampled before the SARS-CoV-2 B1.1.7 variant was first detected in our Health Department in early January 2021. Moreover, the SARS-CoV-2 RNA S-gene was detected in NP from all these participants. The data are depicted for all participants (A); adults (B), children (≤18 years old) (C); adults with COVID-19 (D); children with COVID-19 (E) and asymptomatic adults (F). The absence of non-B.1.1.7-infected asymptomatic children in the cohort precluded meaningful comparison of viral loads between these individuals and those infected by other variants. Differences between medians were compared using the Mann-Whitney *U* test. The Chi-squared test was used for frequency comparisons. Two-sided *P*-values $< 0.05$ were considered statistically significant. Statistical analyses were performed using the SPSS v.25 program. *P* values for comparisons across groups are shown.

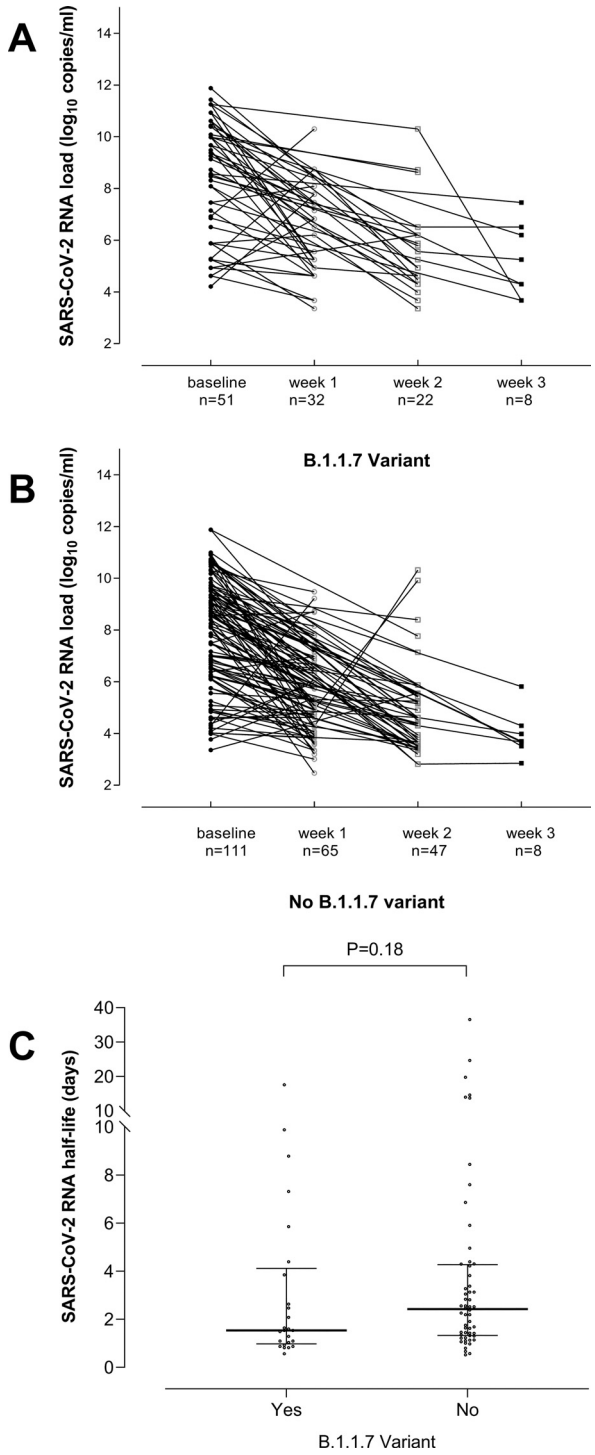


**Fig. 2.** Kinetics of SARS-CoV-2 RNA load in nasopharyngeal specimens from individuals either infected by the B.1.1.7 variant (A) or by other variants (B) through week 3 after diagnosis of SARS-CoV-2 infection. Trajectories of SARS-CoV-2 RNA load in URT were classified as rising or decreasing, as the variation in the viral load between consecutive specimens was > or <0.5 $\log_{10}$ copies/ml (upper limit of intra-assay $\log_{10}$ variation), respectively. SARS-CoV-2 RNA half-life in the upper respiratory tract from individuals either infected by the B.1.1.7 variant or by other variants (C). The kinetics of SARS-CoV-2 RNA clearance followed a logarithmic decay curve in most individuals, expressed by the equation $y_t=y_0e^{-kt}$, where $y_0$ is the initial SARS-CoV-2 RNA load, t is time of follow-up specimen sampling since diagnosis (initial RT-PCR result) and k is the decay constant. SARS-CoV-2 RNA load half-life was then calculated using the equation ln2/k. SARS-CoV-2 RNA half-life in URT was calculated only for individuals (25 infected by the B.1.1.7 variant and 56 controls) that met two criteria: (i) displaying a descending trajectory throughout the study period (three weeks); (ii) the follow-up specimen used for calculations was collected within 4–10 days after the initial one. *P* value for comparisons across groups is shown.

A total of 162 participants (51 infected by the B.1.1.7 variant and 111 controls), had 2 or more follow-up NP specimens collected within 3 weeks after diagnosis of SARS-CoV-2 infection. As shown in Fig. 2, decreasing viral loads were observed in most participants, regardless of whether they were infected with the B.1.1.7 variant (2A) or not (2B). SARS-CoV-2 RNA half-life in URT could be calculated for 25 individuals infected by the B.1.1.7 variant and 56 controls. Most individuals in both comparison groups were adults and were matched ($P > 0.5$) by sex, the presence or absence of symptoms, hospitalization (Supplementary Table 3) and age (median, 38 years in both groups). As shown in Fig. 2C, SARS-CoV-2 RNA load half-life was similar ($P = 0.18$) among groups (B.1.1.7 infected: median 1.54 days; range, 0.5–17 days; controls: 2.42 days; range, 0.27–36 days). In contrast to our findings, Calistri et al.[8] reported longer persistence of SARS-CoV-2 RNA in NP specimens in individuals infected with the B.1.1.7 variant (median 16 days) compared to controls (median 14 days). Nevertheless, the major drawbacks of that study were the lack of information on clinical status of participants and the absence of sequential specimens collected on an individual basis.

The current study has several limitations, most notably its retrospective design, the lack of follow-up specimens from many participants, the low numbers of children with B.1.1.7 symptomatic infection and with non-B.1.1.7 asymptomatic infection and the lack of precise information on the timing of specimen collection in asymptomatic subjects following contact with the index case. Furthermore, the number of individuals from whom viral load half-life could be calculated was limited.

In summary, our data support that initial SARS-CoV-2 load is higher in B.1.1.7-infected adult individuals than in those infected by other variants, regardless of presence or absence of symptoms, but not in symptomatic children. Nevertheless, the data did not suggest an extended duration of SARS-CoV-2 RNA shedding in the URT in individuals infected with the B.1.1.7 variant.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.08.015.

## References

1. Walsh K.A., Jordan K., Clyne B., Rohde D., Drummond L., Byrne P., et al. SARS–CoV-2 detection, viral load and infectivity over the course of an infection. *J Infect* 2020;**81**:357–71.
2. Davies N.G., Abbott S., Barnard R.C., Jarvis C.I., Kucharski A.J., Munday J.D., et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England. *Science* 2021;**372**:eabg3055.
3. Ramanathan M., Ferguson I.D., Miao W., Khavari P.A.. SARS-CoV-2 B1.1.7 and B.1.351 spike variants bind human ACE2 with increased affinity. *Lancet Infect Dis* 2021;**S1473-3099**(21):00262–3.

4. Jones T.C., Biele G., Mühlemann B., Veith T., Schneider J., Beheim-Schwarzbach J., et al. Estimating infectiousness throughout SARS-CoV-2 infection course. *Science* 2021:eabi5273.
5. Kidd M., Richter A., Best A., Cumley N., Mirza J., Percival B., et al. S-variant SARS-CoV-2 lineage B1.1.7 is associated with significantly higher viral load in samples tested by TaqPath polymerase chain reaction. *J Infect Dis* 2021;**223**:1666–70.
6. Ratcliff J., Nguyen D., Fish M., Rynne J., Jennings A., Williams S., et al. Virological and serological characterization of critically ill patients with COVID-19 in the UK: interactions of viral load, antibody status and B.1.1.7 variant infection. *J Infect Dis* 2021:jiab283. doi:10.1093/infdis/jiab283.
7. S.A. Walker, K.D. Vihta, O. Gethings, E. Pritchard, J. Jones, T. House, et al. Increased infections, but not viral burden, with a new SARS-CoV-2 variant. medRxiv 2021.01.13.21249721; 10.1101/2021.01.13.2124972.
8. Calistri P., Amato L., Puglia I., Cito F., Di Giuseppe A., Danzetta M.L., et al. Infection sustained by lineage B.1.1.7 of SARS-CoV-2 is characterised by longer persistence and higher viral RNA loads in nasopharyngeal swabs. *Int J Infect Dis* 2021;**105**:753–5.
9. Tosif S., Neeland M.R., Sutton P., Licciardi P.V., Sarkar S., Selva K.J., et al. Immune responses to SARS-CoV-2 in three children of parents with symptomatic COVID-19. *Nat Commun* 2020;**11**:5703.
10. Albert E., Ferrer B., Torres I., Serrano A., Alcaraz M.J., Buesa J., et al. Amplification of human $\beta$-glucuronidase gene for appraising the accuracy of negative SARS-CoV-2 RT-PCR results in upper respiratory tract specimens. *J Med Virol* 2021;**93**:48–50.



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## Evidence of the reduction of acute circulating communicable viruses during the SARS-CoV-2 pandemic in London

*Dear Editor,*

We read with interest the report by Poole and colleagues who found a sharp reduction in non-SARS-CoV2 respiratory virus infection during the COVID-19 pandemic.[1] To confirm these findings in children as well as adults, and to investigate the effect of the COVID-19 pandemic on gastrointestinal and varicella zoster virus infections, we undertook a review covering two, large hospitals in London: The Royal Free Hospital (RFH; a large teaching hospital in north London) and Great Ormond Street Hospital (GOSH; a specialist hospital for children in central London). We used a retrospective observational design and included all 'first positive' reverse transcriptase polymerase chain reaction (RT-PCR) test results between April 2019 and March 2021 for respiratory viruses (influenza A and B and respiratory syncytial virus (RSV) at RFH and GOSH, with parainfluenza, enterovirus and adenovirus also included from GOSH) and gastrointestinal viruses (norovirus at both RFH and GOSH, with other enteric viruses included at RFH (sapovirus, rotavirus, adenovirus and astrovirus), and varicella zoster virus results from RFH.

Comparing pre-pandemic (April 2019-March 2020) with pandemic periods (April 2020-March 2021) there was an 87.5% reduction in positive tests for gastrointestinal viruses at RFH and GOSH (585 vs. 73; Fig. 1) and a 62.2% reduction in paediatric respiratory viruses at GOSH (2381 vs. 899; Fig. 2). We observed 53.8% reduction in varicella at RFH (65 vs. 30), with the mean monthly number of cases being 5.4 pre-pandemic and 2.7 during the pandemic. There was also a 98.8% reduction in respiratory viruses at RFH (969 vs. 12), however due to the pandemic, demand management of all RT-PCR tests was implemented from October 2020, limiting direct comparisons.

Further analysis is needed to infer causality however the markedly shorter and less severe flu influenza epidemic and rapid decline in respiratory viruses coincide with the introduction of the wide range of non-pharmaceutical public health interventions (NPI) such as: movement restrictions, social distancing, face coverings and increased personal hygiene.[2] It's also possible that the introduction of restrictions including the reduction in global travel may have a part to play. There is also the question of viral interference during a pandemic. However, the evidence we present here suggests the COVID-19 pandemic has had a profound effect on circulating varicella, gastrointestinal, respiratory viruses in the populations covered by two large hospitals in London.

These findings support the growing evidence base covering a range of localities around the world. Data from the WHO Global Influenza Surveillance and Response system shows that across countries in the Southern Hemisphere there has been little Influenza activity since mid-April 2020, despite increased testing in some countries.[3] In the UK, Public Health England (PHE) have also reported that circulating seasonal respiratory viruses, other than SARS-CoV-2 was similar overall compared to levels reported in recent years with lower levels of Respiratory Syncytial Virus (RSV) seen than in previous seasons in England.[4] A significant reduction in RSV cases have also been shown in Australia.[5] In one area of the UK, rhinovirus detection in adults admitted to hospital was significantly lower in summer 2020 compared to 2019, however this increased once schools reopened in September 2020.[6]

There is also evidence of reduced circulation of enteric viruses. PHE has reported an 89% reduction in reported cases of norovirus and rotavirus during the seasons affected by the pandemic, when compared to the average of 2015–2020 seasons. [7] A German study has shown an almost complete reduction in positive norovirus

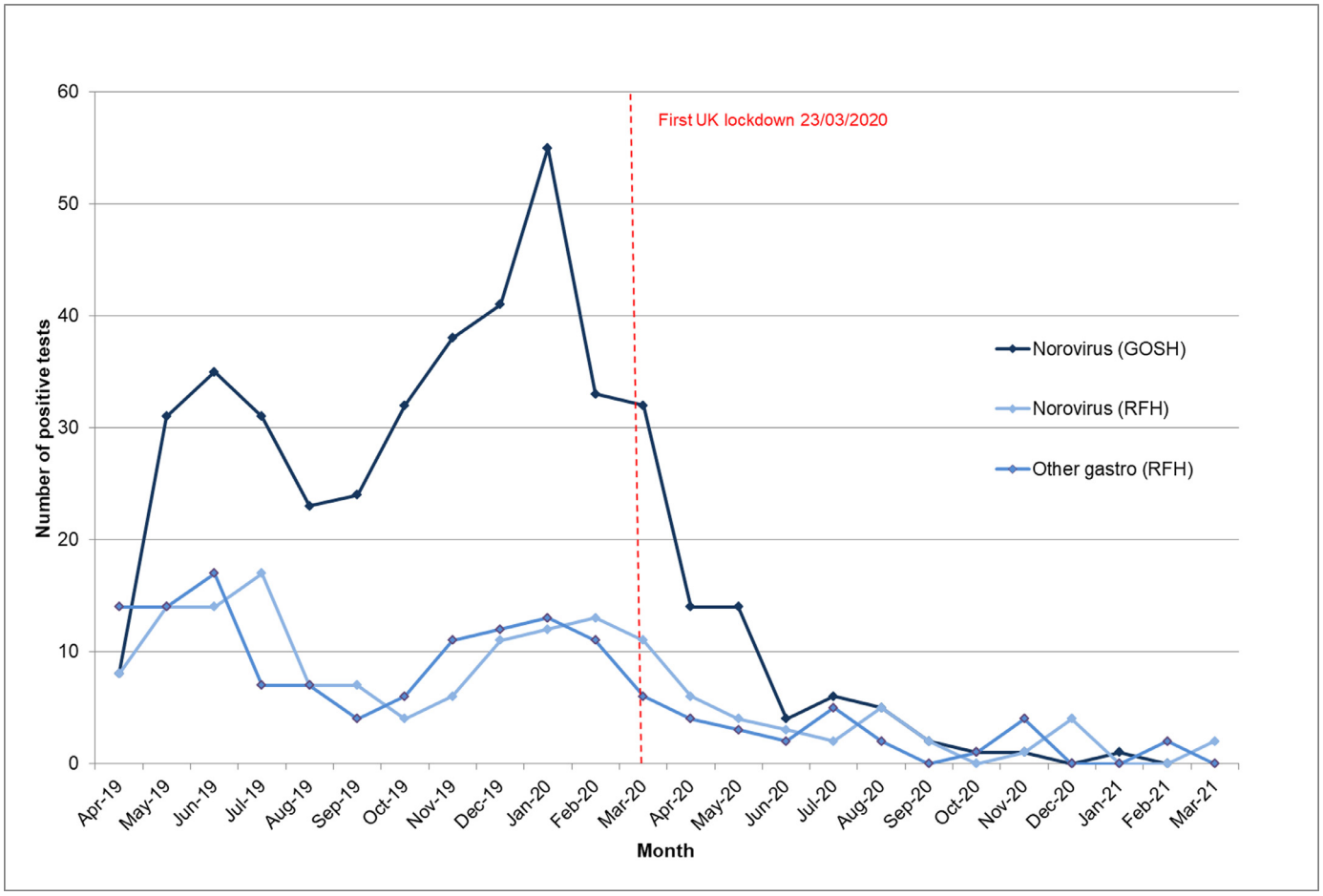


**Fig. 1.** A graph showing the monthly number of positive enteric virus tests between April 2019 and March 2021 at Great Ormond Street and Royal Free Hospitals.

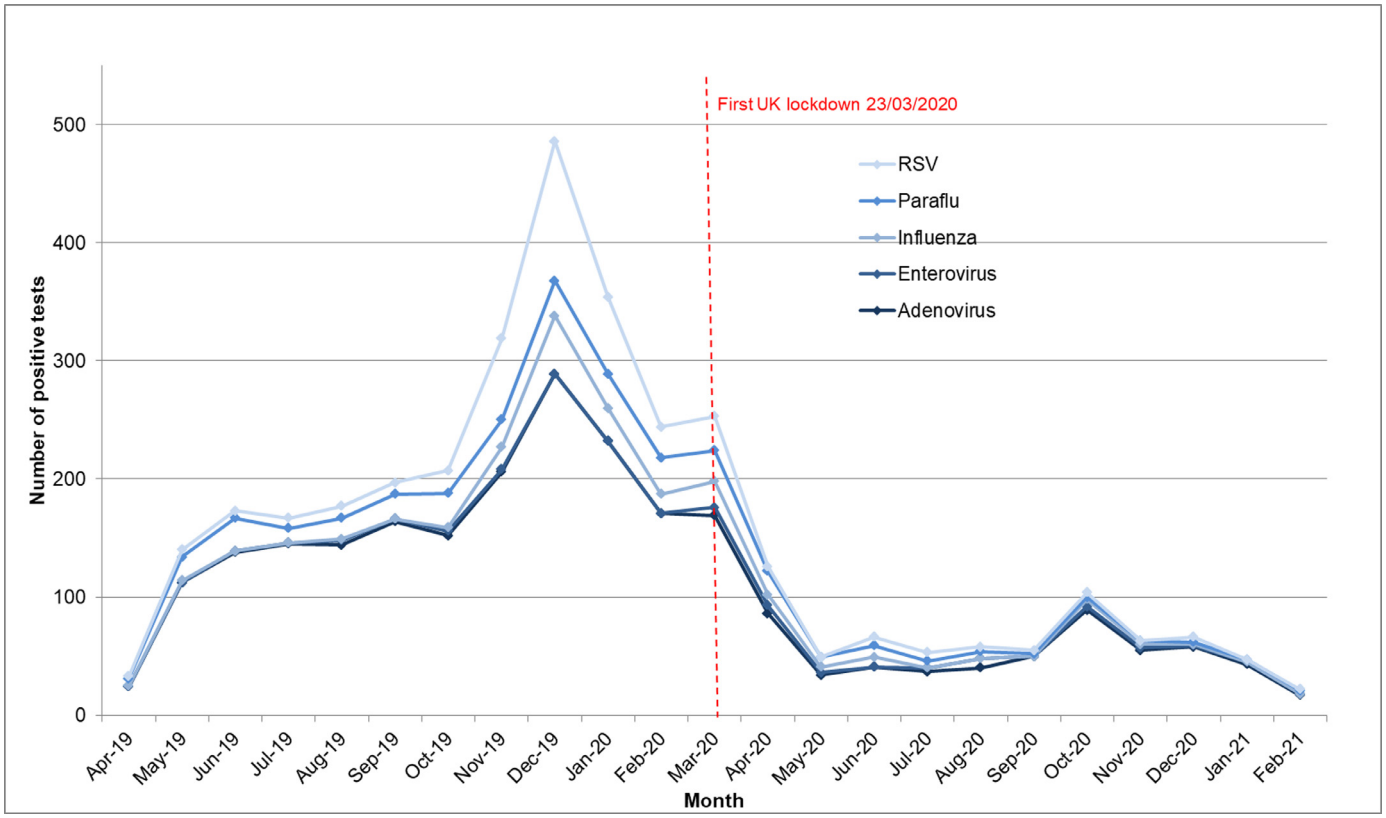


**Fig. 2.** A graph showing the monthly number of positive respiratory virus tests from April 2019 to February 2021 at Great Ormond Street Hospital.

tests;[8] and an Australian study demonstrated an 84.2% reduction in enterovirus detection.[9]

The decrease in varicella cases may lead to potential increases in complications as older children will still be susceptible, as well as a potential rise in cases due to the reduction in boosting of adults with varicella exposure.

These findings are unprecedented and likely to be similar for other transmissible viruses for which data are not routinely collected. Taken together, these data suggest that there is likely to have been a build-up in numbers of children and adults susceptible to these common infections with potential for epidemics to occur following easing of lockdown. We suggest that there is a need for modeling to better understand the possible impact on healthcare services.

## References


1. Poole, S.; Brendish, N.J. and Clark, T.W. SARS-CoV-2 has displaced other seasonal respiratory viruses: results from a prospective cohort study. *J Infect*:81(6):966–972.
2. Jones N.. How coronavirus lockdowns stopped flu in its tracks. *Nature* 2020 May 21Epub ahead of print. PMID: 32440021. doi:10.1038/d41586-020-01538-8.
3. Olsen S.J., Azziz-Baumgartner E., Budd A.P., Brammer L., Sullivan S., Pineda R.F., Cohen C., Fry A.M.. Decreased influenza activity during the COVID-19 Pandemic - United States, Australia, Chile, and South Africa. *MMWR Morb Mortal Wkly Rep* 2020;**69**(37):1305–9 2020 Sep 18.
4. PHE. Surveillance of influenza and other respiratory viruses in the UK. Winter 2019 to 2020. PHE publications gateway number: GW-1328. Pub. Public Health England, London, England.
5. Britton P.N., Hu N., Saravanos G., Shrapnel J., Davis J., Snelling T., Dalby–Payne J., Kesson A.M., Wood N., Macartney K., McCullagh C., Lingam R.. COVID-19 public health measures and respiratory syncytial virus. *Lancet Child Adolesc Health* 2020;**4**(11):e42–3 Nov.
6. Poole S., Brendish N.J., Tanner A.R., Clark T.W.. Physical distancing in schools for SARS-CoV-2 and the resurgence of rhinovirus. *Lancet Respir Med* 2020;**8**(12):e92–3 Dec.
7. Hotham M.. Potential future implications of the COVID-19 pandemic on Norovirus infections in England. *J Infect* 2021;**S0163-4453**(21):00241–3 May 13:.
8. Eigner U., Verstraeten T., Weil J.. Decrease in norovirus infections in Germany following COVID-19 containment measures. *J Infect* 2021;**82**(6):276–316 Jun;.
9. Briggink Leese D, et al. Decreased incidence of enterovirus and norovirus infections during the COVID-19 pandemic, Victoria, Australia, 2020. *Communicable Dis Intell* 2020:44 (2018).



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## Outcomes of Covid-19 organizing pneumonia in critically ill patients

*Dear Editor,*

Efficacy of corticosteroids in COVID-19 pneumonia has been reported in numerous studies.[1] However, Yang et al. concluded that corticosteroids have a negative impact, suggesting that not all patients benefit from the therapy.[2] Features of organizing pneumonia (OP) have been observed in radiological and histopathological studies from these patients.[3–5] OP has usually a good response to corticosteroids. The possible correlation between the radiological pattern and clinical evolution has important implications. This study provides information on patient selection and clinical application of corticosteroids.

Adult patients admitted to a respiratory intermediate care unit (RICU) from November 18th, 2020 to February 18th, 2021 were prospectively enrolled. Institutional review boards authorised the study. Informed consent was waived.

A confirmed case of COVID-19 pneumonia was defined as a patient with compatible symptoms and PCR-confirmed infection. Only patients needing advanced noninvasive respiratory support (NIRS) with high flow nasal cannula (HFNC), continuous positive airway pressure (CPAP) and/or noninvasive ventilation (NIV) were included. All patients had a “do intubate” (DI) or “do not intubate” (DNI) order defined at admission. Information regarding demographics, comorbidities, blood test results and mode and usage of NIRS were recorded. The severity of respiratory failure was assessed by PaO2/FiO2 ratio before NIRS institution. Several outcomes were evaluated: length of stay, need for endotracheal intubation (ETI) and in-hospital mortality.

OP was diagnosed, based on radiology reports, in the presence of bilateral patchy consolidation areas with subpleural and/or peribronchial distribution; perilobular pattern, with thick, ill-defined linear opacities with a polygonal or arcade appearance; and/or reverse halo sign.[3] Bacterial infection was excluded by clinical evaluation, procalcitonin levels, urinary antigens and blood cultures at admission. Pulmonary embolism was excluded using *D*-dimer levels and, if increased, CT pulmonary angiogram.

OP was treated, according to BTS 2008 guidelines[6]: methylprednisolone 500–1000 mg for 3–5 days, followed by 0.75–1 mg/Kg prednisone. Patients improving under 6 mg dexamethasone were switched to prednisone and methylprednisolone pulses were used as a rescue therapy if clinical deterioration and FiO2≥35%. GGO group was treated with 6 mg dexamethasone.[7] Side effects were recorded.

Baseline characteristics of patients with OP and GGO were compared. Continuous and categorical variables were compared using Student’s T test and Chi-squared or Fisher’s exact test, respectively. The association between OP and outcomes was calculated using a logistic regression model adjusted for age, CCI, DNI order and PaO2/FiO2. A two-sided test of $<0.05$ was considered statistically significant.

190 patients were admitted to RICU. At the time of the study, the hospital was attending 10% of the national cases of COVID-19 needing hospitalization. Due to this burden, CT scan was performed in 112. Fig. 1 illustrates patients’ allocation according to CT scan findings and outcomes. Table 1 lists patients characteristics and clinical outcomes according to cohort. Male gender was predominant and the mean age was 67.5 ± 12.1 years. OP group was significantly younger. The PaO2/FiO2 ratio was higher in OP group.

All patients were treated with corticosteroids. Mean time to dexamethasone was 8.5 ± 3.5 days and to OP treatment was 13.0 ± 5.5 days. 75.4% of OP patients were treated with pulse methylprednisolone and the remaining with prednisone 0.75–1 mg/Kg.

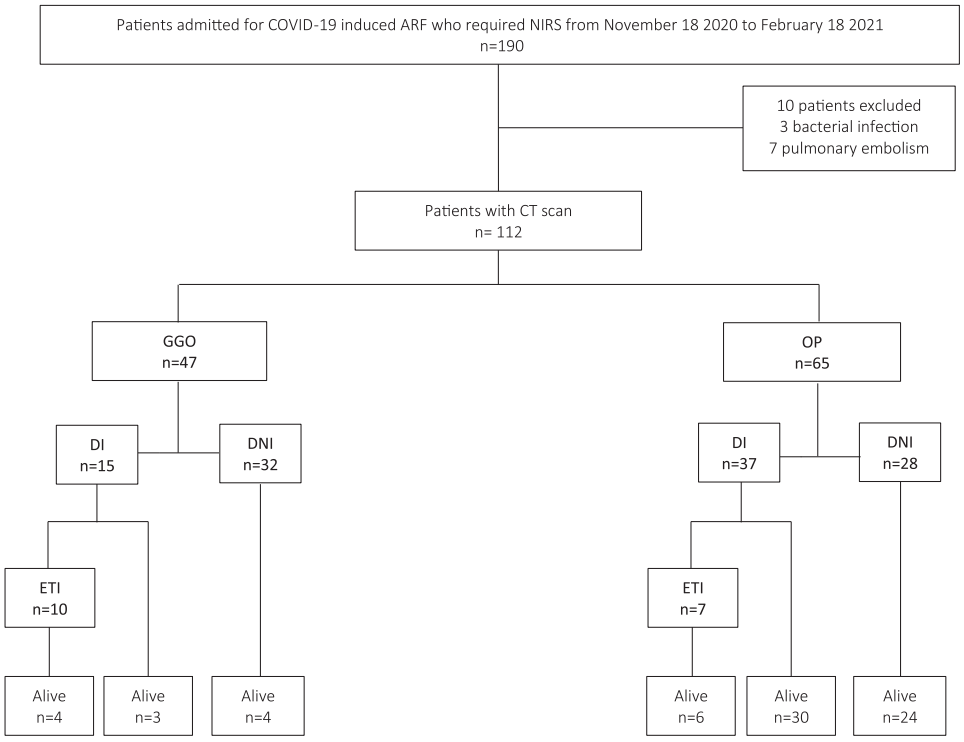


**Fig. 1.** Patient allocation according to chest CT scan findings.
ARF: acute hypoxic respiratory failure; NIRS: noninvasive respiratory support; CT: computed tomography; GGO: ground glass opacities; OP: organizing pneumonia; DI: "do intubate"order; DNI: "do not intubate" order; ETI: endotracheal intubation.

There were no reports of bacterial infection in patients treated with NIRS but 10 patients who needed invasive mechanical ventilation had hospital acquired infection (3/7 in OP group vs. 7/10 in GGO group, $p = 0.350$). Psychosis occurred in 2 patients with OP under methylprednisolone pulses. There were no records of acute diabetic complications or gastrointestinal hemorrhage.

Length of stay was similar between groups. 25.9% needed ICU admission (24.6% in OP group vs. 31.9% in GGO group, $p = 0.394$). The global in-hospital mortality was 36.6%, 76.6% in GGO group vs. 8.6% in the OP group ($p < 0.001$). OP was associated with a significant reduction in need for ETI and in-hospital mortality after adjustment for confounders.

To our knowledge this is the first prospective study to evaluate OP outcomes. Since RECOVERY, many clinical trials have stablished the benefit of corticosteroids in COVID-19 patients.[1,7] However, the specific mechanisms of corticosteroids in treating COVID-19 are not totally understood. The hypothesis that OP in COVID-19 is more frequent than in other viral infections may explain this steroid-responsive effect. We found OP in almost 60% of our patients.

PaO2/FiO2 ratios were extremely low and more than 50% of our patients received a "do not intubate" order, supporting the severity of acute illness and baseline frailty of our population. This might justify the global in-hospital mortality rate. Interestingly, the need for ETI and mortality rate was significantly lower in the OP group, even after adjustment for age, comorbidities, "DNI order" and severity of acute respiratory distress syndrome. Additionally, biological markers associated with worse outcomes, such as leucocyte count, C-reactive protein, lactate dehydrogenase, interleukin-6 and ferritin levels,[8] were significantly lower in the OP group. These results support the theory that OP presence is by itself an independent predictor of good prognosis. In fact, literature reports that approximately 70% of OP patients will respond to corticosteroids.[9]

Identifying and timely treating this condition may avoid unnecessary ETI and drastically reduce mortality. An important concern is that the increasingly adopted dexamethasone protocol may be insufficient, especially in critical care patients, as OP management requires higher doses, prolonged treatment and careful tapering.[6] Considering the critically ill nature of our patients, the positive impact on outcomes and the absence of significant side effects, this approach seems to be beneficial. Nevertheless, the adequate minimal dose of corticosteroids for COVID-19 OP are unknown, particularly because of the secondary nature of the disease.

One limitation of this study is the absence of histopathologic confirmation. Second, as all OP patients were treated, we cannot guarantee that the benefit found was due to OP by it-self or due to the use of high dose of corticosteroids. Finally, larger studies are necessary to confirm these results.

In conclusion, our study showed that radiological features of OP are frequent. Critically ill patients should undergo a chest CT scan in order to identify this condition as treatment with high doses of corticosteroids seems to reduce the need for ETI and hospital mortality.

## Declaration of Competing Interest

None declared.

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**Table 1**
General characteristics and clinical outcomes according to cohort.

| Demographic and clinical characteristics | | | | |
|---|---|---|---|---|
| | Total | Cohort | | *p*-value |
| | | GGO | OP | |
| **Patients** | 112 | 47 | 65 | |
| **Age, years** | 67.5 (12.1) | 73.1 (9.0) | 63.4 (12.4) | <0.001 |
| **Male** | 76 (67.9) | 35 (74.5) | 41 (63.1) | 0.203 |
| **CCI, points** | 3.4 (1.9) | 3.8 (1.6) | 3.1 (2.1) | 0.069 |
| **DNI order** | 60 (53.6) | 32 (68.1) | 28 (43.1) | 0.009 |
| **Length of symptoms, days** | 8.5 (3.7) | 8.0 (3.5) | 8.9 (3.9) | 0.241 |
| **Laboratory findings** | | | | |
| Leucocytes, $10^9$ cells per L | 7.9 (4.1) | 9.0 (5.1) | 7.1 (2.9) | 0.012 |
| Lymphocyte, $10^9$ cells per L | 0.9 (0.4) | 0.9 (0.4) | 1.0 (0.4) | 0.322 |
| C-reactive protein, mg/L | 152.6 (79.9) | 171.3 (82.4) | 139.0 (75.8) | 0.034 |
| Procalcitonin, ng/mL | 0.5 (0.7) | 0.6 (1.0) | 0.4 (0.5) | 0.076 |
| Ferritin>1500 ng/mL | 32 (30.8) | 15 (35.7) | 17 (27.4) | 0.037 |
| Lactate dehydrogenase, U/L | 444.1 (174.2) | 485.9 (178.5) | 413.9 (165.9) | 0.030 |
| Interleukin-6, pg/mL | 138.4 (336.1) | 228.6 (513.8) | 80.2 (96.1) | 0.025 |
| D-dimer, $\mu$g/mL | 2407.0 (8239.4) | 3032.3 (10,023.3) | 1967.3 (6762.8) | 0.509 |
| **PaO2/FiO2, mmHg** | 94.9 (35.1) | 86.3 (29.5) | 101.8 (37.9) | 0.024 |
| **NIRS** | | | | |
| HFNC | 95 (67.0) | 37(78.7) | 58 (89.2) | 0.126 |
| CPAP/NIV | 37 (33.0) | 19 (40.4) | 18 (27.7) | 0.157 |
| **Treatment** | | | | |
| Corticosteroids | 111 (99.1) | 46 (97.9) | 65 (100.0) | 0.420 |
| Remdesivir | 7 (6.3) | 3 (6.4) | 4 (6.2) | 0.961 |

| Clinical outcomes and relative probability | | | | |
|---|---|---|---|---|
| | Total | Cohort | | *p*-value |
| | | OP | OR (95% CI) | |
| **Length of stay, days** | 15.4 (12.1) | 15.6 (9.6) | 1.000 (1.000–1.000) | 0.495 |
| **ETI** | | | | |
| Crude | 17 (18.3) | 7 (10.8) | 0.08 (0.02–0.39) | 0.002 |
| Adjusted[#] | | | 0.06 (0.01–0.44) | 0.003 |
| **Hospital mortality** | 41 (36.6) | 5 (7.7) | | |
| Crude | | | 0.03 (0.01–0.08) | <0.001 |
| Adjusted[#] | | | 0.03 (0.01–0.11) | <0.001 |

Data are presented as *n* (%) or mean (SD). GGO: ground glass opacities; OP: organizing pneumonia; CCI: *Charlson comorbidity index; DNI: "do not intubate"*; PaO2/FiO2: arterial oxygen tension /inspiratory oxygen fraction; NIRS: noninvasive respiratory support; HFNC: high flow nasal cannula; CPAP: continuous positive airway pressure; NIV: nonivasive ventilation; ETI: endotracheal intubation.
[#] : adjusted for age, CCI, DNI order and arterial oxygen tension/inspiratory oxygen fraction ratio.

## References


1. The WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group Association between administration of systemic corticosteroids and mortality among critically Ill patients with COVID-19 a meta-analysis. *JAMA* 2020;**324**(13):1330–41. doi:10.1001/jama.2020.17023.
2. Yang Z., Liu J., Zhou Y., Zhao X., Zhao Q., Liu J.. The effect of corticosteroid treatment on patients with coronavirus infection: a systematic review and meta-analysis. *J Infect* 2020;**81**(1):e13–20 JulEpub 2020 Apr 10. PMID: 32283144; PMCID: PMC7195158. doi:10.1016/j.jinf.2020.03.062.
3. Bao C., Liu X., Zhang H., Li Y., Liu J.. Coronavirus disease 2019 (COVID-19) CT findings: a systematic review and meta-analysis. *J Am Coll Radiol* 2020;**17**:701–9.
4. Polak S.B., Van Gool I.C., Cohen D., von der Thüsen J.H., van Paassen J. A systematic review of pathological findings in COVID-19: a pathophysiological timeline and possible mechanisms of disease progression. [published online ahead of print, 2020 Jun 22]. *Mod Pathol* 2020:1–11. doi:10.1038/s41379-020-0603-3.
5. Wang Y., Jin C., Wu C.C., Zhao H., Liang T., Liu Z., et al. Organizing pneumonia of COVID-19: time-dependent evolution and outcome in CT findings. *PLoS ONE* 2020;**15**(11):e0240347. doi:10.1371/journal.pone.0240347.
6. Bradley B., Branley H.M., Egan J.J., Greaves M.S., Hansell D.M., Harrison N.K., Hirani N., Hubbard R., Lake F., Millar A.B., Wallace W.A., Wells A.U., Whyte M.K., Wilsher M.L.. British Thoracic Society Interstitial Lung Disease Guideline Group, British Thoracic Society Standards of Care Committee; Thoracic Society of Australia; New Zealand Thoracic Society; Irish Thoracic Society. Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. *Thorax* 2008;**63**(Suppl 5):v1–58. doi:10.1136/thx.2008.101691. Erratum in: *Thorax* 2008;63(11):1029.
7. Horby P., Lim W.S., Emberson J.R.. Recovery: collaborative group, dexamethasone in hospitalized patients with Covid-19. *N Engl J Med* 2021;**384**:693–704. doi:10.1056/NEJMoa2021436.
8. Zeng F., Huang Y., Guo Y., Yin M., Chen X., Xiao L., et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *Int J Infect Dis* 2020;**96**:467–74. doi:10.1016/j.ijid.2020.05.055.
9. Cordier J.F.. Cryptogenic organising pneumonia. *Eur Respir J* 2006;**28**:422.



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