Letter to the Editor

Viable SARS-CoV-2 shedding under remdesivir and dexamethasone treatment

Dear Editor,

In a recent systemic literature review, Walsh et al. reported that viral load of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) peaks around symptom onset, and becomes undetectable about two weeks after symptom onset.[1] However, some studies have revealed that infectious SARS-CoV-2 shedding was detectable up to 20 days of symptom onset.[2,3] In patients with severe coronavirus disease 2019 (COVID-19), the duration of SARS-CoV-2 shedding is an issue in infection control and isolation strategies. The current standard treatment (remdesivir and dexamethasone) might influence the duration of infectious viral shedding. However, there have been scarce studies which investigated the duration of viable virus shedding in severe COVID-19 patients, who received anti-inflammatory and antiviral therapy with dexamethasone and remdesivir.

This study analyzed the duration of viable virus shedding in COVID-19 intensive care units of two university hospitals from March 1 to December 31, 2021. We enrolled hospitalized patients with COVID-19 pneumonia who required high-flow oxygen therapy or had a high probability of progression to severe disease due to underlying medical conditions. COVID-19 severity was classified using an ordinal scale proposed by the World Health Organization.[4] Paired nasopharyngeal swab samples were collected on the day of enrollment and every other day after that. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and plaque assays were conducted to detect viable SARS-CoV-2 using the cell culture method (supplementary appendix). In addition, the SARS-CoV-2 rapid antigen test, STANDARD™ Q COVID-19 Ag Test (SD Biosensor, Inc.) was used to evaluate the association between the results of the rapid kit and infectious virus shedding. Serum samples were collected on 7th and 14th day after symptom onset to detect the anti-S immunoglobulin G antibody levels using the Elecsys® Anti-SARS-CoV-2 S assay (Roche, Rotkreuz, Switzerland). The study protocol was approved by the Institutional Review Boards of Korea University Guro Hospital (2021GR0096) and Ajou University Hospital (AJIRB-BMR-SMP-21-030). Informed consent was obtained from the patients or their immediate family members.

Among the 48 enrolled patients with COVID-19 pneumonia, 50% ($n = 24$) were men with a median age of 60 years, while 20 patients (41.7%) had comorbidities (Table 1). High-flow oxygen therapy was required in 85.4% of the patients ($n = 41$). Mechanical ventilation was required in 31.3% of the patients ($n = 15$), while 16.7% ($n = 8$) were supported by extracorporeal membrane oxygenation. Eight patients died during hospitalization. All patients received more than 6 mg/day of dexamethasone, and 46 patients

Table 1
Clinical characteristics and treatment outcomes of patients with COVID-19 pneumonia.

Clinical characteristics and treatment outcomes (N=48)	No. (%)
Sex (men), n (%)	24 (50.0%)
Age, median (IQR)	63 (51-71)
Length of hospital stay, mean days (SD)	20.2 (11.5)
Underlying disease, n (%)	20 (41.7%)
Diabetes, n (%)	15 (31.3%)
Cardiovascular diseases, n (%)	4 (8.3%)
Solid cancer, n (%)	3 (6.3%)
COVID-19 vaccination (any dose), n (%)	12 (25.0%)
WHO COVID-19 ordinal scale at enrollment, n (%)	
Score 4, Oxygen by mask or nasal prongs, n (%)	7 (14.6%)
Score 5, Non-invasive ventilation or high-flow oxygen, n (%)	39 (81.3%)
Score 6, Intubation and mechanical ventilation, n (%)	2 (4.2%)
Mechanical ventilation, n (%)	15 (31.3%)
ECMO, n (%)	8 (16.7%)
Death, n (%)	8 (16.7%)
Dexamethasone treatment, n (%)	48 (100%)
Dexamethasone dose	
6-10 mg/day, n (%)	42 (87.5%)
>10 mg/day, n (%)	6 (12.5%)
Dexamethasone treatment duration	
1-10 days, n (%)	21 (43.8%)
11-20 days, n (%)	19 (39.6%)
>20 days, n (%)	8 (16.7%)
Remdesivir treatment, n (%)	46 (95.8%)
Duration of remdesivir treatment, mean days (SD)	5.5 (1.5)
Interval from symptom onset to remdesivir treatment, mean days (SD)	6.2 (3.3)
†Monoclonal antibody treatment, n (%)	1 (2.1%)

Abbreviations; IQR, interquartile range; SD, standard deviation; COVID-19, coronavirus disease 2019; WHO, World Health Organization; ECMO, extracorporeal membrane oxygenation

† Regdanvimab (Regkirona™), manufactured by Celltrion, Inc.

(95.8%) were treated with remdesivir for five days or more. Among the 160 nasopharyngeal specimens, 17 yielded a positive culture result from days 3 to 18 after symptom onset (Fig. 1A). Infectious virus samples were not detected after the third dose of remdesivir (200 mg intravenously (IV) on day 1, then 100 mg IV daily from day 2) despite high-dose dexamethasone treatment (Fig. 1B). Rapid antigen test (RAT) was positive in 80% (4/5) of culture-positive samples and in 15.6% (10/64) of culture-negative samples (Fig. S1). The positive and negative predictive values of RAT for detecting viable viruses were 28.6% and 98.2%, respectively. The geometric mean titer of immunoglobulin-G anti-S antibody on days 7 and 14 after symptom onset were 28.6 U/mL and 217.8 U/mL, respectively (Fig. S2).

Based on a clinical trial, the cycle threshold (Ct) value of RT-PCR was not significantly reduced by remdesivir.[5] However, this study showed that SARS-CoV-2 shedding significantly decreased after remdesivir treatment, regardless of the Ct value. Early de-

(A)

(B)
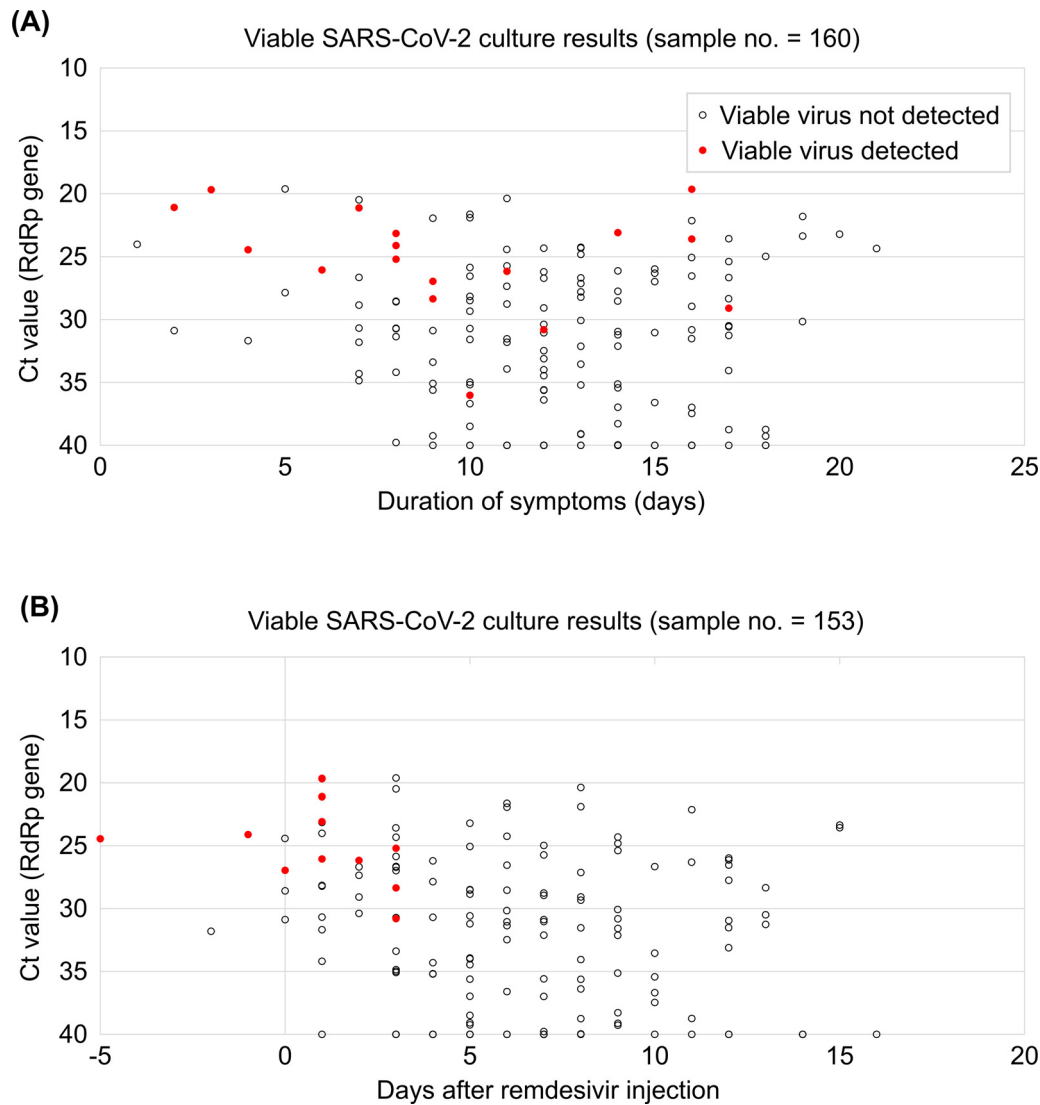
Fig. 1. Correlation between viable severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) shedding and cycle-threshold (Ct) values according to time from symptom onset (A) and start of remdesivir treatment (B).

isolation can be done after remdesivir treatment, even in patients with severe COVID-19. RAT is a viable adjunctive tool that guides the decision to terminate the isolation period.[6]

Declaration of Competing Interest

The authors declare no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.03.022.

References

1. Walsh K.A., Jordan K., Clyne B., et al. SARS-CoV-2 detection, viral load and infectivity over the course of an infection. *J Infect* 2020;**81**(3):357–71. doi:10.1016/j.jinf.2020.06.067.
2. Kim M.C., Cui C., Shin K.R., et al. Duration of culturable SARS-CoV-2 in hospitalized patients with Covid-19. *N Engl J Med* 2021;**384**(7):671–3.
3. van Kampen J.J.A., van de Vijver D., Fraaij P.L.A., et al. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). *Nat Commun* 2021;**12**(1):267.
4. World Health Organization. WHO R&D blueprint: novel coronavirus COVID-19 therapeutic trial synopsys. 2020 [accessed 17 January 2022]. Available from: https://www.who.int/publications/i/item/covid-19-therapeutic-trial-synopsis.
5. Wang Y., Zhang D., Du G., et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020;**395**(10236):1569–78.
6. UK Health Security Agency. Stepdown of infection control precautions and discharging COVID-19 patients and asymptomatic SARS-CoV-2 infected patients. 2022 [accessed 17 January 2022]. Available from: https://www.gov.uk/government/publications/covid-19-guidance-for-stepdown-of-infection-control-precautions-within-hospitals-and-discharging-covid-19-patients-from-hospital-to-home-settings/guidance-for-stepdown-of-infection-control-precautions-and-discharging-covid-19-patients.

Jin Gu Yoon[1]
Division of Infectious Diseases, Department of Internal Medicine,
Korea University College of Medicine, Seoul, South Korea

Jin Sae Yoo[1]
Department of Infectious Diseases, Ajou University School of
Medicine, Suwon, South Korea

Jungmin Lee

Department of Microbiology, Biosafety Center, College of Medicine, Institute for Viral Diseases, Korea University, Seoul 02841, South Korea

Hak-Jun Hyun, Hye Seong, Ji Yun Noh, Hee Jin Cheong, Woo Joo Kim

Division of Infectious Diseases, Department of Internal Medicine, Korea University College of Medicine, Seoul, South Korea

Young Rong Kim, Jung Yeon Heo

Department of Infectious Diseases, Ajou University School of Medicine, Suwon, South Korea

Joon-Yong Bae, Chunguang Cui, Sohyun Lee, Man-Seong Park*[1]

Department of Microbiology, Biosafety Center, College of Medicine, Institute for Viral Diseases, Korea University, Seoul 02841, South Korea

Joon Young Song*[1]

Division of Infectious Diseases, Department of Internal Medicine, Korea University College of Medicine, Seoul, South Korea

*Corresponding authors.
E-mail addresses:* ms0392@korea.ac.kr (M.-S. Park), infection@korea.ac.kr (J.Y. Song)

[1] These authors contributed equally to this work.

Multiplex PCR rapid testing for meningitis/encephalitis

Dear Editor,

We read with great interest the Navarro-Carrera *et al.* article describing a patient receiving ravulizumab, a complement component C5 inhibitor, in whom the BioFire® FilmArray® Meningitis/Encephalitis Panel (BioFire® ME Panel, BioFire Diagnostics, bioMérieux, Marcy l'Etoile, France) successfully identified *Neisseria meningitidis* in cerebrospinal fluid (CSF).[1] The FilmArray® ME panel is designed to detect the 14 most frequent pathogens causing meningitis and/or encephalitis, including six bacteria (*Escherichia coli* K1, *Haemophilus influenzae, Listeria monocytogenes, Neisseria meningitidis, Streptococcus agalactiae,* and *S. pneumoniae*), seven viruses (cytomegalovirus, enterovirus, herpes simplex viruses type 1 and 2 [HSV-1 and HSV-2], human herpesvirus type 6 [HHV-6], human parechovirus, and varicella-zoster virus [VZV]), and one fungus (*Cryptococcus neoformans/gattii*). The FilmArray® ME panel was launched in Taiwan in 2020 and approved for reimbursement by the National Health Insurance Administration of the Ministry of Health and Welfare in 2021.

From January 1 to December 31, 2021, cerebrospinal fluid (CSF) samples were obtained from 443 patients with suspected meningitis/encephalitis who were treated in different departments of China Medical University Hospital, a 2100-bed University-affiliated hospital located in Taichung, Taiwan. All CSF samples were tested for microbial pathogens using the FilmArray® ME panel, in addition to conventional microbiological culture and serological methods (Table 1). Among these, 33 (7.5%) tested positive. The target pathogens detected included seven (21.2%) bacteria (three *E. coli* K1 and four *S. agalactiae*), 20 (60.6%) viruses (seven VZV, five HSV-2, and three HHV-6, four human parechovirus, and one HSV-1). Besides, six samples (18.2%) contained the fungus (*C. neoformans/gattii*). No *N. meningitidis* was detected using the ME panel. To confirm the diagnosis, blood and CSF cultures were used to identify the bacterial pathogens. In addition to CSF cultures for viruses, biochemical tests on CSF (glucose and protein levels and white blood cell [WBC] counts), PCR typing for HSVs, and detection of IgGs and IgMs for VZV, HSV-1, and HSV-2 were used to elucidate the viral pathogens. Cryptococcal antigen and India ink stains were used to detect *C. neoformans/gattii* infection in the blood and CSF cultures (Table 1). In patients with positive ME panel results, two (28.6%) bacterial, four (20%) viral, and six (100%) fungal samples showed abnormal glucose levels in the CSF specimens. Six (85.7%) bacterial, 14 (70%) viral, and six (100%) fungal cases had abnormal CSF protein levels. Five (71.4%) bacterial, 11 (55%) viral, and six (100%) cases had pleocytosis (> 5 WBCs per microliter in the CSF). Interestingly, two of the CSF specimens from patients with bacterial meningitis showed negative bacterial growth, whereas the biochemistry tests remained normal. These false-positive results may be due to a contaminated working area in the clinic. In seven CSF samples identified as VZV infections by ME panels, none were positive in the CSF cultures. However, three patients with VZV infection were confirmed by the detection of VZV IgG in the serum. In five CSF specimens identified with HSV-2 infections, two were further confirmed as HSV-2 infections with HSV PCR tests and serological antibody levels. Interestingly, the patients also showed positive results for HSV-1 antibodies, which may have been caused by previous infection with HSV-1. Among the four specimens infected with human parechovirus, all samples showed increased protein and WBC levels in the CSF, and one of the specimens was positive for enterovirus infection upon CSF culture. Most of the patients with HHV-6 infections were asymptomatic. Infection is usually temporary and can be cured with supportive treatment and antiviral treatment is not necessary. All specimens infected with HSV-1 and *C. neoformans/gattii* were confirmed using PCR tests and CSF cultures.

In this study, no *N. meningitidis* was identified in 2021. In Taiwan, the incidence of invasive meningococcal disease (IMD), a notifiable infectious disease in Taiwan, was low and was estimated to be 0.008–0.192 cases/100,000 persons in 1993–2019.[2] During the COVID-19 epidemic, the number of cases of IMD reported to Taiwan Centers for Disease Control in 2020 and 2021 was four and three, respectively (https://nidss.cdc.gov.tw/nndss/disease?id=025).[3]

Timely recognition of causative pathogens is critical for the appropriate management and improved outcome of patients with central nervous system infections. The conventional microbiological culture of CSF for the detection of bacteria, fungi, and viruses (the gold-standard method for the diagnosis of meningitis/encephalitis), is time-consuming and labor-intensive. In contrast, the BioFire® ME panel using a multiplex PCR technique can provide results within one hour.[4,5] In addition, the sensitivity of microbial cultures of CSF decreases with empirical treatment.[3] This study highlights the importance of rapid diagnostic techniques with high specificity and sensitivity to minimize the cost of time on testing, shorten the length of time in hospital stays, and reduce the use of antibiotics.

Declaration of Competing Interest

The authors declare no conflict of interest.

Ethical approval information

Not required.

Table 1

Diagnosis of pathogens using the BioFire® FilmArray® Meningitis/Encephalitis Panel (BioFire® ME Panel) along with conventional microbiological culture and serological methods.

Target		*E. coli* K1	*S. agalactiae*	VZV	HSV-1	HSV-2	HHV-6	Human parechovirus	*C. neoformans/gattii*
BioFire® ME Panel results (no. of positive tests)		3	4	7	1	5	3	4	6
Abnormal CSF glucose level Normal range: 40–70 mg/dL (no. of positive tests / no. of total tested)		1/3	1/4	5/7	1/1	0/5	0/3	2/4	6/6
Abnormal CSF protein level Normal range: 15–45 mg/dL (no. of positive tests / no. of total tested)		2/3	4/4	5/7	0/1	4/5	1/3	4/4	6/6
Abnormal CSF WBC counts Normal range: < 5 cells/μL (no. of positive tests / no. of total tested)		2/3	3/4	4/7	0/1	4/5	1/3	3/4	6/6
CSF cultures (no. of positive cultures / no. of total tested)		2/3	1/4	0/7	1/1	0/5	0/3	1/4	6/6
India ink stains in CSF (no. of positive stains/no. of total tested)		0/3	0/4	0/7	0/1	0/5	0/3	0/4	6/6
Blood cultures (no. of positive cultures / no. of total tested)		1/3	1/4	0/7	0/1	0/5	0/3	0/4	1/6
PCR for HSVs in CSF (no. of positive cultures / no. of total tested)		0/3	0/4	0/7	1/1	2/5	0/3	0/4	0/6
Serology tests	VZV IgG	0/3	0/4	3/7	0/1	0/5	0/3	0/4	0/6
	VZV IgM	0/3	0/4	0/7	0/1	0/5	0/3	0/4	0/6
	HSV-1 IgG	0/3	0/4	0/7	1/1	2/5	0/3	0/4	0/6
	HSV-1 IgM	0/3	0/4	0/7	0/1	1/5	0/3	0/4	0/6
	HSV-2 IgG	0/3	0/4	0/7	0/1	2/5	0/3	0/4	0/6
	HSV-2 IgM	0/3	0/4	0/7	0/1	1/5	0/3	0/4	0/6

CSF, cerebrospinal fluid; VZV, varicella zoster virus; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; HHV-6, human herpes virus type 6; NR, normal range; WBC, white blood cell.

References

1. Navarro-Carrera P., García-Rodríguez J., Cendejas-Bueno E.. Detection of *Neisseria meningitidis* in a patient receiving ravulizumab by the FilmArray Meningitis/Encephalitis panel- A case report. *J Infect* 2021;**82**(6):e22–3.
2. Aye A.M.M., Bai X., Borrow R., Bory S., Carlos J., et al. Meningococcal disease surveillance in the Asia-Pacific region (2020): the global meningococcal initiative. *J Infect* 2020;**81**(5):698–711.
3. Lai C.C., Chen S.Y., Yen M.Y., Lee P.I., Ko W.C., Hsueh P.R.. The impact of the coronavirus disease 2019 epidemic on notifiable infectious diseases in Taiwan: a database analysis. *Travel Med Infect Dis* 2021;**40**:101997.
4. Lee S.H., Chen S.Y., Chien J.Y., Lee T.F., Chen J.M., Hsueh P.R.. Usefulness of the FilmArray meningitis/encephalitis (M/E) panel for the diagnosis of infectious meningitis and encephalitis in Taiwan. *J Microbiol Immunol Infect* 2019;**52**(5):760–8.
5. Poplin V., Boulware D.R., Bahr N.C.. Methods for rapid diagnosis of meningitis etiology in adults. *Biomark Med* 2020;**14**(6):459–79.

Yu-Chuan Tseng[1]
Department of Laboratory Medicine, China Medical University Hospital, Taichung, Taiwan

Peter Bor-Chian Lin[1]
Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, Indiana, USA

Chiung-Tzu Hsiao
Department of Laboratory Medicine, China Medical University Hospital, Taichung, Taiwan

Po-Ren Hsueh*
Departments of Laboratory Medicine and Internal Medicine, China Medical University Hospital, Taichung, Taiwan
School of Medicine, China Medical University, Taichung, Taiwan

*Corresponding author.
E-mail address:* hsporen@ntu.edu.tw (P.-R. Hsueh)

Protective trend of anti-androgen therapy during the COVID-19 pandemic: A meta-analysis

Dear Editor,

Androgen receptor (AR) is an important transcription factor; thus, androgen and AR-associated pathways play pivotal roles in the progression of several diseases, including prostate cancer (PCa), benign prostatic hyperplasia (BPH), breast cancer, acne, and alopecia [1,2]. Anti-androgen therapy is widely used for the clinical treatment of prostatic diseases, including androgen deprivation therapy (ADT) via surgical castration, pharmacological castration, or androgen receptor blocked therapy for PCa, and 5 alpha-reductase inhibitor (5ARI) for BPH. Influenced by the COVID-19 pandemic, the effects of SARS-CoV-2 on patients receiving anti-androgen therapy have attracted increasing attention. Montopoli *et al.* [3] first reported the potential impact of anti-androgen therapy on SARS-CoV-2 infection, where ADT may provide partial protection of PCa patients from SARS-CoV-2 infection. Lyon *et al.* [4] also demonstrated a reduction in community-acquired SARS-CoV-2 in-

fection with long-term 5ARI administration. However, inconsistent voices were also acknowledged. Notably, several clinical trials (*e.g.*, NCT04446429, NCT04530500, NCT04475601, NCT04354701) also tested the potential protective function of anti-androgen therapy.

In this study, we comprehensively searched publications up to March 15, 2022, in PubMed, Google Scholar, Embase, Cochrane Library and ClinicalTrials, with the following keywords: "androgen", "anti-androgen therapy", "androgen deprivation therapy", "ADT", "5 alpha-reductase inhibitor", "5ARI", "COVID-19", "2019-nCoV", "SARS-CoV-2", "2019 novel coronavirus", and "coronavirus disease 2019". We also searched the reference lists of relevant reviews and studies to avoid any missing articles within the topic. The inclusion criteria were preset: (1) case control study with anti-androgen therapy group and non-therapy group; (2) infection of SARS-CoV-2 detected by reverse transcriptase–polymerase chain reaction test; (3) available data for each group and the accurate number of events; (4) study populations being at least fifteen cases. Case reports, repeated articles, review papers and preprints were eliminated. This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline. Detailed information on the enrolled studies is listed in Tables S1 and S2.

The "*metafor*" R package was employed to perform the meta-analysis. The odds ratio (OR) with 95% confidence interval (CI) generated from the total number and event number was used to evaluate the impact of anti-androgen therapy and was further integrated into the overall OR. The I^2 and Tau^2 values were calculated to quantify the heterogeneity of each subset. Cumulative meta-analysis was used to display the cumulative impact of anti-androgen therapy on events; specifically, we accumulated the studies in the order of fewer to more patients in the control group. Potential publication bias was evaluated by a funnel plot.

For the evaluation of anti-androgen therapy for SARS-CoV-2 infection, seven studies with a total of 53,378 samples were enrolled for the meta-analysis; six studies received ADT treatment, and one study received 5ARI treatment. Five studies showed a protective trend of anti-androgen therapy to avoid SARS-CoV-2 infection, with ORs less than one, while the remaining two studies demonstrated a risky role with ORs higher than one. Additionally, the integrative meta-analysis provided the protective trend of anti-androgen therapy (OR = 0.89, 95% CI: 0.65–1.22, Fig. 1A). To further understand the impact of anti-androgen therapy to avoid SARS-CoV-2 infection, cumulative analysis was further conducted. The studies were accumulated in the order of fewer to more patients in control groups to avoid sparse data bias. We observed that the ORs remained less than one in each step after sample accumulation, which strongly supported the findings that anti-androgen therapy may prevent SARS-CoV-2 infection (Fig. 2A).

Regarding the association assessment between anti-androgen therapy and mortality of COVID-19, a total of 9,619 SARS-CoV-2-infected patients from 14 studies were collected. Out of these patients, 2,615 patients received anti-androgen therapy, and 7,004 patients were treatment free. For the 14 studies, eight collected PCa patients received ADT treatment, including three of enzalutamide; two studies recruited patients (> 18 years old) detected with COVID-19, no matter man or woman, and given proxalutamide treatment; one study applied 5ARI treatment among men older than 20 years and without PCa. A total of 64.29% (9/14) of the studies reported a protective trend of anti-androgen therapy to avoid COVID-19-associated mortality, while the other studies demonstrated the risk of death. Integrative meta-analysis revealed an overall OR of 0.85 (95% CI: 0.41–1.78; Fig. 1B). Of note, the cumulative meta-analysis reported that the ORs remained less than one after the accumulation of each study, which again indicated the protective trend of anti-androgen therapy to prevent COVID-19-associated mortality (Fig. 2B). The funnel plots were also

performed among the SARS-CoV-2 infection subset and COVID-19-caused death subset (Fig. S1). The symmetrical results and Egger's test results (Infection subset: $Z = -0.78$, $P = 0.44$; Death subset: $Z = -0.54$, $P = 0.59$) indicated no potential publication bias.

Angiotensin converting enzyme 2 (ACE2) is the receptor of the SARS-CoV-2 coronavirus S1 domain of the spike protein; the complex further received proteolytic cleavage by the transmembrane protease serine 2 (TMPRSS2), and then the virus entered the host cell [5]. The increased morbidity and mortality in men implicates a sex disparity of the male hormone in SARS-CoV-2 infection and host response. Recently, Leach *et al.* [6] reported that the anti-androgen enzalutamide can reduce TMPRSS2 levels in the lung cells of humans and mice and can also reduce SARS-CoV-2 entry and infection into lung cells. Deng *et al.* [7] revealed the AR-binding sites located in the transcription start sites of the *TMPRSS2* and *ACE2* genes and confirmed that androgen deprivation had an effect on the decreased expression of TMPRSS2 and ACE2, particularly in lung tissues. These biological findings support the clinical results that anti-androgen therapy might prevent humans from the infection and the horrible end caused by SARS-CoV-2.

With the pandemic of the SARS-CoV-2 omicron variant and the clinical trials that have been widely applied to human beings with anti-androgen therapy, it is fortunate that the anti-androgen therapy has not caused greater harm from SARS-CoV-2 to patients. Although Welén *et al.* [8] reported disappointing results among enzalutamide failure to prevent SARS-CoV-2, we still hold a favorable expectation of anti-androgen therapy with the protective trend from the meta-analysis and the biological findings; further studies that enable uncovering the association between anti-androgen therapy and COVID-19 are highly warranted.

Data sharing statement

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

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.03.020.

References

[1]. Shukla G.C., Plaga A.R., Shankar E., Gupta S.. Androgen receptor-related diseases: what do we know? *Andrology* 2016;**4**(3):366–81.
[2]. Marks D.H., Prasad S., De Souza B., Burns L.J., Senna M.M.. Topical antiandrogen therapies for androgenetic alopecia and acne vulgaris. *Am J Clin Dermatol* 2020;**21**(2):245–54.
[3]. Montopoli M., Zumerle S., Vettor R., Rugge M., Zorzi M., Catapano C.V., et al. Androgen-deprivation therapies for prostate cancer and risk of infection by SARS–CoV-2: a population-based study (N = 4532). *Ann Oncol* 2020;**31**(8):1040–5.

A *SARS-CoV-2 infection*

Study or Subgroup	ADT+ Events	Total	ADT− Events	Total	Weight	Odds ratio IV, Random, 95% CI	Odds ratio IV, Random, 95% CI
Montopoli *et al.*	4	5273	114	37161	7.5%	0.25 [0.09, 0.67]	
Jiménez−Alcaide *et al.*	11	156	50	1193	12.7%	1.73 [0.88, 3.41]	
Koskinen *et al.*	6	134	11	218	7.3%	0.88 [0.32, 2.44]	
Klein *et al.*	17	304	85	1475	16.1%	0.97 [0.57, 1.66]	
Kwon *et al.*	18	799	79	4412	16.6%	1.26 [0.75, 2.12]	
Lyon *et al.*	399	944	446	944	27.5%	0.82 [0.68, 0.98]	
Kazan *et al.*	13	138	30	227	12.4%	0.68 [0.34, 1.36]	
Total (95% CI)		**7748**		**45630**	**100.0%**	**0.89 [0.65, 1.22]**	
Total events:	468		815				

Heterogeneity: Tau^2 = 0.09; Chi^2 = 13.21, df = 6 (P = 0.04); I^2 = 55%
Test for overall effect: Z = −0.70 (P = 0.48)

Favours ADT+   Favours ADT−

B *Death*

Study or Subgroup	ADT+ Events	Total	ADT− Events	Total	Weight	Odds ratio IV, Random, 95% CI	Odds ratio IV, Random, 95% CI
Jiménez−Alcaide *et al.*	3	11	17	50	6.6%	0.73 [0.17, 3.10]	
Koskinen *et al.*	1	6	3	11	4.3%	0.53 [0.04, 6.65]	
Klein *et al.*	6	17	13	85	7.2%	3.02 [0.95, 9.61]	
Patel *et al.*	4	22	10	36	6.9%	0.58 [0.16, 2.13]	
Montopoli *et al.*	0	4	18	114	3.6%	0.58 [0.03, 11.23]	
Kwon *et al.*	1	19	7	78	5.0%	0.56 [0.07, 4.88]	
Welen *et al.*.1	21	358	167	4980	8.5%	1.80 [1.13, 2.86]	
Welen *et al.*.2	20	334	167	4980	8.5%	1.84 [1.14, 2.96]	
Welen *et al.*.3	24	152	167	4980	8.5%	5.40 [3.40, 8.58]	
Lyon *et al.*	15	944	19	944	8.2%	0.79 [0.40, 1.56]	
Schmidt *et al.*	25	169	44	308	8.4%	1.04 [0.61, 1.77]	
Cadegiani *et al.*, south	10	106	9	27	7.5%	0.21 [0.07, 0.58]	
Cadegiani *et al.*,north	35	317	162	328	8.6%	0.13 [0.08, 0.19]	
Duarte *et al.*	100	156	32	43	8.1%	0.61 [0.29, 1.31]	
Total (95% CI)		**2615**		**16964**	**100.0%**	**0.85 [0.41, 1.78]**	
Total events:	265		835				

Heterogeneity: Tau^2 = 1.61; Chi^2 = 175.03, df = 13 (P = 0.00); I^2 = 93%
Test for overall effect: Z = −0.42 (P = 0.67)

Favours ADT+   Favours ADT−

Fig. 1. Forest plot showing the odds ratio of SARS-CoV-2 infection and horrible end among the enrolled studies. (A) Association between anti-androgen therapy and SARS-CoV-2 infection; (B) Association between anti-androgen therapy and SARS-CoV-2-induced death.

[4]. Lyon M., Li J., Cullen J., Milinovich A., Kattan M., Jehi L., et al. 5alpha-reductase inhibitors are associated with reduced risk of SARS-CoV-2 infection: a matched-pair, registry-based analysis. *J Urol* 2022;**207**(1):183–9.

[5]. Qiao Y., Wang X.M., Mannan R., Pitchiaya S., Zhang Y., Wotring J.W., et al. Targeting transcriptional regulation of SARS-CoV-2 entry factors ACE2 and TM-PRSS2. *Proc Natl Acad Sci U S A.* 2020.

[6]. Leach D.A., Mohr A., Giotis E.S., Cil E., Isac A.M., Yates L.L., et al. The antiandrogen enzalutamide downregulates TMPRSS2 and reduces cellular entry of SARS–CoV-2 in human lung cells. *Nat Commun* 2021;**12**(1):4068.

[7]. Deng Q., Rasool R.U., Russell R.M., Natesan R., Asangani I.A.. Targeting androgen regulation of TMPRSS2 and ACE2 as a therapeutic strategy to combat COVID-19. *iScience* 2021;**24**(3):102254.

[8]. Welen K., Rosendal E., Gisslen M., Lenman A., Freyhult E., Fonseca-Rodriguez O., et al. A phase 2 trial of the effect of antiandrogen therapy on COVID-19 Outcome: No evidence of benefit, supported by epidemiology and *in vitro* data. *Eur Urol* 2022;**81**(3):285–93.

Jialin Meng[1], Qintao Ge[1], Jiawei Li[1]
Department of Urology, The First Affiliated Hospital of Anhui Medical University, Institute of Urology, Anhui Medical University, Anhui Province Key Laboratory of Genitourinary Diseases, Anhui Medical University, 218th Jixi Road, Hefei, Anhui 230022, China
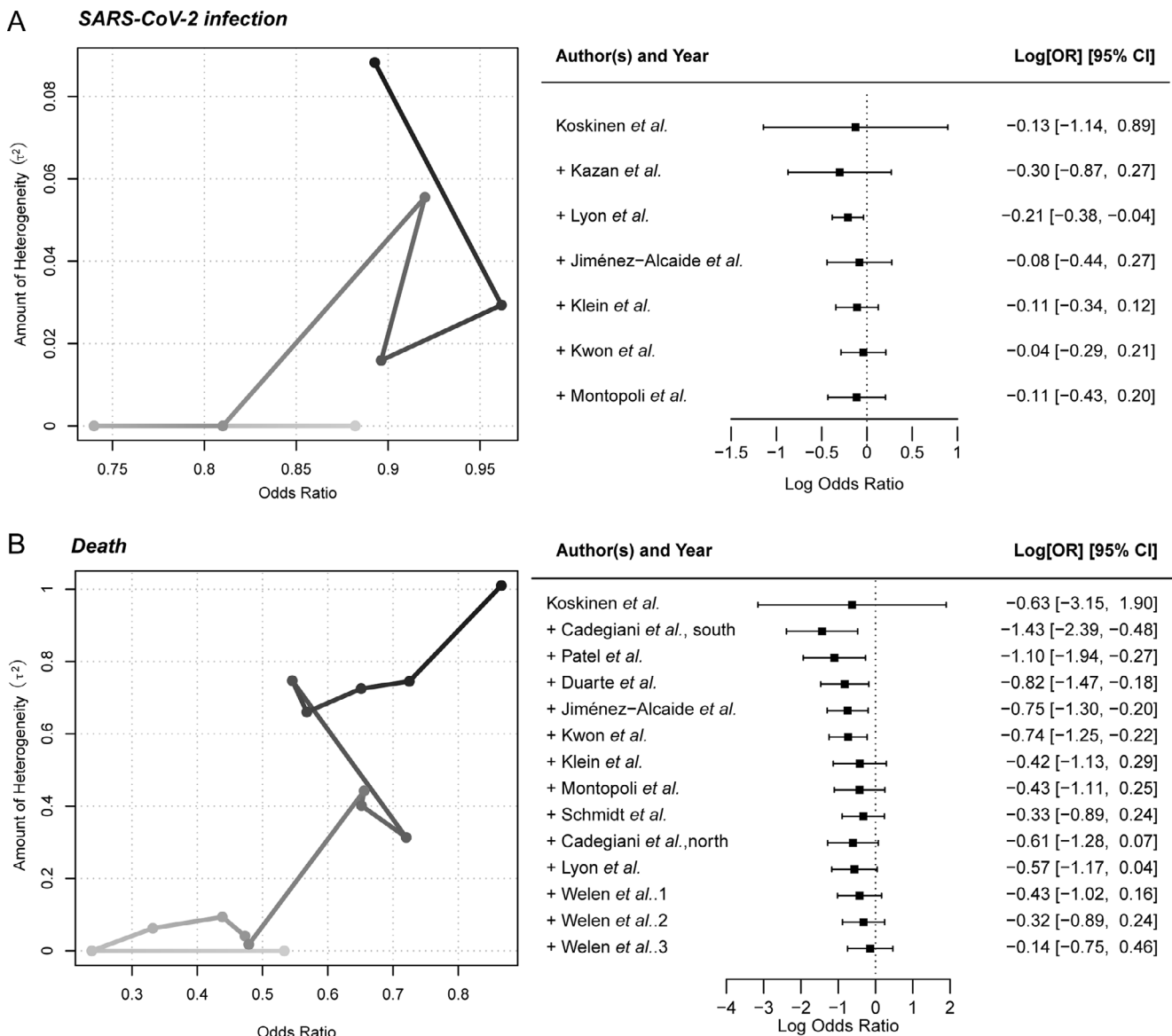
Xiaofan Lu
State Key Laboratory of Natural Medicines, Research Center of Biostatistics and Computational Pharmacy, China Pharmaceutical University, Nanjing 211198, China
Department of Cancer and Functional Genomics, Institute of Genetics and Molecular and Cellular Biology, CNRS/INSERM/UNISTRA, Illkirch 67400, France

Yonghao Chen
Department of Gastroenterology, West China Hospital of Sichuan University, Chengdu, Sichuan 610041, China

Haitao Wang
Center for Cancer Research, Clinical Research/NCI/NIH, Bethesda, MD 20892, United States

Meng Zhang
Department of Urology, The First Affiliated Hospital of Anhui Medical University, Institute of Urology, Anhui Medical University, Anhui Province Key Laboratory of Genitourinary Diseases, Anhui Medical University, 218th Jixi Road, Hefei, Anhui 230022, China

Juan Du
Longgang District People's Hospital of Shenzhen and The Second Affiliated Hospital, The Chinese University of Hong Kong, Shenzhen, Guangdong 518172, China
School of Medicine, The Chinese University of Hong Kong, Shenzhen, Guangdong 518172, China

Li Zhang, Zongyao Hao*, Chaozhao Liang*
Department of Urology, The First Affiliated Hospital of Anhui Medical University, Institute of Urology, Anhui Medical University, Anhui Province Key Laboratory of Genitourinary Diseases, Anhui Medical University, 218th Jixi Road, Hefei, Anhui 230022, China

*Corresponding author at: Department of Urology, The First Affiliated Hospital of Anhui Medical University, Institute of

A *SARS-CoV-2 infection*

Author(s) and Year	Log[OR] [95% CI]
Koskinen *et al.*	−0.13 [−1.14, 0.89]
+ Kazan *et al.*	−0.30 [−0.87, 0.27]
+ Lyon *et al.*	−0.21 [−0.38, −0.04]
+ Jiménez−Alcaide *et al.*	−0.08 [−0.44, 0.27]
+ Klein *et al.*	−0.11 [−0.34, 0.12]
+ Kwon *et al.*	−0.04 [−0.29, 0.21]
+ Montopoli *et al.*	−0.11 [−0.43, 0.20]

B *Death*

Author(s) and Year	Log[OR] [95% CI]
Koskinen *et al.*	−0.63 [−3.15, 1.90]
+ Cadegiani *et al.*, south	−1.43 [−2.39, −0.48]
+ Patel *et al.*	−1.10 [−1.94, −0.27]
+ Duarte *et al.*	−0.82 [−1.47, −0.18]
+ Jiménez−Alcaide *et al.*	−0.75 [−1.30, −0.20]
+ Kwon *et al.*	−0.74 [−1.25, −0.22]
+ Klein *et al.*	−0.42 [−1.13, 0.29]
+ Montopoli *et al.*	−0.43 [−1.11, 0.25]
+ Schmidt *et al.*	−0.33 [−0.89, 0.24]
+ Cadegiani *et al.*,north	−0.61 [−1.28, 0.07]
+ Lyon *et al.*	−0.57 [−1.17, 0.04]
+ Welen *et al.*.1	−0.43 [−1.02, 0.16]
+ Welen *et al.*.2	−0.32 [−0.89, 0.24]
+ Welen *et al.*.3	−0.14 [−0.75, 0.46]

Fig. 2. Cumulative meta-analysis showing the protective trend of anti-androgen therapy against SARS-CoV-2 infection and horrible end. (A) Cumulative line chart and forest plot showing the protective trend of anti-androgen therapy against SARS-CoV-2 infection. (B) Cumulative line chart and forest plot showing the protective trend of anti-androgen therapy against death. Light gray at the beginning, dark gray at the end.

Urology, Anhui Medical University, Anhui Province Key Laboratory of Genitourinary Diseases, Anhui Medical University, 218th Jixi Road, Hefei, Anhui 230022, China.
E-mail addresses: haozongyao@ahmu.edu.cn (Z. Hao), liang_chaozhao@ahmu.edu.cn (C. Liang)

[1] These authors contributed equally to the study.

Elevated neutralization of Omicron with sera of COVID-19 recovered and breakthrough cases vaccinated with Covaxin than two dose naïve vaccinees

Dear Editor,

Yang *et al.*, recently reported the neutralization potential of in-activated vaccines amongst breakthrough cases and vaccinees with regular and booster doses against Omicron variant.[1] The recent emergence of heavily mutated Omicron variant has swamp the world with increased number of COVID-19 infections. Though Omicron doesn't cause severe disease, it has the ability to rapidly spread and evade the immune response.[2] The global public health experts are mainly concerned about the immune escape potential of the Omicron. The immune response generated against COVID-19 vaccines available under emergency user authorization and natural infection with earlier variants has been found to wane over time. This essentially provides little protection against the newly emerging variants with immune escape potential such as Omicron and led to breakthrough infections and reinfections.[3–8] Andrews *et al.*,

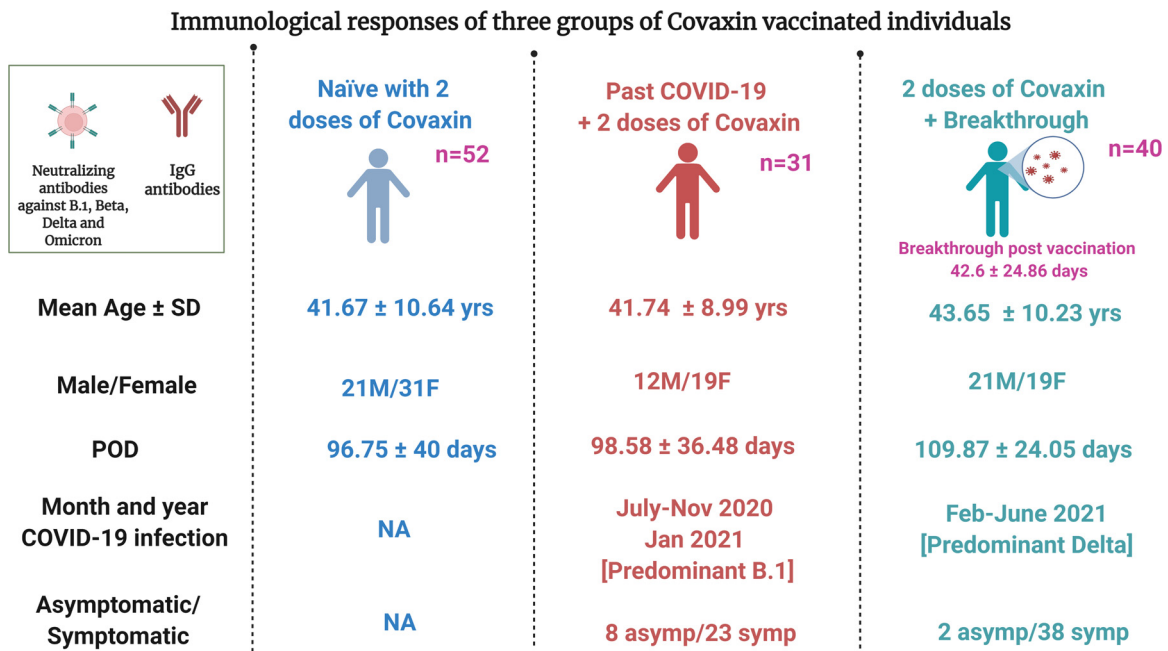## Immunological responses of three groups of Covaxin vaccinated individuals

Fig. 1. Overview of the study design and the participants.

recently reported that the individuals vaccinated with two doses of BNT162b2 or ChAdOx1 nCoV-19 vaccine didn't develop symptomatic disease upon infection with variant. Beside this, booster of any of these vaccines significantly increased protection in vaccinees which unfortunately waned over time.[8] Covaxin, an indigenously developed inactivated COVID-19 vaccine has been used under national vaccination program in India. Till date, millions of doses have been administered to adult population in India. Considering, the impact of third wave of pandemic aroused with Omicron in India, it triggered us to study the effectiveness of Covaxin against Omicron variant. Here, we assessed the sera of naïve, recovered and breakthrough cases vaccinated with Covaxin for its neutralizing ability against Omicron variant.

The study subjects were categorized into three groups i.e., COVID-19 naïve individuals vaccinated with two doses of Covaxin ($n = 52$) with average age of 41.7 years (range 23–65), COVID-19 recovered cases vaccinated with two doses of Covaxin ($n = 31$) with average age of 41.7 years (range 26–64) and breakthrough cases post two dose vaccination with Covaxin ($n = 40$) with average age of 43.7 years (range 27–67). The sera samples of naïve, recovered and breakthrough cases were collected on average 97, 99 and 110 days, respectively. The breakthrough infection found to occur on average 43 days post second vaccination. Majority of the breakthrough cases presented with mild disease (95%) and two were asymptomatic (5%); while 32.5% had co-morbidities like diabetes, hypothyroidism, hypertension, cardiac arrhythmias and allergic asthma (Fig. 1). The IgG antibody response in serum samples of all the subjects were assessed with S1-RBD, N protein and whole inactivated antigen ELISA and the neutralizing antibody titres were determined against Omicron, Delta and Beta variant compared to the prototype B.1 variant with plaque reduction neutralization test (PRNT).[9, 10]

The PRNT50 Geometric mean titre (GMT) of the sera of COVID naïve, recovered and breakthrough cases were determined against B.1 [61.9 (95% CI, 40–96), 126.7 (95% CI, 69–232), 235.6 (95% CI, 126–440)]; Beta (12.6 (95% CI, 6–26), 49.9 (95% CI, 25–99), 66.3(95% CI, 28–157)]; Delta [30.1 (95% CI, 16–58), 79.2 (95% CI, 45–140), 154.1 (95% CI, 61–390)] and Omicron (4.9 (95% CI, 2–10), 15.9 (95% CI, 6–40), 26.6 (95% CI, 14–50)]. The GMT values

were found to be decreasing with Delta, followed by Beta and Omicron variant in all the three groups. Comparative analysis of COVID naïve cases demonstrated fold-reduction of 4.9, 2.06 and 12.49 against Beta, Delta, and Omicron, respectively compared to prototype strain B.1. Similarly, reduction in the neutralizing antibody (NAb) titre was observed with sera of recovered and breakthrough cases against Delta (1.60, 1.53), Beta (2.54, 3.55) and Omicron (7.98, 8.84), respectively with parental virus (Fig. 2A–C). A strain-wise comparative analysis of the recovered and breakthrough cases compared to 2 dose vaccinated cases demonstrated higher neutralization for former scenario (Fig. 2D–G) which were also statistically significant. A two tailed Kruskal Wallis test was used to compare the cases with different strains. Breakthrough cases had highest neutralizing activity against all the variants demonstrating significant increase in the immune response post infection. The recovered cases also showed significant immunity boost post vaccination, but were lower than the breakthrough cases. Apparently, the naïve cases had very low neutralizing titres demonstrating the waning immunity post three months of the second dose. The Omicron variant has shown a pronounced resistance to neutralization with the sera of all the three groups compared to B.1, Beta and Delta variant. The IgG antibody response evaluated with S1-RBD, N-protein and inactivated whole antigen ELISA also demonstrated increasing pattern of GMT titres in recovered (760, 594, 205), naïve (797, 758, 150) and breakthrough cases (2573, 1770, 447), respectively (Fig. 2H–J).

The GMT titres of IgG and NAb clearly demonstrate highest immune response amongst breakthrough cases followed by recovered and naïve cases. Omicron was less effectively neutralized with the sera of naïve cases (12.9 fold) than recovered (7.98 fold) and breakthrough (8.84 fold) compared to B.1 (Fig. 2A–C). Although, the immune response was less against the Omicron, it would still protected the individuals from developing severe disease, hospitalization and mortality.

It is well known that the higher humoral and cellular immune response helps the people to protect from getting seriously ill with SARS-CoV-2. Recently, Vadrevu et al., reported persistent humoral and cellular immune response in individuals vaccinated with two doses of Covaxin against B.1, Alpha, Beta, Delta and Delta plus
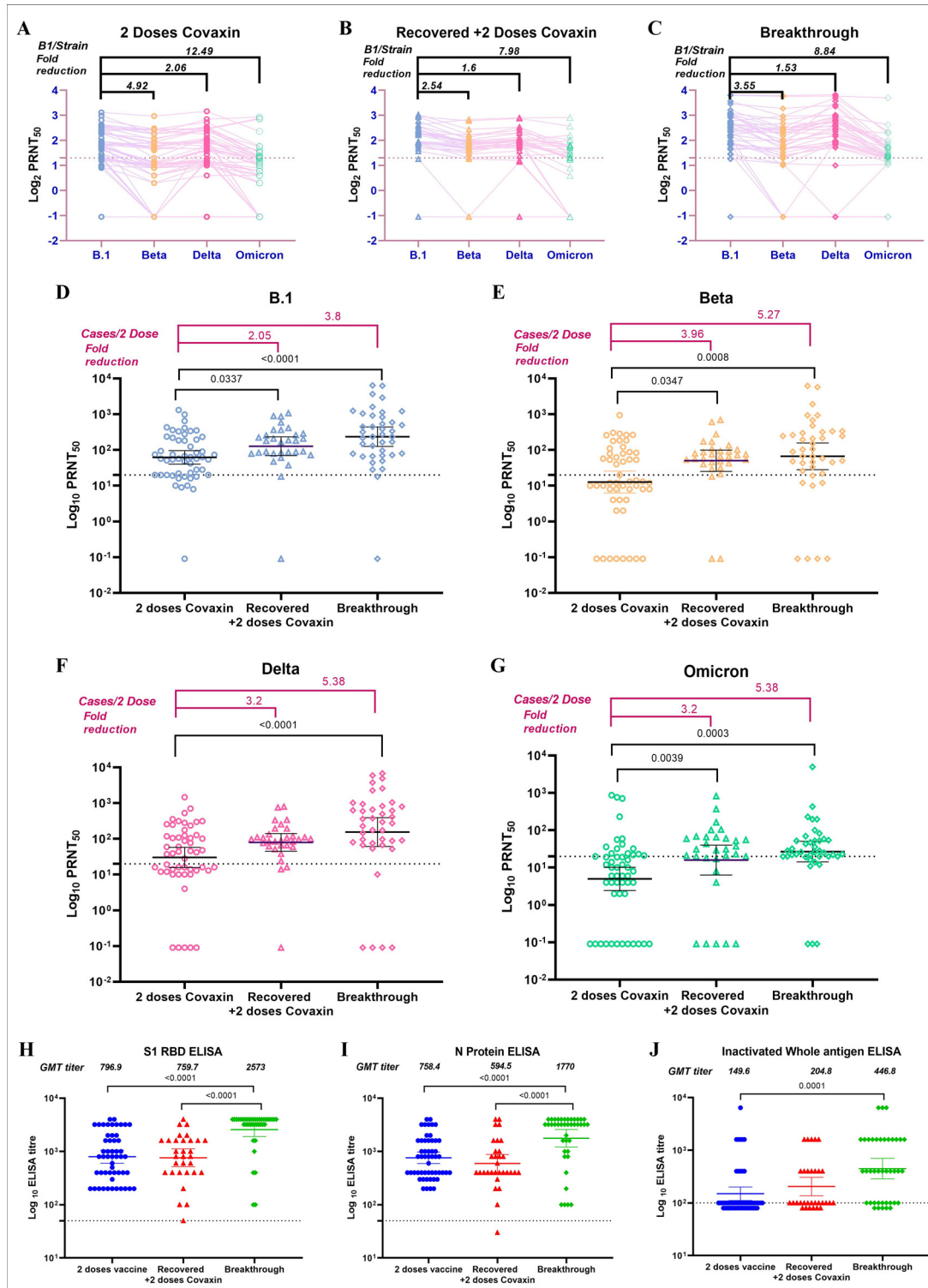
Fig. 2. Plaque reduction neutralization test titer and ELISA titer of sera of COVID naïve, recovered and breakthrough cases: Dot and line plot depicting the plaque reduction neutralization test of the individual sera vaccinated with (A) two doses of Covaxin (B) recovered and two dose Covaxin (C) breakthrough cases. Strain wise scatter plot of the different vaccinated cases (D) prototype B.1 (E) Beta, (F) Delta and (G) Omicron. ELISA titer of the individual sera vaccinated with different vaccinated cases against (H) S1-RBD (I) N protein (J) Whole inactivated virion. A two-tailed pair-wise comparison was performed using the Kruskal Wallis test with a *p*-value of 0.05. The dotted line on the figures indicates the limit of detection of the assay. Data are presented as geometric mean titer values with 95% confidence interval.

variants. Besides this, increased NAb response and protection was demonstrated with booster dose of Covaxin.[11] Hence, the administration of booster or precautionary dose is of much significance as it provides better protection against COVID-19 disease.

Funding

Declaration of Competing Interest

Authors do not have a conflict of interest among themselves.

Acknowledgement

References

1. Yang Y., Gong X., Yang L., Li J., Zhang J., Wei L., et al. Regular and booster vaccination with inactivated vaccines enhance the neutralizing activity against Omicron variant both in the breakthrough infections or vaccinees. *J. Infect.* 2022 Jan 10.
2. Dejnirattisai W., Huo J., Zhou D., Zahradník J., Supasa P., Liu C., et al. SARS–CoV-2 Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. *Cell* 2022.
3. Dimeglio C., Migueres M., Mansuy J.M., Saivin S., Miedougé M., Chapuy-Regaud S., et al. Antibody titers and breakthrough infections with Omicron SARS–CoV-2. *J Infect* 2022 Feb 3.
4. Cele S., Jackson L., Khoury D.S., Khan K., Moyo-Gwete T., Tegally H., et al. SARS–CoV-2 Omicron has extensive but incomplete escape of Pfizer BNT162b2 elicited neutralization and requires ACE2 for infection. *medRxiv* 2021.
5. Roessler A., Riepler L., Bante D., von Laer D., Kimpel J.. SARS-CoV-2 B.1.1.529 variant (Omicron) evades neutralization by sera from vaccinated and convalescent individuals. *medRxiv* 2021.
6. Carreno J.M., Alshammary H., Tcheou J., Singh G., Raskin A., Kawabata H., et al. Activity of convalescent and vaccine serum against SARS-CoV-2 Omicron. *Nature* 2021:1–8.
7. Pulliam J.R., van Schalkwyk C., Govender N., von Gottberg A., Cohen C., Groome M.J., et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. *MedRxiv* 2021 Jan 1.
8. Andrews N., Stowe J., Kirsebom F., Toffa S., Rickeard T., Gallagher E., et al. Effectiveness of COVID-19 vaccines against the Omicron (B.1.1.529) variant of concern. *NEJM* 2022.
9. Ella R., Reddy S., Jogdand H., Sarangi V., Ganneru B., Prasad S., et al. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. *Lancet Infect Dis* 2021 Mar 8.
10. Deshpande G.R., Sapkal G.N., Tilekar B.N., Yadav P.D., Gurav Y., Gaikwad S., et al. Neutralizing antibody responses to SARS-CoV-2 in COVID-19 patients. *Indian J Med Res* 2020;**152**(1–2):82.
11. Vadrevu K.M., Ganneru B., Reddy S., Jogdand H., Raju D., Sapkal G., et al. Persistent immunity and impact of third dose of an inactivated COVID-19 vaccine against 2 emerging variants. *medRxiv* 2022.

Pragya D. Yadav[1], Gajanan N. Sapkal[1], Rima R. Sahay[1], Deepak Y. Patil, Gururaj R. Deshpande, Rajlaxmi Jain, Dimpal A. Nyayanit, Anita M. Shete, Annasaheb Suryawanshi
Indian Council of Medical Research-National Institute of Virology, Pune 411021, India

Ashok Nandapurkar
District Hospital, Aundh Pimpri-Chinchwad, Pune 411027, India

Yogesh K. Gurav, Priya Abraham
Indian Council of Medical Research-National Institute of Virology, Pune 411021, India

*Corresponding author at: Indian Council of Medical Research-National Institute of Virology, Sus Road, Pashan, Pune 411021, India.
E-mail address:* helloppragya22@gmail.com (P.D. Yadav)

[1] These authors contributed equally to this work.
Accepted 16 March 2022
Available online 19 March 2022

Influence of vaccination and prior immunity on the dynamics of Omicron BA.1 and BA.2 sub-variants

Dear Editor,

The B.1.1.529 SARS-CoV-2 lineage, named Omicron, was recently divided into three lineages (BA.1, BA.2 and BA.3). BA.1 and BA.2 are much more dominant than BA.3. BA.1 could cause breakthrough infections in highly immune populations [1]. Preliminary studies indicate that BA.2 can readily overcome the immunity provided by vaccination and/or infection with an earlier variant. We used data for the French city of Toulouse to evaluate the impact of the proportions of the BA.1 and BA.2 variants in positive-testing samples and the impact of vaccination on SARS-CoV-2 proliferation.

Our discretized version of a susceptible infectious and recovered (SIR)-type model has been shown well suited to studies on the spread of SARS-CoV-2 [2,3].

The model includes a diffusion/transmission coefficient R_0 that varies with the likelihood of contagion, and two reduction coefficients $\hat{c}$ and $\hat{q}$ that describe the impact of public health measures on virus transmission. Values of $\hat{c}$ and $\hat{q}$ were estimated in previous studies [2,3]. It also takes into account a parameter p_1 describing the proportion of the BA_1 variant in urban Toulouse, and a similar parameter p_2 for the BA_2 variant; there are also vaccine/immunity efficacy coefficients $\hat{i}_1$ and $\hat{i}_2$ indicating the weight of each variant in the number of new infections. The model predicts how the SARS-CoV-2 virus would have evolved and projects the daily percentage of new positive cases (see Supplementary materials S1).

We set $R_0(D) = 5.9$ for the Delta variant at its peak, based on WHO international data [4]. First elements showed that $R_0(BA_1)$ could reach 10 [5]. We estimated the initial model settings using data collected by Toulouse Virology Laboratory (Table 1).

The nucleic acids in nasopharyngeal swab samples collected at Toulouse University Hospital were extracted with the MGI extraction system and tested using the ThermoFisher TaqPath RT-PCR assay. All positive nasopharyngeal samples with a cycle threshold (Ct) value below 30 (N gene) were tested using the ID solutions screening system for mutations K417N, L452R and E484K. The Omicron BA.1 variant was identified based on TaqPath S gene target failure (SGTF) or S gene target late (SGTL) detection profiles plus the presence of the K417N mutation. The Omicron BA.2 variant was identified based on TaqPath non-SGTF/SGTL detection profiles plus the presence of the K417N mutation. The results for a subset of 1080 positive specimens tested with our VOC screening strategy and those obtained by genome sequencing using Pacific Biosciences Technology [6] were 100% concordant.

In addition to barrier measures, the local authorities decided to make mask wearing compulsory in the Toulouse area from November 24, 2021 (week 47) as this protective measure had been shown to reduce SARS-CoV-2 circulation among Toulouse inhabitants by 27% [3]. The BA.1 variant was the major variant ($> 90\%$) in the Toulouse area from January 1 to February 1 (weeks 1–4, Table 1). The parameters of its R_0 (see Methods; $R_0 = 10$) predicted that the percentage of new positive cases during this period would be 20.9% if 69.8% of the fully vaccinated population was as protected against BA.1 infection as they were against Delta: $<88\%$ [7] (Fig. 1

Table 1.
Model initial parameters.

		Weeks 2021																Weeks 2022						
		36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	1	2	3	4	5	6	7
Variant (%)	Delta	98.5	91.8	87.5	100	100	100	100	100	98.6	100	86.9	97.4	99.5	99.4	90.1	45.1	17.9	2.3	1.2	0.2	0.2	0.1	0	0
	BA.1	0	0	0	0	0	0	0	0	0	0	0	0	0.2	0.6	9.9	54.7	82.1	97.4	97	97.9	94.3	87.7	77.3	60.1
	BA.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.3	1.8	1.9	5.5	12.3	22.7	39.9
	Non Delta/ Non Omicron	1.5	8.2	12.5	0	0	0	0	0	1.4	0	13.1	2.6	0.2	0	0	0.2	0	0	0	0	0	0	0	0
Tests	Total number	1535	1361	1107	947	877	1025	1250	1556	1573	1709	1584	2565	3526	4292	4778	5506	6664	8998	7622	6049	3794	2368	1619	1921
	Positive (%)	5.9%	4.8%	4.1%	4.8%	2.0%	2.9%	3.4%	3.4%	5.3%	5.1%	7.1%	14.5%	16.7%	16.5%	14.3%	21.8%	33.1%	38.4%	42.1%	47.5%	41.6%	32.1%	27.7%	19.6%

A). The vaccine/immunity efficacy coefficient $\widehat{i_1}$ was about 23% after correction based on the observed data (Equation 1, Supplementary S1) (Fig. 1C). The proportion, p_2, of BA.2 variant increased from 5.5% at the end of January 2022 to 39.9% on February 21, 2022 (Table 1). The rate of positive RT-PCR tests should have doubled between February 1 and 21 if the vaccine/immunity efficacy for BA.2 is close to 23%, the same as that for BA.1 (Fig. 1B). However the percentage of positive RT-PCR tests decreased from 44% on February 4 to 19.6% on February 21 (weeks 5–7, Table 1). Correcting the model parameters to bring the predicted data in line with the observed data (Equation 2, Supplementary S1) gave a vaccine/immunity coefficient of 92.8% (Fig. 1C).

The rapid proliferation of BA.1 was different from that of the Delta variant, which became the dominant strain in the summer months, when health measures were relaxed and vaccination coverage lower. This indicates the great capacity of the BA.1 variant to evade antibodies produced in response to infection with an earlier strain of virus and antibodies generated by vaccination. We showed that the Omicron BA.1 variant was more contagious than the Delta variant because of vaccine escape resulting from the spike mutations that alter virus neutralization rather than because of greater virus shedding in the nasopharynx [8]. The "BA.1 wave" seems to induce significant natural immunity against the BA.2 variant. The slowdown in the spread of the SARS-CoV-2 virus could also be due to the vaccine booster campaign that started at the beginning of January 2022 when about 76% of those who were primo-vaccinated had been given 3 doses by mid-February 2022. We could not distinguish between the influence of a booster vaccination and the immunity conferred by a BA.1 infection on the spread of BA.2, because the two events were confounded. These results agree with those showing that BA.2 and BA.1 are similarly able to resist the neutralizing antibodies of people who had been vaccinated or previously infected [9]. A slight difference in neutralizing capacity against Omicron BA.1 and BA.2 of natural or vaccine antibodies could explain a growth advantage of BA.2 over BA.1. In a Danish study, unvaccinated individuals, like vaccinated individuals, were more susceptible to BA.2 infection than to BA.1 infection indicating that viral properties other than immune evasion could also play a role in the growth advantage of BA.2 [10].

We conclude that the increase in the proportion of BA.2 has not led to a faster spread of the virus; which seems to indicate that the immunity induced by BA.1 infection is effective against BA.2. Further studies are needed to determine the contributions of the vaccine booster and a BA.1 infection to protection against BA.2.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgement

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.03.014.

References

1. Kuhlmann C., Mayer C.K., Claassen M., Maponga T., Burgers W.A., Keeton R., Riou C., Sutherland A.D., Suliman T., Shaw M.L., Preiser W.. Breakthrough infections with SARS-CoV-2 omicron despite mRNA vaccine booster dose. *Lancet* 2022 Feb 12;**399**(10325):625–6. doi:10.1016/S0140-6736(22)00090-3.
2. Dimeglio C., Milhes M., Loubes J.M., Ranger N., Mansuy J.M., Trémeaux P., Jeanne N., Latour J., Nicot F., Donnadieu C., Izopet J. Influence of SARS-CoV-2 variant B.1.1.7, vaccination, and public health measures on the spread of SARS-CoV-2. *Viruses* 2021 May 12;**13**(5):898. doi:10.3390/v13050898.
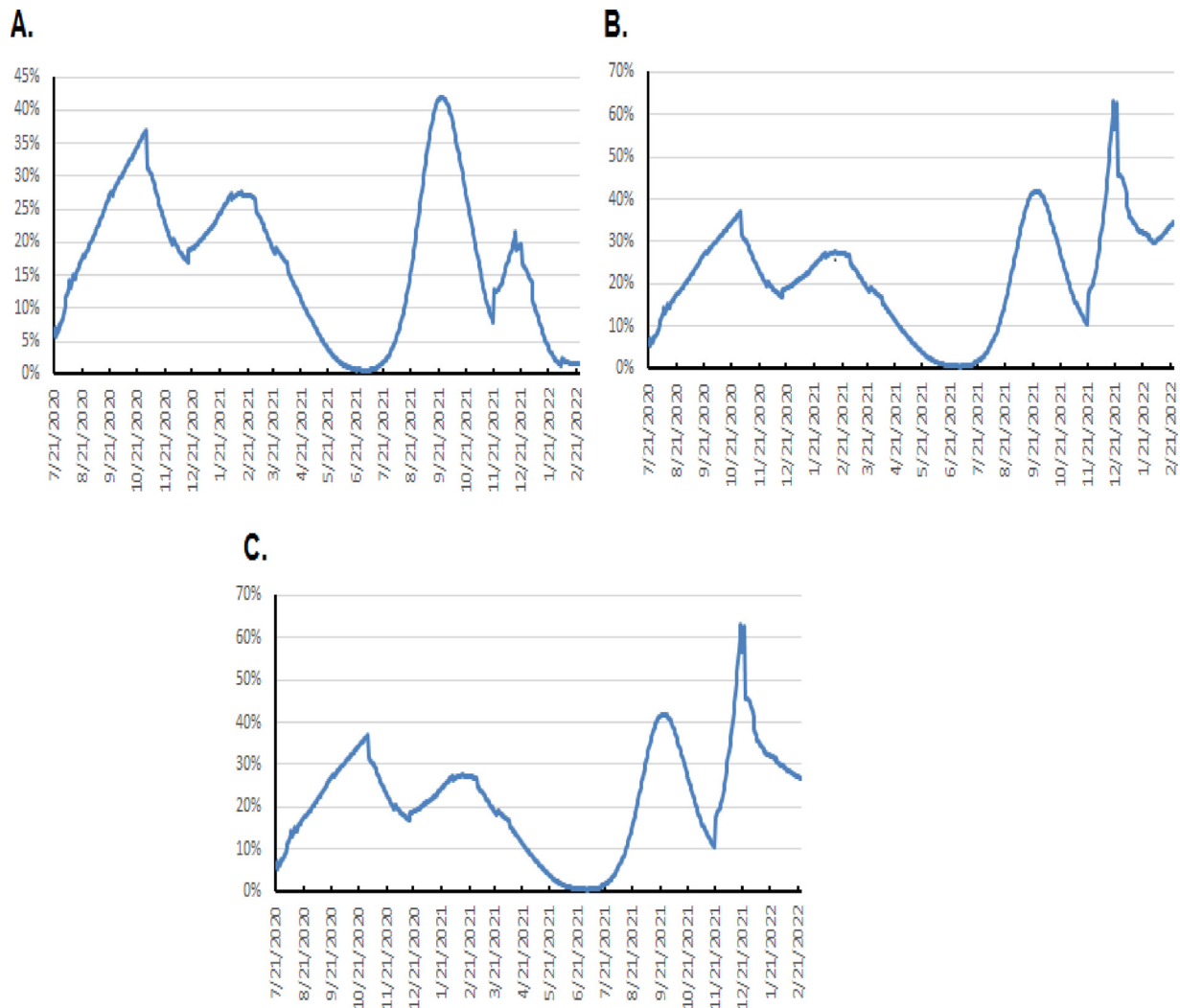
Fig. 1.. Daily dynamics of SARS-CoV-2 infection, July 21, 2020 to February 21, 2022.
A. Assuming that the Omicron BA.1 sub-variant is as sensitive as Delta to vaccination/previous immunity.
B. Assuming that the Omicron BA.2 sub-variant is as sensitive as BA.1 to vaccination/previous immunity.
C. Real daily dynamics of SARS-CoV-2 infection, July 21, 2020 to February 21, 2022.

3. Dimeglio C., Loubes J.M., Mansuy J.M., Izopet J.. Quantifying the impact of public health protection measures on the spread of SARS-CoV-2. *J Infect* 2020.
4. Campbell F., Archer B., Laurenson-Schafer H., Jinnai Y., Konings F., Batra N., Pavlin B., Vandemaele K., Van Kerkhove M.D., Jombart T., Morgan O., le Polain de Waroux O.. Increased transmissibility and global spread of SARS-CoV-2 variants of concern as at June 2021. *Euro Surveill* 2021 Jun;**26**(24):2100509. doi:10.2807/1560-7917.ES.2021.26.24.2100509.
5. Burki T.K.. Omicron variant and booster COVID-19 vaccines. *Lancet Respir Med* 2022 Feb;**10**(2):e17. doi:10.1016/S2213-2600(21)00559-2.
6. Lhomme S., Latour J., Jeanne N., Trémeaux P., Ranger N., Migueres M., Salin G., Donnadieu C., Izopet J. Prediction of SARS-CoV-2 variant lineages using the S1-encoding region sequence obtained by PacBio Single-Molecule Real-Time Sequencing. *Viruses* 2021 Dec 18;**13**(12):2544. doi:10.3390/v13122544.
7. Torgovnick J.. Effectiveness of Covid-19 vaccines against the B.1.617.2 (delta) variant. *N Engl J Med* 2021 Dec 16;**385**(25):e92. doi:10.1056/NEJMc2113090.
8. Migueres M., Dimeglio C., Trémeaux P., Abravanel F., Raymond S., Lhomme S., Mansuy J.M., Izopet J.. Influence of immune escape and nasopharyngeal virus load on the spread of SARS-CoV-2 Omicron variant. *J Infect* 2022 Feb 7(22):00054–8 S0163-4453. doi:10.1016/j.jinf.2022.01.036.
9. Iketani S., Liu L., Guo Y., Liu L., Huang Y., Wang M., Iketani S., Liu L., Guo Y., Liu L., Chan J.F., Huang Y., Wang M., Luo Y., Yu J., Chu H., Chik K.K., Yuen T.T., Yin M.T., Sobieszczyk M.E., Huang Y., Yuen K.Y., Wang H.H., Sheng Z., Ho D.D.. Antibody evasion properties of SARS-CoV-2 Omicron sublineages. *Nature* 2022 Mar 3. doi:10.1038/s41586-022-04594-4.
10. Lyngse F.P., Kirkeby C.T., Denwood M., Christiansen L.E., Mølbak K., Møller C.H., et al. Transmission of SARS-CoV-2 Omicron VOC subvariants BA.1 and BA.2: evidence from Danish Households. Preprint at. *medRXiv* 2022 01.28.22270044; doi: https://doi.org/. doi:10.1101/2022.01.28.22270044.

Chloé Dimeglio*
INSERM UMR1291 – CNRS UMR5051, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), 31300 Toulouse, France
CHU Toulouse, Hôpital Purpan, Virology Laboratory, 31300 France

Jean-Michel Loubes
Université de Toulouse, Institut de Mathématiques de Toulouse, Toulouse 31400, France

Marion Migueres, Karine Sauné, Pauline Trémeaux, Sébastien Lhomme
INSERM UMR1291 – CNRS UMR5051, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), 31300 Toulouse, France
CHU Toulouse, Hôpital Purpan, Virology Laboratory, 31300 France

Noémie Ranger, Justine Latour, Jean-Michel Mansuy
CHU Toulouse, Hôpital Purpan, Virology Laboratory, 31300 France

Jacques Izopet
INSERM UMR1291 – CNRS UMR5051, Toulouse Institute for Infectious and Inflammatory Diseases (INFINITy), 31300 Toulouse, France
CHU Toulouse, Hôpital Purpan, Virology Laboratory, 31300 France

*Corresponding author.
E-mail address:* dimeglio.c@chu-toulouse.fr (C. Dimeglio)

A pilot observational study of CSF vancomycin therapeutic drug monitoring during the treatment of nosocomial ventriculitis

Dear Editor,

We read with interest the report by Shokouhi and Darazam who reported the prospective observation of adequate trough vancomycin cerebrospinal fluid (CSF) concentration in patients with community acquired bacterial meningitis undergoing lumbar puncture examination.[1] In this study, adult patients with bacterial meningitis receiving empiric vancomycin (15 mg/kg loading dose with 30 mg/kg maintenance) with ceftriaxone (4 g / day) underwent lumbar puncture examination with concomitant blood sampling prior to the fourth maintenance dose of vancomycin and during days 8–10 of treatment. Trough vancomycin levels correlated with serum concentrations with little difference between trough samples one and two.[1]

Globally, ventriculitis is a major complication of neurosurgical procedures.[2] Penetration of vancomycin across the blood brain barrier has been cited as leading to suboptimal treatment in some individuals with this condition.[3] We aimed to observe cerebrospinal fluid (CSF) vancomycin concentrations in patients with suspected nosocomial venticulitis and investigate whether the application of CSF therapeutic drug monitoring (TDM) is warranted.

This study was registered as a local service evaluation (registration number = 576). CSF samples collected for routine clinical practice in patients with suspected or confirmed ventriculitis if they were pleocytic (white cell count $>= 5/mm^3$) or culture-positive and the patient was receiving a continuous intravenous (IV) vancomycin infusion at the time of sampling. Demographic, surgical, biochemical, microbiological, and concomitant serum vancomycin TDM data were collected for individuals included in the study. Total vancomycin concentrations were determined in CSF by ThermoFisher QMS (PETINIA immunoassay, lower limit-of-quantification (LLOQ) = 2.0 mg/L) on the Indiko Plus platform and in serum by Abbott Alinity (PETINIA, LLOQ = 1.4 mg/L). The number of patients with a quantifiable CSF vancomycin concentration, and those achieving >4 mg/L (EUCAST Coagulase-negative *Staphylococci* breakpoint) were assessed.

In total, nine individuals were included with 13 CSF samples available for analysis. Six (66%) were male and median (range) age in years was 55 (48–80). Most patients suffered subarachnoid haemorrhage (7/9; 78%), and neurosurgical management was usually external ventricular drainage (8/9; 89%). Vancomycin was given for suspected nosocomial ventriculitis by continuous intravenous (IV) infusion titrated to a target serum concentration 20–25 mg/L (local guidelines) in all cases. Concurrent IV meropenem (8/9; 89%) and/or other gram-negative antimicrobials (2/9; 22%) were also prescribed. Three out of nine individuals (33%) had culture positive CSF (*Staphylococcus epidermidis, Cutibacterium acnes, Klebsiella pneumoniae* with *Morganella morganii*).

CSF vancomycin was quantifiable using our assay (>=2 mg/L) in 3/9 (33%) individuals (6/13 samples, range = 2.1–8.3 mg/L). At the time of CSF sampling, serum vancomycin concentration was >20 mg/L (i.e. considered therapeutic) for 10/13 (77%) samples. For the remaining 3/13 (23%) samples, serum vancomycin concentration was >15 mg/L. An inverse relationship was observed between serum vancomycin concentration and concurrent CSF value, where detectable (R^2=0.362). No adjustments to therapy were made based on the CSF TDM data during the treatment period. There was apparent serum C-reactive protein response to treatment in 6/9 (66%) cases and CSF WCC response in 5/5 (100%) cases where assessable. An exploratory analysis did not show significant association between target attainment and any demographic, clinical, treatment-response, or sample-related variables. Table 1 summarises the clinical and laboratory findings amongst included individuals.

Comparing our observations to prior published data on vancomycin concentration in CSF; a difference is observed between bacterial meningitis and nosocomial ventriculitis.[1] Other studies report vancomycin CSF penetration at around 20% of the serum concentration.[4] A number of potential factors may be responsible for the varition in observations. Sampling from the ventricle close to the choroid plexus via an EVD may not represent the true CSF concentration due to the requirment for mixing and diffusion to occur. Therefore, sampling in the lumbar region may lead to different observations. Discrepancies of CSF sampling between ventricular and lumbar regions is a well known phenomenon. For the treatment of ventriculitis as opposed to bacterial meningitis, sampling from the EVD may be a more accurate representation of target site concentration attainment compared to that of lumbar puncture examination, which is distant from the target site.[5] Several studies of nosocomial ventriculitis have demonstrated variable CSF vancomycin concentrations when sampled via EVD.[6,7] Vancomycin TDM in CSF has been used to improve target concentration[8], often through the addition of intra-thecal vancomycin, with a paucity of high level outcome data to demonstrate benefit.[9]

The pathophysiology of bacterial meningitis and nosocomial ventriculitis may differ, with a number of suspected ventriculitis cases being non-bacterial in aeitiology. For example, chemical meningitis is a common observation following neurosurgey that can mimic bacterial infection. Whilst there may be a level of blood-brain-barrier disruption, in ventriculitis inflammation of meninges may be a local phenomenon. It remains unclear whether this potential difference in inflammation influences the CNS penetration of vancomycin and other antimicrobials, and how this may differ from bacterial meningitis.

Different analytical methods were used between studies with high-performance liquid chromotography mass spectrometry used by Shokouhi and Darazam[1] and immunoassays in our study. Despite the high LLOQ for the assays used in this study, we would expect to be able to detect clinically significant vancomycin concentration towards the breakpoint of common gram-positive causes of nosocomial meningitis.[10]

Finally, the approach to dosing of vancomycin used in our study differed to that used by Shokouhi and Darazam.[1] Evaluation of continuous versus intermittent vancomycin dosing in patients with nosocomial ventriculitis has suggested that continuous infusion is able to achieve and maintain higher CSF concentration when compared to intermittent dosing.[11] Therefore, it is unlikely that the use continuous infusion within our study has led to lower observed CSF concentrations.

This study highlights some of the remaining challenges and gaps in evidence surrounding the use of CSF TDM for the optimisation of vancomycin dosing in nosocomial meningitis. The importance of target site sampling is highlighted, with potential differences in concentration depending on the use of EVD versus lumbar puncture examination for sampling. Whilst data suggest that continuous infusion can achieve superior CSF pharmacokinetic profiles for vancomycin, there is little clinical outcome data compar-

Table 1

Clinical and laboratory findings of patients with suspected nosocomial ventriculitis undergoing vancomycin cerebrospinal fluid therapeutic drug monitoring.

		CSF vancomycin concentration			
		Not quantifiable	At least one sample quantified >=2 mg/l	At least one sample quantified >=4 mg/L	Total
Demographics					
Gender	Male	3	3	1	6
	Female	1	2	1	3
Median (range) age (years)		53 (48–59)	58 (50–80)	51 (50–52)	55 (48–80)
Laboratory					
CSF white cell count (/mm^3)	Median peak value (range)	92.5 (50–230)	72 (4–1750)	19 (4–42)	97 (4–1750)
	Ever >= 5	4	4	1	8
	Never >=5	0	1	1	1
Positive CSF / tissue culture	*S. epidermidis*	0	1	1	1
	C. acnes	1	0	0	1
	K. pneumoniae + M. morganii	0	1	1	1
	Culture negative	3	3	0	6
Infection treatment					
Median duration IV vancomycin in days (range)		6.5 (3–29)	10 (1–12)	7 (6–8)	8 (1–29)
Concurrent antimicrobials	Meropenem 2 g TDS	4	3	1	7
	Ceftriaxone 2 g BD then meropenem 2 g TDS	0	1	0	1
	Colistin + Fosfomycin	0	1	1	1
Treatment response					
CRP response (Day 5 <50% peak)	Yes	2	4	1	6
	No	2	1	1	3
WCC response day 5–15 <50% peak)	Yes	4	1	0	5
	No	0	0	0	0
	Not assessed (only one CSF)	0	4	2	4
TOTAL		**4**	**5**	**2**	**9**

Legend: CSF = cerebrospinal fluid, CRP = C-reactive protein, WCC = white cell count, *S.epidermidis* = *Staphylococcus epidermidis*, *C. acnes* = *Cutibacterium acnes*, *K. pneumoniae* = *Klebsiella pneumoniae*, *M. morganii* = *Morganella morganii*, TDS = three times daily, BD = two times daily.

ing approaches to dosing. Furthermore, if continuously low concentrations are observed within the ventricular system and this is deemed to be the most accurate representation of target site concentration; this may warrant the exploration of alternative dosing methods, such as intra-thecal dosing compared to continuous infusion, or the use of alternative gram-positive antimicrobials with higher and more consistent CSF penetration, for example oxazolidinones.[12] Future research should aim to use gold standard, HPLC methodology to determine both serum and CSF vancomycin concentrations and the influence of total and free drug should also be determined.

In conclusion, our real-world study performing vancomycin CSF TDM via EVDs in nosocomial ventriculitis demonstrated overall poor penetration of vancomycin into the CSF. Prospective clinical studies are required to define appropriate target site for sampling, consider best-practice for routes of vancomycin dosing, and demonstrate association with clinical outcomes. Whilst CSF TDM may provide an opportunity to optimise the treatment of nosocomial ventriculitis, further evidence is required to guide its appropriate use in clinical practice.

Notes

Contribution statement

PA, RW, AH and TMR conceived and designed the study. PA, RW, DBA, MG, MW and KW performed the study. ARN and SCB oversaw sample analysis. PA, RW and TMR performed data analysis. PA and TMR drafted the manuscript with all authors having significant contribution to revisions and finalisations for submission.

Patient consent statement

Ethical approval was not required for this study. This was reviewed and approved by local audit and quality improvement services (Ref no:567).

Declaration of Competing Interest

TMR has received honoraria from Sandoz (2020), bioMerieux (2021–2022), Roche Diagnostics Ltd (2021). MG has received honoraria from Sandoz (2020), Pfizer (2021). All other authors have no conflicts of interest to declare.

Care funded Centre for Antimicrobial Optimisation (CAMO), Imperial College London, provides state-of-the-art research facilities and consolidates multidisciplinary academic excellence, clinical expertise, Imperial's NIHR/Wellcome funded Clinical Research Facility (CRF), and partnerships with the NHS to support and deliver innovative research on antimicrobial optimisation and precision prescribing. (iii) the NIHR/Wellcome Trust Imperial Clinical Research Facility, Imperial College London, UK, where this study took place.; The views expressed in this publication are those of the authors and not necessarily those of the NHS, the National Institute for Health Research, or the UK Department of Health.

References

1. Shervin Shokouhi, Darazam Ilad Alavi. Determination of vancomycin trough level in serum and cerebrospinal fluid of patients with acute community-acquired meningitis: a prospective study. *J Infect* 2014;**69**(5):424–9. doi:10.1016/j.jinf.2014.06.010.
2. Walti Laura N., Anna Conen, Julia Coward, Jost Gregory F., Andrej Trampuz. Characteristics of infections associated with external ventricular drains of cerebrospinal fluid. *J Infect* 2013;**66**(5):424–31. doi:10.1016/j.jinf.2012.12.010.
3. Ashley Marx, Lindsay Daniels, Miller Melissa B., Weber David J.. Vancomycin minimum inhibitory concentration is not a substitute for clinical judgment: response to healthcare-associated ventriculitis and meningitis. *Clin Infect Dis* 2017:1428–9. doi:10.1093/cid/cix552.
4. Roland Nau, Fritz Sörgel, Helmut Eiffert. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. *Clin Microbiol Rev* 2010;**23**(4):858–83. doi:10.1128/CMR.00007-10.
5. Khan Sadid F., Thornton Macauley, Tong Steven Y.C., Ouli Xie, Carly Hughes, Hall Nicholas D.P., et al. When ventricular cerebrospinal fluid assessment misleads: basal meningitis and the importance of lumbar puncture sampling. *Open Forum Infect Dis* 2019;**6**(7). doi:10.1093/ofid/ofz324.
6. Ute Blassmann, Roehr Anka C., Frey Otto R., Cornelia Vetter-Kerkhoff, Niklas Thon, William Hope, et al. Cerebrospinal fluid penetration of meropenem in neurocritical care patients with proven or suspected ventriculitis: a prospective observational study. *Crit Care* 2016;**20**(1):1–9. doi:10.1186/s13054-016-1523-y.
7. Masayuki Ishikawa, Shingo Yamazaki, Takaaki Suzuki, Masashi Uchida, Yasuo Iwadate, Itsuko Ishii. Correlation between vancomycin penetration into cerebrospinal fluid and protein concentration in cerebrospinal fluid/serum albumin ratio. *J Infect Chemother* 2019;**25**(2):124–8. doi:10.1016/j.jiac.2018.10.013.
8. Meritxell Pujal, Dolors Soy, Carles Codina, Josep Ribas. Are higher vancomycin doses needed in venticle-external shunted patients? *Pharm World Sci* 2006;**28**(4):215–21. doi:10.1007/s11096-006-9037-3.
9. Ng Karen, Mabasa Vincent H., Ivy Chow, Ensom Mary H.H.. Systematic review of efficacy, pharmacokinetics, and administration of intraventricular vancomycin in adults. *Neurocrit Care* 2014;**20**(1):158–71. doi:10.1007/s12028-012-9784-z.
10. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. 2016.
11. Morteza Taheri, Simin Dadashzadeh, Shervin Shokouhi, Kaveh Ebrahimzadeh, Masoumeh Sadeghi, Zahra Sahraei. Administration of vancomycin at high doses in patients with post neurosurgical meningitis: a comprehensive comparison between continuous infusion and intermittent infusion. *Iran J Pharm Res* 2018;**17**(Special Issue 2):195–205. doi:10.22037/ijpr.2018.2381.
12. Luque S., Grau S., Alvarez-Lerma F., Ferrández O., Campillo N., Horcajada J.P., et al. Plasma and cerebrospinal fluid concentrations of linezolid in neurosurgical critically ill patients with proven or suspected central nervous system infections. *Int J Antimicrob Agents* 2014;**44**(5):409–15. doi:10.1016/j.ijantimicag.2014.07.001.

Paul Arkell, Richard Wilson
National Institute for Health Research Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom
Centre for Antimicrobial Optimisation, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom

David B Antcliffe
Centre for Antimicrobial Optimisation, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom

Imperial College Healthcare NHS Trust, Dept of Anaesthesia and Critical Care, St Mary's Hospital, Praed Street, London W2 1NY United Kingdom
Division of Anaesthesia, Pain and Critical Care Medicine, South Kensington Campus, Imperial College London SW7 2AZ, United Kingdom

Mark Gilchrist
National Institute for Health Research Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom
Centre for Antimicrobial Optimisation, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom
Imperial College Healthcare NHS Trust, Dept of Pharmacy, St Mary's Hospital, Praed Street, London W2 1NY, United Kingdom

Alan R Noel
Antimicrobial Reference Laboratory, North Bristol NHS Trust, Southmead Hospital, Level 2, Phase 1, Pathology Sciences Building, Bristol BS10 5NB, United Kingdom

Mark Wilson
Imperial College Healthcare NHS Trust, Dept of Anaesthesia and Critical Care, St Mary's Hospital, Praed Street, London W2 1NY United Kingdom
Imperial College Healthcare NHS Trust, Dept of Neurosurgery, St Mary's Hospital, Praed Street, London W2 1NY, United Kingdom

Sophie C Barnes
North West London Pathology (hosted by Imperial College Healthcare NHS Trust), Charing Cross Hospital, Fulham Palace Rd, London W6 8RF, United Kingdom

Killian Watkins
Centre for Antimicrobial Optimisation, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom

Alison Holmes*, Timothy M Rawson
National Institute for Health Research Health Protection Research Unit in Healthcare Associated Infections and Antimicrobial Resistance, Imperial College London, Hammersmith Campus, Du Cane Road, London W12 0NN, United Kingdom
Centre for Antimicrobial Optimisation, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom

Corresponding authors at: Health Protection Research Unit in Healthcare Associated Infections & Antimicrobial Resistance, Hammersmith Hospital, Du Cane Road, London W12 0NN, United Kingdom.
E-mail address: alison.holmes@imperial.ac.uk (A. Holmes)

The effect of canakinumab on clinical outcomes in patients with COVID-19: A meta-analysis

Dear Editor,

We read with interest the report in this journal by Zuo et al. regarding the effectiveness of bamlanivimab in patients with COVID-19.[1] On April 16, 2021, the emergency use authorization for bamlanivimab monotherapy was rescinded by the FDA due to the evolution of SARS-CoV-2 variants. Biologic medications have captured attention as powerful therapeutic options that are engineered from human-synthesized proteins and target specific steps along immune system pathways.

Canakinumab is a human monoclonal antibody that was developed for use in auto-inflammatory syndromes and targets IL-1β, an inflammatory cytokine interleukin that is well-known to be elevated in patients with COVID-19 and plays a crucial role in the initiation of cytokine storm.[2,3] The cytokine storms mediated by overproduction of proinflammatory cytokines have been observed in patients with COVID-19, which is associated with the mortality and severity of COVID-19. IL-1β is thus a potential therapeutic target that can be inhibited by canakinumab to control cytokine storms. Through this mechanism, use of canakinumab may have prognostic benefits regarding patient outcomes with COVID-19 infection and serve as an additional treatment modality. Thus, we aim to perform a meta-analysis in the literature to evaluate the relationship between canakinumab administration and patient outcomes following COVID-19 infection.

An electronic search was performed using the electronic platforms (PubMed, Embase, and Cochrane Library databases) from December 1 2019 to February 21th, 2022. No language or publication restrictions were applied. The following subject heading search terms and key words were searched: ("SARS-CoV-2″ or "COVID-19″ or "2019-nCoV" or "novel coronavirus" or "coronavirus disease 2019″) AND ("canakinumab" or "interleukin 1β antibody" or "ACZ885").

The inclusion criteria for this meta-analysis were as follows: (1) patients with confirmed COVID-19; (2) comparison was reported for clinical outcomes between canakinumab treatment (administered alone) and various control groups (placebo, standard care). Studies were excluded if they were (1) conference abstracts, case reports, editorials, non-clinical studies, and reviews; and (2) duplicated publications. We also extracted baseline information of first author's name, year of publication, study design, country of origin, number of participants, age, gender, dose of canakinumab used, outcomes (mortality, disease severity and change in anti-inflammatory factors).

Meta-analysis was conducted using Review Manager 5.2 (Cochrane Collaboration,

Oxford). We analyzed dichotomous data as a odds ratio (OR) with 95% confidence intervals (CIs) and continuous data as a standardized mean difference (SMD) with 95% CI. Heterogeneity was assessed using Cochran's Q test and the I^2 statistic. We performed sensitivity analyses by sequentially omitting one study each time to assess the stability of the results. A *p*-value below 0.05 is considered to be statistically significant. "PROSPERO (International Prospective Register of Systematic Reviews) database" registration was done with study number as CRD42022314781.

After literature search, a total of 6 studies[4–9] comprising of 1121 adult patients with COVID-19, including 379 in the canakinumab (administered alone) and 742 in the control group arm, were included in this meta-analysis. The study characteristics of the included studies are listed in Table 1. Four studies were from Italy. Two studies were RCTs, three studies were retrospective cohort and one studies was prospective case-control. All studies included mild to severe COVID-19 hospitalized patients. Canakinumab was intra-

venously or subcutaneously administered in the included studies. The eligible studies were published between 2020 and 2021 with different sample patient sizes that ranged from 20 to 520 patients with COVID-19.

The meta-analysis showed the overall mortality was lower in the canakinumab group compared to control group (OR=0.56, 95%CI: 0.35, 0.90, *P* = 0.02; I^2=0%) (Fig 1A). Moreover, canakinumab treatment were not associated with developing severe COVID-19 disease (OR=1.58, 95%CI: 0.73 to 3.41, *P* = 0.24; I^2=66%) (Fig.1B). Compared with control group, CRP levels were significantly decreased in the canakinumab group (SMD=−1.51, 95%CI: −2.33 to −0.96, *P* = 0.0003; I^2=64%) (Fig.1C). In addition, sensitivity analyses by excluding each study at a time did not materially change the overall results, indicating that our results were statistically stable.

In this study, we find that treatment with canakinumab is associated with improvements in overall mortality as well as decreased serum CRP levels, suggesting lower levels of acute inflammation.

The association between treatment with canakinumab and decreased mortality and serum CRP concentration is likely mediated through the mechanism of action of the monoclonal antibody. By inhibiting IL-1β, a key inflammatory response mediator in the cytokine storm triggered by infection by COVID-19, there is a decreased likelihood of systemic hyperinflammation, a well-known predictor of all-cause mortality.[10,11] C-reactive protein (CRP) is an inflammatory biomarker that serves many functions during episodes of acute inflammation, including promoting the secretion of pro-inflammatory cytokines, enhancing leukocyte function and activating the complement cascade.[12] Higher serum concentrations of acute phase reactants indicate more severe inflammatory episodes, allowing for CRP to be used as a marker of inflammation in COVID-19 infection and extrapolated to determine potency and response to canakinumab. Furthermore, by limiting the level of acute inflammation and propensity for the activation of a cytokine storm, canakinumab is thus potentially able to mitigate and even prevent immune-mediated tissue damage and organ dysfunction, both factors which improve overall mortality.[13,14] These restrictions of inflammatory activity are supported by the negative association of canakinumab and serum CRP levels in COVID-19 patients, an outcome that is well documented for other indications of canakinumab as well.[15,16] Altogether, canakinumab serves as a powerful anti-inflammatory therapeutic option that is able to specifically target and limit inflammatory mechanisms.

There are several limitations that should be noted with our study. There was a relatively small sample size for use in the meta-analysis with 6 included articles. There were other inflammatory factors investigated in the included studies, however, the sample size was too small for a meta-analysis to be conducted. However, despite these limitations, our study is the first meta-analysis to explore the association between treatment with canakinumab and patient outcomes following COVID-19 infection.

Additional research is needed to further probe this association and provide a more diverse and sufficiently large sample size to provide a better understanding of what circumstances provide optimal clinical utility.

In conclusion, treatment with canakinumab in patients with COVID-19 infection is associated with a mortality benefit and lower levels of acute inflammation. Additional studies are required to confirm these findings.

Declaration of Competing Interest

The authors declare that they have no competing interest

Table 1
Characteristics of included studies.

Study	Country	Study design	Sample size	Canakinumab	Control	Usage of canakinumab	Patients included	
				Age[a]	Male (%)	Age[a]	Male (%)	
Caricchio[4] 2021	Europe and America	RCT	454	59 (49–69)	135 (59%)	57 (50–68)	132 (58%)	Canakinumab 450 mg for body weight of 40-<60 kg, 600 mg for 60–80 kg, and 750 mg for>80 kg, intravenous	Patients hospitalized with severe COVID-19 without invasive mechanical ventilation
Cremer[5] 2021	America	RCT	45	NR	20 (68.96%)	68.2 (56.1, 83.3)	13 (81.3%)	Canakinumab 300 mg (n = 14), Canakinumab 600 mg (n = 15), intravenous	Hospitalized patients
Generali[6] 2021	Italy	Prospective case-control study	48	70 (29–89)	25 (76%)	69 (44–85)	13 (87%)	canakinumab (150 mg) was administered by subcutaneous injection on day 1 and on day 7	Hospitalized patients
Katia[7] 2021	Italy	Retrospective cohort	34	53 (48, 62)	15 (88.2%)	59 (50, 72)	13 (76.5%)	A subcutaneous single dose of canakinumab 300 mg	Hospitalized mild or severe non ICU patients
Mastroianni[8] 2021	Italy	Retrospective cohort	20	56 (46–82)	4 (50%)	NR	NR	150 mg BID for a body weight of 60–80 kg (or 2 mg/kg for participants weighing ≤40 kg), subcutaneous	Hospitalized patients
Potalivo[9] 2020	Italy	Retrospective cohort	520	NR	NR	NR	NR	NR	Hospitalized patients

[a] Age data presented as median (IQR) or mean (SD); ICU: intensive care units; RCT: randomized controlled trial; NR: not reported.
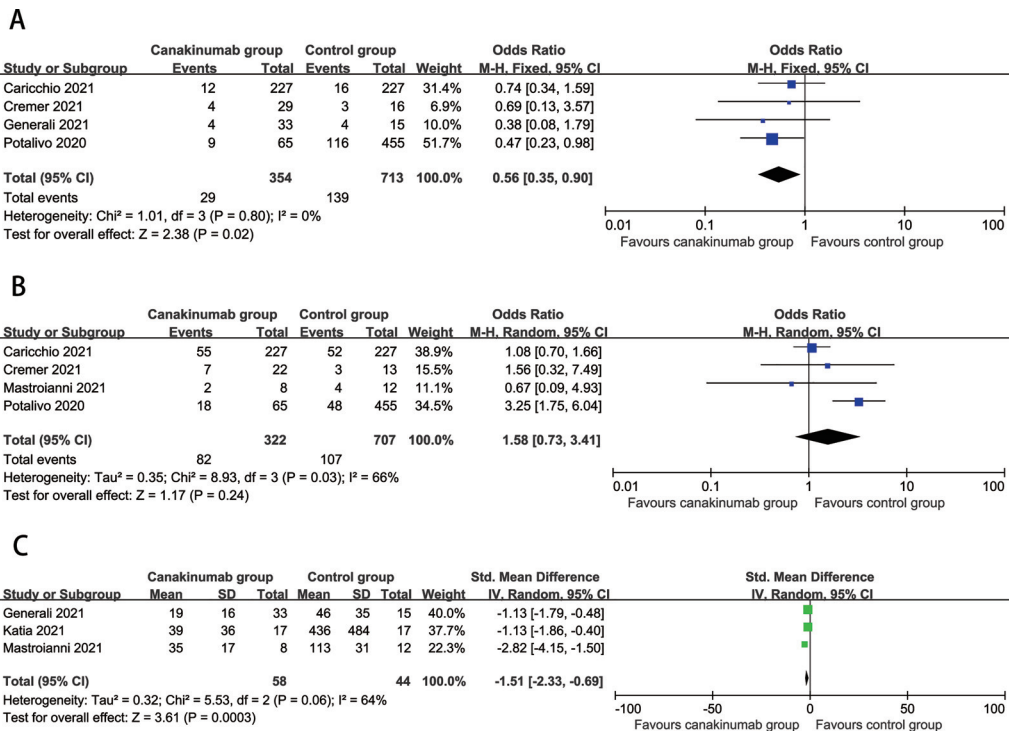
Fig. 1. A Association between canakinumab treatment and mortality, Fig. 1B. Association between canakinumab treatment and developing severe COVID-19, Fig. 1C Association between canakinumab treatment and CRP levels.

References

1. Zuo L., Ao G., Wang Y., Gao M., Qi X.. Bamlanivimab improves hospitalization and mortality rates in patients with COVID-19: a systematic review and meta–analysis. *J Infect* 2022;**84**(2):248–88.
2. Dhimolea E.. Canakinumab. *mAbs* 2010;**2**(1):3–13.
3. Lucas C., Wong P., Klein J., et al. Longitudinal analyses reveal immunological misfiring in severe COVID-19. *Nature* 2020;**584**(7821):463–9.
4. Caricchio R., Abbate A., Gordeev I., et al. Effect of canakinumab vs placebo on survival without invasive mechanical ventilation in patients hospitalized with severe COVID-19: a randomized clinical trial. *JAMA* 2021;**326**(3):230–9.
5. Cremer P.C., Sheng C.C., Sahoo D., et al. Double-blind randomized proof-of-concept trial of canakinumab in patients with COVID-19 associated cardiac injury and heightened inflammation. *Eur Heart J Open* 2021;**1**(1).
6. Generali D., Bosio G., Malberti F., et al. Canakinumab as treatment for COVID-19-related pneumonia: a prospective case-control study. *Int J Infect Dis* 2021;**104**:433–40.
7. Katia F., Myriam D.P., Ucciferri C., et al. Efficacy of canakinumab in mild or severe COVID-19 pneumonia. *Immun Inflamm Dis* 2021;**9**(2):399–405.
8. Mastroianni A., Greco S., Chidichimo L., et al. Early use of canakinumab to prevent mechanical ventilation in select COVID-19 patients: a retrospective, observational analysis. *Int J Immunopathol Pharmacol* 2021;**35**:20587384211059675.
9. Potalivo A., Montomoli J., Facondini F., et al. Sixty-day mortality among 520 Italian hospitalized COVID-19 patients according to the adopted ventilatory strategy in the context of an integrated multidisciplinary clinical organization: a population-based cohort study. *Clin Epidemiol* 2020;**12**:1421–31.
10. Proctor M.J., McMillan D.C., Horgan P.G., Fletcher C.D., Talwar D., Morrison D.S.. Systemic inflammation predicts all-cause mortality: a glasgow inflammation outcome study. *PLoS ONE* 2015;**10**(3):e0116206.
11. Davidson M., Menon S., Chaimani A., et al. Interleukin-1 blocking agents for treating COVID-19. *Cochrane Database Syst Rev* 2022;**1**(1):CD015308.
12. Del Giudice M., Gangestad S.W.. Rethinking IL-6 and CRP: why they are more than inflammatory biomarkers, and why it matters. *Brain Behav Immun* 2018;**70**:61–75.
13. Koritala T., Pattan V., Tirupathi R., et al. Infection risk with the use of interleukin inhibitors in hospitalized patients with COVID-19: a narrative review. *Infez Med* 2021;**29**(4):495–503.
14. Sheng C.C., Sahoo D., Dugar S., et al. Canakinumab to reduce deterioration of cardiac and respiratory function in SARS-CoV-2 associated myocardial injury with heightened inflammation (canakinumab in Covid-19 cardiac injury: the three C study). *Clin Cardiol* 2020;**43**(10):1055–63.
15. Church L.D., McDermott M.F.. Canakinumab, a fully-human mAb against IL-1beta for the potential treatment of inflammatory disorders. *Curr Opin Mol Ther* 2009;**11**(1):81–9.
16. Capodanno D., Angiolillo D.J.. Canakinumab for secondary prevention of atherosclerotic disease. *Expert Opin Biol Ther* 2018;**18**(2):215–20.

Guangyu Ao[1]
Department of Nephrology, Chengdu First People's Hospital, Chengdu, Sichuan, China

Yushu Wang[1]
Chengdu West China Clinical Research Center, Chengdu, Sichuan, China

Anthony Li[1]
School of Medicine, Queen's University, Kingston, Canada

Carolyn Tran
Schulich School of Medicine and Dentistry, Western University, London, Canada

Qing Yang*
Department of Cardiology, West China Hospital, Sichuan University, No.37 Guoxue Lane, Wuhou District, Chengdu, Sichuan 610044, China

*Corresponding author.
E-mail address:* qingyang@scu.edu.cn (Q. Yang)

[1] These author Contributed equally to this work.
Accepted 10 March 2022
Available online 14 March 2022

Table 1
The basic information of the included literature. Total: number of patient included in the study.

Study	PTSD (n)	Total (n)
Zaoxian Mei 2022[2]	23	144
Katharina Beck 2021[4]	10	115
Yumeng Ju 2021[5]	41	114
R. Mendez 2021[6]	45	179
Liqun Huang 2021[7]	64	574
Tarsitani L 2021[8]	12	115
De Lorenzo R 2020[9]	41	185

Meta-analysis of post-traumatic stress disorder and COVID-19 in patients discharged

Dear Editor,

In this journal, Thor Mertz Schou et al. reported that the severity of Coronavirus disease 2019 (COVID-19) has been highlighted as a risk factor for post-traumatic stress disorder (PTSD);[1] thus, suggesting that we should focus on long-term mental illness in COVID-19 patients after discharge. PTSD is a serious mental health condition that is triggered by a terrifying event.[2]

We found that many published studies investigated the occurrence and risk factors of PTSD among discharged COVID-19 patients. They may experience psychosocial difficulties while interacting with others after discharge although patients recover physically in the hospital.[3] It was showed that PTSD is a common psychological problem in patients after discharge, and they found that age, gender, and smoking history can increase the incidence of PTSD in Tianjin, China.[2]

PubMed, Web of Science, Embase, and Cochrane Library databases were extensively searched for all compliant studies published from January 1, 2020, to February 15, 2022. The following keywords were used for the search strategy: "COVID-19," "2019-nCoV," "SARS-CoV-2," "2019 novel coronavirus," "coronavirus disease 2019," "severe acute respiratory syndrome coronavirus 2,""Post-COVID-19," "post-traumatic stress disorder", and "PTSD". Reference lists of the included studies and relevant reviews were searched for additional studies. The inclusion criteria were as follows: (1) adult patients with COVID-19 confirmed by reverse transcriptase–polymerase chain reaction; (2) peer-reviewed original studies in English; (3) individual study populations with at least 100 cases; and (4) key available data of the included studies, four-table data, or effect [95% confidence interval (CI)] clearly stated. Case reports, repeated articles, review papers, and preprints were excluded. After searching the PubMed and other websites, seven eligible studies involving 1426 patients with COVID-19 were included in our meta-analysis. Seven studies reported PTSD symptoms of COVID-19 patients discharged from the hospital. The general information of included studies is summarized in Table 1.[2,4–9]

The results of seven studies listed in Fig. 1 showed the occurrence of PTSD in 18% of the patients (95% CI, 0.12–0.24; *P* < 0.01). It indicated that, out of every 100 patients, 18 experienced PTSD due to some reason after discharge. This suggested that these symptoms might indeed be the sequelae after recovery of COVID-19 survivors. The reasons for PTSD and other symptoms may be as follows: Infection with COVID-19 causes great psychological stress in patients. Factors, such as hospitalization, isolation, and restrictions on family member visits, may create a psychological burden on patients and their families.[4] Exposure to war, physical or sexual assault, disasters, and vehicle accidents are the most common causes of PTSD. In addition, experience of an acute or critical illness is also considered a potential risk factor for PTSD.[8] Meanwhile, it was showed PTSD during follow-up was associated with persistent respiratory symptoms, sleep difficulty, and a diagnosis
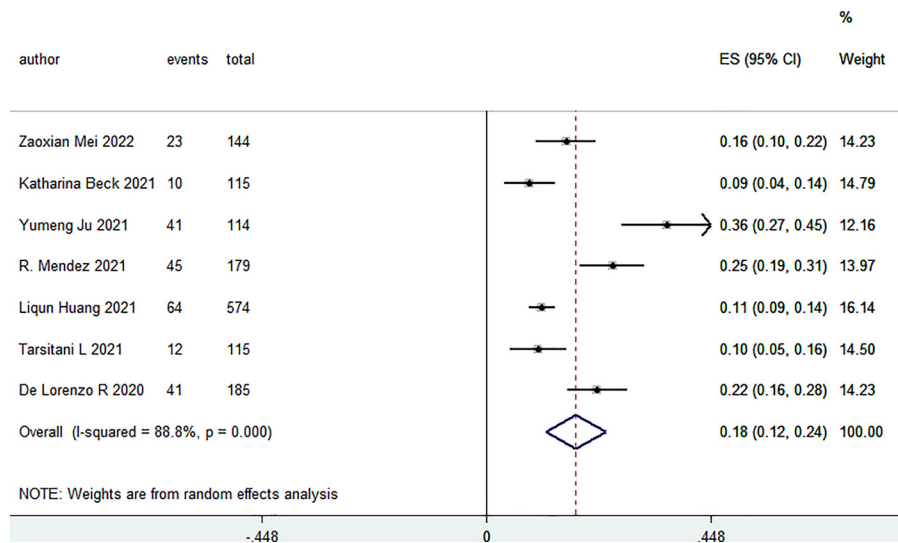
Fig 1. Forest plot of PTSD rates of among COVID-19 in patients discharged from hospital. ES: PTSD rates.

of anxiety. Respiratory manifestations are the main symptoms of COVID-19 patients. The association between PTSD and respiratory symptoms and sleep difficulty was bidirectional. Persistent physical symptoms can lead to mental illness, and conversely, increased mental distress can manifest as physical symptoms. In addition, many patients experience difficulty sleeping, and long-term lack of sleep can also lead to mental and psychological disorders. People with anxiety disorders were 15 times more likely to develop PTSD than people without anxiety disorders.[7] However, further research is needed to confirm the correlation.

The results showed that older age, female gender, current smoking status, and the number of involved pulmonary lobes (≥3) are risk factors for PTSD.[2] A research suggests that obesity predicted the development of PTSD and reasons for this unexpected association should be further investigated,[8] but De Lorenzo R did not observe any impact of body mass index (BMI) or other comorbidities on the development of PTSD.[9] Therefore, the association between obesity and PTSD needs to be investigated further. Lower age, female gender, and positive psychiatric history were significantly associated with the risk of developing PTSD after COVID-19.[9]

At present, our research has certain limitations. Most of the included studies assessed the mental health status of discharged patients through instruments, such as telephone interviews and questionnaires. Neuropsychological evaluation in patients was inconsistent across studies, and the research results were heterogeneous to a certain extent; hence, more research is needed in the future.

In conclusion, our study showed that the occurrence of PTSD was not rare among patients with confirmed COVID-19 infection. This also suggests that we should pay attention to the mental health and social interaction status of patients after discharge, which is very important for disease prognosis and healthy life of patients. PTSD can be treated with medication and psychological intervention.[8] Enhancing emotional support during hospitalization could help prevent PTSD in patients with COVID-19.[5] Therefore, clinicians need to pay more attention to the risk predictors of patients' mental health, and develop corresponding diagnosis and treatment measures in a timely manner during treatment.

Data sharing statement

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

Declaration of Competing Interest

The authors declare no competing interests.

References

1. Schou T.M., Joca S., Wegener G., Bay-Richter C. Psychiatric and neuropsychiatric sequelae of COVID-19 - a systematic review. *Brain Behav Immun* 2021;**97**:328–48 [published Online First: 2021/08/03]. doi:10.1016/j.bbi.2021.07.018.
2. Mei Z., Wu X., Zhang X., Zheng X., Li W., Fan R., et al. The occurrence and risk factors associated with post-traumatic stress disorder among discharged COVID-19 patients in Tianjin, China. *Brain Behav* 2022:e2492 [published Online First: 2022/01/23]. doi:10.1002/brb3.2492.
3. Park H.Y., Jung J., Park H.Y., Lee S.H., Kim E.S., Kim H.B., et al. Psychological consequences of survivors of COVID-19 pneumonia 1 month after discharge. *J Korean Med Sci* 2020;**35**(47):e409 [published Online First: 2020/12/09]. doi:10.3346/jkms.2020.35.e409.
4. Beck K., Vincent A., Becker C., Keller A., Cam H., Schaefert R., et al. Prevalence and factors associated with psychological burden in COVID-19 patients and their relatives: a prospective observational cohort study. *PLoS ONE* 2021;**16**(5):e0250590 [published Online First: 2021/05/06]. doi:10.1371/journal.pone.0250590.
5. Ju Y., Liu J., Ng R.M.K., Liu B., Wang M., Chen W., et al. Prevalence and predictors of post-traumatic stress disorder in patients with cured coronavirus disease 2019 (COVID-19) one month post-discharge. *Eur J Psychotraumatol* 2021;**12**(1):1915576 [published Online First: 2022/01/08]. doi:10.1080/20008198.2021.1915576.
6. Méndez R., Balanzá-Martínez V., Luperdi S.C., Estrada I., Latorre A., González-Jiménez P., et al. Short-term neuropsychiatric outcomes and quality of life in COVID-19 survivors. *J Intern Med* 2021;**290**(3):621–31 [published Online First: 2021/02/04]. doi:10.1111/joim.13262.
7. Huang L., Xu X., Zhang L., Zheng D., Liu Y., Feng B., et al. Post-traumatic stress disorder symptoms and quality of life of COVID-19 survivors at 6-month follow-up: a cross-sectional observational study. *Front Psychiatry* 2021;**12**:782478 [published Online First: 2022/01/28]. doi:10.3389/fpsyt.2021.782478.

8. Tarsitani L., Vassalini P., Koukopoulos A., Borrazzo C., Alessi F., Di Nicolantonio C., et al. Post-traumatic stress disorder among COVID-19 survivors at 3-month follow-up after hospital discharge. *J Gen Intern Med* 2021;**36**(6):1702–7 [published Online First: 2021/03/31]. doi:10.1007/s11606-021-06731-7.

9. De Lorenzo R., Conte C., Lanzani C., Benedetti F., Roveri L., Mazza M.G., et al. Residual clinical damage after COVID-19: a retrospective and prospective observational cohort study. *PLoS ONE* 2020;**15**(10):e0239570 [published Online First: 2020/10/15]. doi:10.1371/journal.pone.0239570.

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

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

Mengyun Zhou
Department of Molecular & Cellular Physiology, Shinshu University School of Medicine, 390-0803, Japan

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

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

Dong Yang*
Hangzhou Institute of Cardiovascular Diseases, Affiliated Hospital of Hangzhou Normal University, Hangzhou 310015, China

*Corresponding authors.
E-mail addresses:* h9450203@126.com (Z. Feng), xsyd0724@163.com (D. Yang)

[1] These authors contributed equally to this work.
Accepted 7 March 2022
Available online 10 March 2022

Acute cholecystitis associated with sepsis-induced ischemic cardiomyopathy

Dear Editor,

Various non-cardiac conditions, including cholecystitis, pancreatitis, and pneumonitis, have been reported to mimic ischemic heart disease manifesting both clinically and as electrocardiography (ECG) changes.[1,2] These conditions usually lead to nonspecific T-wave inversion or ST-segment elevation.[1,2] Acute cholecystitis complicating sepsis-induced ischemic cardiomyopathy is rare. Here, we report the case of a female patient with symptoms consistent with chest pain, fever, nausea, and vomiting. ECG showed unexpected changes with inferolateral ST-segment elevation (Fig. 1A) indicative of an inferolateral myocardial infarct.

A 74-year-old woman with underlying hypertension and rheumatic heart disease received medical drug therapy. She experienced chest pain, fever, epigastric discomfort, nausea, and vomiting. The patient complained of cold sweats and then suddenly collapsed. Emergency intubation and CPCR were performed with mechanical ventilator support, and inotropic drugs were administered for septic shock. Repeat ECG showed ST-T changes over leads II, III, aVF, V1, and V2, suggestive of acute inferolateral myocardial infarction. After discussion with the family, emergency coronary angiography was performed, which revealed 50% stenosis of the middle segment of the left anterior descending coronary artery. Laboratory data showed leukocytosis with neutrophil predominance and elevated cardiac enzyme and lactate levels. Abdominal computed tomography revealed a thickened gallbladder wall with multiple gallstones (Fig. 1B). Percutaneous transhepatic gallbladder drainage (PTGBD) was then performed.

After admission, the patient's condition stabilized with fluid resuscitation, vasopressors, antibiotics, and mechanical ventilator support. Ventilation was weaned off smoothly on the third hospital day. Bile culture was positive for *Enterococcus faecalis* and *Escherichia coli*. After antibiotic therapy for a few days, the patient's condition improved, and ECG showed recovery of ischemic changes. The PTGBD tube was removed, and the patient was discharged on the 10th hospital day. A delayed elective surgery was performed during an outpatient visit.

Discussion

Although chest pain with ST-segment elevation is often indicative of cardiac ischemia, it has also been reported in other conditions such as acute cholecystitis.[1–3] The differential diagnosis of ST-segment elevation includes four major conditions: ST-segment elevation myocardial infarction (STEMI), early repolarization, pericarditis, and ST-segment elevation secondary to an abnormality of the QRS complex (left bundle branch block, left ventricular hypertrophy, and preexcitation).[2–4] Other conditions include hyperkalemia, pulmonary embolism, and Brugada syndrome. The pathophysiology of cardiovascular impairment in sepsis-associated cardiac dysfunction has two phases.[1–5] Initially, an inflammatory disorder, with a high oxygen demand from the periphery, induces a hyperdynamic circulation phase with high frequency, high cardiac index, and normal or high output; patients present with warm, red extremities despite the frequent occurrence of low systolic pressure (warm shock).[2–4] As sepsis progresses, the scenario switches to "cold shock," with reduced cardiac output, which contributes to peripheral hypoperfusion, tissue hypoxemia, acidosis, and organ failure.[5] Acute inflammatory and ulcerative conditions involving the gallbladder or duodenum cause irritation and spasticity in the surrounding structures. This can create reflex stimuli through autonomic pathways to restrict or alter the coronary blood supply.[2,5] Postulated mechanisms of pulsatile diaphragmatic contraction include direct stimulation of the diaphragm by the inferior wall of the left ventricle or triggering of the left leaf of the diaphragm by the left phrenic nerve.[1,2,4,5] A unique pattern of apparent STEMI is critical illness with a very high risk of in-hospital death.[1,2,5] However, the exact pathophysiological mechanism underlying ECG changes remains unclear. Younger age, higher lactate level on admission, and history of heart failure are risk factors.[2–5] If initial diagnostic interventions do not yield expected results, alternative diagnoses, including intra-abdominal infections, should be considered.[5,6]

Conclusion

It is important for clinicians to be aware of other uncommon causes of ST-segment elevation of ECG. Furthermore, delay in the diagnosis of cholecystitis or intra-abdominal infections may lead to serious complications. When the initial diagnostic interventions for chest pain with ST-segment elevation do not yield the expected results (i.e., persisted fever, leukocytosis, high C-reactive protein), an alternative diagnosis, such as intra-abdominal illness, should be considered.
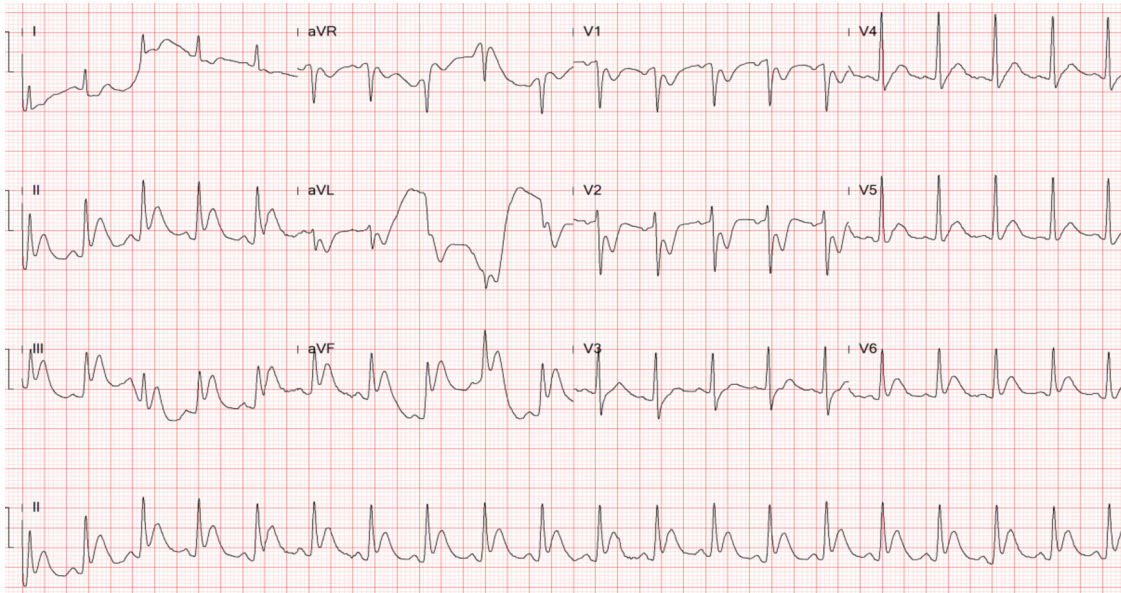
Fig. 1. (A) Electrocardiography showed acute myocardial infarction at the inferolateral wall with ST-segment elevation and change in leads II, III, aVF, V1, and V2. (B). Computed tomography of the abdomen revealed gallbladder wall thickening with pericholecystic fat stranding in favor of acute calculus cholecystitis.
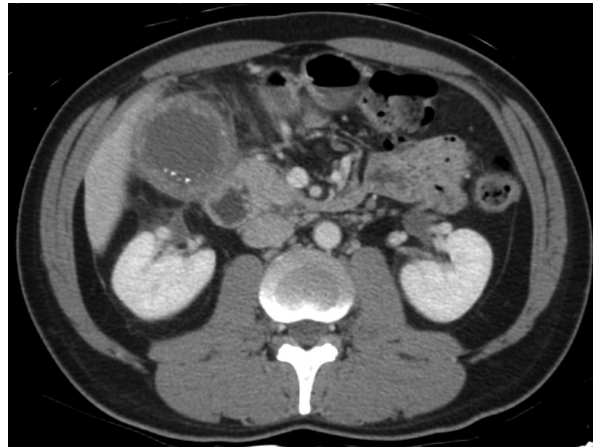
Fig. 1. Continued

Ethics approval

Ethics approval was not required for this study

Declaration of Competing Interest

The authors have no competing interests to declare

References

1. Leoni D., Rello J.. Cardiac arrest among patients with infections: causes, clinical practice and research implications. *Clin Microbiol Infect* 2017;**23**:e735–6.
2. Patel N., Ariyarathenam A., Davies W., Harris A.. Acute cholecystitis leading to ischemic ECG changes in a patient with no underlying cardiac disease. *JSLS* 2011;**15**(1):105–8. doi:10.4293/108680811X13022985131534.
3. Shih Y.N., Chen Y.T., Shih C.J., Ou S.M., Hsu Y.T., Chen R.C., et al. Association of weekend effect with early mortality in severe sepsis patients over time. *J Infect* 2017;**74**:345–51.
4. Shebl R.E., Hamouda M.S.. Outcome of community-acquired pneumonia with cardiac complications. *Egypt J Chest Dis Tuberc* 2015;**64**:633–8.
5. Habimana R., Choi I., Cho H.J., Kim D., Lee K., Jeong I.. Sepsis-induced cardiac dysfunction: a review of pathophysiology. *Acute Crit Care* 2020;**35**(2):57–66. doi:10.4266/acc.2020.00248.
6. Cao Y., Ma W.F., Liu Z.G., Pei Y.F., Zhu Y.M., Chen F.. Early predictive value of platelet function for clinical outcome in sepsis. *J Infect* 2022. doi:10.1016/j.jinf.2022.02.004.

Ke-Shiuan Ju
Department of Internal Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan

Ying-Shih Su
Department of Internal Medicine, Division of Infectious Diseases, Wan Fang Hospital, Taipei Medical University, Number 111, Section 3, Hsing Long Road, Taipei 116, Taiwan

Wen-Sen Lee*
Department of Internal Medicine, Division of Infectious Diseases, Wan Fang Hospital, Taipei Medical University, Number 111, Section 3, Hsing Long Road, Taipei 116, Taiwan
Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan

*Corresponding author at: Department of Internal Medicine, Division of Infectious Diseases, Wan Fang Hospital, Taipei Medical University, Number 111, Section 3, Hsing Long Road, Taipei 116, Taiwan
E-mail address: 89425@w.tmu.edu.tw (W.-S. Lee)

Available online 10 March 2022

https://doi.org/10.1016/j.jinf.2022.03.007

Co-administration of treatment for rifampicin-resistant TB and chronic HCV infection: A TBnet and ESGMYC study

Dear Editor,

We have been inspired by the report of excellent outcomes of concomitant treatment for human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infection.[1] With the present study, we aimed to increase evidence on the optimal management of another common and clinically relevant co-infection, that of HCV and tuberculosis. Chronic HCV-infection affects worldwide 71 million people.[2] Direct-acting antivirals (DAA) revolutionised HCV clinical management since their introduction. Tuberculosis is responsible of 1.4 estimated million deaths per year and multidrug-resistant/rifampicin-resistant tuberculosis (MDR/RR-TB) is a major public health issue worldwide.[3] Chronic HCV-infection is estimated at 7% among active tuberculosis patients,[4] reaching up to 30% in some settings among MDR/RR-TB,[5] and is associated with liver-related toxicity during anti-tuberculosis treatment. Currently, the World Health Organization (WHO) recommends treating all HCV patients above 12 years with pan-genotypic DAA.[6] Concomitant HCV and rifampicin-susceptible tuberculosis treatment is contraindicated due to drug-drug interactions. Conversely, no interactions are expected between second-line anti-tuberculosis drugs and DAA.[7] However, since only limited evidence is available on the co-administration of these drugs,[8,9] the WHO makes no specific recommendation.[6] The objective of our study was to assess safety and effectiveness of concomitant treatment of chronic HCV-infection and MDR/RR-TB.

We performed an observational cohort study across centres affiliated to the Tuberculosis Network European Trialsgroup (TBnet),[10] and the Study Group on Mycobacteria of the European Society of Clinical Microbiology and Infectious Diseases (ESGMYC). Consecutive patients with confirmed active MDR/RR-TB and HCV-infection who started DAA during or up to four weeks before MDR/RR-TB treatment since January 1, 2015, were included and followed-up until February 2021. Primary endpoints were sustained virologic response at 12 weeks and 24 weeks after finishing HCV-treatment, MDR/RR-TB treatment outcome, rates of Grade 3 or higher liver-related adverse events, and total rates of serious adverse events (SAE). SAEs were defined as events which were life-threatening or resulted in permanent disability, prolonged hospitalization, or death. De-identified data were collected retrospectively and collated in a secured database at the coordinating center. Continuous variables were described using median with interquartile range (IQR), and categorical variables with frequency and proportions. Statistical analysis was performed using STATA 12.0 (StataCorp, USA). Ethical approval was provided by the Institutional Review Board of Bligny Hospital (Briis-sous-Forges, France).

Overall, 23 patients were enrolled across six centres in France, Belarus, Italy, and Spain (Table 1). Two patients were described in a case report.[9] Twenty were men (87%), median age was 42 years (IQR 39–45). Nine patients were HIV-infected, with a median CD4 lymphocyte count of 85 cells/mm^3 (IQR 77–626): among four for whom HIV-treatment status was known, three were receiving antiretrovirals (tenofovir disoproxil fumarate/emtricitabine plus darunavir [$n = 2$] or raltegravir [$n = 1$]) and one was not treated. One patient had HBV/HDV chronic hepatitis. Median body mass index and serum albumin were 20 kg/m^2 (IQR 18–21) and 38 mg/dl (IQR 34–42). The predominant HCV genotype was 3 (40%, $N = 20$). Liver fibrosis was absent (F0) or mild (F1) in the majority of patients (73%). Two patients (9%) had liver cirrhosis; none had hepatocellular carcinoma. The most frequently used DAA were velpatasvir/sofosbuvir (39%) and sofosbuvir/daclatasvir (35%), with a median treatment duration of 84 days (IQR 83–91). DAA were started mostly due to previous MDR/RR-TB treatment hepatotoxicity (30%) or because of elevated transaminases before starting MDR/RR-TB treatment (26%). DAA were usually started during MDR/RR-TB treatment (65%), a median of 267 days (IQR 69–584) after MDR/RR-TB treatment start. All patients completed DAA treatment without interruptions. Baseline plasmatic HCV-RNA was detected in all patients (median: 5,7log IU/ml (IQR 5.2–6.3) and became undetectable for all from week 12 (Fig. 1). Sustained virological response at week 4, 12, and 24 was achieved for all patients with available results ($N = 11$).

All patients had pulmonary tuberculosis, with extrapulmonary involvement in 17%. Bilateral lung involvement, lung cavitations, and positive baseline sputum smear were present in 61%, 46%, and 50%, respectively. Overall, 52% of patients had received previous tuberculosis treatment. Thirty percent had additional resistance to any fluoroquinolone or second-line injectable, and 17% to both. All patients received linezolid, most received clofazimine (87%), cycloserine (78%), and bedaquiline (65%). At censoring, 52% of patients were still ongoing MDR/RR-TB treatment. Among the other 11 patients, 10 (91%) achieved cure and one (9%) died of accidental causes.

Overall, 18 liver-related adverse events were reported in 48%, the majority (94%) during MDR/RR-TB treatment but before DAA were started. Most adverse events were Grade 1. No liver-related SAEs or grade 4 adverse events were reported. Blood transaminases were increased at DAA treatment start (AST: median 60 U/l [IQR 37–102], ALT: median 79 U/l [IQR 37–167]) but decreased into normal range from week 4 (Figure). The median duration until resolution was 90 days (IQR 50–147). Other non-liver-related SAE were observed in 30% of patients.

In our multicentre, retrospective cohort study, concomitant HCV and MDR/RR-TB treatment was effective and well-tolerated. DAA treatment led to achieve undetectable HCV-RNA and sustained virological response for all patients with available data. Similarly, treatment success was achieved for 91% of patients who completed MDR/RR-TB treatment. These results are particularly important considering the high prevalence of infection with HIV and HCV genotype 3. Similar, encouraging outcomes have been reported in a cohort from Armenia.[8] Liver-related adverse events were mostly mild or moderate, and occurred mainly before DAA start. Moreover, blood transaminases decreased into normal range for all patients from week 4 of DAA treatment. Since HCV-treatment was often started because of previous hepatotoxicity, our results suggest that co-administration of DAA may prevent liver toxicity during MDR/RR-TB treatment.

Our study is limited by the small sample, retrospective data collection, and lack of MDR/RR-TB outcome in some patients. However, the results show the safety of the association of second-line anti-tuberculosis drugs and DAA. DAA treatment should be considered in MDR/RR-TB patients to reduce tuberculosis-treatment-related hepatotoxicity, minimizing the risk of prolonged treatment interruption, and prevent progression of HCV-mediated liver disease. Integrated services for the management of tuberculosis, HIV, and HCV, should be promoted widely and collaboration with surveillance, prevention and control programs should be strengthened.

Table 1
Characteristics and treatment outcome of patients treated for chronic HCV infection and active rifampicin-resistant tuberculosis.

N	TB form	HIV	HBV	HCV genotype	Liver fibrosis	Liver cirrhosis	Baseline HCV RNA (log IU/ml)	HCV treatment	TB treatment regimen	HCV treatment (days)	TB treatment (days)	HCV RNA at end of HCV treatment	TB treatment outcome	Liver-related AE, grade$
1	P	Yes	No	3	F1	No	5,2	Sof/Vel	E, Z, Lfx, Mfx, Amk, Cs, Lzd, Cfz	89		Neg	Ongoing	
2	P + E	No	No	1b	F1	No	4,2	Sof/Led	Mfx, Amk, Eto, Cs, PAS, Lzd, Cfz	101	571	Neg	Cure	1
3	P	No	No	4	F2	No	5,7	Sof/Vel	E, Z, Lfx, Amk, Eto, Cs, Lzd, Imp/Amx/Clv	121		Neg	Death	2
4	P	No	No	1b	F1	No	5	Sof/Led	E, Mfx, Eto, Cs, Lzd, Cfz	91	1126	Neg	Cure	3
5	P	No	No	1b	F1	No	6,1	Gle/Pib	E, Z, Lfx, Eto, Cs, PAS, Lzd, Cfz, Bdq, Imp/Amx/Clv		852	Neg	Cure	3
6	P + E	Yes	No	1a	F1	No	6,3	Gle/Pib	E, Z, Lfx, Mfx, Amk, Cs, Lzd, Cfz, Dlm	61	740	Neg	Cure	1
7	P	No	No	1a	F1	No	Pos#	Sof/Dac	Lfx, Cs, Lzd, Cfz, Bdq	70		Neg	Ongoing	3
8	P	Yes	No	1a	F1	No	Pos#	Sof/Dac	Cs, Lzd, Cfz, Bdq, Dlm	84		Neg	Ongoing	
9	P	No	No	3	F1	No	Pos#	Sof/Dac	Lzd, Cfz, Bdq, Dlm	90		Neg	Ongoing	
10	P	Yes	No	3	F1	No	Pos#	Sof/Dac	Lzd, Cfz, Bdq, Dlm, Imp/Amx/Clv	83		Neg	Ongoing	
11	P	No	No	1a	F1	No	Pos#	Sof/Dac	Lfx, Cs, Lzd, Cfz, Bdq	84		Neg	Ongoing	
12	P	Yes	No	3	F1	No	Pos#	Sof/Dac	Lfx, Cs, Lzd, Cfz, Bdq	83		Neg	Ongoing	
13	P	Yes	No	1a	F0	No	Pos#	Sof/Dac	Lfx, Cs, Lzd, Cfz, Bdq	95		Neg	Ongoing	2
14	P	Yes	No	3	F0	No	Pos#	Sof/Dac	Lfx, Cs, Lzd, Cfz, Bdq	83		Neg	Ongoing	
15	P	No	No	3	F1	No	5,98	Sof/Led	E, Lfx, Mfx, Amk, Cs, PAS, Lzd, Cfz, Bdq, Dlm	81	725	Neg	Cure	
16	P + E	Yes	Yes*	1b	F4	No	5,66	Sof/Vel	Z, Mfx, Amk, Cs, Lzd, Bdq, Dlm	91	669	Neg	Cure	2
17	P	No	No	3	F1	Yes	6,98	Sof/Vel	Amk, Cs, Lzd, Cfz, Bdq, Dlm	89		Neg	Ongoing	3
18	P + E	Yes	No	1b	F3	No	6,38	Sof/Vel	Z, Mfx, Eto, Cs, Lzd, Cfz, Bdq, Dlm	90	731	Neg	Cure	
19	P	No	No	1a	F2	Yes	4,82	Sof/Vel	Lfx, Mfx, Amk, Cs, PAS, Lzd, Cfz, Bdq, Dlm	84	431	Neg	Treatment completed	1
20	P	No	No	NA	NA	No	4,82	Gle/Pib	Amk, Lzd, Cfz, Bdq, Imp/Amx/Clv	55		Neg	Ongoing	
21	P	No	No	NA	F2	No	6,89	Sof/Vel	E, Lfz, Amk, Cm, Lzd, Cfz, Dlm	83	NA	Neg	Cure	1
22	P	No	No	NA	F2	No	Pos#	Sof/Vel	E, Lfx, Lzd, Cfz, Dlm	84	NA	Neg	Cure	
23	P	No	No	3	F1	No	Pos#	Sof/Vel	E, Z, Lfx, Amk, Cm, Cs, Lzd, Cfz	55		Neg	Ongoing	

TB = tuberculosis; AE = adverse event; NA = not available; P = pulmonary tuberculosis case; P + E = pulmonary and extrapulmonary tuberculosis case; Sof/Vel = sofosbuvir/velpatasvir; Sof/Led = sofosbuvir/ledispavir; Gle/Pib = glecaprevir/pibrentasvir; Sof/Dac = sofosbuvir/daclatasvir; H = isoniazid; E = ethambutol; Z = pyrazinamide; Lfx = levofloxacin; Mfx = moxifloxacin; Amk = amikacin; Eto = ethionamide; Cs = cycloserine; PAS = para-aminosalicylic acid; Lzd = linezolid; Cfz = clofazimine; Bdq = bedaquiline; Dlm = delamanid; Ipm = imipenem; Amx/Clv = amoxicillin/clavulanic acid.
* = co-infection with HDV; # = quantitative results available only; $ = if multiple liver-related adverse events occurred, the highest grade is reported.
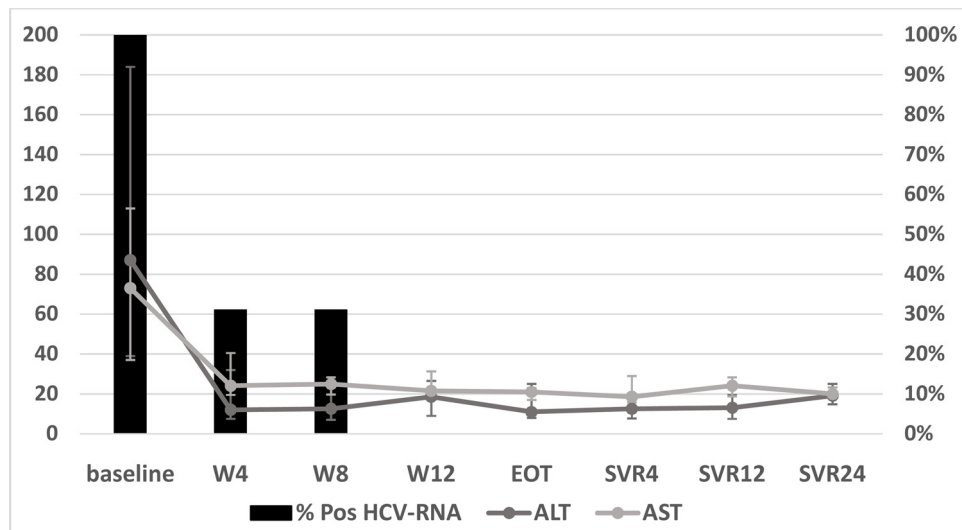
Fig. 1. Plasma HCV-RNA positivity rates (%) and blood transaminase levels (U/L, with standard deviations bars) during treatment with direct-acting antivirals in a cohort of 23 HCV-infected patients with active rifampicin-resistant tuberculosis. W4, W18, W12 = 4, 8, and 12 weeks after starting HCV treatment; EOT = at end of HCV treatment; SVR4, SVR12, SVR24 = 4, 12, and 24 weeks after the end of HCV treatment.

Author contributions

LG and ST made a substantial contribution to the conception and design of the work, to the acquisition, analysis and interpretation of data for the work, performed statistical analysis, wrote the manuscript, critically revised the manuscript for important intellectual content, gave final approval of the current version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

DLD and MJF made a substantial contribution to the conception and design of the work, to the acquisition and interpretation of data for the work, critically revised the manuscript for important intellectual content, gave final approval of the current version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

GG, JPM, and AS made a contribution to the conception and design of the work, to the acquisition and interpretation of data for the work, critically revised the manuscript for important intellectual content, gave final approval of the current version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

All other authors made a substantial contribution to the conception and design of the work, to the acquisition and interpretation of data for the work, critically revised the manuscript for important intellectual content, gave final approval of the current version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of Competing Interest

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.03.004.

References

1. Carvalho L., Pillai S., Daniels E., Sellers P., Whyte R., Eveson L., et al. Higher sustained virological response rates at 12 weeks in HIV-HCV co-infection; a tertiary centre experience. *J Infect* 2020;**80**:232–54.
2. WHO, Global Hepatitis Report 2017. Geneva: World Health Organization; 2017. Licence: CC BY-NC-SA 3.0 IGO.
3. WHO. *Global Tuberculosis Report 2020*, Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
4. Behzadifar M., Heydarvand S., Behzadifar M., Bragazzi N.L.. Prevalence of hepatitis C Virus in tuberculosis patients: a systematic review and meta-analysis. *Ethiop J Health Sci* 2019;**29**:945–56.
5. Seung K.J., Franke M.F., Hewison C., Huerga H., Khan U., Mitnick C.D.. High prevalence of hepatitis C infection among multidrug-resistant tuberculosis patients. *J Hepatol* 2020;**72**:1028–9.
6. WHO. *Guidelines for the Care and Treatment of Persons Diagnosed with Chronic Hepatitis C Virus Infection*. Geneva: World Health Organization; 2018.
7. Kempker R.R., Alghamdi W.A., Al-Shaer M.H., Burch G., Peloquin C.A.. A pharmacology perspective on simultaneous tuberculosis and hepatitis C treatment. *Antimicrob Agents Chemother* 2019. [Internet][cited 2021 Apr 10]; 63Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6879218/.
8. Melikyan N., Huerga H., Atshemyan H., Kirakosyan O., Sargsyants N., Aydinyan T., et al. Concomitant treatment of chronic hepatitis C with direct-acting antivirals and multidrug-resistant tuberculosis is effective and safe. *Open Forum Infect Dis* 2021;**8**:ofaa653.
9. Musso M., Mosti S., Gualano G., Mencarini P., Urso R., Ghirga P., et al. Hepatitis C virus infection: a challenge in the complex management of two cases of multidrug-resistant tuberculosis. *BMC Infect Dis* 2019;**19**:882.
10. van Leth F., Brinkmann F., Cirillo D.M., Dheda K., Duarte R., Guglielmetti L., et al. The tuberculosis network European trials group (TBnet) ERS clinical research collaboration: addressing drug-resistant tuberculosis through European cooperation. *Eur Respir J* 2019;**53**.

Simone Tunesi
For the *Infectious Diseases Unit, ASST Valle Olona, Busto Arsizio, Italy*

Damien Le Dû
Sanatorium, Centre Hospitalier de Bligny, Briis-sous-Forges, France

Gina Gualano[1]
National Institute for Infectious Diseases L. Spallanzani-IRCCS, Rome, Italy

Joan-Pau Millet
CIBER of Epidemiology and Public Health (CIBERESP), Barcelona, Spain
Epidemiology Service, Public Health Agency of Barcelona

Aliaksandr Skrahin[1]

The Republican Research and Practical Center for Pulmonology and TB, Minsk, Belarus

Graham Bothamley
Homerton University Hospital, London E9 6SR, UK
London School of Hygiene and Tropical Medicine, Queen Mary University of London, UK

Xavier Casas
Epidemiology Service, Public Health Agency of Barcelona, Spain

Delia Goletti
National Institute for Infectious Diseases L. Spallanzani-IRCCS, Rome, Italy

Christoph Lange
Research Center Borstel, Clinical Infectious Diseases, Borstel, Germany
German Center for Infection Research (DZIF) Tuberculosis Unit, Borstel, Germany
Respiratory Medicine & International Health, University of Lübeck, Lübeck, Germany
Global TB Program, Baylor College of Medicine and Texas Children's Hospital, Houston, TX, USA

Maria Musso, Fabrizio Palmieri
National Institute for Infectious Diseases L. Spallanzani-IRCCS, Rome, Italy

Valérie Pourcher
APHP, Service des Maladies Infectieuses et Tropicales, Sorbonne Université, Site Pitié, Paris, France
Inserm UMR_S 1136, Institut Pierre Louis d'Epidémiologie et de Santé Publique (iPLESP), Paris, France

Christophe Rioux
APHP, Service des Maladies Infectieuses et Tropicales, Hôpital Bichat-Claude Bernard, Paris, France

Alena Skrahina
The Republican Research and Practical Center for Pulmonology and TB, Minsk, Belarus

Nicolas Veziris
Département de Bactériologie, APHP, Groupe Hospitalier Universitaire Sorbonne Université, Site, Paris, Saint-Antoine, France
Laboratoire de Bactériologie-Hygiène, APHP, Groupe Hospitalier Universitaire Sorbonne Université, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, 91 Boulevard de l'hôpital, Paris Cedex 13 75634, France
INSERM, U1135, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, Équipe 13, Sorbonne Université, Paris, France

Dzmitry Viatushka
The Republican Research and Practical Center for Pulmonology and TB, Minsk, Belarus

Mathilde Jachym-Fréchet
Sanatorium, Centre Hospitalier de Bligny, Briis-sous-Forges, France

Lorenzo Guglielmetti*
Laboratoire de Bactériologie-Hygiène, APHP, Groupe Hospitalier Universitaire Sorbonne Université, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, 91 Boulevard de l'hôpital, Paris Cedex 13 75634, France
INSERM, U1135, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, Équipe 13, Sorbonne Université, Paris, France

Dhiba Marigot-Outtandy
Bligny

Xavier Lescure, Marie Dubert, Yazdan Yazdanpanah
Paris-Bichat

Eric Caumes, Pascaline Choinier, Elie Haddad
Paris-Pitié-Salpetrière

Jakub Kowalczyk
Mulhouse

Hélène Laurichesse, Olivier Lesens
Clermont Ferrand

Alexandra Aubry, Isabelle Bonnet, Florence Morel
Paris-CNR Mycobactéries

*Corresponding author at: Laboratoire de Bactériologie-Hygiène, APHP, Groupe Hospitalier Universitaire, Faculté de Médecine -Sorbonne Université, Hôpital Pitié-Salpêtrière, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, 91 Boulevard de l'hôpital, Paris Cedex 13 75634, France.
E-mail address: lorenzo.guglielmetti@aphp.fr (L. Guglielmetti)

[1] These authors contributed equally to this work.
[2] Tuberculosis Network European Trialsgroup.
[3] Study Group on Mycobacteria of the European Society of Microbiology and Infectious Diseases.
[4] Members of the French MDR-TB Group are the following: Dhiba Marigot-Outtandy (Bligny), Xavier Lescure, Marie Dubert, and Yazdan Yazdanpanah (Paris-Bichat), Eric Caumes, Pascaline Choinier and Elie Haddad (Paris-Pitié-Salpêtrière), Jakub Kowalczyk (Mulhouse), Hélène Laurichesse and Olivier Lesens (Clermont Ferrand), Alexandra Aubry, Isabelle Bonnet, and Florence Morel (Paris-CNR Mycobactéries).

Wild poliovirus outbreak in Afghanistan: A wake-up call for global health experts

Dear Editor,

In a recent article titled "War-torn Afghanistan-potential risk to the polio eradication efforts: A call for global concern!"[1], we previously reported that Afghanistan's turmoil and war pose a risk of infection spreading globally owing to a lack of health management policies. Regrettably, the wild poliovirus (WPV) outbreaks in Afghanistan have alarmed the bells for global health experts to revisit the polio eradication at all levels and identify the loopholes the virus exploited for spread polio-endemic Pakistan-Afghanistan area has been documented recently. This paper will discuss the possible limitations and potential solutions for strengthening polio eradication efforts in this endemic region.

Afghanistan is now at a potentially historic crossroads due to years of violence and war-torn territories linked to humanitarian crises. Health workers and disease experts are departing Afghanistan following the Taliban's quick re-accession to power and political chaos, potentially hindering regional polio eradication efforts. In 2021, Pakistan confirmed only one case of wild poliovirus (WPV), and it is anticipated that Pakistan may not report

Fig. 1. Polio outbreak in Afghanistan in 2022. Emerging of wild poliovirus after the political upheaval in bordering areas in Afghanistan is extremely alarming and at high risk for nearby country Pakistan.

any new case of WPV in 2022.[2, 3] Alarmingly, a polio case was reported in a 24-month-old female child who developed paralysis near the Pakistan-Afghan border village of Minzi (Rohani) in Dila district, Province Paktika (Fig. 1).[4]

The UN Refugee Agency (UNHCR) pleaded with the international community to increase its assistance to People in Afghanistan who were affected by the recent humanitarian disasters.[5] Pakistan responded to the UNCHR appeal by opening its borders to Afghans resulting in a massive wave of immigrants entering Pakistan. The most alarming situation is that most of the children are unvaccinated.

Despite Pakistan's established polio immunization initiatives, unrestrained immigrants put the country's already overburdened healthcare system and vaccination programs at risk. Keeping in view the cross-border movement of the general population, the adjacent border areas of Pakistan are now at high risk of WPV transmission and future outbreaks.[6] Dreadfully, it appears as the tremendous efforts of global and Pakistani public health experts toward polio eradication have been thwarted.

Even though vaccination has never been convenient in Afghanistan, it may become more difficult in the future if unrest and political chaos continue. To reach migratory communities, Taliban collaboration is essential to ensure the security of polio workers in Afghanistan, which will help them cover secluded locations and remote areas. Pakistan's policymakers should collaborate with Global Polio Eradication Initiative partners to expedite environmental surveillance, cross-border immunization initiatives, and polio eradication campaigns in Pak-Afghan territories. Strengthening these activities will assist in eradicating polio recurrence in high-risk areas and accomplishing the long-awaited goal of a polio-free world—or else global efforts would be in vain.

Author's contribution

UAA and MWM conceived and designed the study, analyzed, and interpreted the data. MSA, SK, MH and HA were involved in writing the first draft, statistical analysis of the manuscript, and interpretation of results. UAA supervised and did the final correction of the manuscript. All authors approved the final version of the manuscript.

Role of funding source

There is no role of any funding source for this manuscript.

Declaration of Competing Interest

We declare no competing interest.

References

1. Awan U.A., Malik M.W., Ahmed H., Hassan U., Khan A.S., Bashir S., et al. War–torn Afghanistan-potential risk to the polio eradication efforts: a call for global concern!. *Journal of Infection* 2021;**83**:709–37.
2. Pakistan Polio Eradication Program. District Wise Polio Cases2021 10-02-2022. Available from: https://www.endpolio.com.pk/polioin-pakistan/district-wise-polio-cases.
3. Awan U.A., Malik M.W., Khattak A.A., Ahmed H., Khan M.I., Qureshi H., et al. Emerging polio hotspots in Pakistan: challenges and the way forward. *Journal of Infection* 2021;**83**:496–522.
4. Polio Global Eradication Initiative. Afghanistan2022 11-02-2022. Available from: https://polioeradication.org/where-we-work/afghanistan/.
5. (UNHCR) U.R.A. UNHCR warns Afghanistan's conflict taking the heaviest toll on displaced women and children2021 12-02-2022. Available from: https://www.unhcr.org/news/briefing/2021/8/611617c55/unhcr-warns-afghanistans-conflict-taking-heaviest-toll-displaced-women.html.
6. Pakistan Polio Eradication Program. Vaccinating Pak-Afghan border-Stepping towards a Polio free region2021 12-02-2022. Available from: https://www.endpolio.com.pk/media-room/field-stories/vaccinating-pak-afghan-border-stepping-towards-a-polio-free-region.

Usman Ayub Awan*[1]

Department of Medical Laboratory Technology, The University of Haripur, Haripur, Khyber Pakhtunkhwa, Pakistan

Muhammad Wasif Malik[1]

Field Epidemiology & Disease Surveillance Division, National Institute of Health (NIH), Islamabad, Pakistan

Shehrish Kamran

Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

Haroon Ahmed

Department of Biosciences, COMSATS University, Islamabad, Pakistan

Mohsina Haq

Department of Pathology, Peshawar Medical College, Prime Foundation, Riphah International University, Islamabad Pakistan

Muhammad Sohail Afzal

Department of Life Sciences, School of Science, University of Management and Technology (UMT), Lahore, Pakistan

*Corresponding author.
E-mail address: usman.ayub111@gmail.com (U.A. Awan)

[1] These authors contributed equally.
Accepted 1 March 2022
Available online 6 March 2022

SARS-CoV-2 adaptive immunity in nursing home residents up to eight months after two doses of the Comirnaty® COVID-19 vaccine

Dear Editor,

There is scant information as to how SARS-CoV-2 antibody and T-cell immune responses elicited by mRNA COVID-19 vaccines evolve in the general population, and in particular in elderly nursing home residents, who are at increased risk of developing severe clinical forms of COVID-19. We read with interest the work by Tré-Hardy and colleagues who reported a significant antibody decrease at around 6 months after full vaccination in healthcare workers, that was more marked in SARS-CoV-2 naïve vaccinees [1]. The authors suggested that in a supply-limited environment, booster dose schemes may be spared for SARS-CoV-2-experienced individuals. The data presented herein extend this observation to elderly nursing home residents. The current prospective cohort study included 680 (478 female; median age, 87 years; range 65–100) of a cohort of 881 nursing home residents initially recruited from a representative sample of Valencian Community nursing homes ($n = 13$) for assessment of SARS-CoV-2 immune responses at a median of 3 months (3 M) following full-dose Comirnaty® COVID-19 vaccination [2] who were re-examined at a median of 219 days (range, 139–246) after vaccination (7 M). Out of the 680 participants, 238 had been infected by SARS-CoV-2 prior to receiving the first vaccine dose, as recorded in the electronic Valencia Health System Integrated Databases. Two residents contracted the infection (Delta variant, as documented by whole-genome sequencing) between sampling times (3 M and 7 M). The remaining 440 participants were presumably naïve for SARS-CoV-2 at the time of sampling (7 M).

The current study was carried out under the epidemiological surveillance competences of the Valencia Government Health Department (Law 16/2003/May 28 on Cohesion and Quality of the National Health System, and Law 10/2014/ December 29 on Public Health of the Valencian Community), without requiring informed consent or ethics approval by an institutional review board. Likewise, in accordance with local law and regulations, data publication is exempt from the research ethics committee approval. Personal data from nursing homes and residents were processed in accordance with European data protection regulations.

All participants were initially examined for presence SARS-CoV-2-Spike (*S*)-specific antibodies in whole blood obtained by finger-stick using a lateral flow immunochromatographic assay (LFIC): the OnSite COVID-19 IgG/IgM Rapid Test (CTK BIOTECH, Poway, CA, USA) [2]. As shown in Table 1, a total of 148 of the 680 (21.7%) residents tested negative by LFIC at 7 M. The percentage of residents without detectable anti-S LFIC responses at 7 M was roughly double the proportion at 3 M. Moreover, overall, the strength of antibody reactivity [2] in LFIC among those who tested positive at both sampling times ($n = 520$) tended to decrease by 7M: 169, 84 and 267 residents showed decreased, increased or similar antibody reactivity grades, respectively. Interestingly, SARS-CoV-2-experienced participants were more likely to display detectable and higher grade antibody responses at 7 M than SARS-CoV-2-naïve participants (Fig. 1A). Indeed, negative LFIC results were registered in 11/238 (4.6%) and 137/440 (31%) of SARS-CoV-2-recovered and naïve residents, respectively ($P<0.001$; Fisher exact test), while antibody reactivities grade ≥ 2 were present in 181/238 (76%) and 118/440 (26.8%) of SARS-CoV-2-experienced and naïve residents, respectively ($P<0.001$).

Participants testing negative by LFIC underwent for quantitation of receptor binding domain (RBD)-reactive total antibodies using an (Electro)chemiluminescent –(E)CLIA- immunoassay (Roche Elecsys® Anti-SARS-CoV-2-S, Roche Diagnostics, Pleasanton, CA, USA), and IgG antibodies against a trimeric S-protein antigen by employing CLIA (LIAISON® SARS-CoV-2 TrimericS IgG assay; DiaSorin S.p.A, Saluggia, Italy) in plasma. Antibody testing could be performed in 144 of the 148 residents, of which 138 (95.8%) tested positive by RBD ECLIA and 108 (75%) by S-trimeric assay. Taking the above data together, 670/676 residents undergoing testing by LFIC and (E)CLIA (99.1%) exhibited detectable S-reactive antibody responses by 7 M, a similar figure (98%) to that reported in the original cohort at 3 M after vaccination [2].

A total of 100 residents had 3 M/7 M paired plasma specimens analyzed by RBD ECLIA. As shown in Fig. 1B, overall antibody levels declined over time, but particularly at the expense of SARS-CoV-2-naïve participants.

Participants testing negative for SARS-CoV-2 antibodies by all the above assays ($n = 6$) with available specimens ($n = 5$) were examined for presence of SARS-CoV-2-S-reactive IFNγ-producing T cells by whole-blood flow cytometry for intracellular cytokine staining (ICS), as previously described [2,3]. Four residents had detectable S-targeted CD8+ T cells (median, 0.47%; range, 0.16–3.94%), whereas none had CD4+ T cells. We next examined 28 randomly selected participants (25 SARS-CoV-2-naïve and 3 experienced) testing negative by LFIC but positive by (E)CLIA: 23 displayed detectable CD8+ T-cell responses (median, 0.24%; range, 0.01–2.88%), 3 had both CD8+ and CD4+ T-cell (median, 0.44%; range, 0.03–0.77%) responses and 2 had neither.

Paired 3 M/7 M whole-blood specimens were available from 24 residents (Supplementary Table 1). Examining SARS-CoV-2-S-reactive IFNγ-producing CD8+ T cells, we observed that 8 residents who had not detectable responses at 3 M acquired them by 7 M, whereas 16 had documented responses at 3 M, which

Table 1

Anti-SARS-CoV-2-Spike (S) antibody reactivity as determined by lateral flow immunochromatography in nursing home residents by 3 and 6 months after full-dose vaccination with the Comirnaty® COVID-19 vaccine.

Anti-S antibody reactivity by 3 months (median) after full-dose vaccination	Anti-S antibody reactivity by 7 months (median) after full-dose vaccination (number of residents)			
	0	1+	2+	3+
0	73	8	2	2
1+	57	119	35	6
2+	15	90	101	43
3+	3	15	64	47

The IgG line intensity was scored visually using a 4-level scale, as previously reported [3]: 0 = negative result; 1+ = intensity of test band lower than control band; 2+= intensity of test band equal to control line; 3+ = intensity of test band greater than control line. Reactivities ≥2 in the LFIC assay corresponded roughly to antibody levels ≥250 IU/ml as measured by Elecsys® Anti-SARS-CoV-2 S-total antibody assay (Roche Diagnostics, Pleasanton, CA, USA).

Supplementary Table 1

SARS-CoV-2-S reactive antibodies and T-cells in nursing home residents with paired specimens collected at 3 and 7 months after full Comirnaty® COVID-19 vaccination.

Patient code[a]	Sex	SARS-CoV-2 infection status	Anti-SARS-CoV-2 RBD Antibody level (IU/ml)[b]		SARS-CoV-2-S-reactive IFN-γ-producing T cells (3 M)		SARS-CoV-2-S-reactive IFN-γ producing-T cells (7 M)	
			3M	7M	CD4$^+$ (%)	CD8$^+$ (%)	CD4$^+$ (%)	CD8$^+$ (%)
1	F	Recovered (infection acquired 90 days prior the first vaccine dose	30	16	1.07	0.62	ND	0.11
2	F	Naïve	ND	ND	ND	ND	ND	ND
3	M	Naïve	ND	6.9	ND	ND	0.03	1.26
4	F	Naïve	11.9	2	2.10	0.85	0.44	0.80
5	F	Naïve	9.68	10	1.88	0.48	ND	0.87
6	F	Naïve	51.6	39	0.06	0.02	ND	1.43
7	M	Naïve	49.9	22.4	0.47	0.40	ND	0.13
8	F	Naïve	ND	ND	0.02	ND	ND	3.94
9	M	Naïve	11.7	5.9	0.03	ND	ND	1.08
10	F	Naïve	17.5	16	0.10	ND	ND	0.54
11	F	Naïve	97.4	82	0.54	0.34	ND	0.16
12	F	Naïve	54.8	65	1.17	0.15	ND	0.04
13	F	Naïve	32.3	23	0.69	0.37	ND	0.62
14	F	Naïve	53	36	0.51	0.11	ND	2.88
15	F	Naïve	25.3	28	1.27	0.67	ND	0.01
16	F	Naïve	168	142	0.41	ND	ND	0.19
17	M	Naïve	58.6	47	0.08	ND	ND	0.28
18	F	Naïve	81	29	0.03	ND	ND	0.08
19	F	Naïve	120.7	48	0.45	0.08	ND	0.90
20	F	Naïve	49.1	33.6	0.90	0.08	ND	0.05
21	M	Naïve	1.9	2.8	1.67	0.81	ND	ND
22	M	Naïve	12.4	11.5	0.47	0.28	ND	ND
23	M	Naïve	15.8	20	2.68	0.33	ND	0.09
24	M	Naïve	35.5	31	1.30	0.61	ND	0.50

3 M, a median of 3 months after full-dose vaccination; 7 M, median of 7 months after full-dose vaccination; ND, not detectable; RBD, receptor binding domain of Spike (S) protein.

[a] Paired 3 M/7 M whole-blood specimens were available from 24 residents (23 SARS-CoV-2-naïve and 1 recovered).

[b] Elecsys® Anti-SARS-CoV-2 S-total antibody assay (Roche Diagnostics, Pleasanton, CA, USA).

were maintained in 14 and lost in 2. Regarding SARS-CoV-2-S-reactive IFNγ-producing-CD4$^+$ T cells, most responders at 3 M (21/22) no longer had detectable responses at 7 M, whereas 1 out of 2 residents acquired them by 7 M Fig. 1.C illustrates that while SARS-CoV-2-S-reactive IFNγ-producing CD8$^+$ T-cell levels increased slightly over time (P = 0.12), those of CD4$^+$ T cells declined dramatically (P<0.001). That most residents maintained detectable S-targeted CD8$^+$ T-cell responses at 7 M was in contrast to previously published data [4] reporting positive SARS-CoV-2 T-cell responses as determined by the QuantiFERON assay in only 5% of SARS-CoV-2-naïve participants at 24 weeks after full vaccination with the Comirnaty® vaccine. Nevertheless, it is uncertain how SARS-CoV-2 QuantiFERON assay and our flow cytometry ICS method compare analytically.

Limitations of the current study included the use of a semi-quantitative LFIC for front-line antibody testing and that functional specificities of SARS-CoV-2-S-reactive T cells beyond IFN-γ production were not explored.

In conclusion, our data indicated that both antibody and peripheral blood CD4$^+$ T-cell levels measured after Comirnaty® vaccination in elderly nursing home residents wane over time in line with previous findings [2–8], declining significantly by 7 M after vaccination, particularly in SARS-CoV-2 naïve individuals.
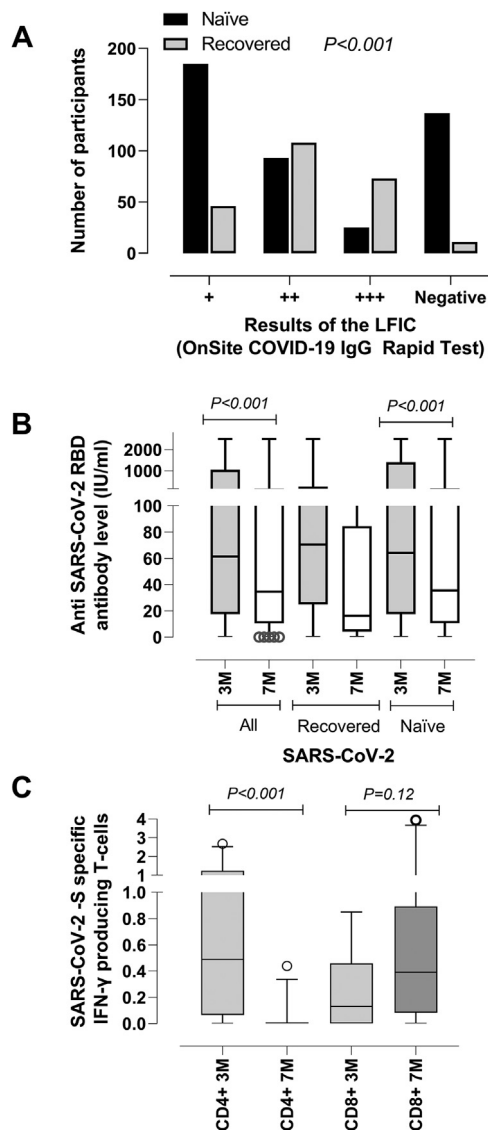
Financial Support

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

EG, EA, JSB, SP, DS, HV, RL, MJA, JS-P, JD, IC and FG-C: Methodology and data validation. JSB, SP, and DN: Conceptualization and

Fig. 1. SARS-CoV-2 antibody and T-cell immunity in nursing home residents up to eight months after two doses of the Comirnaty® COVID-19 vaccine. (A) SARS-CoV-2-Spike IgG reactivity of plasma from SARS-CoV-2-naïve and experienced nursing home residents at a median of 7 months after full vaccination with the Comirnaty® vaccine, as determined by the OnSite COVID-19 IgG/IgM Rapid immunochromatography Test (CTK BIOTECH, Poway, CA, USA). The IgG line intensity was scored visually using a 4-level scale as previously reported: 0 = negative result; 1+ = intensity of test band lower than control band; 2+ = intensity of test band equal to control line; 3+ = intensity of test band greater than control line. (B) SARS-CoV-2-Spike total antibody levels as measured by Roche Elecsys® assay (Roche Diagnostics, Pleasanton, CA, USA) in paired plasma specimens collected from 100 either SARS-CoV-2-naïve or -experienced nursing home residents at a median of 3 months (3 M) and 7 months (7 M) after full Comirnaty® vaccination. Both assays are calibrated to the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody [9] and provide quantitative values that strongly correlate with SARS-CoV-2 neutralizing antibody titers [10]. (C) SARS-CoV-2-Spike-reactive IFNγ-producing CD4$^+$ and CD8$^+$ T cells, as enumerated by flow cytometry for intracellular staining in paired whole-blood specimens collected from 24 nursing home residents at a median of 3 months (3 M) and 7 months (7 M) after full Comirnaty® vaccination. Differences between medians were compared using the Mann–Whitney *U test* or the Wilcoxon test, as appropriate. Two-sided exact *P*-values are reported. A *P*-value <0.05 was considered statistically significant. The analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA).

data analysis. DN: writing the original draft. All authors reviewed the original draft.

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.02.035.

References

1. Tré-Hardy M., Cupaiolo R., Wilmet A., Antoine-Moussiaux T., Della Vecchia A., Horeanga A., et al. Immunogenicity of mRNA-1273 COVID vaccine after 6 months surveillance in health care workers; a third dose is necessary. *J Infect* 2021;**83**:559–66.
2. Albert E., Burgos J.S., Peiró S., Salas D., Vanaclocha H., Giménez E., et al. Immunological response against SARS-CoV-2 following full-dose administration of Comirnaty COVID-19 vaccine in nursing home residents. *Clin Microbiol Infect* 2022;**28**:279–84.
3. Torres I., Albert E., Giménez E., Alcaraz M.J., Botija P., Amat P., et al. B and T cell B and T cell immune responses elicited by the Comirnaty COVID-19 vaccine in nursing-home residents. *Clin Microbiol Infect* 2021;**27**:1672–7.
4. Van Praet J.T., Vandecasteele S., De Roo A., Vynck M., De Vriese A.S., Reynders M.. Dynamics of the cellular and humoral immune response after BNT162b2 mRNA Covid-19 vaccination in Covid-19 naive nursing home residents. *J Infect Dis* 2021 Sep 13:jiab458.
5. Salmerón Ríos S., Mas Romero M., Cortés Zamora E.B., Tabernero Sahuquillo M.T., Romero Rizos L., Sánchez-Jurado P.M., et al. Immunogenicity of the BNT162b2 vaccine in frail or disabled nursing home residents: COVID-A study. *J Am Geriatr Soc* 2021;**69**:1441–7.
6. Blain H., Tuaillon E., Gamon L., Pisoni A., Miot S., Rolland Y., et al. Antibody response after one and two jabs of the BNT162b2 vaccine in nursing residents: the CONsort-19 study. *Allergy* 2022;**77**:271–81.
7. Naaber P., Tserel L., Kangro K., Sepp E., Jürjenson V., Adamson A., et al. Dynamics of antibody response to BNT162b2 vaccine after six months: a longitudinal prospective study. *Lancet Reg Health Eur* 2021;**10**:100208.
8. Giménez E., Alberola J., Torres I., Albert E., Alcaraz M.J., Botija P., et al. Evolution of SARS-CoV-2 immune responses in nursing home residents following full dose of the Comirnaty® COVID-19 vaccine. *J Infect* 2021 S0163-4453(21)00542-9.
9. Mattiuzzo G., Bentley E.M., Hassall M. Establishment of the WHO International Standard and Reference Panel for anti-SARS-CoV-2 antibody. WHO/BS/2020.2403, December 10, 2020.
10. Poljak M., Oštrbenk Valenčak A., Štamol T., Seme K. Head-to-head comparison of two rapid high-throughput automated electrochemiluminescence immunoassays targeting total antibodies to the SARS-CoV-2 nucleoprotein and spike protein receptor binding domain. *J Clin Virol* 2021;**137**:104784.

Estela Giménez, Eliseo Albert
on behalf of the *Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Valencia, Spain*

Javier S. Burgos
General Directorate of Research and Healthcare Supervision, Department of Health, Valencia Government, Valencia, Spain

Salvador Peiró

Foundation for the promotion of health and biomedical research of the Valencian Community (FISABIO), Valencia, Spain

Dolores Salas

Foundation for the promotion of health and biomedical research of the Valencian Community (FISABIO), Valencia, Spain
General Directorate of Public Health, Department of Health, Valencia Government, Valencia, Spain

Hermelinda Vanaclocha

General Directorate of Public Health, Department of Health, Valencia Government, Valencia, Spain

Ramón Limón

General Directorate of Healthcare. Department of Health, Valencian Government, Valencia, Spain

María Jesús Alcaraz

Microbiology Service, Clinic University Hospital, INCLIVA Health Research Institute, Valencia, Spain

José Sánchez-Payá

Preventive Medicine Service, Alicante General and University Hospital, Alicante, Spain
Alicante Institute of Health and Biomedical Research (ISABIAL), Alicante, Spain

Javier Díez-Domingo

Foundation for the promotion of health and biomedical research of the Valencian Community (FISABIO), Valencia, Spain

Iñaki Comas

Biomedicine Institute of Valencia, Spanish Research Council (CSIC)

Fernando Gonzáles-Candelas

CIBER in Epidemiology and Public Health, Spain, Joint Research Unit "Infection and Public Health" FISABIO-University of Valencia, Valencia, Spain; Institute for Integrative Systems Biology (I2SysBio), CSIC-University of Valencia, Valencia, Spain

*Corresponding author at: Microbiology Service, Hospital Clínico Universitario, Instituto de Investigación INCLIVA. Av. Blasco Ibáñez 17, 46010 Valencia, Spain.

Vaccine Ab neutralization against Omicron and SARS-CoV-2 variants using neutralization and specific ELISA assays

Dear Editor,

In this Journal, Murray and colleagues recently described their validation of a commercially available indirect assay for SARS-CoV-2 neutralizing antibodies using a pseudotyped-virus assay [1]. Here, we report the use of different assays to determine the vaccine-induced antibodies ability to neutralize Variant Of Concerns (VOCs). Recently, it has been shown that Omicron is able to escape vaccine antibodies; one month after the third BNT162b2 (BioNTech-Pfizer) dose, neutralizing activity was 75% for Delta and below 50% for Omicron [2]. The better characterization of the humoral response beyond the level of antibodies is crucial.

The present study was performed on sera samples from nine healthcare workers (HCWs) from Bichat-Claude Bernard Hospital Virology department (Paris, France) vaccinated with BNT162b2. Samples were collected between first vaccine dose and up to one month after third dose. Anti-N serology was negative at all time-points (SARS-CoV-2 IgG kit, Abbott, IL, USA). All HCWs provided informed consents. Following viral supernatant production, SARS-CoV-2 was titrated by a lysis-plaque assay and live virus neutralization assay was performed as previously described [3,4]. A pseudoneutralization method, based on ACE-2 receptor binding inhibition (iFlash®−2019-nCoV Nab, YHLO, Shenzhen, China), measured the ability of antibodies to bind the RBD of the ancestral SARS-CoV-2 spike protein. The Meso-Scale-Discovery® (V-PLEX SARS-CoV-2 Panel13) assay enabled to assess pseudoneutralization activity of sera against different VOCs using a multiplexed Spike antigens technology. The CoViDiag kit (Innobiochips®, Loos, France) is a quantitative ELISA test detecting IgG against different VOCs including Omicron.

Nine BNT162b2-vaccinated HCW, were followed up to one month after the third vaccine dose, received in median 8.6 months (IQR=8.5–9.2) following the second dose. Kinetics of anti-S SARS-CoV-2 antibodies is described in Supplementary Fig. 1. We report the results of live virus neutralization for Omicron and others VOCs until one month after the third vaccine dose (Fig. 1A), extending our previous data [3,5]. We showed that Omicron neutralization titers were in median 16- and 8-fold lower than the Delta ones, one month after the second and one month after the third dose, respectively. We also extending our previous data [3] with the iFlash® pseudoneutralization assay showing an increased median of antibody titers from 44 BAU/mL (IQR=38–57) to 3134 (IQR=758–5109) before and one month after the third dose, respectively. The median antibody titer was significantly higher after the third than after the second dose (669 vs 3134 BAU/mL, $p = 0.02$) (Fig. 1B, Supplementary Table 1). One month after the second dose, using Meso-Scale-Discovery® assay, we showed a median percentage of inhibition of 63% (IQR=62–80), 60% (IQR=44–61), 42% (IQR=36–54), 29% (IQR=16–37) and 21% (IQR=14–40) for B, Alpha, Delta, Beta and Gamma variants, respectively. The median percentage of inhibition at the last available sample before the third dose was 24% (IQR=17–52), 19% (IQR=14–38), 9% (IQR=6–26), 12% (IQR=3–22), and 8% (IQR=0–19) for B, Alpha, Delta, Beta and Gamma variants, respectively (Fig. 1C). Using the CoViDiag® assay, one month after the second dose, antibodies median titers were 2500 BAU/mL (IQR=2500–6250), 2500 (IQR=2500–5437), 1695 (IQR=1459–2007), 1486 (IQR=1237–2192), 1918 (IQR=1852–2942), and 603 (IQR=545–1192) for B, Alpha, Delta, Beta, Gamma and Omicron variants, respectively (Fig. 1D). Then, a decrease was observed until right before the third dose, at which point median titers were 186 BAU/mL (IQR=161–250), 169 (IQR=120–250), 90 (IQR=48–111), 103 (IQR=69–166), 124 (IQR=71–241), and 50 (IQR=35–90) for B, Alpha, Delta, Beta, Gamma and Omicron, respectively. One month after the third dose the median titers were 6250 BAU/mL (IQR=4861–6724), 6250 (IQR=4928–6250), 3189 (IQR=2659–4868), 3757 (IQR=2451–6250), 4150 (IQR=2957–6250), and 1648 (IQR=1207–2700) for B, Alpha, Delta, Beta, Gamma and Omicron, respectively. The antibody titer for Omicron after the second dose was significantly lower than the titer for Delta (median fold-change=2.8; $p = 0.0008$), difference no longer observed after the third dose (median fold-change=1.9; $p = 0.12$). The median antibody titer after the third dose was higher than the titer reached after the second for all VOCs (Omicron: $p<0.0001$). The Spearman's correlation coefficient

A.

B.

C.

D.
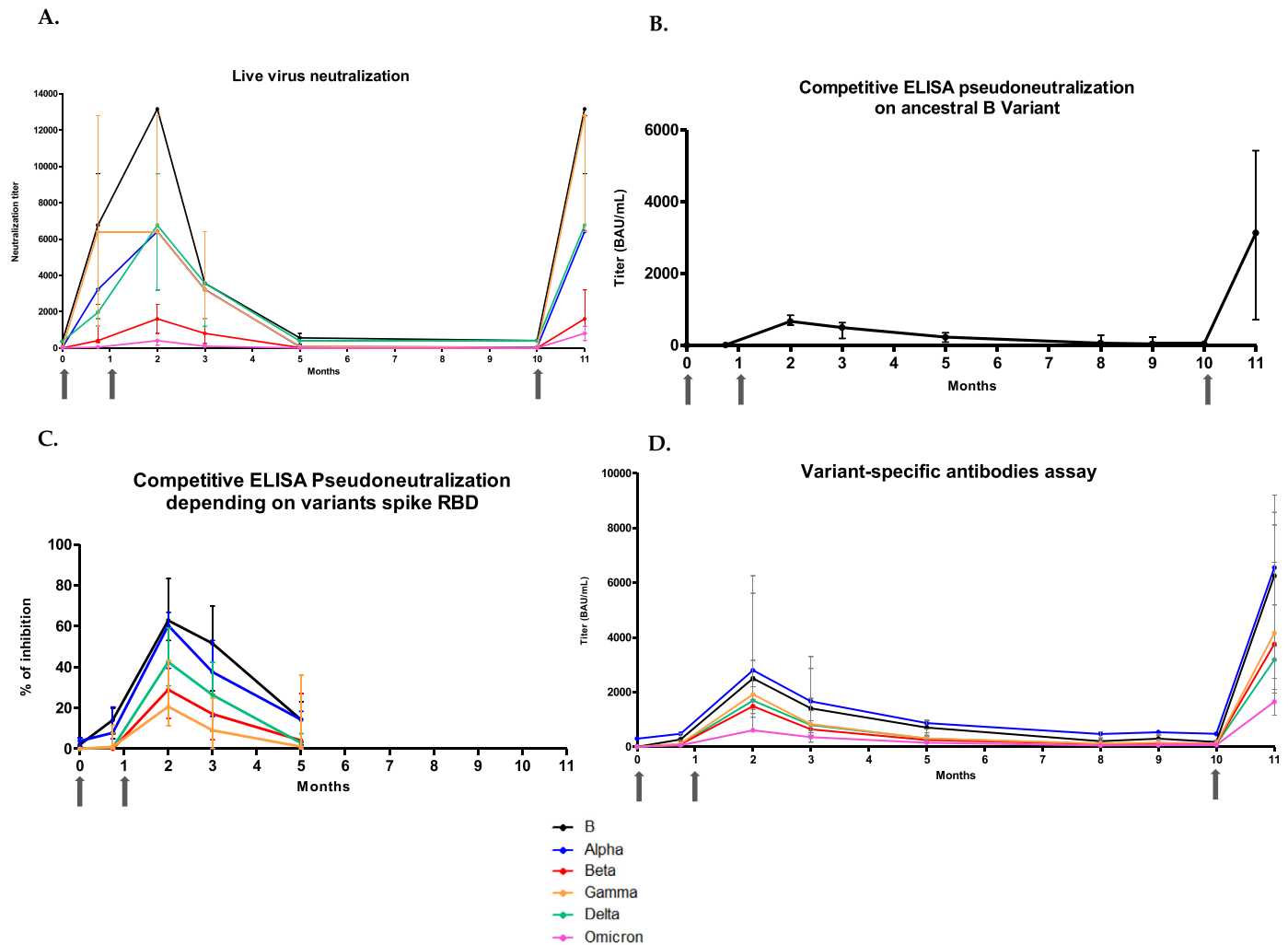
Fig. 1. Longitudinal follow-up of the humoral response of nine BNT162b2-vaccinated healthcare workers using: **A.** Live virus neutralization assay, **B.** iFlash® pseudoneutralization assay, **C.** Meso-Scale-Discovery® pseudoneutralization assay, and **D.** CoViDiag® variant-specific ELISA assay. The arrows represent the time of each vaccine dose.

between iFlash® and CoViDiag® titers for B variant was $\rho=0.95$ ($p < 0.0001$) (Fig. 2). The correlation coefficient between Meso-Scale-Discovery® percentages and CoViDiag® titers were $\rho=0.94$ ($p < 0.0001$), $\rho=0.90$ ($p < 0.0001$), $\rho=0.87$ ($p < 0.0001$), $\rho=0.80$ ($p < 0.0001$), and $\rho=0.81$($p < 0.0001$) for B, Alpha, Delta, Beta and Gamma (Fig. 2).

In this study, among nine BNT162b2-vaccinated HCW, the lowest neutralizing antibodies titers, after the second and third doses, were observed with Omicron as well with live virus neutralization as with a variant-specific ELISA assay. Furthermore, neutralizing antibodies titers were reduced with Delta and Omicron, responsible of the two last COVID-19 waves, in agreement with previous studies [6,7]. The present study showed that the humoral response level was significantly higher after the third dose than after the second dose, whatever the assay used, confirming the boosting effect of the third dose [8]. We showed a very good correlation between Meso-Scale-Discovery® pseudoneutralization and CoViDiag® variant-specific ELISA assays for all VOCs. We also showed a very good correlation for B variant between iFlash® pseudoneutralization and CoViDiag® assay. Furthermore, for Omicron, CoViDiag® titers agree with live virus neutralization, showing that Omicron is the variant that escapes the most to the post-vaccine humoral immunity.

To our knowledge, these are the first data comparing various serological assays, pseudoneutralization or variant-specific ELISA assays, to the reference method, live virus neutralization. All these assays, despite using different technologies and measuring different variables, showed the same trend in terms of humoral response magnitude and of VOC neutralizing ability levels. Beta VOC, already known to escape to vaccine-induced immunity [9], is indeed weakly neutralized by post-vaccine antibodies with the different assays used. This low neutralizing ability is also observed for Omicron, confirming first data observed with the current circulating VOC [6,7]. These data showed that the nature of humoral response can be characterized using more easy-to-use or automated assays than the live virus neutralization. Indeed, these assays could be interesting to monitor highly immunocompromised patients, populations for whom a serological follow-up is recommended by French Health Authorities [10].

In conclusion, since the good correlation observed with the live virus neutralization assay, pseudoneutralization or variant-specific ELISA assays could be useful to monitor the humoral response.

Funding

A.

B.

C.

D.

E.

F.
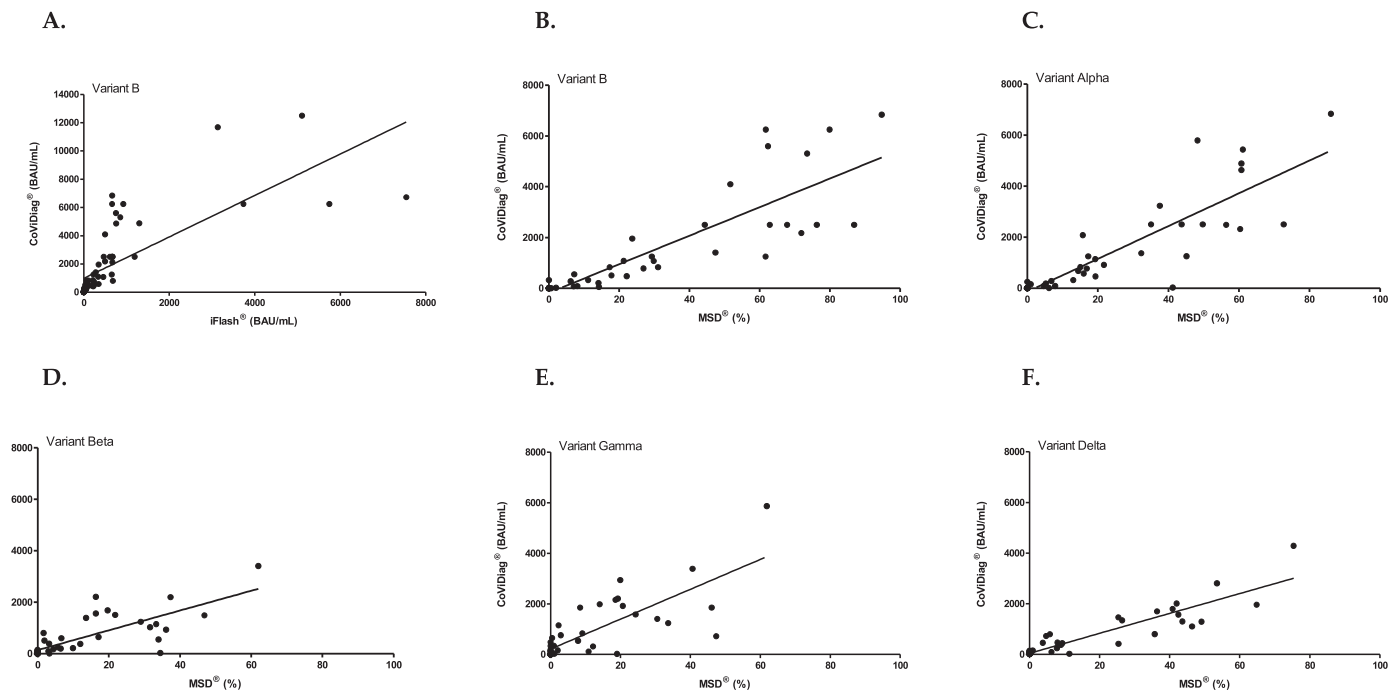
Fig. 2. Relationship between iFlash® and CoViDiag® assays for the historical B variant (Panel A), between Meso-Scale-Discovery® and CoViDiag® assays for the historical B variant (Panel B), Alpha variant (Panel C), Beta variant (Panel D), Gamma variant (Panel E), and Delta variant (Panel F) among nine BNT162b2-vaccinated healthcare workers.

Declaration of Competing Interest

The authors have no commercial or other associations that might pose a conflict of interest.

Acknowledgements

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.02.031.

References

1. Murray M.J., McIntosh M., Atkinson C., Mahungu T., Wright E., Chatterton W., et al. Validation of a commercially available indirect assay for SARS-CoV-2 neutralising antibodies using a pseudotyped virus assay. *J Infect* 2021;**82**:170–7.
2. Muik A., Lui B.G., Wallisch A.K., Bacher M., Mühl J., Reinholz J., et al. Neutralization of SARS-CoV-2 omicron by BNT162b2 MRNA vaccine–elicited human sera. *Science* 2022 Online ahead of print.
3. Ferré V.M., Lebourgeois S., Menidjel R., Collin G., Chenane H.R., Guindi M.O., et al. Decreasing humoral response among healthcare workers up to 4 months after two doses of BNT162b2 vaccine. *J Infect* 2021:S0163–4453.
4. Gordon D.E., Jang G.M., Bouhaddou M., Xu J., Obernier K., White K.M., et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature* 2020;**583**:459–68.
5. Lebourgeois S., Menidjel R., Chenane H.R., Ferré V.M., Collin G., Damond F., et al. Alpha (B.1.1.7) and delta (B.1.617.2 - AY.40) SARS-CoV-2 variants present strong neutralization decay at M4 post-vaccination and a faster replication rates than D614G (B.1) lineage. *J Infect* 2021 Online ahead of print.
6. Planas D., Saunders N., Maes P., Guivel-Benhassine F., Planchais C., Buchrieser J., et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. *Nature* 2021 Online ahead of print.
7. Khan K., Karim F., Cele S., San J.E., Lustig G., Tegally H., et al. Omicron infection enhances neutralizing immunity against the delta variant. *medRxiv* 2021 2021.12.27.21268439.
8. Accorsi E.K., Britton A., Fleming-Dutra K.E., Smith Z.R., Shang N., Derado G., et al. Association between 3 doses of mRNA COVID-19 vaccine and symptomatic infection caused by the SARS-CoV-2 omicron and delta variants. *JAMA* 2022 Online ahead of print.
9. Planas D., Bruel T., Grzelak L., Guivel-Benhassine F., Staropoli I., Porrot F., et al. Sensitivity of infectious SARS-CoV-2 B1.1.7 and B.1.351 variants to neutralizing antibodies. *Nat Med* 2021;**27**:917–24.
10. https://solidarites-sante.gouv.fr/IMG/pdf/cosv_-_recommandations_pour_la_protection_des_personnes_severement_immunodeprimees_-_19_novembre_2021.pdf

Valentine Marie Ferré
Samuel Lebourgeois
Houssem Redha Chenane
Reyene Menidjel
Christelle Masson
Gilles Collin
Benoit Visseaux
Diane Descamps
Nadhira Fidouh
Charlotte Charpentier*
Service de Virologie, Université de Paris, INSERM, IAME, UMR 1137, AP-HP, Hôpital Bichat-Claude Bernard, 46 Rue Henri Huchard, Paris F-75018, France
*Corresponding author.
E-mail address: charlotte.charpentier@aphp.fr (C. Charpentier)

Humoral and cellular responses to vaccination with homologous CoronaVac or ChAdOx1 and heterologous third dose with BNT162b2

Dear Editor,

We read with interest the recent manuscript of Mak et al. reporting SARS-CoV-2 antibody and T cell responses one year after COVID-19 convalescence and vaccination.[1] The authors showed that T cell and humoral responses persisted for up to one year after mild to moderate COVID-19, and that a single dose of COVID-19 vaccine induced robust responses, irrespective of the vaccine type (Ad26.CoV2, ChAdOx1, mRNA-1273 or BNT162b2). A second dose did not further increase cellular and humoral responses.

Despite the benefits of vaccination and immune protection due to convalescence, the spread of new variants such as Omicron[2] raise new questions about vaccine efficacy, immunity waning and the need of booster doses.[3] Studies on heterologous third dose with BNT162b2 after two doses of CoronaVac[4] (CV/CV/BNT) or ChAdOx1[5] (Ch/Ch/BNT) showed increased immunogenicity in both cases. Here, we corroborate these data and show that both heterologous vaccination schemes, largely adopted in Brazil[6], produced consistent increases in humoral and cellular responses after the third booster dose in our cohorts.

We conducted an observational study with a non-randomized cohort of 48 healthcare workers of regional hospitals and institutions, vaccinated with two homologous doses of CoronaVac (CV, $n = 25$, 72% females) or ChAdOx1 (Ch, $n = 23$, 87% females), and with a third heterologous dose of BNT162b2. For CV/CV/BNT and Ch/Ch/BNT, blood samples were collected according to the scheme shown on Fig. 1A. The dates of sample collection could not be completely synchronized between the CoronaVac and ChAdOx1 groups due to delays in vaccine distribution and due to differences in the vaccination schedules. Participants were tested for anti-S1 IgG at all time points. Neutralizing antibodies and cellular responses were evaluated at t3, t3', t4, and t4'. Assays were performed with Anti-S1 QuantiVac IgG, NeutraLISA and SARS-CoV-2 IGRA kits (EUROIMMUN). Statistical significance tests were performed using the non-parametric Wilcoxon–Mann–Whitney test (details on Supplementary Methods). All individuals enrolled in this study provided written informed consent as part of the protocols approved by the Ethics Committee of the Federal University of São Paulo and by the National Ethics Committee (CONEP, study number CAAE: 32,571,720.0.0000.5505).

The median age was 30 (25th–75th percentile: 24–41) years and 40 (35–53) years for the CV/CV/BNT and Ch/Ch/BNT groups, respectively. In the CV/CV/BNT group, the median anti-S1 IgG values increased from 19.8 BAU/ml (6.0–38.7, 7/24 positives) at t1 after the first dose to 429.0 BAU/ml (227.3–578.5, 25/25 positives) at t2 after the second dose ($p < 0.0001$) (Fig. 1B). From t2 to t3, the concentrations significantly decreased ($p < 0.01$) to 115.7 BAU/ml (62.3–184.5, 22/25 positives) (Fig. 1B). However, after the third booster dose at t4, the anti-S1 IgG concentration increased 25-fold ($p < 0.0001$) to 2843.0 BAU/ml (2738.2–2956.0, 19/19 positives) (Fig. 1B). The levels of neutralizing antibodies significantly increased ($p < 0.0001$) from 23.5% (13.4%–38.3%, 8/25 positives) at t3 to 99.3% (99.2%–99.3%, 19/19 positives) at t4 (Fig. 2A).

In the Ch/Ch/BNT group, the median anti-S1 IgG responses increased from 86.8 BAU/ml (53.0–280.1, 16/19 positives) at t1' to 648.9 BAU/ml (588.3–721.4, 21/21 positives) at t2' ($p < 0.0001$) (Fig. 1B). The anti-S1 IgG levels also decreased significantly ($p < 0.01$) to 390.9 BAU/ml (231.6–484.9, 19/20 positives) from t2' to t3' (Fig. 1B). After the third booster dose at t4', anti-S1 IgG levels increased 7-fold ($p < 0.0001$) to 2799.2 BAU/ml (2182.8–2832.3, 11/11 positives) (Fig. 1B). The levels of neutralizing antibodies increased ($p < 0.0001$) from 63.2% (46.8%–87.6%, 16/20 positives) at

t3' to 98.9% (range 98.6%–99.1%, 11/11 positives) at t4' (Fig. 2B). Additional anti-S1 IgA and anti-NCP IgG assays were also performed (Supplementary Figure).

Both CoronaVac and ChAdOx1 vaccines induced high cellular responses at t3 and t3', presenting median IFN-γ values of 778.9 mIU/ml (340.0–1092.2 mIU/ml, 21/25 positives) and 1232.7 mIU/ml (579.3–2663.2 mIU/ml, 19/20 positives) (Fig. 2B), respectively. The third booster dose with BNT162b2 significantly increased the IFN-γ levels to 4906.4 mIU/ml (4423.1–4928.6 mIU/ml, 19/19 positives) and 12,197.2 mIU/ml (3041.7–12,277.3 mIU/ml, 9/11 positives) in the CoronaVac ($p < 0.0001$ from t3 to t4) and ChAdOx1 ($p < 0.01$ from t3' to t4') groups, respectively (Fig. 2B).

A large population study in Brazil showed the importance of the massive vaccination campaign and of all vaccines for the prevention of severe COVID-19 and deaths.[7] However, it also indicated immune senescence for three of the vaccines currently in use in the country: CoronaVac, ChAdOx1, and BNT162b2, especially for CoronaVac.[7] We observed waning immunity >75 days after the second dose in CoronaVac or ChAdOx1 groups (Fig. 1B). However, the heterologous vaccination schemes CV/CV/BNT or Ch/Ch/BNT resulted in consistent increases in humoral (Figs. 1B and 2A) and cellular responses (Fig. 2B) after the third booster dose in both groups.

This study has several limitations. The small sample size of our cohorts did not allow the analysis of possible differences between sexes and stratification of age groups. Information on previous medical conditions was not systematically collected. The dates of sample collection could not be completely synchronized between the CoronaVac and ChAdOx1 groups. The trial is non-randomised and unblinded, which inhibits direct comparisons between the two vaccine groups. The age differences between the two groups also limits comparisons.

In conclusion, our study provides evidence that waning immunity after >75 days of the second doses of CoronaVac or ChAdOx1 vaccines can be strongly recovered by the administration of a heterologous booster dose of BNT162b2.

Declaration of Competing Interest

Acknowledgments

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.02.026.

References

1. Mak Willem A., Koeleman Johannes G.M., van der Vliet Marijke, Keuren Frans, Ong David S.Y. SARS-CoV-2 antibody and T cell responses one year after COVID-19 and the booster effect of vaccination: a prospective cohort study. *J Infect* 2022;**84**(2):171–8. doi:10.1016/j.jinf.2021.12.003.
2. Salim Karim, Abdool S., Abdool Karim Quarraisha. Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic. *Lancet* 2021;**398**(10317):2126–8. doi:10.1016/S0140-6736(21)02758-6/ATTACHMENT/B8C47DC8-B485-4240-95DD-FF7ADBB31E18/MMC1.PDF.
3. Smriti Mallapaty. China's COVID vaccines have been crucial — now immunity is waning. *Nature* 2021;**598**(7881):398–9. doi:10.1038/D41586-021-02796-W.
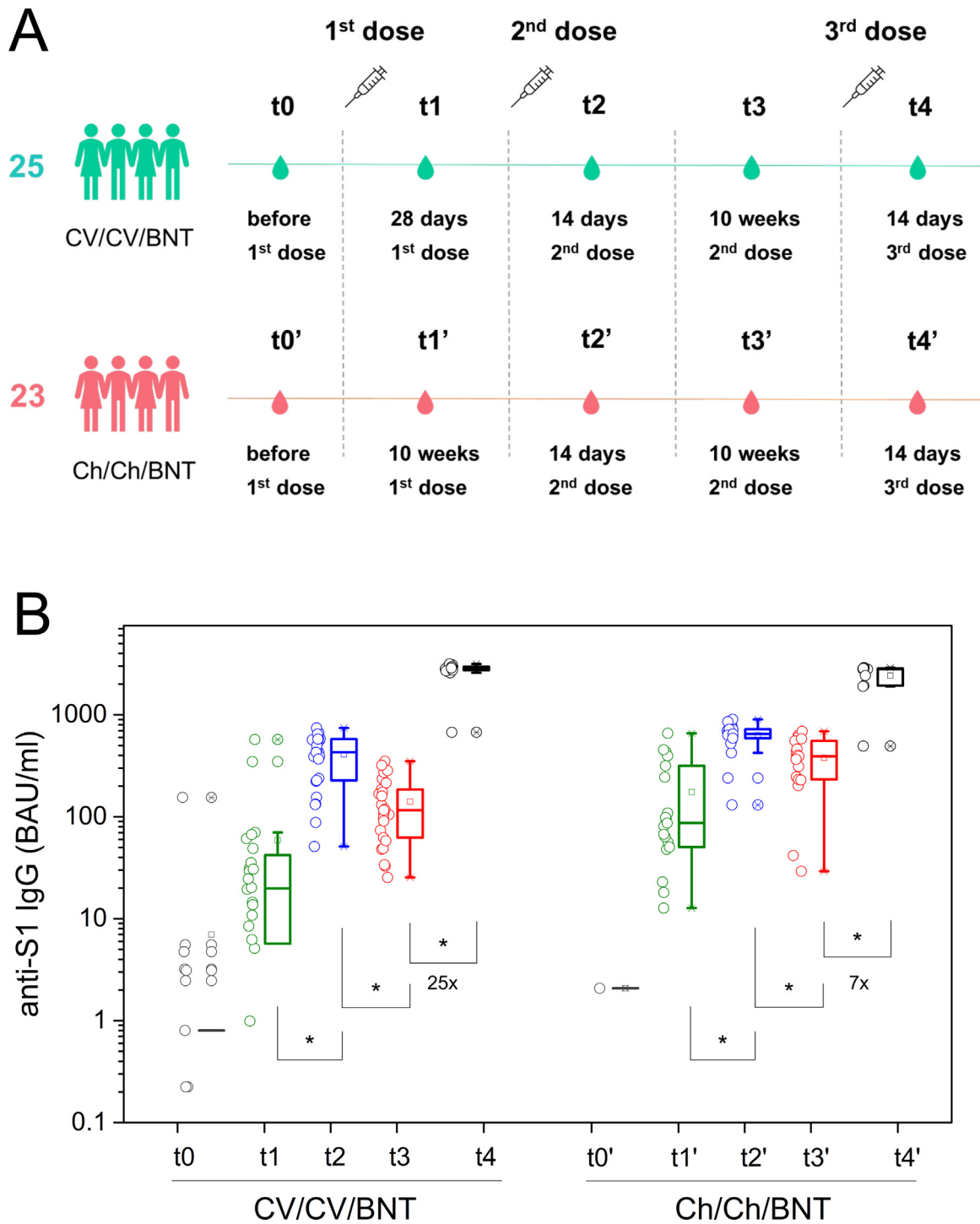
Fig. 1. (A) Experimental design of this study. Healthcare professional volunteers vaccinated with two homologous doses of CoronaVac and with a heterologous third dose of BNT162b2 (CV/CV/BNT) or with two homologous doses of ChAdOx1 and with a heterologous third dose of BNT162b2 (Ch/Ch/BNT). Blood samples were collected at t0–t4 for CV/CV/BNT and at t0'–t4' for Ch/Ch/BNT. Humoral and cellular responses were evaluated by the levels of anti-S1 IgG, neutralizing antibodies, and IFN-γ. (B) Dynamics of humoral responses in vaccinated volunteers after two homologous doses of CoronaVac followed by a third dose with BNT162b2 (CV/CV/BNT) or after two homologous doses of ChAdOx1 followed by a third dose with BNT162b2 (Ch/Ch/BNT).

4. Mok Chris Ka, Pun Cheng, Samuel M.S., Chunke Chen, Karen Yiu, Tat-On Chan, Cheung Lai Kiu, et al. A RCT of a third dose CoronaVac or BNT162b2 vaccine in adults with two doses of CoronaVac. *MedRxiv* 2021 2021.11.02.21265843. doi:10.1101/2021.11.02.21265843.

5. Munro Alasdair P.S., Leila Janani, Victoria Cornelius, Aley Parvinder K., Gavin Babbage, David Baxter, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *Lancet* 2021;**398**(10318):2258–76. doi:10.1016/S0140-6736(21)02717-3.

6. Thiago Cerqueira-Silva, Vittal Katikireddi Srinivasa, Vinicius de Araujo Oliveira, Renzo Flores-Ortiz, Bertoldo Júnior Juracy, Paixão Enny S., et al. Vaccine effectiveness of heterologous CoronaVac plus BNT162b2 in Brazil. *Nat Med* 2022:1–6. doi:10.1038/s41591-022-01701-w.

7. Villela Daniel A.M., Noronha Tatiana Guimarães de, Bastos Leonardo S., Pacheco Antonio G., Cruz Oswaldo G., Max Carvalho Luiz, et al. Effectiveness of mass vaccination in Brazil against severe COVID-19 cases. *MedRxiv* 2021 2021.09.10.21263084. doi:10.1101/2021.09.10.21263084.
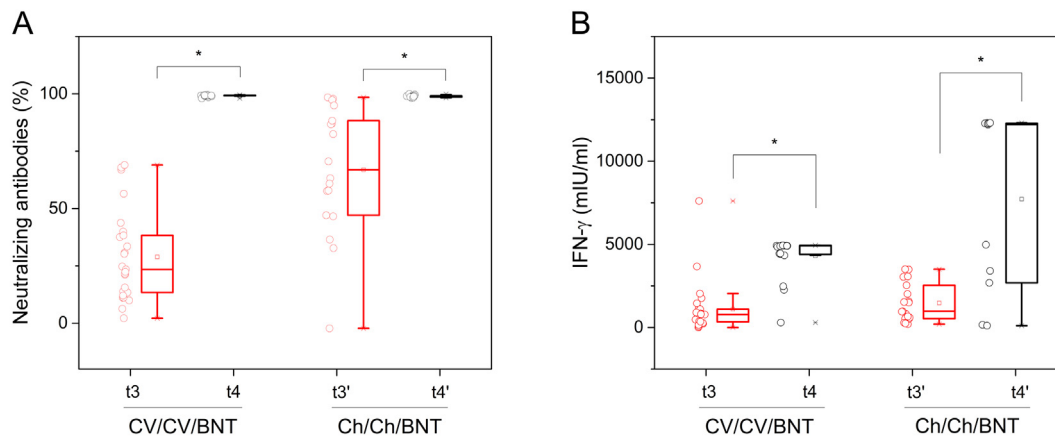
Fig. 2. (A) Levels of neutralizing antibodies and (B) IFN-γ after homologous vaccination with CoronaVac followed by a third dose with BNT162b2 (CV/CV/BNT) or after homologous vaccination with ChAdOx1 followed by a third dose with BNT162b2 (Ch/Ch/BNT).

Jackelinne Y. Hayashi, Adriana Simizo, Jackson G. Miyamoto
*Department of Biochemistry, Escola Paulista de Medicina,
Universidade Federal de São Paulo, São Paulo, Brazil*

Lucas V.S. Costa, Olívia F. Souza
*Department of Microbiology, Immunology and Parasitology, Escola
Paulista de Medicina, Universidade Federal de São Paulo, São Paulo,
Brazil*

Tassia Chiarelli
*Department of Biochemistry, Escola Paulista de Medicina,
Universidade Federal de São Paulo, São Paulo, Brazil*

Natália B.S. Bacarov, Rafaela Hidalgo, Letícia D. Garcia, Michel
M. Soane
EUROIMMUN Brasil, São Caetano do Sul, Brazil

Carla T. Braconi, Juliana T. Maricato, Luiz M. Janini
*Department of Microbiology, Immunology and Parasitology, Escola
Paulista de Medicina, Universidade Federal de São Paulo, São Paulo,
Brazil*

Reinaldo Salomão
*Division of Infectious Diseases, Department of Medicine, Escola
Paulista de Medicina, Universidade Federal de Sao Paulo, São Paulo,
Brazil*

Ana F. Popi
*Department of Microbiology, Immunology and Parasitology, Escola
Paulista de Medicina, Universidade Federal de São Paulo, São Paulo,
Brazil*

Isaias Glezer
*Department of Biochemistry, Escola Paulista de Medicina,
Universidade Federal de São Paulo, São Paulo, Brazil*

Tatiana Bonetti
*Division of Gynecology, Department of Medicine, Escola Paulista de
Medicina, Universidade Federal de Sao Paulo, São Paulo, Brazil*

Alexandre K. Tashima*
*Department of Biochemistry, Escola Paulista de Medicina,
Universidade Federal de São Paulo, São Paulo, Brazil*

*Corresponding author.
E-mail address:* aktashima@unifesp.br *(A.K. Tashima)*

https://doi.org/10.1016/j.jinf.2022.02.026

SARS-CoV-2 Omicron infection is associated with high nasopharyngeal viral load

Dear Editor,

We read with interest the article of Zheng et al.[1], who reported that a high breath emission rate of viral particles may be one of the most important reasons for justifying the higher transmissibility of the new severe acute respiratory coronavirus 2 (SARS-CoV-2) Omicron variant. Unfortunately, no direct comparison of viral load with previous SARS-CoV-2 variants has been reported in the work of Zheng et al., so that this conclusion remains intriguingly speculative.

In order to provide a direct comparison of the viral load in nasopharyngeal specimens of patients infected by Omicron and former SARS-CoV-2 variants, we retrospectively reviewed the results of SARS-CoV-2 testing conducted during two corresponding periods of years 2021 and 2022 (i.e., between 3 and 9 January), characterized by local prevalence of Alpha (> 95%; January 2021) and Omicron (>90%; January 2022) SARS-CoV-2 variants, respectively. Briefly, the study population consisted of all patients with a positive SARS-CoV-2 test performed between 3 and 9 January 2021 or 2022, respectively. Routine nasopharyngeal samples were collected according to standardized practice in subjects referred to the Pederzoli Hospital of Peschiera del Garda (Italy) for undergoing routine SARS-CoV-2 testing, for presenting symptoms of coronavirus disease 2019 (COVID-19) or for reporting close contact with SARS-CoV-2 positive subjects. In both these two years SARS-CoV-2 viral load was quantified using the same method and instrumentation (Seegene Allplex SARS-CoV-2 Assay; Seegene Inc., South Korea). This multiplex real-time polymerase chain reaction (PCR) assay detects four target genes of SARS-CoV-2 (*E, RdRP/S* and *N*) within a single sample, providing test results as cycle threshold (Ct) values. Additional technical and analytical characteristics of this method are comprehensively described elsewhere[2]. The cumulative viral

---

Abbreviations: SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; COVID-19, Coronavirus Disease 2019.
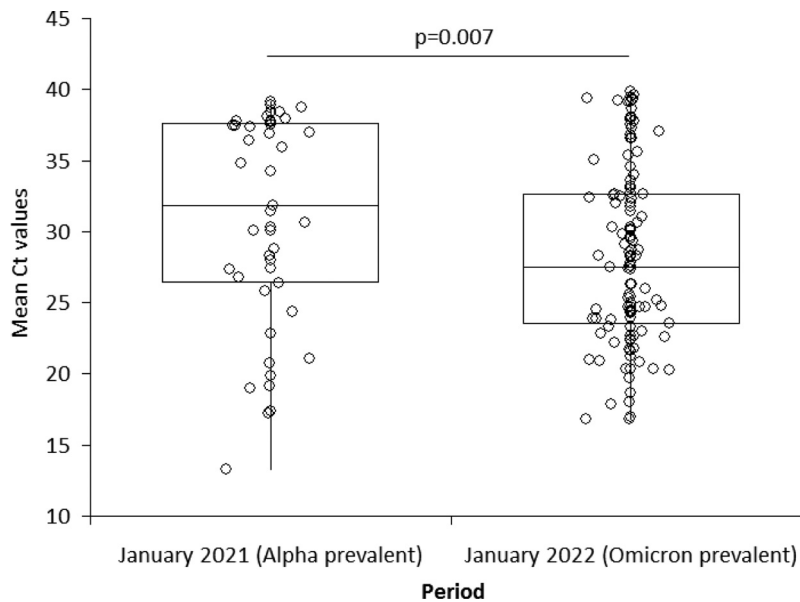
Fig. 1. Nasopharyngeal viral load measured in subjects infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) between 3–9 January 2021 (Alpha variant prevalent) and 3–9 January 2022 (Omicron variant prevalent)
Ct, cycle threshold.

load that we measured in nasopharyngeal swabs was expressed as mean Ct value of the different SARS-CoV-2 genes. Final results were reported as median and interquartile range (IQR), and compared with Mann-Whitney or Chi square tests, when appropriate, using Analyse-it software (Analyse-it Software Ltd, Leeds, UK). This retrospective study was conducted as part of routine clinical laboratory operations, using pre-existing nasopharyngeal specimens collected for systematic SARS-CoV-2 diagnostic testing at the local facility, so that patient informed consent and Ethical Committee approval were unnecessary. All test results were anonymized before statistical analysis. The study was conducted in accordance with the Declaration of Helsinki, under the terms of relevant local legislation.

The total number of patients with positive SARS-CoV-2 tests was 47 in January 2021 and 118 in January 2022, respectively, thus mirroring the dramatic surge of infections seen after local spread of the Omicron variant. Subjects with positive SARS-CoV-2 tests in 2021 were significantly older (median age, 63 years; IQR, 47–80 years) compared to those testing positive in 2022 (median age, 42 years; IQR, 25–53 years; $p < 0.001$), whilst the sex distribution was similar (females: 68% in 2021 vs. 58% in 2022; $p = 0.107$). The mean viral load of the genes measured with the Allplex SARS-CoV-2 Assay is reported in Fig. 1, showing that the Ct values in January 2022 (median Ct value, 27.5; IQR, 23.5–32.7) when the Omicron variant was prevalent were significantly lower than those measured during the same period of the year 2021 (median Ct value, 31.8; IQR, 26.4–37.6; $p = 0.007$), when the Alpha variant was prevalent. Importantly, the rate of subjects with high nasopharyngeal viral load (i.e., Ct values < 25) was over 2-fold higher in January 2022 than in January 2021 (45/118 vs. 10/47; Odds ratio, 2.28 and 95%CI, 1.03–5.03; $p = 0.041$).

In conclusion, the results of our retrospective analysis provide convincing support to the suggestion that aerosols released by patients infected by SARS-CoV-2 Omicron variant may contain higher viral particles than those released by subjects infected with previous SARS-CoV-2 strains, thus providing a solid biological background to justify enhanced transmissibility of this new linage and higher prevalence of upper respiratory tract symptoms reported in other studies.[3,4]

Declaration of Competing Interest

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

Funding

None declared.

Acknowledgments

None

References

[1]. Zheng J., Wang Z., Li J., et al. High amounts of SARS-CoV-2 in aerosols exhaled by patients with Omicron variant infection. *J Infect* 2022 Feb 17:S0163-4453(22)00075-5Epub ahead of print. doi:10.1016/j.jinf.2022.02.015.

[2]. Liotti F.M., Menchinelli G., Marchetti S., et al. Evaluation of three commercial assays for SARS-CoV-2 molecular detection in upper respiratory tract samples. *Eur J Clin Microbiol Infect Dis* 2021;**40**:269–77.

[3]. Migueres M., Raymond S F., Lhomme S., Mansuy J.M., Izopet J., et al. Influence of immune escape and nasopharyngeal virus load on the spread of SARS-CoV-2 Omicron variant. *J Infect* 2022 Feb 7:S0163-4453(22)00054-8Epub ahead of print. doi:10.1016/j.jinf.2022.01.036.

[4]. Lippi G., Nocini R., Henry B.M.. Is SARS-CoV-2 Omicron (B.1.1.529) variant causing different symptoms? *J Infect* 2022 Feb 17:S0163-4453(22)00077-9Epub ahead of print. doi:10.1016/j.jinf.2022.02.011.

Gian Luca Salvagno
Section of Clinical Biochemistry, University of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy
Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy

Brandon M. Henry
Disease Intervention & Prevention and Population Health Programs, Texas Biomedical Research Institute, San Antonio, TX, USA
Section of Clinical Biochemistry and School of Medicine, University of Verona, Italy

Laura Pighi, Simone De Nitto
Section of Clinical Biochemistry, University of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy

Service of Laboratory Medicine, Pederzoli Hospital, Peschiera del Garda, Italy

Martina Montagnana, Giuseppe Lippi*
Section of Clinical Biochemistry, University of Verona, Piazzale L.A. Scuro, 10, Verona 37134, Italy

*Corresponding author.
E-mail addresses:* giuseppe.lippi@univr.it, ulippi@tin.it (G. Lippi)

National mortality data for Germany before and throughout the pandemic: There is an excess mortality exceeding COVID-19-attributed fatalities

Dear Editor,

An increased all-cause mortality beyond previous years' levels was reported for several European countries within the first half of 2020 corresponding to the early phase of the Severe acute respiratory syndrome-Coronavirus-2 (SARS-CoV-2) pandemic. [1] However, more recent studies suggested that excess mortality was only partly to be explained by deaths directly attributed to the Coronavirus Disease 2019 (COVID-19).[2] In this light, our group investigated temporal evolution of excess deaths showing an additional increase in mortality in the second half of 2020 in Germany, which did not correspond with the number of deaths directly related to SARS-CoV-2-infections.[3] With the current letter, we expand this analysis by examining data through December 2021 in the ongoing pandemic situation.

Nationwide open access data from the German Federal Bureau of Statistics depicted daily and weekly all-cause deaths.[4] Data of full weeks from January to December 2021 was extracted and compared to averaged death counts from corresponding weeks in 2016–2019 representing the pre-pandemic control period. Emerging SARS-CoV-2-infections and deaths attributed to COVID-19 were daily reported by the Robert-Koch-Institute (RKI).[5] Information on SARS-CoV-2-infections was gathered from July 2020 on to examine a lagged influence on mortality. Incidences of infections per 100,000 inhabitants within a federal state were used to calculate tertiles defining low, intermediate and high COVID-19 case volume. Locally Estimated Scatterplot Smoothing curves (LOESS, degree of smoothing $\alpha = 0.25$) with 95% confidence intervals (CI) were fitted illustrating daily fatalities. Inferential statistics were based on generalized linear mixed models (GLMM) and Poisson GLMMs with log link function. Effects were estimated with the lme4 package (version 1.1–26) in the R environment for statistical computing (version 4.0.2). Varying intercepts were specified for random factors. Another model was employed for each factor with the variables period, treatment contrasts for the factor levels and the corresponding interactions. Incidence rate ratios (IRRs, calculated by exponentiation of the regression coefficients) were reported with 95% CIs. Mortality prediction implementing lagged SARS-CoV-2-infection rates and the computation of sliding IRRs were performed via Poisson GLMMs. For the calculation of sliding IRRs for a 12-week range, the dataset has been expanded by mortality data of 2020.

Compared to previous years, there was an overall excess all-cause mortality (IRR 1.090, 95%CI 1.087–1.093, $p < 0.01$) that persisted after the exclusion of deaths officially attributed to COVID-19 (IRR 1.022, 95%CI 1.019–1.025, $p < 0.01$). The latter was driven by an increase in daily deaths between the end of April and July and between August and December as illustrated by LOESS curves (Fig. 1, Panel A). Calculating sliding IRRs for 12-week intervals (Fig. 1, Panel B), a reduced risk of all-cause death excluding COVID-19-attributed fatalities was shown for the first months in 2021 (period 1: 01/04/2021–03/21/2021, IRR 0.922, 95%CI 0.916–0.927, $p < 0.01$), while there was an ongoing increase of IRRs from the end of March on (period 2: 03/29/2021–01/02/2022, IRR 1.059, 95%CI 1.056–1.063, $p < 0.01$). Non-COVID-19-attributed mortality was associated with higher age (≥ 80 years, IRR for interaction 1.154, 95%CI 1.122–1.187, $p < 0.01$), male sex (IRR for interaction 1.033, 95%CI 1.027–1.039, $p < 0.01$) and treatment in a region with higher COVID-19 case volume (IRR for interaction 1.018, 95%CI 1.012–1.025, $p < 0.01$). These associations were consistent when analyzing the two different periods in 2021 that were based on the sliding IRR analysis. When calculating prediction models for all-cause mortality including age, gender, weekly indices and the number of SARS-CoV-2-infections with different preceding time intervals, lagged infection incidences had a significant influence on mortality with the strongest influence after a delay of 4 weeks (Fig. 2). Another increase in IRRs for mortality was related to the incidence of SARS-CoV-2-infections after a lag of 19 weeks and more.

We present an up-to-date analysis comparing mortality statistics for 2021 and a pre-pandemic control period that shows an excess mortality in 2021 even after the exclusion of deaths attributed to COVID-19 cases. This corresponds to the results of our earlier work, although the effect is currently even more pronounced with a significant excess mortality in the second half of 2021.[3] Interestingly, an analysis of inpatient data showed no increased in-hospital mortality in a German multicentric hospital database, which could led to the assumption of a shift of deaths to the outpatient setting.[6] Both an assumed avoidance of patients to enter the health care system fearing nosocomial viral transmission and extensive changes in patient pathways throughout different disease groups including the widespread postponement of planned procedures could contribute to our findings. This is supported by the observation of a parallel increase of cardiovascular excess mortality and COVID-19-related deaths and the fact that non-COVID-19 excess mortality has been linked to the prevalence of comorbidities in datasets from the United States.[7,8] The reduction of acute hospitalizations for cardiovascular diseases and the deficit of cardiovascular interventions during the pandemic indicating a lower quality of overall patient care could contribute to the increased cardiovascular death rates. Postponed treatments were also reported in other medical fields including oncology.

Another possible explanation would be an underestimation of COVID-19-related deaths due to a lacking detection of infections.[9] In this light, a recent study from Sanmarchi and colleagues found an association of viral test capacity and a divergence of excess mortality and counts of fatal SARS-CoV-2-infections.[10] This might be an explanation for the observation that infection incidences are associated with all-cause mortality with a time lag of few weeks even after the exclusion of COVID-19-attributed deaths. Lastly, delayed effects and sequelae of past SARS-CoV-2-infections may contribute to an increased mortality at a later time by different mechanisms,
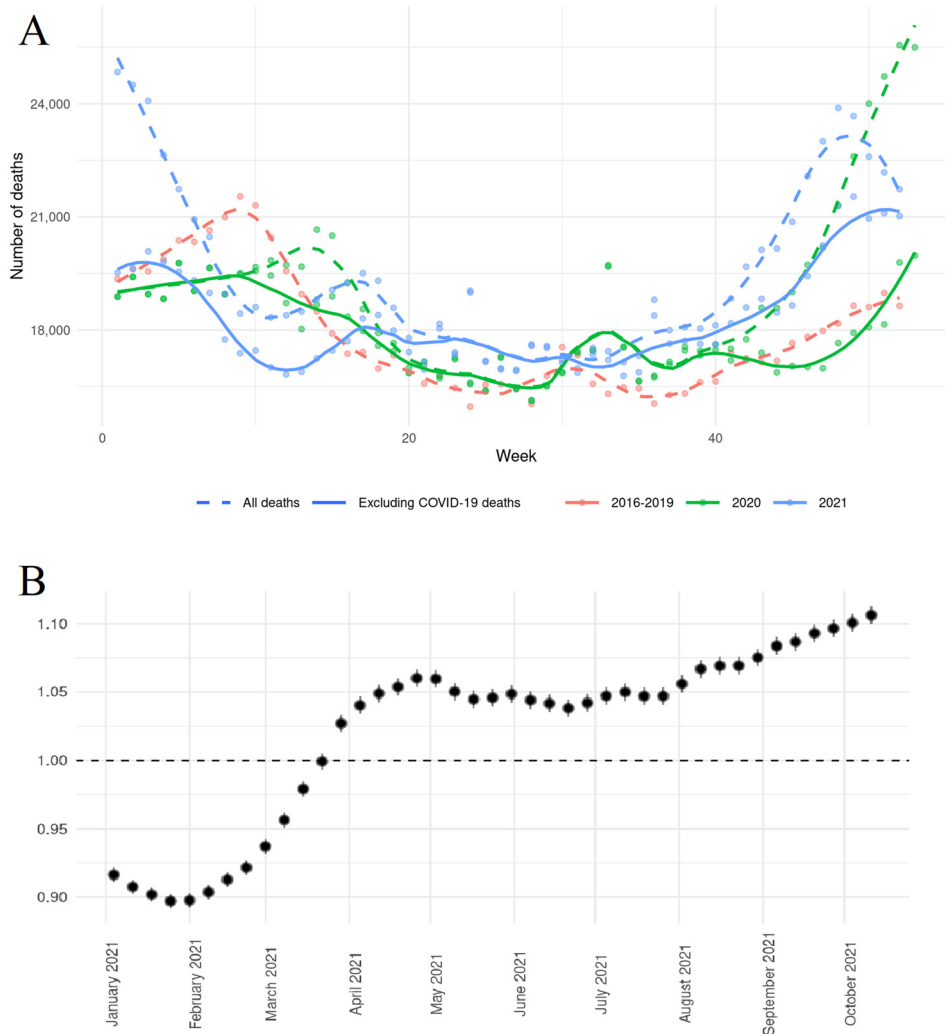
Fig. 1. LOESS curves for weekly mortality counts comparing 2016–2019 with 2020 and 2021 (Panel A). Sliding IRR analysis of all-cause mortality for 12-week intervals (dots are placed at the starting date of the time interval, bars represent 95% CIs) excluding deaths attributed to SARS-CoV-2 (Panel B).

but were likely not counted as COVID-19-related deaths. The late re-increase of IRRs in the analysis of lagged effects of SARS-CoV-2 infections on mortality may reflect such sequelae.

Whatever the reason is, these observations are noteworthy and require a further evaluation in order to adequately respond to it in the future.

## Declaration of Competing Interest

Nothing to declare

Funding

There has been no funding in connection with this study.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

References

1. Vestergaard L.S., Nielsen J., Richter L., Schmid D., Bustos N., Braeye T., et al. Excess all-cause mortality during the COVID-19 pandemic in Europe - preliminary pooled estimates from the EuroMOMO network. March to April 2020. *Euro Surveill* 2020;**25**(26).

2. Gallo E., Prosepe I., Lorenzoni G., Acar A.S., Lanera C., Berchialla P., et al. Excess of all-cause mortality is only partially explained by COVID-19 in Veneto (Italy) during spring outbreak. *BMC Public Health* 2021;**21**(1):797.

3. Konig S., Hohenstein S., Ueberham L., Hindricks G., Meier-Hellmann A., Kuhlen R., et al. Regional and temporal disparities of excess all-cause mortality for Germany in 2020: is there more than just COVID-19? *J Infect* 2021;**82**(5):186–230.

4. Statistisches Bundesamt. Wöchentliche Sterbefallzahlen 2021 [Available from: https://www.destatis.de/DE/Themen/Querschnitt/Corona/Gesellschaft/bevoelkerung-sterbefaelle.html. [last access February 3rd 2022]

5. Robert-Koch-Insitut. COVID-19: case numbers in Germany https://www.rki.de/DE/Content/InfAZ/N/Neuartiges_Coronavirus/Fallzahlen.html: Robert-Koch-Institut; [last access February 3rd, 2022]

6. Konig S., Pellissier V., Hohenstein S., Leiner J., Hindricks G., Meier-Hellmann A., et al. A comparative analysis of in-hospital mortality per disease groups in Germany Before and during the COVID-19 pandemic from. 2016 to 2020. *JAMA Netw Open* 2022;**5**(2):e2148649.

7. Stokes A.C., Lundberg D.J., Elo I.T., Hempstead K., Bor J., Preston S.H.. COVID-19 and excess mortality in the United States: a county-level analysis. *PLoS Med* 2021;**18**(5):e1003571.

8. Zhu D., Ozaki A., Virani S.S.. Disease-specific excess mortality during the COVID-19 pandemic: an analysis of weekly US death data for 2020. *Am J Public Health* 2021;**111**(8):1518–22.

9. Iuliano A.D., Chang H.H., Patel N.N., Threlkel R., Kniss K., Reich J., et al. Estimating under-recognized COVID-19 deaths, United States, March 2020-May 2021 using an excess mortality modelling approach. *Lancet Reg Health Am* 2021;**1**:100019.

10. Sanmarchi F., Golinelli D., Lenzi J., Esposito F., Capodici A., Reno C., et al. Ex-
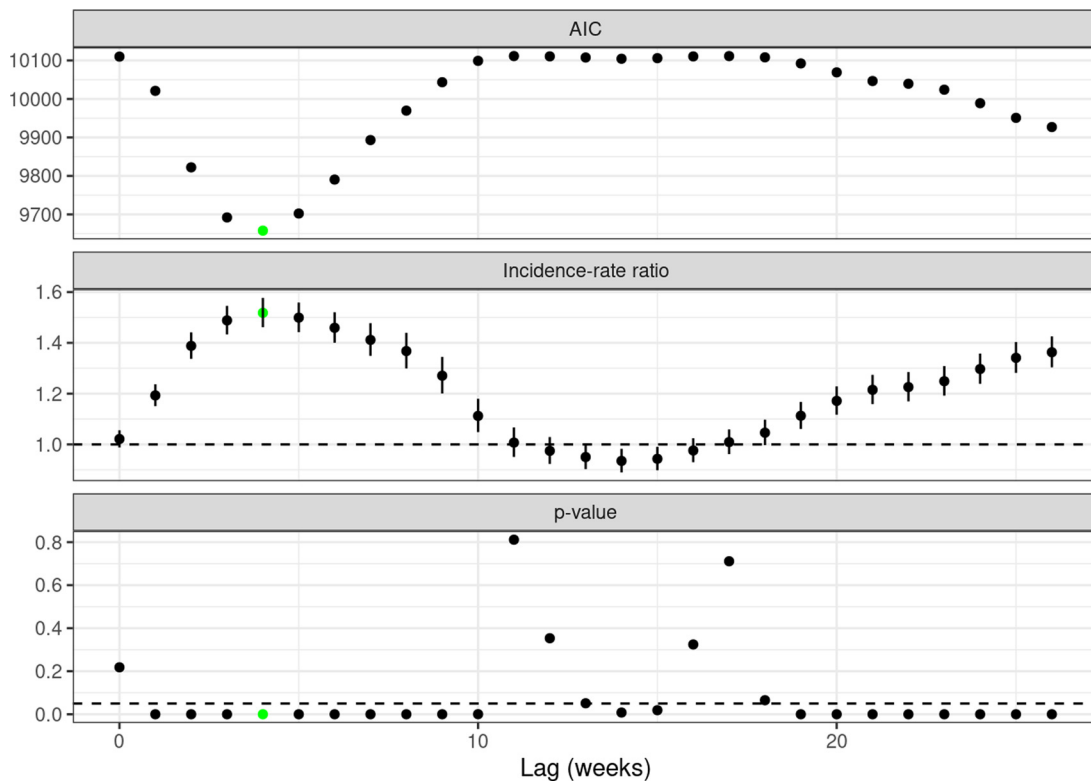
Fig. 2. Prediction model for all-cause mortality (excluding SARS-CoV-2-attributed deaths) implementing different lags for incidences of SARS-CoV-2-infections. IRRs are given for an increase of 100,000 SARS-CoV-2-infections.
AIC: Akaike's information criterion (lower values indicate better model performance).

ploring the gap between excess mortality and COVID-19 deaths in 67 countries. *JAMA Netw Open* 2021;**4**(7):e2117359.

Sebastian König*
Department of Electrophysiology, Heart Center Leipzig at University of Leipzig, Strümpellstraße 39, Leipzig 04289, Germany
Leipzig Heart Institute, Leipzig, Germany

Sven Hohenstein
Leipzig Heart Institute, Leipzig, Germany

Johannes Leiner, Gerhard Hindricks
Department of Electrophysiology, Heart Center Leipzig at University of Leipzig, Strümpellstraße 39, Leipzig 04289, Germany
Leipzig Heart Institute, Leipzig, Germany

Andreas Meier-Hellmann
Helios Hospitals, Berlin, Germany

Ralf Kuhlen
Helios Health, Berlin, Germany

Andreas Bollmann
Department of Electrophysiology, Heart Center Leipzig at University of Leipzig, Strümpellstraße 39, Leipzig 04289, Germany
Leipzig Heart Institute, Leipzig, Germany

*Corresponding author at: Department of Electrophysiology, Heart Center Leipzig at University of Leipzig, Strümpellstraße 39, Leipzig 04289, Germany.
E-mail address: sebastian.koenig@helios-gesundheit.de (S. König)