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

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Letters to the Editor

Correspondence on "dengue and dementia risk: A nationwide longitudinal study" by Chu et al.

Dear Editor,

We read with great interest the Chu et al. article investigating whether patients with dengue have an association with the increasing risk of getting dementia.[1] The result is interesting and provocative. Yet, some points remain to be discussed.

Firstly, robust study design and case ascertainment play an essential role in reaching solid conclusions in any longitudinal population based study. To ensure the diagnostic validity of dengue, only those who were hospitalized for the principal diagnosis of dengue were included in the Chu et al. study. However, it is better to link the NHIRD with the Notifiable Disease Datasets of confirmed cases through an encrypted dataset.[2] Through such comprehensive linkage, dengue cases would be validated with a positive predictive value of almost 100%. Additionally, the laboratory confirmation test for dengue cases is suggested to be implemented in this study instead of ICD-9 codes to avoid any detection bias in this study. For instance, in the Chien et al. study, the suspected dengue cases are identified and confirmed through the certified laboratory confirmation test in accordance with any criteria mentioned by Taiwan CDC.[3] By implementing the laboratory confirmation test on any dengue cases, the accuracy of the study is assured.

Secondly, dengue consists of variable clinical symptoms ranging from inapparent symptoms, mild fever to severe hemorrhage and shock, and it might have different pathogenesis related to neuro inflammation.[4] However, in the Chu et al. study, dengue group only included inpatients dengue cases that tend to have more severe dengue compared with outpatients dengue cases. Therefore, a subgroup analysis with outpatients and inpatients dengue cases might need to be conducted to gain some insights. Besides, to avoid any surveillance bias, consideration of the frequency of all outpatient visits and inpatients days is recommended.[5]

Thirdly, a previous study has revealed that dengue is an endemic disease, and outbreaks of dengue mainly occurred in southern Taiwan.[6] As a result of spatial-temporal patterns of dengue, it would be better to include dengue cases in a specific region with high prevalence of dengue in order to be well representative of whole dengue cases in Taiwan.

Even if the *e*-value is utilized to assess the potential confounders in Chu et al. study, the residual confounders from omitting confounders such as comorbidities have not been solved precisely. Regarding to the residual confounders mentioned above, there is convincing evidence revealing that patients with rheumatoid diseases are vulnerable to getting dementia compared to those without rheumatic diseases .[7] Therefore, there is a necessity of considering the comorbidities to ensure the study's accuracy.

In conclusion, for the above mentioned points, we believe that more pieces of information should be confirmed and elucidated to enhance credibility.

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Patient and public involvement

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Patient consent for publication

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Provenance and peer review

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Ethical approval information

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Data sharing statement

Not required.

Declaration of Competing Interest

The authors declare no conflict of interest.

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[1] Dear Editor, I am Renin Chang, an author of this Letter. Here I request a change of rearrangement of authors list. Professor James Cheng-Chun Wei (JCW) indeed devotes a lot for this Letter. However, we did not include him into the co-first authors in the previous version. We apologize for our mistake. Now, we provide all authors' confirmation as attached filed. All authors agree this rearrangement: AK and PEK and JCW contributed equally to the manuscript.

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Suspected reinfections of SARS-COV-$_2$ in Khyber Pakhtunkhwa, Pakistan – analysis of province-wide testing database

Dear Editor,

SARS-CoV-2 reinfection is a relatively new phenomenon in the ongoing coronavirus disease (COVID-19) pandemic. Although, the first confirmed case of reinfection was identified in August 2020, studies reporting reinfection have been scarce, and limited only to single-case studies, or smaller case-series and cohorts.[1] Only few studies have determined incidence of reinfection in large, population-based settings based on epidemiological evidence.[2–8] If genotyping is unavailable, Centres for Disease Control (CDC) recently set an investigative criteria, defining SARS-CoV-2 reinfection as a second positive real time reverse transcription-polymerase chain reaction (RT-PCR) test, taken ≥90 days after the primary result.[9] However, not all published work follows a uniformly set criteria for what constitutes confirmed reinfection.

Studies which follow the CDC criteria indicate high level of protection following recovery from infection, with reinfection suspected in less than 1.0% of those previously infected.[2,5–8] However, further detailed prospective studies are needed to assess frequency of recurring infection. There is a possibility that a large proportion of reinfections are undetected, which could explain the low risk of SARS-CoV-2 reinfection reported in literature. This may especially be true in lower-middle-income settings, such as ours, where testing for asymptomatic infection is rare, and carrying out mass-scale genomic sequencing is not always logistically feasible.

Pakistan is currently experiencing a fourth epidemic wave of SARS-CoV-2 transmission. This provided us an opportunity to retrospectively determine incidence of suspected reinfections after three global waves in the pandemic. Using epidemiological criteria set by CDC, we undertook this study to identify suspected cases of SARS-CoV-2 reinfection, in the province of Khyber Pakhtunkhwa (KP), using the provincial government's contact tracing database.

Methods

This descriptive cross-sectional study was approved by the Khyber Medical University (KMU) Ethical Committee. Due to retrospective nature of our analysis, we were granted a waiver from obtaining informed consent.

In KP, testing has been extended to symptomatic and suspected cases, those presenting at hospitals, people identified through contact tracing, contacts of confirmed cases, people with travel history, or anyone using the free COVID-19 response hotline. Samples are sent to one of the 28 public and private sector diagnostic laboratories, serving all regions of KP province, which has a population of 35.53 million people. This network of laboratories is regulated by the provincial health department, and all SARS-CoV-2 viral RNA RT-PCR tests are conducted under guidelines from National Institute of Health (NIH), Islamabad. To ensure uniformity in sample processing and data analysis, all laboratories in KP are periodically assessed through an External Quality Assurance program mandated by NIH. Individual-level test results are logged onto, and maintained on a centralised contact tracing database.

We extracted retrospective data for all SARS-CoV-2 RT-PCR tests carried out from March, 2020, to July, 2021, in KP. For purpose of this study, primary infection was defined as a positive RT-PCR test for SARS-CoV-2. Suspected reinfection was defined as a second positive test taken for SARS-CoV-2, ≥90 days after the primary positive test result. Analyses were performed using MS Excel. Comparison between demographic variables of primary infections and suspected reinfections were made using Chi-square test.

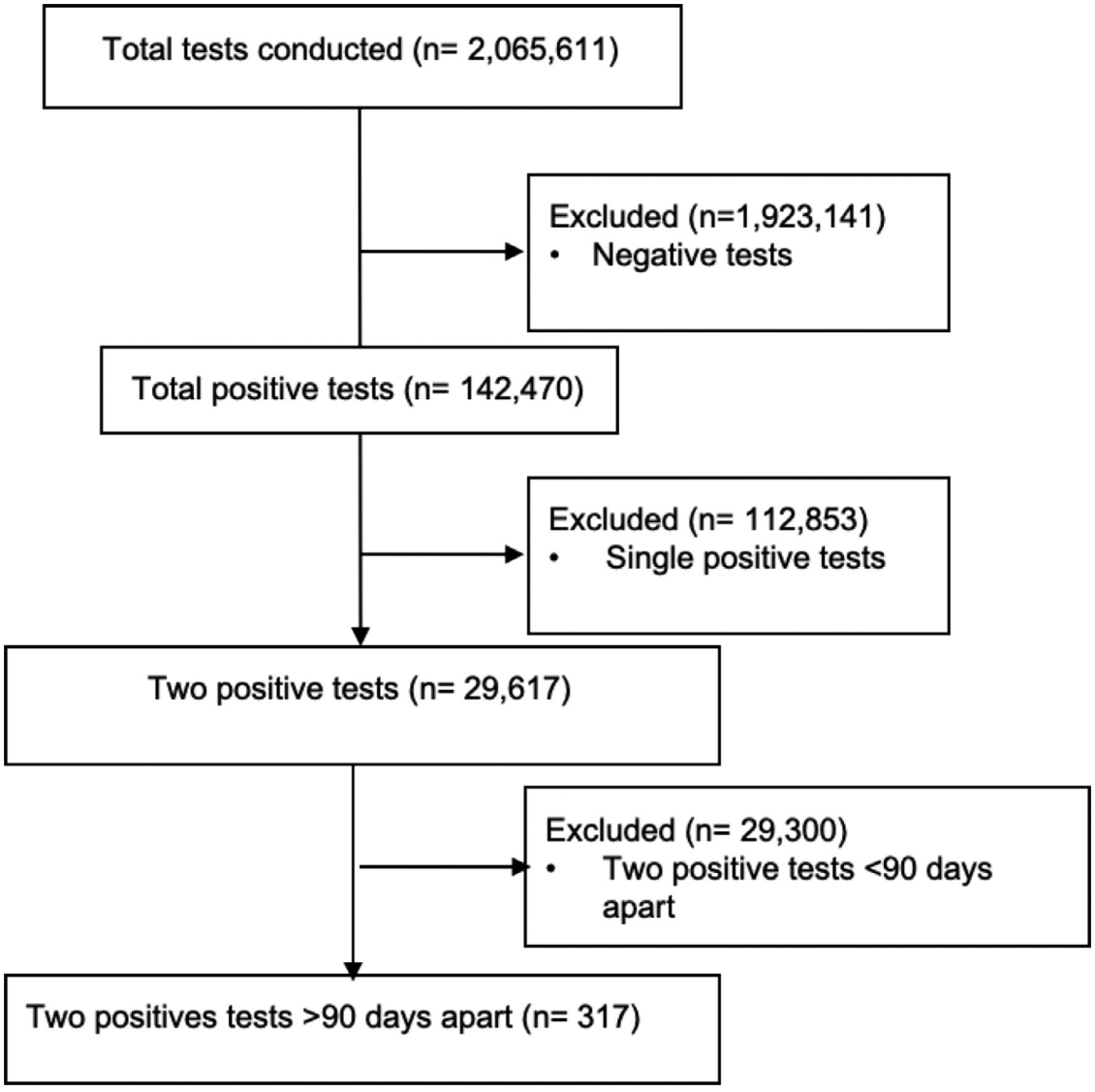

Fig. 1. Schematic diagram showing selection of suspected reinfection cases from total conducted tests.

Results

Between March 2020 and July 2021, a total of 2065,611 SARS-CoV-2 RT-PCR tests were conducted in KP. We found 142,787 individuals had tested positive for SARS-CoV-2 once, out of which 58.2% of primary infections were hospital referrals, while 41.8% were identified through KP's comprehensive contact tracing program. A subset of 29,617 individuals were identified, who had received repeat RT-PCR testing for SARS-CoV-2. Results of the primary infection were used as start of the 90 day interval, and multiple positive test results obtained within 90 days of each other were discounted. Overall, we observed 317 (0.22% of total positive cases) suspected cases of SARS-CoV-2 reinfections (Fig. 1).

Mean age of individuals with suspected reinfection was slightly lower compared to those infected once (36.94 ± 14.34, vs 38.41 ± 18.59, *p*-value 0.004). Suspected reinfection was found to be more common in males (74.8%, *p*-value < 0.001), and in young and middle-aged adults, between 19 and 45 years old (54.1%, *p*-value < 0.001). Our analysis shows that 73.1% (231/317) of reinfections were detected through hospital referrals, while 26.9% (85/317) reinfections were identified through contact tracing (*p*-value < 0.001). Higher suspected reinfections were observed in urban centres where more testing is conducted (supplementary Fig. 1), with Peshawar division reporting highest proportion (189, 59.6%) of suspected reinfection cases (*p*-value <0.001) (Table 1).

Discussion

These findings suggest that SARS-CoV-2 reinfection could be common in age groups often presumed less likely to suffer from severe, or critical COVID-19. This is in contradiction to evidence which shows symptomatic COVID-19 results in enhanced, long-lasting protective immunity following recovery from primary infection.[10] Our results reveal that more than two thirds (73.1%) of recurring infections were picked up through hospital referrals, which could indicate that people with severe symptoms are more likely to seek medical attention.

Studies have demonstrated that a large proportion of recurring infections are asymptomatic, and reinfection is more common in individuals whose primary infections were also asymptomatic.[1,7] In Pakistan, the testing strategy has largely relied on targeting of symptomatic and suspected cases. Therefore, it is impossible to accurately predict the true number of SARS-CoV-2 reinfections,

Table 1
Demographic characteristics of SARS-COV-2 primary and suspected reinfection from Khyber Pakhtunkhwa province.

Characteristics		Infection status n (%)		*P*-Value
		Primary infection	Reinfection	
Gender	female	49,416 (34.7)	80 (25.2)	<0.001
	male	93,054 (65.3)	237 (74.8)	
Age, Years	Mean ± SD	38.41 (±18.59)	36.04 (±14.340)	*0.004
Age categories	≤ 18 years	15,815 (11.4)	17 (5.4)	<0.001
	19 - 30 years	35,066 (25.3)	123 (38.2)	
	31 - 45 years	39,903 (28.8)	109 (34.4)	
	46 - 60 years	30,040 (21.7)	46 (14.5)	
	>60 years	17,775 (12.8)	22 (6.9)	
Sample type	Contact tracing	59,592 (41.8)	85 (26.9)	<0.001
	Hospital referral	82,878 (58.2)	231 (73.1)	
Region	Malakand	29,478 (20.8)	58 (18.3)	<0.001
	Hazara	16,596 (11.7)	29 (9.1)	
	Mardan	14,270 (10.1)	18 (5.7)	
	Peshawar	61,706 (43.5)	189 (59.6)	
	Kohat	12,036 (8.5)	16 (5.0)	
	Bannu	4307 (3.0)	3 (0.9)	
	DI Khan	3550 (2.5)	4 (1.3)	

* calculated using independent samples *t*- test.

which may be significantly larger than what has been reported in literature.

There were several limitations to this work. Since data was gathered retrospectively, we were unable to obtain medical history, clinical demographics, or personal details, regarding individuals whose test results were utilised in our analysis. Thus, we could not correlate role of any pre-existing health conditions with suspected reinfection. Similarly, the proportion of individuals employed in high-risk professions, such as healthcare workers, was unknown. Our future work aims to address these limitations. To best of our knowledge, this is the largest multi-centre analysis (n = 142,787) of its kind carried out to date, reporting one of the highest event-rates (317 suspected reinfections) in published literature.[6] Our results fall within range of reinfections reported in literature, despite limited testing being carried out in Pakistan for identification of asymptomatic cases. This analysis suggests that recurring infection with SARS-CoV-2 may indeed be a widespread phenomenon, although the risk is known to be low.

Declaration of Competing Interest

The authors declare no conflict of interest.

Ethics approval

Ethical approval of the study was granted by Ethics Board, Khyber Medical University (Reference number: Dir/KMU-EB/PR/000879).

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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.2021.10.005.

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Serological profile of first SARS-CoV-2 reinfection cases detected within the SIREN study

Dear Editor,

A study published by Hanrath and colleagues[1] in this Journal found no SARS-CoV-2 reinfection cases between the first two waves of the pandemic in a cohort of healthcare workers. However, several SARS-CoV-2 reinfection cases have been reported during the second wave, although reinfection definitions are not consistent.[2,3]

It is crucial to understand whether SARS-CoV-2 antibody titres could be used as a correlate of protection in assessment of disease susceptibility. In the SIREN study, a large national longitudinal cohort of more than 44,000 healthcare workers, participants are followed for at least 12 months using fortnightly symptom and exposure questionnaires and nucleic acid amplification testing (NAAT), with monthly antibody testing against SARS-CoV-2.[4] Potential reinfections are flagged when meeting the following criteria: two positive RT-PCR tests at least 90 days apart (with no additional intervening positives) or a new RT-PCR positive test at least four weeks after a positive SARS-CoV-2 antibody test. Additional total antibody testing is performed at Public Health England laboratory using the semi-quantitative Elecsys Anti-SARS-CoV-2 nucleocapsid (N) protein assay and fully quantitative Elecsys Anti-SARS-CoV-2 spike (S) protein assay which targets the receptor binding domain (RBD) (Roche Diagnostics).[5]

We here describe two reinfection cases in which additional serological assays were performed: in-house recombinant SARS-CoV-2 IgG spike (S) protein RBD indirect ELISA,[6] live virus microneutralisation using SARS-CoV-2 isolate England/02/2020.[7] and pseudovirus neutralisation.[8] Semi-automated multiplexed immuno-blotting assay was performed to detect RBD-, N-, S1-, S2- and S-specific IgG, IgA and IgM antibodies.[8]

Case 1

A 45-year-old female nurse, with history of asthma and treated breast cancer, was SARS-CoV-2 antibody positive on 7th August 2020. She reported COVID-19 symptoms in March 2020 (dry cough, fever, headache and myalgia, followed by anosmia and ageusia), however RT-PCR was not performed. On 10th October, during a nosocomial outbreak of SARS-CoV-2, she became SARS-CoV-2 PCR positive, however asymptomatic at the time of testing. Four days later, she reported headache followed by sore throat, myalgia, arthralgia, ageusia and a productive cough. She reported milder symptoms during the second episode.

SARS-CoV-2 was successfully cultured from the earliest of several samples taken between 10th to 23rd October. A phylogenetic analysis was undertaken to compare sequences derived from the PCR positive swabs with circulating SARS-CoV-2 strains in the UK, using cluster investigation and viral epidemiology tools (Pangolin COVID-19 Lineage Assigner). Infection was due to SARS-CoV-2 lineage B.1.523 with exact concordance between all sequences obtained from the individual. Sequences segregated to the same lineage, within one or two SNPs as samples from 18 other individuals involved in the nosocomial outbreak.

Prior to reinfection, S binding antibodies (RBD ELISA and Roche S/RBD ECLIA) and neutralising antibodies (live virus and pseudovirus) were at or below the limit of detection but were boosted significantly following reinfection, with neutralising antibodies increased to high titres > 1:1000 33 days after reinfection (Fig. 1).

The immuno-blotting results (Fig. 2a) demonstrated N-specific IgG was clearly detectable at the time of reinfection, whereas the intensity of the S-specific band was weak, consistent with other serological results. IgM levels were undetectable. In contrast, all antigens except S2 were clearly detectable by IgA 30 days after reinfection.

Case 2

A 37-year-old female administrator had SARS-CoV-2 antibodies on 28th August 2020. She described COVID-19 symptoms in March 2020, (fever, shortness of breath, flu-like symptoms, anosmia and ageusia) that lasted 4 weeks, when no RT-PCR test was performed. A surveillance RT-PCR test undertaken on 06th October 2020 was positive, when she had coryzal symptoms and diarrhoea that lasted less than 24 h, with no other symptoms.

Genomic analysis identified from swabs on 6th and 9th October were identical and belonging to B.1.258.4 lineage. Serology demonstrated an increase in antibody reactivity in all assays following the second infection (Fig. 1). Prior to the reinfection, both N and S antibodies were detectable with low levels of neutralising antibodies (live virus and pseudovirus). However, within three days of reinfection, neutralising antibodies increased to a titre of >1:200.

Previous exposure to SARS-CoV-2 was evident from the immuno-blot (Fig. 2b). Thus, IgG and IgA specific to the Nucleocapsid protein, but not other antigens, could be detected prior to the onset of symptoms. Two weeks later, an increase in antibody responses was observed, against the N antigen, Spike antigen, RBD and S1 sub-domains. IgM was detected against N and S1, albeit weaker against the latter antigen.

The mechanisms of failure of immune protection from reinfection have not been clearly elucidated. In these two cases, an anamnestic antibody response was observed using virus neutralisation, antibody binding and immuno-blotting assays. All investigations showed an increase in antibody levels following the onset of symptoms in both cases and low or absent levels of neutralising antibodies at time of reinfection. This might be due to a lack of effective antibody response after the first infection or decrease in neutralising antibody titres over time, as observed in other studies.[9]

Our data are consistent with the hypothesis that absence or low levels of neutralising antibody titres are likely correlate with a lack of protection against SARS-CoV-2 reinfection. There is strong evidence that neutralising antibodies play a critical protective role. Was estimated that neutralising antibody titres can offer an accurate prediction of immune protection, with neutralisation level for 50% protection being 54 U/mL, which equates to a titre of 1:10 or 1:30 in most virus neutralisation assays.[10] The sera from both

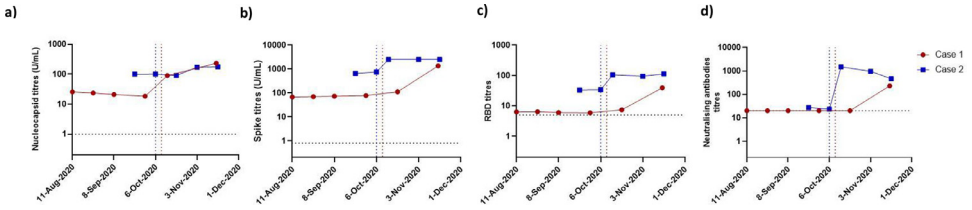

Fig. 1. Serological response in Case 1 and Case 2 against SARS-CoV-2, including anti-N, anti-S, anti-RDB and neutralising antibodies. Vertical dashlines represent the re-infection events for Case 1 (red) and Case 2 (blue). Horizontal dashline represents cutoff values. (a) Anti-SARS-CoV-2 nucleocapsid (N) protein assay (Roche Diagnostics - Cutoff ≥ 1.0 U/mL). (b) Fully quantitative Elecsys Anti-SARS-CoV-2 spike (S) protein assay (Roche Diagnostics - Cutoff ≥ 0.8 U/mL).[5] (c) In-house recombinant SARS-CoV-2 IgG spike (S) protein receptor binding domain (RBD) indirect ELISA (Cutoff ≥ 5.0).[6] (d) Neutralising antibodies were detected using a live virus microneutralisation assay, using England/2/2020 virus (Cutoff ≥ 20.0).[7]

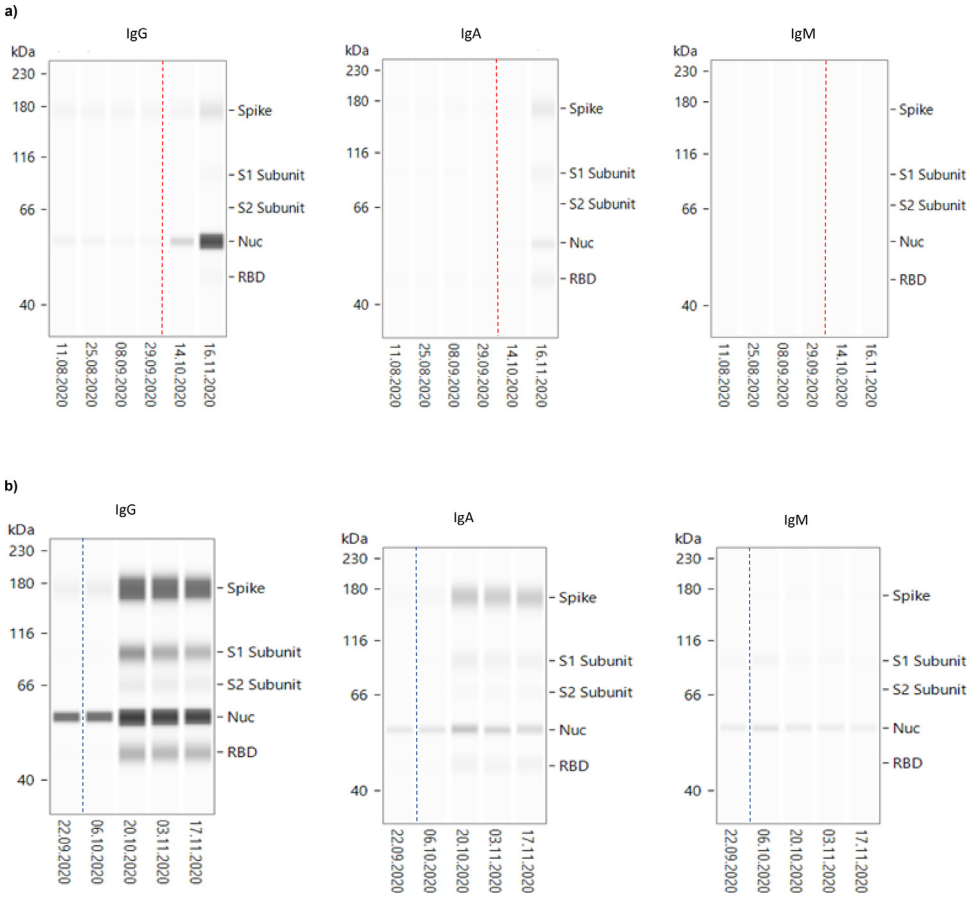

Fig. 2. Immuno-blotting of Case 1 (a) and Case 2 (b) plasma samples showing the reactivity of IgG (left), IgA (middle) and IgM (right) against the Spike, S1, S2, N and RBD antigens of SARS-CoV-2. Dashed lines represent the reinfection events.

cases had virus neutralising antibody levels below this threshold at the time of SARS-CoV-2 reinfection.

There are some limitations in this study: no samples from the first infection episodes were available, thus no comparative genomic analysis of infecting viruses was possible. However, the sequences obtained in October were genetically distant from SARS-CoV-2 viruses from March 2020 but closely related to viruses circulating locally at time of reinfection. Secondly, symptoms from the first infection episodes are subject to recall bias. Finally, our analysis was restricted to two cases; therefore, our hypothesis will require support from more extensive studies. Further analysis using a case-control design is essential to clarify the potential role of neutralising antibodies in SARS-CoV-2 reinfection.

Declarations of Competing Interest

None.

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Decreasing humoral response among healthcare workers up to 4 months after two doses of BNT162b2 vaccine

Dear Editor,

We read with interest the articles by Harris Ross et al.,[1] and its comment by Capetti Amedeo et al.,[2] showing an antibody persistence up to 24 weeks and one year among COVID-19 infected individuals. In response to this journal, Tré-Hardy et al.,[3] and Salvagno Gian et al.,[4] presented slightly decreasing anti-S IgG decrease among vaccinated healthcare workers (HCWs) three months after mRNA vaccine first dose suggesting the need for potential new vaccine injection. Here we want to both provide new elements that strenghen this point with the analysis of vaccinated HCWs response up to five months after the first vaccine dose and add live virus neutralization data on several SARS-CoV-2 variants.

Thus, in the current study, we tested 138 sera samples from 17 BNT162b2 vaccinated HCWs up to 5 months after the first vaccine dose for anti-N and anti-S IgG (Abbott Diagnostics, Chicago, US) and pseudoneutralization activity (iFlash-2019-nCoV Nab assay, YHLO, Shenzen, China). Live virus neutralization assays with B, Alpha, Beta and Gamma SARS-CoV-2 strains were undertaken for a subset of 45 sera from 9 vaccinated HCWs. Decomplemented sera were subjected to serial two-fold dilution (1:25 to 1:12800), incubated with 50 μL of diluted virus (2×10^3 PFU/mL) in a 96-well plate at 37°C, 5% CO2 for 60 min. Then 3×10^4 cells Vero E6 cells (ATCC, reference R CRL-1586) were added and incubated (37°C, 5% CO_2) until cytopathic effect assessment at day 4.

Among our vaccinated HCWs (median age 57 years old [IQR=24-52]), humoral response demonstrated a median anti-S titer at 728 AU/mL [343–1612] one month after the first dose and an antibody peak one month after the second dose at 11720 AU/mL [8350-20056] (Fig. 1). Then it gradually decreased, reaching a plateau 4 months after second dose (3059 AU/mL [2314-5124]). This decrease, five months after first vaccine dose, confirmed the trends observed by Tré-Hardy et al.,[3] and Salvagno Gian et al.,[4] at three months. The anti-S titers decrease was also confirmed by pseudoneutralization titers with a peak at 1593.1 AU/mL [788.4–1659.6], one month after the second dose followed by a plateau

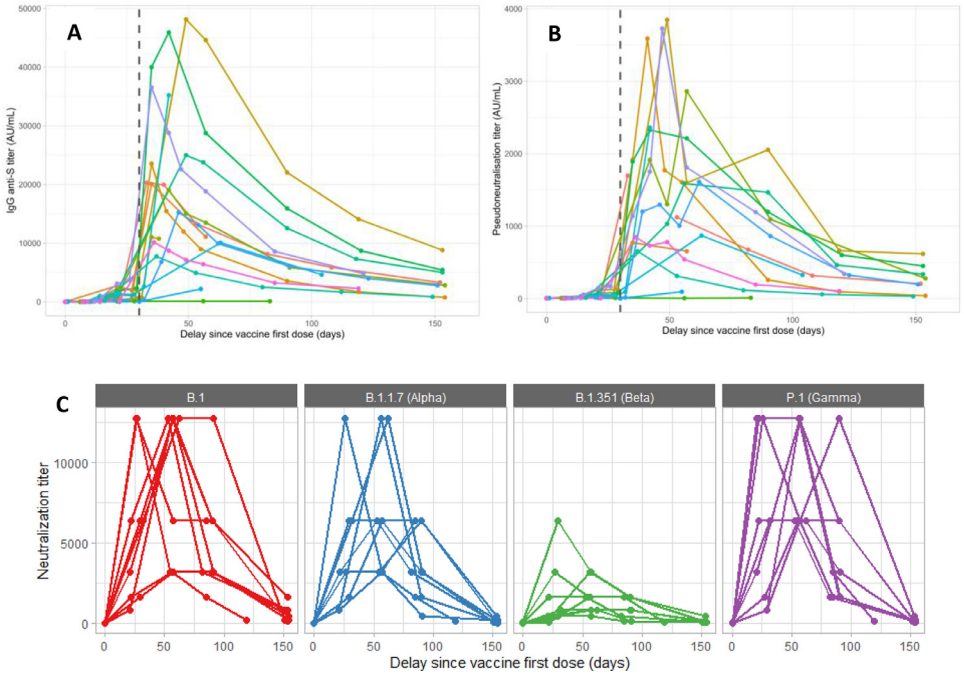

Fig. 1. Antibody kinetics and Neutralization titers among vaccinated HCWs up to 5 months after BNT162b2 vaccination initiation. Panel A depicts the anti-S IgG, panel B the Spike RBD-pseudoneutralization titers and panel C the live virus neutralization assay titers according to viral lineages.

at 242.0 AU/mL [157.7-365.1]. When confirming those observations on live virus neutralization assay (Fig. 1). We also observed a pic of neutralizing anti-SARS-CoV-2 antibodies one month after the second vaccine dose (median neutralizing antibody titer of 1:12800 [1:3200–1:12800], 1:6400 [1:3200–1:6400] and 1:6400 [1:6400–1:12800] for B.1, Alpha and Gamma variants, respectively). However, we observed an even stronger decrease in neutralizing activity of sera, for all variants, leading to very low neutralizing titers at around 1:50 four months after the second vaccine dose for Alpha and Beta variants (1:50 [1:25–1:200] and 1:50 [1:25–1:100], respectively). A lower decline in neutralizing titers was also observed for ancestral strain and Gamma variant with median titers of 1:400 [1:200–1:800] and 1:100 [1:100–1:200], respectively. Regarding the Beta variant, neutralizing capacity of sera stayed significantly lower at 1 and 2 months after the second vaccine injection compared to ancestral SARS-CoV-2 strain (p=0.009 and p=0.014 respectively), Alpha variant (p=0.009 and p=0.034, respectively) and Gamma variant (p=0.009 and p=0.014, respectively). This difference of neutralization activity faded as neutralization titers dropped for all lineages 3 months after the second dose.

To help routine antibody response follow-up, we evaluated the global concordance between anti-S IgG measurement, pseudoneutralization assay and live virus neutralization assay. The comparison between pseudo-neutralizing antibodies titers and anti-S titers was conducted on the 138 samples from vaccinated HCWs, 48 additional samples from COVID-19 patients and 16 pre-epidemic samples. We observed an overall agreement of 96% among vaccinated HCWs (n=133/138) and 100% among COVID-19 patients (n=48/48) and pre-pandemic samples (n=16/16) associated to a strong correlation between anti-S IgG and pseudo-neutralization titers with a Spearman coefficient Rho = 0.95 (p<0.0001) (Fig. 2). Regarding the 5 non concordant results in the HCWs group, all were anti-S IgG positive but negative for pseudoneutralization. They all were obtained less than 7 days after the first vaccine dose, compatible with an early low-RBD affinity antibody production. Interestingly, CPE neutralization assay demonstrated a strong neutralizing activity of vaccinated HCWs one month after the first vaccine dose against all variants (median IQR x4) despite relatively low anti-S (median IQR) and pseudoneutralization titers (median IQR) (Fig. 2). After the second vaccine dose, the neutralization titers were correlated with anti-S and pseudoneutralization titers (p<0.0001 for all lineages with rho's Spearman correlation test at 0.90, 0.86, 0.77 and 0.89 for B, Alpha, Beta and Gamma lineages respectively).

In the current study on BNT162b2 vaccine among HCWs, a strong antibody response was observed two months after the first vaccine dose (i.e. one month after the second dose), decreasing drastically afterwards from two months up to four months after the first dose. From four to five months after the first dose, the antibody titers kept to slightly decrease while still presenting detectable anti-S IgG and pseudoneutralization positive results. If live-virus neutralization assays demonstrated the same kinetic, its results were more concerning as 0/9, 5/9, 6/9 and 2/9 HCWs presented no or low neutralization activities (i.e. with an effective dilution below 1:50) for the historical B, Alpha, Beta and Gamma strains, respectively.

These findings are of importance as another recent study on vaccinated HCWs in Israel demonstrated that reinfections were associated with lower antibody neutralizing titers and that lower neutralizing titers were also associated with higher viral load during infection that could lead to higher risk of transmission.[5] Thus, the decrease in antibody and seroneutralization responses observed in the current work highlights the needs for additional vaccine doses evaluation. This is of peculiar importance for HCWs population which is at risk of transmission to fragile patients.

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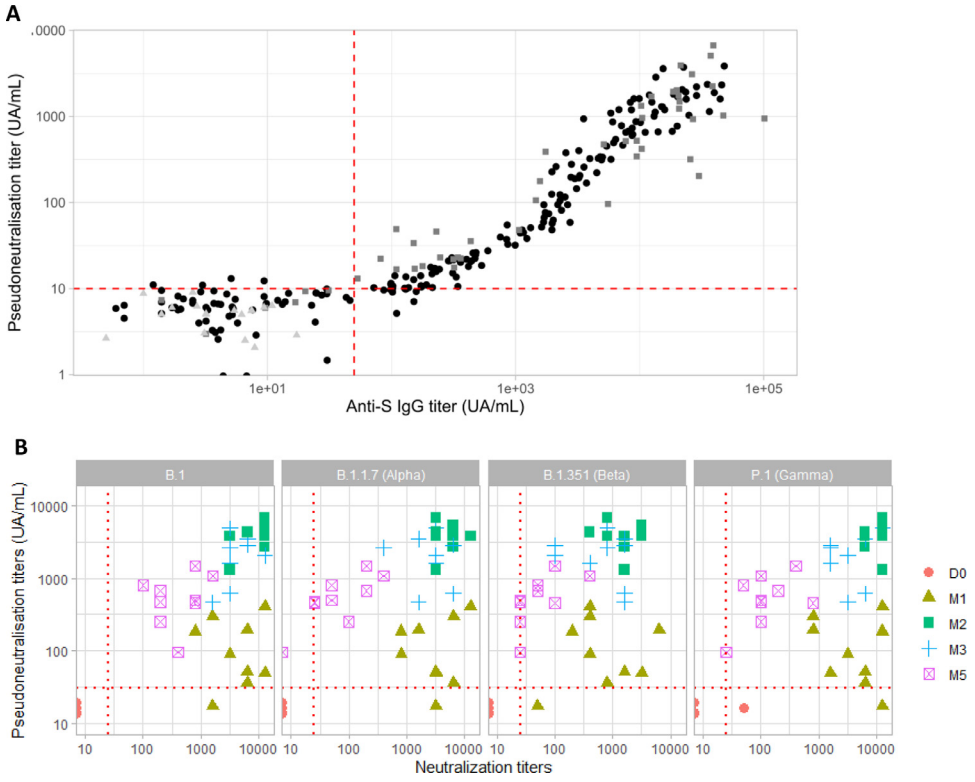

Fig. 2. Correlation between IgG anti-S with pseudoneutralization and live virus neutralization assays. Panel A depicts the correlation with pseudoneutralisation assay for vaccinated HCWs, depicted with black circles, COVID-19 diagnosed patients, depicted with dark gray squares, and prepandemic samples, depicted with light gray triangles. Panel B depicts the correlation with neutralization assay titers for tested SARS-CoV-2 lineages. Sampling timepoints after the vaccine first dose are indicated by the dots' shapes and colors (D0: time of first vaccine dose, M1-2-3-5: one, two, three and five months after first vaccine dose).

Declaration of Competing Interest

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

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Antibody titers and protection against a SARS-CoV-2 infection

Dear Editor,

Recent studies indicate that binding and neutralizing SARS-CoV-2 antibodies elicited by natural infection or vaccination persist for more than 6 months although their concentration decreases over time.[1] The passive transfer of neutralizing antibodies (NAb) and protection are correlated in non-human primates.[2] While such a link has not yet been defined in humans, individuals with a high NAb titer could well be better protected against SARS-CoV-2. A recent letter in Journal of Infection indicated that indeed neutralizing antibodies are considered linked to protective immunity due to their ability to block the viruses from entering the host cells. The authors discussed the decrease in neutralizing antibodies after vaccination without being able to provide a threshold below which protection against SARS-CoV-2 infection is no longer guaranteed.[3]

We measured the antibody titers in 8758 healthcare workers (HCWs), vaccinated and unvaccinated, soon after the first epidemic wave had occurred in France (10 June–10 July, 2020). Total SARS-CoV-2 antibodies were measured in longitudinal samples with a quantitative enzyme-linked immunosorbent assay (ELISA) (Wantai Biological Pharmacy Enterprise Co., Ltd, China) and a live-virus neutralization assay using Vero cells and a B.1.160 strain (GISAID EPI-ISL-804372).[4] Symptomatic and asymptomatic infections were detected with a nucleic-acid amplification method (Aptima™ SARS-CoV-2 assay, Panther™ system, Hologic, USA).[5] This study was approved by the French Research Ethics Committee Est-III (COVID BioToul, ID-RCB 2020-A01292-37, ClinicalTrials.gov Identifier: NCT04385108).

The median age of the 8758 HCWs (7039; 80.4% females) was 40 years (interquartile range [IQR] 32–50). Over half of them (4811; 54.9%) had been given one (2244; 46.6%) or two (2567; 53.4%) doses of vaccine between January and April 15, 2021. Of these, 1290 (26.8%) had one dose of the Oxford–AstraZeneca ChAdOx1 nCoV-19 (AZD1222) vaccine, 954 (19.8%) had one dose of the Pfizer-BioNTech COVID-19 mRNA (BNT162b2) vaccine and 2567 (53.4%) had two doses. An average of 9.65% (range [7.2–12.1%]) of the HCW who had no NAbs became infected after a median follow-up of 275 days (IQR: 265–281), as did 2.2% [95% CI: 0.4–4%] of those with a NAb titer well below 64. In contrast only 0.6% [95% CI: 0–1.5%] of those with NAb titers of 64 to 128 became infected together with none of those with NAb titers of 256 and above (Fig. 1, $p < 0.01$, Chi^2 test). The correlation between the ELISA total antibody values expressed in binding antibody units (BAU) per ml using the WHO international standard (NIBSC code 20/136)[6] and the neutralizing antibody titers from July 2020 to April 2021 was 0.8 for unvaccinated HCWs and 0.79 for vaccinated HCWs. Analysis of ELISA total antibody concentrations indicated that 12.1% [95% CI: 11.5–12.8%] of HCWs with a negative ELISA or an ELISA concentration below 13 BAU/ml became infected between July 2020 and April 2021, as did 10.6% [95% CI: 6.5–16.1%] of HCWs that had an ELISA concentration between 13 and 141 BAU/ml. In contrast only 1.3% [95% CI: 0.03–7.2%] of those with an ELISA concentration between 141 and 1700 BAU/ml became infected and none of those with an ELISA concentration of 1700 BAU/ml and above ($p < 0.01$, Chi2 test). Analysis of all the data indicated that a NAb titer well below 64 provided 76.8% protection against SARS-CoV-2, a titer of 64 to 128 gave 94% protection and a NAb titer of 256 or more provided full (100%) protection (Fig. 2A). In the same way, an ELISA concentration between 13 and 141 BAU/ml provided only 12.4% protection against SARS-CoV-2, a concentration between 141 and 1700 BAU/ml provided 89.3% protection and a concentration of 1700 BAU/ml and above provided full protection (Fig. 2B). In our cohort, none of the two doses-vaccinated HCWs had an ELISA concentration below 141 BAU/ml one month after the second injection, unlike 79.3% of the HCWs three months after a natural infection.

Our study did not assess cell-mediated immunity and all the subjects were HCWs. However, the data suggest that monitoring the neutralizing antibody response but also total antibody concentrations, logistically more feasible, can be used to optimize vaccination strategies by estimating the duration and degree of protection provided by vaccines. The thresholds of protection found in our study should be compared to those obtained in further studies on other populations. It is also essential to estimate the influence of an antibody's reduced neutralizing capacity against new emerging viruses variants [7,8].

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Declaration of Competing Interest

The authors declare no conflict of interest

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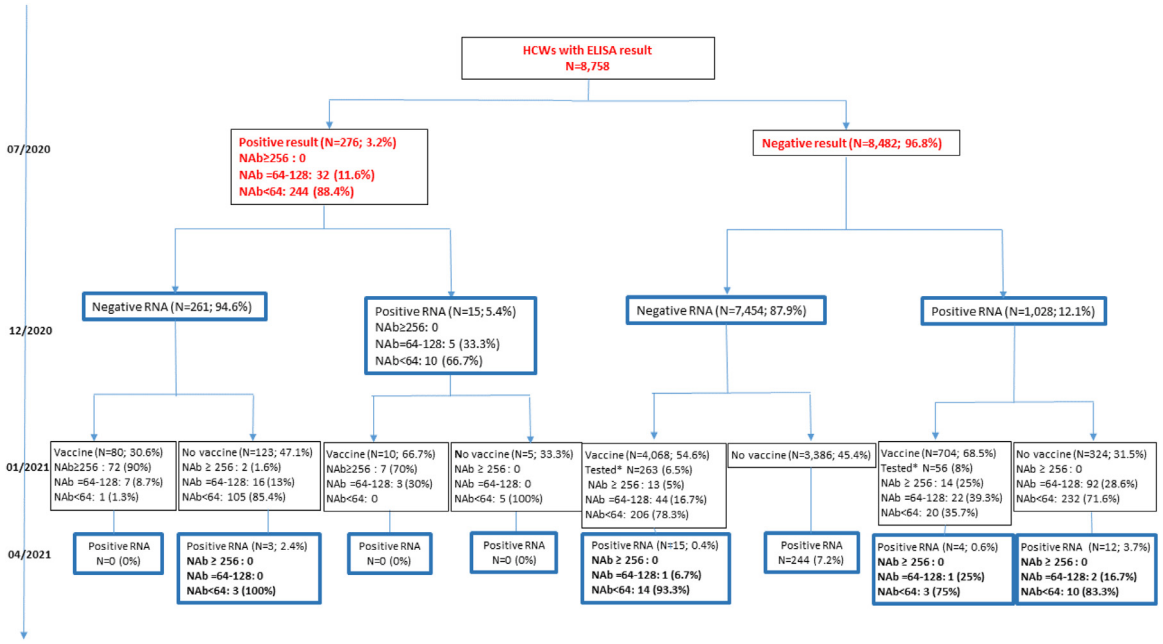

Fig. 1.. Study flowchart. The NAb titers were obtained at each time of ELISA screening (July 2020, January 2021) for all ELISA-positive samples. The January 2021 NAb titers for vaccinated HCWs were obtained three weeks after administration of the first or second vaccine dose. Finally, the NAb titers reported for December 2020 and April 2021 were those obtained at the previous screening (July 2020 and January 2021, respectively) and for which an infection occurred during the following three months.
Source: *Representative sample based on age and gender

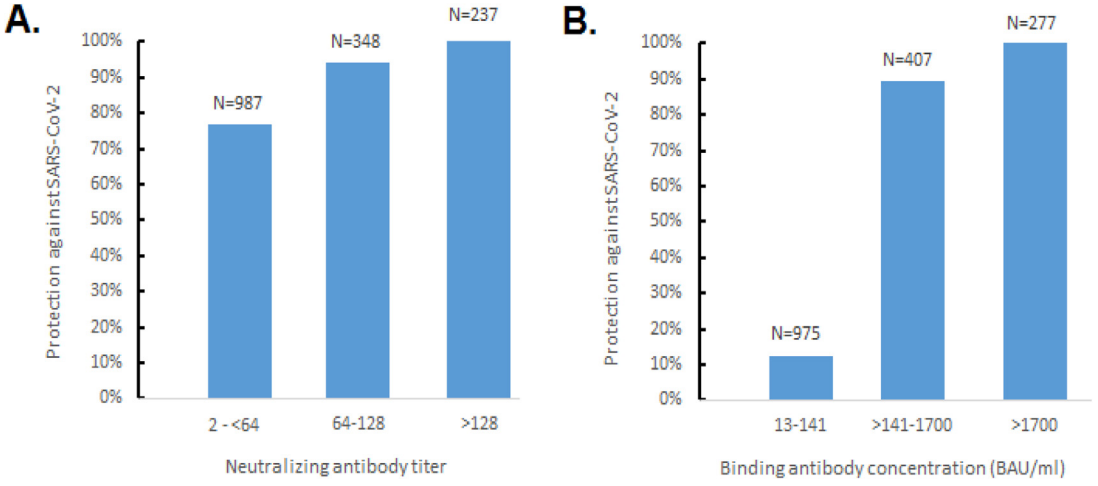

Fig. 2.. Protection against SARS-CoV-2 according to neutralizing (A) or binding (B) antibody classes.

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Endogenous *Candida* endophthalmitis: Has it been discerned from a cotton wool spot in the retina?

Dear Editor,

I read the paper by Seidelman et al. with concern.[1] The authors hypothesize that a variety of risk factors may predict the development of endophthalmitis in the setting of *Candida* septicemia with potential for guiding ophthalmologic screening. However, the data and interpretation presented demonstrate severe flaws that prohibitively limit their conclusions, including but not limited to inaccurate disease classification, selection bias, lack of control for confounding variables, lack of visual outcomes data, any details regarding changes in management based on ocular findings, lack of microbial confirmation from ocular tissue, and lack of a control group for comparison. While the Infectious Diseases Society of America (IDSA) Clinical Practice Guideline for the Management of Candidiasis advocates for routine screening of patients with *Candida* septicemia (with self-admittedly "low-quality evidence"),[2] the authors here fail to provide data from their results, or other adequate evidence from the literature, to support this practice pattern. The authors' claim that endophthalmitis incidence is "underappreciated" in the setting of *Candida* septicemia is also unsubstantiated as it perpetuates this low-value practice of screening and potential harm.[3]

The authors defined endophthalmitis without requiring vitreous involvement as a universal criterion in their analyses. As previously defined, endophthalmitis necessitates vitreous inflammation.[4,5] Its observation in patients with *Candida* bloodstream infection is rare (less than 1%) as found in routinely screened patients based on thousands of prospectively and retrospectively screened patients by systematic review[6], which is absent in the discussion. Instead, this study inflates the true rate of endophthalmitis by including patients with less severe disease including chorioretinitis (and those with non-specific lesions, such as cotton wool spots in the retina). Grouping these findings together is akin to classifying both simple urinary tract infection and pyelonephritis as pyelonephritis, despite each having distinct prognoses and approaches to management. This alarmingly poor methodology is further apparent when non-specific findings associated with genitourinary irritation are also included in this pyelonephritis category, despite the lack of an infectious etiology.

The unusually high rate of endophthalmitis or ocular involvement (16%) reported by the authors[1] and the IDSA[2] can be explained by the underlying comorbidities associated with *Candida* septicemia. This statistic is similar to those with abnormal retinal findings in sick patients from a critical care unit (19%), including those without *Candida* septicemia.[7] There is no mention by Seidelman et al. how retinal findings consistent with medical comorbidities were distinguished from intraocular infection with *Candida* organisms. While some of the sub-analyses appear to identify differences between cases of chorioretinitis without vitreous involvement and those with vitreous involvement, there is no explanation as to how chorioretinitis was defined. Many lesions that are classified as chorioretinitis can be clinically indistinguishable from retinal findings that are non-specific, including cotton wool spots, even with advanced imaging modalities including optical coherence tomography.[8] There is also no mention of any *Candida* species or other infectious organisms isolated from any ocular samples of any of these patients.

Interestingly, the authors ascribe race as a risk factor for the development of endophthalmitis in this setting, but without substantiation. As retinal lesions are typically explained by underlying comorbidities, this finding is also confounded by known differences in outcomes from associated medical problems including diabetes based on race, regardless of infection.[9] The authors mention assessing "if an ophthalmologic examination was performed" as an "exposure of interest" across their institutions, but in the results section, fail to report the denominator of patients with *Candida* septicemia including those who did not receive this examination, subjecting this study to selection bias. Given the lack of utility of these screening examinations,[6] it is common for physicians to forego this low-value practice, as evidenced by rates of non-adherence exceeding 30% for patients with *Candida* septicemia.[3]

The authors advocate for targeted screening of patients with *Candida* septicemia, yet no visual outcomes data are presented to substantiate this recommendation. In addition to lack of microbial confirmation of organisms from ocular tissue, there is no evidence of any change in systemic or ophthalmologic management based on a diagnosis of endophthalmitis. The authors also claim that systemic antifungal therapy may change based on the presence of ocular findings, however neither their data nor other prospective, multicenter investigations have substantiated this hypothetical benefit.[1,3,6] There is also no report of any vitreous biopsy, intravitreal injection, or vitrectomy surgery for any patient, or the course following treatment, if performed. Screening has not been shown to improve outcomes, and instead the risks from potentially unnecessary interventions have been demonstrated without showing benefit.[3,6,10] The practice of immediate systemic antifungal administration for minimum two weeks following negative blood culture growth and exchange of indwelling catheters with infectious source control, as advocated by the IDSA,[2] appears sufficient for the resolution of incidentally associated ocular findings with *Candida* septicemia.[3,6] Consistent with these findings, the American Academy of Ophthalmology recommends against routine ophthalmologic screening of patients solely on the basis of *Candida* septicemia.[3]

In summary, Seidelman et al. fail to construct a methodologically sound investigation, provide critical data, and sufficiently analyze their results in the context of retinal findings in patients with *Candida* septicemia to support their claims, which includes overinflation of true endophthalmitis and retinal findings due to infection. Many of the limitations within this study are also shared by other reports in recent literature.[3,10] At least until robust clinical data with rigorous disease classification, control groups, and long-term visual outcomes are provided, the authors should refrain from inappropriately escalating endophthalmitis incidence and endors-

ing this low-value screening practice (regardless of modification) that can subject harm to patients.

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Integrated mobile container PCR laboratory (IMCPL): A novel strategy to reduce unnecessary rapid antigen testing

Dear Editor,

Kretschmer et al. reported in this journal that SARS-CoV-2 lateral flow tests (LFT) for rapid antigen testing resulted in an unnecessary burden in Germany with statistics on rapid antigen testing conducted in Cologne, in May 2021.[1] In this study, they used the PCR test to determine the false positive rate of the antigen test. The results showed that the total false positive rate of rapid antigen detection was as high as 46.28%. Andreas and colleagues concluded that "From a pure laboratory and diagnostic point of view it has to be concluded that the usage of an antigen testing strategy with a false detecting rate of about 50% is unacceptable."[1]

To obtain the insights into the economic burden of rapid antigen testing, they calculated the direct costs arising from the performed tests and the indirect costs arising from the subsequent quarantine. The authors further concluded that "the use of rapid antigen testing only appears appropriate in high prevalence/incidence situations, because a (too) low prevalence may increase the risk of false-positive results leading to unnecessary quarantine and high economic burden."[1]

Currently, the diagnosis of COVID-19 is mainly based on testing SARS-CoV-2 RNA load using quantitative real-time polymerase chain reaction (qRT-PCR).[2] Although the detection method using qRT-PCR has been considered as the benchmark among the currently available diagnostic approaches, strategies to rapidly scale up the testing for SARS-CoV-2 must be considered for future diagnostic testing, particularly dealing with the circumstances encountered worldwide in the past year. Nucleic acid testing usually requires considerable laboratory equipment and staff, and test results may take several days to become available to the physician.[3] Therefore, rapid antigen testing is a crucial supplement to nucleic acid testing for COVID-19 management with the test results obtained rather quickly.[4] However, taking the experience of Kretschmer et al.[1] into account, with the rapid antigen testing leading to unnecessary quarantine and high economic burden, the novel rapid and inexpensive testing methods are therefore valuable.

To deal with these difficulties in detecting the SARS-CoV-2, the Integrated Mobile Container PCR Laboratory (IMCPL) unit was developed in China, which was a standard PCR laboratory established inside a walk-in container and can be quickly set up at any convenient locations to carry out the large-scale diagnostic testing.

Two main characteristics of the IMCPL make this medical unit more suitable for diagnostic detection than the traditional laboratories in the event of major public health emergencies. First, the IMCPL unit is extremely sturdy and mobile (Fig. 1). The ~~square~~ rectangle cabin is 17.5 m in length, 3 m width, and 3 m height, built using steel plates of 3 mm thickness with each part further reinforced to ensure the safety during transportation. In order to be deployed rapidly, the IMCPL unit can be conveniently carried and transported by using a logistics 17.5 m semi-trailer (Fig. 1A), enabling the long-distance and cross-regional laboratory support facilitated in a short time. Second, the installation requirements of the IMCPL unit are minimal. The IMCPL unit can be installed quickly at any convenient sites under most circumstances in less than 2 h with the availability of both water and electricity.

To assess the operating efficiency of the IMCPL unit, we carried out the tests in the IMCPL unit at the Shandong Provincial Hospital in China. The processing speed of the laboratory completing the sample testing depends largely on the proficiency of the personnel members and the operational efficiency of the supporting equipment.[5] In our study, several skilled medical laboratory technicians performed individually a load test on the supporting equipment installed in the IMCPL unit, including the hot thermostat pan (used to inactivate viruses), nucleic acid extractor, and the fluorescent quantitative PCR analyzer (Fig. 2A-C). The experimental procedure was performed according to the manual of the kits.

The results showed that it took a skilled technician ~35 min to complete the sample inactivation, 20–40 min to finish the nucleic acid extraction, and 90–120 min to run the nucleic acid amplification (Fig. 2D). These results showed evidently that the operation efficiency on the equipment was greatly improved in the IMCPL unit in comparison to normal process performed in a regular molecular laboratory. Overall, it took a skilled technician 135–195 min to complete the detection of viral nucleic acid in a batch of 96 samples in the IMCPL unit.

In order to maintain the high work efficiency, we organized four working shifts every day with a group of staff members replaced and the instruments disinfected every 6 h. The results showed that an IMCPL unit equipped with 12 PCR analyzers completed the nucleic acid detection of ~8000–10,000 clinical samples within 24 h

Fig. 1. The IMCPL units in China. (A) An IMCPL unit loaded onto a truck and later transported to Korla, Xinjiang. (B) An IMCPL unit being unloaded from the truck and installed locally. (C) An IMCPL unit set up at the Hongkong West Road in Qingdao, Shandong, next to the buildings of Guoxin Haitian Center. (D) An IMCPL unit installed at the Qingdao Navy Special Service Sanatorium, Qingdao, Shandong.

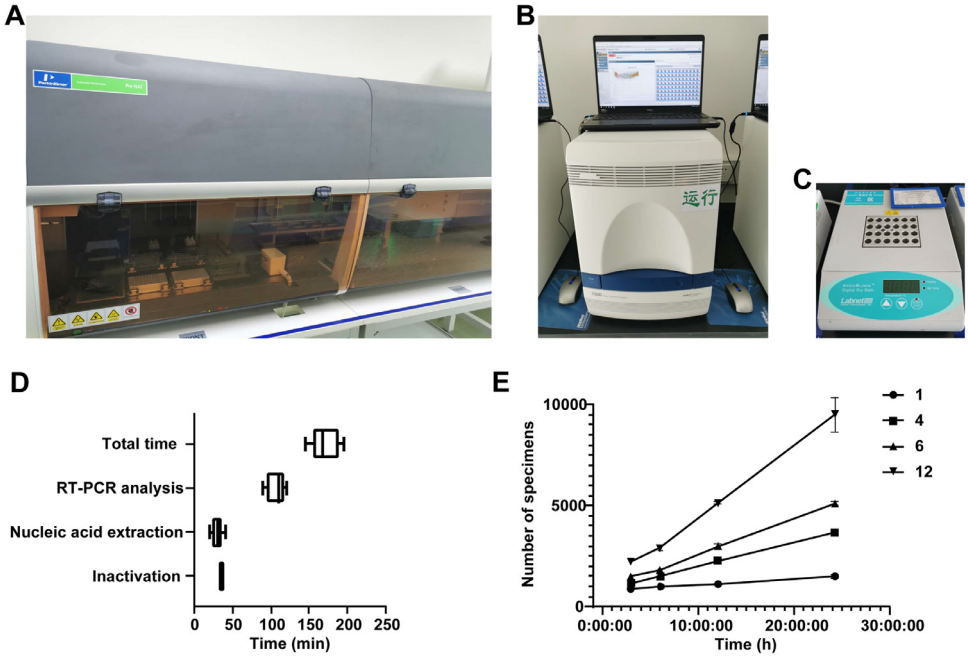

Fig. 2. The high operating load of the IMCPL unit. (A) Nucleic acid extractor. (B) PCR analyzer. (C) Dry thermostat. (D) A statistical chart of the time required by a skilled laboratory doctor to complete the steps of COVID-19 testing. (E) Statistical graph of the number of specimens tested vs. number of PCR analyzers operated.

(Fig. 2E), demonstrating evidently the enhanced SARS-CoV-2 detection capability of the IMCPL unit in a high-throughput manner.

It is worth noting that under the condition of low incidence, IMCPL can be used as an independent laboratory configured by the hospital to complete the daily testing work. The Shandong Provincial Hospital began to operate the IMCPL unit on January 7, 2021, with a total of 132,027 samples processed by August 14, 2021, significantly alleviating the workload pressure on the clinical laboratory at the hospital.

The large-scale SARS-CoV-2 screening is necessary to prevent the spread of the disease COVID-19, to help government make relevant public health policies, and to test people and their close contacts with a history of travel or residence in the epidemic areas. The IMCPL unit can be used as a reserve force for the health and epidemic prevention programs, ultimately reducing the unnecessary high positive rate and economic burden caused by the application of rapid antigen testing. Under the current unpredictable situations in many countries globally, the IMCPL unit could be used in combination with the local medical laboratories to eliminate the blind spots in the areas with epidemic.

Declaration of Competing Interest

The authors declare that no competing interests exist related to this submission.

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Clinical features of COVID-19 by SARS-CoV-2 Gamma variant: A prospective cohort study of vaccinated and unvaccinated healthcare workers

Dear Editor,

In this Journal, Gidari and colleagues showed that sera from BNT162b2 vaccinated humans had lower neutralization potency on the SARS-CoV-2 Gamma variant and that convalescent patients that had been infected by the Gamma variant were less protected from other SARS-CoV-2 strains.[1]

The Gamma variant has been considered the predominant SARS-CoV-2 lineage in Brazil during the first half of 2021.[2] In our tertiary-care hospital in São Paulo, Brazil, all healthcare workers (HCW) with suspected COVID-19 are clinically evaluated by the HCW Health Services and tested using RT-PCR for SARS-CoV-2. We aimed to characterize the clinical features of COVID-19 caused by the Gamma variant in comparison with strains that were not variants of concern (non-VoC).

In this prospective cohort we included symptomatic COVID-19 cases among HCW from January 22 to May 15, 2021. All positive samples for SARS-CoV-2 RT-PCR underwent whole genome sequencing using the MinION platform (additional details on supplementary material).[3,4] Clinical data were collected using a structured data formulary.

Proportions and medians were compared using the chi-square test and Mann-Whitney U test. Risk factors for Gamma variant infection were evaluated using a multivariate logistic regression with COVID-19 immunization, previous COVID-19, age, and month of diagnosis as independent variables. Risk factors for the presence of COVID-19 symptoms on diagnosis were evaluated using a multivariate logistic regression including Gamma variant infection, COVID-19 immunization, previous COVID-19, duration of symptoms on diagnosis, sex, and age as independent variables. Since we evaluated risk factors for 11 symptoms, these analyses were adjusted for multiple comparisons according to Bonferroni's correction. A HCW was considered to be fully immunized after at least 14 days after the second dose of any COVID-19 vaccine. Statistical analyses were two-tailed with an alpha error of 0.05. The software SPSS (version 17.0) and R (version 4.1.0) were used for the analyses. This study was approved by the Hospital's Ethics Committee (CAAE:42,708,721.0.0000.0068).

During the study period there were 523 symptomatic COVID-19 cases among HCW and 423 of them were included in the study, of which 415 (98%) had mild disease (Table 1 and Supplementary Fig. 1). The median (25th–75th percentile) age of our study group was 38 (29–48) years, 70% were female. Of the 175 (41%) fully immunized patients, 173 (99%) had received CoronaVac with a median (25th–75th percentile) interval between the second dose of the vaccine and onset of symptoms of 61 (37–75) days. Seventeen patients had previous COVID-19 with a median (25th–75th percentile) interval between the onset of symptoms of each episode of 245 (230–276) days. The most prevalent symptoms were coryza (73%) and headache (72%).

Among the 423 SARS-CoV-2 isolates, 313 (74%) were of the Gamma variant and 110 (26%) were non-VoC. The distribution of SARS-CoV-2 lineages varied over the study period with predominance of non-VoC cases in the first months and a posterior increase in cases caused by the Gamma variant (Supplementary Fig. 2).

Table 1
Clinical characteristics of 423 healthcare workers with COVID-19, and bivariate analysis comparing cases caused by the Gamma variant with cases caused by strains that are not variants of concern (non-VoC) (Hospital das Clínicas, São Paulo, Brazil. January 22 - May 15, 2021).

	Gamma variant ($N = 313$) N (%) or median (25th–75th percentile)	Non-VoC ($N = 110$)	p [a]
Age (years)	37 (29–49)	39 (31–46)	0.452
Age strata (years)			0.890
16–40	189 (60%)	67 (61%)	
41–60	106 (34%)	38 (35%)	
> 60	18 (6%)	5 (5%)	
Female	220 (70%)	76 (69%)	0.814
Pre-existing conditions			
Hypertension	35 (11)	12 (11)	0.938
Hyperlipidemia	17 (5)	7 (6)	0.716
Chronic pulmonary disease	16 (5)	2 (2)	0.176
Type 2 diabetes mellitus	15 (5)	4 (4)	0.791
Heart disease	14 (5)	6 (6)	0.676
Obesity	7 (2)	1 (1)	0.686
Chronic kidney disease	3 (1)	0	0.571
Immunosuppressive drug use	2 (1)	2 (2)	0.278
Cancer	2 (1)	1 (1)	1.000
Solid-organ transplantation	1 (0)	0	1.000
Pregnancy	4/220 (2)	1/76 (1)	0.932
Work category			0.628
Nursing technician	79 (25)	25 (23)	
Administrative staff	66 (21)	23 (21)	
Physician	58 (18)	16 (14)	
Auxiliary health team[b]	43 (14)	20 (18)	
Nurse	27 (9)	11 (10)	
Multidisciplinary health team[c]	18 (6)	10 (9)	
Others	22 (7)	5 (5)	
Vaccination status and type of vaccine			< 0.001
Unvaccinated	44 (14)	22 (20)	
Partially immunized [d]	99 (32)	83 (75)	
CoronaVac	71 (72)	81 (98)	
ChAdOx1	19 (19)	1 (1)	
Unknown	9 (9)	1 (1)	
Fully immunized [e]	170 (54)	5 (5)	
CoronaVac	168 (98)	5 (100)	
ChAdOx1	1 (1)	0	
BNT162b2	1 (1)	0	
Symptoms on diagnosis			
Coryza	236 (75)	71 (65)	0.028
Headache	230 (74)	76 (69)	0.376
Cough	227 (73)	74 (67)	0.296
Sore throat	170 (54)	52 (47)	0.203
Myalgia	159 (51)	43 (39)	0.034
Asthenia	137 (44)	43 (39)	0.393
Fever	94 (30)	43 (39)	0.81
Hyposmia/anosmia [f]	78 (25)	51 (46)	< 0.001
Dysgeusia [f]	66 (21)	42 (38)	< 0.001
Gastrointestinal tract symptoms[g]	63 (20)	33 (30)	0.033
Dyspnea	31 (10)	8 (7)	0.412
Duration of symptoms (days)	3 (2 - 4)	3 (2–4)	0.823
COVID-19 diagnosed previously	7 (2)	10 (9)	0.002
Previous positive RT- PCR	3 (43)	0	
Previous positive serology	4 (57)	10 (100)	
Hospitalization	5 (2)	3 (2)	0.434
Oxygen supplementation	4 (1)	3 (3)	0.383
Admission to intensive care unit	3 (1)	2 (2)	0.608
Invasive mechanical ventilation	3 (1)	0	0.571
Vasoactive drug support	2 (1)	0	1.000
Thromboembolic event	2 (1)	1 (1)	0.772
Death	3 (1)	0	0.571

[a] Calculated by Chi-square test or Mann Whitney U test.
[b] Included workers of pharmacy, laboratory, radiology, security, and medical students.
[c] Included physiotherapist, dentist, psychologist, nutritionist, and social services.
[d] Recipients of at least one dose of a COVID-19 vaccine and recipients of 2nd dose in the last 13 days.
[e] Recipients of two doses of a COVID-19 vaccine at least 14 days after the second dose.
[f] The simultaneous dysfunction of taste and smell was present in 93 (30%) Gamma variant cases and 58 (53%) non-VoC cases.
[g] Abdominal pain, diarrhea, nausea, or vomiting.

Although in the bivariate analysis COVID-19 immunization was associated with infection by the Gamma variant, in the multivariate analysis immunization status was not associated with Gamma variant infection after adjustment for the month of occurrence of COVID-19, age, and history of previous COVID-19 (Supplementary Table 1 and Fig. 3). The month in which COVID-19 occurred was the only factor associated with having an infection by the Gamma variant.

In the unadjusted bivariate analysis, coryza and myalgia were more frequent in Gamma variant cases; and hyposmia/anosmia,

dysgeusia, and gastrointestinal tract symptoms in non-VoC cases. However, in the multivariate analysis after adjustment for multiple comparisons, hyposmia/anosmia (OR = 0.304, adj $p < 0.001$) and dysgeusia (OR = 0.385, adj $p = 0.011$) were the only symptoms significantly associated with the Gamma variant (Table 2 and Supplementary Table 2).

We showed important differences in the clinical presentation between Gamma variant and non-VoC infection with a decreased frequency of hyposmia/anosmia and dysgeusia in Gamma variant cases. The increased occurrence of taste and smell disorders in COVID-19 has been considered a useful tool for the clinical triage of respiratory infections during the pandemic, with a frequency varying from 5 to 87% in COVID-19.[5–7] Thus, COVID-19 caused by the Gamma variant may present more often with cold-like symptoms. It has also been described that hyposmia/anosmia and dysgeusia are more common among younger and female patients.[6,8] However, there was no association between these symptoms and age or sex in our study.

The increase in the proportion of COVID-19 cases caused by the Gamma variant in early 2021 was temporally associated with the beginning of the vaccination campaign in Brazil.[2] This context raised the concern that Gamma variant could evade previous SARS-CoV-2 immune response, as suggested in a previous *in vitro* study.[9] Our results do not corroborate this hypothesis since we found no association between immunization status or previous COVID-19 diagnosis, and Gamma variant infection. However, our study does not have enough power to analyze this association due to restricted circulation of Gamma variant soon after the vaccination campaign.

The rise in Gamma variant cases in Brazil was also coincident with an increased COVID-19 incidence in younger patients.[10] Nonetheless, there was no association between age and Gamma variant infection in our study. This difference is probably explained by the COVID-19 vaccination rollout in Brazil, which prioritized elderly and HCW regardless of age.

Our study has limitations. Our results might not be generalizable to severe cases of COVID-19 since our cohort comprised mainly mild cases. The temporal association between the vaccination campaign and the rise in Gamma variant cases might have impaired the evaluation of the association between vaccination status and the occurrence of Gamma variant infection by unadjusted or residual confounders.

In conclusion, COVID-19 caused by the Gamma variant presents with different symptoms compared to non-VoC infection. The increase in Gamma variant cases should raise the awareness that COVID-19 may present more often with cold-like symptoms because of a decrease in the frequency of hyposmia/anosmia and dysgeusia.

Table 2
Evaluation of factors associated with hyposmia/anosmia and dysgeusia in 423 healthcare workers with COVID-19 (Hospital das Clínicas, São Paulo, Brazil. January 22 - May 15, 2021).

	Hyposmia/anosmia						Dysgeusia					
	Bivariate analysis			Multivariate analysis			Bivariate analysis			Multivariate analysis		
	OR(95%CI)[a]	*p*	Adj p[b]	OR(95%CI)[a]	*p*	Adj p[b]	OR(95%CI)[a]	*p*	Adj p[b]	OR(95%CI[a]	*p*	Adj p[b]
Gamma variant	0.433(0.270–0.693)	< 0.001	< 0.001	0.303(0.176–0.522)	< 0.001	< 0.001	0.384(0.244–0.605)	< 0.001	< 0.001	0.385(0.221–0.672)	0.001	0.011
Previous COVID-19	1.630(0.606–4.381)	0.333	1.000	1.317(0.465–3.734)	0.604	1.000	1.626(0.586–4.507)	0.350	1.000	1.321(0.458–3.806)	0.607	1.000
Fully immunized[c]	0.856(0.561–1.306)	0.470	1.000	1.657(0.989–2.779)	0.055	0.605	0.784(0.500–1.230)	0.290	1.000	1.334(0.779–2.286)	0.294	1.000
Duration of symptoms	1.084(0.990–1.186)	0.081	0.891	1.119(1.020–1.227)	0.017	0.187	1.097(1.000–1.204)	0.50	1.000	1.125(1.023–1.236)	0.015	0.165
Female	1.250(0.801–1.951)	0.326	1.000	0.802(0.503–1.279)	0.354	1.000	0.975(0.605–1.573)	0.918	1.000	1.071(0.652–1.759)	0.788	1.000
Age strata (years)												
16–40	1 (ref)			1 (ref)			1 (ref)			1 (ref)		
41–60	0.734(0.468–1.151)	0.178	1.000	0.639(0.397–1.026)	0.064	0.704	0.821(0.512–1.318)	0.414	1.000	0.730(0.446–1.196)	0.212	1.000
>60	0.416(0.137–1.261)	0.121	1.000	0.281(0.083–0.947)	0.041	0.451	0.559(0.184–1.702)	0.306	1.000	0.408(0.121–1.377)	0.149	1.000

[a] Odds ratio (95% confidence interval).
[b] Adjusted *p*-value for multiple comparisons (11 symptoms) according to Bonferroni's correction.
[c] Recipients of two doses of a COVID-19 vaccine as of 14 days after the second dose.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.09.005.

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Bamlanivimab improves hospitalization and mortality rates in patients with COVID-19: A systematic review and meta-analysis

Dear Editor,

The coronavirus disease 2019 (COVID-19) pandemic has spread globally and poses a serious threat to the world. As of August 9, 2021, there were over 200 million COVID-19 cases and 4 million deaths. The clinical manifestations of COVID-19 range from mild to severe, the incidence of illness and mortality is high in some vulnerable subgroup of patients. Until now, treatment option for

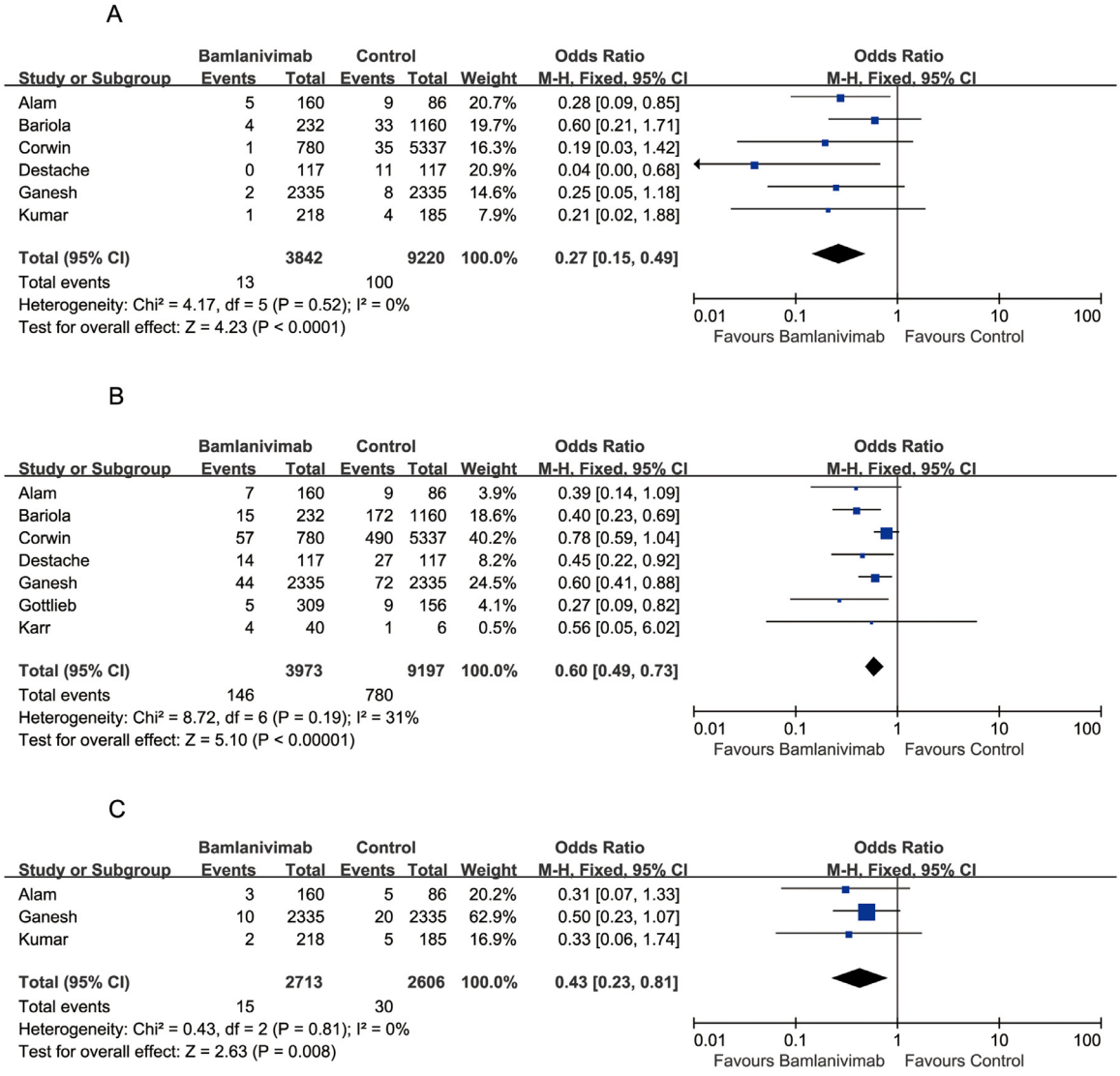

Figure. 1A. Association between Bamlanivimab treatment and mortality
Figure. 1B Association between Bamlanivimab treatment and hospitalization
Figure. 1C Association between Bamlanivimab treatment and developing severe COVID-19
Figure. 1D Pooled analysis of adjusted results of association between Bamlanivimab treatment and mortality
Figure. 1E Pooled analysis of adjusted results of association between Bamlanivimab treatment and hospitalization

patients to prevent progression to severe COVID-19 is limited. Although, vaccination is considered as an effective method to stop the spread of the COVID-19 pandemic, the newly emerged SARS-CoV-2 variants have led to breakthrough infections after completion of vaccination regimen.[1]

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) attaches to host cells by binding its spike protein to angiotensin-converting enzyme 2 (ACE2) receptors on target cells.[2] Bamlanivimab (also knowns as LY-CoV555 or LY3819253), a recombinant human IgG1 monoclonal antibody, binds to the receptor binding domain of the spike protein of SARS-CoV-2 and blocks viral entry into host cells. Bamlanivimab(BAM) was granted emergency use authorization by the Food and Drug Administration (FDA) in November 2020.[3] Currently, there were a few studies that evaluated the effect of BAM on clinical outcomes in patients with mild-to-moderate COVID-19. Thus, we aim to perform a systematic review and meta-analysis of the available evidence to investigate the efficacy of BAM in COVID-19.

An electronic search of the PubMed, Embase, and Cochrane Library databases was conducted from December 1 2019, to August 9 2021, with no language restrictions. The following key words and/or medical subject heading terms searched were used: ("COVID-19″ or "novel coronavirus" or "2019-nCoV" or "coronavirus disease 2019″ or "SARS-CoV-2″) AND ("Bamlanivimab" or "LY3819253" or "LY-CoV555"). A manual search of possible articles that were relevant to the topic were carried out. "PROSPERO (International Prospective Register of Systematic Reviews) database" registration was done with study number as CRD42021274064.

The inclusion criteria in our meta-analysis were as follows: (1) Patients with confirmed COVID-19; (2) reported information of comparison of clinical outcomes between BAM treatment (administered alone) and various control groups (no treatment, usual care according to the hospital guidelines, NIH guidelines). Studies were excluded if they were (1) case reports, conference abstracts, non-clinical studies, editorials and reviews; and (2) duplicated publications. All data from studies were extracted by independent investigators (GA and YW) onto prespecified. Any discrep-

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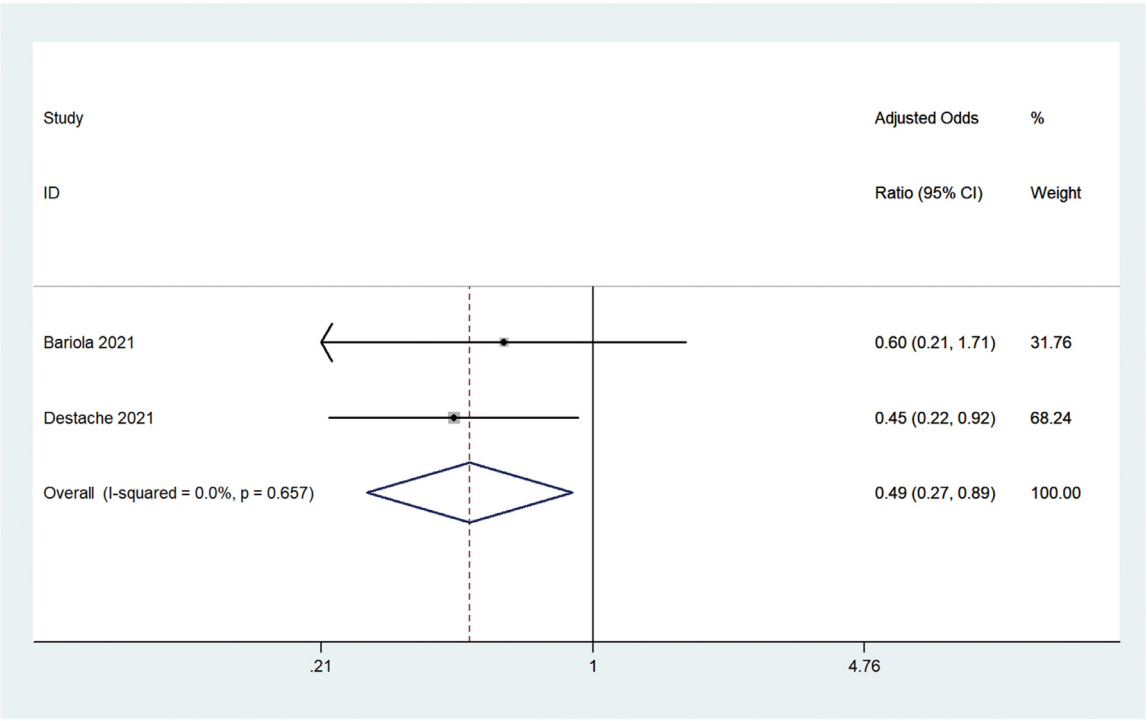

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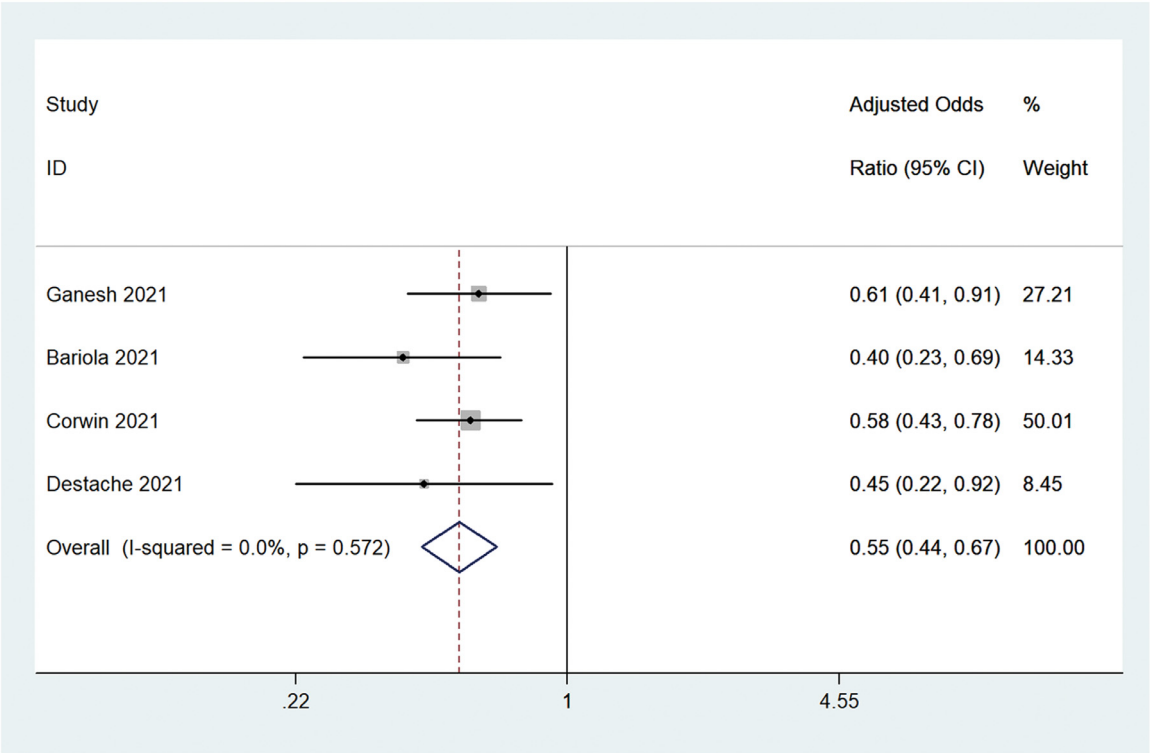

Figure. 1A. Continued

ancies were resolved through discussion in group conference. We also extracted baseline information of name of the first author, study design, country, the participants, number of participants, demographics data (mean/median age, male gender), dose of Bamlanivimab used, outcomes (mortality, hospitalizations, deterioration and progression to the complications). The quality of randomized controlled studies (RCTs) was evaluated using the Jadad scale. The quality of observational studies was assessed using a nine-item Newcastle-Ottawa Quality scale. High-quality studies were defined

Table 1
Characteristics of included studies.

Study	Country	Study design	Study population	Definition of severity used	Dose of bamlanivimab	Sample size	Bamlanivimab group		Control group	
							Age	Male (%)	Age	Male (%)
Alam[4]	America	Case-control	Mild-to-moderate COVID-19 in nursing homes and long-term care facilitie	Requiring mechanical ventilation	700 mg	246	81.42 ± 0.68	82 (51.2)	84.42 ± 0.89	35 (40.7)
Bariola[5]	America	Retrospective cohort	Mild to moderate COVID-19 nonhospitalized patient	NR	700 mg	1392	67.3 ± 13	108 (46.6)	67.1 ± 13.4	510 (44)
Corwin[6]	America	Retrospective cohort	Outpatients with mild to moderate COVID-19	NR	700 mg	6117	62.6 ± 15.6	352 (45.1)	56.7 ± 2.0	2309 (43.3)
Destache[7]	America	Retrospective cohort	Mild to moderate COVID-19 nonhospitalized patient	NR	NR	234	72 (65–80)	55 (47.0)	72 (65–80)	55 (47.0)
Ganesh[8]	America	Retrospective cohort	Mild to moderate COVID-19 do not hospitalize	ICU admissions	Single-dose bamlanivimab infusion	4670	63 (52,71)	1183 (50.7)	63(52,72)	1181 (50.6)
Gottlieb[9]	America	RCT	Mild-to-moderate COVID-19 nonhospitalized patient	NR	700 mg, 2800 mg, 7000 mg	465	39 (31–58)	38 (37.6)	46 (35–57)	71 (45.5)
Karr[10]	America	Retrospective cohort	Mild-to-moderate COVID-19 nonhospitalized patient	NR	700 mg	46	69	26 (65)	69	3 (50)
Kumar[11]	America	Case-control	Inpatient and outpatient	ICU admissions	700 mg	403	66 (57–74)	115 (52.8)	62 (50–72)	95 (51.4)

RCT: randomized controlled study; ICU: intensive care unit; NR: not reported.

as a study with a Jadad score of $\geq$ 2 (maximum, 5), or a modified Newcastle-Ottawa score (NOS) of $\geq$ 6 (maximum, 9).

The analysis was done using Review Manager and Stata software version 15.1. Reported odd ratios (ORs) and 95% CIs were extracted from each study. We selected OR as an effect estimate. The heterogeneity of outcomes was calculated using Cochran's Q test and the I^2 statistic. The I^2 value of 25%, 50%, and 75% represented low, moderate, and high degrees of heterogeneity, respectively. If $I^2 \leq 50\%$, the meta-analysis was performed using the fixed effect model (Mantel-Haenszel). If $I^2 > 50\%$, the random-effect model (DerSimonian and Laird) was preferred. Sensitivity or subgroup analyses were performed to identify potential sources of heterogeneity. Sensitivity analysis was performed to evaluate the stability of the results by sequentially omitting one study at a time. $P < 0.05$ was considered to be statistically significant.

The search strategy yielded 263 potentially eligible literatures. Among them, 47 literatures were excluded due to duplicated searches. 168 studies were subsequently screened and regarded as absolute irrelevant studies by examining titles and abstracts. 48 studies were identified for full-text review and 40 studies were excluded because they had no comparison groups between Bamlanivimab treatment and control or outcomes were not available. A total of 8 studies[4–11] comprising of 13,573 adult patients with COVID-19, including 4191 in the BAM (administered alone) and 9382 in the control group arm, were included in this meta-analysis.

The study characteristics of the included RCT, cohort studies and case-controls are shown in Table 1. All studies were from America. Four studies were retrospective cohort, two studies were case-control and only one study was RCT. Most studies included mild to moderate COVID-19 nonhospitalized patient and used a dosage of 700 to 7000 mg infusion of Bamlanivimab. All of the eligible studies were published in 2021 with different sample patient sizes that ranged from 46 to 6117 patients with COVID-19. The details of quality assessment are presented in the Supplemental Tables 1 and 2. All studies included in our meta-analysis were considered as high quality (RCT with a Jadad score of $\geq$ 2 and observational studies with a modified NOS score of $\geq$ 6).

The meta-analysis showed the overall mortality was lower in the BAM group compared to control group (OR = 0.27, 95%CI: 0.15, 0.49, $P < 0.0001$, $I^2 = 0\%$) (Fig. 1A). Moreover, BAM treatment were associated with lower risk of hospitalization (OR = 0.60, 95%CI: 0.49 to 0.73, $P < 0.00001$; $I^2 = 31\%$) (Fig. 1B) and developing severe COVID-19 disease (OR = 0.43, 95%CI: 0.23 to 0.81, $P = 0.008$; $I^2 = 0\%$) (Fig. 1C). Pooled analysis of adjusted results revealed that BAM group had a lower risk of mortality (OR = 0.49, 95%CI: 0.27 to 0.89, $P = 0.019$; $I^2 = 0\%$) (Fig. 1D) and hospitalization compared with control group (OR = 0.55, 95%CI: 0.44 to 0.67, $P < 0.001$; $I^2 = 0\%$) (Fig. 1E). In addition, sensitivity analyses by excluding each study at a time did not significantly alter the overall results.

COVID-19 remains a public health emergency. Although people have taken many measures to control the virus, the treatment of COVID-19 remains a challenge. Until now, vaccines are still the primary option for COVID-19 prevention.[12] Unlike vaccine-derived immunity that develops over time, the use of neutralizing monoclonal antibodies is an immediate and passive immunotherapy. In the current meta-analysis, our results demonstrated that patients received BAM treatment had a better outcome in hospitalization and mortality. With limited therapeutic options for COVID-19, BAM provide an effective treatment option.

Prevention of hospitalization is an important part of COVID-19 management. High patient volumes in the hospital from COVID-19 strain medical resources and supplies. The shortage of personal protective equipment and air isolation wards will bring greater risks to health care workers. As for mild-to-moderate COVID-19,

treatments administered in outpatient is a more reasonable approach to preserver hospital supplies and reduce overcrowding. Our study affirmed the efficacy of BAM in the treatment of mild-to-moderate COVID-19, which was consistent with previous clinical trials (BLAZE-1 and −2).[9,13] Before monoclonal antibodies treatment, there was no strategies to reduce the rates of hospitalization or death in outpatients with COVID-19.

SARS-CoV-2 infection is mediated by the interaction between the viral spike and the ACE2 receptors. BAM can specifically block this event. With the evolution of SARS-CoV-2 spike protein, the effect of monoclonal antibody therapy may be affected.[14] Due to the evolution of SARS-CoV-2 variants, the emergency use authorization (EUA) for Bamlanivimab monotherapy was rescinded by the FDA on April 16, 2021. Another dual monoclonal antibody treatment, Bamlanivimab plus Etesevimab remains in place.[15] Despite this, the results of this study indicated that treatment with neutralizing antibody was an effective way to mitigate the current COVID-19 pandemic.

Several limitations of our study should be noted. Most of the studies included were retrospective in design and had relatively small sample size, which were subject to potential confounders that may weaken the overall results. Moreover, all studies were conducted in America, which may not provide sufficient statistical power to explore accurate correlations. Furthermore, lack of data of individual patient, it is uncertain which patients will benefit most when treated with BAM. Therefore, large clinical RCTs are needed to overcome these limitations Despite these limitations, there were advantages of our meta-analysis. First, to our best knowledge, this is the first meta-analysis assess the clinical impact of Bamlanivimab monoclonal antibody monotherapy on mortality and disease severity in patients with COVID-19. In addition, the heterogeneity across the studies was low, which enhances the reliability of our results.

In conclusion, our meta-analysis provide evidence that BAM is effective in the treatment in COVID-19 patients. There is an urgent need for well-designed randomized trials to determine the effectiveness and safety of BAM in severe COVID-19.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.09.003.

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Mendelian randomization reveals potential causal candidates for COVID-19 in 123 blood metabolites

Dear Editor,

We read with interest the recently published letter in Journal of Infection by Yang et al., who conducted a quantitative meta-analysis of twenty-seven studies containing 146,364 cases and found a lack of association between dyslipidemia and COVID-19 mortality.[1] However, Hilser et al. investigated the relationship between metabolic syndrome-related serum biomarkers and the severity of COVID-19 in UK Biobank and found that an increase of 10 mg/dl HDL-Cholesterol reduced the risk of suffering from COVID-19 by 13% (OR = 0.87, 95% CI: 0.79–0.94, P = 1.2E-03).[2] In fact, most studies collected by Yang et al. neglected to distinguish the subtypes of dyslipidemia in detail, such as the levels

Table 1
MR analysis of blood metabolites and COVID-19.

Metabolites	Methods	N_{SNP}	Beta	SE	*P*	OR
Citrate	IVW	3	0.213	0.106	0.0452	1.24 (1.01–1.52)
	MR-Egger	3	0.914	1.67	0.681	2.49 (0.09–65.84)
M.VLDL.C	IVW	11	0.121	0.0573	0.0349	1.13 (1.01–1.26)
	MR-Egger	11	−0.0653	0.134	0.6374	0.94 (0.72–1.22)
S.VLDL.P	IVW	12	0.0944	0.0462	0.0411	1.10 (1.00–1.20)
	MR-Egger	12	−0.0664	0.119	0.590	0.94 (0.74–1.18)

VLDL: Very low density lipoprotein; M.VLDL.C: Total cholesterol in medium VLDL; S.VLDL.P: Concentration of small VLDL particles. SE: standard error. The statistically significant association of IVW is defined to be $P < 0.05/123 = 0.00041$. The statistically significance of no horizontal pleiotropy is defined to be $P > 0.05$.

of low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride and cholesterol, etc.[1] Inconsistent findings from previous studies sparked our interest to further investigate the causal relationship between blood metabolite levels and COVID-19.

In this study, we aimed to clarify causal candidates for influencing COVID-19 infection rates among 123 blood metabolites. In stage 1, we obtained the genome-wide association study (GWAS) summary statistics of 123 blood metabolites on nearly 25,000 individuals by Kettunen et al.[3] We obtained COVID-19 GWAS summary statistics from COVID-19 Host Genetics Initiative Round 4, including 14,134 COVID-19 cases and 1,284,876 controls. All the participants were of European descent. In stage 2, according to the assumptions of Mendelian randomization (MR) model, we chose SNPs that were strongly correlated with exposure (blood metabolites) as instruments ($P < 5E\text{-}08$).[4] We only kept the blood metabolites with more than 2 instruments, allowed SNPs with LD Rsq value > 0.8 as proxy SNPs in the outcome (COVID-19), and aligned strands for palindromic SNPs.[4] In stage 3, we performed MR analysis using the inverse variance weighted (IVW) method, and assessed the horizontal pleiotropy of the instruments using a sensitivity test[4]. MR analysis results showed that three blood metabolite concentrations were causally related to the higher risk of COVID-19, increasing the COVID-19 infection rate by 24%, 13%, and 10%, respectively, but none of them passed the adjusted significance threshold ($P < 0.05/123 = 0.00041$) (Table 1).

In conclusion, we supported that the blood metabolite concentration might not be related to the risk of COVID-19, and therefore patients with dyslipidemia might not need specific treatment and medication, so as to avoid potential waste of medical resources.[1]

Acknowledgment

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SARS-CoV-2 variants with shortened incubation periods necessitate new definitions for nosocomial acquisition

Dear Editor,

In the last issue, Lumley et al.[1] provide compelling evidence that indeterminate cases of nosocomial SARS-COV-2 infection, who develop symptoms 3–7 days after hospital admission,[2] are indeed genuine nosocomial acquisitions. By applying genomic data, they found 26/33 (79%) of sequenced indeterminate nosocomial cases share genomic similarity with their putative nosocomial transmission clusters formed through epidemiological linkage.

Interestingly, the definitions of nosocomial acquisition are based on the incubation period of ancestral strains of SARS-CoV-2. There is increasing evidence that SARS-CoV-2 variants of concern may have increased viral fitness, such as higher viral loads,[3] longer viral shedding,[4] and also shorter incubation periods. Also reported in this journal, the median incubation period for the alpha variant has been estimated at around 3 days, compared to around 5 days for ancestral strains.[5,6] In another study, delta variant was similarly shown to have a shorter incubation period compared to ancestral strains (4 days vs 6 days).[7] A shorter median incubation

period increases the likelihood that indeterminate cases as defined by current definitions are indeed nosocomial acquisitions. Notably, Lumley et al. study captures the second wave of the pandemic in the UK which was dominated by cases of the alpha variant.

As viral variants with increased fitness replace ancestral strains of SARS-CoV-2, definitions of nosocomial acquisition may therefore need to be altered to reflect the shorter incubation time. Moreover, a narrower distribution of incubation periods may make judging the probability of nosocomial acquisition vs community-onset acquisition more difficult, especially when using epidemiology alone. This further suggests genomic linkage may be required for accurate resolution of putative nosocomial transmission clusters.

In addition, in the context of shortened incubation periods and continued high prevalence of infection, methodology for using genomic-linkage to resolve transmission clusters can be strengthened. Genomic analysis of nosocomial transmission could include not just similarity of genomes to other epidemiologically-linked cases, but also a measure of how common this haplotype (or variant) is in the community. Confidence of transmission between epidemiologically-linked cases can be increased not only if the cases share haplotypes, but also if this haplotype is rare compared to those circulating in the community. This measure has been introduced previously,[8] perhaps most robustly by Stirrup et al.[9] who provide a statistical tool for this purpose.

Declaration of Competing Interest

No competing interests to declare. No funding applicable to this correspondence.

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Antibody formation against SARS-CoV-2 in imatinib-treated COVID-19 patients

Dear Editor,

The article by Gobbi and colleagues[1] exemplifies the general interest in understanding the dynamics of immune response to SARS-CoV-2 vaccine, which is currently being widely investigated. However, the study of this response after COVID-19 infection should not be left aside, particularly in those patients who have been treated with drugs that may alter the normal functioning of the immune system. This is the case of imatinib, the principal BCR-Abl inhibitor for the treatment of chronic myeloid leukaemia (CML), which has been proposed as a potential treatment for SARS-CoV-2 infection, as it may exhibit both antiviral and immunomodulatory properties.[2] Indeed, a recent randomised controlled trial on the use of imatinib in severe COVID-19 has yielded encouraging results.[3] However, concerns have been raised regarding possible interferences of this kind of tyrosine kinase inhibitors (TKI) with *B*-cell response. [4]

Given the increasing attention on imatinib in COVID-19, it is a matter of interest to assess the presumed negative effect of this drug in the production of antibodies against SARS-CoV-2 in real-world patients. Thus, we have analysed the first 30 consecutive subjects from the ongoing COVINIB clinical trial, which evaluates the activity of imatinib and baricitinib in COVID-19 inpatients (NCT04346147), who were treated with the former drug. According to the study protocol, patients assigned to this group received imatinib 400 mg daily for 7 days as part of their treatment for COVID-19. Nuclear acid amplification test (transcription-mediated amplification or real-time polymerase chain reaction) for SARS-CoV-2 in nasopharyngeal swab was positive and pneumonia was confirmed by chest radiography in all patients before entering the clinical trial. Epidemiological, clinical, radiological and laboratory data were collected, as well as the main concomitant treat-

Table 1
Baseline characteristics, clinical data and main laboratory results.

Patient	Age (yr)	Sex	Day of symptoms*	Oxygen therapy		Laboratory results at imatinib onset				Other therapies	Days of hospitalization
				Day 1 of imatinib	Highest†	C-reactive protein (mg/dL)	Interleukin-6 (pg/mL)	Lymphocyte count (x10^9/L)	D-dimer (ng/mL)		
1	47	M	9	No need	No need	4.1	3.2	1.3	392	–	8
2	56	M	8	LFNC	LFNC	16.4	53.9	0.8	349	DXM, TCZ	9
3	57	M	3	No need	LFNC	14.4	–	0.7	328	DXM	7
4	61	M	10	No need	No need	9.8	26.8	1.2	729	–	8
5	53	M	6	No need	No need	2.5	–	0.6	952	DXM	7
6	49	M	5	LFNC	LFNC	4.6	16.0	1.1	302	DXM	7
7	57	F	8	No need	No need	2.7	6.2	1.6	306	–	6
8	64	M	9	LFNC	LFNC	8.5	10.2	1.0	910	–	5
9	56	M	5	No need	LFNC	10.6	21.7	1.4	445	–	7
10	53	M	8	LFNC	LFNC	2.1	37.4	1.4	453	DXM	8
11	50	M	10	No need	No need	6.4	25.7	1.0	409	–	4
12	39	M	8	LFNC	Mask	6.5	26.9	1.5	307	DXM, TCZ	13
13	56	F	6	LFNC	LFNC	14.4	3.6	1.0	709	DXM	7
14	61	F	5	LFNC	LFNC	11.1	1.5	1.2	475	DXM, RDV	9
15	53	M	3	LFNC	Mask	30.6	237.0	0.9	630	DXM, TCZ	18
16	65	M	5	LFNC	IMV	15.1	91.0	1.0	396	DXM, TCZ	41
17	40	M	7	No need	No need	6.2	24.5	1.3	911	–	7
18	65	F	8	No need	LFNC	13.2	29.3	1.2	882	DXM	11
19	53	F	10	No need	No need	7.7	18.6	1.2	571	–	5
20	53	M	9	No need	No need	4.6	12.6	1.0	313	DXM	7
21	50	M	7	LFNC	LFNC	8.6	27.0	1.4	561	–	5
22	51	M	10	No need	LFNC	13.4	109.2	0.6	238	DXM, TCZ	9
23	64	F	8	No need	LFNC	4.5	19.0	1.4	449	DXM	7
24	54	M	9	LFNC	LFNC	10.9	106.0	1.1	445	DXM, TCZ	6
25	60	M	5	No need	LFNC	6.0	18.0	1.2	551	DXM, RDV	7
26	51	M	6	LFNC	LFNC	9.2	145.5	0.8	458	DXM, TCZ	6
27	60	M	6	No need	LFNC	2.1	26.4	1.3	150	DXM, TCZ	12
28	69	F	9	LFNC	LFNC	12.3	–	0.7	505	DXM	8
29	57	M	7	LFNC	Mask	8.4	35.1	0.8	390	DXM, TCZ	12
30	35	M	6	No need	No need	7.1	391.0	1.2	522	DXM	6

Abbreviations: *yr* = years; *M* = male; *F* = female; LFNC = low-flow nasal cannula; Mask = Venturi mask or nonrebreather face mask; IMV = invasive mechanical ventilation; DXM = dexamethasone; TCZ = tocilizumab; RDV = remdesivir. Reference ranges for C-reactive protein, interleukin-6 and D-dimer: < 0.5 mg/dL, < 7 pg/mL and < 500 ng/mL, respectively.

* Days from symptom onset to treatment with imatinib.

† Highest oxygen therapy support needed after imatinib onset.

ments for COVID-19 during follow-up. SARS-CoV-2 serology was performed at the last control visit, which was scheduled at 70 ± 5 days from inclusion. In this regard, serum antibodies against SARS-CoV-2 were measured using Maglumi 2019-nCoV chemiluminescence immunoassay for specific IgG against viral spike and nucleocapsid antigens (Snibe Diagnostic) and/or Access SARS-CoV-2 IgG Antibody Test for IgG against the receptor binding domain of the S1 protein subunit of the viral spike (Beckman Coulter). Immunoglobulin concentrations were obtained in AU/mL, and the result was considered positive or negative according to manufacturer's instructions.

The main baseline characteristics of the patients are included in Table 1, as well as clinical and laboratory data regarding SARS-CoV-2 infection. To describe quantitative data, mean and standard deviation or median and interquartile range were used as appropriate, while percentages were calculated for qualitative data. 76% of patients were male, and the mean age reached 54.6 ± 7.8 years. Three subjects (10.0%) were current smokers and 16.7% had a previous history of alcohol consumption. The prevalence of dyslipidaemia, hypertension and diabetes mellitus was 40.0%, 30.0% and 16.7%, respectively, and the most common preadmission medications were statins (23.3%), angiotensin-converting enzyme inhibitors (16.7%) and oral hypoglycaemic drugs (16.7%). Four patients had been previously diagnosed with obstructive sleep apnea syndrome, and one subject had asthma. The mean of days from symptom onset to initiation of imatinib was 7.2 ± 2.0. Median C-reactive protein and interleukin-6 levels at imatinib start were 8.5 mg/dL (interquartile range 12.5–4.6 mg/dL) and 26.4 pg/mL (interquartile range 53.9–16.0 pg/mL), respectively. Mean lymphocyte count at that moment was 1.1 ± 0.3 × 10^9/L, while mean D-dimer value was 501.3 ± 208.5 ng/mL. Regarding concomitant therapies for COVID-19, dexamethasone, tocilizumab and remdesivir were administered to 70.0%, 30.0% and 6.7% of patients, respectively. Prophylactic enoxaparin was received by 93.3% of subjects. Only one patient (number 16) required intensive care unit admission, and all subjects were alive at the conclusion of the study. Anti-SARS-CoV-2 IgG positivity reached 100% at the end of follow-up; no patient had received any dose of any SARS-CoV-2 vaccine. Neither readmissions related to reactivation of COVID-19 nor reinfections were documented.

Previous evidence from *in vitro* studies supports the putative deleterious effect of BCR-Abl inhibitors on humoral response, as both decreased serum levels of immunoglobulins and a reduced number of memory *B*-cells have been observed in CML patients treated with this kind of TKI.[5,6] Moreover, treatment with such drugs has also been linked with a suppressed *B*-cell activation,[7] as well as with impaired isotype switching in a murine model.[8] Although there is not robust correlation between these observations and an increased risk of infection, concerns have been raised about the potential interference of these TKI with antibody formation after vaccination[6] or some infections, including COVID-19.[4] Regarding serological response to SARS-CoV-2 vaccine, studies of hematological patients receiving BCR-Abl inhibitors have shown conflicting data: while anti-SARS-CoV-2 immunoglobulins are de-

tected in most of these subjects after vaccination,[9,10] antibody synthesis may be negatively affected in some degree, as one study found that median IgG antibody concentration in TKI-treated patients was around half of that reported in healthy controls.[10]

To our knowledge, this is the first report of non-hematological COVID-19 patients who received treatment with imatinib for this infection and in whom the subsequent production of IgG against SARS-CoV-2 has been assessed. Even though the short duration of imatinib treatment might have prevented this TKI to display its hypothetical detrimental effects on humoral immunity, the finding that anti-SARS-CoV-2 antibodies were present in all analysed subjects after infection contributes to encouraging the research on the role of imatinib in COVID-19.

In conclusion, although our results are preliminary and related to a low number of cases, they suggest that a short course of imatinib does not interfere with IgG formation in hospitalised COVID-19 patients. Further investigation with this and other BCR-Abl inhibitors would be needed to elucidate the potential impact of these drugs on humoral immunity against SARS-CoV-2.

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Ethical statement

This study was approved by the Regional Ethics Committee (Comité de Ética de la Investigación con Medicamentos Regional de la Comunidad de Madrid)

Declaration of Competing Interest

David Bernal-Bello is the principal investigator of the COVINIB trial, a non-sponsored randomised trial investigating the therapeutic role of imatinib and baricitinib in COVID-19 patients (NCT04346147; EudraCT 2020–001321–31). The rest of the authors are sub-investigators in this project. All authors declare no other competing interests.

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Emergence of Q493R mutation in SARS-CoV-2 spike protein during bamlanivimab/etesevimab treatment and resistance to viral clearance

Dear Editor,

Data from clinical trials suggest that monoclonal antibody (mAb) treatments can prevent deaths and severe disease among people with mild to moderate COVID-19. Recently, a cocktail of two mAbs- bamlanivimab and etesevimab- was reported to cut the risk of hospitalization and death by 87%.[1] The European Medicine Agency (EMA) recommends the use of this combination in patients who do not require supplemental oxygen, and who are at high risk of progression to severe disease.[2] Since SARS-CoV-2 Spike protein is the target of antibody-based therapeutics, they all suffer from one major risk: mutational escape of the Spike protein.[3]

Table 1
Virological data during treatment follow-up.

Virological data	Bamlanivimab/Etesevimab treatment						
Day post treatment	−2 (diagnosis)	0	7	15	21	27	39
CT value (RDRP gene, Allplex assay)	18.6	9.9	20.6	15.5	15.1	14.3	24.8
SARS-CoV-2 lineage	NA	B.1.1.7	B.1.1.7	B.1.1.7	B.1.1.7	NA	B.1.1.7
Presence of E484K/Q mutation (Yes/No)	No	No	No	No	No	No	No
Amino-acid at Spike position 493	NA	Q	Q	R	R	NA	R
Number of reads at Spike nucleotide position 23,040 (total number of reads)	NA	177A (177)	142A (142)	169 G (169)	2506 G (2516)	NA	935 G (936)
GISAID reference for viral genome sequence	NA	EPI_ISL_ 2,143,435	EPI_ISL_ 2,227,230	EPI_ISL_ 2,361,537	EPI_ISL_ 2,444,359	NA	EPI_ISL_ 2,646,207
Total antibody testing (qualitative, Wantai) (Index)	NA	NA	11.6	13	NA	10.3	12.5
IgG quantification (Euroimmun) (binding activity units/mL)	NA	NA	>1920	>1920	NA	>1920	>1920

We here report a well-characterized case of the emergence of SARS-CoV-2 Spike escape.

Q493R mutation during bamlanivimab/etesevimab treatment.

A 63-year-old patient was diagnosed in July 2017 with cutaneous T-cell lymphoma (mycosis fungoides). The patient underwent several treatments including carmustine, methotrexate, bexarotene, and extracorporeal photopheresis. Tumoral extension to lymph nodes (stage III) led to a CHOEP chemotherapy regimen. An allogeneic hematopoietic stem cell transplantation was then performed in January 2021, complicated by the occurrence of a graft-versus-host disease.

A COVID-19 screening was performed in April 2021, due to an intrafamilial exposure, and a positive RT-PCR result was found on a nasopharyngeal (NP) specimen. The patient developed mild symptoms such as runny nose and dry cough. A treatment with bamlanivimab (700 mg) /etesevimab (1400 mg) was initiated. Sequential NP samples were collected for treatment follow-up.

SARS-CoV-2 RNA detection and E484K mutation screening were performed with the AllplexTM SARS-CoV-2 Variants I Assay (Seegene, Eurobio®). For the viral whole genome sequencing, total RNA extraction was performed using the MGIEasy Nucleic Acid Extraction Kit on the MGISP-960 instrument (BGI®). The libraries were prepared using Illumina® COVIDSeq protocol, and paired-end sequencing with 150 bp read length was carried out on NextSeq 550 platform. Data were processed using DRAGEN COVIDSeq Test Pipeline 1.0.0 (Illumina®). Clades and lineages were assigned to the genomes according to the Nextstrain nomenclature (version 1.0.0) and the PANGOLIN package (version 2021.06.15), respectively. Mutation analysis of the Spike region was provided by Nextstrain and GISAID CoVsuver tool.

Serum samples were also collected for antibody testing. Qualitative detection of anti-S antibodies was done with the Wantai SARS-CoV-2 Ab ELISA kit (Eurobio®) and the Quantivac.

ELISA Anti SARS-CoV-2 (Euroimmun®) was used for antibody quantification.

Overall, the clinical evolution was good regarding COVID-19. The patient was discharged from hospital just after the initiation of bamlanivimab/etesevimab treatment which was well tolerated, and was followed as outpatient in the infectious disease department. She experienced prolonged respiratory symptoms with dyspnea and cough; however, no worsening of the status was observed and patient did not require oxygen therapy or hospitalization for COVID-19 symptoms. Furthermore, chest X-ray and biological inflammatory markers remained normal.

Virological data are summarized in Table 1, and show that the viral clearance was not efficient, with a viral load which remains high almost 40 days after the initiation of treatment. Viral whole genome sequencing identified the widely circulating B.1.1.7 lineage (20I/501Y.V1) SARS-CoV-2. The spike mutation analysis did not detect the E484K mutation during the follow-up; however, an A → G mutation was observed at nucleotide position 23,040 from day 15 post-treatment leading to Q493R amino-acid substitution in the spike protein. Good quality sequences were obtained in all tested samples, with a genome coverage between (99.99 and 100%), and a median number of reads per nucleotidic position between 174 and 3465. In addition, a focus on the specific position showed a very good coverage between 142X and 2516X (See Table 2).

Furthermore, antibody testing confirmed the presence of high levels of anti-Spike IgG antibodies.

This observation is clearly compatible with a reduced efficacy or a lack of efficacy of the bamlanivimab/etesevimab treatment. In the BLAZE-1 trial (phase 2), a combination treatment with 2800 mg of bamlanivimab and 2800 mg of etesevimab led to viral load change of −4.37 and −17.91 from baseline to day 11 and day 29 post-treatment, respectively. A Ct value lower than 27.5 on day 7 post-treatment was considered as persistently high viral load.[1] The resistance to treatment observed is this case is probably associated with the Q439R substitution that emerged in the Spike protein. The high initial viral load and the viral on-treatment replication could have contributed to the occurrence of this mutation.

Changes in the spike protein can significantly alter the efficacy of mAbs, and the most dangerous for immune escape are the ones occurring within the receptor binding domain (RBD).[3]

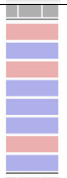
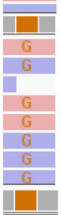
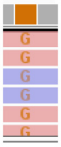
In-vitro studies have identified mainly six amino-acid substitutions corresponding to four positions (E484D/K/Q, F490S, Q493R, and S494P) that led to a reduced susceptibility to bamlanivimab, while six amino-acid substitutions at three positions (K417N, D420N, and N460K/S/T/Y) were shown to be critical for etesevimab. More interestingly, a retention of susceptibility to the other antibody alone was observed, with the exception of the Q493R substitution. A pseudotyped virus-like particle assay showed for variants harboring E484K, E484Q, and Q493R substitutions, a reduced susceptibility to bamlanivimab and etesevimab combination of 17-fold, 22-fold, and > 100-fold, respectively.[2,4]

The escape site Q493 seems thus to be the most critical for the bamlanivimab and etesevimab cocktail. It was recently reported that this site is not in the receptor-binding ridge, but in a region of joint structural overlap by both antibodies, such that a substitution leading to a positively charged residue (R, K) may directly affect binding by each antibody.[5]

As we prepare this manuscript, another team also described a similar case using Sanger sequencing of the viral spike region, with a follow-up period of 2 weeks.[6]

In conclusion, the emergence of the spike protein Q493R mutation can lead to virological failure during bamlanivimab/etesevimab treatment, and should be rapidly screened in nonresponders, especially when immunocompromized.

Table 2
A→G mutation at nucleotidic position 23,040.

Day post-treatment	GISAID sequence reference	Nucleotide position 23040	
		C A A	Detailed number of reads
0	EPI_ISL_2143435		NC_045512.2:23 040 Total count: 177 A : 177 (100%, 85+, 92-) C : 0 G : 0 T : 0 N : 0
7	EPI_ISL_2227230		NC_045512.2:23 040 Total count: 142 A : 142 (100%, 62+, 80-) C : 0 G : 0 T : 0 N : 0
15	EPI_ISL_2361537		NC_045512.2:23 040 Total count: 169 A : 0 C : 0 G : 169 (100%, 78+, 91-) T : 0 N : 0
21	EPI_ISL_2444359		NC_045512.2:23 040 Total count: 2516 A : 8 (0%, 5+, 3-) C : 1 (0%, 0+, 1-) G : 2506 (100%, 1042+, 1464-) T : 1 (0%, 0+, 1-)
39	EPI_ISL_2646207		NC_045512.2:23 040 Total count: 936 A : 0 C : 0 G : 935 (100%, 353+, 582-) T : 1 (0%, 0+, 1-) N : 0

Ethical statement

This report was approved by the Institutional data protection authority of CHU Lille. Informed consent was obtained from the patient and data have been anonymized as much as possible.

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False positive rapid antigen tests for SARS-CoV-2 in the real-world and their economic burden

Dear Editor,

Ladhani and coworkers reported in this journal that false positive SARS-CoV-2 assays may cause serious complications for elderly care homes.[1] In their report they presented a number of such false-positive cases that had a significant impact on the care homes, including a temporary unnecessary isolation of vulnerable residents and a loss of workforce leading to reduced care provision. Ladhani and colleagues concluded that "*repeated unnecessary interventions are also likely to be detrimental to the long-term mitigation strategy in care homes, have significant resource implications and impact on the wellbeing of residents and staff*"[1]. The authors further conclude that "*in addition, there is the danger of behavioural fatigue so that, when strict infection control measures are required in a genuine outbreak, recommended measures may not be adhered to.*"[1]

Taking the experience of Ladhani et al.[1] into account we here present evidence that the problem of false positive SARS-CoV-2 testing goes even beyond these latter risks, especially if the screening strategy is based on latera flow antigen tests (rapid tests).

A recent German strategy to overcome the COVID-19 pandemic is the broad usage of SARS-CoV-2 lateral flow tests (LFT) for rapid antigen testing. These assays are intended to detect SARS-CoV-2 in the upper airways of infected people and are approved for the usage in symptomatic patients.

While some initial reports on the usage of lateral flow antigen assays based on controlled clinical studies were promising,[2–4] the real-world performance of LFTs remains controversial.[5–8]

Nevertheless, the Federal testing strategy in Germany includes an off-label usage of these assays for liberal screening of asymptomatic people[9] due to the probability of infection establishment with the intention to detect and isolate infected individuals as soon as possible and since 8th March the state bears the incurred costs for at least one rapid antigen test per week for German citizens.[9] These direct cost are accompanied by indirect costs as the off-label usage may result in a high rate of false positive results, which might be associated with a high economic burden. This can be calculated on the basis of data we have obtained for the City of Cologne in May 2021. Between 1st and 31st of May a total number of 1.245.962 rapid antigen assays were performed in approximately 800 test centers certified for rapid antigen testing according to recent emergency edicts by the German government.

The daily rates of positive and false positive lateral flow assays are shown in Fig. 1 (a and b). In summary, out of the 1.245.962 rapid antigen tests performed in the city of Cologne in May 2021 a total number of 2.906 specimens were tested positive by lateral flow assays, of which only 1.345 could be confirmed by PCR, thus the overall false-positive rate was 46.28%. 52% (1561/3060) of all positive cases identified during May 2021 in Cologne were identified by rapid antigen screening. It can be excluded that there is any bias regarding test providers or assay used, because positive rapid antigen tests were uniformly reported from all over the city, as cross checked by postcodes (data not shown). Considering that this number probably includes persons who used a test reasonably (e.g. because of possible contact) and thus have a higher pre-test probability and persons who would had made a PCR test otherwise, this number is a conservative estimate for the effectiveness of liberal antigen screening.

On the basis of the data it is not possible to calculate other diagnostic accuracy measures than the positive predictive values (PPV), as only positive tests were verified, i.e. negative lateral flow tests were not confirmed by PCR testing. The overall PPV for May is PPV = 0.53, whilst the PPV for the last week of May with already lower incidence is PPV = 0.47. This indicates that with decreasing pre-test probability the PPV likely further decreases (Fig. 1a and b). This assumption is confirmed by the fact that at the end of May the percentage of positive cases correctly identified by LFTs decreases to 39% (313/796).

To get insights into the economic burden of rapid antigen testing we calculated the direct costs arising from the performed tests and the indirect costs arising from the subsequent quarantine. The associated costs of false negative tested persons could not be included because no data on false-negatives were available. The assumptions for resource use and prices are listed in Table 1a. As the data on new infections (*R*-value) and hospitalization rate were highly variable in the last months we decided to use the moving average before implementation of rapid antigen testing for the R-value, hospitalizations, respectively.

Based on the demographic data available for Cologne the cost were initially calculated and then extrapolated to Germany based on the number of citizens and the average household size. For this purpose, we assumed that the average incidence as well as quarantine and discharge management apply to the entire Federal Republic of Germany. However, this simplification may cause a bias as in other parts of Germany the incidence at that time, the contact tracing, and the diagnostic turn-over times may vary, especially in rural areas. Consequently, the resulting numbers should only be considered as rough approximations.

The costs to be reimbursed for the assays performed in Cologne in May summed up to 22.427.316 €. Depending on the length of preventive quarantine before availability of PCR-test results, the total costs for the rapid antigen testing strategy ranged from 24.742.608 € (minimum = 22.427.316 € test costs plus 2.315.292 € productivity loss) to 27.057.899 € (maximum = 22.427.316 € direct test costs plus 4.630.583 € productivity loss) (Table 1b). Merely assuming three days quarantine the indirect costs of quarantine due to false negative results were about 1.607.399 €. Based on this cost, it can be estimated that the "cost per identified case" were 16.592 €, the "cost per infection avoided" were about 11.061 €, and the "cost per hospitalization avoided" were about 110.610 €.

When the costs are extrapolated to the entire Federal Republic of Germany, the costs ranged between 1.884.178.754 € (minimum =1.714.084.799 € test costs plus 170.093.955 € productivity loss) to 2.054.272.708 € (maximum =1.714.084.799 € test costs plus 340.187.909 € productivity loss) (Table 1c).

Table 1a
Resource usage, and prices/costs used for the calculation, based on data available for 2019 and/or 2020.

Parameter	Price/Value/ Percentage/Number (as applicable)	Reference
Performed tests	1,245,962	Data collected by the authors
Test Costs	18 €	https://www.bundesgesundheitsministerium.de/fileadmin/Dateien/3_Downloads/C/Coronavirus/Verordnungen/Corona-TestV_BAnz_AT_09.03.2021_V1.pdf
Households in Cologne (2020)	564.973	https://www.stadt-koeln.de/mediaasset/content/pdf15/statistik-einwohner-und-haushalte/kurzinformation_bev%C3%B6lkerung_3_2021.pdf
Households in Germany (2020)	41.506.000	https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Bevoelkerung/Haushalte-Familien/Tabellen/1-1-privathaushalte-haushaltsmitglieder.html;jsessionid=B26A308FE15449D60BC0E692DECFC23B.live742
Average quarantine time	Median: 3 days, range 2 to 4 days	Data collected by the authors
Average weekly working time per household (2019)	35.6 h	https://www.destatis.de/DE/Themen/Arbeit/Arbeitsmarkt/Qualitaet-Arbeit/Dimension-3/woechentliche-arbeitszeitHaushaltl.html#:~:text=Pro%20Haushalt%20wurden%20im%20Jahr,53%2C0%20Stunden%20pro%20Woche.
Average cross value per person (2019):	55,95 € per hour	https://www.destatis.de/DE/Themen/Wirtschaft/Volkswirtschaftliche-Gesamtrechnungen-Inlandsprodukt/Publikationen/Downloads-Inlandsprodukt/inlandsprodukt-vorlaeufig-pdf-2180140.pdf?__blob=publicationFile
Average R-value before implementation of comprehensive anti-gen testing	about 1.5	https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Projekte_RKI/Nowcasting.html
Hospitalization rate before implementation of comprehensive anti-gen testing	about 10%	https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Daten/Klinische_Aspekte.html

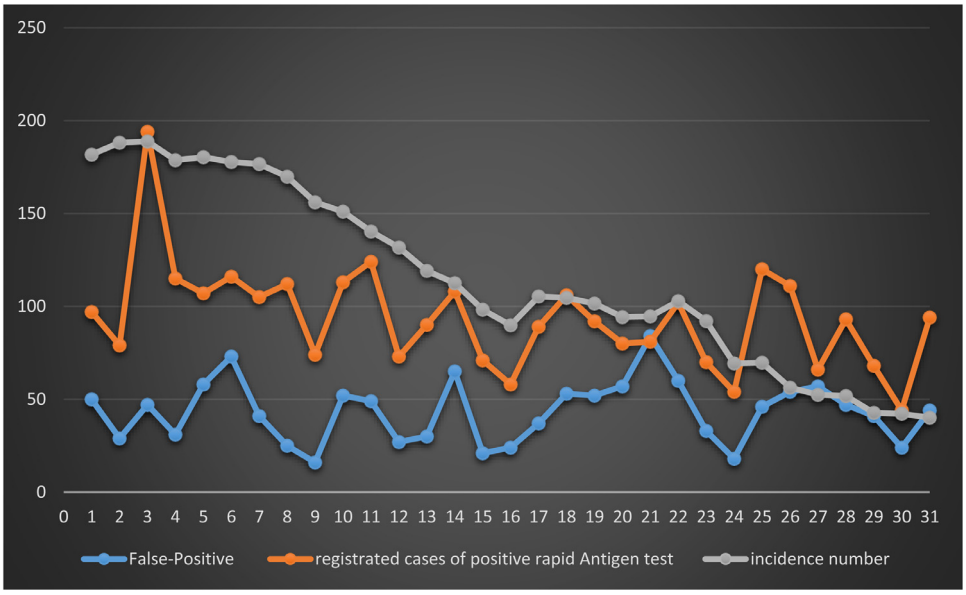

Fig. 1a. Daily incidence of newly detected SARS-CoV-2 infections per 100.000 people, total registered positive rapid antigen tests and false positive rapid antigen tests in Cologne (May 2021).

Table 1b
Analyses of costs arising from rapid antigen tests, Cologne.

	3 days quarantine	2 days quarantine	4 days quarantine
Test-cost	22.427.316 €	na	na
Productivity loss total	3.472.937 €	2.315.292 €	4.630.583 €
Productivity loss false negative	1.607.399 €	1.071.599 €	2.143.198 €

na: not applicable.

Table 1c
Extrapolation of costs analyses arising from rapid antigen tests, Germany.

	3 days quarantine	2 days quarantine	4 days quarantine
Test-cost	1.714.084.799 €	na	na
Productivity loss total	255.140.932 €	170.093.955 €	340.187.909 €
Productivity loss false negative	118.088.284 €	78.725.523 €	157.451.045 €

na: not applicable.

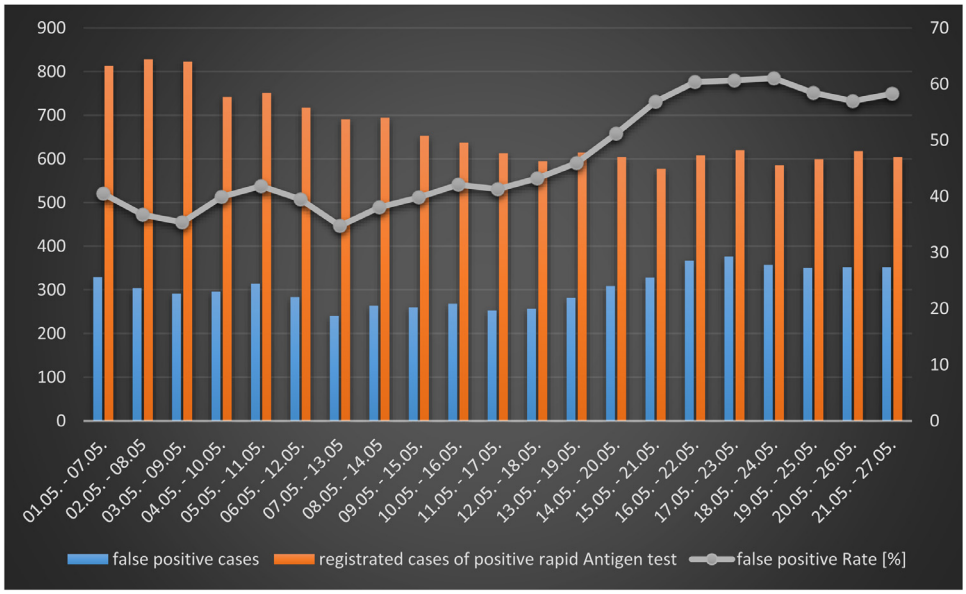

Fig. 1b. False positive and registered positive rapid antigen tests in a 7-day interval in May 2021 (Cologne, Germany).

Noticeable, all cost data refer to May 2021. In the view of the subsequently declining incidence and consequently higher rate of false positives, the cost per infection and hospitalization avoided are likely higher in June when the incidence was even much lower.

From a pure laboratory and diagnostic point of view it has to be concluded that the usage of an antigen testing strategy with a false detecting rate of about 50% is unacceptable, although false positive results have no direct negative effect on the healthy individual. Concurrently, from the public health perspective it is indisputable to ideally identify all positive cases to curb possible outbreaks, but therefore, the most important test accuracy measure in the current phase of the pandemics is the negative predictive value.

Our data suggest that the use of rapid antigen testing only appears appropriate in high prevalence/incidence situations, because a (too) low prevalence may increase the risk of false-positive results leading to unnecessary quarantine and high economic burden. Although the Covid-19 prevalence was quite high (compared to target incidences foreseen by the governmental guidelines) in May in Cologne the cost per hospitalization avoided were 110.610 €. Therefore, as the prevalence and the morbidity from Covid-19 decreases due to vaccination coverage and seasonal effects, and in parallel the results of false positive results increases, the negative consequences from false positive results become more and more important. Consequently, the question arises whether a universal population screening of asymptomatic people is a reasonable measure or if the antigen testing strategy should be considered more strongly to the incidence to increase the PPV and to improve cost-effectiveness, especially as PCR-based test strategies appear feasible in low incidence situations regarding laboratory capacities in western countries.

Moreover, the effectiveness of a universal antigen screening has to be considered against alternative screening strategies. Taking into account that further infection waves can be expected for the upcoming autumn and winter season and that morbidity from Covid-19 further decreases there appears to be a need to find other, more valid and more targeted testing strategies. These strategies must be superior to rapid antigen assays regarding diagnostic test accuracy and thus are more cost-effectiveness. An example of a promising concept is the so-called “lolly-assays” performed in high-risk settings with high personal on interior contact settings as actually performed in schools of North-Rhine Westphalia. For these assays entire school classes are tested twice weekly, while the individual children have to suck on a swab for 30 s before all class swabs are collected in a single transport vehicle with stabilizing transport buffer and then are PCR tested by a pool testing specialized laboratory. Positive pools are individually resolved and lead to single testing of all pupils in the positive class. This concept could easily be expanded to other settings which high transmission rates such as workplaces.

Declaration of Competing Interest

The authors declare that no competing interests exist related to this submission.

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COVID-19 case-clusters and transmission chains in the communities in Japan

Dear Editor,

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged at the end of 2019 and has caused substantial morbidity and mortality in many parts of the world. Clusters of COVID-19 cases associated with superspreading events have often been reported and considered important mechanisms for the spread of the infection.[1–3] In the review article in this journal, Majra et al. summarized the relationship between superspreading events and community spread.[4] To afford collateral evidence, we herein illustrate concrete instances of COVID-19 case-clusters and their transmission chains in the communities in Japan.

Contact tracing to elucidate COVID-19 transmission chains

In Japan, all COVID-19 cases were reported to public health centers of local governments and the Ministry of Health, Labor and Welfare. Contact tracing by public health officers or public health nurses was performed if at all possible, in two directions as follows: 1) backward investigation to figure out the possible source of infection of a case under investigation; this consisted of questions on travel and contact history of 14 days prior to illness onset or infection confirmation by laboratory diagnosis and 2) forward investigation to list possible persons who got infected from a case under investigation; public health officers or public health nurses asked the patient about the contacts encountered within two days leading to the illness onset until infection confirmation. Details of the methodology are available in.[5,6]

Transmission chains of 28 case-clusters in Japan

There were 213 case-clusters with five or more associated cases between January and July 2020 in Japan. Of these, we report 28 instances in which more than 10 cases were associated, the transmission was sustained for at least three transmission-generations, and detailed information about transmission events and venues was available.

For example, consider the instance in Fig. 1. Backward contact tracing enabled a public health authority to notice that several COVID-19 patients had attended party A before illness onset, thereby identifying this party as a possible source of the SARS-CoV-2 infection. Subsequently, the rest of the party participants were tested, and more persons were detected positive for the virus. A patient from party A transmitted the virus to one's co-worker, and another patient from the party spread the infection to three more people at dinner C. A patient that contracted the virus at dinner C further transmitted the virus to one's family member. Concurrently, a nosocomial outbreak of SARS-CoV-2 occurred in hospital B. Through backward contact tracing, it was discovered that a patient in the hospital had an epidemiological link with party A. There was further transmission to a family member of a patient in hospital B, and the one was the last case related to the cluster.

From here, we describe the qualitative findings from the 28 instances of COVID-19 case-clusters. First, superspreading events and places where many people contracted the virus played significant roles in COVID-19 transmission in communities (Supplementary Figs. 1–28). It is worth noting that the first case confirmed by laboratory diagnosis in a case-cluster was different from the case with the earliest illness onset in the cluster in 12 instances (Supplementary Figs. 1–3,7,12,13,15,19,21–23,28). This underscores the power of backward contact tracing to detect superspreading events and places. Identification of superspreading events and places led to the detection of more associated cases and their contacts. Consequently, transmission chains were blocked off, and the infection spreads were contained within five weeks for all 28 instances.

Many transmission chains started from parties, restaurants, or bars (Supplementary Figs. 1–12). Eating and drinking together may have increased the chance of viral transmission because many people gather, chat for a long time in close proximity, and do not wear a facemask. Gymnasiums and music-related events are also common places for superspreading (Supplementary Figs. 5,11,13–15). Heavy breathing and singing could enhance the viral transmission risk via droplets or aerosols.[7] Consecutive superspreading events occurred at such superspreading-prone sites generating a large number of cases in communities (Supplementary Figs. 1–6,13,14). In addition, case-clusters among co-workers were frequently observed (Supplementary Figs. 2,3,5,7,12,15–18). However, it is difficult to discern if the virus was transmitted among co-workers at the workplace or during social interactions.

Many COVID-19 cases were also ascertained in hospitals, care facilities, and schools including nurseries (Supplementary Figs. 1,7–11,15,19–28). Given that these are the areas where the same people stay or gather every day, it is unknown whether one or a few superspreading events occurred or long sequential transmission chains existed. Frequent and close contacts and the presence of vulnerable people in those places could prolong the outbreaks and make the case-clusters very large. In some cases, the same patients, staff, or visitors visited different facilities,

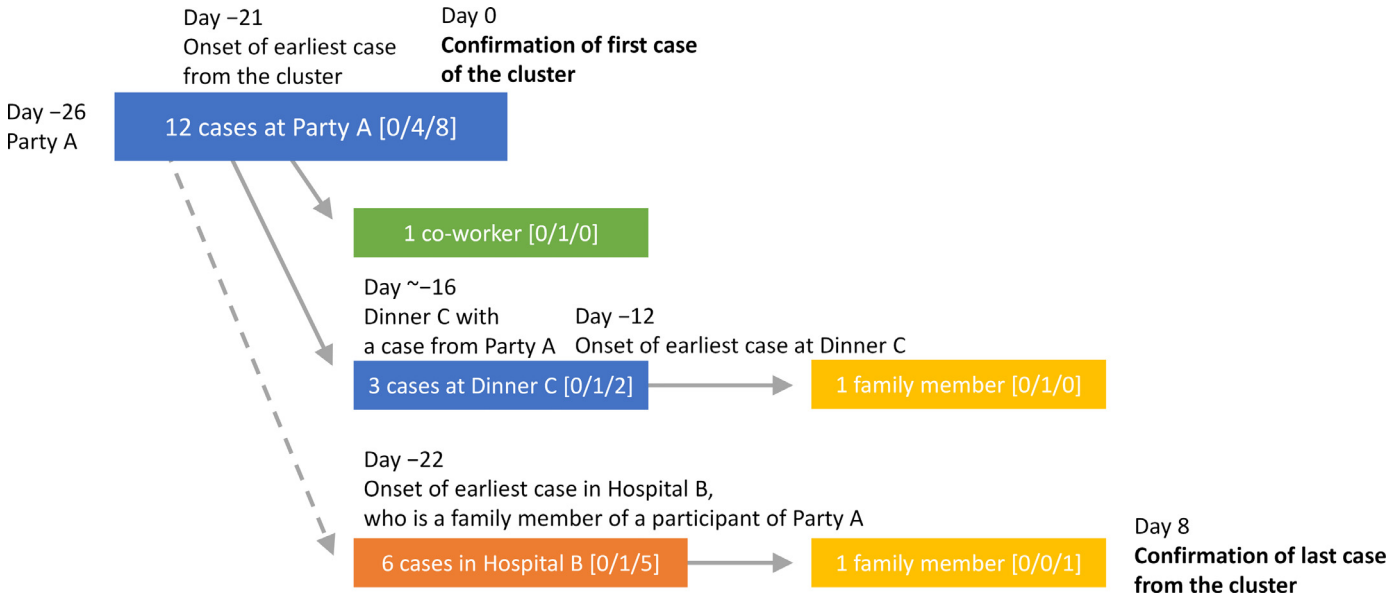

Fig. 1. An example of a case-cluster and transmission chains
Transmission dynamics in a case-cluster are illustrated. The blue, green, orange, and yellow boxes represent cases at community superspreading events, cases among co-workers, cases at hospitals/care facilities/schools, and cases among family members, respectively. The three values in square brackets denote the number of patients aged 0–19, 20–59, and 60 or more. Arrows indicate infector-infectee transmission pairs, and different arrow lines mean different sources of infection. A dashed arrow line indicates an indirect transmission chain.

leading to transmissions between those facilities (Supplementary Figs. 9,21–23).

"Spillover" transmissions from hospitals, care facilities, and schools were observed, most of which were household transmissions (Supplementary Figs. 1,8,10,19–26). Outbreaks in hospitals, care facilities, and schools rarely led to community superspreading events such as those at parties. Conversely, we noticed the introduction of infections into hospitals, care facilities, and schools in some instances by cases from community superspreading events (Supplementary Figs. 1,7–11,15). Although most household transmissions were observed at the edge of transmission chains, they could sometimes lead to further spread outside the household to communities or healthcare/care facilities (Supplementary Figs. 3,12,14,19,24).

As described earlier, transmissions to hospitals, care facilities, schools, and family members are generally located at the edge of transmission chains. Therefore, the infections in children and older adults were usually observed at a later stage of local spread. This corroborates the previous findings that people in their 20s–50s may be the driving force of the COVID-19 epidemic.[7–9] Nevertheless, because of the super-aged society in Japan, it was also observed that community superspreading took place from the resident community of older adults and the transmissions were sustained among people in that age group (Supplementary Figs. 1,10,13,14).

Conclusion

This study showed examples of how SARS-CoV-2 was transmitted in communities and described the common features of COVID-19 transmission chains as schematized in Fig. 2. Through our investigation, we figured out that "three Cs"—closed spaces with poor ventilation, crowded places with many people nearby, and close-contact settings—were the conditions leading to a high risk of viral transmission.[10] Because the epidemiological investigation was conducted by interviews and relied on the voluntary cooperation of patients, there could be missed transmission chains. Still, the combination of backward and forward contact tracing enables us to understand the mechanisms of transmission dynamics and prevent the further spread of the infection.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.08.016.

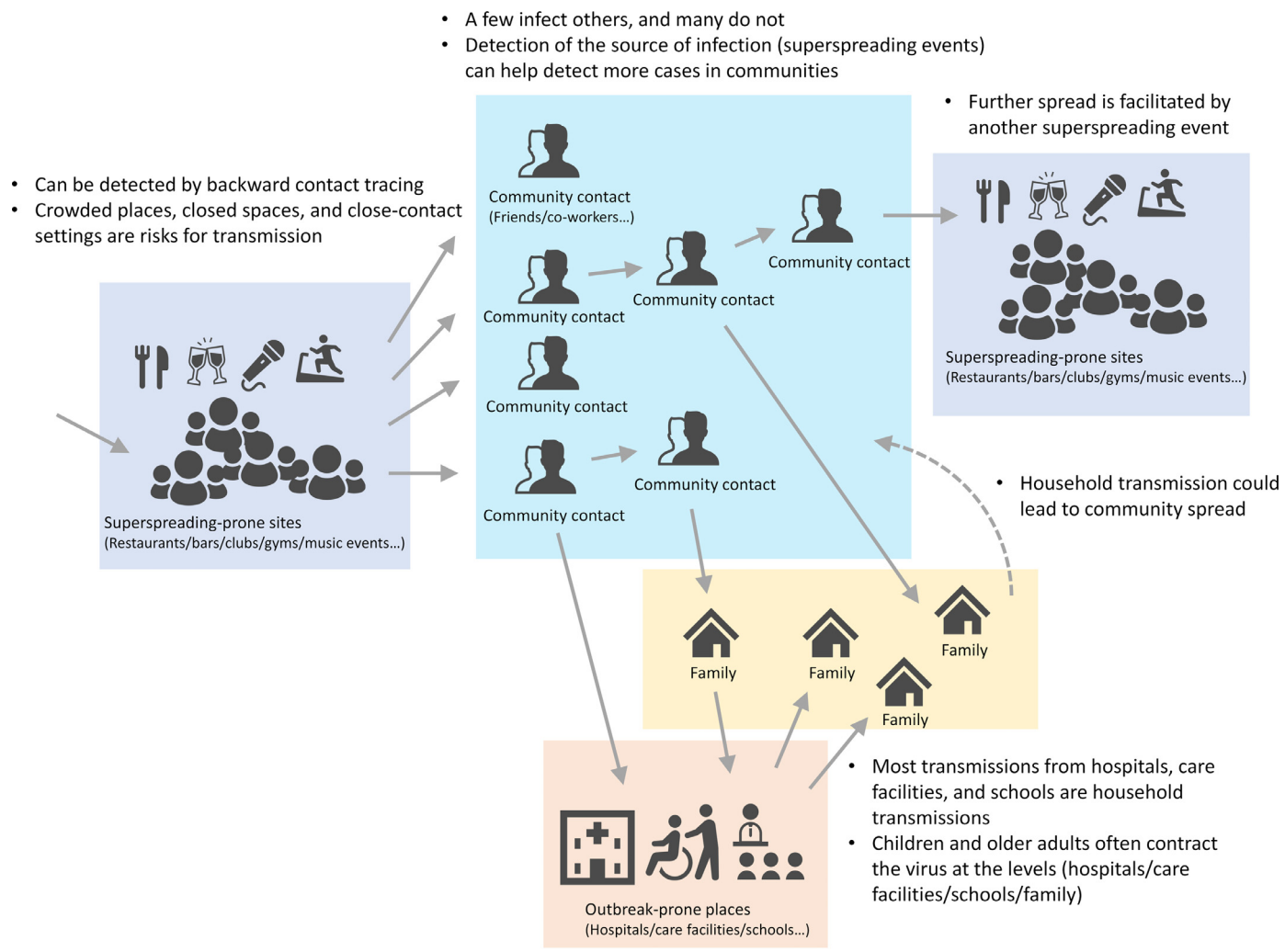

Fig. 2. Schematic summary of COVID-19 transmission chains
A schematic overview of the common features of COVID-19 transmission chains is illustrated.

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Predicting COVID-19 incidence in Pakistan: It's time to act now!

Dear Editor,

We read with great interest the article entitled "A quick prediction tool for unfavorable outcome in COVID-19 inpatients: Development and internal validation" by Salto-Alejandre et al.[1] Authors of this article forecast the outcomes of COVID-19. The current paper highlights the prediction of pandemics and potential catastrophes of COVID-19 in Pakistan.

COVID-19 is a severe and ongoing public health emergency in Pakistan that has infected over 01 million people with COVID-19, leading to 23,000 deaths so far.[2,3] The number of confirmed cases varies according to each country's epidemiological surveillance and detection capacities. As a result, an estimate of the total number of confirmed cases and anticipated future cases is integral to maintaining demand and resource allocation to the health system. Short- and long-term case estimates require mathematical and statistical modeling tools to determine the magnitude and type of measures necessary to confined an outbreak.[4]

Auto-Regressive Integrated Moving Average (ARIMA) models effectively simulate the time-dependent structure of a time series by accounting for evolving trends, regular changes, and random distortions. The ARIMA approach is mainly devoid of mathematics and statistics.[5] Predictive models for end-users have been established in this manner and may be used further in the decision-making process.

This study evaluates the fourth mode of COVID-19 epidemic propagation in Pakistan, which is the deadliest. The data considered in this study spans the period 1st July 2021 to 31st July 2021 (31 days) and is used to forecast the next 30 days (August month 2021). The data set was utilized to implement and analyze cases and an estimating model for fatalities using several ARIMA models. Thus, in addition to providing insight into the epidemic's transmission patterns, its objective is to use models based on basic quantitative models to give authorities realistic estimations of the epidemic's peak period and severity. These models can aid in forecasting future medical infrastructure and material requirements for patients in these countries.

Figs. 1 and 2 depict predicting graphs of COVID-19 cases and deaths for the next 30 days, respectively. The findings indicate that the expected daily infection rate for the next 30-days (end of August 2021) might reach 8320 (CI 95%: 3289–13,350), while daily mortality could get 47 (CI 95%: −61–156). The anticipated number indicates an increase in infected cases and deaths during the next 30 days. Prediction errors were used to validate the model. We considered the mean absolute error (MAE) and R2 parameters to determine the ARIMA model's significance for COVID-19 pandemic data in Pakistan. ARIMA (0,1,0) daily registered cases models with R-squared (0.86), RMSE (445.40), and MAE (351.66), as well as ARIMA (0,1,0) daily deaths models with R-squared (0.81), RMSE (435.30), and MAE (332.45), were verified and adequately predicted.

Pakistan's deteriorating healthcare system may become overwhelmed by the growing number of fourth-wave patients, and the country may become an epicenter in Asia. Medical, public health and policymakers' responses will need to be led by statistics that determine the locations, dates, and populations affected by new instances. This data can be used to drive the country's health advocacy and the kind and extent of government actions to require public health behaviors or managing commercial activities to contain the spread. Forecasting infected cases are crucial for organizing healthcare resources and ensuring that communities confronting the unpredictability of a quickly growing infectious disease throughout a pandemic response have access to care and the best possible results. Careful management and distribution of COVID-19 patients are critical; otherwise, the consequences will be more severe and catastrophic.

Declaration of Competing Interest

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

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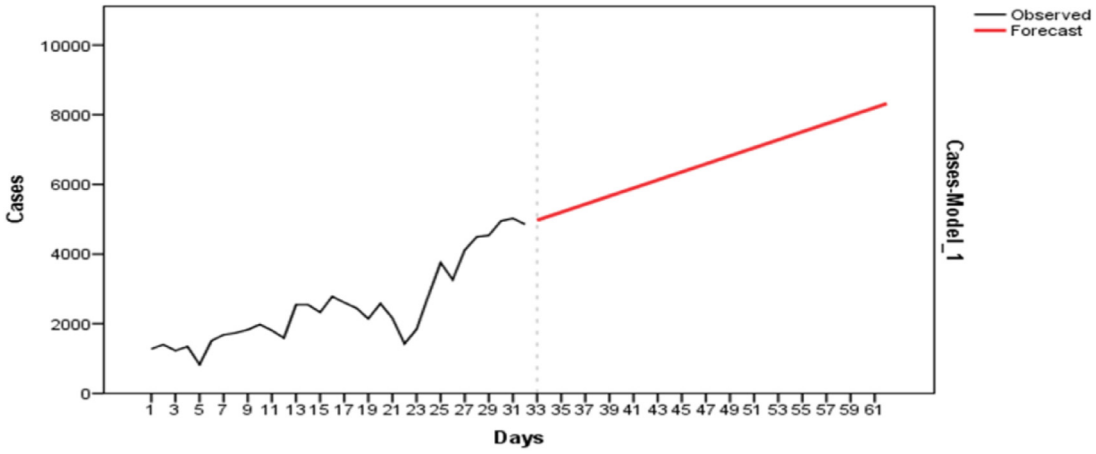

Fig. 1. ARIMA (0,1,0) daily registered cases validated and accurate predictive model with R-squared 0.86, RMSE: 445.40, and MAE: 351.66. Predictive line for the next 30-days shows significant increase in new cases.

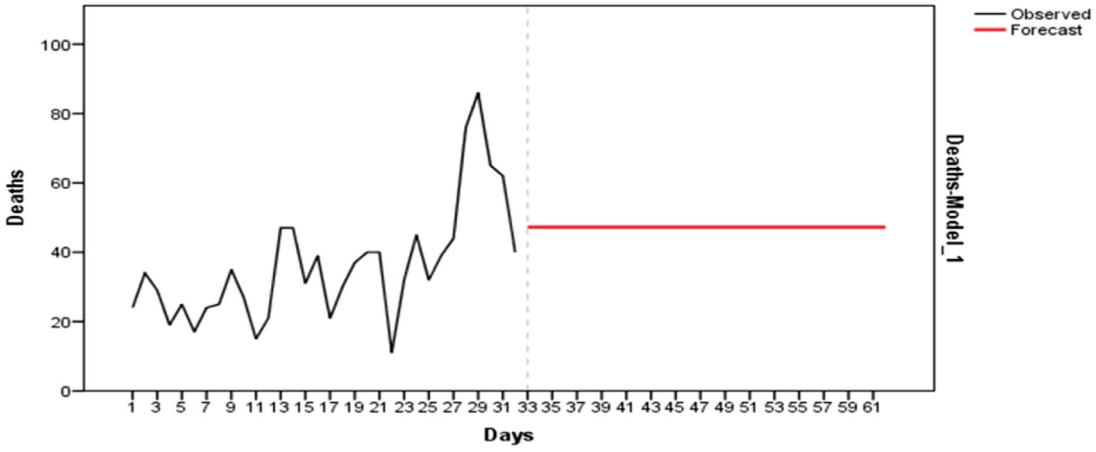

Fig. 2. ARIMA (0,1,0) daily registered deaths validated and accurate predictive model with R-squared: 0.81, RMSE: 435.30, and MAE: 332.45. Predictive line for the next 30-days shows significant increase in new deaths.

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Post-COVID functional limitations on daily living activities are associated with symptoms experienced at the acute phase of SARS-CoV-2 infection and internal care unit admission: A multicenter study

Dear Editor,

Evidence supports that almost 60% of COVID-19 survivors will experience post-COVID symptom during the first 6-months following the infection.[1] These symptoms lead to a decrease in health-related quality of life and decreased function.[2,3] In a letter to the editor in *Journal of Infection*, Taboada et al. found that female sex, age, length of hospital stance and internal care unit (ICU) admission were associated with limitations in functional status.[4] These authors evaluated the Functional Status Scale (PCFS), a scale assessing global patient-functional limitation by using a 6-points scale.[5] The PCFS did not differentiate on which daily life activity the limitation is present since it uses a global score. Due to the complexity of COVID-19, it is possible that functional limitations and their associated risk factors are different depending on the type e.g., social, occupational, basic or instrumental, of daily living activity. In addition, previous studies have included limited numbers of participants ($n < 300$) recruited from just one single

center.[2–4] We describe a multicenter study investigating those risk factors associated with the presence of functional status differentiating occupational, leisure/social, basic or instrumental daily living activities in a sample of hospitalized COVID-19 survivors at a long-term follow-up period after hospital discharge.

This multicenter study included patients hospitalized in five hospitals of Madrid (Spain) with a diagnosis of SARS-CoV-2 infection during the first wave of the pandemic. A sample of 400 individuals from each hospital was randomly selected for this study. Local Ethics Committee of each participating hospital approved the study (HUFA 20/126, HCSC20/495E, HSO25112020, HUIL/092-20, HUF/EC1517). All participants provided their informed consent. Clinical features (i.e., gender, age, height, weight, medical co-morbidities), symptoms at hospital admission, and hospitalization data (days at hospital, ICU admission) were collected from medical records. Participants were scheduled for a telephone interview conducted by experienced healthcare professionals to evaluate the functional status of the patient. Participants were asked for self-perceived limitations in occupational, leisure/social activities, instrumental, and basic daily living activities. They s were asked for determining their functional status at the moment of the interview (post-COVID) in comparison with their previous status before hospitalization.

Mean and standard deviation (SD) or percentages were calculated. Missing values were imputed using median imputation. Multivariate logistic regressions were conducted to analyze associations between clinical and hospitalization variables with the presence of limitations in occupational, leisure/social activities, instrumental, and basic activities of daily living (dependent variables) using Python's library statsmodels 0.11.1. Adjusted odds ratio (OR) with their confidence intervals (95%CI) were calculated.

From 2000 patients randomly selected from the five hospitals and invited to participate, a total of 1969 (46% women, age: 61, SD: 16 years) were assessed a mean of 8.4 months (SD 1.5) after hospital discharge. Fever (74.6%), dyspnea (31.5%) and myalgia (30.7%) were the most prevalent symptoms at hospital admission (Table 1). The mean number of COVID-19 symptoms at hospital admission was 2.2. (SD 0.8). Almost 57.5% of patients (n = 1133) reported at least one comorbidity. The mean number of medical co-morbidities was 0.8 (SD 0.9). Hypertension (26.2%), diabetes (12.0%), and cardiovascular disorders (11.9%) were the most common medical co-morbidities (Table 1). Between 20 and 30% of participants reported limitations during at least one daily living activity (Table 1).

The regression model revealed that the number of COVID-19 symptoms at hospital admission (occupational activities: OR1.51, 95%CI 1.14–2.01, P = 0.004; leisure/social activities: OR1.59, 95%CI 1.25–2.03, P < 0.001; basic daily live activities: OR1.61, 95%CI 1.21–2.13, P < 0.001; instrumental daily live activities: OR1.57, 95%CI 1.22–2.01, P < 0.001) and ICU admission (occupational activities: OR1.79, 95%CI 1.16–2.78, P = 0.009; leisure/social activities: OR1.83, 95%CI 1.22–2.73, P = 0.003; basic daily living activities: OR3.30, 95%CI 2.16–5.05, P < 0.001; instrumental daily living activities: OR2.37, 95%CI 1.57–3.58, P < 0.001) were significantly with limitations in all activities. Additionally, age was also associated with limitation in leisure/social activities (OR1.009, 95%CI 1.003–1.016, P = 0.006), limitations basic daily living activities (OR1.016, 95%CI 1.008–1.024, P < 0.001), and limitations in instrumental daily living activities (OR1.020, 95%CI 1.013–1.028, P < 0.001). Female sex was only associated with limitations in leisure and social activities (OR1.46, 95%CI 1.13–1.89, P = 0.003) and limitations instrumental daily living activities (OR1.66, 95%CI 1.28–2.16, P < 0.001).

Our multicenter study found that at least 20% of COVID-19 survivors self-reported limitations on daily living activities eight months after hospitalization. Current data agree with previous studies[2–4]; nevertheless, we differentiated the type of activity perceived as limited, a distinction that is not commonly conducted in former post-COVID literature. Identification of risk factors is needed for an early identifying and monitoring of patients at a high risk of developing post-COVID sequelae.[6] This multicenter study found that a higher number of symptoms at hospital admission and ICU admission were associated with functional limitations at all daily living activities. Our findings the assumption that a higher symptom load at the acute phase of the infection leads to a greater likelihood of suffering long-term functional disability at least in hospitalized COVID-19 survivors.

We also observed that other risk factors, e.g., female sex and age, which have been associated with post-COVID symptoms,[7] were associated with the limitations of daily living activities, although clinical relevance of age could be questioned due to their lower adjusted OR. Our findings agree with those found by Taboada et al. except for the longer hospital stay.[4] Increasing evidence suggests that post-COVID fatigue, probably one of the symptoms most associated with functional status, is not associated with the severity of the initial infection[8] or damage in lung function at 3- and 6-month after.[9] It would be probably that hospitalization data can exert less influence on the development of post-COVID sequelae than expected.

Although our study provides further evidence to the current literature about post-COVID functional status with a large, multicenter design evaluating specific daily living activities, potential weaknesses should be considered. First, only hospitalized COVID-19 patients participated. Second, the number of patients requiring ICU admission was small.

Third, the cross-sectional design did not permit to determine cause -and-effect association between suffering COVID-19 and functional status. Fourth, we did not collect objective data of

Table1
Clinical, hospitalization and functional status data of the sample (n = 1969).

Age, mean (SD), years	61 (16)
Gender, male/female (%)	1054 (53.5%) / 915 (46.5%)
Weight, mean (SD), kg.	75 (15)
Height, mean (SD), cm.	165 (16.5)
Main Symptoms at hospital admission, n (%)	1469 (74.6%)
Fever	620 (31.5%)
dyspnea	604 (30.7%)
Myalgia	549 (27.9%)
Cough	332 (16.9%)
Headache	210 (10.7%)
diarrhoea	167 (8.5%)
Anosmia	102 (5.2%)
Throat Pain	66 (33.5%)
Ageusia	55 (2.8%)
Vomiting	
Medical co-morbidities	
Hypertension	514 (26.1%)
Diabetes	236 (12.0%)
Cardiovascular Disease	234 (11.9%)
Asma	126 (6.4%)
Obesity	88 (4.5%)
Chronic Obstructive Pulmonary Disease	77 (3.9%)
Stroke	38 (2.0%)
Rheumatological Disease	31 (1.6%)
Other (Cancer, Kidney Disease)	332 (16.9%)
Stay at the hospital, mean (SD), days	11.3 (11.4)
Intensive Care Unit (ICU) admission Yes/No, n (%)	130 (6.6%) / 1839 (93.4%)
Functional Limitations n (%)	
Limitation in Occupational Activities	418 (21.2%)
Limitation in Leisure/Social Activities	604 (30.6%)
Limitation in Basic Activities of Daily Life	542 (27.5%)
Limitation in Instrumental Activities of Daily Life	389 (19.7%)

COVID-19 disease and measures of lung damage, although these factors seem to be not related to post-COVID sequelae.[8,9]

Declaration of Competing Interest

None.

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No funds were received for this study.

Author contributions

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

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A brief smartphone-based intervention significantly improved pre-exposure prophylaxis adherence among Chinese men sex with men: Findings of a randomized controlled trial

Dear Editor,

Optimal adherence to the pre-exposure prophylaxis (PrEP) regimen is critical to prevent HIV transmission. However, high proportion of PrEP users reported poor adherence. Previous digital telehealth interventions showed encouraging results in supporting PrEP adherence.[1] However, there was a lack of data on ED-PrEP (event-driven) users[2] and studies in low and middle-income countries.[3]. The use of asynchronous videos instead of clinical visits demonstrated potential advantages in promoting PrEP adherence.[4] Therefore, we developed a brief asynchronous-video-monitored (AVM) intervention and investigated its effectiveness for both D-PrEP (daily use) and ED-PrEP users as compared to the standard of care (SOC) over a 12-month period.

We conducted a two-arm parallel-group non-blinded randomized controlled trial (RCT) between December 2018 and October 2020 in Shenyang, China. This project was registered with clinical trials registration number ChiCTR1900024545. Participants were men who have sex with men (MSM) recruited from a multicenter real-world demonstration study (CROPrEP). The recruitment strategies and inclusion eligibility were previously described.[5] Participants were randomly assigned to either the AVM or the SOC group and chose either D-PrEP or ED-PrEP regimen. All participants were provided free Truvada (composed of 200 mg FTC and 300 mg TDF per pill) for up to 12 months. The SOC group received a risk assessment, PrEP education, and risk-reduction counseling provided by a physician. Each participant received reminders for PrEP taking during the face-to-face clinic visits every 3 months. On top of the SOC, participants in the AVM group recorded their medication process when taking PrEP, and uploaded these videos to WeChat APP in the first 30 days of PrEP initiation. Videos were only viewed by designated research staff. ED-PrEP users followed the "2–1–1″ schedule: 2 pills 2–24 h before sexual intercourse, one pill 24 h after the first dose, and one pill 24 h after the second dose. Other non-video forms (such as picture, text, voice messages, etc.) were not encouraged (please find detailed video requirements in supplementary file1). Participants completed an online self-administered survey at baseline and follow-up visits and answered a weekly 3-minute online questionnaire to assess sexual behaviors and PrEP adherence. All questionnaires were accessible through QR codes that were sent through WeChat. All participants received free laboratory tests, including HIV, syphilis, routine blood and urine tests at all visits. The study participation was voluntary and participants could withdraw from this RCT at any time.

This study was approved by the ethical committee of the First Affiliated Hospital of China Medical University ([2018]2015–139–5). Each participant provided written informed consent before participation (supplementary file2)

A pharmacy administrator recorded the amount of the medication given and returned by each participant at follow-up visits. An adherence score was calculated using the number of pills taken divided by the number of pills need to be taken within the follow-up period. An adherence score of ≥90% was defined as good adherence.[6] Chi-square tests and Fisher Exact tests were used to inspect the between-group differences of baseline characteristics. The association between the AVM intervention and good adherence was performed by Generalized estimating equations (GEE). We included all adherence data of the 5 follow-up points (month 1, 3, 6, 9, 12) into the GEE model. Missing data was handled by multiple imputation. All analyses were conducted using IBM SPSS v26.0 (Armonk, NY, USA).

196 eligible participants were randomly assigned to the AVM or SOC group. The majority of the participants were younger than 35 years (65.3%), and without tertiary education (73.5%). Most of the participants (62.2%) have had casual sex in the past three months. There was no statistically significant differences between AVM and SOC in sociodemographic characteristics, except for education level ($p = 0.02$) (Table 1). No new HIV infections were reported during the follow-up period. GEE analyses showed that participants who record their medication-taking process in the AVM group were five times more likely to have good adherence than the SOC group (adjusted odds ratio or AOR: 4.73, $p<0.001$). The sub-groups of D-PrEP users (AOR: 7.65, $p<0.001$) and ED-PrEP users (AOR: 2.72, $p<0.001$) showed similar results.

As compared to SOC, the smartphone-based AVM intervention significantly improved PrEP adherence among Chinese MSM. Similar efficacy applied to the sub-groups of D-PrEP and ED-PrEP users. Although video-taking was only conducted for the first 30 days, it significantly improved PrEP initiation and the overall adherence rates across a year. The prevalence of good adherence in the AVM group was similar to or even slightly higher than other digital health approaches.[7] Moreover, the intervention targeted MSM with high HIV risk behaviors such as condomless receptive anal intercourse, causal sex, and recreational drug use. Therefore, the intervention would substantially reduce the risk for HIV acquisition.

Previously, although some complex interventions with more than five components had the strongest effect in improving adherence, it was unclear whether and how well each component worked.[8] Moreover, it might be difficult and costly to scale up such complex interventions in “real world” and resource-limited settings. In contrast, strategies that are easy to implement might be more feasible and sustainable in resource-limited settings. This AVM intervention addressed potential barriers and manifested advantages of easy-performing, low intensity, and less stigmatized. Therefore, this intervention could be scaled up and used in future MSM PrEP programs for HIV prevention.

This study was conducted among MSM in a large Chinese city; cautious should be made when generalizing the results to other populations and other regions. Although randomization was used to allocate participants to each group, two groups showed statistical differences in the baseline education level. It might be due to the small sample size. To reduce the possible effects on study results, we controlled for this covariate in the GEE model. Future studies could reevaluate the intervention effect in multiple study settings and diverse groups. Furthermore, self-reported data may not be accurate and could affect the study results, especially for the ED-PrEP group.

In conclusion, this smartphone-based AVM intervention significantly improved PrEP adherence over a 12-month period among Chinese MSM PrEP users. The intervention has the advantages of high efficacy, convenience, easy-performing, thus could be adopted by future HIV prevention PrEP programs Fig. 1.

Table 1
Baseline characteristics, sexual debut with male sexual partner, and sexual behaviors in the past three months in AVM and SOC group ($N = 196$).

	AVM		SOC		
Variables	***n* = 98**	**%**	***n* = 98**	**%**	**P Values**
Sociodemographic characteristics					
Age group (years)					
18–24	20	20.4	13	13.3	0.09
25–34	51	52.0	44	44.9	
35–65	27	27.6	41	41.8	
Ethnicity					
Han ethnic	82	83.7	78	79.6	0.72
Manchu	12	12.2	16	16.3	
Others	4	4.1	4	4.1	
Education					
University or higher	19	19.4	33	33.7	0.02
High school or below	79	80.6	65	66.3	
Income (per month)					
<4000 CNY	60	61.2	51	52.1	0.06
4000–6000 CNY	17	17.3	31	31.6	
>6000 CNY	21	21.4	16	16.3	
PrEP dosing regimens					
Event-driven regimen	40	40.8	47	48.0	0.12
Daily regimen	58	59.2	51	51.2	
Sexual debut with male sexual partners					
Age at sexual debut					
<20 years	46	46.9	49	50.0	0.67
≥20 years	52	53.1	49	50.0	
Main venue to seek male sexual partners					
GSN app	78	79.6	77	78.6	0.86
Others	20	20.4	21	21.4	
Sexual behavior in the past three months					
Sexual roles with male					
Oral	1	1.0	3	3.1	0.11
Top	36	36.7	36	36.7	
Bottom	33	33.7	20	20.4	
Versatile	28	28.6	39	39.8	
Number of male sexual partners					
≤4	61	62.2	53	54.1	0.25
>4	37	37.8	45	45.9	
Casual sex with male					
Yes	60	61.2	62	63.3	0.77
No	38	38.8	36	36.7	
Drunk sex with male					
Yes	21	21.4	29	29.6	0.19
No	77	78.6	69	70.4	
Recreational drug use before sex					
Yes	47	48.0	56	57.1	0.20
No	51	52.0	42	42.9	

AVM=asynchronous-video-monitored, SOC=standard of care, GSN= Geosocial Networking (a widely-used app for MSM socialization). CNY, Chinese Yuan.

Declaration of Competing Interest

The authors declared no conflict of interests.

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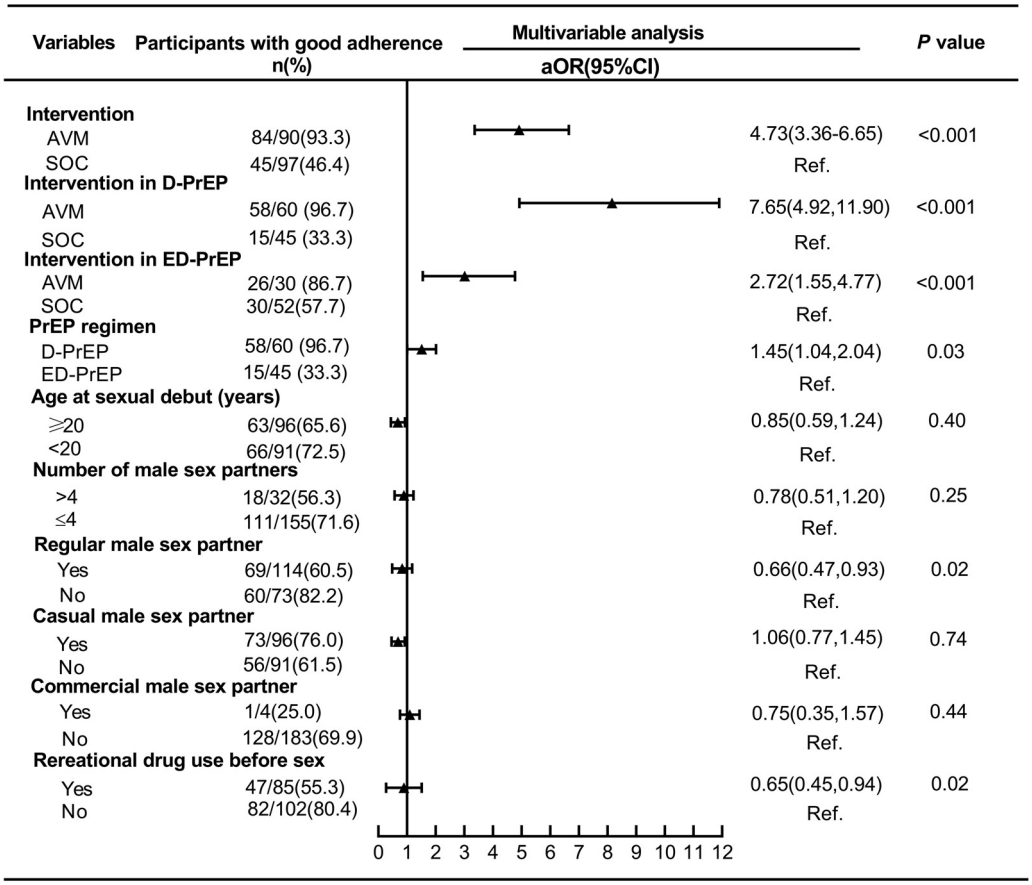

Fig. 1. Generalized estimating equation analyses of factors associated with good adherence in AVM and SOC group (AVM=asynchronous-video-monitored, SOC=standard of care, AOR: adjusted for age, ethnicity, education level, living status, and income).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.12.045.

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