Letters to the Editor

Probable close contact transmission in a restaurant in China

Dear Editor,

Recently, we published a study[1] regarding the probable long-range airborne transmission in a restaurant in China by filtering out both close contact and fomite routes. In this specific context, we found that close contact transmission may predominate a COVID-19 outbreak caused by the B.1.617.2 (Delta) variant in a China's restaurant via 133,446 human behavior data points relevant to virus transmission (surface touching and close contacts) from analysis of video recordings.
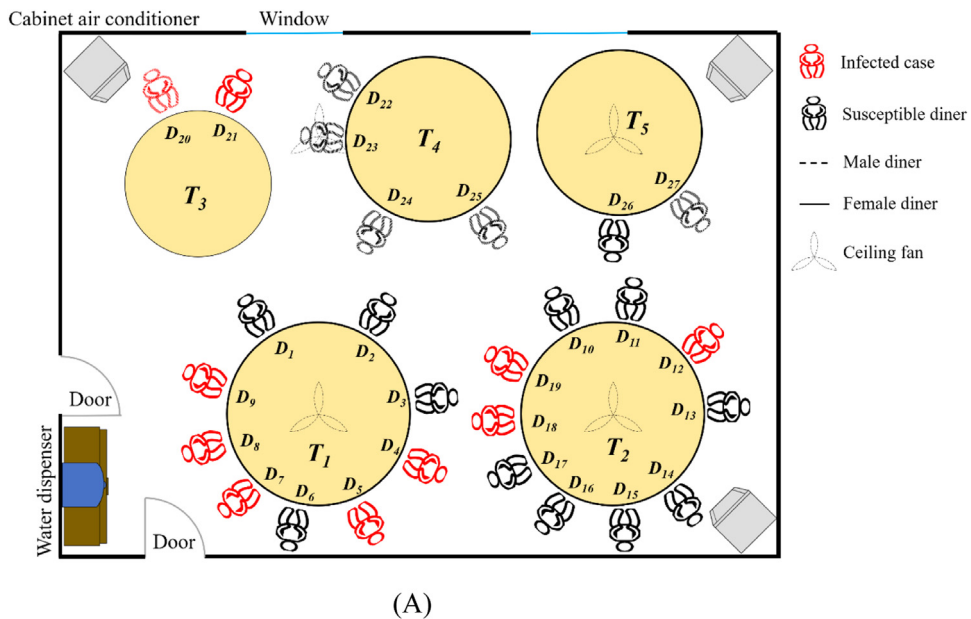
(A)
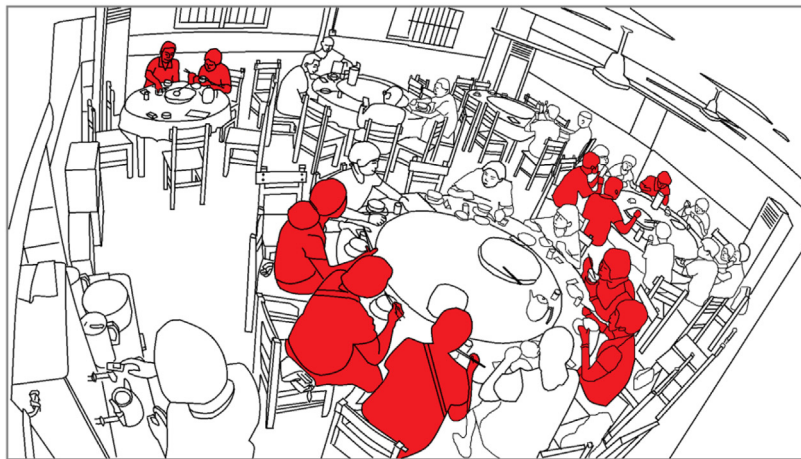
(B)

Fig. 1. Setting of the restaurant. (A) Floor plan; (B) image obtained from the video camera.

(A)

(B)

Fig. 2. Virus transmission potential via close contact and fomite routes. (A) total duration between possible source infectors (D_4 and D_{20}) and other diners and staff; (B) Virus transmission via the fomite route for different source infectors.

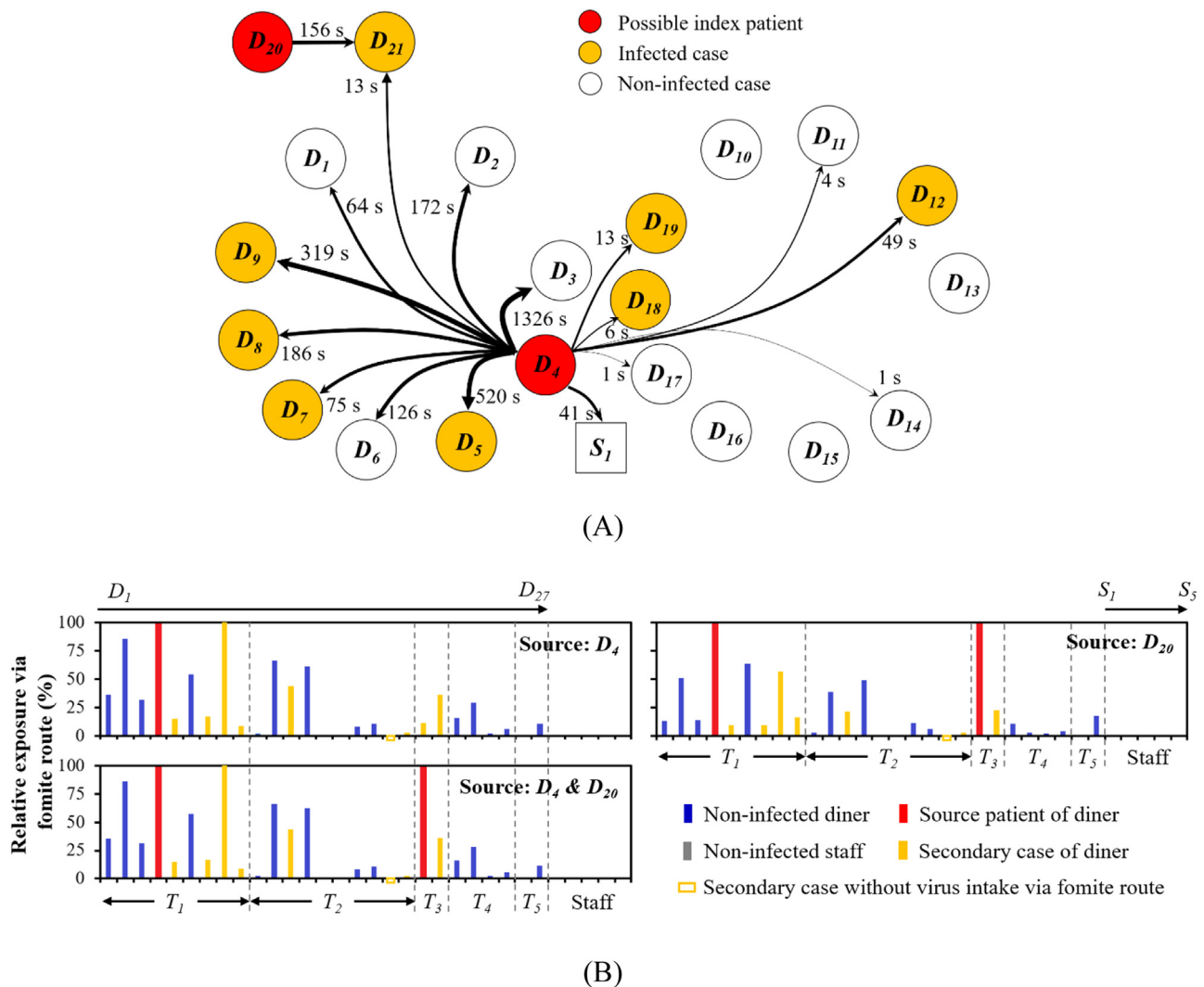Restaurants are a high-risk indoor environment for SARS-CoV-2 transmission, due to frequent close contact, crowding and masking impossibility.[2,3] However, it is difficult to identify whether it was close contact or fomite transmission that dominated COVID-19 outbreaks in restaurants, due to a lack of data of indoor human behavior.

A COVID-19 outbreak caused by the B.1.617.2 (Delta) variant in a restaurant in Guangzhou on May 23, 2021 resulted in 10 diners being infected (Fig. 1, Appendix A). Human behaviors on close contact and surface touching were collected by video recordings during the lunch time (Appendix B). Combining the data on human behaviors and established indoor transmission model (Appendix C), the virus exposure via close contact, fomite, and long-range airborne route of all diners and staff was estimated.

According to the epidemiological investigation of the outbreak and the gene sequencing results, D_4 was the most likely infectious source for the outbreak. However, since D_{20} may be an infectious source, we considered a total of 15 scenarios (Appendix D).

A total of 3108 close contacts with the total duration of 29,362 s were identified (Appendix E). D_{12} and D_{13} were involved in the most close-contacts (110), while D_{26} and D_{27} had the longest close contact duration of 2340s. Focusing on the possible source infectors (D_4 and D_{20}), we noted no close contact between them and diners sitting at Tables 4 and 5, and no diners sitting at Tables 4

and 5 were infected. D_4 had a close contact of only 41 s with S_1, no other close contacts between D_4/D_{20} and staff were identified (Fig. 2A). Close contact was likely to be the dominant virus transmission route in the restaurant when only D_4 or both D_4 and D_{20} were the infectious source (with $p < 0.01$ or < 0.05). D_{20} was not the only source of infection in the restaurant outbreak. If D_4 was the source, D_3 would have had the highest virus exposure via both short-range inhalation and mucous deposition during the lunch, but D_3 did not get infected. If D_{20} was the only source, only D_{21} would have been exposed to the virus via the close contact route, which was also an impossibility. If D_4 or both D_4 and D_{20} were the sources, D_{18} would have had the lowest exposure via both short-range inhalation (0.35% of exposure of D_4) and mucous deposition (0.30% of exposure of D_4).

A total of 14,687 touches with a total duration of 206,334 s were identified (Appendix F). Diners and staff on average spent 84.4% (91.5%) and 78.9% (85.6%) of their indoor time touching surfaces with their left (right) hand. The simulation results for virus intake fraction via the fomite route for different source infectors is shown in Fig. 2B. Only one out of five staff (S_4) had any fomite route virus exposure, because the other four staff members did not touch their mucosa during the lunch period. Infected diner, D_{18} never touched her mucosa during the lunch period either. We found that the average intake fraction by infected people was only

80.5% (30.8% - 154.4%) higher than it of susceptible people, and that there was no significant difference in virus exposure between the susceptible and the infected for all scenarios.

With an indoor ventilation rate of more than 5 ACH (6 L/s per person), the indoor virus concentration was no more than 1400/m³. Compared with exposure via short-range inhalation, long-range inhalation contributed relatively little (0.3%) (Appendix G). The fomite route was not the main transmission route in this outbreak, but that close contact may be. D_4 or both D_4 and D_{20} were the most likely source infectors of this outbreak.

It is believed that close contacts, which include short-range airborne, are a high risk transmission route.[4,5] Restaurants may have an extremely high rate of SARS-CoV-2 exposure via the close contact route because of high close contact rate (20%) without masks.[1] In offices, people spend around 10% of their time in close contact.[6]

To quickly track close contacts in crowded and poorly ventilated indoor environments such as a restaurant, QR codes (location codes), which are unique to each public place, can be used. After people scan the location code with a mobile device, an electronic record including basic information such as arrival and departure times, would be generated for a specific indoor environment. This information could be used solely for public health responses. On the other hand, previous studies[7] have shown that vaccination is effective in COVID-19 prevention and control. To reduce exposure via the close contact route in restaurants, some cities implemented strategies such as limiting the number of diners (e.g. restaurant cannot fill more than 50% of its capacity), tables were required to be at least 1.5 m apart,[8] and dividers to be put on tables to separate diners.[9]

In general, this study showed that close contact was the main transmission route of the outbreak (B.1.617.2 (Delta) variant) in the restaurant, although a previous study[1,10] showed that long-range airborne route dominated the outbreak (initial strain: NC_045512) in another restaurant. This may be explained in the difference in ventilation. the indoor ventilation in the previous outbreak was very poor, at 0.9 L/s per person.[10] In this newly studied outbreak the ventilation is much better in the restaurant. The restaurant has large doors, which were open. Obviously, involved variants of SARS-CoV-2 are different in the two outbreaks. One would expect some differences in the main transmission route, but there is no supporting evidence.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.08.029.

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Comparison of the amount of SARS-CoV-2 exhaled by Delta and Omicron patients

Dear Editor,

In this Journal, Salvagno GL and colleagues supposed Omicron variant patients might exhale more viral particles than previous variant patients based on the higher nasopharyngeal viral load in Omicron infections.[1] Unfortunately, they did not directly compare the viral load of exhaled breath from COVID-19 patients. In this study, the amount of SARS-CoV-2 exhaled by Omicron and Delta patients was compared. Our findings may help to explain why the Omicron variant is more transmissibility than the Delta variant.

The Omicron SARS-CoV-2 variant (B.1.1.529) was first reported by the World Health Organization (WHO) from South Africa on 24 November 2021 (https://www.who.int/news/item/26–11–2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern). The Delta variant began decreasing, whereas the Omicron variant continued its increase. The Omicron variant has replaced the Delta variant and became clearly a dominant variant.[2] It was reported that the effective reproduction number (R_e) and basic reproduction number (R_0) of the Omicron variant elicited 3.8 and 2.5 times higher transmissibility than the Delta variant, respectively.[3] The Omicron variant possesses 100-fold greater than the Delta variant in transmissibility the increased transmissibility of SARS-CoV-2 Delta variants was related to the higher viral loads.[4,5] However, the reasons driving the increased transmissibility of the Omicron variant are still not well understood, and the viral load in exhaled breath of patients with Omicron and Delta has not been compared.

Here, serial exhaled breath specimens and nasopharyngeal swabs were taken at 1, 3, 5, 7, 9, 11, and 13 days post hospitalization (dph) from 5 Delta and 8 Omicron patients. The Delta patients were selected from the group of COVID-19 patients in our previous study.[6] Exhaled breath specimens were collected for 5 min from all the patients by using a BioScreen device (Dingblue Technology Co., LTD, Beijing, China). SARS-CoV-2 RNA was extracted by using a viral RNA extraction kit (Tguide, TIANGEN) and detected by a SARS-CoV-2 nucleic acid detection kit (PCR-Fluorescence Probing) (Sansure Biotech, Hunan, China), targeting both the N and ORF1ab genes. A standard curve was fitted using a series of 10-fold dilutions of a standard plasmid DNA containing N genes of SARS-CoV-2. The numbers of viral RNA copies in the samples were estimated from the measured cycle threshold (Ct) values.
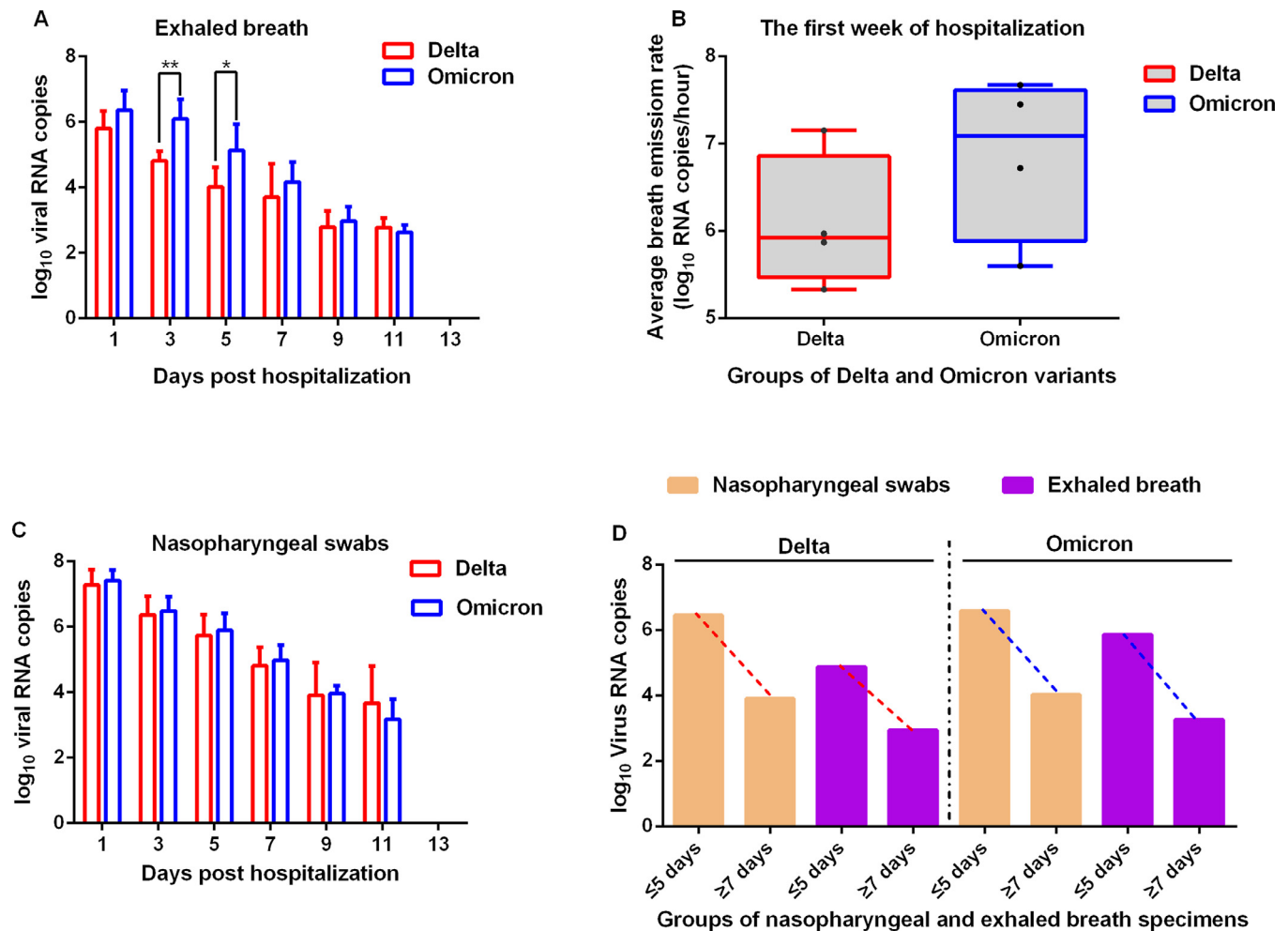
Fig. 1. Viral load of exhaled breath and nasopharyngeal swabs from COVID-19 patients with Delta or Omicron. (A) Comparison of SARS-CoV-2 exhaled by Delta and Omicron variant patients. (B) The average breath emission rate of patients with Delta or Omicron after the first week of hospitalization. (C) Comparison of nasopharyngeal viral load of patients with Delta and Omicron. (D) Variation trends of viral load in nasopharyngeal swabs and exhaled breath from patients with Delta or Omicron. Statistical analysis was performed using GraphPad Prism software (San Diego, CA, United States). The error bars indicate the standard deviation. Statistical significance was calculated by Student's *t*-test. *$P < 0.05$, **$P < 0.01$.

As shown in Fig. 1A, the Omicron variant patients exhaled more SARS-CoV-2 virions than the Delta variant patients at 1, 3, 5, 7 and 9 dph, and it showed significantly higher at 3 and 5 dph (*, $P < 0.05$; **, $P < 0.01$). Both the Omicron and Delta variant patients exhaled ten million SARS-CoV-2 particles per hour (10^7 viral RNA copies) at 1 dph (Fig. 1A). At the first week of hospitalization, the overall breath emission rate of Delta variant patients was from $10^{5.33}$ to $10^{7.15}$ copies per hour and Omicron variant patients was from $10^{5.60}$ to $10^{7.67}$ copies per hour (Fig. 1B). The average breath emission rate of Omicron variant patients ($10^{7.3}$ copies/h) was higher than the Delta variant patients ($10^{6.6}$ copies/h) at the first week of hospitalization.

To explore whether the higher viral load exhaled by Omicron patients is related to an increased nasopharyngeal viral load. The viral loads in nasopharyngeal swabs of the Delta and Omicron variants patients were also measured. The Omicron variant patients did not contain higher nasopharyngeal viral load than the Delta variant patients (Fig. 1C). Furthermore, we also analyzed the trend of viral load in nasopharyngeal swabs and exhaled breath from patients with Delta or Omicron, respectively. The viral loads in nasopharyngeal samples from patients with Delta or Omicron declined with increasing duration of illness after hospitalization, and the exhaled breath specimens had the similar variation trend with nasopharyngeal samples (Fig. 1D).

It was reported that Omicron variant lead to more significant immune escape compared with Delta variant,[7] which might one reason for the increased transmissibility of Omicron. In this study, we found Omicron patients exhaled more virus particles than Delta patients at the first week of hospitalization, which provided another reason for the increased transmissibility. We also found that the Omicron and Delta variants had similar viral load in nasopharyngeal swab samples as previously reported.[8] These data suggest the increased SARS-CoV-2 particles exhaled by the Omicron patients is not caused by the higher nasopharyngeal viral load as reported for previous variants,[9] but might be by other factors, such as enhanced immune escape and virus shedding ability. The viral load in exhaled breath had the similar variation trend with nasopharyngeal specimens from COVID-19 patients (Delta or Omicron), suggesting that COVID-19 patients who infected with the same SARS-CoV-2 strain and had higher nasopharyngeal viral load might exhibit higher virus excretion.

In conclusion, more SARS-CoV-2 particles were exhaled by the Omicron patients than the Delta patients, which provide a direct evidence for the enhanced transmissibility of the Omicron variant.

Ethics statement

Regarding the ethical requirements of human subjects, all specimen collections were obtained with the informed consent of the patients. None of the specimens recorded patient identifiers. The Ethics and Scientific Review Committee of Sierra Leone approved the study.

Declaration of Competing Interest

The authors declare no competing interests.

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Clinical impact of next-generation sequencing on laboratory diagnosis of suspected culture-negative meningitis and encephalitis

Dear Editors,

We read with interest an article published by Yin et al. on the performance of metagenomics next-generation sequencing (mNGS) in sepsis[1]. Here, we describe the clinical impact of NGS in confirming the microbiological cause and excluding an infective etiology as well as stopping unnecessary antimicrobials and adding specific treatment in patients with suspected culture-negative meningitis and encephalitis.

This study was approved by the Institutional Review Board of The University of Hong Kong - Shenzhen Hospital ([2022]120), and the informed consent was exempted. The study was conducted over a three-year-and-two-month period (1st March 2019 to 30th April 2022) in The University of Hong Kong - Shenzhen Hospital.

During the study period, cerebrospinal fluid (CSF) samples from a total of 14 patients with suspected meningitis/encephalitis were submitted to mNGS analysis (Table 1). All the 14 patients had clinical features suggestive of meningitis and/or encephalitis, including fever ($n = 12$), headache ($n = 9$), confusion ($n = 4$), nausea and vomiting ($n = 2$), dizziness ($n = 2$), hyperalgesia ($n = 1$), limb convulsion ($n = 1$), neck stiffness ($n = 1$) and sleepiness ($n = 1$). The CSF cell counts were abnormal in 12 patients (Table 2).

Among these 14 samples submitted for mNGS, all were positive for sequences of one or more microorganisms (Table 2). Judging from the clinical information, one or more of the microorganisms detected in 10 of the 14 cases were considered as clinically significant. These included *Angiostrongylus cantonensis* (case 1), *Mycobacterium tuberculosis* (case 2), varicella-zoster virus (case 3), adenovirus (case 5), human herpes virus-6A (case 6), herpes simplex virus I and II (HSV-I and HSV-II) (case 7), HSV-I and HSV-II and *Streptococcus pneumoniae* (case 9), *Haemophilus influenzae* (case 10), *Listeria monocytogenes* (case 11), and HSV-I (case 14). In these 10 cases, nine of them also contained sequences considered as contaminants. The Epstein-Barr virus sequences detected in two patients (cases 7 and 12) were considered as latent infection.

HSV DNA was detected in the CSF samples of cases 7 and 9 and *M. tuberculosis* DNA was detected in the CSF sample of case 2. For case 1, *A. cantonensis* antibody was detected in the patient's serum by enzyme-linked immunosorbent assay. In cases 4 and 8, immunoglobulin-like cell adhesion molecule 5 (IgLON5) and myelin-oligodendrocyte glycoprotein (MOG) antibodies were detected in the patients' CSF and serum samples respectively. For case 11, *L. monocytogenes* was isolated from the blood of the patient[2].

Among the 14 patients, unnecessary antimicrobial treatment was stopped in 12 (cases 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, and 13) whereas specific antimicrobial and/or other adjunctive treatment was added in 11 (cases 1, 2, 4, 5, 6, 8, 9, 10, 11, 12, and 13) when NGS results were available (Table 1). All 14 patients survived with 13 of them recovered fully.

In this study, we describe the diverse microbiological causes of suspected culture-negative meningitis/encephalitis confirmed by NGS. Since NGS is a one-technology-for-all-pathogen laboratory test,[2-6] it is a particularly useful tool for this clinical scenario. Among the 14 patients in this study, 10 (71.4%) had their microbiological causes confirmed by NGS, involving microbes that are localized to Southern China (e.g. *A. cantonensis*), those that are more prevalent in developing than developed countries (e.g. *M. tuberculosis*) and those that are globally important (e.g. HSV, *H. influenzae*, *S. pneumoniae*). It is also interesting to note that one patient (case 6) with transient ischemic attack had 428 reads of HHV-6A detected in his CSF sample. Since HHV-6 could infect vascular endothelial cells, induce angiopathy and cause focal infarction or necrosis in the central nervous system,[7-9] we speculate that the transient ischemic attack of this patient could be a result of HHV-6A reactivation.

In addition to its usefulness in confirming the microbiological diagnosis of suspected culture-negative meningitis/encephalitis, NGS is also helpful in excluding an infective cause of these clinical syndromes in some cases. Case 4 was a 60-year-old man with diabetes mellitus and *Klebsiella pneumoniae* invasive liver abscess syndrome with cerebral abscess and endophthalmitis 2–4 months before the development of fever again. NGS analysis of the CSF sample did not show any pathogens, but only 88 reads of *Pseudomonas aeruginosa*, which were likely contaminants. Subsequently, IgLON5 antibodies were detected in his CSF and serum samples. Case 8 was a 28-year-old-woman presented with fever and headache for one week. NGS analysis of the CSF sample did not show any pathogens, but only 1–2 reads each of *P. aeruginosa*, *Stenotrophomonas maltophilia*, *Escherichia coli* and *Haemophilus parainfluenzae*. Subsequently, MOG antibodies were detected in her CSF and serum samples. In both cases, autoimmune encephalitis was diagnosed and they responded promptly to corticosteroid treatment. In these cases, NGS served as a one-test-for-all-pathogen platform to exclude an infective cause of the meningitis/encephalitis syndrome, giving the clinician more confidence to commence corticosteroid for the treatment of their autoimmune encephalitis. After all, we have shown that NGS has major clinical impact on patient management, as unnecessary antimicrobials were stopped and specific treatment was commenced in 12 and 11 of the 14 cases respectively after NGS results were available (Table 2).

Despite its high clinical impact and usefulness, NGS results must be interpreted discreetly. Since NGS is such a sensitive technology, it is able to detect the nucleic acids of any microorganism present in the clinical sample, no matter whether they are from the pathogens causing the infection, colonizers or contaminants. From the data in the present study, it was shown that among the 38 microorganisms detected in the 14 patients, sequences from 27 (71.1%) were considered as contaminants. All these sequences were from bacteria, presumably as a result of contamination from the skin flora (e.g. *Staphylococcus epidermidis*) of the patient and/or the hospital environment (e.g. *P. aeruginosa*) during collection of the CSF samples. It is important to note that even for the 10 CSF samples where one or more clinically significant microorganisms were detected, contaminants were also present in nine of them. Therefore, similar to PCR and other microbiological tests, results of NGS must be interpreted with reference to the clinical context, in order to avoiding over-treating patients with unnecessary antimicrobials.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Table 1

Clinical and radiological characteristics of patients in the present cohort.

Case No.	Sex/age	Underlying disease/past history	Key symptoms on admission	Diagnosis	Imaging studies of brain	Treatment stopped after NGS report	Treatment kept after NGS report	Treatment added after NGS report	Outcome
1	F/63	Hypertension	Hyperalgesia for 2 weeks, fever and headache for 10 days	*Angiostrongylus cantonensis* meningoencephalitis	Small ischemic lesions seen in bilateral paraventricular areas; sporadic micro hemorrhagic foci seen on SWI, bilateral parietal lobes predominantly	Doxycycline, acyclovir	Valproate, levetiracetam, mannitol	Prednisone, dexamethasone, albendazole, external right ventricular drainage	Recovered
2	M/32	None	Fever for 10 days, headache for 4 days	Tuberculous meningitis	No abnormality seen in the brain	Cefuroxime, acyclovir, doxycycline	Mannitol	Isoniazid, rifampin, ethambutol, pyrazinamide	Recovered
3	M/69	Hypertension, lung adenocarcinoma, pulmonary tuberculosis	Abdominal pain, nausea and vomiting for 3 days, fever for 1 day	VZV meningoencephalitis, grand mal, acyclovir induced neurotoxicity	Abnormal signals seen in bilateral cerebral hemispheres, cerebella and brainstem, predominantly in the cortex and subcortex, with meningeal thickening and enhancement of bilateral frontotemporal lobes and tentorium cerebelli	Ceftriaxone	Acyclovir	None	Recovered
4	M/60	Diabetes mellitus; sequelae stage of invasive liver abscess syndrome caused by *Klebsiella pneumoniae*	Fever for 2 days, unconsciousness for 1 day	Autoimmune encephalitis	An enhancement nodule seen in right centrum semiovale; leptomeningeal thickening and enhancement seen in left frontal and parietal lobes; reduced volume of the right eye	Ceftriaxone	None	Ceftazidime, dexamethasone, TMP-SMX	Recovered
5	M/38	None	Recurrent unconsciousness and limb convulsion for 2 weeks, fever for 1 day	Adenovirus meningoencephalitis, secondary epilepsy	Diffuse swelling of gyri of the right frontal, temporal and parietal lobes with enhancement of adjacent pia mater	Acyclovir, doxycycline, ceftriaxone	Levetiracetam	IVIG	Recovered
6	M/68	Hypertension	Dizziness for half a month	TIA	Ischemic foci seen in bilateral corona radiata and pons; arteriosclerosis of intracranial segments of bilateral internal carotid arteries and vertebrobasilar arteries	Amoxicillin-clavulanate, doxycycline	Irbesartan, celecoxib, valsartan, pantoprazole, alprazolam, rosuvastatin, aspirin, ebastine	Foscarnet	Recovered
7	F/72	None	Dizziness, nausea and vomiting for 3 days, fever, mental and behavior disorder for 1 day	HSV meningoencephalitis	Abnormal signals of the left thalamus and temporal insula lobe; a softening lesion seen in the right cerebellum	None	Valproate, acyclovir	None	Recovered

Table 1 (*continued*)

Case No.	Sex/age	Underlying disease/past history	Key symptoms on admission	Diagnosis	Imaging studies of brain	Treatment stopped after NGS report	Treatment kept after NGS report	Treatment added after NGS report	Outcome
8	F/28	None	Headache and fever for 1 week	Autoimmune encephalitis	Bilateral diffuse leptomeningeal enhancement	Acyclovir, ceftriaxone, doxycycline	None	Methylprednisolone	Recovered
9	M/31	None	Fever and headache for 1 week, slurred speech for 1 day	HSV meningoencephalitis and *Streptococcus pneumoniae* meningitis, secondary epilepsy, subarachnoid hemorrhage	Swelling of the left temporal lobe and bilateral hippocampi with hyperintensity and dotted lesions seen in the left hippocampus	Amoxicillin-clavulanate	Acyclovir, valproate	Ceftriaxone, vancomycin, meropenem, dexamethasone	Recovered
10	F/33	Hearing loss of the right ear	Fever, headache and neck stiffness for 2 days	*Haemophilus influenzae* meningitis	Long T2 signals seen in the right mastoid; no abnormal finding in brain	Acyclovir, vancomycin	Ceftriaxone, dexamethasone	Ciprofloxacin	Recovered
11	M/59	Adult-onset immunodeficiency due to interferon gamma autoantibodies	Fever, headache and fatigue for 1 day	Listeria bacteremia and meningitis	Sporadic white matter hyperintensities in bilateral frontoparietal lobes; abnormal signals of basilar clivus and upper cervical vertebrae	Piperacillin-tazobactam	None	Ampicillin	Recovered
12	M/29	None	Fever and headache for 8 days, sleepiness for 1 day	Tuberculous cerebrospinal meningitis	Multiple linear enhanced foci of the surface of brain and spinal cord, pons, right cerebellum and cervical spinal cord predominantly; suspected patchy enhancement of medulla oblongata	Meropenem, vancomycin, doxycycline, ampicillin, tigecycline, acyclovir	Valproate	Isoniazid, rifampicin, linezolid, moxifloxacin, synchronous plasmapheresis, IVIG, dexamethasone, enoxaparin	Recovered with sequelae
13	F/51	Adult-onset immunodeficiency due to interferon gamma autoantibodies	Fever, fatigue, skin rash and lymphadenopathy for 1 month, headache for 3 days	Disseminated *Mycobacterium abscessus* infection: lymphadenitis, meningoencephalitis	Linear enhancement of the surfaces of bilateral frontoparietal lobes; softening lesions of the heads of bilateral caudate nuclei; mildly thickening of bilateral ethmoidal mucosa	Acyclovir	None	Imipenem-cilastatin, ceftazidime, amikacin, clarithromycin, clofazimine, minocycline, cyclophosphamide	Recovered
14	M/39	None	Headache for 1 day	HSV meningitis	No abnormality seen in the brain	None	Acyclovir	None	Recovered

NGS: next-generation sequencing; SWI: susceptibility weighted imaging; VZV: varicella-zoster virus; TMP-SMX: trimethoprim-sulfamethoxazole; IVIG: intravenous immunoglobulin; HSV: herpes simplex virus.

Table 2
Laboratory findings of patients in the present cohort.

Case No.	CSF NGS analysis (number of reads)	Opening pressure (mmH$_2$O)	Protein (mg/L)	Glucose (mmol/L)	RBC (× 10^6/L)	Nucleated cell (× 10^6/L)	NEU%	LYM%	MON%	EOS%	HSV DNA	VZV DNA	MTB workup	Other lab test supporting diagnosis	ESR (mm/h)	CRP (mg/L)
1	*Angiostrongylus cantonensis* (159), *Acinetobacter* species (65), *Staphylococcus hominis* (26), *Staphylococcus epidermidis* (9), *Cutibacterium acnes* (20), *Moraxella osloensis* (9)	300	617	3.21	144	274	11	10	40	38	–	–	–	Serum *Angiostrongylus cantonensis* antibodies +	27	14.31
2	*Mycobacterium tuberculosis* complex (2), *C. acnes* (16), *S. hominis* (7), *Staphylococcus haemolyticus* (4), *Acidovorax* species (6), *M. osloensis* (5)	>320	1014	2.55	1	68	81	17	2	<1	–	–	GeneXpert weakly +	IGRA +	28	5.12
3	VZV (137,150), *Bradyrhizobium elkanii* (36), *Bradyrhizobium japonicum* (8), *Burkholderia vietnamiensis* (15), *Sphingomonas echinoides* (13), *Staphylococcus* species (4)	155	8632	4.25	1000	699	5	64	31	<1	–	Not done	–	None	7	1.23
4	*Pseudomonas aeruginosa* (88)	185	1074	4.99	1	28	21	72	7	<1	–	–	–	CSF and serum IgLON5 +	44	52.51
5	Adenovirus (1), *Corynebacterium simulans* (1), *Escherichia coli* (1)	320	985	2.48	0	817	28	65	4	2	–	–	–	None	14	4.96
6	Human herpes virus-6A (428)	120	816	3.47	0	1	N/A	N/A	N/A	N/A	–	–	–	None	58	4.89
7	HSV-I (730), HSV-II (5), *Corynebacterium striatum* (7), EBV (1), *P. aeruginosa* (5), *E. coli* (4), *S. haemolyticus* (3), *Streptococcus sanguinis* (1)	150	432	4.32	10	49	21	64	15	<1	+	–	–	None	42	1.59
8	*P. aeruginosa* (2), *Stenotrophomonas maltophilia* (2), *E. coli* (1), *Haemophilus parainfluenzae* (1)	225	570	3.02	10	241	52	13	36	<1	–	–	–	Serum and CSF MOG antibodies +	47	8.25
9	HSV-I (866), HSV-II (6), *Streptococcus pneumoniae* (4), *C. striatum* (1)	330	785	4.22	5	121	14	70	16	<1	+	–	–	None	Not done	3.08
10	*Haemophilus influenzae* (8), *S. epidermidis* (8)	202	1151	3.39	None	1010	58	20	22	<1	–	–	–	None	53	142.1
11	*Listeria monocytogenes* (7), *P. aeruginosa* (6), *Micrococcus luteus* (1)	160	373	2.81	3	387	8	10	82	<1	–	–	–	Blood culture: *L. monocytogenes*; bone culture: *Mycobacterium kansasii*	110	282.4
12	EBV (6), *C. acnes* (169), *S. epidermidis* (14), *Staphylococcus capitis* (7), *S. hominis* (7), *Staphylococcus saccharolyticus* (6), *Corynebacterium accolens* (6), *M. osloensis* (25), *M. luteus* (4)	155	1600	2.22	1230	201	6	70	23	1	–	–	–	None	11	120.6
13	*Pseudomonas stutzeri* (46), *Corynebacterium urealyticum* (5)	155	299	3.3	9	0	N/A	N/A	N/A	N/A	–	–	–	Lymph node culture: *Mycobacterium abscessus*	117	66.79
14	HSV-I (107), *M. osloensis* (19), *Staphylococcus warneri* (11), *Acinetobacter johnsonii* (10)	142	737	3.03	125	90 (Cytolysis, not able to categorize)	N/A	N/A	N/A	N/A	–	–	–	None	6	2.25

CSF: cerebral spinal fluid; NGS: next-generation sequencing; RBC: red blood cell; NEU: neutrophil; LYM: lymphocyte; MON: monocyte; EOS: eosinophil; VZV: varicella-zoster virus; HSV: herpes simplex virus; EBV: Epstein-Barr virus; MTB: *Mycobacterium tuberculosis*; IGRA: interferon gamma release assay; IgLON5: immunoglobulin-like cell adhesion molecule 5; MOG, myelin-oligodendrocyte glycoprotein; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; -: negative; +: positive; N/A: not applicable.

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Diagnostic value of serum KL-6 and IL-6 levels in critically ill patients with COVID-19-associated pneumonia
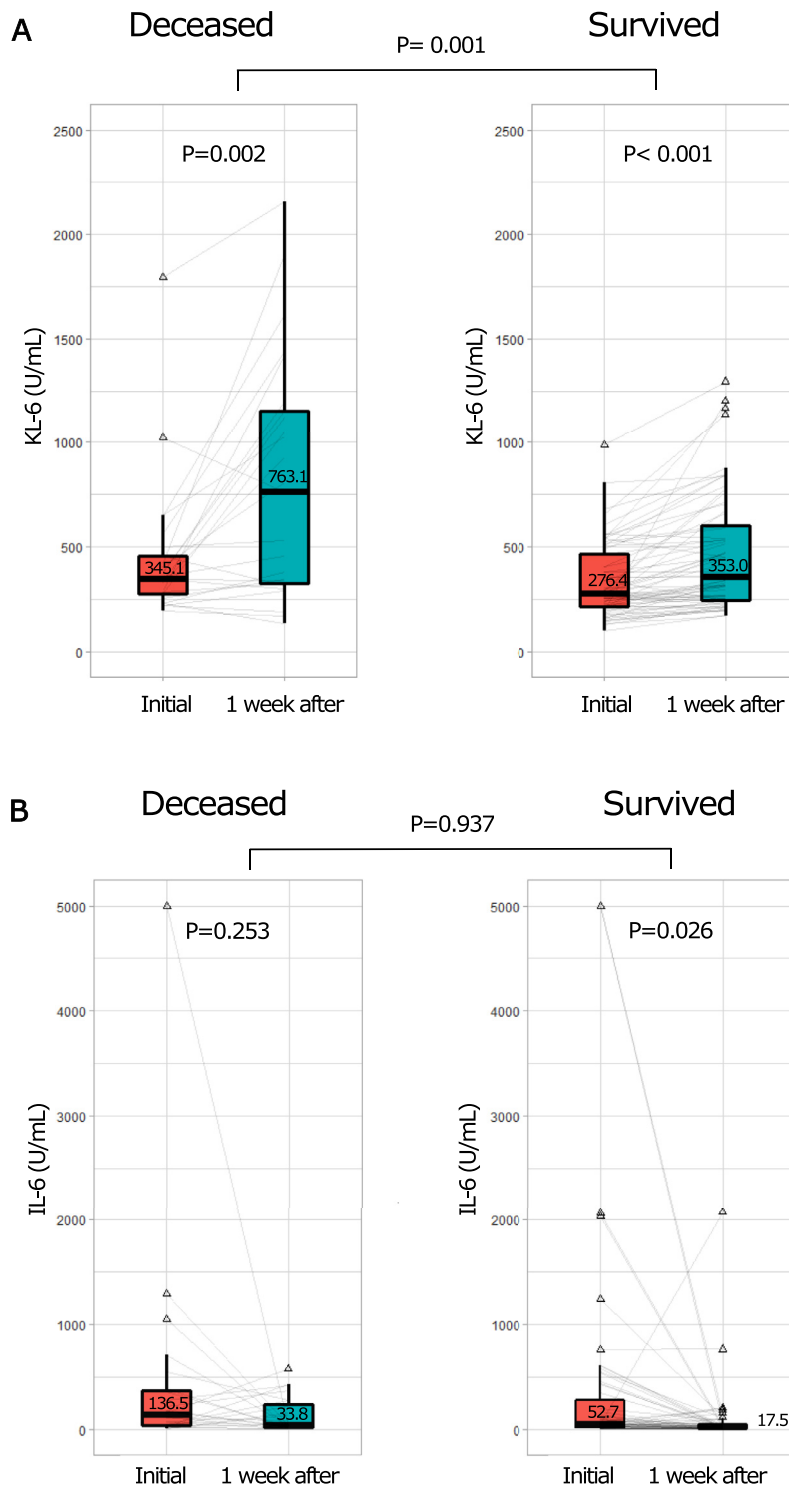
Dear Editor,

We recently read the article by Grifoni et al. with great interest, which reported the diagnostic value of serum interleukin-6 (IL-6) level at admission for predicting severe COVID-19 progression and/or in-hospital mortality.[1] The pathophysiology of COVID-19 is characterized by early direct viral invasion of lung alveolar epithelial cells and a late hyperinflammatory period caused by cytokine cascades, such as IL-6.[2] In addition, Krebs von den Lungen-6 (KL-6) is a representative serum marker for alveolar damage.[3] Therefore, we conducted a retrospective Cohort study evaluating the diagnostic value of serum KL-6 and IL-6 levels for predicting patient in-hospital mortality among critically ill patients with COVID-19-associated pneumonia.

Adult patients ($\geq$ 18 years) with respiratory organ support, who were admitted to Samsung Changwon Hospital - a 760-bed tertiary teaching hospital, between January 2021 and March 2022 were included. Respiratory organ support was defined as mechanical ventilation or high-flow nasal cannula ($FiO_2 \geq 0.3$ with a flow rate $\geq$ 30 L/min). KL-6 and IL-6 measurements were recommended for COVID-19 patients; these biomarkers were measured at admission and weekly thereafter (Methods for tests were described in supplementary material). The objective of this study was to evaluate the association between mortality and serum KL-6/IL-6 in critically ill patients with COVID-19. In addition, subgroup analysis was performed for patients with $\geq$ 2 serial KL-6 and/or IL-6 measurements to analyze and compare trend changes in KL-6 or IL-6 levels measured at admission and one week after admission in deceased and survived patients. The study was approved by the Samsung Changwon Hospital Institutional Review Board (IRB number: SCMC 2022–06–016).

A total of 102 critically ill patients were included in this study (Supplementary Fig. 1). In-hospital mortality was 28.4% (29/102). In the deceased and survived groups, initial KL-6 level (median 345.1 U/mL (interquartile range 252.3–477.1) vs. 276.4 (211.4–435.2), $P = 0.064$)) and initial IL-6 level (106.0 pg/mL (24.9–340.8) vs. 50.1 (11.2–196.0)) tended to be higher in the deceased group without statistical significance. Meanwhile, peak KL-6 level (499.9 U/mL (344.3–1317.8) vs. 353.6 (248.1–577.4), $P = 0.008$)) and peak IL-6 level (178.5 pg/mL (75.6–457.5) vs. 67.4 (21.1–352)) were significantly higher in the deceased group. In addition, lower

A

Deceased · P= 0.001 · Survived

P=0.002 (left panel) · P< 0.001 (right panel)

KL-6 (U/mL)

345.1 → 763.1 (Deceased: Initial, 1 week after)

276.4 → 353.0 (Survived: Initial, 1 week after)

B

Deceased · P=0.937 · Survived

P=0.253 (left panel) · P=0.026 (right panel)

IL-6 (U/mL)

136.5 → 33.8 (Deceased: Initial, 1 week after)

52.7 → 17.5 (Survived: Initial, 1 week after)

Fig. 1. Difference between serum KL-6 and IL-6 levels measured at admission and one week after admission.
(a) KL-6
KL-6, Krebs von den Lungen-6
*Serial data of KL-6 measurements from 25 to 65 deceased and survived patients, respectively.
(b) IL-6
IL-6, interleukin-6
*Serial data of IL-6 measurements from 24 to 59 deceased and survived patients, respectively.

Table 1
Predictors of mortality in critically ill COVID-19 patients.

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P-value	OR (95% CI)	P-value
Age ≥ 68 years	6.08 (1.92–19.22)	0.002	31.85 (3.48–291.10)	0.002
Modified CCI per point	1.15 (0.94–1.41)	0.174		
Inotrope /vasopressor use	9.63 (3.52–26.19)	< 0.001	8.88 (2.35–33.49)	0.001
Peak KL-6 ≥ 1019.2 (U/mL)	9.07 (2.99–27.54)	< 0.001	14.19 (2.33–86.34)	0.004
Peak IL-6 ≥ 101.5 (pg/mL)	5.01 (1.81–13.87)	0.002		
Initial Platelet ≤ 222 (K/μL)	3.73 (1.36–10.23)	0.011		
Initial Lactate ≥ 2.2 (mmol/L)	4.78 (1.86–12.29)	0.001	4.31 (1.07–17.44)	0.040
AKI	3.08 (1.24–7.63)	0.015		

COVID-19, coronavirus disease-19; ECMO, extracorporeal membrane oxygenation; CCI, Charlson Co-morbidity Index; KL-6, Krebs von den Lungen-6; IL-6, interleukin-6; AKI, acute kidney injury.
*Data from 97 patients with complete laboratory data (70 and 27 for survived and deceased patients, respectively).

platelet count, higher serum lactate, and presence of AKI were more frequently observed in the deceased group (Supplementary Table 1). Before performing multivariable analysis, the following cut-off values for age and laboratory results were defined based on Youden index: 68 years, 1019.2 U/mL for peak KL-6, 101.5 pg/mL for peak IL-6, 222 K/μL for platelet count, and 2.2 mmol/L for serum lactate. In multivariate analysis, peak KL-6 ≥ 1019.2 U/mL was associated with mortality and showed the highest odds ratio (OR 14.19, 95% confidence interval (CI) 2.33–86.34, $P = 0.004$) (Table 1).

In subgroup analysis, serum KL-6 level tended to be higher in the deceased group (345.1 U/mL (274.8–454.3)) than in the survived group (276.4 (213.5–465.3)) at admission and was significantly higher one week after admission (763.1 U/mL (323.6–1154.1) and 353.0 (243.2–599.2), respectively). However, the magnitude of increase was higher in the deceased group than in the survived group ($P = 0.001$; Fig. 2A). When serum IL-6 level was compared in the deceased group (136.5 pg/mL (37.9–368.0)) and the survived group (52.7 (17.8–274.0)) at admission and one week after admission (33.8 pg/mL (12.6–232.3) and 17.5 (4.4–48.4), respectively), a decrease in IL-6 level was observed in the survived group. No tendency was observed in the deceased group. In addition, statistical difference was not found in increased or decreased tendency between the deceased and survived groups ($P = 0.937$) (Fig. 1).

Critically ill COVID-19 patients were described as experiencing sequential disease progression to three stages: early infection, pulmonary phase, and hyperinflammation phase.[2] The hyperinflammation phase results from dysregulated host inflammatory response, and IL-6 is the representative disease marker during this period.[4] In a meta-analysis, the diagnostic role of IL-6 in COVID-19 also showed that patients admitted to the intensive care unit had a higher IL-6 level (ratio of means = 3.24; 95% CI 2.54–4.14).[4] The results in the present study are in agreement with the previous studies.

KL-6 is a specific marker in ILD for disease activity, prediction for progression, and mortality.[5] This high-molecular-weight glycoprotein is mainly expressed on the surface of type II alveolar epithelial cells, and air-blood barrier disruption by inflammation enhances permeability to elevate KL-6 influx into the bloodstream.[6] Therefore, elevated serum KL-6 level can be observed in all causes of inflammatory lung damage.[7] In the present study, peak serum KL-6 level was higher in the deceased group compared with the survived group among critically ill patients. The serum KL-6 level cut-off value of 1019.2 U/mL for predicting mortality in this study was similar to other studies predicting poor outcomes among COVID-19 patients.[8] Therefore, 1000 IU/mL might be an important level of serum KL-6 for predicting mortality in COVID-19 patients.

In the present study, both serum peak KL-6 and IL-6 levels were higher in the deceased group than the survived group. However, the kinetics of these biomarkers differed. IL-6, an important biomarker for hyperinflammation, was frequently elevated at admission, and a decrease was not observed. KL-6, an important biomarker for inflammatory lung injury, was increased one week after admission. Therefore, this finding appeared to reflect the pathophysiology of critically ill patients with COVID-19.[9] Therefore, serial measurement of these serum biomarkers is important to predict poor outcomes of critically-ill patients with COVID-19-associated pneumonia.

In conclusion, peak serum KL-6 and IL-6 levels were associated with mortality. In particular, KL-6 level of 1000 IU/mL could be an important cut-off value for predicting mortality in COVID-19 patients. Although KL-6, reflecting lung injury resulting from hyperinflammation, was more highly increased during serial measurement in the deceased group compared with the survived group, IL-6, reflecting hyperinflammation, was higher in the deceased group and often increased at admission but did not show significant change during serial measurements.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Sensitivity of anti-SARS-CoV-2 nucleocapsid protein antibody for breakthrough infections during the epidemic of the Omicron variants

Dear Editor,

Seroepidemiological data are useful to estimate the spread of SARS-CoV-2 infection, which can contribute to the planning and evaluation of measures against coronavirus disease 2019 (COVID-19). As the target of mRNA vaccine is spike protein, anti-nucleocapsid antibody (Ig-N) has been used to monitor natural infection after vaccine rollout.[1] Despite high vaccine uptake, we are facing a marked increase of milder forms of COVID-19, characterized by lower levels of and faster waning of antibodies over time.[2] In this regard, we read with interest a letter in this journal by Allen

et al.,[3] who raised concerns for the use of Ig-N as a marker of natural infection in the post-vaccine era.

Using data of repeat serosurveys and COVID-19 registry among the staff of a tertiary referral hospital in Tokyo,[4] we compared the proportion of Ig-N positives among the patients with PCR or antigen-confirmed COVID-19 (sensitivity) across different periods and vaccine doses. We measured Ig-N (total Ig) using the Roche Elecsys® Anti-SARS-CoV-2 N assay, which can reliably detect mature Ig-N over 6 months.[5] In this cohort, we identified 224 patients who were infected for the first time after the second or third dose of mRNA vaccine, mainly BNT162b2 (Pfizer-BioNTech), and subsequently attended a serosurvey without receiving the additional dose. All the infections were mild or asymptomatic.

Patients who were infected within 2 months after the booster (during Omicron BA.1 epidemic) showed a higher proportion of asymptomatic infection (14%) and significantly lower Ig-N index (median, 7.4) and sensitivity (78%), as compared with other groups (sensitivity, 94–100%) (Fig. 1). In contrast, patients who were infected 4 to 5 months after the booster (during Omicron BA.2 epidemic) had Ig-N index (median, 18.6) and sensitivity (97%) comparable to those who were infected before vaccination rollout or after the second dose (during Delta or Omicron BA.1 epidemic). Similarly, patients who were infected 5 to 8 months after the second vaccine had high index (median, 15.1) and sensitivity (96%). These results support that the level of immunity (largely determined by vaccine dose and time since vaccination), rather than Omicron subvariant, accounts for the discrepancy in test performance.

Earlier serological studies in the pre-vaccine era showed that anti-SARS-CoV-2 Ig-N detection rate is higher among symptomatic patients than asymptomatic patients.[4] In the present analysis among patients who were infected within 2 months after the booster, however, the sensitivity did not change (78%) after excluding asymptomatic cases. Among vaccine recipients who might have acquired strong immunity during short period after the booster, the presence of symptoms may not reflect SARS-CoV-2 Ig-N production.

In this well-defined cohort with repeat serological assessments and rigorous registration of COVID-19, the performance of the Roche Ig-N assay was decreased by approximately 20-percentage points for the breakthrough infections occurred within 2 months after the booster dose, whereas it performed well for those occurred 3 months or more after the second or third dose. The decreased performance observed shortly after the booster needs to be considered in the planning of seroepidemiological study and interpretation of the results.
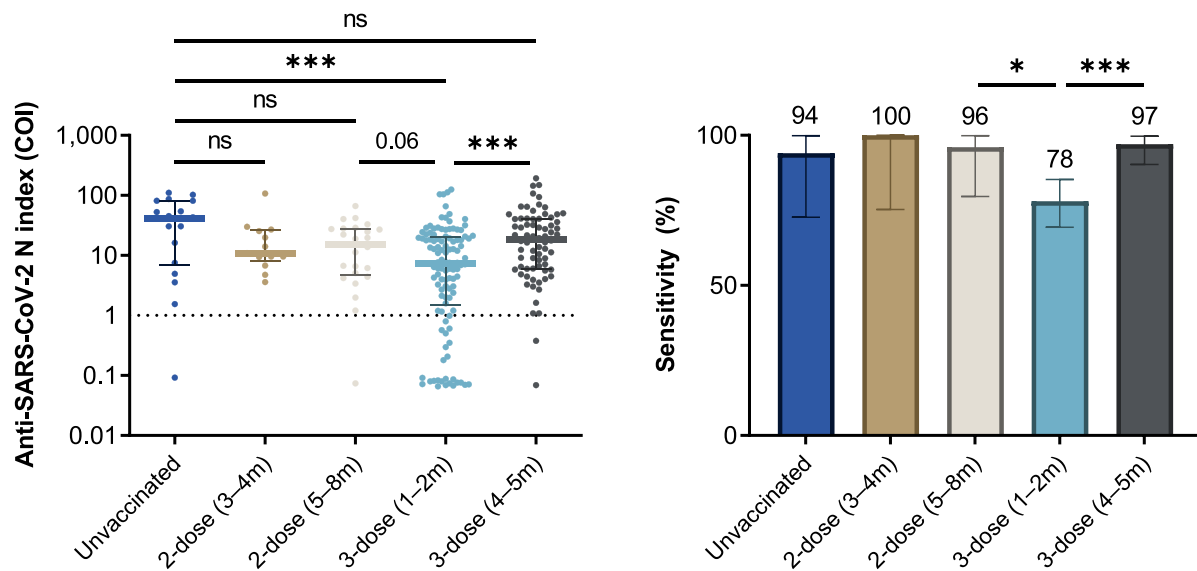
Ethical approval

Financial support

Declaration of Competing Interest

All authors: No reported conflicts of interest.

Fig. 1. Roche anti-SARS-CoV-2 nucleocapsid index (**Left**) and the proportion of seropositive among those with previously confirmed COVID-19 (**Right**) according to epidemic phase and vaccine status *Timing of infection (dominant virus type)*: Unvaccinated: before vaccination rollout (Wild-type, Japan-specific B.1.1.214), $n = 18$ 2-dose (3–4 m): 3–4 months after the second dose (Alpha, Delta), $n = 13$ 2-dose (5–8 m): 5–8 months after the second dose (Omicron BA.1), $n = 25$ 3-dose (1–2 m): 1–2 months after the third dose (Omicron BA.1), $n = 114$ 3-dose (4–5 m): 4–5 months after the third dose (Omicron BA.2), $n = 72$

For the Left panel, the bars indicate the median value, and I-shaped bars indicate its interquartile range. The dushed horizontal line in the Left panel indicates the threshold of the seropositive (≥ 1.0 COI).

For the Right panel, the sensitivity with 95% confidence intervals is calculated using the exact binomial technique.

Statistical significance was tested using Kruskal–Wallis test (Left) and chi-squared test (Right). *** $p < 0.001$, * $p < 0.05$, ns $P > 0.1$.

Acknowledgment

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Ocular involvement in monkeypox: Description of an unusual presentation during the current outbreak

Dear Editor,

We read with great interest the manuscript by Orviz et al.[1] recently published in the *Journal of Infection.* In this paper, the authors describe the clinical and virological characteristics of the first 48 cases of monkeypox (MPX) observed in a reference centre for sexual transmitted infections/HIV infection in Spain during the multi-national outbreak started in May 2022 in non-endemic countries.

As discussed by the authors, during the current outbreak, which counts, as of August 4, 2022, 13,022 confirmed cases from 27 EU/EEA countries,[2] the disease has mainly occurred in men who had sex with men (MSM) who had no epidemiological link to the endemic countries. Additionally, in the above manuscript,[1] consistent with previous reports,[3] the clinical presentation of the disease appeared mild and self-limited, with rare severe manifestations or complications. In this regard, in the course of this outbreak, ocular complications of the disease were described rarely (<1%),[2] compared with reports from endemic areas where ocular involvement, including corneal scarring and endophthalmitis, ranged from 9 to 23%.[4,5]

Here we reported a clinical case of ocular involvement in a MSM, in which MPXV-DNA was detected by real-time PCR both in eyelid and conjunctival swabs, and MPXV was also isolated in cell culture as replication-competent, infective virus. This case highlights some elements that may have implications for the transmission and pathogenesis of this unusual complication.

In May 2022, a 26-year-old Italian male attended the outpatients' department for the appearance of two papular lesions in the suprapubic area and he was diagnosed as infected by MPXV by real-time PCR performed on skin lesions. He reported a protected sexual intercourse five days before the visit with a male partner who subsequently tested positive for MPXV.

Two days after the first visit, he was admitted to our hospital for fever, general malaise, headache, painful inguinal lymphadenopathy and multiple papular lesions in the right eyelid with progressive periorbital and conjunctival involvement. The ophthalmologist's evaluation ruled out visual impairment and corneal involvement, and, on suspicion of bacterial superinfection, topical steroid therapy was started along with intravenous antibiotic therapy.

Due to the clinical worsening (increased number of lesions in upper and lower eyelids and the eye fornix with periorbital oedema and conjunctival hyperaemia), swabs from periorbital lesions and conjunctiva were collected and tested for MPXV. Viral DNA was extracted by Qiamp Viral RNA mini kit (Qiagen), and two real time PCRs were used to assess the presence of MPXV DNA: Real-Star *Orthopoxvirus* PCR Kit (Altona Diagnostics GmbH), that recognizes a region common to all *Orthopoxviruses* without distinction of species, was used as screening PCR; the second PCR (G2R_G assay) published by Li et al. targets the tumour necrosis factor (TNF) receptor gene and was used as confirmatory PCR.[6] The Rotor-Gene Q (Qiagen) platform was used to run both assays. We also measured the viral quantification cycle (Cq) in positive samples. Both eyelid and conjunctival samples were positive for MPXV DNA by both PCRs. Moreover, the conjunctival swab was successfully inoculated in Vero E6 cells, a clear cytopathic effect was observed 48 h after the inoculum and MPXV replication was confirmed by real-time PCR on DNA purified from cell growth medium collected after 48, 72, and 96 h.[7]

The patient was treated with two doses of intravenous cidofovir (5 mg/kg weekly associated with oral probenecid and fluid support) and anti-inflammatory and vitamin A-based eye drops; steroid local therapy was stopped. Slow clinical improvement was observed with asynchronous evolution of the lesions and their total disappearance at approximately two months after onset (Fig. 1).

Our case confirms the possibility of ocular involvement during MPXV infection in a non-endemic setting. We detected MPXV-DNA by real time PCR and were able to isolate MPXV as a replication-competent virus from conjunctival swabs. Unlike previously described, in our patient systemic symptoms and ocular involvement were subsequent to the appearance of the first skin lesions by a few days. This time course suggests that eye localization might be
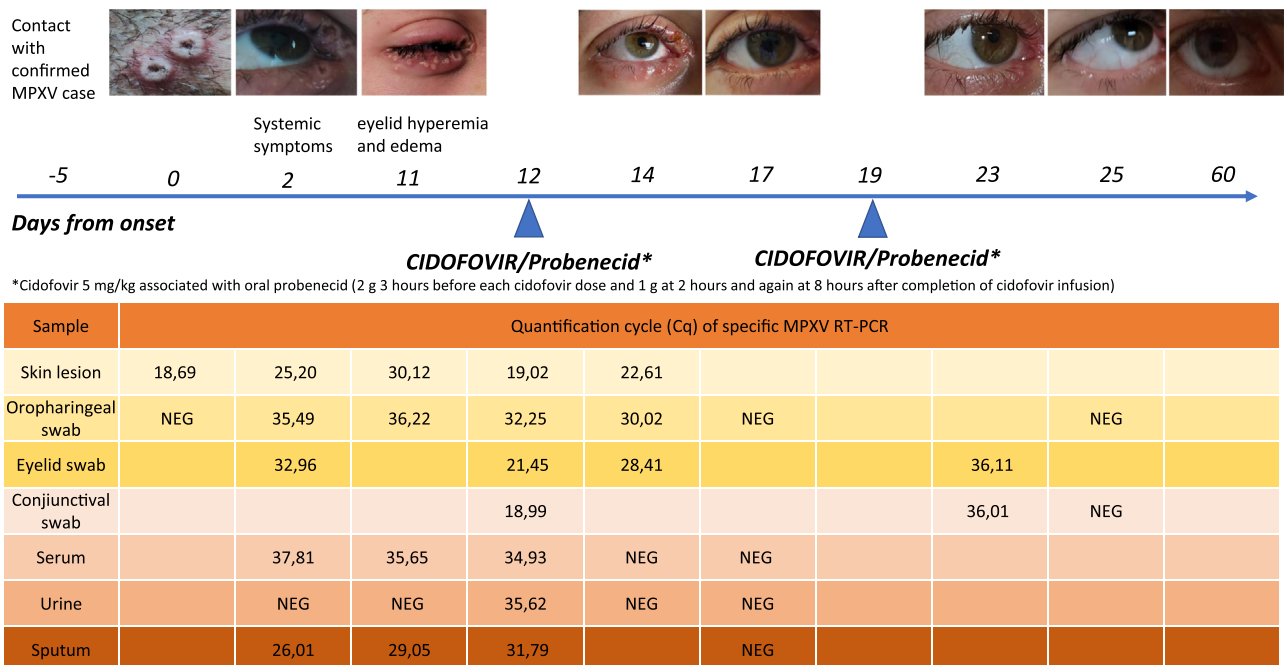
Days from onset

CIDOFOVIR/Probenecid*   CIDOFOVIR/Probenecid*

*Cidofovir 5 mg/kg associated with oral probenecid (2 g 3 hours before each cidofovir dose and 1 g at 2 hours and again at 8 hours after completion of cidofovir infusion)

Sample	Quantification cycle (Cq) of specific MPXV RT-PCR									
Skin lesion	18,69	25,20	30,12	19,02	22,61					
Oropharyngeal swab	NEG	35,49	36,22	32,25	30,02	NEG			NEG	
Eyelid swab		32,96		21,45	28,41			36,11		
Conjiunctival swab				18,99				36,01	NEG	
Serum		37,81	35,65	34,93	NEG	NEG				
Urine		NEG	NEG	35,62	NEG	NEG				
Sputum		26,01	29,05	31,79		NEG				

Fig. 1. Timeline of clinical evolution and PCR positivity in biological samples collected.

related to self-inoculation, rather than conjunctival spread during the early viraemic phase of infection.

Although the passage of the virus into the conjunctival secretion from the plasma compartment has been hypothesized, previously described cases of ocular complications of MPX were mainly related to bacterial superinfection.[8] In contrast, we showed that both eyelid and conjunctival specimens were positive for MPXV DNA, and a replication-competent MPXV was isolated in culture from conjunctival swab, in absence of bacterial growth.

MPX in the current outbreak had usually mild to moderate and self-limited clinical presentation[9] and observed cases generally did not require specific antiviral therapies.[3] However, antivirals may be considered in severe disease, or complicated lesions localized in areas at high risk of sequelae.[10] Tecovirimat is currently the only drug approved by the European Medicines Agency (EMA) for the treatment of MPXV, but it is not yet readily available. Although only data *in vitro* and from mouse models are available on systemic antiviral therapy with cidofovir,[11] we used this drug due to the worsening clinical picture to avert the risk of sequelae on vision. Complete recovery and intraocular viral clearance occurred slowly, suggesting that the antiviral activity of cidofovir only partially contributed to clinical resolution. Finally, our case suggests a pathogenetic mechanism of ocular localization based on the spread from a local inoculum. Therefore, appropriate counselling on hygiene measures to reduce the risk of virus self-spreading should be also carried out.

Author contributions

CP, VM, AM, and AA followed the patients during the diagnostic and therapeutic path, conceived the study, drafted the first manuscript, and revised the final version. FB, MM, SGT, MC, SV, and EN followed the patients during the diagnostic and therapeutic path and discussed the results of the study. FC, SM, CM, GM and FM provided for virological assay on samples. RS performed eye follow-up and obtained eyelid and conjunctival samples. EG and FV reviewed and supervised the manuscript. All authors gave their final approval of the version to be submitted.

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Declaration of Competing Interest

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Is SARS-CoV-2 an oncogenic virus?

Dear Editor,

Recently, in this journal, Wu et al. (1) and Gao et al. (2) have both indicated that host genetic variation related to COVID-19 might be associated to endometrial cancer. We here add evidence from gene expression analysis supporting that the connection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and cancer could be more general, in line with several other viral infections that represent serious risks for carcinogenesis in humans. The SARS-CoV-2 has developed similar strategies to Epstein-Barr virus (EBV) and hepatitis B virus (HSV1) to control p53 by hijacking the protein *via* virus antigens, and ultimately leading to its degradation (3, 4). Specifically, the Nsp2 viral protein of the SARS-

CoV-2 interacts with the prohibitin 1 and 2 (PHB1, PHB2) that are primarily located in the mitochondrion and play an essential role in maintaining mitochondrial DNA activity. Their depletion triggers a chain of cell responses that lead to a leakage of reactive oxygen species (ROS) to the nucleus and oxidative damage, that ultimately provokes the impairment of the transactivation of p53-dependent genes. In addition, the Nsp3 SARS-CoV-2 protein binds and activates the RING finger and CHY zinc finger domain-cotainin protein 1 (RCHY1) and E3 ubiquitin ligase, promoting p53 degradation (5). Therefore, SARS-CoV-2 has the ability to trigger external and internal apoptotic pathways of the host cells, facilitating its spread. Impairment of p53 could be seen as a strategy of the virus to take advantage of the cell pathways controlled by this protein for its own benefit during acute phase of infection, therefore evading host immune response and facilitating its replication (3). In this context, a reduced expression of p53 during the acute phase of infection is also a biomarker of severe disease.

Although it has not been demonstrated yet, it has been hypothesized that a long-term inhibition of p53 by the SARS-CoV-2 could be carcinogenic. The onco-suppressive protein p53 is a key player within the apoptotic signaling pathway and regulates the expression of about 500 target genes; therefore, it plays a role in cell cycle arrest, cell aging, cell death, etc. (6). We examine three gene expression datasets to demonstrate that p53 is downregulated during acute SARS-CoV-2 infection and long coronavirus-disease 19 (COVID-19); a long-term reduction of p53 could be interpreted as a risk factor in carcinogenesis.

We analyzed *TP53* gene expression in blood from COVID-19 patients stratified by severity as well as healthy controls using the RNAseq data from Jackson et al. (7) and *n*-Counter (overlapping) data from Gómez-Carballa et al. (8) ($n = 65$ and $n = 30$, respectively). Processing of raw data was carried out as in the original articles. In addition, gene expression data of long-COVID-19 patients and healthy controls ($n = 44$) from another RNAseq convalescence study (9) (12-, 16-, and 24-weeks post infection) were also analyzed. For the three studies, severity was determined during the acute phase of the infection. Data normalization was undertaken as in (7). Wilcoxon test was used to assess statistical significance between groups, and Spearman test for the computation of the correlation indices (r) and *P*-values. We also evaluated whether the overall expression of a p53 related pathways is up- or down-regulated using the Fast Approximation to ROAST Gene Set Test with Mean Aggregated Set Statistics (*fry*; https://f1000research.com/slides/5-2605) by selecting Gene Ontology (GO) processes including the term 'p53' in the description as well as *TP53* gene within the gene-set. We carried out two independent comparisons: (*i*) severe patients *vs.* non severe patients (including healthy controls) from the acute COVID-19 cohort, and (*ii*) 24 w.p.i severe/critical patients *vs.* healthy controls from the long-COVID-19 cohort. We used *edgeR* package (https://bioconductor.org/packages/release/bioc/html/edgeR.html) to process raw count data for the *fry* analysis.

The data show that *TP53* is downregulated in patients with the highest WHO severity scores in both the RNAseq (Fig. 1A) and the *n*-Counter datasets (Fig. 1B). These differences are statistically significant when compared against controls and mild patients. In addition, we have also observed that *TP53* gene expression is negatively correlated with the length of symptoms until sample collection only in severe patients (Fig. 1A and 1B).

We further re-analyzed *TP53* blood gene expression data available in a follow-up study of long-COVID-19 patients (9) also stratified by severity (in the acute phase) and sampled at different time-points, namely, 12-, 16- and 24-weeks post-infection (w.p.i) (Gene Expression Omnibus acc. n°: GSE169687). Mild / moderate patients showed statistically significant downregulation of *TP53* expression when compared to healthy controls at 16 w.p.i, and a reactivation
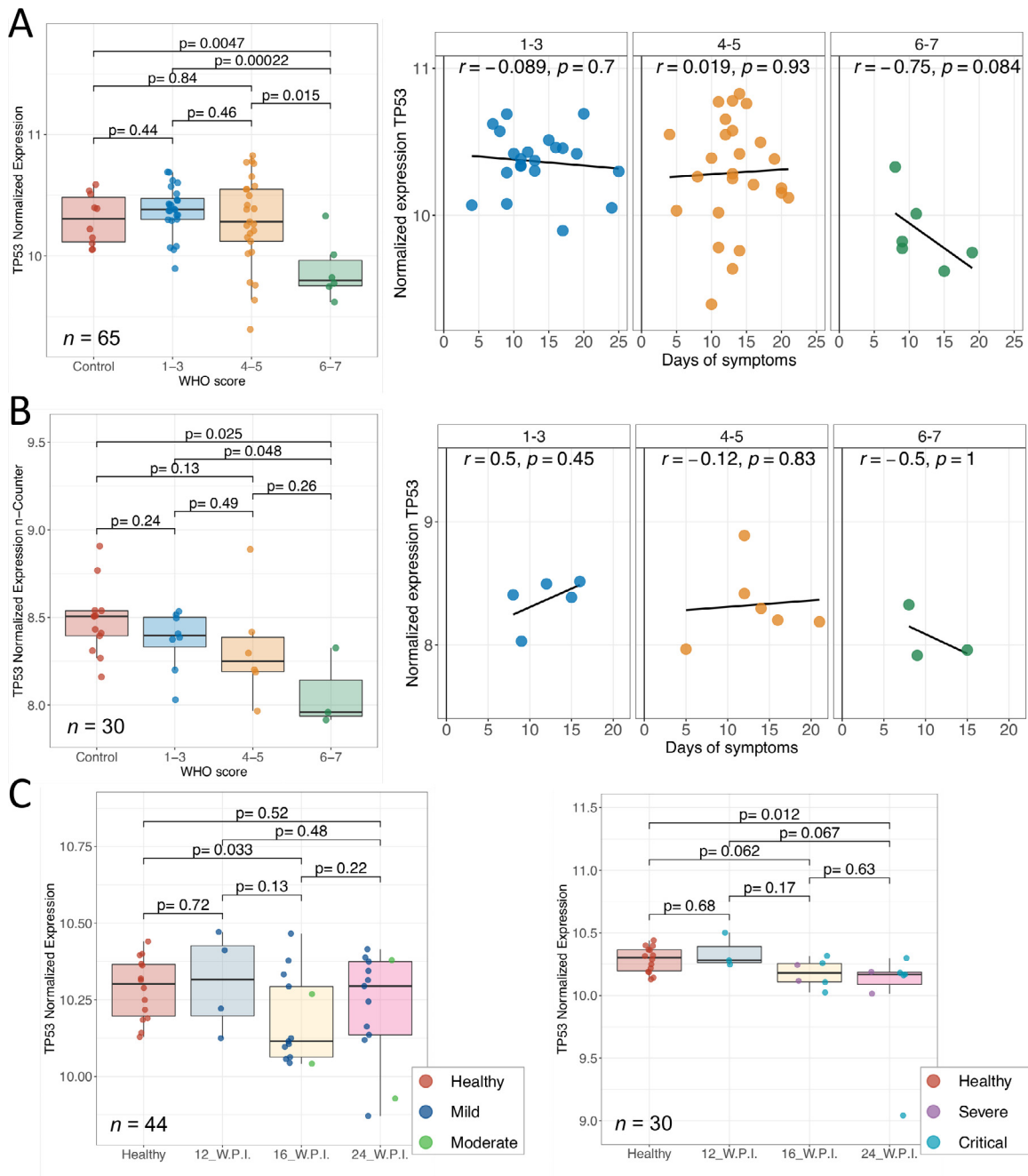
Fig. 1. (A) *TP53* gene expression in blood samples from COVID-19 patients and healthy controls from the RNAseq dataset (7) and stratified by WHO severity score (left). Correlation between days of symptoms to sample collection and *TP53* expression in the RNAseq dataset (right). (B) *TP53* gene expression in blood samples from COVID-19 patients and healthy controls from the *n*-Counter dataset (8) and stratified by WHO severity score (left); patients from this cohort partially overlap with those in the RNAseq dataset. Correlation between days of symptoms to sample collection and *TP53* expression in the *n*-Counter dataset (right). Asymptomatic patients were not included in the correlation analyses (*r* = spearman correlation coefficient). (C) *TP53* gene expression in blood samples from mild/moderate (left) and severe/critical (right) long-COVID-19 patients as well as healthy controls from Ryan et al. (9) collected at different timepoints post infection (w.p.i = weeks post infection); here we used the authors' scores of severity (9).

towards normal values at 24 w.p.i (Fig. 1C). However, in severe / critical patients *TP53* is progressively downregulated to at least 24 w.p.i (statistically significant when compared to controls) with no evidence of recovery to the *TP53* expression level observed in controls (Fig. 1D).

In addition, the data indicate that the downregulation of *TP53* has a significant impact on a number of its interacting genes. Thus,

there are a total of eight pathways (GO terms) related to *TP53* that are significantly up- and down-regulated in acute severe patients and in 24 w.p.i long-COVID-19 severe / critical patients when compared to non-severe (including healthy controls) and a healthy control group, respectively (Table S1). The affected pathways are related to e.g., apoptosis, DNA damage response and signal transduction (Table S1).

We show convergent evidence from three different transcriptomic datasets and techniques that represent a molecular proof of concept that p53 may be acutely and persistently reduced after severe SARS-CoV-2 infection. A persistent reduction of the p53 tumor suppression functions, as might be the case in long-COVID-19 severe patients, may constitute a risk factor for oncogenesis comparable to pathogenic mutations in *TP53*. Such long-term reduction of p53 might trigger cancer onset or contribute to worsen the course of patients with an ongoing tumoral process (1, 2). Future efforts should target larger cohorts and follow-up time, and assess additional types of samples, including lung tissue. A causal relationship between SAR-CoV-2 and cancer has not been demonstrated but, if confirmed, it would have enormous impact on public health.

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Declaration of Competing Interest

The authors report no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.08.005.

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Performance of existing definitions and tests for the diagnosis of invasive aspergillosis in critically ill, non-neutropenic, adult patients: An update including COVID-19 data [☆]

Dear Editor,

Almost three years ago, we reported the results of a systematic review on the performance of existing definitions and tests for the diagnosis of invasive aspergillosis (IA) in critically ill, non-neutropenic, adult patients.[1] The final qualitative synthesis was aimed at providing the expert panel of the FUNgal infections definitions in intensive care unit (ICU) patients (FUNDICU) initiative with the necessary baseline evidence to guide the discussions over the development of a standard set of definitions for invasive fungal diseases (IFD) in critically ill, adult patients outside classical, immunocompromised populations at risk.[2] The main results stemming from our systematic review were as follows: (*i*) against histology/autopsy as reference, the diagnostic performance for invasive pulmonary aspergillosis (IPA) of the AspICU definition was promising, although with the limitations of small samples and applicability only in the presence of positive respiratory cultures; (*ii*) there was a consistently better diagnostic performance for IPA of bronchoalveolar lavage fluid (BALF) galactomannan (GM) than serum GM across studies; (*iii*) the specificity of BALF and serum (1,3)-β-$_D$-glucan (BDG) for the diagnosis of IPA was suboptimal.[1] After two years into the coronavirus disease 2019 (COVID-19) pandemic, which temporarily slowed down the FUNDICU project, our initiative is now ready to move forward to the next step of discussing and developing IFD definitions. Before doing that, an update of our previous systematic review was necessary to account for possible novel evidence that became available in the past three years. Using the same methodology (see supplementary methods for details), the literature search was expanded from 2018 up to 31 March 2022.

From an initial total of 461 records, we extracted 34 full texts and eventually selected 11 studies for inclusion in the present update (Fig. 1). As in the original search, included studies allowed to review the diagnostic performance of tests for the diagnosis of IPA and not of other forms of IA. No studies included in the present update evaluated the diagnostic performance of existing definitions against histology as reference. Overall, the diagnostic performance of serum GM was assessed in 5 studies, of which 4 overlapped with the 6 studies that assessed the diagnostic performance of BALF GM, whereas the diagnostic performance of tracheal aspirate (TA) GM was assessed in 1 study (supplementary Table 1). Regarding tests other than GM, the performance of BALF culture, serum BDG, BALF *Aspergillus* lateral-flow device (AspLFD), BALF GM-lateral flow assay (GM-LFA), TA GM-LFA, and BALF polymerase chain reaction (PCR) was assessed in 4, 2, 2, 3, 1, and 2 non-mutually exclusive studies, respectively (supplementary Table 2). Overall, GM-LFA was the most investigated test besides GM and culture, showing variable performance based on different cut-offs and reference definitions (supplementary Table 2). The diagnostic performance of

combinations of laboratory tests and radiology was assessed in 4 and 1 studies, respectively (supplementary Tables 2 and 3). Finally, 7/11 (64%), 4/11 (36%), and 0/11 (0%) studies had a risk of bias of ⟨ 3, 3–4, and ⟩ 4 points according to the scoring system designed for the project (Fig. 2).

Three major considerations stem from the present literature search update: (*i*) the updated evidence is in line with the conclusions of the original study on the better performance of BALF GM than serum GM and the suboptimal specificity of serum BDG for the diagnosis of IPA; (*ii*) four studies assessing the diagnostic performance for IPA of GM-LFA met our inclusion criteria, providing a structured baseline evidence for guiding panel discussion on this test in the next phases of the FUNDICU project; (*iii*) six of the included studies (55%) assessed the diagnostic performance of laboratory markers for the diagnosis of COVID-19-associated pulmonary aspergillosis (CAPA), an entity which was obviously unknown three years ago. Regarding CAPA, incorporation bias (i.e., presence of the evaluated laboratory test in the reference mycological criteria for CAPA) was predominant in studies conducted in critically ill patients with COVID-19 and may have been relevant in biasing the diagnostic performance of mycological tests in the respiratory tract, with overestimation of their diagnostic accuracy for CAPA in some of the main analyses. Considering that in many studies different mycological tests were performed on the specimens from the respiratory tract (where their positivity may also reflect either colonization or growth without invasive disease), the mostly appreciable consequence due to incorporation bias was likely that of overestimating specificity (frequently very close to 100% for both GM and culture, see supplementary tables), although concomitant, more subtle biases on sensitivity cannot be excluded.

Since recent data suggest a possible unfavorable prognostic effect of positive mycological markers (serum GM and/or the combination of positive BALF GM and BALF culture) in critically ill patients with COVID-19,[4,5] overdiagnosis of CAPA in colonized patients (or in patients with early localized disease unlikely to progress to manifest invasive disease) might be considered, at least in part, a somewhat acceptable compromise from a clinical perspective (pending more precise diagnostic algorithms), aiming not to delay or miss the treatment of true CAPA cases.[6] However, for the goal of improving comparability and standardization of research findings, an overestimation of diagnostic accuracy remains an important limitation. The concept of improving diagnostic accuracy by combining classical mycological markers with PCR or other innovative tests is certainly promising, but the related evidence is still preliminary, as also testified by the heterogeneity of evaluated combinations across the few studies that met the inclusion criteria for the present review. In addition, the lack of included studies on the diagnostic performance of mycological tests against histology further precludes a firm assessment of their true accuracy for the diagnosis of CAPA. For all these reasons, the evidence resulting from the present review may ultimately not add to expert considerations and opinions that already allowed to develop shared definitions of CAPA during the pandemic.[7] Consequently, the expert panel of the FUNDICU project will evaluate whether to develop a novel definition of CAPA or to support already existing definitions pending further evidence. For similar reasons, the possibility of supporting already existing definitions will also be considered for influenza-associated pulmonary aspergillosis (IAPA).[8]

In conclusion, the present update mostly confirms the statements from our original review and will serve as the necessary baseline evidence for the development of standard definitions of IPA in critically ill, nonneutropenic patients in ICU.

Fig. 1. Flow diagram of the study selection process, Modified from Moher et al.[3].
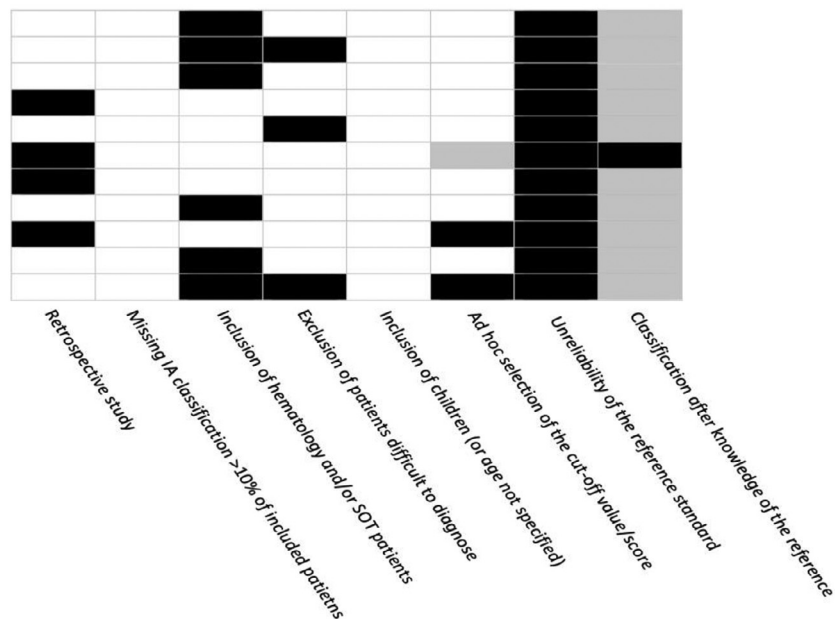
Fig. 2. Risk of bias in included studies.

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Ethical statement

No ethical approval was required as the research in this systematic review is based on previously published data.

Declaration of Competing Interest

Outside the submitted work, DRG reports investigator-initiated grants from Pfizer, Shionogi, and Gilead Italia, and speaker

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.08.003.

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Clinical and virological characteristics of SARS-CoV-2 Omicron BA.2.2 variant outbreaks during April to May, 2022, Shanghai, China

Dear Editor,

Shanghai, as the largest economic center and metropolitan city in China, is always facing greater pressure of SARS-CoV-2 importation from abroad. Although the strict implementation of dynamic zero strategies against SARS-CoV-2 infection is in effect in China, the sudden surge of SARS-CoV-2 Omicron variant caused a large wave of COVID-19 pandemic in Shanghai, China, since the late February, 2022.[1,2] The outbreaks of Omicron variant peaked in April, despite the strict implementation of lockdown. As of June 30th, 2022, more than 630,000 confirmed cases and nearly 600 fatal cases have been reported in Shanghai.[3] Although Zhang et al. reported that SARS-CoV-2 BA.2.2 was the predominant strain circulating in Shanghai,[4] little is known about its clinical and virological characteristics as yet. Thus, we carried out the study to understand the clinical features, viral shedding and genetic characteristics of SARS-CoV-2 Omicron variant in Shanghai.

Herein, a total of 191 child-parent pairs' households including 230 children and 465 parents with SARS-CoV-2 infection admitted in the designated Children's Hospital of Fudan University for COVID-19 in Shanghai, during April 1st to May 5th, 2022, were included. Nasopharyngeal swab samples were collected from these patients. Viral RNA was extracted from 200 μl of the samples using viral RNA extraction kit (DAAN, China). SARS-CoV-2 RNA was detected by the dual-target (ORF1ab and N genes) detection kits (DAAN), and the C_t value < 35 in either target gene was determined as a positive detection.

The median age of patients was 23.5 years and 45.6% of them was male. Most of patients presented to be mild (91.8%) to asymptomatic (8.2%) (Table 1). A vast majority of the children had fever (92.2%), followed by cough (36.5%), nausea/vomiting/diarrhea (22.2%), nasal congestion (10.9%), sore throat (9.1%) and loss of taste or smell (1.7%), while adults were predominated with fever (68.4%) and cough (37.4%), followed by sore throat (22.8%), fatigue (12.7%), nasal congestion (4.3%) and loss of taste or smell (3.2%) (Table 1). Of the patients, 51 (22.2%) children and 362 (77.8%) adults have received at least one dose vaccines. These findings were consistent with the other studies that the Omicron BA.2 variant caused milder diseases.[5–7]

After the symptom onset, the viral RNA load in patients showed a rapid increase in about one to two days, being faster in children with about one-day interval compared with 2 days in adults (Fig. 1A). During the first week, the median viral RNA load remained high levels, and then had a significant decline both in children and adults (Fig. 1A), which was similar to those of other studies regarding Omicron variants.[8,9] We observed that the median peak viral RNA loads ($p = 0.0008$) were significantly higher and the median duration days of viral RNA shedding (C_t value < 35) ($p = 0.0009$) were 2 days longer in children than in adults (Fig. 1B, C). Notably, higher median peak viral RNA loads and longer median duration days of shedding were found in children aged < 1 year than most of the other age groups of children with significant differences (Fig. 1D, E). It emphasized the importance of stringent infection control and early treatment for high-risk young children.

Using the sequencing strategy, hybrid capture based enrichment of SARS-CoV-2,[10] we succeeded in obtaining 42 viral genomes from the SARS-CoV-2 cases. The cases represented throughout 12 districts of Shanghai, between April and May, 2022. All these viral genome sequences belonged to the 21 L/BA.2.2 Omicron variant. Combining the previous report,[3] we concluded that COVID-19 pandemic in Shanghai were predominately attributable to Omicron
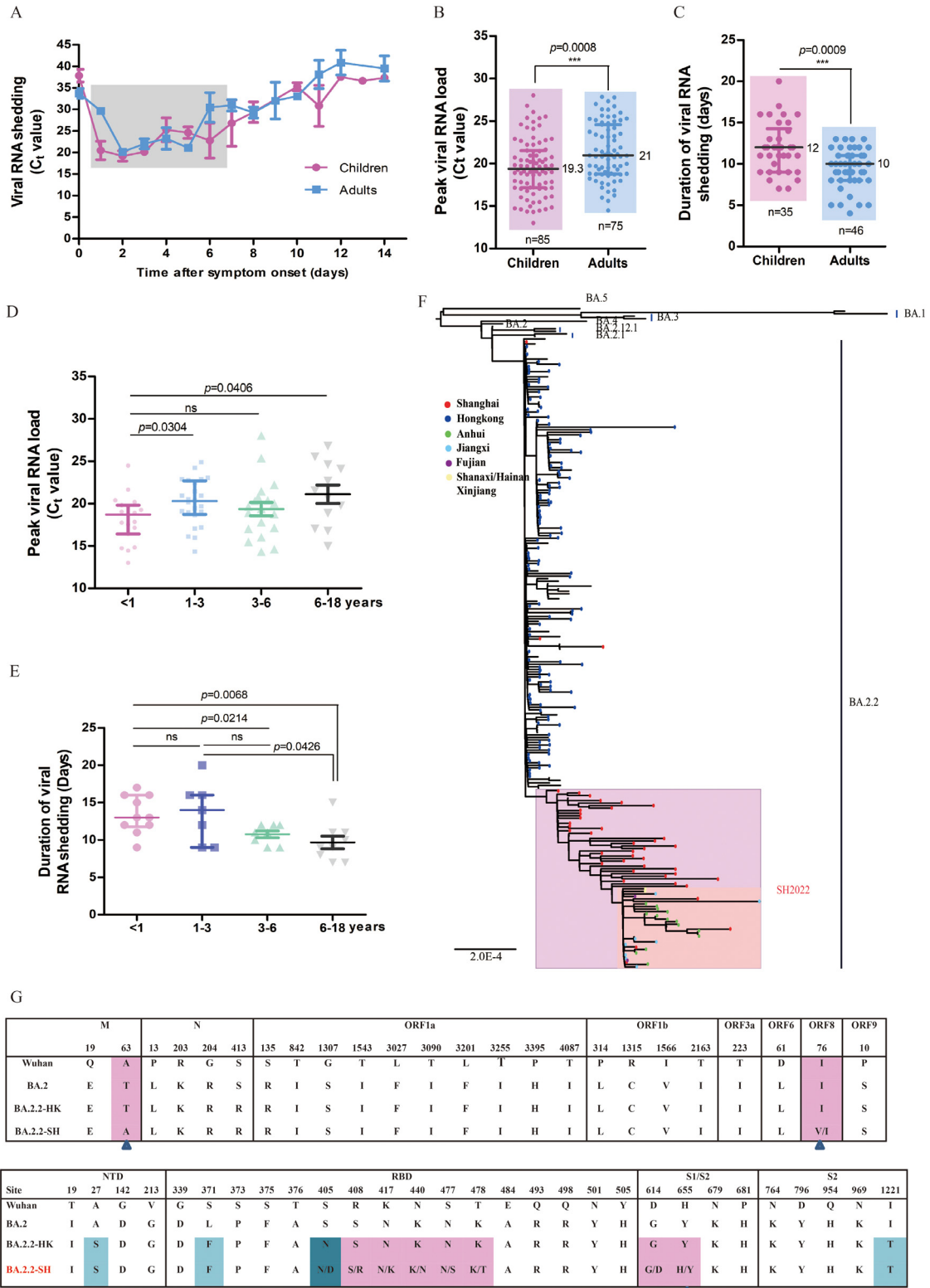
Fig. 1. Viral shedding and genetic characteristics of SARS-CoV-2 Omicron BA.2.2 variants, Shanghai, China. (A) The temporal profile of serial viral RNA shedding in nasal swab from children and adults. (B, C) Comparison of peak viral RNA load and duration of viral RNA shedding between children and adults. Each dot represented a case; box tops and bottoms indicated interquartile range, while horizontal lines denoted medians. A lower Ct value (cycle threshold) indicated a higher viral load. P value were calculated by the Kruskal-Wallis test. *$P < 0.05$, ****$P < 0.0001$; ns, not significant. (D, E) Comparison of peak viral RNA load and duration of viral RNA shedding between different age group of children. (F) Maximum likelihood phylogenetic tree of genomic sequences from BA.2.2 strains. A total of 251 sequences were included for both analyses. Each strain was represented by a circle and colored based on their identified locations in China, while those identified from other counties were not indicated. The new emergent BA.2.2 cluster was indicated in pink shading. (G) Schematic illustration of proteins and their interesting mutations in Shanghai BA.2.2 strains. The Wuhan-Hu-1/2019 (MN908947) was used as the compared reference sequence. The common mutations in all BA.2.2 strains were indicated by cyan shading; the unique mutations in Shanghai BA.2.2 strains were highlighted by pink shading; the unique mutations with a high occur frequency in Shanghai BA.2.2 strains were indicated by black strangle.

Table 1
Epidemiological and clinical characteristics of SARS-CoV-2 Omicron BA.2.2 infection in Children and adults.

	Total (n = 695)	Children (n = 230)	Adults (n = 465)	P value
Age, median (IQR), years	23.5 (3, 35)	3 (1.1, 6)	34 (31, 39)	
Gender				
M	317 (45.6%)	135 (58.7%)	182 (39.1%)	
F	378 (54.4%)	95 (41.3%)	283 (60.9)	
Clinical type				
Asymptomatic	57 (8.2%)	8 (3.5%)	49 (10.5%)	0.001
Symptomatic-Mild	638 (91.8%)	222 (96.5%)	416 (89.5%)	
Vaccination status				
Un-vaccination	282 (40.6%)	179 (77.8%)	103 (22.2%)	
vaccination	413 (59.4%)	51 (22.2%)	362 (77.8%)	
Co-morbidities	32 (0.27%)	17 (7.4%)	15 (3.2%)	
Symptoms				
Fever	530 (76.3%)	212 (92.2%)	318 (68.4%)	< 0.001
Cough	258 (37.1%)	84 (36.5%)	174 (37.4%)	0.818
Nasal congestion	45 (6.5%)	25 (10.9%)	20 (4.3%)	0.001
Sore throat	127 (18.3%)	21 (9.1%)	106 (22.8%)	< 0.001
Nausea/Vomiting/Diarrhea	63 (9.1%)	51 (22.2%)	12 (2.6%)	< 0.001
Fatigue	60 (8.6%)	1 (0.4%)	59 (12.7%)	< 0.001
Loss of taste or smell	19 (2.7%)	4 (1.7%)	15 (3.2%)	0.258

BA.2.2 sub-lineage. Furthermore, a maximum likelihood (ML) tree was reconstructed using 254 BA.2.2 genomic sequences including 42 strains in our study and 212 strains available from GISAID. The ML tree showed that the BA.2.2 strains obtained in our study and identified in other provinces from mainland of China clustered together, but separated from the strains in other regions, indicated that BA.2.2 strain had regionally evolved into an emerging cluster (SH2022) circulating in Shanghai (Fig. 1F). Besides, SH2020 was most closely related to the strains from Hong Kong, indicating that SH2022 strain was most likely originated from the strain of Hong Kong. Additionally, the tree showed that the BA.2.2 strains identified in other provinces in China were transmitted from Shanghai (Fig. 1F), suggesting that the BA.2.2 variant in Shanghai had spread across the provinces in China.

Analysis of those BA.2.2 genomic sequences obtained in the study showed the presence of a mean of 51 aa substitutions and 12 aa deletions, compared with the reference SARS-CoV-2 strain Wuhan-Hu-1/2019 (MN908947). These included 29, 2, 1 and 4 aa mutations in the S, M, E and N proteins, respectively; 10, 4, 1, 1 and 1 aa mutations in the ORF1a, ORF1b, ORF3a, ORF6 and ORF9 proteins (Fig. 1G), respectively. However, compared with other known BA.2.2 strains, eight reversion mutations (N405D, S408R, N417K, K440N, N477S, K478T, G614D and Y655H) in S protein and one mutation (T63A) in M protein were found as Shanghai BA.2.2 strain-specific (Fig. 1G). Among these reversion mutation residues, Y655H in S protein and T63A in M protein presented with a high occur frequency of 42.9% and 88.1% in 42 strains, while other reversion mutations were almost shown at the frequency of more than 7.1%. Furthermore, a unique new mutation I76V within the ORF8 protein was identified in 21.4% of Shanghai BA.2.2 strains. In addition, three unique new nucleotide silent mutations of G7393T in ORF1a, C26789T in M and T28897A in N genes were also found, with the occur frequency of 42.7%, 33.3% and 92.9%, respectively. Thus, the roles of these unique mutations, especially when combined, deserve urgent and further investigations in Shanghai BA.2.2 strains.

Collectively, we not only provide more virologic evidence for the recent upsurge in Shanghai COVID-19 outbreaks caused by the newly identified BA.2.2 variants, but also offer the important understandings for the epidemiology of BA.2.2 variant. Continuing surveillance on complete genome information could facilitate the understandings of SARS-CoV-2 evolutionary patterns and of genotype replacement and, ultimately, in the control and development of effective vaccines and antiviral drugs against SARS-CoV-2 variant infections.

Findings

Author contributions

J. X. and M. Z. led the design , conduct and analysis of the clinical study and the development of the manuscript. Y.Y. A., performed the trial. J. J. L., Z.Q. W., Z. L. W., H. T., Y. Q., X. M. F., W. J. M., and L. T. L. collected the clinical samples and data. Y.Y. A. and J. J. L. contributed to the protocol design and data analysis. All authors were involved in interpreting the data and in ensuring the accuracy and completeness of the data and the fidelity of the trial to the protocol. Y.Y. A. wrote the first draft of the manuscript. The manuscript was subsequently revised by J. X. and M. Z., and approved by all authors, who agreed to submit the manuscript for publication.

Declaration of Competing Interest

All the authors declared no conflicts of interest.

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Community-acquired bacterial meningitis in patients with inflammatory bowel diseases

Dear Editor,

Inflammatory bowel diseases (IBD), ulcerative colitis and Crohn's disease, are chronic inflammatory disorders of the gastrointestinal tract. Higher incidence of common infections has been reported in IBD patients compared to matched controls[1]. Here, we report characteristics of IBD patients with community-acquired bacterial meningitis that were identified in a large nationwide observational cohort study and performed a review of the literature.

From March 2006 through December 2021, patients of 16 years of age or older with community-acquired bacterial meningitis were included in a prospective nationwide cohort in the Netherlands (MeninGene)[2,3]. In this cohort, community-acquired bacterial meningitis was defined by a positive CSF culture or when CSF showed at least one individual predictor of bacterial meningitis according to the Spanos criteria[4]. Detailed study methods have been published previously[2]. Outcome was scored using the Glasgow Outcome Scale (GOS) score. A favorable outcome was defined as a score of 5, and an unfavorable outcome was defined as a score of 1 to 4. Informed consent was obtained during admission from all participating patients or their legally authorized representatives. The study was approved by the Medical ethical committee of the Amsterdam University Medical Center, location Academic Medical Center (AMC).

Pubmed was searched up to December 2021 using the following search strategy for the literature review: ("Meningitis"[M*esh*]) AND ((("Inflammatory Bowel Diseases"[M*esh*]) OR "Crohn Disease"[M*esh*]) OR "Colitis, Ulcerative"[M*esh*]). As a part of data screening, the references of relevant publications were also reviewed.

Continuous data were described with medians and interquartile range and categorical variables with frequency and percentage. The Fisher's exact test and Chi-square tests were used for categorical variables, and Mann Whitney U test was used for continuous variables, as appropriate. A P-value < 0.05 was considered as statistically significant.

From 2006 through 2022, 27 of 2704 (1%) community acquired bacterial meningitis episodes were recorded in 27 IBD patients, including 13 (48%) patients with Crohn's disease and 14 (52%) with ulcerative colitis (Table). The median age of IBD patients was 56 (interquartile range [IQR] 45–67) years, and 9 (33%) were female.

Ten of 27 (37%) patients received treatment with 5-aminosalicylates, 9 (33%) corticosteroids, 8 (30%) purine analogues (i.e., 6-mercaptopurine, 6-thioguanine or azathioprine), 7 (26%) TNF inhibitors (i.e., infliximab or adalimumab), and cyclosporine in one patient (4%). Eight of 27 patients (30%) used a combination of immunosuppressants. Twelve of 27 patients (44%) did not receive immunosuppressive medication. Median time from diagnosis of IBD to meningitis was 3 years (IQR 2–7). Ten (37%) IBD cases experienced preceding gastrointestinal symptoms (consisting of watery or bloody diarrhea, and vomiting); these symptoms were initially attributed to IBD exacerbation in 8 patients.

Common presenting features of meningitis were fever (24 of 26 patients; 92%), headache (20 of 24; 83%), neck stiffness (19 of 24; 79%), and impaired level of consciousness (14 of 24; 59%). The classic triad of meningitis consisting of fever, neck stiffness and altered mental status was present in 11 of 21 patients (52%). On admission, focal neurological deficits were present in 5 of 25 patients (20%) and seizures in 2 of 24 patients (8%). Neuroimaging was performed in 23 of 27 patients (85%) showing a brain abscess and cerebral infarction, each in one patient (4%). Median cerebrospinal fluid (CSF) leukocyte count was 1432 cells per µL (IQR, 554–3468).

CSF cultures grew *Listeria monocytogenes* in 13 of 27 patients (48%), *Streptococcus pneumoniae* in 9 (33%) and *Neisseria meningitidis* in 2 (7%). All 7 patients using TNF inhibitors had listeria meningitis. All 10 cases with preceding gastrointestinal symptoms were diagnosed with listeria meningitis. IBD patients with bacterial meningitis were at higher risk of listeria infection as compared to patients without IBD (13 of 27 [48%] versus 156 of 2677 [6%]; odds ratio 15.01; 95%CI 6.93–32.47; *P*<0.001). One patient with pneumococcal meningitis died (4%); all other 25 patients survived, of whom 8 (32%) had unfavorable outcomes. The outcome of one patient remained unknown.

The literature search revealed 61 IBD cases with meningitis in 50 publications; 31 patients were excluded leaving 30 patients with IBD and community-acquired bacterial meningitis (supplementary table). Nineteen patients (63%) had CD and 11 (37%) UC. All patients but one received immunosuppressive medication: corticosteroids (*n* = 27, 77%), TNF- inhibitors (*n* = 21, 70%), purine analogues (*n* = 16, 53%), and 5-aminosalicylates (*n* = 12; 44%).

Table

Characteristics of IBD patients with bacterial meningitis in the cohort and in the literature.

Characteristics[a]	Cohort (N = 27)	Literature (N = 30)	Combined (N = 57)
Age- years	56 (45–67)	40 (26–65)	52 (36–66)
Gender-female	9/27 (33)	11/28 (39)	20/55 (36)
Time from IBD to meningitis (years)	3 (2–7)	4 (2–16)	3 (2–8)
IBD Type			
Crohn's disease	13/27 (48)	19/30 (63)	32/57 (56)
Ulcerative colitis	14/27 (52)	11/30 (37)	25/57 (44)
Anti-inflammatory and immunomodulating treatment			
Corticosteroids	9/27 (33)	23/30 (77)	32/56 (57)
TNF-alpha inhibitors	7/27 (26)	21/30 (70)	24/56 (43)
Purine analogues	8/27 (30)	16/30 (53)	28/56 (50)
5-aminosalicylates	10/27 (37)	12/27 (44)	22/53 (42)
Clinical manifestations			
Headache	20/24 (83)	16/25 (64)	36/49 (73)
Fever	24/26 (92)	22/25 (88)	46/51 (90)
Impaired level of consciousness	14/24 (59)	16/25 (64)	30/49 (61)
Meningeal signs	19/24 (79)	11/25 (44)	30/49 (61)
Seizure	2/24 (8)	1/25 (4)	3/49 (6)
Focal neurological deficits	5/25 (20)	5/25 (20)	10/50 (20)
Bacteremia	11/19 (58)	12/21 (57)	23/40 (58)
CSF parameters			
Leukocyte count (cells/µL)	1432 (554–3468)	763 (106–3499)	1109 (361–3462)
Granulocyte (%)	87 (82–98)	83 (59–92)	84 (71–95)
Protein concentration (mg/dL)	201 (99–547)	150 (73–355)	189 (91–454)
Glucose concentration (mg/dL)	29 (9–56)	27 (20–50)	27 (13–54)
Causative pathogens			
Listeria monocytogenes	13/27 (48)	21/29 (72)	34/56 (61)
Streptococcus pneumoniae	9/27 (33)	1/29 (3)	12/56 (21)
Neisseria meningitidis	2/27 (7)	1/29 (3)	3/56 (5)
Other pathogens	0/27 (0)	5/29[b] (17)	5/56 (9)
No pathogen isolated	1/27 (4)	1/29 (3)	2/56 (4)
Favorable outcome[c]	17/26 (65)	NR	NR
Death	1/26 (4)	6/28 (21)	7/55 (13)

[a] Numbers are represented by the number of patients with specific characteristics/ the number of patients evaluated (percent) for nominal variables and by median (25th quartile, 75th quartile) for quantitative variables.

[b] Including *Bordetella bronchiseptica*, *Campylobacter fetus*, *Pseudomonas stutzeri*, Peptostreptococcus, *E. coli*, and Bacteroides. NR=not reported.

[c] Favorable outcome was defined as a Glasgow Outcome Scale score of 5.

Fever was reported in 22 of 25 patients (88%), headache in 16 patients (64%), and impaired consciousness in 16 patients (64%). The most common causative pathogen was L. *monocytogenes* occurring in 21 (72%) of 29 patients. Fatal outcome was reported in six (21%) of 28 patients (Table).

IBD is an uncommon risk factor for bacterial meningitis patients occurring in 1% of bacterial meningitis cases. L. *monocytogenes* is the most common causative pathogen, especially in those using anti-TNF agents. One single-center laboratory surveillance study estimated an elevated incidence of listerial bacteriemia for IBD patients with an odds ratio of 7.4 compared to the general population, irrespective of anti-TNF therapy treatment[5]. Listeriosis is common in biologics-treated patients, especially related to anti-TNF therapy treatment use given concomitantly with other immunosuppressive therapies[6]. Indeed, all patients with meningitis using TNF inhibitors were infected with L. *monocytogenes*. TNF is a proinflammatory cytokine that plays a key role in IBD pathogenesis[7] and in host resistance against various microorganisms, particularly intracellular pathogens[8].

Meningitis developed concomitantly or shortly after development of gastrointestinal symptoms that were often interpreted as flares of IBD. It remains unclear whether these symptoms were actually due to IBD flares or caused by the intestinal listerial infection preceding invasive disease. Bacterial meningitis in IBD was associated with high rates of unfavorable outcome (33%), but the morality rate was relatively low (4%).

Our study has several limitations. We selected our patients based on patients' underlying conditions in the CRF, after which discharge letters were analyzed for more detailed information. Since these data and letters were not available for all patients, IBD cases may have been missed. Nevertheless, to the best of our knowledge, this is the largest cohort of IBD patients with bacterial meningitis published to date.

Data availability

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

Authors' contributions

F.S. performed the research and analyzed the data. F.S., D.v.d.B and M.C.B. designed the study. F.S. wrote the first draft of the paper, D.v.d.B., M.L, and M.C.B. revised the paper. F.S., D.v.d.B., M.C.B., and M.L. have read and approved the final manuscript.

Potential conflicts of interest

All authors no conflicts.

Supplementary materials

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Robust neutralizing antibody responses after single-dose BNT162b2 vaccination at long intervals from prior SARS-CoV-2 infection and ceiling effect with repeated vaccination

Dear Editors,

In response to the continued emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants and the time-dependent decline in neutralizing antibodies (nAbs) after coronavirus disease 2019 (COVID-19) vaccination, various strategies for repeated vaccination have been adopted (1-3). There may be significant differences in vaccine immune response depending on whether the person is previously infected with SARS-CoV-2, the time elapsed after recovery from infection, and the interval between vaccinations. Those are very important to establish an optimal vaccination strategy in a situation where the COVID-19 transitions to endemic spread and repeated vaccinations must be considered. In the previous study by Mak et al., a second COVID-19 vaccination in prior-infected individuals did not further increase anti-SARS-CoV-2 immunoglobulin G responses in comparison to single-dose vaccination (4). Thus, we evaluated anti-SARS-CoV-2 neutralizing antibody responses in wild-type (WT) SARS-CoV-2-infected individuals who received the primary series and booster dose of BNT162b2.

This prospective cohort study was conducted on 36 individuals infected with the ancestral Wuhan-1 strain of SARS-CoV-2 who received three doses of the BNT162b2 COVID-19 vaccine. We investigated the kinetics of anti-SARS-CoV-2 nAbs by measuring anti-SARS-CoV-2 nAbs 3 weeks after the first dose (V1–3 w), 1 month after the second dose (V2–1 m), 3 months after the second dose (V2–3 m), and 3 weeks after the third dose of BNT162b2 (V3–3 w). The median day (range) from the COVID-19 diagnosis to each dose of BNT162b2 vaccination was 17.2 months (498–536 days) for the first dose, 18.6 months (533–578 days) for the second dose, and 22.5 months (652–696 days) for the third dose. Cross-reactive immunogenicity was also evaluated against delta and omicron variants. The study protocol was approved by the Institutional Review Board of Korea University Guro Hospital (approval no.:2021GR0099), and written informed consent was obtained from all participants.

For the nAb analysis, a plaque reduction neutralization test (PRNT) was performed using WT SARS-CoV-2 (hCoV/Korea/KCDC03/2020), delta variant (B.1.617.2 lineage, hCoV-19/Korea/KDCA229079/2021), and omicron variant (lineage B.1.1.529, hCoV-19/Korea/KDCA447321/2021). The mixture of serum dilution/virus (40 PFU/well) was incubated at 37 °C for 2 h, added to a plate seeded with Vero E6 cells, and incubated at 37 °C for 1 h, followed by the addition of 0.5% agarose (Lonza, Basel, Switzerland). After 2–3 days of incubation, the cells were fixed with 4% paraformaldehyde and stained to visualize plaques. A reduction in plaque count of 50% (PRNT50) was then calculated for the median neutralizing titer (ND50) using the Spearman–Karber formula, and an ND50 $\geq$ 1:20 was considered positive. As for the comparison of geometric mean titer (GMT) of nAbs from paired sera at each time point, the Wilcoxon signed rank test was performed.
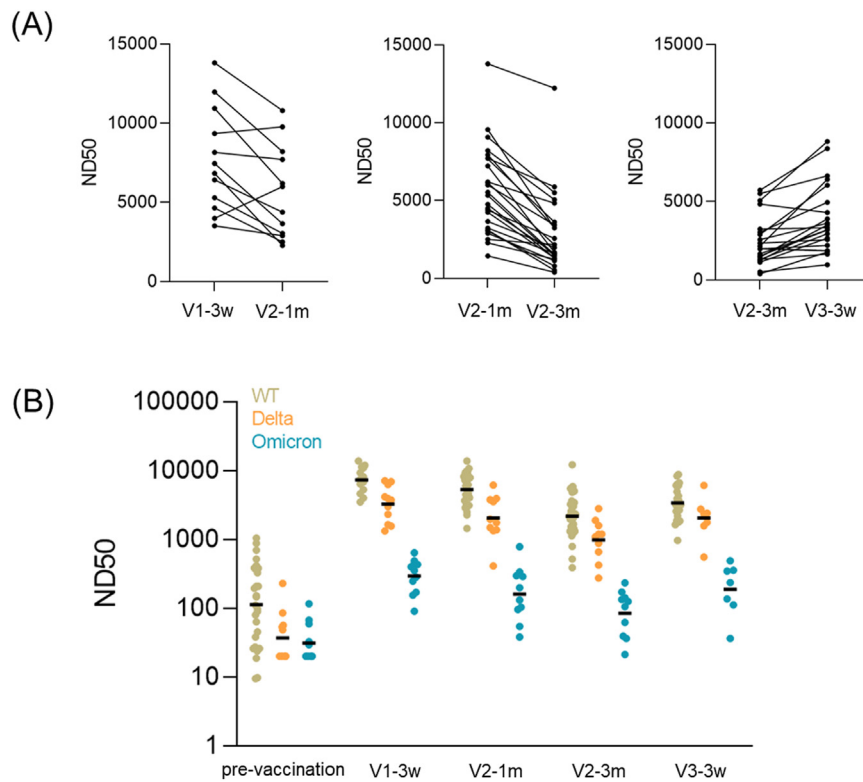
Fig. 1. (A) Comparison of paired sera which were collected longitudinally from the study subjects. The geometric mean titer (GMT) of neutralizing antibodies (nAbs) of V1–3 w was higher than that of V2–1 m ($n = 12$, 7067.7 [95% CI, 5354.1–9329.7] vs. 4916.6 [95% CI, 3470.9–6964.4], $P = 0.008$) (left). The GMT of nAbs waned from 4954.4 (95% CI, 3986.7–6157.0) at V2–1 m to 2094.9 (95% CI, 1514.3–2898.0) at V2–3 m ($n = 25$, $P<0.001$) (middle). The GMT of nAbs of V3–3 w was higher than that of V2–3 m ($n = 22$, 3320.9 [95% CI, 2585.0–4266.4] vs. 1994.8 [95% CI, 1468.2–2710.2], $P<0.001$) (right) (B) Kinetics of nAbs against SARS-CoV-2 wild-type, delta, and omicron strains. All available data of titers of nAbs from the study subjects are shown. Black line indicates the GMT of nAbs. V1–3 w, 3 weeks after the first dose; V2–1 m, 1 month after the second dose; V2–3 m, 3 months after the second dose; V3–3 w, 3 weeks after the third dose of BNT162b2; CI, confidence interval.

All participants were women, and the median age was 50 years (range, 38–57 years). SARS-CoV-2 infection was diagnosed by real-time polymerase chain reaction. Although the viral sequences of SARS-CoV-2 were not investigated at the time of diagnosis, our study population was assumed to be infected with the ancestral Wuhan-1 strain of SARS-CoV-2 because they were diagnosed with COVID-19 during the early phase of the pandemic in March 2020.

The GMT of nAbs of V1–3 w against SARS-CoV-2 WT was the highest among serial time points (112.9 at pre-vaccination, 7324.4 in V1–3 w, 5287.6 in V2–1 m, 2173.2 in V2–3 m and 3409.3 in V3–3 w) (Table S1). Interestingly, in the comparison of paired sera collected longitudinally from each subject, the GMT of V1–3 w nAbs was significantly higher than that of V2–1 m ($P = 0.008$, Fig. 1A), indicating that the second dose of vaccination at 3-week intervals did not raise the nAb titers sufficiently in individuals infected with SARS-CoV-2 18 months ago. Although the third dose of the vaccine showed a booster effect on the antibodies neutralizing SARS-CoV-2 ($P<0.001$), the titers in V3–3 w were rather lower than those of V1–3 w and V2–1 m.

As for cross-reactive immunogenicity, the titers of nAbs against the omicron strain were significantly attenuated compared with those against WT (21–30 fold) in individuals previously infected with SARS-CoV-2 who received the primary series and booster dose of BNT162b2, while nAb titers against the delta strain were 1.9–2.5 fold lower than those against WT (Fig. 1B and Table 1). In our study, the fold difference of titers of nAbs between the WT and omicron was larger than the results of other studies (5-7). Multiple factors may be involved in this difference, including the heterogeneity in neutralization assays, characteristics of the study population, and the timing of prior SARS-CoV-2 infection and vaccination. However, the fold difference between the ancestral strain and variants was found to be smaller after the booster dose of BNT162b2, which was consistent with the results of other studies (7, 8).

There has been a lack of information regarding the optimal intervals of vaccination. Determination of the interval required for repeated COVID-19 vaccination is essential to induce an optimal immune response that protects against SARS-CoV-2 (9, 10). When vaccinated at a sufficiently long interval after natural SARS-CoV-2 infection and when memory B-cells were fully mature, single-dose vaccination induced robust neutralizing antibody responses, and cross-reactive immunity was also induced against the omicron variant. However, repeated vaccination at short-term intervals may elicit a limited boosting effect (ceiling effect) on the nAb response, especially in individuals who have already gained substantial levels of nAbs. It is necessary to investigate whether the same phenomenon will occur in the case of booster vaccinations at longer intervals following the primary vaccination series without prior infection.

In conclusion, although the number of samples is small and a longitudinal analysis was not performed at all time points in all subjects, we found that robust antibody response would be induced even with a single-dose COVID-19 vaccination when vaccinated at long intervals (more than 12 months) after SARS-CoV-2 infection. However, remarkable ceiling effects were observed with repeated vaccination.

Author contributions

JYN and JYS conceived and designed the study. JYN and JYS prepared the manuscript and analyzed the data. All authors con-

Table 1

Longitudinal comparison of geometric mean titers of neutralizing antibodies against SARS-CoV-2 wild-type, delta, and omicron in the BNT162b2-vaccinated individuals with prior SARS-CoV-2 infection.

	Pre-vaccination ($n = 11$)	V1–3 w ($n = 11$)	V2–1 m ($n = 10$)	V2–3 m ($n = 10$)	V3–3 w ($n = 7$)
Wild-type, GMT	196.2	7207.5	4861.3	2452.7	3928.1
(95% CI)	(84.2–457.2)	(5469.6–9497.6)	(3404.6–6941.3)	(1495.1–4023.7)	(2448.2–6302.4)
Delta variant, GMT	37.1	3260.6	2039.5	987.9	2039.7
(95% CI)	(21.4–64.2)	(2171.7–4895.3)	(1176.4–3535.7)	(601.7–1622.1)	(1048.6–3967.7)
Omicron variant,	31.3	294.1	161.5	84.6	186.7
GMT (95% CI)	(20.6–47.6)	(198.5–435.7)	(83.9–311.0)	(48.3–148.1)	(81.3–428.3)

V1–3w: three weeks after the first dose; V2–1m: one month after the second dose; V2–3m: three months after the second dose; V3–3w: three weeks after the third dose of BNT162b2; GMT: geometric mean titer; CI: confidence interval.

tributed to the acquisition of the clinical and laboratory data. JYN, BK and JYS contributed to data interpretation and statistical analysis. All the authors critically reviewed the manuscript for intellectual content and approved the final draft for submission.

Declaration of Competing Interest

The authors declare no conflict of interest.

Supplementary materials

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Synergism of interferon-beta with antiviral drugs against SARS-CoV-2 variants

Dear Editor,

In their recent article, Vellas et al. reported that tixagevimab-cilgavimab treatment of COVID-19 patients induces resistance mutations in SARS-CoV-2 Omicron BA.2,[8] contributing to concerns that resistance formation may affect the efficacy of anti-SARS-CoV-2 therapies. In this context, more effective combination therapies are anticipated to reduce resistance formation[9].

Interferons are potential anti-SARS-CoV-2 drugs but displayed limited efficacy in initial clinical trials for the treatment of COVID-19.[10] Based on findings that Omicron variant BA.1 isolates replicated less effectively in interferon-competent cells and were more sensitive to interferon treatment than a Delta isolate,[2,3] we here systematically compared the sensitivity of Delta, BA.1, and BA.2 isolates to betaferon (a clinically approved interferon-β preparation) alone or in combination with the approved anti-SARS-CoV-2 drugs remdesivir (RNA-dependent RNA polymerase inhibitor), EIDD-1931 (the active metabolite of molnupiravir that induces 'lethal mutage-
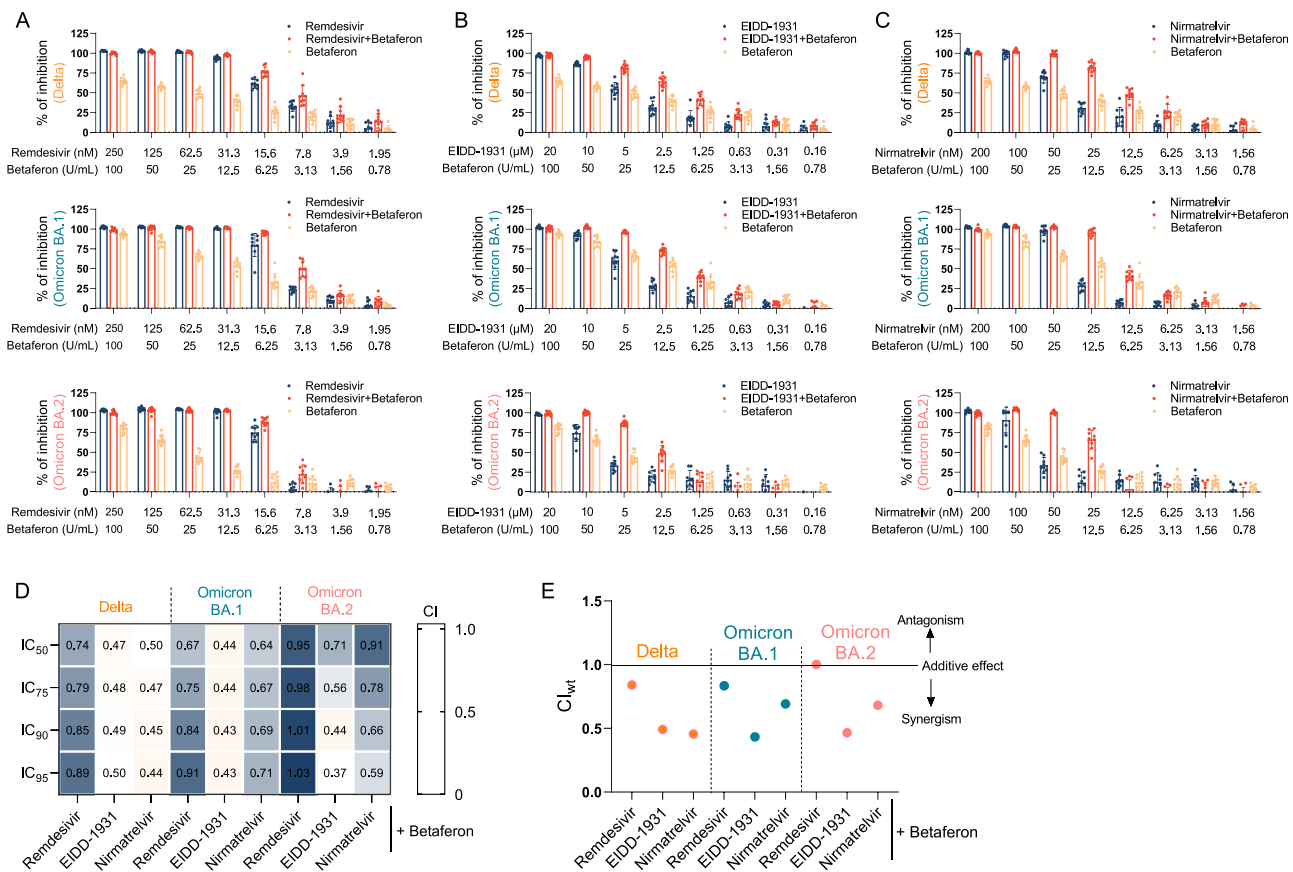
Fig. 1. Antiviral effects of approved anti-SARS-CoV-2 drugs in combination with interferon-β (betaferon) against Delta, Omicron BA.1, and Omicron BA.2 isolates. Betaferon was tested in fixed combinations combination with remdesivir (A), EIDD-1931 (B), or nirmatrelvir (C) in SARS-CoV-2 (MOI 0.01)-infected Caco-2-F03 cells. Values represent mean ± S.D. of three independent experiments. D) Combination indices were calculated at the IC_{50}, IC_{75}, IC_{90}, and IC_{95} levels following the method of Chou and Talalay. E) The weighted average CI value (CI_{wt}) was calculated according to the formula: CI_{wt} [CI_{50} + $2CI_{75}$ + $3CI_{90}$ + $4CI_{95}$]/10. A CI_{wt} <1 indicates synergism, a CI_{wt} =1 indicates additive effects, and a CI_{wt} >1 suggest antagonism.

nesis' during virus replication), nirmatrelvir (inhibitor of the SARS-CoV-2 main/ 3CL protease, the antivirally active agent in Paxlovid), and aprotinin, a protease inhibitor that inhibits SARS-CoV-2 replication[1] and that was recently reported to be effective in COVID-19 patients in a clinical trial.[7]

A comparison of sequence variants in Delta, Omicron BA.1, and Omicron BA.2 virus isolates identified 96 sequence variants in putative viral interferon antagonists that differed from the reference genome of the original Wuhan strain (Suppl. Table 1). The overlap in sequence variants between BA.1 and BA.2 was larger (49) than between Delta and BA.1 (21) and Delta and BA.2 (18). Moreover, Delta displayed more unique sequence variants (54) than BA.1 (23) or BA.2 (26) (Suppl. Figure 1A). These findings appear to reflect the closer relatedness of BA.1 and BA.2 relative to Delta. However, the variant overlaps are complex (Suppl. Figure 1B, Suppl. File 1), and it is not clear, which of them drive the virus response to interferons. Of the 45 of the 96 sequence variants that could be modelled on protein structures or models (Suppl. File 1), only two were proposed to have a likely impact on interferon signaling based on an *in silico* structural analysis (Suppl. Fig. 1, Suppl. Table 1, Suppl. File 1). These findings warrant the further comparison of Delta, BA.1, and BA.2 variants for their responses to interferon treatment. Indeed, a BA.2 isolate replicated more effectively than BA.1 but less effectively than Delta in Caco-2-F03 cells, a Caco-2 subline that is highly susceptible to SARS-CoV-2 infection[4] (Suppl. Fig. 2).

Next, we tested the effects of remdesivir, EIDD-1931, and nirmatrelvir on Delta, BA.1, and BA.2 replication. Delta and BA.1 displayed similar sensitivity to the approved anti-SARS-CoV-2 drugs

remdesivir, nirmatrelvir, and EIDD-1931, whereas BA.2 was less sensitive to EIDD-1931 than Delta and BA.1 (Suppl. Fig. 3).

In agreement with previous findings[2] the clinically approved interferon-β preparation betaferon (Bayer) was more effective against BA.1 than against Delta (Suppl. Fig. 3). Interestingly and perhaps unexpectedly, the betaferon response of BA.2 more closely resembled that of Delta and not that of the more closely related BA.1 (Suppl. Fig. 3). This confirmed our previous findings (Suppl. Fig. 1) that the impact of amino acid sequence differences in different SARS-CoV-2 isolates on the viral interferon response is not easily predictable and can differ even between closely related virus variants.

Among the tested antiviral drugs, remdesivir was the only one that did not display synergistic effects in combination with betaferon (Fig. 1), which may reflect clinical findings indicating that the addition of interferon does not increase remdesivir efficacy in COVID-19 patients.[5] While EIDD-1931 and nirmatrelvir treatment resulted in similar levels of synergism with betaferon against Delta, combined EIDD-1931 and interferon treatment was associated with a more pronounced synergism against BA.1 and BA.2 than the combination of nirmatrelvir and betaferon (Fig. 1).

Aprotinin inhibited Delta (IC50: 0.66 µM) and BA.1 (IC50: 0.64 µM) in a similar concentration range as the original Wuhan strain isolates [1] (Suppl. Figure 4). Effects against BA.2 were less pronounced (IC50: 1.95 µM) but still in the range of clinically achievable plasma concentrations after systemic administration, which have been shown to reach 11.8 µM.[6] Moreover, aerosol preparations like the one used in the clinical trial that demon-
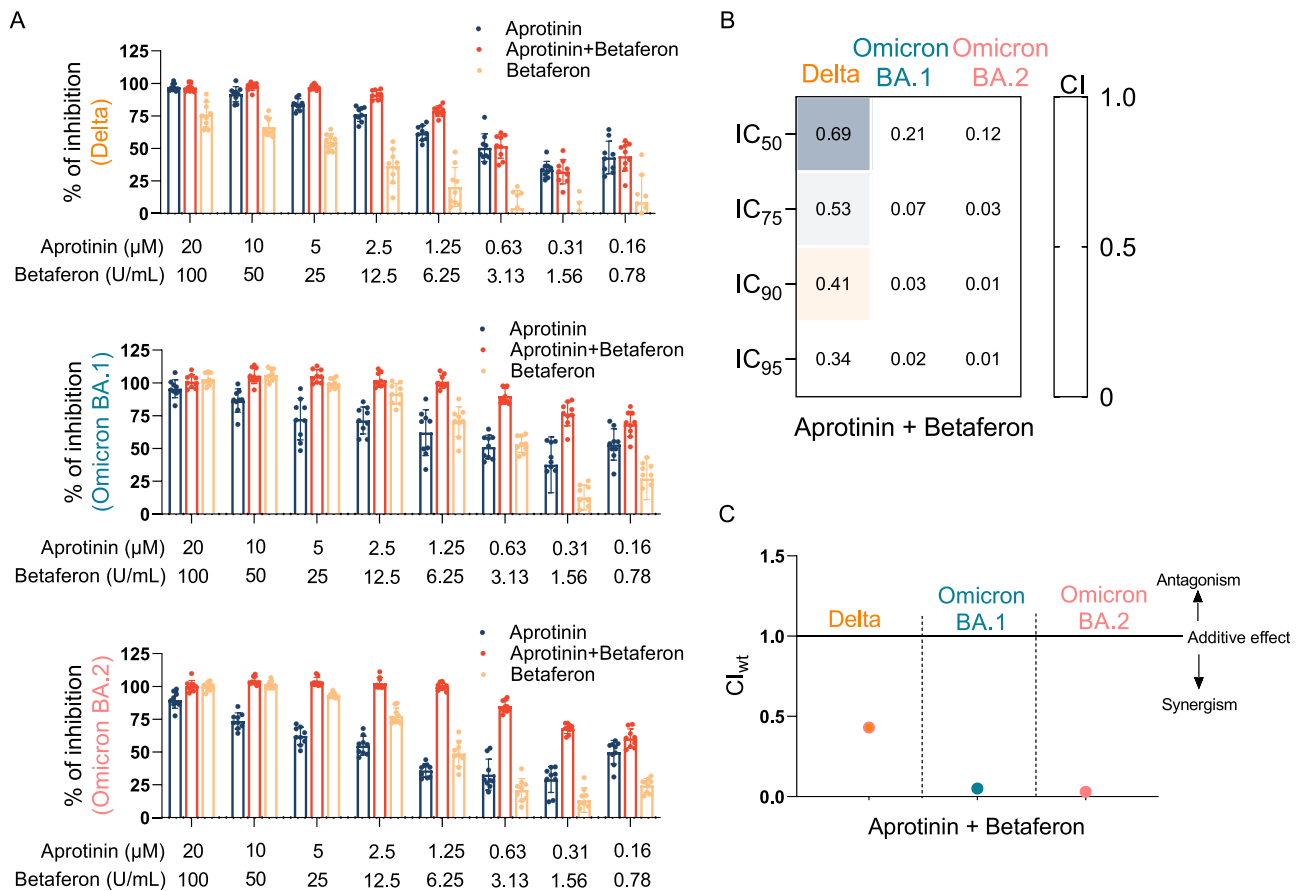
Fig. 2. Antiviral effects of aprotinin in combination with interferon-β (betaferon) against Delta, Omicron BA.1, and Omicron BA.2 isolates. Betaferon was tested in a fixed combination with aprotinin in SARS-CoV-2 (MOI 0.01)-infected Caco-2-F03 cells. Values represent mean ± S.D. of three independent experiments. B) Combination indices were calculated at the IC_{50}, IC_{75}, IC_{90}, and IC_{95} levels following the method of Chou and Talalay. C) The weighted average CI value (CI_{wt}) was calculated according to the formula: CI_{wt} $[CI_{50} + 2CI_{75} + 3CI_{90} + 4CI_{95}]/10$. A CI_{wt} <1 indicates synergism, a CI_{wt} =1 indicates additive effects, and a CI_{wt} >1 suggest antagonism.

strated therapeutic efficacy of aprotinin against COVID-19 [7] are expected to result in substantially higher local aprotinin concentrations in the lungs.

Aprotinin displayed the strongest synergism with betaferon against BA.1 and BA.2 among all tested drugs. Against Delta, the level of synergism of aprotinin/ betaferon was similar to that of EIDD-1931/ betaferon (Fig. 2).

In conclusion, even closely related SARS-CoV-2 (sub)variants can differ in their biology, as indicated by different BA.1 and BA.2 replication kinetics, and in their response to antiviral treatments, as indicated by differences in the virus responses to betaferon, EIDD-1931/ molnupiravir, and aprotinin and differing levels of synergism of betaferon combinations with other antiviral drugs. Betaferon combinations with nirmatrelvir and, in particular, with EIDD-1931 and aprotinin displayed high levels of synergism, which makes them strong candidates for clinical testing.

Declaration of Competing Interests

The authors declare no competing interests.

Supplementary materials

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Monkeypox infection among men who have sex with men: PCR testing on seminal fluids

Dear Editor,

in this journal recently J Heskin et al. described how sexual transmission is becoming predominant among new monkeypox (MPX) cases, Awan UA et al. identified as critical to achieve infection control raising public knowledge of risk factors and informing of the strategies they may take to decrease their exposure to the virus,[1,2] and we provided an example of an atypical MPX proctitis following sexual intercourse.[3]

In this case-series we presented data on MPX virus detection on seminal fluids to corroborate the hypothesis of sexual transmission.

Overall, 36 men who have sex with men (MSM) diagnosed with MPX infection at the Infectious Diseases Unit of San Raffaele Scientific Institute, Milan, Italy, were tested on seminal fluids.

Real-time (RT) PCR (RealStar® Orthopoxvirus PCR Kit 1.0 – altona DIAGNOTICS) targeting *variola virus* and *non-variola* Orthopoxvirus species (*cowpox, monkeypox, raccoonpox, camelpox, vaccinia virus*) was used to detect *non-variola* DNA on swabs, serum, plasma, seminal fluids and urines and a specific RT PCR targeting Monkeypox virus DNA (Liferiver - SHANGHAI ZJ BIO-TECH CO., LTD) subsequently confirmed MPX infections.

All individuals had a positive oropharyngeal, cutaneous, genital or rectal MPX swab and were at different time points tested for MPX also on seminal fluids and urines.

All reported high-risk sexual behaviors in the 3 months before diagnosis, with unprotected sexual intercourse with >10 partners. 15/36 (42%) were people living with HIV (PLWH) and 15/36 (42%) were HIV pre-exposure prophylaxis (PrEP) users.

Among 22/36 (61%) individuals MPX virus was detected also in seminal fluids, whereas in 8/36 (22%) in urines. Median (interquartile) cycle thresholds (CT) of MPX were 34 (29–36.5) in seminal fluids and 34 (33–36) in blood.

Individuals with positive seminal fluids tested positive for MPX on plasma or serum in 17/22 (77%) of cases, whilst 7/14 (50%) of those with negative seminal fluids tested positive on plasma or serum. Full details on virologic data are presented in Table 1.

Table 1
Virologic data on different specimens of monkeypox (MPX) virus.

Cases	MPX swab[§]	Seminal fluids	Urines	Serum/Plasma
Case 1	+	+	+	+
Case 2	+	–	–	–
Case 3	+	+	+	+
Case 4	+	+	–	+
Case 5	+	–	–	–
Case 6	+	+	–	+
Case 7	+	–	–	–
Case 8	+	+	–	+
Case 9	+	–	–	–
Case 10	+	–	–	+
Case 11	+	+	–	+
Case 12	+	–	–	–
Case 13	+	–	–	+
Case 14	+	+	–	+
Case 15	+	–	–	–
Case 16	+	–	–	+
Case 17	+	+	–	+
Case 18	+	+	–	–
Case 19	+	+	–	+
Case 20	+	–	–	+
Case 21	+	+	+	–
Case 22	+	–	–	–
Case 23	+	+	+	+
Case 24	+	+	+	+
Case 25	+	–	–	+
Case 26	+	+	+	–
Case 27	+	+	–	+
Case 28	+	+	–	+
Case 29	+	–	–	+
Case 30	+	+	–	+
Case 31	+	+	–	+
Case 32	+	+	–	–
Case 33	+	+	–	+
Case 34	+	+	–	–
Case 35	+	–	+	+
Case 36	+	+	+	+

§ : "MPX -swab" refers to either cutaneous, rectal, oropharyngeal or genital swabs.

Table 2
Individuals' characteristics among those who tested positive or negative for MPX on seminal fluids.

Characteristics	Overall (n = 36)	Positive seminal fluids (n = 22)	Negative seminal fluids (n = 14)	p-value
Age (IQR)	41.5 (31.25–35.5)	37.5 (35–45)	35 (31–37)	0.303 a
HIV infection	15 (42%)	7 (32%)	8 (57%)	0.175 b
PrEP users	15 (42%)	9 (41%)	6 (43%)	1 b
Number of sexual partners				0.947 b
10–20	20 (55%)	15 (68%)	5 (36%)	
20–30	10 (28%)	6 (27%)	4 (28%)	
>30	6 (17%)	4 (18%)	2 (14%)	
Type of intercourse:				0.975 b
Receptive	4 (11%)	2 (9%)	2 (15%)	
Insertive	4 (11%)	2 (9%)	2 (15%)	
Both	26 (72%)	18 (82%)	10 (71%)	
Previous STIs	36 (100%)	22 (100%)	14 (100%)	1 b
Concurrent STIs	4 (11%)	1 (4%)	3 (21%)	0.277 b
Presence of genital lesions	13 (36%)	10 (45%)	3 (21%)	0.175b
Presence of rectal lesions°	18 (50%)	10 (45%)	8 (57%)	0.730 b
Presence of cutaneous lesions°	20 (55%)	10 (45%)	10 (71%)	0.176 b
Positive serum or plasma	24 (66%)	17 (77%)	7 (50%)	0.147 b
CT (median, IQR)	34 (33–36)	33.5 (30–36)	34.5 (34–36)	0.263 a

ᵃ : by Mann-Whitney test;.
ᵇ : by chi-square or Fisher's exact test.
° : without genital lesions
Abbreviations: STI: sexually transmitted infection; IQR: interquartile; CT: cycle threshold.

Genital lesions were present among 10/22 (45%) of MSM with positive seminal fluids and 3/14 (21%) with negative. Among individuals without genital lesions, 18/23 (78%) had rectal lesions, 20/23 (87%) cutaneous lesions and 15/23 (65%) both: 10/18 (55%), 10/20 (50%), 8/15 (53%), respectively, tested positive on seminal fluids for MPX.

Overall, only one individual with positive seminal fluids had a concurrent sexually transmitted infection (STI), whilst three with negative seminal fluids had a concurrent one.

Individuals' characteristics among those who tested positive or negative for MPX on seminal fluids are presented in Table 2.

In this case-series positive seminal fluids were frequent among MPX cases, in line with other previously reported preliminary results.[4] A higher proportion of MSM tested positive on seminal fluids than urines, suggesting that MPX testing on seminal fluids rather than urines could be possibly included in clinical assessment of individuals.

We observed that also individuals who presented without genital lesions or who did not engage in insertive intercourse had positive seminal fluids. Most MSM with positive seminal fluids tested positive for MPX also on serum or plasma. Given these two factors, we hypothesize that MPX presence in seminal fluids follows bloodstream dissemination of the virus.

Furthermore, concurrent STIs seemed to not have an effect on the positivity rate of seminal fluids.

A key question remains the duration of viral shedding in seminal fluids following clinical healing of MPX lesions, especially among those living with an immune impairment, such as PLWH. Moreover, the significance of detecting MPX DNA on seminal fluids and urines is still uncertain, as to this day no data are available regarding viral culture on seminal fluids. However, we believe that these data, in our high-risk population, corroborate the hypothesis of sexual transmission.

Contributorship statement

S.N., A.R.R. and E.B. visited the individuals and contributed to writing the article. C.C and D.C. visited the individuals and contributed to the reviewing of the article. A.C. coordinated clinical activities and contributed to the reviewing of the article. D.M. and A.R. coordinated virologic activities and performed PCR tests for MPX. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2022.07.022.

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## Remdesivir modifies interferon response in hospitalized COVID-19 patients

Dear Editor,

We have read with great interest the article by Lucijanic et al. that was recently published in Journal of Infection.[1] In a retrospective matched case-control analysis they showed an increased occurrence of bacteremia in patients treated with remdesivir and, notably, they found no clinical benefit of remdesivir in patients with bacteremia. In fact, whereas the use of certain immunomodulating drugs has been documented to be beneficial in patients with severe COVID-19 disease,[2] the clinical effect of anti-viral therapy is still debated.[3] Thus, in the recently published final report from the WHO Solidarity trial, the meta-analysis of the published literature confirms a modest survival effect of remdesivir in non-ventilated patients, but also a potential harm in ventilated patients.[4] Previous published trials that evaluated SARS-CoV-2 virus clearance did not find any effect of remdesivir,[5–7] despite clinical benefit when given in early disease.[7] The background for these seemingly conflicting effects is not clarified, although treatment initiation at the tail of the viral phase in hospitalized patients with critical disease could partly explain the lack of clinical effect in this patient group. An alternative explanation could be disparate interacting effects of remdesivir and host immunity in different stages of the disease, representing off-target effects of remdesivir.

To elucidate any potential immunomodulating effects of remdesivir, in the present study we performed transcriptome analyses of peripheral blood mononuclear cells (PBMC) in 15 hospitalized COVID-19 patients (mean age±SD: 60,9 ± 13 years, 8 males [53%]) that were included In the NOR Solidarity trial,[5] an independent sub-study of the WHO Solidarity trial.[4] Seven patients received standard of care (SoC) and eight patients received SoC +200 mg of intravenously remdesivir on day 1, then 100 mg daily up to 9 days. Three days after inclusion, peripheral blood was collected in BD CPT^{TM} Cell Preparation Tube containing sodium heparin (BD, Franklin Lakes, New Jersey, USA) and PBMC were isolated according to the manufacturerś instructions. Total RNA was isolated from PBMC with miRNeasy Kit (Qiagen, Hilden, Germany). Novogene (UK) Company Limited performed the stranded library preparation and sequencing on the Illumina platform. Differentially expressed genes were obtained using the DESeq2 R package. The study was approved by the Committee for Medical Research Ethics Region South East Norway (REK no. 118,684) and registered on Clinical-Trials.gov March 25th 2020 (NCT04321616). All participants gave informed consent prior to inclusion, either directly or through a legally authorized representative.

Functional enrichment analysis of isolated PBMC transcriptomes revealed alteration of hallmark gene sets related to interferon (INF)γ response in patients randomized to remdesivir compared to SoC (Fig. 1A). To determine the association of genes in-
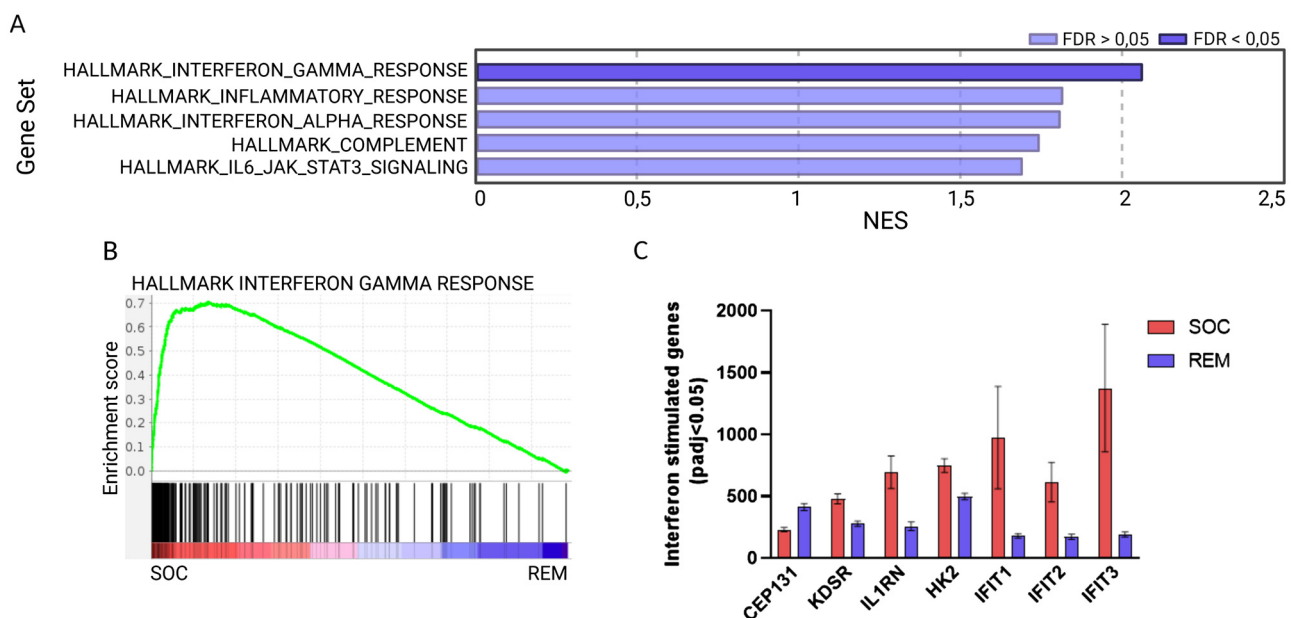
Fig. 1. RNA-seq and hallmark gene set enrichment analysis of RNA isolated from peripheral mononuclear cells reveals alterations of "interferon gamma response" in patients randomized to remdesivir (REM, *n* = 8) compared to standard of care (SOC, *n* = 7) (**A**), with dampened enrichment of this pathway in patients receiving remdesivir (**B**), and differential expression analysis of interferon-stimulated genes between the two treatment arms (**C**).

volved in "Hallmark interferon gamma response" pathway between the two treatment arms we ran a gene set enrichment analysis (GSEA). Of note, this pathway was dampened in immune cells from the remdesivir-treated patients compared to SoC treated patients (Fig. 1B) and differential expression analysis revealed that six of the 13 differentially regulated genes (p$_{adj}$<0.05) were IFN-regulated genes (Fig. 1C).

Although it has been speculated that remdesivir metabolites that are adenosine analogues may alter innate and specific immunity in the same fashion as adenosine does,[1,8] the mechanisms for the potential immune modulating effects of remdesivir is at present not clear. Whatever mechanisms, our results suggest an impaired INFγ response by remdesivir treatment that could have different clinical implications depending on the disease stage. In early and moderate disease (i.e., non-hospitalized and moderate disease in hospitalized patients), an impaired INFγ response could prevent an overshooting immune response and potentially explain the clinical benefit by preventing hospitalization and death despite lack of viral clearance in nasopharyngeal samples.[7] In more advanced disease, such effects could be potentially harmful, including lack of disease control in patients with prolonged immune exhaustion and reduced capacity for adaptive immune response.[9] Remdesivir has not yet been recommended in WHO treatment guidelines despite regulatory approval by EMA and FDA. Targeting the right patient population is critical in treatment algorithms, and our results suggest that off-target effects interacting with host immunity could be of relevance for the seemingly disparate clinical effects of remdesivir in different stages of COVID-19.

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Declaration of Competing Interest

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