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

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Letters to the Editor

Outcome prediction by serum calprotectin in patients with COVID-19 in the emergency department

Dear Editor,

The coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 virus, becomes clinically manifest in a broad range from mild symptoms to life-threatening multi-organ failure (MOF). We have read with interest the recent letter by García de Guadiana Romualdo and colleagues[1] and the review by Skevaki and colleagues[2] examining the significance of biomarkers for risk assessment and prognosis of COVID-19.

A severe course of disease is characterized by a dysregulated immune response, suspected to be initiated by dysregulation of innate immune cells of the granulomonocytic lineage.[3] The pro-inflammatory mediator calprotectin (S100A8/A9, MRP 8/14) is reported to be an early signal, mediating the cytokine storm associated with an increased severity of COVID-19.[1,3,4]

The expression of calprotectin is predominantly restricted to the intracellular compartment of neutrophil granulocytes, where it presents about half of the total cytosolic protein content. In contrast to routinely used inflammatory biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT), it is released into the bloodstream without need for *de novo* protein biosynthesis. Thereby, circulating calprotectin possibly has a decisive kinetic advantage in that it might be one of the first responses of an organism to an inflammatory disease.

Previous studies have reported significantly elevated levels of calprotectin in patients with severe COVID-19 and the possible ability of calprotectin to discriminate between mild and severe form of the disease.[3–5] In addition, elevated fecal calprotectin has been shown to associate with thromboembolic events in COVID-19 in the absence of gastrointestinal manifestations.[6] However, it is not yet fully elucidated whether changes of calprotectin serum levels occur prior to the progression to severe disease and therefore might be detectable already at an early stage of COVID-19, e.g. in patients in the emergency department (ED), and whether calprotectin is superior compared to traditional biomarkers. Thus, here we evaluated calprotectin levels with regard to prediction of prognosis (subsequent intensive care unit (ICU) admission, MOF, mortality) in ED patients.

We prospectively enrolled a total of 66 patients presenting to the ED with suspected SARS-CoV-2 infection and isolated serum samples for further investigation. Using PCR testing in pharyngeal swabs, 47 patients were tested negative, and 19 patients were tested positive for SARS-CoV-2 and diagnosed with COVID-19. Main characteristics of SARS-CoV-2 positive patients are presented in Table 1A. The disease course was evaluated with regard to the clinical endpoints i) MOF, defined as the clinical need for organ replacement of at least two organ systems in regard to the SOFA-Score (Sequential Organ Failure Assessment),[7] within either 72 h after admission or ii) during the total hospital stay (total MOF), iii) admission to the ICU, and iv) death, defined as 90-day mortality. These definitions lead to subgroups of $n = 8$ for ICU admission, $n = 4$ for MOF within 72 h, $n = 6$ for total MOF, *and* $n = 2$ for 90-day mortality. The study design is presented in Fig. 1A and the frequency of the described endpoints is depicted in Table 1. In all patients we quantified blood levels and calculated the Area Under the Receiver Operating Characteristic curve (AUROC) and the 95% confidence interval (CI) for calprotectin (measured by turbidimetric method, Gentian AS, Norway) within these subgroups in comparison with the biomarkers routinely used for clinical evaluation of patients admitted to the ED (Table 1B). Routine biomarkers were determined immediately as standard of care and calprotectin was measured in directly isolated serum, which was centrifuged within 30 min after blood withdrawal. With regard to the endpoint MOF incurring within 72 h, calprotectin showed the highest AUROC (0.87) as compared to lactate (0.79), CRP (0.70) and PCT (0.75) (Table 1C). Indeed, patients suffering from MOF within 72 h displayed a two-fold increase in median serum calprotectin at presentation to the ED compared to patients not experiencing MOF within 72 h (5.14 mg/L vs. 2.08 mg/L, $p = 0.03$, Table 1B). In patients with total MOF i.e. incurring within the total hospital stay, the AUROC of calprotectin was even higher at 0.91 with a comparable difference in median serum values between patients with and without this outcome (4.79 mg/L vs. 2.07 mg/L, $p < 0.01$, Table 1B). Again, calprotectin displayed the highest AUROC for this endpoint compared to the other biomarkers (Table 1C). With regard to ICU admission, calprotectin was better than CRP and PCT (0.80 vs 0.66 and 0.60 respectively), yet inferior to lactate, although hyperlactatemia being a widely used biomarker for admitting patients to the ICU.[8] Thus, the routine in-house strategy presents a potential source of bias in favor for lactate. However, the discriminatory capacity of calprotectin with regard to death was lower compared to lactate, CRP and PCT. Yet, these results should be interpreted with caution based on the low number of patients for this endpoint (90 day mortality: $n = 2$). Indeed, others have shown a predictive value of calprotectin levels also for in-hospital mortality.[1] Values of calprotectin serum levels and AUROCs are visualized within all study groups and endpoints (Figure 2B).

Taken together, our study evaluated the association of calprotectin serum levels at the earliest possible moment, which is when patients are presented to the ED, with COVID-19 disease progression. Our data strongly argue for calprotectin representing a valuable biomarker for risk stratification, in particular with regard to subsequent MOF. Indeed, measurement of calprotectin might add to the biomarker repertoire in the ED since it seems to perform better than traditional markers such as lactate, CRP and PCT. Further, both CRP and PCT may be of low informative value with regard to early patient management in COVID-19 patients evaluated in the ED.

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Table 1
Demographics and Biomarkers by Outcome Groups.

	All	ICU Treatment			Multi-Organ Failure within 72 h			Multi-Organ Failure - Total			90-Day Mortality		
	($n = 19$)	No($n = 11$)	Yes($n = 8$)	p	No($n = 15$)	Yes($n = 4$)	p	No($n = 13$)	Yes($n = 6$)	P	No($n = 17$)	Yes($n = 2$)	p
(A) Demographics													
Age [years]	67.6 (53.9–72.0)	67.6 (54.0–70.8)	65.6 (54.8–76.4)	0.44	67.6 (53.3–71.6)	67.7 (57.5–78.0)	0.36	57.3 (51.0–70.5)	74.0 (62.5–77.2)	0.06	67.6 (52.3–71.9)	68.5 (63.9–73.0)	0.42
Female	11 (58%)	6 (55%)	5 (62%)	1.00	9 (60%)	2 (50%)	1.00	8 (62%)	3 (50%)	1.00	10 (59%)	1 (50%)	1.00
BMI [kg/m^2]	17; 27.0 (22.3–30.5)	10; 24.9 (21.9–29.6)	7; 29.4 (26.4–30.9)	0.19	14; 28.5 (22.6–30.8)	3; 25.7 (23.9–26.4)	0.43	12; 27.0 (22.1–30.3)	5; 27.0 (25.7–30.9)	0.51	15; 26.2 (22.2–30.4)	39.1 (33.0–45.1)	0.29
(B) Biomarkers													
Calprotectin [mg/L]	2.4 (1.4–3.2)	2.08 (1.36–2.59)	3.77 (1.90–5.16)	0.15	2.08 (1.205–2.81)	5.14 (3.98–10.48)	0.03	2.07 (1.20–2.49)	4.79 (3.36–5.51)	<0.01	2.13 (1.21–2.98)	13.91 (8.43–19.40)	0.14
Lactate [mg/dL]	15.0 (11.6–21.5)	13.00 (11.15–15.00)	21.50 (16.75–31.25)	0.03	13.50 (11.65–18.00)	32.50 (25.25–44.00)	0.09	13.00 (11.30–15.00)	26.00 (19.00–33.75)	0.03	13.50 (11.30–18.00)	28.50 (25.25–31.75)	0.10
CRP [mg/L]	37.7 (22.2–93.4)	36.60 (23.60–65.90)	93.40 (22.50–175.70)	0.27	36.60 (22.25–72.20)	93.40 (61.58–165.25)	0.26	35.90 (18.70–63.00)	126.80 (85.65–237.70)	0.02	36.60 (18.70–75.60)	301.50 (285.10–317.90)	0.01
PCT [µg/L]	0.1 (0.1–0.2)	0.11 (0.07–0.16)	0.15 (0.07–0.70)	0.51	0.09 (0.065–0.16)	0.455 (0.18–0.93)	0.15	0.09 (0.06–0.13)	0.45 (0.12–0.71)	0.08	0.09 (0.06–0.19)	1.19 (0.96–1.43)	0.03
(C) AUROCs by Outcome													
AUROC Calprotectin		0.70 (0.42–0.99)			0.87 (0.63–1.00)			0.91 (0.77–1.00)			0.85 (0.54–1.00)		
AUROC Lactate		0.80 (0.58–1.00)			0.79 (0.38–1.00)			0.82 (0.56–1.00)			0.88 (0.71–1.00)		
AUROC CRP		0.66 (0.36–0.96)			0.70 (0.34–1.00)			0.83 (0.58–1.00)			1		
AUROC PCT		0.60 (0.29–0.90)			0.75 (0.37–1.00)			0.76 (0.47–1.00)			1		

Continuous variables are represented with median and IQR, nominal variables with frequency and column percentage (of valid cases).
For continuous variables, where not all cases have data, the number of valid cases is shown.
P-values are calculated with Mann-Whitney-U test for continuous variables and Fisher's Exact Test for nominal variables.
Area under the Receiver Operating Characteristics (AUROCs) are shown for each biomarker, for each outcome, including 95% confidence intervals. BMI – body mass index; CRP – C-reactive protein; PCT – procalcitonin; AUROC – area under the receiver operating characteristic.

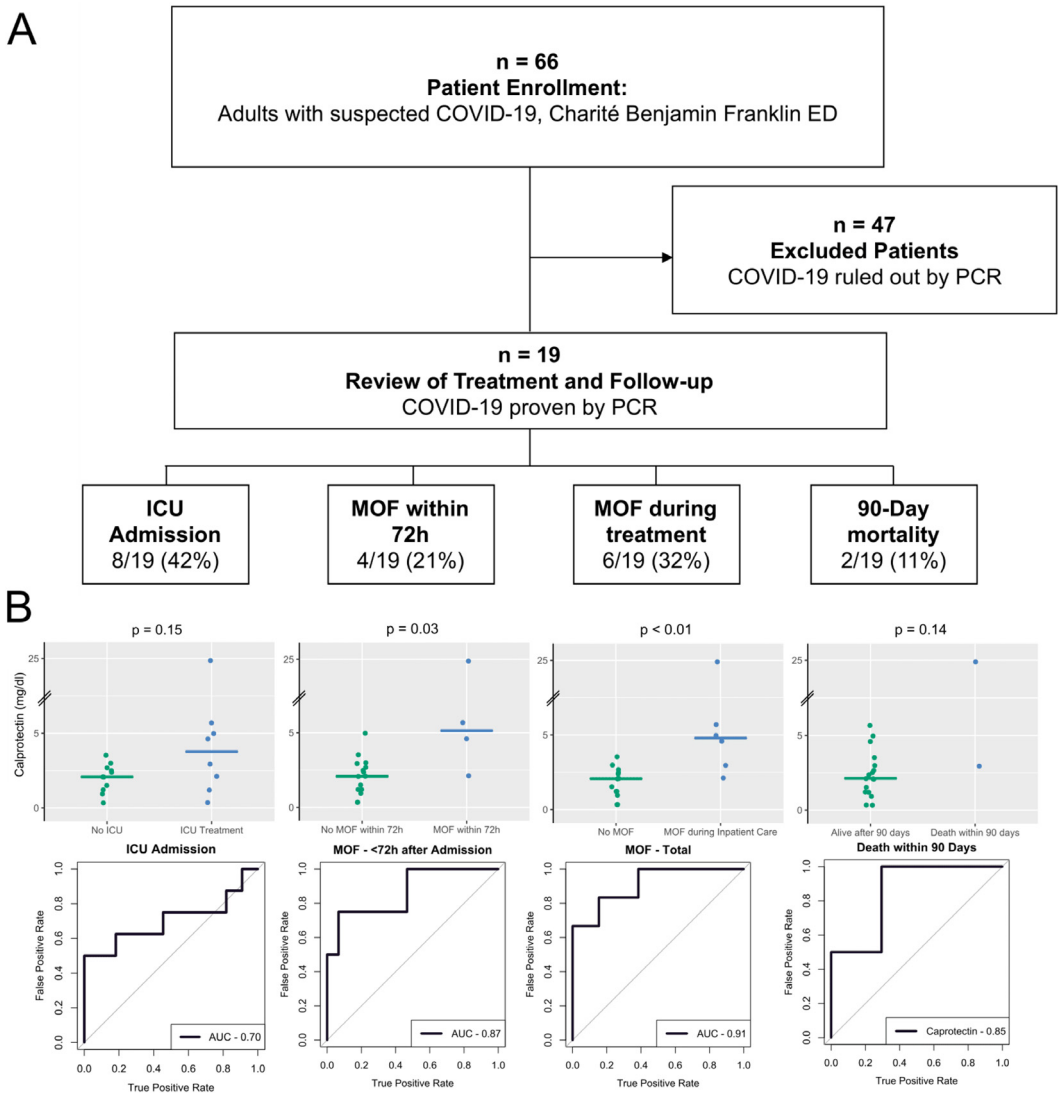

Fig. 1. (A) Patient enrollment and outcomes and (B) receiver operating characteristics with scatterplots for calprotectin predicting those outcomes. The scatterplots include a broken y axis. The horizontal bar on the scatterplot represents the median calprotectin concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Therefore, we believe that calprotectin represents a novel and useful discriminator in COVID-19 patients admitted to the ED with respect to disease outcome, in particular MOF, with calprotectin measurement in blood samples being easily applicable in routine laboratories.

Declaration of Competing Interest

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Comparison of saliva and nasopharyngeal swab SARS-CoV-2 RT-qPCR testing in a community setting

Dear Editor,

Several groups[1–4] have reported that saliva specimens perform as well or better than nasopharyngeal swabs (NPS) in hospital, emergency care and mass screening settings when testing for SARS-CoV-2 with reverse transcriptase real time PCR (RT-qPCR). In contrast, others found saliva less sensitive than NPS in community[5] or mildly infected outpatient settings[6]. To better understand saliva's performance in the SARS-CoV-2 RT-qPCR assay, we collected paired NPS and saliva from self-reported mild symptomatic or asymptomatic individuals at two community testing sites in Tucson, Arizona between late July and early September 2020. The study was reviewed and approved by the Advarra Institutional Research Board.

Table 1
Demographic distribution of double positive, NPS-only positive, and saliva-only positive individuals

	NPS+Saliva+	NPS+Saliva-	NPS-Saliva+	p-value*
Symptom				
No	18	30	5	0.226
Yes	27	24	3	
Age				
18–29	28	15	3	0.003
30–49	10	25	1	
50 or above	8	14	4	
Sex				
Male	22	16	2	0.201
Femal	22	34	4	
Race				
NH White	15	17	3	0.378
Hispanic	30	31	4	
other or unknown	1	6	1	
Saliva device				
SDNA1000	27	40	6	0.235
Conical	19	14	2	
Collection center				
Site one: n = 637	39	50	3	0.001
Site two: n = 335	6	3	5	

* Fisher exact test via Monte carlo simulation

Self-collection of saliva was performed using either the SDNA-1000 Saliva Collection device (Spectrum Solutions LLC, USA) or a sterile dry 50 ml conical vial followed by NPS collection within 10 min by medical staff. All samples were processed within 12 h of collection using the Beckman RNAdvance Viral XP Reagent kit and the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel with One Step PrimerScript™ III RT-PCR Kit (Takara Bio Inc. Japan). Using this platform, the viral yield of saliva was comparable to that of NPS using contrived samples and serial dilution of positive saliva samples.

A total of 943 pairs of samples were collected and tested, of which 108 pairs had positive results (11.5%). This included n = 54 samples in which only NPS samples were positive ("NPS-only"), n = 8 in which only saliva was positive ("saliva-only") and n = 46 in which both NPS and saliva were positive ("double positive").

The overall positive agreement of saliva to NPS (saliva sensitivity) was 46% (95% CI: 36.6%–55.7%). The average saliva cycle threshold (Ct) value was 26.8 ± 5.9 (N1, same for all below) which was significantly higher than 23.2 ± 8.5 (paired t-test, $p < 10^{-4}$) of NPS, consistent with several earlier reports[4–6]. Of 843 NPS negatives, 8 saliva specimens were positive. The saliva specificity relative to NPS was 99.1% (n = 843, 95% CI: 98.1% to 99.5%) with an average Ct of 34.4 ± 3.4.

Saliva sensitivity varied inversely with NPS Ct. When NPS Ct was lower than 26, saliva was positive in all NPS positive samples (n = 27, sensitivity 100%, 95% CI: 87.5%–100%). When NPS Ct was between 26 and 33, only 48.0% of the positive NPS samples had paired positive saliva (n = 25, 95% CI: 30.0%–66.5%). When NPS Ct was greater than 33, the saliva sensitivity further decreased to 14.6% (n = 48, 95% CI: 7.3%–27.2%) (Fig. 1). Although the lowest NPS Ct groups had a significantly higher number of symptomatic individuals ($p = 0.04$, Fig. 1 pie chart), the overall saliva sensitivity is not related to the symptoms (Table 1).

Among NPS-only positive individuals, a total of 22 had onset of symptoms 14 days earlier or SARS-COV-2 positivity longer than 14 days whereas there was only one such case among double positives. After excluding these individuals, the saliva sensitivity reached 86.7% (n = 45, 95% CI: 73.8%–93.7%) for NPS Ct of 33 and lower (Fig. 1). Our results corroborate early reports that saliva

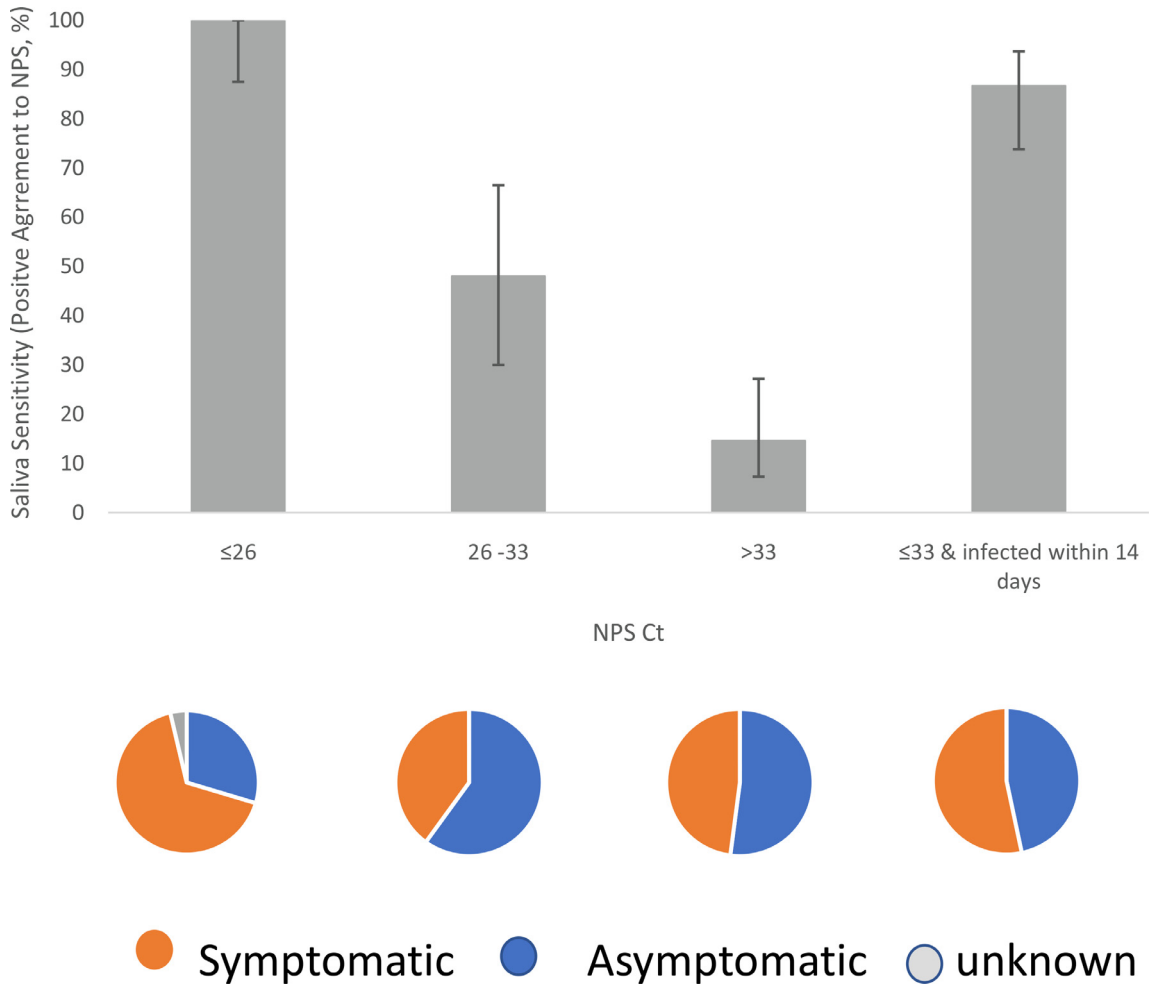

Fig. 1. Bar graph: The relationship between saliva sensitivity (or positive agreement to NPS) and the NPS Ct range. Saliva sensitivity from left to right: 100% (n = 27), 48.0% (n = 25), and 14.6% (n = 48), corresponding to the NPS Ct range of 26 and lower, between 26 and 33, and higher than 33. After excluding individuals infected longer than 14 days, saliva sensitivity was 86.7% when NPS Ct was 33 and lower (n = 45) (the right most bar). Error bar represents the 95% confidence interval. Pie chart: The composition of symptomatic (orange) and asymptomatic (blue) individuals in the corresponding NPS Ct range. Percentage of symptomatic individuals from left to right: 66.7%, 40.0%, 47.9%, and 53.3%. The lowest NPS Ct group (the left most pie) had a significant number of symptomatic individuals ($p = 0.04$).

positivity declines more rapidly than that of NPS after two weeks of infection[2,6].

We examined the effects of other factors on saliva sensitivity, including age, gender, race, saliva collection device, and collection site (Table 1). The double positive group had a significant number of individuals younger than age 30 (p=0.003). Gender, race, and collection device had no significant impact on saliva sensitivity. Of note, the two specimen collection sites had significantly different testing result profiles. Site 1 had the majority of positive cases (positivity rate of NPS and saliva combined was 14.4%), whereas Site 2 had a much lower positivity rate of 4.5%. Saliva appeared to be more sensitive than NPS at Site 2. Site 2 had 4 NPS-only positives and 5 saliva-only positives, whereas Site 1 had 50 NPS-only positives and 3 saliva-only positives. Although the total number of positive cases at Site 2 was small (Table 1), the difference is significant ($p = 0.001$). Moreover, of 3 NPS-only positives at Site 2, one was inconclusive on saliva testing and two were known positive for more than 14 days at the time of this study. The two sites generated two different results with saliva more sensitive at one site, and less at another.

The differences cannot be readily attributed to procedural variations. The two collection sites had the same rotating medical staff, followed the same collection and transportation protocol, and collected the paired specimens on the same days. The samples from two sites were randomly batched together for the lab analysis. The population at Site 2 was more suburban and socially distanced with an average age of 47.8 years, compared to 34.4 years at Site 1. Since the performance of laboratory tests can vary as a function of the prevalence of the disease, the disparate results profiles across sites could be related to the differences in the SARS-CoV-2 positivity at the two testing sites. It is also possible that the observed differences were due to demographic differences or a chance occurrence.

Nearly half (47%) of all NPS positives in our cohort had Ct higher than 33. Some had prolonged presence of the virus and other had unknown date of initial infection, likely a true picture of many communities. Most of those people were tested negative by saliva. Previous studies have shown there is much lower likelihood of isolating live SARS-CoV-2 virus from test samples when Ct > 33–35[7,8]. Detecting viral RNA does not equate with infectious virus being present and transmissible. Further work is needed to establish the relationship between RT-qPCR Ct values in saliva and viral infectivity[9], particularly in populations with a high prevalence rate[10].

Because its collection is non-invasive and does not require trained medical staff, saliva is a desirable specimen for COVID-19 screening and diagnostics. Our results indicate that RT-qPCR testing of saliva in a community-based population can effectively identify infected individuals with the high viral loads in a timely fashion, which is important for identifying those who may have the greatest potential to spread the virus.

Declaration of Competing Interest

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Clinical characteristics of 51,815 patients presenting with positive and negative SARS-CoV-2 swab results in primary health care settings: Priority populations for vaccination

Dear Editor,

We read with interest Brendish et al. study on the comparison between patients with SARS-CoV-2 positive and negative swab results.[1] The study compared the clinical characteristics of adult hospitalised patients with SARS-CoV-2 infections positive and negative results. Brendish et al. reported that different health comorbidities including, hypertension, diabetes, chronic kidney disease, chronic liver disease did not differ significantly between both groups. On the other hand, patients with COPD, smokers were less often presented among patients with positive rt-PCR swab results in hospitalised patients. They suggested that the lower the presentation of current smokers and patients with COPD may be linked, and they have noted that other studies associated smoking with worse disease outcomes.

The study is of significance as earlier research studies reported the characteristics of hospitalised patients with no comparator

Table 1
Characteristics of the population with recurrent positive results.

	Overall ($N = 51,815$)
PCR	
Negative	37,143 (71.7%)
Positive	14,672 (28.3%)
Gender	
N-Miss	12
Female	21,567 (41.6%)
Male	30,236 (58.4%)
Age	
Mean (SD)	35.8 (11.4)
Range	18.3 - 99.0
Age Groups	
18–30 Yrs.	19,200 (37.1%)
30–40 Yrs.	18,096 (34.9%)
40–50 Yrs.	8647 (16.7%)
50–60 Yrs.	3929 (7.6%)
>60 Yrs.	1943 (3.7%)
HTN	
No	46,244 (89.2%)
Yes	5571 (10.8%)
DM	
No	46,096 (89.0%)
Yes	5719 (11.0%)
Dyslipidemia	
No	46,780 (90.3%)
Yes	5035 (9.7%)
CKD	
No	51,298 (99.0%)
Yes	517 (1.0%)
CVD	
No	50,679 (97.8%)
Yes	1136 (2.2%)
Asthma	
No	47,777 (92.2%)
Yes	4038 (7.8%)
COPD	
No	51,763 (99.9%)
Yes	52 (0.1%)
Smoking Status	
N-Miss	26,479
Nonsmoker	19,269 (76.1%)
Former Smoker	1438 (5.7%)
Smoker	4629 (18.3%)
Pregnancy	
N-Miss	30,248
Negative	20,889 (96.9%)
Positive	678 (3.1%)

Table 2
Cross table for comparison of patient characteristics between groups with positive and negative rt-PCR swab results

	N	Negative ($N = 37,143$)	Positive ($N = 14,672$)	Test Statistic	Rate ratio	95% CI
Gender: Male	51,803	0.6 21,213/37,135	0.6 9023/14,668	X21=83.42, $P<0.01$	**1.2**	**1.15–1.25**
Age Groups	51,815			X24=502.86, $P<0.01$		
18–30 Yrs.		0.4 14,646/37,143	0.3 4554/14,672		Ref	
30–40 Yrs.		0.3 12,967/37,143	0.3 5129/14,672		**1.27**	**1.21–1.33**
40–50 Yrs.		0.2 5846/37,143	0.2 2801/14,672		**1.54**	**1.48–1.63**
50–60 Yrs.		0.1 2478/37,143	0.1 1451/14,672		**1.88**	**1.75–2.03**
>60 Yrs.		0.0 1206/37,143	0.1 737/14,672		**1.96**	**1.78–2.2**
HTN: Yes	51,815	0.1 3547/37,143	0.1 2024/14,672	$\chi 2 = 197.55$, $P<0.01$	**1.52**	**1.43–1.61**
DM: Yes	51,815	0.1 3584/37,143	0.1 2135/14,672	$\chi 2 = 257.42$, $P<0.01$	**1.60**	**1.51–1.69**
Asthma: Yes	51,815	0.1 3100/37,143	0.1 938/14,672	$\chi 2 = 55.83$, $P<0.01$	**0.75**	**0.7–0.81**
COPD: Yes	51,815	0.0 43/37,143	0.0 9/14,672	$\chi 2 = 3.11$, $P = 0.08$	0.52	0.26–1.09
CVD: Yes	51,815	0.0 753/37,143	0.0 383/14,672	$\chi 2 = 16.68$, $P<0.01$	**1.29**	**1.14–1.47**
CKD: Yes	51,815	0.0 340/37,143	0.0 177/14,672	$\chi 2 = 9.02$, $P<0.01$	**1.32**	**1.1–1.59**
Dyslipidemia: Yes	51,815	0.1 3350/37,143	0.1 1685/14,672	$\chi 2 = 72.86$, $P<0.01$	**1.31**	**1.23–1.39**
Smoking Status	25,336			$\chi 2 = 302.59$, $P<0.01$		
Nonsmoker		0.7 13,147/17,917	0.8 6122/7419		Ref	
Former Smoker		0.1 1012/17,917	0.1 426/7419		0.9	0.80–1.02
Smoker		0.2 3758/17,917	0.1 871/7419		**0.5**	**0.46–0.54**
Pregnancy 18–50 Yrs. of age: Yes	18,863	0.0 435/14,079	0.1 243/4784	$\chi 2 = 40.80$, $P<0.01$	**1.68**	**1.43–1.97**

groups.[2–6] To our knowledge, there are no studies to report on the clinical characteristics of patients with SARS-CoV-2 in primary care settings with comparison groups. The SARS-CoV-2 presentations in primary care settings reflect mild-to-moderate form of the disease, which presents different cohort of patients to hospitalised patients.

Following the methodology used by Bendish et al. study, this record-based study compares the demographics and comorbidities among patients attending primary health care corporation (PHCC) with a suspected diagnosis of SARS-CoV-2 infection. PHCC is a governmental institution that runs 27 health centres in Qatar.

The study population included all adult patients attending primary health care corporation from February 10th, 2020 to July 30th, 2020. Inclusion criteria included all adult patients with a documented diagnosis of a suspected diagnosis of SARS-CoV-2 infection and a documented rt-PCR swab result during the study period. Comparison groups are based on rt-PCR positive and negative results. Patients with more than one result were considered positive if any of the results is positive. Patients with inconclusive results were excluded from the analysis.

The study examines and compares demographics and clinical characteristics of adult patients presenting to primary health care settings testing positive and negative for SARS-CoV-2 rt-PCR, using electronic medical records. The comparison might reflect which clinical characteristics might increase the risk of infection rather than predict the outcomes. The result might inform both public health policies and vaccination guidelines.

Overview

During the study period, we retrieved 63,444 patient records. 51,815 adult patients had documented positive or negative swab results. The mean of the population age was 35.8 ± 11.4. (Median: 34 [Min: 18.3 - Max: 99]). Male patients were more represented in the sample (30,236/51,815; 58%). More than a quarter of the sample had a positive SARS-CoV-2 PCR result (14,672/51,815, 28%).

Comparison of patient characteristics between groups with positive and negative rt-PCR swab results

Age had the most significant difference between both groups with increasing odds ratio. Patients over 60 years of age were nearly twice as likely to have positive rt-PCR results compared to patients younger than 30 years of age (odds ratio = 1.96; 95% CI = 1.78, 2.2). Pregnancy was the second most common condition associated with an increased frequency of positive swab results (odds ratio = 1.68; 95% CI = 1.43, 1.97). Diabetes and hypertension and gender were also associated with increased risk, but to a lesser extent. Smoking and asthma were associated with less presentation among the population with positive results (Table 2).

Summary

The study results report on the risk of infection associations with different population characteristics. Patients over 40 years of age, pregnant women and patients with diabetes and hypertension seem to be at higher risk.

Earlier studies suggest that older age is associated with higher mortality.[7] However, authors of the same report suggested that there are no studies to report on the age-dependence in susceptibility to infection. Other studies have suggested that high mortality is associated with higher comorbidities rather than age[8]. Besides the fact that patients of over 60 years of age had the highest risk of infection, the population showed a progressive increase in risk among different age groups. The findings are supportive of age as an independent variable plays a key role in susceptibility to infection.

Also, in our results, pregnancy, diabetes, and hypertension had an increased risk of infection. There are no studies that have reported on the association between pregnancy and risk of SARS-CoV-2 infections.[9] Our results are the first to support the increased risk among this cohort of patients. The increased risk of infection in patients with diabetes and hypertension may be linked to the increased disease prevalence in older age groups. Patients with diabetes and hypertension had higher rates of ICU admissions and mortality.[10,11]

Patients with current smoking status and asthma were less likely to have positive swab results. Similar to the findings of earlier studies, we think both diagnoses are linked. Further research must explore causes for the lower risk among this cohort of patients.

In conclusion, there are differences between patients with positive and negative rt-PCR SARS-CoV-2 swab results presenting to primary health care settings in Qatar. Older age, pregnancy, and diabetes are among the most associated with increased frequency of positive results. Our results should complement the earlier evidence from secondary care, which suggested that they also influence disease outcomes. Of interest, the list of characteristics and comorbidities had no impact on recurrence rates.[12]

In view of the current surge of numbers, the list may inform prediction models for diagnosis, public health measures, and vaccination prioritisation policies. In our view's vaccination should be prioritised for patients older than 50 years of age, pregnancy, and patients with cardiovascular risk factors.

Strengths and limitations

The study reports on the large sample size, 51,815, which instils confidence in the significance of our findings. Also, the study reports on characteristics of mild-to-moderate presentations in primary health care settings. The study compares patients with positive SARS-CoV-2 swab results with patients with negative results as a control group. However, the data points do not report on patients' outcomes. Regression analysis is required to further examine the association between variables and including assessment of disease susceptibility risk (Table 1).

Informed consent

Data request and analysis were anonymous, and no patient consent was required.

Authorship

All authors have contributed to the drafting and critical revision of the article. The final version approved by all authors.

Ethics statement

Anonymous data request approved by the department of clinical research, primary health care corporation with reference number PHCC/DCR/2020/04/031.

Declaration of Competing Interest

There are no competing interests for any author.

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Clinical and laboratory characteristics of recovered versus deceased COVID-19 patients in Islamabad, Pakistan

Dear Editor,

We read with great interest the article published in this journal entitled "Clinical features of critically ill patients with confirmed COVID-19" by Yanan Chu and colleagues.[1] The authors comprehensively described the epidemiological and clinical characteristics of confirmed COVID-19 patients admitted to the intensive care unit at Zhejiang hospital in China. Here we present a comparative analysis of clinical and laboratory features associated with recovered and deceased COVID-19 patients in Pakistan.

Table 1
Demographic, clinical and laboratory characteristics of recovered and deceased COVID-19 patients.

Patient characteristics	COVID-19		*P*-Value
	Recovered; $n=82$(%)	Expired; $n=18$(%)	
A. Demographic Parameters			
Age in years; Mean + SD	45.32±16.47	60.83± 11.08	**0.003**
Age Groups			
20–40	37 (45.1)	0	**0.0003**
41–60	22 (26.8)	08 (44.4)	0.138
>60	23 (28.1)	10 (55.6)	**0.024**
Recovery/Death Days (Mean ± SD)	18.14 ± 4.22	26.75 ± 2.6	**<0.0001**
Gender			
Male	64 (78.1)	11 (61.1)	0.133
Female	18 (29.9)	07 (38.9)	0.133
Socioeconomic status			
High	28 (34.1)	07 (38.9)	0.703
Middle	41 (50.0)	08 (44.4)	0.667
Low	13 (15.9)	03 (16.7)	0.928
B. Signs and Symptoms			
Fever	78 (95.1)	18 (100)	0.337
Fatigue	63 (76.8)	17 (94.4)	**0.091**
Cough	47 (57.3)	13 (72.2)	0.242
Rash	05 (6.10)	08 (44.4)	**<0.0001**
Runny Nose	13 (15.8)	07 (38.8)	**0.027**
Sore throat	21 (25.6)	08 (44.4)	0.111
Shortness of breath	39 (47.5)	16 (88.9)	**0.001**
Loss of smell and taste	51 (62.1)	15 (83.3)	0.087
Conjunctivitis	11 (13.4)	06 (33.3)	**0.041**
Headache	45 (54.8)	14 (77.7)	**0.073**
Chest Pain	11 (13.4)	14 (77.7)	**<0.0001**
Nausea & Vomiting	21 (25.6)	10 (55.5)	**0.012**
Diarrhea	23 (28.1)	11 (61.1)	**0.007**
C. Hematological markers			
WBC ($4–10 \times 10^9$/L)			
Increased	11 (13.4)	07 (38.8)	**0.010**
Decreased	37 (45.1)	03 (16.2)	**0.025**
Neutrophils ($2–7 \times 10^9$/L)			
Increased	13 (15.8)	09 (50.0)	**0.0004**
Decreased	34 (41.4)	4 (22.2)	0.128
Lymphocyte ($1–3 \times 10^9$/L)			
Decreased	56 (68.2)	15 (83.3)	0.204
Platelets ($150–400 \times 10^3$/μL)			
Decreased	63 (76.8)	17 (94.4)	0.091
Hemoglobin (g/dL; M: 13.0–18.0, F: 11.5 to 16.5)			
Decreased	09 (10.9)	04 (22.2)	0.201
Coagulation Markers			
PT (≤13 s)			
Increased	66 (80.4)	18 (100)	**0.04**
APTT (≤36 s)			
Increased	61 (74.3)	18 (100)	**0.015**
D. Biochemical Markers			
i. LFTs			
Total Bilirubin (Normal Range= 0.2–1.0 mg/dL)			
Increased	27 (32.9)	12 (66.7)	**0.007**
ALT (<50 U/L)			
Increased	42 (51.2)	18 (100)	**0.0001**
AST (<40 U/L)			
Increased	48 (58.5)	18 (100)	**0.0007**
ALP (65–306 U/L)			
Increased	38 (73.1)	15 (83.3)	**0.004**
ii. RFTs			
Urea (10–52 mg/dl)			
Increased	17 (20.7)	12 (66.7)	**0.0001**
Creatinine (upto 1.2 mg/dl)			
Increased	24 (29.2)	16 (88.9)	**<0.0001**
iii. Electrolytes			
Sodium (135–150 mmol/L)			
Decreased	59 (71.9)	15 (83.3)	0.317
Potassium (3.5–5.0 mmol/L)			
Decreased	53 (64.6)	13 (72.2)	0.535
Chlorides (98–108 mmol/L)			
Decreased	43 (52.4)	12 (66.7)	0.271
iv. Cardiac Enzymes			
CPK (upto 190 U/L)			

(*continued on next page*)

Table 1 (*continued*)

Patient characteristics	COVID-19		*P*-Value
	Recovered; $n = 82$(%)	Expired; $n = 18$(%)	
Increased CK-MB (upto 25 U/L)	35 (42.6)	13 (72.2)	**0.023**
Increased v. LDH (150–250 U/L)	37 (45.1)	15 (83.3)	**0.003**
Increased vi. Glucose Random (90–160 mg/dl)	47 (57.3)	14 (77.7)	0.107
Increased vii. Serum Albumin (3.5–5 g/dL)	43 (52.4)	13 (72.2)	0.126
Decreased viii. CRP (0–6 mg/dL)	27 (32.9)	14 (77.7)	**0.0004**
Increased	73 (89)	18 (100)	0.141
E. ABO-Blood Grouping			
A-	32 (39.0)	02 (11.1)	**0.023**
A+	02 (2.40)	01 (5.50)	0.483
B-	08 (9.70)	02 (11.1)	0.865
B+	04 (4.90)	0	0.333
AB-	11 (13.4)	08 (44.4)	**0.002**
AB+	05 (6.1)	04 (22.2)	**0.03**
O-	03 (3.60)	0	0.412
O+	17 (20.7)	01 (5.50)	0.128
F. Comorbidities			
Diabetes	11 (13.4)	08 (44.4)	**0.002**
Chronic Renal Disease	04 (4.80)	03 (16.6)	0.075
Sepsis	07 (8.50)	08 (44.4)	**0.0001**
CVD	19 (23.1)	05 (27.7)	0.681
Pulmonary diseases	13 (15.8)	09 (50.0)	**0.001**
Cancer	0	01 (5.50)	**0.031**

WBC= White blood cells, PR= Prothrombin time, APTT= Activated partial prothrombin time, LFTs= Liver function tests, ALT= Alanine amino transferase, AST= Aspartate amino transferase, ALP= Alkaline phosphatase, RFTs= Renal function tests, CPK= Creatine phosphokinase, CK-MB= Creatine kinase-MB, LDH= Lactate Dehydrogenase, CRP= C-Reactive Protein, CVD= Cardiovascular Disease, SD= Standard deviation.

The first case of SARS-CoV-2 emerged in Wuhan, China and later became a serious public health threat with rapid spread to 213 countries across the world. The World Health Organization declared this pandemic as a public Health Emergency of International concern on 30 January 2020. As of 6 September 2020, over 49 million confirmed cases and over 12 million deaths have been reported across the globe.[2]

First case of COVID-19 in Pakistan, was detected on February 26, 2020; the toll then reached at 3,40,251 confirmed cases including 6923 deaths as of September 6, 2020.[2]

It is important to know the difference among the demographic, clinical and laboratory characteristics of recovered and deceased COVID-19 patients for the proper case management, which will be helpful to reduce the rate of mortality.

For this retrospective single center study, we included 100 critical COVID-19 confirmed patients admitted to the intensive care unit at a tertiary care hospital in Islamabad Pakistan, from June 12 to July 4, 2020. Laboratory confirmation of SARS-CoV-2 was done at the department of Virology, National Institute of Health (NIH) Islamabad through real-time reverse-transcriptase polymerase chain reaction (PCR) assay using nasopharyngeal swab specimens. We obtained demographic features, clinical symptoms, laboratory test and outcome data from patient's electronic medical records. Clinical outcomes were followed up to 17 July 2020. The SPSS Statistics 23.0 software (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis of the data. A value of $P < 0.05$ was considered to be statistically significant. The study was approved by the internal review board of NIH and written informed consent was obtained from the patients (or their caretakers) enrolled for the study.

A total of 100 confirmed COVID-19 patients were enrolled in the study, including 82 of whom were fully recovered later and 18 who died at the hospital. The mean age of recovered patients was 45.32±16.47 years, while that of the deceased group was 60.83± 11.08 years ($p < 0.003$). In the recovered group, 78% subjects were male and 29.9% were female whereas in deceased group, 61% subjects were male and 38.9% were female. There was no significant difference found in the socioeconomic status of both groups. Comaprison of clinical features between recovered/deceased individuals indicated that majority of the patients exhibited fever (95/100%), fatigue (74/94%), cough (57/72%), loss of taste and smell (62/83%) breathing difficulties (47/88%), headache (54/77%), chest pain (12/77%) and diarrhea (28/61%) respectively. The shortness of breath and chest pain were significantly different and more severe in deceased patients comapred to the survived patients ($p < 0.001$).

Most of the laboratory parameters, including white blood cell (WBC) count, neutrophil, lymphocytes, prothrombin time (PT), partial thromboplastin time (APTT, bilirubin, Alanine Aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine were significantly different between the recovered and deceased group ($p < 0.001$). The decreased level of electrolytes such as sodium, potassium and chloride was found in both groups without any significant difference. The level of cardiac enzyme, creatine phosphokinase (CPK) and creatine kinase (CK-MB) were significantly different between the recovered and deceased group ($p < 0.001$). High percentage (44%) of deceased patients belonged to the blood group AB^- followed 22% who had AB^+ whereas the high percentage (39%) in the recovered group matched to blood group A^- followed by 20% with O^+ blood group. Presence of common comorbidities such as diabetes, sepsis and chronic pulmonary disorder were significantly different between the both group *($p < 0.001$)*. The mean duration from the onset to recovery was 18.14 ± 4.22 days whereas the deceased patients survived for 26.75 ± 2.6 days after confirmed diagnosis. Up to 17 July 2020, 82% patients were fully recovered and discharged from the hospital while 18% patients had died at the hospital. Details of demographic, clinical and laboratory features are summarized in Table 1.

In the present study, the mean age of patients expred due to COVID-19 was significantly higher than that of the recovered patients as reported a in the previous study.[3] We observed a greater number of male patients compared to females in our reported cases infected by SARS-CoV-2 as already observed during SARS-CoV-2 pandemic.[4] In term of laboratory tests, lymphocytopenia, thrombocytopenia, low WBC count, reduced hemoglobin and elevated coagulation markers were observed in most of the COVID-19 infected patients. The elevated level of biochemical markers such as cardiac enzymes, LDH, glucose, CRP and decreased level of electrolytes were noted in study patients coinciding with the results of previous study.[5] Majority (39%) of COVID-19 patients who recovered belonged to A^- blood group whereas 44% of deceased group belonged to AB^- blood group however the blood group related impact of COVID-19 has not been reported extensively. Results of present study suggest that SARS-CoV-2 more likely infect older men suffering from chronic comorbidities such as diabetes, sepsis, pulmonary diseases and with AB blood group that may result in severe and even fatal outcome. The median time from the disease onset to recovery and deaths was 18.14 ± 4.22 and 26.75 ± 2.6 days respectively and similar findings have been reported from China.[6] Older age, male sex, comorbidities and AB blood group are believed to be the major risk factors for critical illness and deaths from COVID-19 infection.

This study has several limitations such as small number of cases, single center study, lack of radiological findings and treatment details. The preliminary data derived from the present study permits an early assessment of demographic, clinical and laboratory features of recovered and deceased COVID-19 patients in Islamabad Pakistan.

The actual picture of recovered and deceased COVID-19 patients in Pakistan warrants further investigation at the country level.

Declaration of Competing Interest

All contributing authors stated that there are no conflicts of interest to declare.

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Author contribution

MSR, MU, MMA, AK, SSZZ and MS design the study MSR, MU and MMA search literature, collected data, MU, MSR analyzed data, MSR, MU and MMA wrote paper. All authors approved the final draft.

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Evidence of SARS-CoV-2 re-infection with a different genotype

Dear Editor,

We read with great interest in this journal the description by Tomassini et al. of six possible cases of re-infection with SARS-CoV-2 in England.[1] The characterization and extent of such re-infections are currently increasingly investigated, and their implications are a growing concern.[2] Indeed, the emergence of SARS-CoV-2 in December 2019 in China was followed by the worldwide spread of the virus and its circulation for several months (https://coronavirus.jhu.edu/map.html). In several European countries, including France, the outbreak almost ended during spring, but a second COVID-19 outbreak occurred in late summer (https://covid19-country-overviews.ecdc.europa.eu/). We observed such an evolution of SARS-CoV-2 diagnoses at the Méditerranée Infection Institute in Marseille, France, where we have performed more than 300,000 SARS-CoV-2 qPCR since end of January 2020 and have detected the first infection at the end of February (https://www.mediterranee-infection.com/covid-19/). As the SARS-CoV-2 pandemic is still on-going, a current major issue is whether or not and how long immune responses to the virus are protective. In this regard, it is important to prove the cases of reinfection, which were first reported in August in Hong Kong.[2] Tomassini et al. defined re-infection as qPCR positivity at least 28 days after a previous qPCR-positive Covid-19 episode that was followed by clinical recovery and at least one negative qPCR.[1] We report here a patient with two infections at a 105 days interval despite seroconversion. In addition to Tomassini et al.' criteria for re-infection, we demonstrated by genotypic analyses that the two successive infections involved distinct viral variants and that samples tested were collected from the same individual.

The patient is a 70-year-old immunocompetent man living in a retirement home due to behavioral and memory disorders. On April 22nd, 2020, he developed fever and cough. His oxygen saturation was 95%. SARS-CoV-2 PCR performed on a nasopharyngeal swab[3] was positive (cycle threshold value (Ct)= 27). A low-dose chest CT-scan highlighted minimal ground glass images in both lungs. The patient subsequently fully recovered and further nasopharyngeal samples, collected on May 8th, 14th and 18th, were PCR-negative. Serological testing performed by a chemiluminescent immunoassay (CLIA) on a Liaison DiaSorin XL instrument (DiaSorin Inc., Saluggia, Italy) showed IgG seroconversion. Indeed, a serum sample collected on May 5th, two weeks after the onset of clinical symptoms and PCR diagnosis was IgG-negative, whereas a serum sample collected two weeks later, on May 18th, was IgG-positive

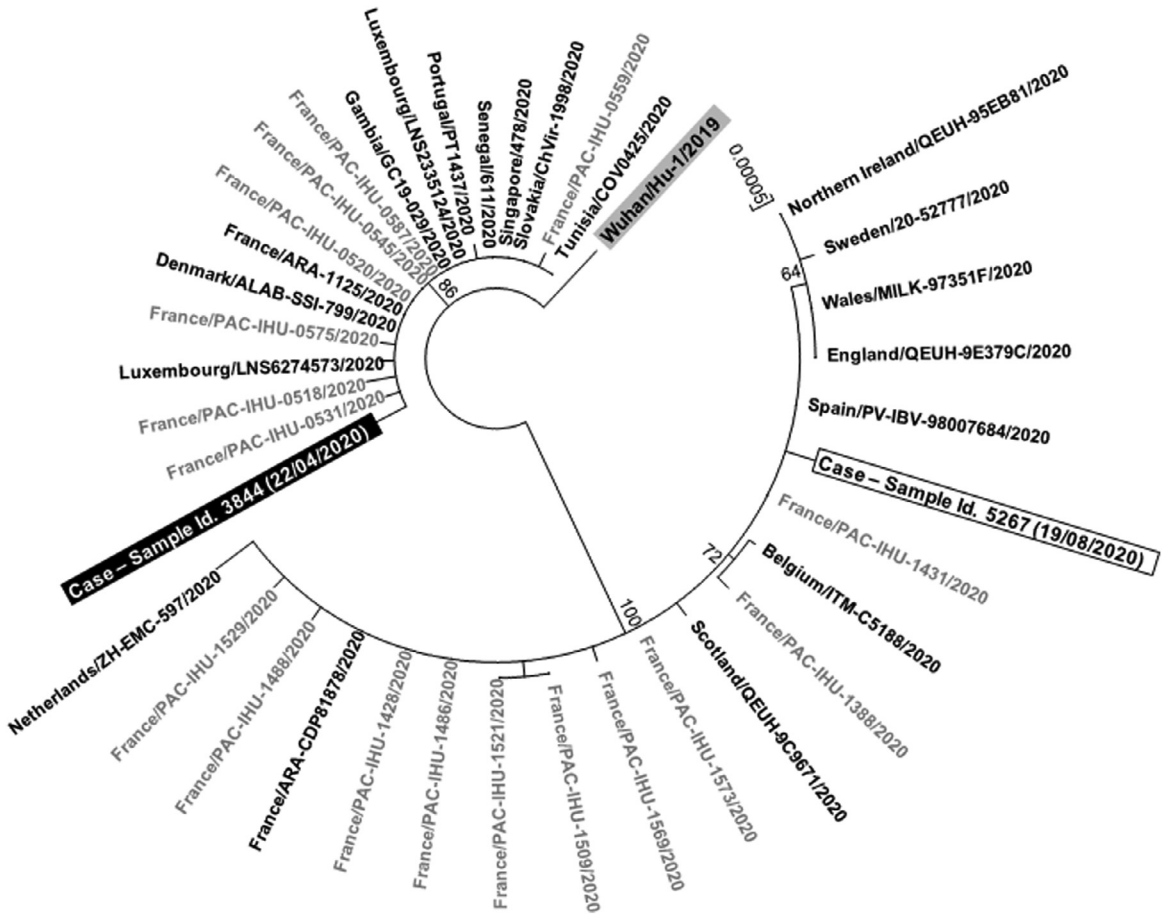

Fig. 1. Phylogeny reconstruction based on SARS-CoV-2 genomes recovered during the first and second infections. Phylogenetic tree was reconstructed using the MEGA X software (https://www.megasoftware.net/) based on SARS-CoV-2 genome sequences, with a total of 29,703 positions in the final dataset. This analysis incorporated the genome sequences the most similar through BLASTn searches (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) to the two genome sequences recovered from the case-patient in April and August 2020 (indicated by a black background and a white bold font and a framed white background and a black bold font, respectively) among those obtained in our center from respiratory samples collected since end of February 2020 until end of September (indicated by a gray bold font) and those from the GISAID database (https://www.gisaid.org/) (indicated by a black bold font). Among top hit sequences from the GISAID database, a single one was kept by country. The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Bootstrap greater than 50% are indicated in the tree. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All nucleotide positions with less than 80% site coverage were discarded (partial deletion option). Prior nucleotide sequence alignment was performed using Muscle.

(signal= 21; positivity threshold= 15). On August 19th, the patient was tested PCR-positive again (Ct= 18), when sampled during a systematic screening performed in his retirement home while he was asymptomatic.

Next-generation sequencing (NGS) of SARS-CoV-2 genomes was carried out using Illumina (San Diego, CA, USA) technology as previously described.[3] Genome consensus sequences were generated with the CLC Genomics workbench v.7 (https://digitalinsights.qiagen.com/) by mapping NGS reads on the Wuhan-Hu-1 SARS-CoV-2 genome (GenBank accession no. NC_045512) with 0.8 and 0.9 as coverage and similarity thresholds, respectively. The genome sequence (20,879 non-contiguous nucleotides; IHU-3844/2020) from April 22nd was most closely related to those from strains of Nextrain clade 20A that circulated during the first outbreak in our geographical area[4] (Fig. 1). The SARS-CoV-2 genome (deposited in the GISAID database (https://www.gisaid.org/) with no. France/PAC-IHU-1347/2020) from August 19th belonged to the Marseille 4 lineage that emerged in our geographical area during the second outbreak[4] (Fig. 1), and 11 mutations that are hallmarks of the Marseille 4 lineage (C4543U, G5629U, G9526U, C11497U, G13993U, G15766U, A16889G, G17019U, G22992A, G28975C, G29399A) were absent from the genome obtained from the first sample. In contrast, 2 mutations (C2416U, G8371U) that are hallmarks of the genotype identified in the first sample were absent in the second genome (Supplementary Table S1). In order to prove that samples were from the same patient, we confirmed genetically that each of 24 independent short tandem repeat markers analyzed (Supplementary Material) identified identical alleles.

Here, we demonstrate that the same patient was infected in April, cleared the virus, seroconverted, but was re-infected four months later with a new viral variant. The two infections reflect the circulating strains in Marseille at the same time.[4] It is the most comprehensive studied as it documented seroconversion following the first infection, showed drastically different viral genomes with 34 nucleotide differences, and ruled out errors of samples by techniques commonly used for forensic identifications. The present case adds to 13 previously reported cases of re-infection with a different SARS-CoV-2 strain that occurred in China, Belgium, the Netherlands, India, Ecuador and the USA[2,5–9] (Supplementary Table S2) documented with varying degrees of robustness (Supplementary Table S3). Mean age (± standard deviation) of the cases was 40±20 years (range, 24–89), and patients were mostly immunocompetent individuals (in 12 cases (86%)). The 14 reports involved men in 9 of 13 documented cases (69%). The mean delay between the two diagnoses was 81±36 days (19–142). The symptomatology of the first and second infections was

much variable. In eight cases, symptoms were reported in both infections, re-infection being less severe in two cases and more severe in four, including one death. In two cases, both infections were asymptomatic, in two only re-infection was asymptomatic, and in two only re-infection was symptomatic. Serology was performed in three cases following the first infection and was positive. Serology was performed in 11 cases following the second infection and was negative in four and positive in seven.

Such early re-infections with SARS-CoV-2 is surprising, as we are used with a majority of respiratory viruses to observe a single, annual epidemic episode.[10] This atypical epidemiological pattern is particularly relevant in our geographical area where the second outbreak that started during the summer was linked to multiple distinct variants having accumulated mutations that differed from viral mutants that circulated during the first outbreak.[4] This deserves conducting further studies to figure out whether or not this would make sense to include several viral variants in future vaccines.

Declaration of Competing Interest

The authors have no conflict of interest to declare. Funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Ethics

The study was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (N°: 2020-029). Access to the patients' biological and registry data issued from the hospital information system was approved by the data protection committee of Assistance Publique-Hôpitaux de Marseille (APHM) and was recorded in the European General Data Protection Regulation registry under number RGPD/APHM 2019-73.

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Supplementary materials

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SARS-CoV-2 detection by nasal strips: A superior tool for surveillance of paediatric population

Dear Editor,

A reliable, simple and safe sampling method applicable to a wide-age-range is required for community-based SARS-CoV-2 surveillance. Nasopharyngeal/oropharyngeal specimens require trained-personnel to perform and trigger sneezing, and coughing, which pose a risk of disease transmission.[1] Self-administered sampling methods[2–4] have been assessed in adult in-patients, but these methods do not translate well to the community setting, e.g., saliva collection is difficult in young children and the elderly, and

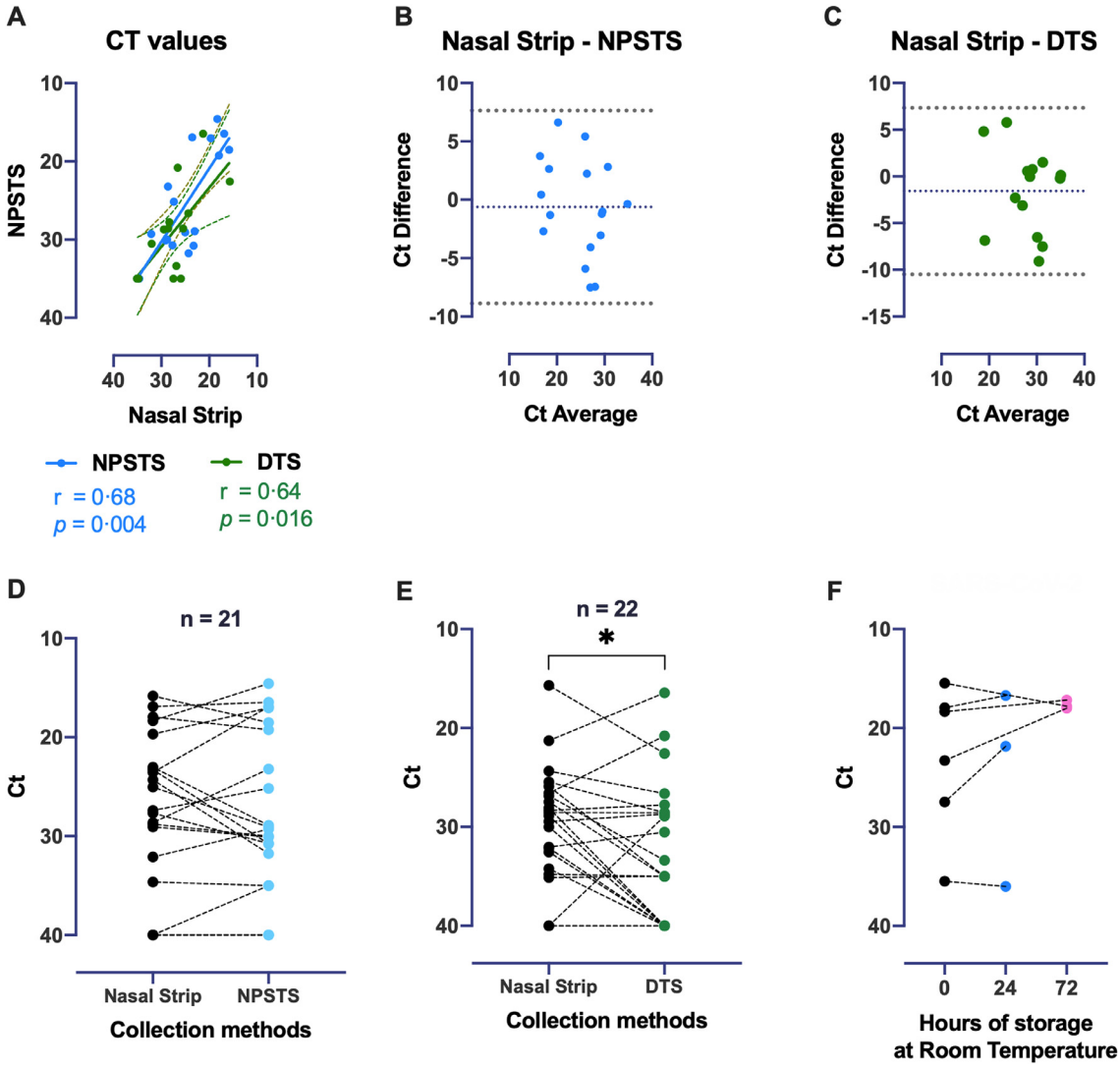

Fig. 1. Correlation, agreement and comparison of the cycle threshold (Ct) values from nasal strip, NPSTS and DTS, and the stability of nasal strip sample at room temperature. The correlation coefficients of NPSTS and DTS are superimposed on the panel with trend lines estimated using simple linear regression (Panel A). Plot shows the available Ct values of 31 samples which had positive test results from both tests. Data on three samples with negative result in both nasal strip and NPSTS, one sample with negative result in nasal strip but a positive result in NPSTS (Ct value = 35), one sample with negative result in both nasal strip and DTS, one sample with negative result from nasal strip but positive result in DTS, and six samples with positive result in nasal strip but negative result DTS were excluded from the Spearman correlation analysis. Bland-Altman Plots indicate the agreement of nasal strip versus NPSTS (Panel B) and DTS (Panel C), respectively. The differences between the two measurements are plotted against their average Ct values. Almost all observations are located within 2 standard deviations of the mean difference, and no bias is shown. The plots show that the nasal strip gives consistent and comparable measurements versus the NPSTS and DTS. SARS-CoV-2 RNA load in nasal strip and NPSTS (n = 21) (Panel D) and DTS (n = 22) (Panel E). Samples were obtained from 36 in-patients who had a diagnosis of COVID-19. Panel A shows SARS-CoV-2 RNA Ct in the nasal strip and NPSTS; panel B shows SARS-CoV-2 RNA Ct in the nasal strip and DTS. The lines indicate samples from the same patient obtained within 24 hours. Negative result is arbitrarily set as Ct = 40 and results were compared with the use of a Wilcoxon signed-rank test ($p < 0.05$). Panel F shows the stability of nasal strip samples for the detection of SARS-CoV-2 (n = 6). Comparison of Ct upon 24 (blue) and 72 (pink) hours RT storage from nasal strips directly lysed after sample collection.

variably reduces test sensitivity.[5] A recent study published in *the Journal of Infection* reviewed the methodologies used in the estimation of diagnostic accuracy of SARS-CoV-2 real-time reverse transcription polymerase chain reaction (RT-PCR) and other nucleic acid amplification tests for COVID-19 and pointed out the importance in employing standardized guidelines for study designs and statistical methods.[6]

Here, we compared different sample collection methods and introduced nasal strip as a sensitive and low-risk collection method and assessed its application in both paediatric and adult subjects at the Prince of Wales Hospital, Hong Kong. Thirty-eight asymptomatic and symptomatic subjects hospitalized with COVID-19 were recruited prospectively by convenience sampling. The disease status was confirmed by two RT-PCR tests targeting different regions of the RdRp gene performed by the local hospital and Public Health Laboratory Service. Twenty infected adults (range: 22–74 years old) and eighteen children/adolescents (range: 6–17 years old) were recruited of whom ten were asymptomatic. Adult subjects or guardians of participants below 18 years old provided informed consent (see the Methods section in the Supplmentary Appendix).

We obtained nasal epithelial lining fluid (NELF) by nasal strip (n = 43), to compare against pooled nasopharyngeal and throat swabs (NPSTS) (n = 21) or deep throat saliva (DTS) (n = 22) collected within 24 h of the nasal strip. 13 paired nasal swabs were also collected right before the collection of nasal strip to evaluate their SARS-CoV-2 detection performance. All samples were subjected to viral RNA quantitation by real-time PCR targeting the nucleoprotein gene.[7]

Table 1
Number of tested samples and performance of the collection methods

Reference		Nasal strip Positive	Negative	Total	Agreement (%)
NPSTS	Positive	17	1	18	94.44
(n = 21)	Negative	0	3	3	100.00
	Value (95% CI)				
Accuracy	95.2 (76.18–99.88)				
DTS	Positive	14	1	15	93.33
(n = 22)	Negative	6	1	7	14.29
	Value (95% CI)				
Accuracy	68.18 (45.13–86.14)				

No significant difference in the detection rate was observed between NPSTS (McNemar's test $p = 1.000$) or DTS ($p = 0.13$) for the qPCR test (NPSTS and DTS, $p = 0.29$). CT value equal or below 35 is defined as positive.

Spearman's test demonstrated significant correlation between nasal strip and NPSTS ($p = 0.0003$) and between nasal strip and DTS ($p = 0.01$) (Fig. 1A). The agreement between nasal strip samples and NPSTS was 94.44% (17/18) and 100% (3/3) for NPSTS positive and negative samples (Table 1, Fig. 1B). In contrast, the agreement between nasal strip specimens and DTS was 93.33% (14/15) and 14.29% (1/7) for DTS positive and negative samples, respectively (Table 1, Fig. 1C). Eight discrepant samples were identified (Table S1, Figure S1) of which seven were DTS specimens. Nasal strip outperformed DTS on six occasions, where negative result was reported in the latter. Four of these DTS specimens were collected from paediatric patients (Patients 1 to 4). Nasal strip samples were tested negative on two occasions when the reference test revealed Ct values of 35 and 28.92 (Figure S1). Wilcoxon signed rank test revealed that nasal strip and NPSTS gave similar Ct values (Fig. 1D, $p = 0.76$) while a lower Ct was detected in nasal strip compared to paired DTS (Fig. 1E, $p = 0.016$).

Of the 43 nasal strips collected, 13 were paired with a nasal swab sample obtained concurrently by a healthcare worker. A significant correlation was found between Ct values from the nasal strip and nasal swab specimens ($r = 0.88$, $p = 0.0031$, Figure S2A). Though nasal swab missed two positive cases detected by nasal strip and nasal strip missed one positive case detected by nasal swab, there was no significant difference detected between Ct values of the 13 paired samples (Figure S2B).

Finally, we collected nasal strip pairs from six patients to determine viral stability over time, viral RNA remained detectable after 24- and 72 h storage at room temperature (Fig. 1F).

The high correlation of nasal strip samples with the standard sampling methods is likely the result of steady NELF absorption with the strip in close contact with the nasal mucosa which reduces sample variability. This study also indicated the possible insensitivity of DTS, particularly in paediatric patients who are less able to provide DTS with consistent quality (Table S1) and how nasal strip would be a superior tool for surveillance of paediatric populations. Nasal strip is also a better collection method than NPSTS as it is less traumatic and irritating. The application of nasal strip reduces the risk of any sneezes and coughs and therefore lessens the risk of virus transmission. Nasal strip is a more comfortable and easier to apply sampling method than the other available standard sampling tools. Repeat nasal strip sampling as part of a community-based surveillance program is feasible in children and adults and likely to succeed as a result of its non-invasive nature (Video 1).

Compared with NPSTS, nasal strip sampling achieved an accuracy of 95.2% (Table 1). Nasal strip sample is comparable if not superior to other sampling methods reported in the literature, including self-administered tongue, lower- and mid-nasal specimens.[4] Apart from its good accuracy, we assessed the validity of the nasal strip samples after prolonged room temperature storage so as to mimic the duration needed to post the specimens to the laboratory. This aspect was not assessed in previous studies, albeit an important criterion if a sampling method is adopted for community-based testing purposes. Our findings suggest that nasal strip would provide at least consistent qualitative results (positive or negative), as long as the Ct value is within the range of an inferred infectivity.[8] This would be sufficient to identify potentially infectious individuals and susceptible contacts for further management and quarantine.

There are several limitations in this study. This prospective study presents the cross-sectional data performed in a single hospital. The clinical sample pairs (n = 6) that underwent 24- to 72 h room temperature storage remained stable in terms of viral detection. However, the involvement of protease and RNase activity of individual subjects and its contribution to sample stability has not been fully elucidated. The current method provides detection of SARS-CoV-2 at the gene level but no information was obtained regarding the infectious titer.

Conclusion

Our nasal strip collection method serves as an excellent sampling method with comparable performance with NPSTS, DTS and nasal swab specimens in identifying subjects infected with SARS-CoV-2. This reliable, non-invasive, self-administered method with its extended sample stability makes it uniquely suited for repeated sampling and large-scale community study, especially for paediatric population.

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Supplementary materials

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Co-occurrence of *mcr-9*, extended spectrum β-lactamase (ESBL) and AmpC genes in a conjugative IncHI2A plasmid from a multidrug-resistant clinical isolate of *Salmonella diarizonae*

Dear Editor,

The recently published studies in this journal reported that the emergence of Enterobacterales carrying Extended-Spectrum β-Lactamase (ESBL) enzymes and Carbapenem Resistance has limited the antimicrobial arsenal available for Urinary Tract Infections (UTIs) which are the most common bacterial infections requiring antibiotic treatment.[1, 2] Colistin was reintroduced into clinical medicine as last line of defense against infections such as UTIs caused by extremely-drug-resistant (XDR) and multidrug-resistant (MDR) organisms that resistant to carbapenems, other β-lactams, and other antibiotics.[3] Unfortunately, the clinical efficacy of colistin was seriously threatened by the emergence of the plasmid-mediated mobile colistin resistance (*mcr*) gene family.

In May 2019, a novel *mcr* homologue, *mcr-9* was identified in an MDR *Salmonella Typhimurium* isolated from a human patient.[4] Here, we report the complete genome sequence of a conjugative plasmid pXXB1403 that co-harboring *mcr-9*, $bla_{\text{TEM-1B}}$ (ESBL gene) and $bla_{\text{DHA-27}}$ (AmpC β-lactamase gene) in a clinical *Salmonella enterica* subsp. *diarizonae* strain XXB1403 isolated from urine sample of a 56-year-old male patient with UTI after urethral stricture who did not receive colistin treatment before, in May 2017, Shanghai, China.

Susceptibility testing by Vitek-2 system showed that isolate XXB1403 was resistant to most of the antimicrobials tested (Table 1), including members of the carbapenem class of β-lactam antibiotics [imipenem (MIC 4 mg/L), ertapenem (MIC 4 mg/L)] and cephalosporins [cefotetan (MIC 64 mg/L), ceftazidime (MIC 64 mg/L)] which are used routinely in the treatment of UTIs. The MIC of colistin was determined to be 2 mg/L by using the broth microdilution method according to CLSI guidelines.

The transferability of *mcr-9* gene was verified by conjugation assay, *mcr-9* could be transferred into Sodium-azide resistant *Escherichia coli* J53, *Pseudomonas aeruginosa* ATCC 9027 and cefepime-resistant *Acinetobacter baumannii* ATCC 19606 at the frequencies of 10^{-4}, 10^{-1} and 10^{-6} respectively, suggesting pXXB1403 has the potential to transfer to other clinically relevant pathogens.

To fully understand the genetic characteristics of pXXB1403, total DNA of *S. diarizonae* XXB1403 was extracted and sequenced by Nanopore GridION and Illumina HiSeq platforms. pXXB1403 is a circular plasmid in size of 277,539 bp with 47.56% GC-content (Fig. 1A), and was identified as the IncHI2A plasmid by PlasmidFinder. IncHI2 plasmids were reported to be predominant in antibiotic-resistant *Salmonella* isolates,[5] and was the dominant replicon type carrying *mcr-9*.[6]

BLASTn analysis showed that pXXB1403 displayed 96% query coverage and 99.98% identity with plasmid pIHI2-233 (CP049047.1) from *Enterobacter hormaechei* strain Y233 harboring a novel aminoglycoside resistance gene *aac(3)-IIg*, which was isolated from a teaching hospital in Wenzhou, China; displayed 95% query coverage and 99.70% identity with plasmid p505108-MDR (KY978628.1) from a MDR *Cronobacter sakazakii* strain 505,108 which was isolated from a sputum specimen of a neonate with severe pneumonia.[7] Interestingly, the *mcr-9*, $bla_{\text{TEM-1B}}$ and $bla_{\text{DHA-27}}$ genes on these three plasmids shown 100% identity and 100% coverage (Fig. 1A), suggesting that these MDR plasmids have circulated in China.

Except pXXB1403, only three complete genome sequences of *mcr-9*-carrying plasmid (MK933279, CP051135.1, CP041734.1) were reported. We performed BLASTn search of *mcr-9* in all (834) complete genomes of *Salmonella* spp. in NCBI database (Access on 30

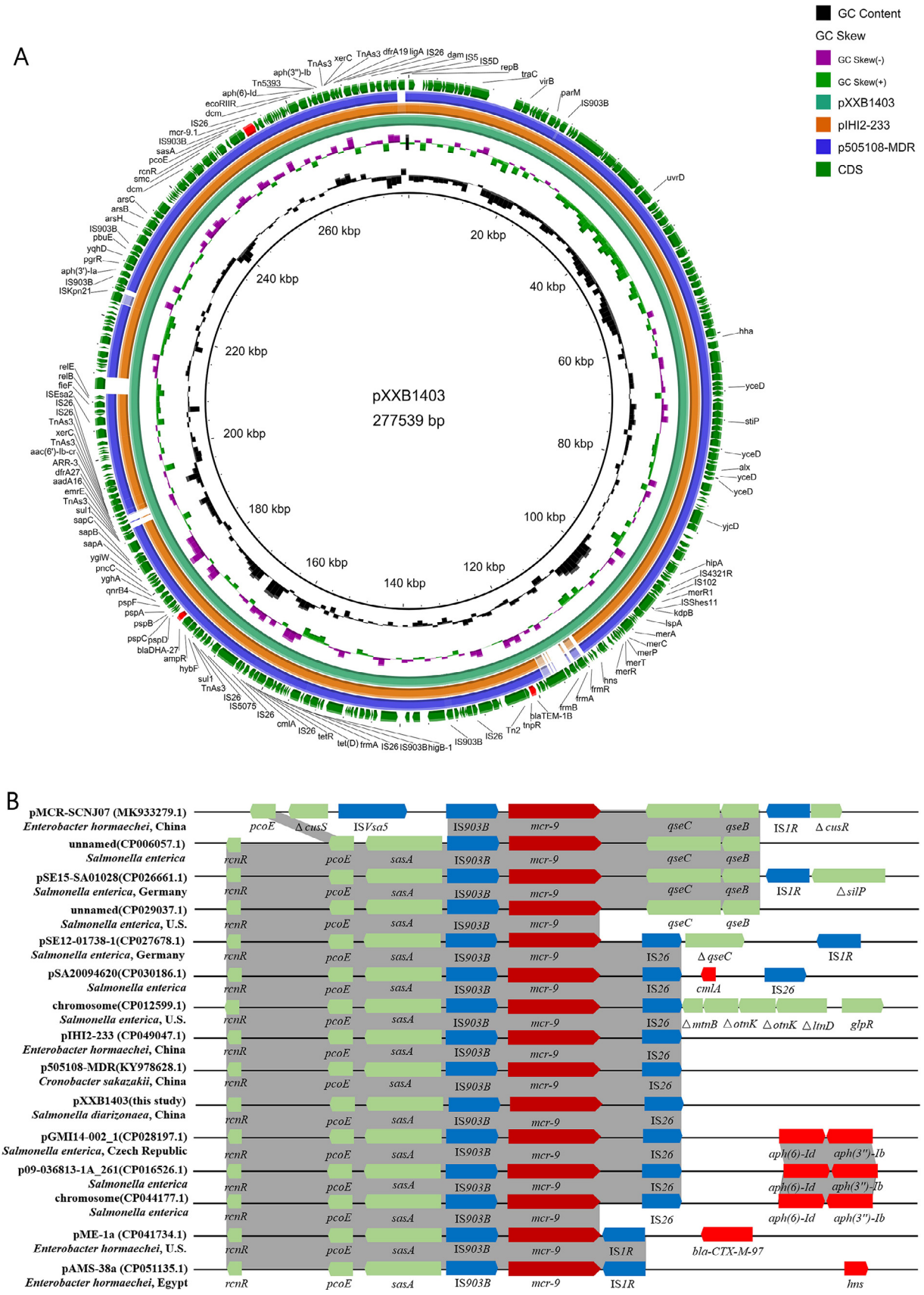

Fig. 1. The genetic contexts of *mcr-9*-carrying plasmids. **(A)** Circular comparison of plasmids pXXB1403, pIHI2-233 (CP049047.1) and p505108-MDR (KY978628.1). Plasmid pXXB1403 was used as the reference genome sequence. GC-content, GC-skew and Coding sequences (CDS) of pXXB1403 are indicated **(B)** Comparison of the genetic environments of *mcr-9* gene. Genetic environment of pIHI2-233, p505108-MDR, pME-1a (CP041734.1), pAMS-38c (CP051135.1) and pMCR-SCNJ07 (MK933279.1) and additional 7 *mcr-9*-containing complete plasmid genomes (CP016526.1, CP028197.1, CP030186.1, CP027678.1, CP026661.1, CP029037.1 and CP006057.1) and 2 *mcr-9*-containing chromosome sequences (CP012599.1 and CP044177.1) in GenBank were extracted. Arrows indicate the positions and directions of the genes. △indicates the truncated gene. Regions with >99 % homology are indicated in the light grey shadow.

Table 1
Antibiotic susceptibility profile of *Salmonella diarizonae* XXB1403.

Antibiotics	MIC (mg/L)	Antibiotics	MIC (mg/L)	Antibiotics	MIC (mg/L)
Ampicillin (A)	32	Amikacin (AN)	16	Ampicillin-Sulbactam (U)	32
Aztreonam (ATM)	64	Cefazolin (CFZ)	64	Cefepime (CPE)	64
Cefotetan (CTT)	64	Ceftazidime (CAZ)	64	Ceftriaxone (CFT)	64
Ciprofloxacin (CFX)	4	Colistin (CL)	2	Ertapenem (ETP)	4
Gentamycin (G)	2	Imipenem (IMP)	4	Levofloxacin (LVX)	2 (S)
Nitrofurantoin (NIT)	64	Piperacillin-Tazobactam (PTZ)	128	Tobramycin (MN)	16
Trimethoprim-Sulfamethoxazole (SXT)	320				

April, 2020) and identified additional 7 *mcr-9*-containing complete plasmid genomes and 2 *mcr-9*-containing chromosome sequences. We then, performed comparative alignments of the genetic environments of *mcr-9* gene in these 13 plasmid sequences and 2 chromosome sequences (Fig. 1B). We found that most (14/15) of *mcr-9* genetic environment showed 100% homology to the backbone that was composed by *rcnR-pcoE-sasA*-IS*903-mcr-9.* Among them, more than half (9/14) were found having IS*26* in the downstream of *mcr-9* (Fig. 1B). Interestingly, the genetic context of *mcr-9* on the two chromosome sequences were consistent with the structure (*rcnR-pcoE-sasA*-IS*903-mcr-9*-IS*26*) that on the plasmid sequences, suggesting that *mcr-9* gene may be transferred as a gene cassette between the chromosomes and plasmids.

It is reported that the *rcnR-rcnA-pcoE-pcoS-IS903-mcr-9-wbuC* structure was consistent in most *mcr-9* cassettes,[6] however, gene *rcnA* and *wbuC* were absent and *pcoS* was replaced by *sasA* in our plasmid. Other insertion elements such as IS*5*, IS*5075*, IS*102*, IS*4231R*, IS*5D*, IS*kpn21*, IS*Shes11*, Tn*As3,* Tn*5393* and Tn*2* were found in pXXB1403 (Fig. 1A), which may indicate the recombinational activity of this plasmid.

Notably, in consistent with its resistance to cambepem and cephalosporin (Table 1), in addition to *mcr-9*, pXXB1403 harbored both $bla_{\text{TEM-1B}}$ and $bla_{\text{DHA-27}}$. TEM-1 is one of the most well-known ESBLs which are mainly plasmid-encoded enzymes capable of hydrolysing extended-spectrum cephalosporins, rendering first-line antimicrobial therapy ineffective.[8] DHA-27 is a recently identified variant of DHA-1 enzyme which is plasmid-mediated and inducible AmpC β-lactamase that can confer resistance to carbapenems when combined with decreased outer membrane permeability and that they are not neutralized by ESBL inhibitors, which limits the therapeutic approaches.[9]

Besides, other six classes of antibiotic resistance genes were also identified in pXXB1403, including aminoglycosides resistant genes *aadA16, aac(6")-lb-cr, aph(3")-la, aph(3")-lb, aph(6′)-ld*, sulfonamide-trimethoprim resistant gene *sul1*, tetracyclines resistant gene *tetD and tetR*, fluoroquinolone resistant gene *qnrB4*, rifamycin resistant gene *arr-3* and diaminopyrimidines resistant genes *dfrA27, dfrA19* (Fig. 1A).

In conclusion, to the best of our knowledge, this is the first report of a clinical colistin-resistant MDR *Salmonella diarizonae* isolate from an UTI patient that co-harbored *mcr-9*, ESBL and AmpC genes in a conjugative plasmid in China. This isolate showed resistance to various antimicrobials including the last-resort antibiotics colistin and carbapenems (imipenem and ertapenem), reinforcing the need for enhanced continuous surveillance of antimicrobial resistance in *Salmonella* isolates from patients with UTIs, and alerts us to prevent possible failure in antimicrobial treatment of severe UTIs.

Data availability

Genome sequence of *Salmonella diarizonae* XXB1403 and plasmid pXXB1403 has been deposited into GenBank under the accession number PRJNA649845.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Immune responses and residual SARS-CoV-2 in two critically ill COVID-19 patients before and after lung transplantation

Dear Editor,

A small number of COVID-19 patients develop critical illness resulting in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Although lung transplantation (LT) can be used to rescue patients from COVID-19-related ARDS,[1,2] current information concerning the immune statues and pathogenic conditions of such transplant patients is lacking. In this study, we assessed the immune responses and the residual SARS-CoV-2 nucleic acids in patients before and after LT, including the first COVID-19 lung transplant patient in the world.

There were two critically ill COVID-19 patients in Wuxi of China, from January 25 to March 31, 2020, who eventually recovered after LT (Ethics No. 2020–014).[2,3] For comparison purposes, we analyzed the whole blood lymphocytes, immunocyte subclasses (T, B and NK cells), blood cytokines and Ag-specific IgM and IgG of hospitalized COVID-19 patients in Wuxi (Ethics No. 2020–010–1). We focused on the analyses of the two critically ill COVID-19 patients (Patient 1: a 58-year-old male had COVID-19-associated ALI and ARDS; Patient 2: a 73-year-old male had COVID-19-associated multiple organ failure and ARDS).

In view of the limited number of critically ill patients, we merged severe and critical illness, designated as severe illness (Fig 1). Most of the severely ill patients had low levels of blood lymphocytes during hospitalization, and in particular, the blood lymphocytes in the two critically ill patients remained below the normal value before and after LT ($<1.1 \times 10^9$/L) (Fig. 1A). There were statistically significant lower levels of blood $CD3^+$ $CD45^+$ T (<60%) (Fig. 1B and 1C), $CD4^+$ T, $CD8^+$ T and NK cells in individuals with critical clinical manifestations ($P < 0.05$), and a reduction of $CD8^+$ T cells was the most statistically significant in the severely ill patients ($P < 0.01$) (Fig. 1B and 1D). Compared with untreated mildly ill patients, no significant increase of T and NK cells was observed in blood before and after LT, and only B cells increased slightly in the two critically ill patients, likely owing to mesenchymal stem cell infusion therapy (Fig. 1B and 1D). The two critically ill patients developed mildly positive SARS-CoV-2-specific IgM and IgG before LT, and such humoral immune responses became negative post LT (data not shown), likely due to blood transfusion therapy in the absence of new pathogen stimulation. It was reported that IL-6 and IL-10 play distinct roles in immune tolerance.[4,5] In our study of the two critically ill patients, long-lasting IL-6 and IL-10 levels in plasma exceeded the upper limits of normal values, accompanied by viral replication. The concentrations of proinflammatory cytokines (IL-6, IFN-γ, and TNF-α), anti-inflammatory cytokine IL-10 and B-/T-cell stimulating factor IL-4 in the severe period were significantly higher than those in the recovery period ($P < 0.05$), especially for the critically ill patients post LT (Fig. 1E). The above findings together indicate that SARS-CoV-2 infection in critically ill patients results in lower levels of cellular and humoral immune responses.

Pathological analyses were performed by immunostaining for $CD3^+$ T, IgA^+ and SARS-CoV-2 S $protein^+$ cells in the diseased lungs. In immunohistochemistry, critically ill patient' lungs (Patient 2) showed obscure mature $CD3^+$ T cells in tissues, and extensively fibrosis (Fig. 1F), interstitial hemorrhage (Fig. 1G) and mucous exudative necrosis in the bronchioles (Fig. 1H), as well as alveolar epithelial atrophy, hyperplasia and shedding in the alveolar cavity (Fig. 1I). The number of IgA^+ cells from alveoli epithelial cells decreased in both the right and the left pulmonary lobes (Fig. 1J-1M). Residual SARS-CoV-2 in the lungs has been suggested to be the main reason for viral positivity of discharged COVID-19 patients.[6,7] We observed a direct evidence of residual SARS-CoV-2 in excised lungs (Fig. 1N-1Q), suggesting that antiviral therapy may not completely eliminate the virus in the dysfunctional lungs. For the two critically ill COVID-19 patients under therapies, convalescent plasma and mesenchymal stem cell infusions appeared unable to restore a systemic immunity, including cellular and mucosal (IgA) immune responses in the diseased lungs. A previous pathologic study showed that SARS-CoV-2 was highly destructive to the immune system, resulting in reduced splenic T and B cell compositions due to necrosis and apoptosis.[8] This may account for the long-term low systemic immunity of COVID-19 transplant patients.

SARS-CoV-2 RNA can be detected in the gastrointestinal tract using swabs and stool sampling,[9] and in particular, SARS-CoV-2 particles can be found in the gut endothelium,[10] suggesting the potential significance of the gut in viral transmission and pathogenesis. Prior to LT, the two critically ill patients in the current study were under treatments with convalescent plasma infusion, mesenchymal stem cell infusion and antiviral agents until SARS-CoV-2 nucleic acid turned into negative in blood and nasopharyngeal and anal swabs. Post LT, residual SARS-CoV-2 in nasopharyngeal and anal swabs was also examined. In anal swabs, SARS-CoV-2 was mildly positive at day 26, 30, and 43 post LT in Patient 1 (Fig. 2A), and mildly positive at day 28 post hospitalization and day 17 post LT in Patient 2 (Fig. 2B). Of note, in the absence of antiviral treatment and under immune suppression therapy (doses of drugs were only 1/6 of those for

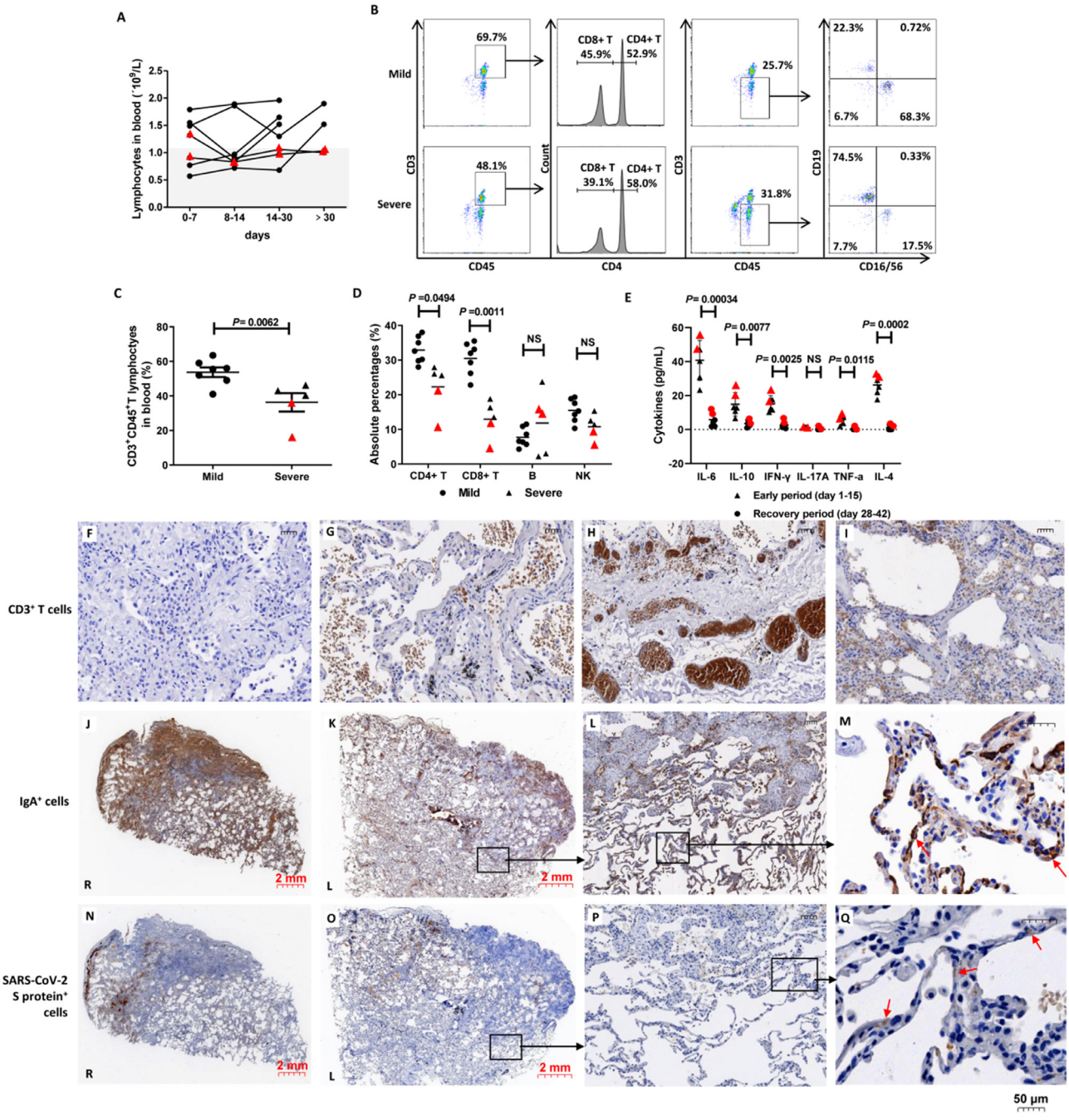

Fig. 1. Immune responses of critically ill COVID-19 patients and immunohistochemistry of the diseased lungs. **A.** Blood T lymphocyte counts. Blood T lymphocyte counts were detected by clinical blood cell analyzer. The gray area is the location of the dangerous values ($<1.1 \times 10^9$/L). **B.** Gating strategies for blood immunocytes. $CD3^+$ and $CD45^+$ lymphocytes were gated for $CD4^+$ and $CD8^+$ T cells. $CD3^-$ and $CD45^+$ immunocytes were gated for B ($CD19^+$) and NK ($CD16/56^+$) cells. **C.** Percentage comparison of blood $CD3^+$ and $CD45^{++}$lymphocyte levels. **D.** Absolute percentage comparison of blood immunocytes. **E.** Plasma cytokine levels. Plasma samples from COVID-19 patients ($n = 6$) were collected during the early and recovery periods (mildly and severely ill patients were at around day 30; transplant patients were before being transferred to the general ward). IL-6, IL-10, IFN-γ, IL-17A, TNF-α, and IL-4 beads were used for staining the cytokines in plasma samples. Quantitative detection and comparison of inflammatory cytokine expression levels in different periods were conducted by flow cytometry. Mildly ill patients (Mild): individuals who had mild manifestation and no apparent or long-term decrease in lymphocyte levels. Severely ill patients (Severe): individuals who had lasting lower levels of lymphocytes and needed supplemental oxygen and intensive care. Critically ill patients: individuals who had failure of respiratory organs leading to dependency on ventilators. The red solid symbol (▲ or ●) stands for critically ill patients. The results are expressed as the mean ± SEM; NS, not statistically significant, $P < 0.05$ was considered statistically significant. **F-I.** Hominine CD3 immunostaining of diseased lung tissues. **J-M.** Hominine IgA immunostaining. **N-Q.** SARS-CoV-2 S protein immunostaining. Post LT, approximately 2-cm segments from excised human lungs were collected and fixed in formalin for 24 h. After fixation, tissue was embedded and sectioned. For the detection of CD3-, IgA- and SARS-CoV-2 S protein-positive cells in lungs, the slides were stained with CD3ε rabbit monoclonal antibody (Cell Signaling Technology, U.S.A, 1: 1000, E4T1B), human IgA heavy chain rabbit polyclonal Ab (Proteintech, U.S.A., 1: 400), and SARS-CoV-2 S protein rabbit polyclonal Ab (Sino Biological Inc., China, 1: 50), respectively, overnight at 4 °C. After labeling with a goat anti-rabbit secondary IgG Ab, a color reaction was developed with the addition of 3, 3′-diaminobenzidine free base (DBA), followed by counterstaining with hematoxylin. Data shown are representative immunohistochemistry results. S protein: spike protein; R, right upper lobe; L, left lower lobe; The red arrow shows the positive cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

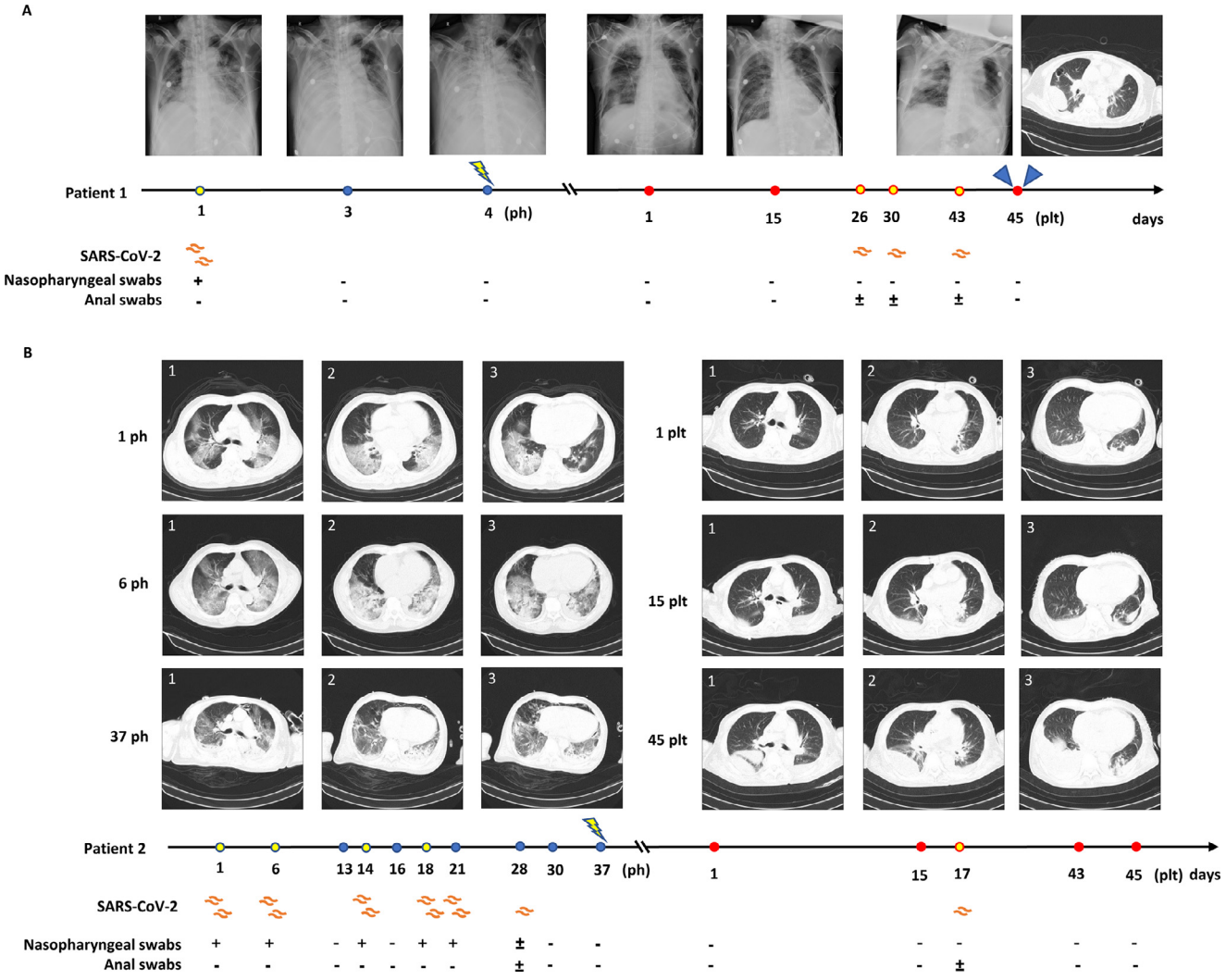

Fig. 2. Chest imaging and viral nucleic acid detection before and after LT. **A.** Chest radiographic images and SARS-CoV-2 nucleic acid results of Patient 1. Chest radiographic images were obtained before and post LT. Axial CT images on day 45 post LT, and SARS-CoV-2 nucleic acid results. **B.** Axial chest CT images before and after LT and SARS-CoV-2 nucleic acid results of Patient 2. Nasopharyngeal and anal swabs were placed in viral transport medium at a low temperature. Total RNA extraction and SARS-CoV-2 detection were performed using the commodity reagents of Da An Gene (Da An Gene, China) according to the manufacturer's instructions. Briefly, SARS-CoV-2 RNA was detected by real-time RT-PCR. Target genes (ORF1ab + N) were set as described in the reagent instructions. A cycle threshold value (Ct value) less than 36 was defined as a positive (+), and Ct value between 36 and 37 was defined as mildly positive (±). 1, 2 and 3 on CT images mean the different axial images within the lungs. ph: post-hospitalization; plt: post LT.

ordinary transplant patients) post LT, there was no indication of SARS-CoV-2 infection in the new (donor) lungs according to the chest radiographs and axial pulmonary CT graphs in the negative-pressure ward up to 45 days (Fig. 2A and 2B), while viral nucleic acid remained negative after the patients were transferred to the general ward. There was no medical staff infected by SARS-CoV-2 during medical care in general ward. The above results indicated that the detection of viral positive nucleic acids by anal swapping does not necessarily reflect a contagious SARS-CoV-2 in the gut.

In conclusion, following LT, the two critically ill COVID-19 patients in the absence of antiviral treatment have not had a second SARS-CoV-2 infection in the new lungs. For the first time, our study provides information relating to the immune status and SARS-CoV-2 positivity of lung transplant patients.

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.

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The under-representation of BAME patients in the COVID-19 Recovery trial at a major London NHS Trust

Dear Editor,

A recent letter in this journal by Kakkar and colleagues noted how, during the first wave of the coronavirus pandemic, black, Asian and minority ethnic (BAME) inpatients were significantly more likely to be admitted to intensive care compared to white inpatients at Sheffield Teaching Hospitals.[1] This is just one piece of a growing body evidence that illustrates how BAME individuals have been disproportionately affected by the novel coronavirus pandemic, with excess mortality due to COVID-19 in BAME populations in England now a well-established phenomenon.[2] The precise makeup of factors responsible for this disparity remains unknown.

An historical under-representation of BAME patients in medical research[3] has prompted concerns that COVID-19 studies may suffer from the same pitfall[4]. Numerous studies concerning potential vaccines and treatments for COVID-19 are already underway and it is essential they reflect the populations they hope to serve. The extent to which these studies are fulfilling adequate representation in their study cohorts is unclear.

Several weeks after the initial COVID-19 surge, we conducted a retrospective analysis of the ethnicity of inpatients enrolled onto the six COVID-19 interventional treatment trials at Imperial College Healthcare NHS Trust (ICHT) in London, UK (Table 1). Patient records were insufficiently granular to provide ethnicity data beyond 'BAME', 'white', and 'unknown'.

In total, 179 patients were enrolled onto the six trials. The trial with the largest cohort was Recovery ($n = 83$), which recently demonstrated that amongst inpatients hospitalised with COVID-19, dexamethasone reduced 28-day mortality among those receiving invasive mechanical ventilation or oxygen at randomization.[5] Ethnicity was not included as a subgroup in this analysis.

Of the total 179 patients, 61 (34%) were BAME, 80 (45%) were white and for 38 (21%), ethnicity was unknown. Of the 83 patients

Table 1
Interventional COVID-19 trials at ICHT.

Trial	Interventions	Indication
RECOVERY	Dexamethasone; azithromycin; tocilizumab; convalescent plasma; REGN-COV2 (monoclonal antibodies)	Suspected or confirmed COVID-19
C19-ACS	Early acute coronary syndrome therapy	Suspected or confirmed COVID-19
Gilead Moderate	Remdesivir	Moderate COVID-19
Gilead Severe	Remdesivir	Severe COVID-19
COVACTA	Tocilizumab	Severe COVID-19
REMAP-CAP	Multiple domains including antivirals and immunoglobulin therapy	Severe COVID-19

enrolled into Recovery, 24 (29%) were BAME, 40 (48%) were white and for 19 (23%), ethnicity was unknown.

To understand if BAME representation within the trials was reflective of the total cohort of inpatients with COVID-19, we consulted the best available standard of comparison: a retrospective cohort study of all patients hospitalised with confirmed SARS-CoV-2 infection at ICHT between February 25 and April 5, 2020. Of the 520 patients in this cohort, 209 (40%) were BAME, 196 (38%) were white, and for 115 (22%) ethnicity was unknown.[6]

There was no statistically significant difference between the proportion of BAME patients across all trials compared to the total inpatient cohort (chi square test, $p = 0.089$). However, there was a statistically significant difference between the proportion of BAME patients in the Recovery trial compared to the total inpatient cohort ($p = 0.036$).

The reasons for this observation of an apparent under-representation of BAME patients in the Recovery trial at ICHT are unclear. Existing evidence suggests that the reasons for an under-representation of BAME individuals in medical research are complex and include a range of subject, clinician/researcher, societal and cultural factors.[7]

Patients were eligible for enrolment into Recovery provided they met three eligibility criteria: (i) hospitalization, (ii) clinically suspected or laboratory confirmed SARS-CoV-2 infection and (iii) no medical history that might, in the opinion of the attending clinician, put the patient at risk.

It is plausible that BAME inpatients were more likely to have co-morbidities that clinical teams believed could put them at risk if they were enrolled. For example, type 2 diabetes is disproportionately prevalent in South Asians and dexamethasone is known to interfere with glycaemic control. We also know that BAME patients with COVID-19 are disproportionately admitted to critical care settings[1,2] where staff are required to wear FIT-tested PPE. During the initial surge and before research was announced as a key pillar of the government's plans, clinical teams were understandably prioritised for FIT testing, meaning the local research team initially experienced difficulty in accessing and recruiting patients being treated on intensive care. The Recovery trial also permitted clinician consent if a patient lacked capacity, raising the possibility that recruitment may have been skewed towards older patients who, as a population, are more likely to lack capacity. At ICHT, white COVID-19 patients had a higher age composition than their BAME counterparts.[6]

Furthermore, the research team was not provided with non-English language patient information sheets, and there was minimal access to interpreting services. Mandatory reporting of ethnicity was not a component of the Recovery enrolment protocol and it was only after the intense initial surge that a more robust screening log, tracking reasons for exclusion, was introduced.

A Public Health England review into the disparities in COVID-19 outcomes highlighted how a lack of ethnicity data hindered the scope of analysis[2] and one systematic review of the impact of ethnicity on clinical outcomes in COVID-19 published in June found that of 1518 COVID-19 studies registered on ClinicalTrials.gov at the time, only six were recording ethnicity data.[8] The National Institutes of Health (NIH) in the US has required researchers to replicate the ethnic composition of their study population since 1994, and phase III clinical trials must include subgroup analysis to assess ethnic differences in treatment efficacy.[7] Although this practice is recommended in the UK, it is not mandated.

Whilst there is a paucity of evidence regarding effective interventions to increase BAME representation in clinical trials,[9] mandatory ethnicity recording in COVID-19 research studies in the UK would ensure inclusivity could be more accurately tracked. Furthermore, in 2018 the National Institute for Health Research (NIHR) launched the Innovations in Clinical Trial Design and Delivery for underserved groups project (INCLUDE), which has issued guidance to support inclusive COVID-19 research.[10] Accordingly, the government, public health bodies and funders should commit to ensuring local research teams are able to invest in the necessary resources to maximize trial inclusivity.

The issue of BAME under-representation in medical research is complex and will take considerable time and effort to overcome, including addressing societal and cultural factors. But the disproportionate impact of COVID-19 on BAME individuals and a second surge in cases and hospitalisations mean there is an urgent need for the government, researchers and healthcare professionals to do everything possible to ensure inclusivity in COVID-19 research studies.

Declaration of Competing Interest

None.

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Comparing SARS-CoV-2 and influenza A(H1N1)pdm09-infected patients requiring ECMO – A single-centre, retrospective observational cohort experience

Dear Editor,

We read with interest the recent report from Fiore and colleagues,[1] describing their experience with ECMO on COVID-19 cases. Here we share our own ECMO–COVID-19 experience, and compare this with our previous experience of ECMO use in influenza patients.[2]

ECMO is a resource-intensive, highly specialised, and expensive form of life support with the potential for significant complications with unknown benefits, in the management of COVID-19. Thus its clinical utility during the current pandemic has been uncertain.[3] An early retrospective case series from China described the role of ECMO in COVID-19 as 'unpromising', with nearly half of patients treated with ECMO dying from septic shock and multiple organ failure.[4]

To investigate this further, we describe the clinical characteristics and outcomes of 34 patients presenting with severe COVID-19 pneumonitis who received respiratory ECMO support during the COVID-19 pandemic to one of the commissioned UK respiratory ECMO centres. Single-centre, retrospective data collection was performed for all patients receiving respiratory ECMO support for severe respiratory failure secondary to SARS-CoV-2 infection during the peak of the UK pandemic (1st April to 31st May 2020). Various ECMO-related and laboratory parameters were extracted and statistically compared between patients who died ($n = 18$) and those who survived ($n = 16$) using the *t*-test or Mann-Whitney test for continuous variables and the Fisher-exact test for categorical variables. Correlation between duration on ECMO and laboratory parameters was assessed using Spearman's rank correlation coefficient.

A total of 268 patients were referred for consideration of respiratory ECMO support in Leicester (patient eligible criteria are shown in **Fig. S1**). Of the 268 referrals, 38 were retrieved for consideration of ECMO. Thirty-four went on to receive ECMO support, with 4 patients managed with advanced conventional ventilatory support as part of the Severe Acute Respiratory Failure (SARF) pathway (**Table S1**).

The mean age of the 34 patients receiving ECMO support was 46 years (range: 28–58). The majority were male (27/34, 79%), with a mean body mass index (BMI) of 31.9 (range: 22.4–45), and most patients (88%) had at least one comorbidity (hypertension, diabetes or obesity).

The most frequently occurring COVID-19 symptoms in this patient group included fever (26/34, 76%), cough (25/34, 74%) and myalgia (10/34, 29%). Anosmia was a relatively uncommon finding, being recognised in only 2 patients (6%). Black and Minority Ethnic (BAME) groups accounted for 59% (20/34) of patients, with 38% (13/34) of the cohort being healthcare workers. Co-morbid conditions were present in the majority of patients (30/34, 88%), with the most common being: obesity (23/34, 68%), hypertension (8/34, 24%) and diabetes (4/34, 12%).

The majority of patients had a dual-lumen cannula configuration of the right internal jugular vein (28/34, 82%), as is routine practice for our unit. Renal replacement therapy was initiated in 26% (9/34) of patients. The mean duration of ventilation prior to initiation of ECMO was 4.9 days (range: 2–8), and the mean dura-

Table 1
Demographic and clinical characteristics of the COVID-19 patients on ECMO.

Demographics	Survivor ($n = 18$)	Died ($n = 16$)	p-value
Age (years)	45.6 (6.7)	47.0 (8.4)	0.596
Sex - M	15 (83%)	12 (75%)	0.682
Ethnicity			
Asian	8 (44%)	8 (50%)	0.893
Black	2 (11%)	2 (13%)	
White	8 (44%)	6 (38%)	
HCW	6 (33%)	7 (44%)	0.725
Medical history			
Any comorbidity	17 (94%)	13 (81%)	0.323
Hypertension	6 (33%)	2 (13%)	0.233
Diabetes	3 (17%)	1 (6%)	0.604
Obese	15 (83%)	8 (50%)	0.066
Haematology and biochemistry parameters			
White cell count (10^9/L)	14.1 (5.7)	10.5 (2.9)	0.027
Neutrophils (10^9/L)	12.3 (5.4)	9.1 (2.9)	0.035
Lymphocytes (10^9/L)	1.1 (0.4)	0.9 (0.5)	0.220
C-reactive protein (mg/L)	195.4 (113.0)	260.6 (95.5)	0.080
Haemoglobin (g/L)	105.3 (11.2)	103.4 (11.8)	0.623
LDH (IU/L)	656.4 (277.2)	603.5 (243.7)	0.684
ALT (IU/L)	51 (35 - 73)	47.5 (34 - 79)	0.931
GGT (IU/L)	154.5 (78.5 - 251)	104 (73 - 156)	0.097
AST (IU/L)	74 (59.5 - 85)	69 (65 - 94)	0.728
Total bilirubin (μmol/L)	13 (8 - 31)	10.5 (7 - 17.5)	0.284
Urea (mmol/L)	9.4 (6.7 - 13)	10.3 (7.4 - 15)	0.504
Na (mmol/L)	143.0 (4.6)	146.2 (4.2)	0.043
K (mmol/L)	5.3 (0.7)	4.8 (0.5)	0.045
Creatinine (μmol/L)	74.5 (52 - 190)	68 (45.5 - 124)	0.704
Troponin (ng/L)	15 (4 - 41)	29 (17 - 83)	0.142
BNP (pg/mL)	384 (253 - 566)	532 (341 - 1127)	0.365
D-dimers (ng/mL)	7.9 (3.3 - 9.6)	6.2 (3.5 - 18)	0.772
ECMO parameters			
PaO_2:FiO_2 ratio	9.0 (7.3 - 10)	8.3 (7 - 9.8)	0.398
PEEP (cm H_20)	12.8 (3.5)	13.8 (3.2)	0.373
Tidal volume (mL)	476.5 (78.3)	476.1 (154.6)	0.992
Respiratory rate (RR, bpm)	20.4 (4.3)	19.3 (5.3)	0.498
Peak RR (bpm)	32.9 (3.7)	32.3 (3.6)	0.612
pH	7.3 (0.1)	7.3 (0.1)	0.823
PaO_2 (kPa)	7.9 (1.2)	8.0 (1.2)	0.689
$PaCO_2$ (kPa)	7.7 (1.9)	9.6 (4.3)	0.112
Base excess (mmol/L)	2.4 (4.5)	3.0 (2.9)	0.702
Lactate (mmol/L)	1.5 (0.6)	1.5 (0.5)	0.911
Bicarbonate (HCO_3, mmol/L)	27.2 (4.8)	28.7 (4.3)	0.409
MAP (mm Hg)	79.6 (12.1)	77.3 (10.7)	0.553
Days ventilated pre-ECMO	5.2 (1.8)	4.6 (1.7)	0.278
Days on ECMO	11.1 (4.9)	15.6 (5.6)	0.017

LDH - lactate dehydrogenase; ALT – alanine aminotransferase; GGT – gamma glutamyl transferase; AST – aspartate aminotransferase; BNP - brain natriuretic peptide; PEEP – positive end expiratory pressure; H_2O – water; bpm - breaths per minute; $PaO_2/PaCO_2$ – partial pressure of arterial oxygen/carbon dioxide; MAP – mean arterial pressure; ECMO – extra-corporeal membrane oxygenation.

Table 2
Comparison of demographic and clinical characteristics of COVID-19 (2020) and influenza (2018–2019) patients on ECMO.

	COVID-19 ($n=34$)	A(H1N1)pdm09 ($n=26$)	p-value
Demographics			
Age (yrs)	46.3 (7.5)	43.1 (8.7)	0.133
Sex - male	27 (79%)	18 (69%)	0.386
Ethnic White	14 (41%)	24 (92%)	<0.001
Body-mass index (BMI)	31.9 (6.0)	30.6 (7.8)	0.475
Medical history			
Comorbidity	30 (88%)	18 (69%)	0.104
Hypertension	8 (24%)	5 (19%)	0.760
Diabetes	4 (12%)	2 (8%)	0.689
Obese	23 (68%)	12 (46%)	0.118
Haematology and biochemistry parameters			
White cell count (10^9/L)	12.4 (4.9)	5.8 (4.6)	<0.001
C-reactive protein (mg/L)	226.1 (108.7)	259.4 (140.3)	0.323
Haemoglobin (g/L)	104.4 (11.3)	129.7 (23.0)	<0.001
ALT (IU/L)	47.5 (35 - 78)	57 (31 - 78)	0.883
Total bilirubin (µmol/L)	11.5 (8 - 22)	18 (9 - 30)	0.702
Urea (mmol/L)	10.2 (7.1 - 13)	9.05 (4.9 - 15.1)	0.405
ECMO parameters			
Creatinine (µmol/L)	70.5 (51 - 149)	100 (71 - 181)	0.070
PaO_2:FiO_2 ratio	8.6 (7.3 - 9.9)	8.3 (7.2 - 10.1)	0.994
PEEP (cm H_20)	13.3 (3.3)	12.8 (3.3)	0.657
Tidal volume (mL)	476.3 (118.4)	495.0 (108.6)	0.548
Respiratory rate (RR, bpm)	19.9 (4.8)	20.0 (6.9)	0.954
Peak RR (bpm)	32.6 (3.6)	30.3 (5.2)	0.058
pH	7.3 (0.1)	7.3 (0.2)	0.337
PaO_2 (kPa)	7.9 (1.2)	8.1 (1.8)	0.722
$PaCO_2$ (kPa)	8.6 (3.3)	7.8 (3.0)	0.361
Base excess (mmol/L)	2.7 (3.8)	−0.8 (6.6)	0.023
Lactate (mmol/L)	1.5 (0.6)	2.2 (1.4)	0.023
Bicarbonate (HCO_3, mmol/L)	27.8 (4.6)	23.7 (5.4)	0.004
MAP (mm Hg)	78.5 (11.4)	72.4 (11.8)	0.048
Days ventilated pre-ECMO	4.9 (1.7)	2.4 (2.5)	<0.001
Outcomes			
Days on ECMO	13.2 (5.6)	12.3 (8.0)	0.601
RRT	9 (26%)	24 (92%)	<0.001
Death	16 (47%)	8 (31%)	0.288

ALT – alanine aminotransferase; PEEP – positive end expiratory pressure; H_2O – water; bpm - breaths per minute; $PaO_2/PaCO_2$ – partial pressure of arterial oxygen/carbon dioxide; MAP – mean arterial pressure; ECMO – extra-corporeal membrane oxygenation; RRT – renal replacement therapy.

tion of ECMO support was 13.2 days (range: 4–26). The survival to discharge in this cohort of patients was 53% (18/34).

Table 1 compares COVID-19 patients who survived (18/34, 52.9%) versus those who died (16/34, 47.1%), with both groups being evenly matched with respect to demographic data and medical history. White cell count ($p=0.027$) and neutrophil count ($p=0.035$) were both significantly lower in those patients who died (10.5 vs. 14.1 and 9.1 vs. 12.3, respectively). The duration of ECMO was also significant, 11.1 days (survivors) versus 15.6 days (deaths). The slight differences in Na/K concentrations between the two groups, though just statistically significant ($p<0.05$), were of little clinical significance.

When comparing this COVID-19 cohort to a previous cohort presenting for ECMO support with influenza A(H1N1)pdm09 during the 2018–2019 season (**Table 2**), a number of significant differences were noted. Most significantly, severe influenza requiring ECMO support occurred more frequently in ethnically white (Caucasian) compared to the COVID-19 patients ($p \leq 0.001$). White cell count also appears significantly lower in the influenza cohort (5.8 vs. 12.4, $p \leq 0.001$). A significant proportion of the patients in the influenza group required renal replacement therapy (24/26, 92%), which was not seen in the COVID-19 patients. The number of days ventilated prior to initiation of ECMO was also significantly shorter in the influenza group (2.4 vs. 4.9, $p \leq 0.001$). More deaths were recognised in the COVID-19 group (47% vs. 31%), although this did not reach statistical significance.

In this single-site, retrospective observational cohort study, we present the data from 34 patients admitted to one of the six UK severe respiratory failure centres who received ECMO support for COVID-19 pneumonitis. Earlier speculation by some teams about the potential application of ECMO suggested that it may be useful in severe cases of COVID-19, though this would depend very much on case selection. This speculation has since been borne out by more recent reports on single[5,6], small[7] and larger[8,9] case series of patients with severe COVID-19, where the authors have been cautious about the potential benefits of ECMO in such patients, emphasising the limited capacity for this complex treatment.

A recent ECMO consensus document[10] states this succinctly as: *"ECMO is a highly technical therapy and is resource intensive. Although the distribution of this therapy should be as equitable as possible, during a pandemic such as COVID-19, distribution should focus on optimal candidates for recovery."*

In our case series presented here, it is difficult to conclude exactly how much the ECMO therapy contributed to the survival of these severely ill COVID-19 patients versus if they only had standard intensive care unit (ICU) support, as it was not possible for each patient to act as his/her own control in this comparison. However, as per our previous experience reported on seasonal influenza patients requiring ECMO[2] it is likely that ECMO was more beneficial than not in this highly selected patient cohort.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.11.003.

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Early, low-dose, short-term methylprednisolone decreased the mortality in critical COVID-19 patients: A multicenter retrospective cohort study

Dear Editor,

We read with interest the recent paper by Yang et al., concluding that corticosteroids overall have a negative impact on COVID-19 outcomes from a meta-analysis [1]. Critical COVID-19, characterized by refractory hypoxemia caused by acute respiratory distress syndrome (ARDS), is a life-threatening multi-organ dysfunction syndrome resulted from host response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Glucocorticoid (GC) was one of the anti-inflammatory medications used in critical patients [2]. Efficacy of glucocorticoids has been reported in numerous clinical studies in the treatment of coronavirus pneumonia [3]. Yang and colleagues demonstrated that patients treated with GC had a higher mortality [1], suggesting that not all patients could benefit from GC treatment. Present study aimed to evaluate the effect of GC on different patient population. Since critical patients were more likely to receive GC therapy, only severe type and critical type patients, according to clinical classification of the *Chinese Recommendations for Diagnosis and Treatment of Novel Coronavirus (SARSCoV2) infection (Trial 7th version)*[4], were enrolled in present study. We retrospective collected the clinical and outcome data of critical COVID-19 patients, and taking methylprednisolone (MP) treatment, the most used GC during clinical treatment, as an exposure factor analyzed the outcome. This study provides information on MP clinical application in treatment of SARS-CoV-2 infection, including patient selection and administration time and dosage.

Present multicenter retrospective cohort study was performed in 4 government designated treatment centers for COVID-19 patients in 3 cities in China, Wuhan, Guangzhou, and Shenzhen. The data collection period was from December 2019 to March 2020. This study was approved by the Research Ethics Commission of General Hospital of Southern Theater Command of PLA (HE-2020–08) and the requirement for informed consent was waived by the Ethics Commission. Inclusion Criteria for all patients: (1) Adult aged >= 18 years old; (2) Laboratory (RT-PCR) confirmed SARS-COV-2 infection in throat swab and/or sputum and/or lower respiratory tract samples; or conformed plasma positive of specific antibody (IgM or/and IgG) against SARS-COV-2; (3) In-hospital treatment ≥72 h. The patients in who meet any one of the following criteria were enrolled in severe type group [4]: (a) Respiratory rate >= 30/min; or (b) Rest SPO_2 <= 90%; or (c) PaO_2/FiO_2 <= 300 mmHg. The patients in who meet any one of the following criteria were enrolled in critical type group [4]: (a) Respiratory failure and needs mechanical ventilation; or (b) Shock occurs; or (c) Multiple organ failure and needs ICU monitoring. Exclusion Criteria: (1) Exist of other evidences that can explain pneumonia including but not limited to influenza A virus, influenza B virus, bacterial pneumonia, fungal pneumonia, noninfectious causes, etc.; (2) Women who are pregnant or breast-feeding.

Data from 338 patients diagnosed as critical COVID-19 were collected in present study. According to whether methylprednisolone was employed during treatment, 164 in Non-MP group and 174 in MP group. For all cases, MP treatment did not show benefit in prognosis (Fig. 1). The effects of MP differed between different clinical classification and baseline lactate concentration. MP treatment on the critical type patients could decrease the 60-day fatality (HR: 0.409, 95% CI: 0.238–0.704, *p*-value: 0.001), while it has no influence on the fatality of severe type patients. In addition, patients with higher lactate concentration on baseline could get more benefit from MP treatment. For the patients with lactic acid concentration over 2 mmol/L, MP treatment could significantly decrease the 60-day fatality (HR: 0.150, 95% CI: 0.055–0.408, *p*-value: < 0.001). These results showed that MP treatment have more efficiency on the patients with serious condition. Glucocorticoid could suppress lung inflammation and but also inhibit immune responses. Therefore, balancing the risk and benefit is crucial during the treatment, that is, not all patients could benefit from GC therapy. Critical type COVID-19 patients showed excessive inflammatory responses and MP treatment could alleviate the cytokine storm by inhibiting the inflammatory cells activation [5]. In addition, critical illness-related corticosteroid insufficiency occurs across a broad spectrum of critical illness due to the impairment of the hypothalamic pituitary axis [6]. Inadequate endogenous glucocorticoid resulted in insufficient anti-inflammatory activity, and MP treatment could adverse this issue. Moreover, MP could improve the microcirculation in critical patients [7]. That explained that our subgroup analysis results, which showed that MP treatment reduced the fatality of the patients with increased lactate.

Few studies have discussed the application time, dosage and duration of MP, which were mostly based on the physician experience. To further clarify when and how to employ MP application on the critical type patients, the hazards ratios were analyzed in each group according to the starting time, dosage, and treatment duration (Fig. 2). In all 107 critical type patients, 33 of them were not received MP treatment, 59 of them received MP treatment in 7 days after admission to hospital and 12 of them were received after 7 days. 3 patients received MP treatment, but the starting time were missed, and they were not enrolled in analysis. Our results showed MP treatment in 7 days after admission could decrease the 60-day fatality (HR: 0.294, 95% CI: 0.159–0.543, *p*-value < 0.001), while MP treatment after 7 days has no effect on the fatality. Subgroup with different doses of MP (=< 80 mg/d or > 80 mg/d) were also analyzed. We found that small dose MP showed significant effect on the fatality (HR: 0.329, 95% CI: 0.178–

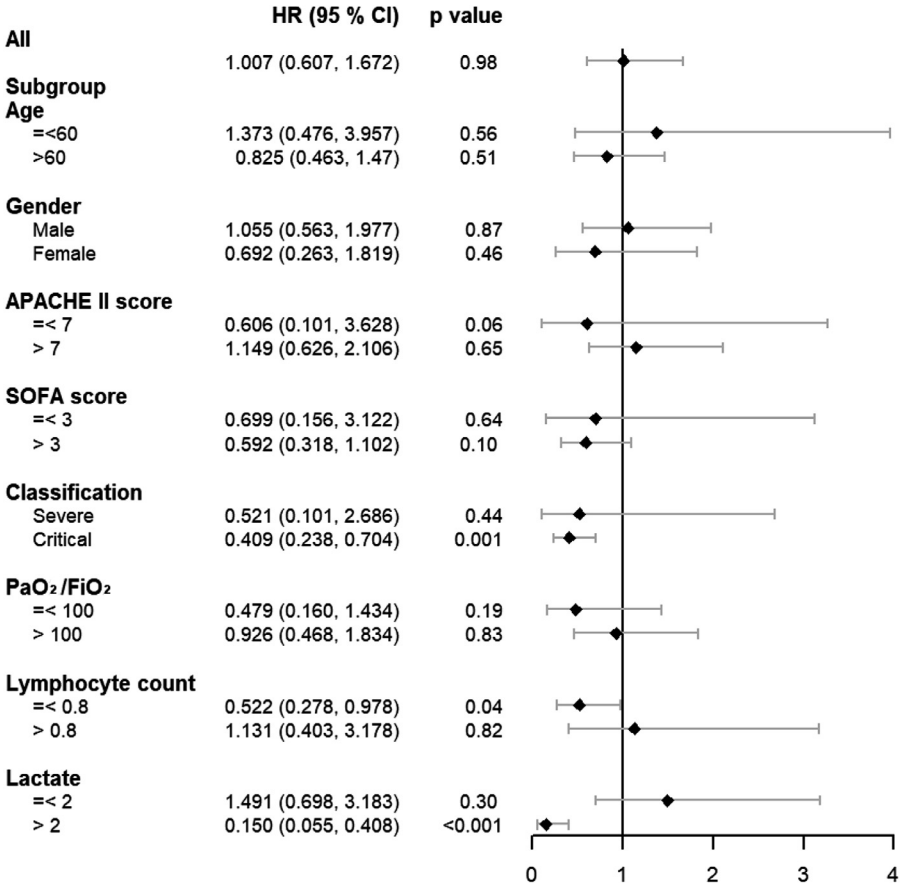

Fig. 1. Effect of methylprednisolone therapy on the 60-day fatality in different subgroup ($n = 338$).

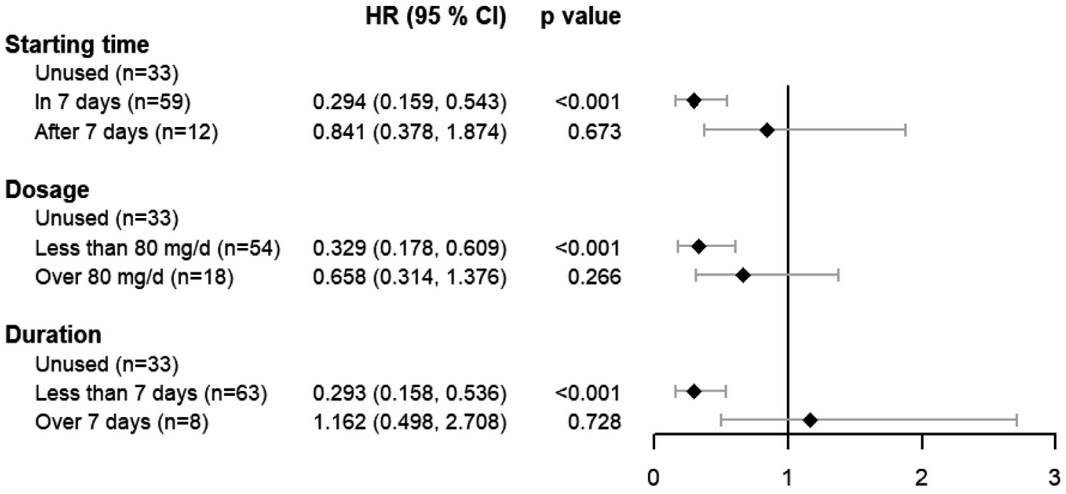

Fig. 2. Effect of different starting time, dosage, and duration of methylprednisolone on the on the 60-day fatality of critical type patients ($n = 107$).

0.605, *p*value < 0.001). In addition, most patients benefited from MP were received treatment no more than 7 days. MP long-term treatment might increase the death risk.

Present multicenter retrospective cohort study showed that methylprednisolone therapy could decrease the 60-fatality for the COVID-19 patients diagnosed as critical type, that is, those occurred respiratory failure and needs mechanical ventilation, or shock, or multiple organ failure and needs ICU monitoring. Early (starting in 7 days after admission), low-dose (no more than 80 mg/d), and short-term (no more than 7 days) methylprednisolone therapy could significant decrease the 60-day fatality.

Declarations

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Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Ethics approval

The study was approved by the Research Ethics Commission of General Hospital of Southern Theater Command of PLA.

Consent to participate

The requirement for informed consent was waived by the Ethics Commission.

Consent for publication

All authors reviewed the manuscript and approved the publication.

Availability of data and material

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Zhifeng Liu was responsible for study concept and design. Ziyun Shao, Ming Wu, Qifeng Xie, Zheying Liu, Zhifeng Liu, Li Zhong, and Conglin Wang were responsible for collecting the data. Jingjing Ji, Ming Wu, Zhifeng Liu and Li Zhong were responsible for statistical analysis. Zhifeng Liu, Jingjing Ji and Ming Wu were responsible for drafting the manuscript. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Seropositivity and risk factors for SARS-CoV-2 infection in staff working in care homes during the COVID-19 pandemic

Dear Editor,

We recently reported in the *Journal of Infection* very high rates of SARS-CoV-2 infection in four London care homes experiencing prolonged outbreaks of COVID-19 during the peak of the pandemic in England.[1] These outbreaks were associated with two deaths and

Table 1
Summary statistics and Chi-squared test p-value for potential risk factors for SARS-CoV-2 infection.

Risk factor	N	N Seropositive	Chi-squared test p-value
DEMOGRAPHIC FACTORS			
Sex			0.357
Female	254/325 (78.2%)	123 (48.4%)	
Male	71/325 (21.8%)	30 (42.3%)	
Age			0.014
0–39	142/327 (43.4%)	55 (38.7%)	
40–59	149/327 (45.6%)	83 (55.7%)	
60+	36/327 (11.0%)	16 (44.4%)	
Co-morbidity			0.742
No comorbidity	231/308 (75.0%)	110 (47.6%)	
At least 1 comorbidity	77/308 (25.0%)	35 (45.5%)	
Ethnic group			0.185
Asian	70/282 (24.8%)	38 (54.3%)	
Black	87/282 (30.9%)	49 (56.3%)	
White	111/282 (39.4%)	46 (41.4%)	
Mixed	6/282 (2.1%)	2 (33.3%)	
Other	8/282 (2.8%)	3 (37.5%)	
EMPLOYMENT FACTORS			
Care Home			0.100
Home A	135/327 (41.3%)	56 (41.5%)	
Home B	63/327 (19.3%)	31 (49.2%)	
Home C	77/327 (23.5%)	35 (45.5%)	
Home D	52/327 (15.9%)	32 (61.5%)	
Contract type			0.637
Permanent	261/311 (83.9%)	121 (46.4%)	
Temporary	50/311 (16.1%)	25 (50.0%)	
Work in another care home			0.390
FALSE	219/229 (95.6%)	101 (46.1%)	
TRUE	10/229 (4.4%)	6 (60.0%)	
TRAVEL FACTORS			
Car	80/327 (24.5%)	32 (40.0%)	0.144
Bus	145/327 (44.3%)	78 (53.8%)	0.030
Train	64/327 (19.6%)	33 (51.6%)	0.425
Underground	40/327 (12.2%)	17 (42.5%)	0.534
Bicycle	12/327 (3.7%)	5 (41.7%)	0.701
Walk	34/327 (10.4%)	16 (47.1%)	0.996
Other	44/327 (13.5%)	17 (38.6%)	0.227
HOUSEHOLD FACTORS			
Resides at place of work			0.075
FALSE	311/327 (95.1%)	143 (46.0%)	
TRUE	16/327 (4.9%)	11 (68.8%)	
Household member works in a care home			0.044
FALSE	265/310 (85.5%)	116 (43.8%)	
TRUE	45/310 (14.5%)	27 (60.0%)	
Household member works in hospital/GP surgery			0.144
FALSE	273/305 (89.5%)	131 (48.0%)	
TRUE	32/305 (10.5%)	11 (34.4%)	

two additional hospitalisations among staff members before the onset of this investigation.

In order to control the outbreak in the four care homes, weekly nasal swabbing was performed for all residents and staff for four weeks starting 3 May 2020 to rapidly identify and isolate infected individuals. The results of weekly swabbing and antibody testing in residents have already been reported.[2] The staff took their own nasal swab and completed a detailed questionnaire on employment, work patterns, household composition and travel pattern (**Supplement Figure S1**). In total, 443/596 staff were on shift when swabs were taken, and provided at least one swab and completed a questionnaire. None of the 1305 nasal swabs from 443 staff tested positive for SARS-CoV-2 RNA by RT-PCR.[1] After the four-week period, a blood sample was taken from consenting staff ($N = 327$) for SARS-CoV-2 anti-N IgG antibody testing, performed according to manufacturer instructions,[3] and 154 (47.1%) were seropositive (**Supplement Figure S2**). We explored risk factors for antibody positivity in staff including age, gender, co-morbidities, ethnicity, employment, household composition and travel to work using Pearson's Chi-squared test and included factors with $p<0.25$ in a multivariate logistic regression model (Table 1). All analyses were performed using the *tidyverse* (version 1.3.0) and *arsenal* (version 3.5.0) packages in *R* (version 4.0.2).

Chi-squared tests showed that age group ($p = 0.014$), ethnic group ($p = 0.185$), care home of employment ($p = 0.100$), travelling to work by car ($p = 0.144$), bus ($p = 0.030$) or other (0.227) method, residing at their place of work ($p = 0.075$), having a household member who works at a care home ($p = 0.044$) and having a household member who works at a hospital/GP surgery ($p = 0.144$) were predictors of positive antibody status at $p<0.25$ (Table 1). When these were reanalysed in a multivariate analysis, we found statistically significant associations with having a household member who works in a care home (OR 2.97, 95%CI 1.33–7.04), Asian ethnicity (OR 2.03, 95%CI 1.04–4.05) or having a household member who works in a hospital/GP surgery (OR 0.36, 95%CI 0.13–0.92), and weak evidence for 40–59 age group (OR 1.78, 95%CI 1.00 – 3.22), Black ethnicity (OR 1.79, 95%CI 0.94–3.43) or working at care home D (OR 2.17, 95%CI 0.98–4.88) (Table 2). The characteristics

Table 2
Multivariate analysis of significant risk factors for SARS-CoV-2 in univariate analysis (Table 1). Controlling for ethnicity and having a household member who works in a care home, travel to work by bus is not a significant predictor of seropositivity.

Risk factor	OR	95% CI – lower OR	95% CI – higher OR	p-value	Concordance
(Intercept)	0.364	0.163	0.792	0.012	0.694
Age category					
40–59	1.784	0.998	3.215	0.052	
60+	1.105	0.435	2.741	0.831	
Ethnic group					
Asian	**2.034**	**1.038**	**4.049**	**0.040**	
Black	1.788	0.941	3.425	0.077	
Mixed	0.807	0.101	4.781	0.820	
Other	0.917	0.150	5.211	0.921	
Care home of employment					
Home B	1.164	0.513	2.640	0.716	
Home C	1.186	0.581	2.428	0.639	
Home C	2.166	0.984	4.875	0.057	
Travel by car	0.797	0.375	1.679	0.552	
Travel by bus	1.154	0.615	2.163	0.654	
Travel by 'other'	0.890	0.376	2.089	0.790	
Resides at place of work	2.802	0.848	10.336	0.100	
Household member works in care home	**2.973**	**1.326**	**7.041**	**0.010**	
Household member works in hospital/GP surgery	**0.356**	**0.125**	**0.915**	**0.039**	

of staff members that had an antibody test result ($N = 327$) did not differ from staff who completed the risk factor questionnaire ($N = 443$) but did not provide a blood sample.

In the four care homes under investigation, nearly half the staff members had antibodies against SARS-CoV-2. Since no staff had a positive RT-PCR test result for SARS-CoV-2 during the 4 weeks prior to the antibody test, it is likely that the vast majority of infections occurred early in the course of the pandemic in London (March-April), at a time when there was widespread community infection and limited personal protective equipment (PPE) and SARS-CoV-2 testing available in care homes.[5] Both PPE and testing for SARS-CoV-2 are now available for care homes across the UK.

Our findings are consistent with other care home investigations reporting very high seropositivity rates among staff irrespective of their PCR-positivity or symptom development.[4] Seropositivity rates among care home staff are several fold higher than reported in any other occupational setting, including frontline hospital healthcare staff, highlighting the degree of virus exposure experienced by care home staff and residents.[2]

Antibody testing after four weeks of negative nasal swabs demonstrates its usefulness is assessing past exposure to the virus. It is increasingly clear that all nearly all adults who are exposed to SARS-CoV-2 have an detectable antibody response within 28 days of infection, and the majority of people who have detectable antibodies also have neutralizing antibodies,[6,7] as reported in staff and residents in other London care homes..[6] High rates of seropositivity may provide a degree of protective immunity in these settings, although the duration of such protection is at present unclear. Close monitoring of further cases and outbreaks will be important to help contribute to our understanding of protective immunity.

The negative weekly nasal swabs following the high infection rates prior to testing (as evidenced by the staff seropositivity rate) and small sample sizes meant that we were not able to identify additional risk factors for SARS-CoV-2 infection *per se*, such as working across different care homes as reported in other investigations.[4] The independent association of SARS-CoV-2 antibody positivity with Asian ethnicity, however, is consistent with other healthcare settings and the community.[8,9] This is compounded by having other members of the household – usually a spouse – also working in a care home, for which there was strong evidence of an increased risk in our cohort. The reduced risk associated with having a household member who works in a hospital/GP surgery likely reflects less exposure to potentially infected individuals (other staff or patients) when compared to the close contact with residents in care homes. Non-occupational risk factors such as travel to place of work, which have been identified as important risk factors for SARS-CoV-2 infection in other cohorts[8,9], were not significantly associated with seropositivity in this cohort.

In summary, we found SARS-CoV-2 seropositivity rates among staff working in care homes affected by COVID-19 outbreaks to be several times higher than community seroprevalence in London.[10] The high seropositivity rates precluded more detailed assessment of risk factors for SARS-CoV-2 infection, especially in relation to non-occupational risk factors. SARS-CoV-2 antibody positivity was, however, significantly, independently and positively associated with Asian ethnicity and having a household member who also worked in care homes. Further studies are needed to assess the level and duration of protection offered by the antibodies against SARS-CoV-2 re-infection and onward transmission in institutional settings.

Ethics: PHE has legal permission, provided by Regulation 3 of The Health Service (Control of Patient Information) Regulations 2002, to process patient confidential information for national surveillance of communicable diseases and as such, individual patient consent is not required.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.10.035.

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The presence of Pneumocystis jirovecii in critically ill patients with COVID-19

Dear Editor,

We read with interest the recent review on co-infections in coronavirus disease-2019 (COVID-19) patients[1] and believe that fungal co-infections as evaluated from selected studies are underestimated. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV2) is still spreading pandemically. Approximately 5–10% of COVID-19 patients may require intensive care unit (ICU) management and 30% may develop secondary pneumonia without identified etiology.[2] Hospital-acquired bacterial or fungal superinfections, as described in critically ill patients with *Influenza* virus, can be suspected.[3] Since pneumocystosis is usually reported in patients with T-cell immunodepression,[4] less attention has been paid to *Pneumocystis jirovecii* in non-immunocompromised ICU patients although it accounts for 7% of the co-infections reported in those admitted with Influenza.[5] Interestingly, COVID-19 patients may develop lymphocytopenia and acute respiratory distress syndrome (ARDS) requiring adjunctive steroids and/or immunomodulatory therapies, well-known susceptibility factors for developing pneumocystosis.[5] We designed this observational cohort study to investigate the prevalence of *P. jirovecii* acid nucleic detection in respiratory specimens sampled to identify co-infections in COVID-19 patients in the ICU.

All consecutive patients admitted to the ICU between 2020/03/15 and 2020/05/01 with a positive SARS-CoV-2 PCR (Cobas® SARS-CoV-2 Test, Roche, France) and ≥1 respiratory sample (bronchoalveolar lavage (BAL), tracheal aspirate, sputum) sent to the mycology department. This study was part of the COVID-ICU and French COVID-19 cohort registries. Our institutional ethics committee approved the study (IDRCB, 2020-A00256-33; CPP, 11-20-20.02.04.68737). When possible, signed informed consent was obtained from the patients or the next of kin.

Whenever possible, bronchoalveolar lavage (BAL) was performed in the middle lobe by a trained pneumologist in the ICU using at least 120 ml saline (yield, ∼50%). Upon reception, specimens including BAL fluids and dTT-treated aspirations (dTT 1X at 37 °C for 15 min) were centrifuged, suspended in 200µL of water and submitted to extraction (whole nucleic acids extraction) using the GeneLead-VIII extractor-thermocycler™ (Precision System Science, Japan). *P. jirovecii* reverse transcriptase quantitative PCR (RTqPCR) was performed to amplify mtSSU and mtLSU RNA and DNA of *P. jirovecii* using the new R-DiaPnJ kit™ (Diagenode, Belgium). Serum β-D-glucan was tested using the Fungitell kit™ (Cape Cod Inc, US) as recommended by the manufacturer. Data are presented as median [25th-75th percentiles] or percentages as appropriate. Comparisons were performed using Mann-Whitney or exact Fisher tests as required. *P*-values ≤0.05 were considered as significant.

One hundred-and-eight successive HIV-negative COVID-19 patients (Male/Female sex ratio, 4.4; age, 62 years [56–68]) with the usual risk factors for severe COVID-19 presentation were included (Table 1). All except three patients were intubated on admission. Thirty-four patients (31.4%) who developed ARDS received at least one day of corticosteroids before BAL sampling. Respiratory samples included 80 BALs (74.1%), 22 tracheal aspirates (20.4%), 4 sputa (3.7%) and two bronchial aspiration fluids (1.9%). In 10/108 patients (9.3%), *P. jirovecii* RTqPCR was positive. Median delay between sampling and ICU admission was 2days [1–2]. The median quantitative cycle value was 32.6 [30.8–34.7]. Serum β-D-glucan were measured in nine patients and was negative (>80pg/mL) in seven patients.

Clinical characteristics of the patients carrying *P. jirovecii* did not significantly differ from the other patients except for lower

Table 1
Characteristics of hundred-and-eight critically ill COVID-19 patients according to *Pneumocystis jirovecii* detection in the respiratory samples. Data are presented as percentages or medians [25th-75th percentiles]. Comparisons were performed using Mann-Whitney (○) or Fisher exact tests (*), as appropriate.

	Total ($N=108$)	No detection of *P. jirovecii* ($N=98$)	Detection of *P. jirovecii* ($N=10$)	*P*
Male gender, N (%)	88 (81.5%)	80 (81.6%)	8 (80.0%)	1*
Age (years)	62 [56–68]	62 [56–68]	59 [46–68]	0.40○
COVID-19 risk factors				
Past hypertension, N (%)	64 (59.3%)	58 (59.2%)	6 (60.0%)	1*
Diabetes, N (%)	40 (37.0%)	37 (37.8%)	3 (30.0%)	0.74*
Obesity, N (%)	35 (32.4%)	32 (32.7%)	3 (30.0%)	1*
Coronary disease, N (%)	15 (13.9%)	14 (14.3%)	1 (10.0%)	1*
Body-mass index (kg/m^2)	28 [25–31]	28 [25–31]	28 [27–32]	0.61○
Other remarkable comorbidities				
Asthma, N (%)	5 (4.6%)	4 (4.1%)	1 (10.0%)	0.39*
Chronic obstructive pulmonary disease, N (%)	2 (1.9%)	2 (2.0%)	0 (0.0%)	1*
Immunocompromised patient, N (%)	10 (9.3%)	10 (10.2%)	0 (0.0%)	0.59*
Long-term corticosteroids, N (%)	11 (10.2%)	8 (8.2%)	3 (30.0%)	0.06*
Biological data of interest on admission				
PaO_2/FiO_2 (mmHg)	137 [83–247]	134 [83–239]	177 [108–253]	0.60○
Serum creatinine (µmol/L)	80 [64–111]	80 [63–111]	80 [67–104]	0.99○
Plasma d-dimer (ng/mL)	2,2395 [1193–4635]	2610 [1405- 4700]	1270 [750–2390]	0.03○
Serum lactate dehydrogenase (IU/L)	687 [540–901]	687 [568–903]	708 [436–893]	0.65○
Bronchoalveolar lavage characteristics				
% BAL macrophages	28 [15–46]	27 [14–42]	51 [49–55]	0.13○
% BAL polymorphonuclear cells	37 [26–81]	46 [26–81]	29 [18–32]	0.54○
% BAL Lymphocytes	13 [6–32]	14 [5–34]	13 [10–23]	0.81○
Specific anti-COVID-19 therapy, N (%)				
Azithromycin, N (%)	34 (31.5%)	30 (30.6%)	4 (40.0%)	0.72*
Hydroxychloroquine, N (%)	34 (31.5%)	30 (30.6%)	4 (40.0%)	0.72*
Hydroxychloroquine + Azithromycin, N (%)	29 (26.9%)	26 (26.5%)	3 (30.0%)	1*
Lopinavir-ritonavir, N (%)	16 (14.8%)	12 (12.2%)	4 (40.0%)	0.04*
Polyvalent immunoglobulins, N (%)	3 (2.8%)	3 (3.1%)	0 (0.0%)	1*
Sarilumab, N (%)	1 (0.9%)	1 (1.0%)	0 (0.0%)	1*
Eculizumab, N (%)	6 (5.6%)	4 (4.1%)	2 (20.0%)	0.10*
Tocilizumab, N (%)	4 (3.7%)	4 (4.1%)	0 (0.0%)	1*
Dexamethasone, N (%)	53 (49.1%)	46 (46.9%)	7 (70.0%)	0.19*
Dexamethasone cumulative dose >100 mg, N (%)	16 (14.8%)	15 (15.3%)	1 (10.0%)	1*
Severity during hospitalization and outcome				
SAPS II on admission	37 [31–49]	38 [31–51]	34 [28–37]	0.16○
SOFA on admission	6 [3–8]	6 [3–8]	5 [2–7]	0.43○
Lowest PaO_2/FiO_2 (mmHg)	71 [58–89]	71 [59–89]	65 [53–102]	0.79○
Vasopressors, N (%)	89 (82.4%)	81 (82.7%)	8 (80.0%)	1*
Renal replacement therapy, N (%)	38 (35.2%)	35 (35.7%)	3 (30.0%)	1*
ECMO, N (%)	10 (9.3%)	9 (9.2%)	1 (10.0%)	1*
SAPS II on admission	37 [31–49]	38 [31–51]	34 [28–37]	0.16○
ICU length of stay (days)	20 [12–32]	20 [12–33]	10 [6–19]	0.03○
Mortality, N (%)	47 (43.5%)	44 (44.9%)	3 (30.0%)	0.51 *

BAL, bronchoalveolar lavage; ECMO, extracorporeal membrane oxygenation; ICU, intensive care unit; SOFA, Sepsis-related Organ Failure Assessment; SAPS II, Simplified Acute Physiology Score II.

plasma D-dimer (1270 ng/mL [750–2390] vs 2610 ng/mL [1405–4700], $P=0.03$) and more frequent lopinavir/ritonavir administration (40.0% vs 12.2%, $P=0.04$), while long-term corticosteroid prescription tended to be more frequent (30.0% vs 8.2%, $P=0.06$). Of note, among our 10 *P jirovecii* carriers, five concomitantly met the criteria for COVID-19-associated pulmonary aspergillosis.[6] Out of these patients, four (40%) received co-trimoxazole as prophylaxis (80/400 mg once daily) whereas six including four who rapidly improved did not. One co-trimoxazole-treated and two non-treated patients died while the seven remaining patients were discharged. Mortality was similar in both groups.

We found an unexpectedly high proportion of critically ill COVID-19 patients detected with *P. jirovecii* (10/108 patients; 9.3%), similarly to previous findings in influenza patients (3/45; ~7%).[5]

The presence of *P. jirovecii* in the healthy adult population has been measured using oropharyngeal wash samples obtained by gargling and examined by conventional or nested PCR methods.[7] However, experts agree that the reported prevalence (~20%) has been overestimated due to technical issues such as contamination with amplicons responsible for false positives.[4] In our center managing almost exclusively immunocompromised patients, prevalence of qPCR-positive respiratory specimens with fungal load as low as in our COVID-19 patients, is ~13% (unpublished data), as reported elsewhere.[3] COVID-19 patients mostly exhibited marked lymphopenia and alterations in lymphocyte functions,[8] likely explaining the high-rate of *P. jirovecii* detection.

Since serum β-D-glucan is advocated in pneumocystosis diagnosis,[4] we measured its concentrations in four of our five *P. jirovecii* RTqPCR-positive patients and obtained low values (<120pg/mL) in accordance with the low nucleic acids fungal loads in the lung alveoli.[9] Of note, in two out of our nine tested *P. jirovecii* RTqPCR-negative patients, higher B-D-glucan concentrations (450 and 500pg/ml) lead to the diagnosis of pulmonary aspergillosis, another fungal infection of risk in COVID-19 patients.[6] Although a recent meta-analysis questioned its sensitivity in non-HIV patients,[10] β-D-glucan has been widely used to rule out pneumocystosis because of its high negative predictive value. This finding may support the hypothesis that our patients were carrying *P. jirovecii,* yet not being infected *per se*. Thus, although interesting in the context of invasive fungal infections diagnosis, serum β-D-glucan should be interpreted with caution when excluding the diagnosis of pneumocystosis.

Here, four out of ten *P. jirovecii* RTqPCR-positive patients received co-trimoxazole as prophylactic regimen, based on the treat-

ing physician's decision. Whether a positive result should be an indication to consider administering co-trimoxazole, at least at prophylactic dosage in COVID-19 patients remains questionable.

Our study limitations include the relatively small number of patients, the bi-center setting, and the short study period. However, to the best of our knowledge, this is the first study evaluating the prevalence of *P. jirovecii* in COVID-19 patients. Because we focused on critically ill COVID-19 patients, *P. jirovecii* prevalence in less severe patients remains to be determined.

In conclusion, an unexpectedly high proportion of *P. jirovecii*-positive pulmonary samples is observed in critically ill COVID-19 patients. Based on our findings, we advocate systematically searching for *P. jirovecii* in deep respiratory specimens in these patients. We believe that this strategy may be useful in limiting enhanced inflammation due to the presence of *P. jirovecii* in the lung and avoiding inter-patient *P. jirovecii* transmission.

Ethics approval and consent to participate

This study was part of the French COVID-19 cohort registry conducted by the REACTing consortium (REsearch and ACTion targeting emerging infectious diseases) and directed by INSERM (Institut national de la santé et de la recherche médicale) and ISARIC (International Severe Acute Respiratory and Emerging Infection Consortium). Our institutional ethics committee approved the study (N, IDRCB, 2020-A00256-33; CPP, 11-20-20.02.04.68737).

Availability of data and materials

Drs. Alanio, Mégarbane and Bretagne conceived of and designed the study. Drs. Voicu, and Mégarbane managed the patients. Drs. Alanio, Dellière and Bretagne performed the microbiological analysis. All authors acquired, analyzed the data and interpreted the results. Drs. Alanio, Mégarbane and Bretagne drafted the manuscript. All authors participated to the critical revision of the manuscript for important intellectual content. Dr. Alanio has full access to all data and takes responsibility for the data integrity and its analysis accuracy.

Consent for publication

All the authors agree to publish.

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Declaration of Competing Interest

The authors declare that they have no competing interests.

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First and second COVID-19 waves in Japan: A comparison of disease severity and characteristics

Dear Editor,

We read with interest the recent article published in your journal detailing the severity of coronavirus disease 2019 (COVID-19) in China during the first wave of the pandemic[1]. COVID-19 has become a global pandemic, occurring in forming several peaks in waves[2,3]. This study compared the severity and characteristics of the first and second waves in Japan.

We obtained the study data from the COVID-19 Registry Japan (COVIREGI-JP). The COVIREGI-JP includes data from a observational cohort study using medical records in Japan. The criteria for enrol-

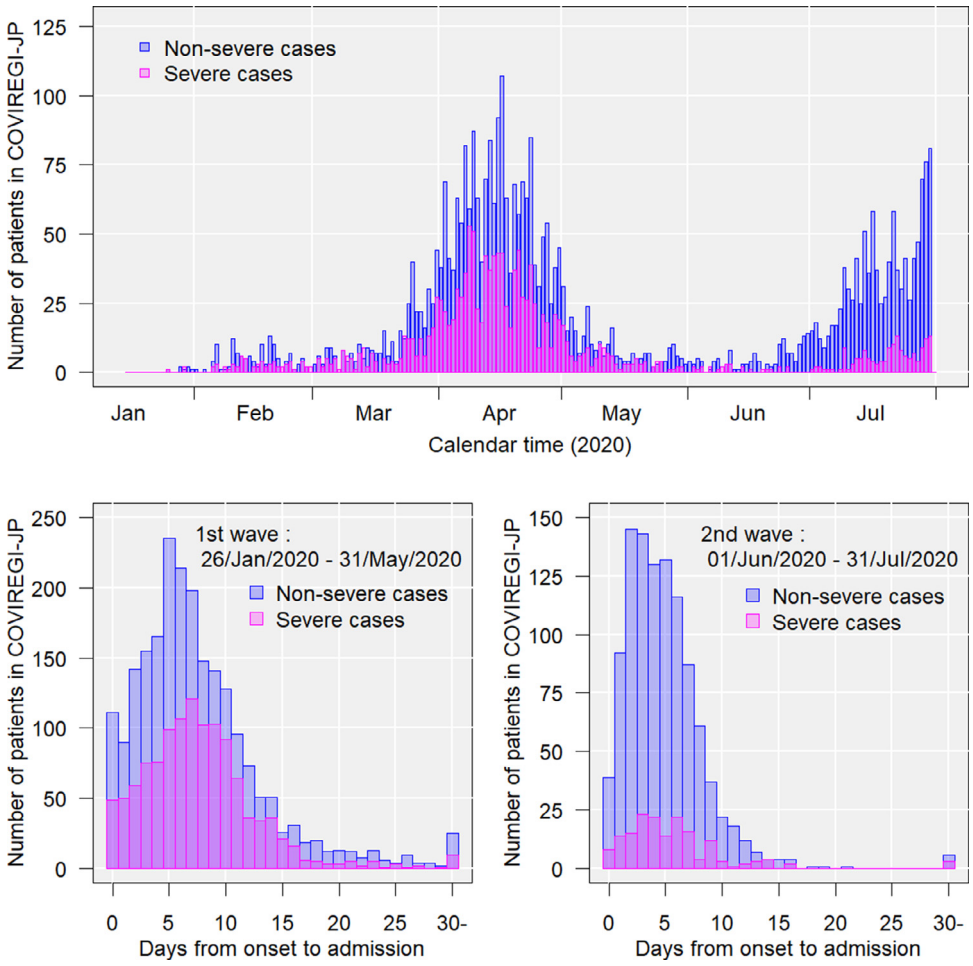

Fig. 1. Epidemic curve, severity, and days from onset to admission in COVID-19 patients in Japan
(a) Epidemic curve and severity from 26 January to 31 July 2020, as recorded in the COVID-19 Registry Japan.
(b) Number of days from onset to admission from 26 January to 31 May (first wave)
(c) Number of days from onset to admission from 1 June to 31 July (second wave)
Abbreviations: COVIREGI-JP, COVID-19 Registry Japan; COVID-19, coronavirus disease.

ment were (1) tests such as polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP) that turned positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and (2) inpatient treatment at a health care facility. We evaluated age, sex, comorbidities, disease severity at admission, supportive care, medications, and the outcome on discharge. A patient's condition was denoted as "severe" on fulfilment of one or more of the following criteria: the need for invasive or non-invasive mechanical ventilation, need for supplemental oxygen, an oxygen saturation (SpO_2) of $<$ 94% at room air, and tachypnoea (respiratory rate of $>$24 breaths per minute). Patients who did not meet these criteria were classified as "non-severe" at admission. Patients admitted between 26 January and 31 May 2020, were included in the first wave, and those admitted between 1 June and 31 July 2020, were included in the second wave[4] (frozen data as of 2 September 2020). Continuous variables were expressed as medians and interquartile ranges, and categorical variables were expressed as numbers (%). All statistical analyses were conducted using R version 4.0.2 (R core Team).

Data of 5194 cases from 327 facilities were included in the analysis: 3833 and 1361 cases from the first and second waves, respectively. At admission, the second wave had a smaller proportion of severe cases (12.0% vs 33.1%, Fig. 1a); the duration from onset to admission was also shorter (median, 4 vs 7 days) than that in patients in the first wave (Fig. 1b, 1c). Patients in the second wave tended to be younger (median age, 37 vs 56 years), were less frequently transferred from other hospitals (3.8% vs 15.0%) and were less likely to have comorbidities such as cardiovascular diseases (1.9% vs 5.9%), and cerebrovascular disease (1.8% vs 6.1%). Mortality (1.2% vs 7.3%) in hospitalized or discharged patients was also lower in the second wave; the same trend was observed on stratification according to age and severity at admission (Table 1).

Our study showed that the proportion of cases involving severe disease at admission was smaller in the second wave. Considering the lower percentage of patients transferred from other hospitals in the second wave, it is likely that the first wave had a more critical effect on the ability of healthcare institutions to receive patients. Moreover, the number of PCR tests performed was greater in the second wave than in the first wave [5]. Earlier admission of patients in the second wave may reflect the increase in the number of PCR tests performed and the number of beds available to COVID-19 patients. Data from the second wave indicated a demographic shift toward a younger population with fewer comorbidities, a lower proportion of severe patients at admission, and decreased mortality. However, the mortality was lower in second wave even if stratifying age and severity at admission. This may be because of the shorter time between disease onset and admission, differences in patient background, comorbidities, and advances in treatment methods.

Although this registry gathers information on a large number of patients, it does not cover all patients in Japan, and data from the second half of the second wave was not included in this study;

Table 1
Demographics, characteristics, comorbidities, treatments, and outcomes of the patients.

			1st wave: From 26 January to 31 May			2nd wave: From 1 June to 31 July		
		All	All	Non-severe	Severe	All	Non-severe	Severe
No. (%)[a]		5194	3833 (73.8)	2563 (66.9)	1270 (33.1)	1361 (26.2)	1198 (88.0)	163 (12.0)
Demographics								
Sex (Male)		3068 (59.2)	2272 (59.5)	1398 (54.8)	874 (68.9)	796 (58.6)	687 (57.5)	109 (66.9)
Age, y	Median [IQR]	52 [34, 68]	56 [40, 71]	51 [35, 66]	67 [53, 79]	37 [25, 53]	35 [25, 50]	57.5 [39.25, 72.75]
	0–64	3609 (69.8)	2447 (64.0)	1871 (73.2)	576 (45.4)	1162 (86.1)	1066 (89.7)	96 (59.3)
	≥65	1565 (30.2)	1377 (36.0)	685 (26.8)	692 (54.6)	188 (13.9)	122 (10.3)	66 (40.7)
Ethnicity (Japanese)	4928 (95.2)	3634 (95.1)	2412 (94.4)	1222 (96.4)	1294 (95.3)	1140 (95.4)	154 (94.5)	
Transfer from other hospitals	624 (12.1)	573 (15.0)	218 (8.6)	355 (28.0)	51 (3.8)	29 (2.5)	22 (13.5)	
Days from onset to admission	Median [IQR]	6 [3, 9]	7 [4, 10]	6 [4, 10]	7 [4, 10]	4 [2, 7]	4 [2, 6.25]	5 [3, 7]
Comorbidity								
Cardiovascular diseases[b]	251 (4.8)	225 (5.9)	87 (3.4)	138 (10.9)	26 (1.9)	16 (1.3)	10 (6.1)	
Cerebrovascular disease	257 (4.9)	232 (6.1)	105 (4.1)	127 (10.0)	25 (1.8)	15 (1.3)	10 (6.1)	
Chronic respiratory diseases[c]	184 (3.5)	155 (4.0)	45 (1.8)	110 (8.7)	29 (2.1)	13 (1.1)	16 (9.8)	
Severe renal diseases or dialysis	65 (1.3)	56 (1.5)	29 (1.1)	27 (2.1)	9 (0.7)	7 (0.6)	2 (1.2)	
Diabetes		738 (14.2)	630 (16.4)	312 (12.2)	318 (25.0)	108 (7.9)	71 (5.9)	37 (22.7)
Obesity[d]		249 (4.8)	192 (5.0)	107 (4.2)	85 (6.7)	57 (4.2)	41 (3.4)	16 (9.8)
Solid tumour		193 (3.7)	173 (4.5)	88 (3.4)	85 (6.7)	20 (1.5)	16 (1.3)	4 (2.5)
Immunosuppression[e]	131 (2.6)	122 (3.2)	74 (2.9)	48 (3.9)	9 (0.7)	8 (0.7)	1 (0.6)	
Supportive care[f]								
Oxygen therapy		1664 (32.1)	1487 (38.8)	452 (17.7)	1035 (81.6)	177 (13.0)	89 (7.4)	88 (54.0)
Invasive mechanical ventilation / ECMO	389 (7.5)	371 (9.7)	47 (1.8)	324 (25.6)	18 (1.3)	5 (0.4)	13 (8.0)	
Medication[f]								
Favipiravir		1806 (68.3)	1542 (70.0)	781 (64.0)	761 (77.5)	264 (59.7)	192 (55.8)	72 (73.5)
Steroid (excluding Ciclesonide)	468 (9.1)	374 (9.9)	99 (3.9)	275 (21.8)	94 (6.9)	51 (4.3)	43 (26.4)	
Anticoagulant		420 (8.1)	363 (9.5)	92 (3.6)	271 (21.3)	57 (4.2)	28 (2.3)	29 (17.8)
Remdesivir		40 (1.5)	11 (0.5)	2 (0.2)	9 (0.9)	29 (6.6)	14 (4.1)	15 (15.5)
Ciclesonide		1373 (52.1)	1142 (52.1)	663 (54.8)	479 (48.8)	231 (52.1)	192 (55.7)	39 (39.8)
Nafamostat		224 (9.8)	189 (10.3)	78 (8.0)	111 (13.0)	35 (7.9)	23 (6.7)	12 (12.2)
Outcome								
Death		295 (5.7)	279 (7.3)	63 (2.5)	216 (17.0)	16 (1.2)	4 (0.3)	12 (7.4)
	Age: 0–9	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Age: 10–19	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Age: 20–29	1 (0.1)	1 (0.3)	0 (0)	1 (2.9)	0 (0)	0 (0)	0 (0)
	Age: 30–39	1 (0.1)	1 (0.2)	1 (0.3)	0 (0)	0 (0)	0 (0)	0 (0)
	Age: 40–49	4 (0.5)	4 (0.7)	1 (0.2)	3 (2.5)	0 (0)	0 (0)	0 (0)
	Age: 50–59	10 (1.1)	10 (1.4)	1 (0.2)	9 (4.1)	0 (0)	0 (0)	0 (0)
	Age: 60–69	47 (6.8)	46 (7.7)	10 (2.9)	36 (14.1)	1 (1.1)	0 (0)	1 (4.5)
	Age: 70–79	78 (12.1)	75 (13.3)	18 (6.2)	57 (20.6)	3 (3.7)	1 (2.0)	2 (6.2)
	Age: ≥80	154 (27.1)	142 (28.0)	32 (14.1)	110 (39.1)	12 (20.0)	3 (8.3)	9 (37.5)
Selfcare ability (Worsened)[g]	398 (8.2)	361 (10.2)	123 (4.9)	238 (22.8)	37 (2.8)	14 (1.2)	23 (15.3)	
Walking ability (Worsened)[g]	384 (9.0)	344 (11.8)	110 (5.4)	234 (26.0)	40 (3.0)	17 (1.4)	23 (15.3)	
Transfer to long-term care facility[g]	84 (1.6)	73 (1.9)	39 (1.5)	34 (2.7)	11 (0.8)	6 (0.5)	5 (3.1)	

[a] Since the number of missing values varies for each parameter, the number of cases in the severity category for each parameter was used as the denominator to calculate the percentages.
[b] Cardiovascular diseases include myocardial infarction, congestive heart failure, and peripheral vascular disease.
[c] Chronic respiratory diseases include chronic obstructive pulmonary disease and chronic lung diseases, which were defined as pulmonary diseases resulting in dyspnoea upon slight activity.
[d] Obesity was based on physician diagnosis.
[e] Immunosuppression includes neutropenia (< 500 neutrophils/μL), use of glucocorticoids/steroids within 1 month (doses greater or equal to an equivalent of 20 mg of prednisone per day for at least 1 month), chemotherapy or radiation therapy or the use of immunosuppressants (such as antitumor necrosis factor-α therapy, anti-IL-6 receptor/anti-CD20 monoclonal antibodies, selective T-cell co-stimulation blockers, methotrexate, tacrolimus) within the past 3 months, post hematopoietic stem cell transplantation, post organ transplantation, asplenia, and primary immunodeficiency syndrome or HIV infection.
[f] Patients who received these treatments at least once during their hospitalization were included.
[g] Data were counted only for patients who were alive at discharge.Abbreviations: COVID-19, coronavirus disease; ECMO, extracorporeal membrane oxygenation; IQR, interquartile range.

this may be a source of bias in this study. In addition, since data are updated daily, there is a possibility that future findings will differ from the current results.

The findings of our study indicated that in the first wave, the medical system was under greater strain with more severe cases on admission. In the second wave, patients were younger with fewer underlying diseases and lower mortality rates.

Declaration of Competing Interest

H.O. reports personal fees as a statistician and as an external consultant for clinical trials from EPS International, outside the submitted work. All other authors report no potential conflicts.

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Rates of recurrent positive SARS-CoV-2 swab results among patients attending primary care in Qatar

Dear Editor,

We read with interest the work of COCOREC (Collaborative study COvid RECurrences) study group.[1] Recurrent positive rt-PCR results for SARS-CoV-2 infection were reported from early in the epidemic.[1–8] Viral genomic sequences provided concrete evidence for reinfection by distinct SARS-CoV-2 infection.[9–12] The number of days in between both infections in viral genomic proven reports ranged from 48 to 142 days.

While viral genomic sequencing provides robust evidence, it does not lend itself well to everyday practice. The COCOREC study identified 11 cases of reinfection using well-defined criteria.[1] The group suggested recurrent positive rt-PCR results of more than 21 days following the resolution of symptoms as criteria for reinfection. The criteria though less specific, are more feasible to use in primary health care settings.

Utilising the criteria set by the COCOREC study group, this record-based study reports on the cases with recurrent positive RT-PCR nasopharyngeal swab for SARS-CoV-2 results in primary health care corporation (PHCC) settings in Qatar. PHCC is the largest primary care provider in Qatar with 27 health centers covering all the country. The organisational employs an electronic medical record (EMR), which links all public primary health care centers. For this study, all electronic data were extracted from the primary healthcare setting visits, and no sampling was needed.

Table 1
Characteristics of the population with recurrent positive results.

	Overall ($N = 62$)
Number of days between positive results	
Mean (SD)	29.2 (11.6)
Range	21.0 - 84.0
Age	
Mean (SD)	37.3 (12.2)
Range	11.0 - 74.0
Gender	
Female	13 (21.0%)
Male	49 (79.0%)
HTN	
No	54 (87.1%)
Yes	8 (12.9%)
DM	
No	50 (80.6%)
Yes	12 (19.4%)
Asthma	
No	55 (88.7%)
Yes	7 (11.3%)
COPD	
No	62 (100.0%)
CVD	
No	62 (100.0%)
CKD	
No	62 (100.0%)
Dyslipidemia	
No	51 (82.3%)
Yes	11 (17.7%)
Smoking Status	
N-Miss	22
Never Smoker	29 (72.5%)
Former Smoker	3 (7.5%)
Current Smoker	8 (20.0%)
Pregnancy	
N-Miss	49
Not Pregnant	11 (84.6%)
Pregnant	2 (15.4%)

Table 2

Number of Days between recurrent positive	Counts	% of recurrent positive (62)	% of the total swabs (63,444)
21–30 Days	41	66.1 %	0.07%
30–42 Days	14	22.6 %	0.02%
>42 Days	7	11.3 %	0.01%

The study population included patients attending with documented SARS-CoV-2 rt-PCR results during the study period. The study period was from February 10th, 2020, to July 30th, 2020, a total of 171 days. The recurrent positive population included all patients with a minimum number of 2 positive swabs and a minimum number of 21 days in between positive swab results. Inconclusive and reactive rt-PCR results were considered negative. During the study period, patients were entitled to a repeat swab if they are attending with new symptoms following the resolution of initial symptoms. A maximum number of days in between any positive swab results was calculated for those who met our definition criteria.

The study aims to answer the following questions. What is the maximum number of days in between positive swab results? What are the rates of recurrent rt-PCR SARS-CoV-2 positive results of more than 21 days, and what are the population characteristics?

Overview

During the study period, we retrieved a total of 63,444 patient records with 76,742 swab results. Only 62 patients met our inclusion criteria (62/63,444;0.1%).

The population was predominantly young. The mean age is 37.3 ± 12.2. (Median: 35 [Min: 11-Max: 74]). Male patients were more represented in the sample (49/62;70.6%). 2 female patients were pregnant (2/13; 15%). The percentage of current smokers was high (8/40;20%) (Table 1).

The maximum number of days and rates of recurrent positives among the study population

84 days was the maximum number of days for recurrent positive and the mean of the maximum number of days between recurrent positive results is 29.2 ± 11.6. (Median: 25 [Min: 21-Max: 84]).

The rates for recurrent positive results are reported for the total recurrent positive (62) and the total study population, (63,444). The recurrent positive results of more than 42 days were rare (7/63,444,0.01%) (table 2).

Summary

Recurrent positive findings could occur in all age groups and different population types, including paediatric, elderly, and pregnant patients. Current smoking status was highly prevalent among patients with recurrent positive results.

No previous studies reported to the rates of recurrent positive rt-PCR for SARS-CoV-2 infections. Given the extensive reporting of the SARS-CoV-2 infections, the number of case reports of recurrent positive and reinfection to date is extremely low, which agrees with our findings. Earlier studies reported that viral shedding is dynamic and continue in most cases 20–22 days but positive results were generally rare beyond 30 days.[13] So, one could theorise that recurrent positive results in symptomatic patients should be considered reinfection, especially if more than 42 days.

The rare occurrences of recurrent infections are reassuring to the world given the current surge and in favour of immunity. However, it does not allude to the length of that immunity. Given the rarity of recurrent positive results which is supported by our findings, vaccination should be recommended for patients with no earlier SARS-CoV-2 infection.

Strengths and limitations

The study utilised centralised database records that allowed for large sample size, 63,444 and long study period of 6 months. However, the record-based study does not report on the severity or the resolution of symptoms or the patients' outcomes.

Informed consent

Data request and analysis were anonymous, and no patient consent was required.

Ethics statement

Anonymous data request approved by the department of clinical research, primary health care corporation with reference number PHCC/DCR/2020/04/031.

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Pentraxin 3: Potential prognostic role in SARS-CoV-2 patients admitted to the emergency department

Coronavirus disease 2019 (COVID-19) was caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and spread all over the world.[1] Patterns of hematologic, biochemical, inflammatory, and immune biomarker abnormalities have been identified in patients with severe disease compared to mild systemic disease and differently combined in risk stratification models. Current clinical practice suggests determining IL-6, D-dimer, lactate dehydrogenase (LDH), and transaminases in addition to routine laboratory tests, in order to identify patients at risk of fatal complications.[2] However, several biomarkers have been investigated as a possible role for prognosis outcome, like Presepsin (PSP) that in a recent analysis has emerged as a potential prognostic marker in SARS-CoV-2 patients.[3] We read with interest a recent manuscript by Hansen C et al.[4] that evaluated the role of complement related pattern recognition molecules, including C-reactive protein (CRP) and Pentraxin 3 (PTX3), as markers of short-term mortality in intensive care patients. PTX3 and CRP are the well-known, prototypic short and long pentraxin, respectively, differing for gene organization, protein oligomerization and expression pattern. CRP is produced by the liver, whereas PTX3 is an inflammatory mediator produced by various cells in peripheral tissues.[5] CRP is a typical acute phase biomarker since it is produced as a result of systemic inflammatory responses. Conversely, PTX3 defines early and local acute phases, being rapidly produced and released by mononuclear phagocytes, neutrophils, fibroblasts, and epithelial and endothelial cells in response to primary inflammatory signals (e.g. IL-1 and TNF-α).[6] In patients with community-acquired pneumonia (CAP), the plasma concentration of PTX3, but not CRP, was correlated with the severity of CAP based on the pneumonia severity index (PSI), CURB-65, Acute Physiology and Chronic Health Evaluation (APACHE) II scores, and the length of hospital stay.[7] In order to evaluate the potential prognostic value of PTX3 and its correlation with the severity of SARS-CoV-2, measurement of PTX3 in serum samples of patients ($n = 75$, male/female 47/28, age 69 years (median) 59–75 years (IQR)) with COVID-19 microbiology proven infection (from March to May 2020) was carried out using an enzyme-linked immunosorbent assay (ELISA) (DSX, Technogenetics srl, Milano, Italy), in addition to routine laboratory tests performed at admission. Forty patients were admitted in the intensive care unit (ICU), 35 patients in infectious disease division or in pneumology division (nICU). According to the severity and the evolution of the disease, 37 ICU patients died and 3 were moved to nICU divisions for improved clinical conditions. In our cohort, routine laboratory tests showed an increase of CRP (Dimension Vista, Siemens Healthcare Diagnostics Inc, Tarrytown USA), D-dimer (CS 5100, Siemens Healthcare Diagnostics Inc), PSP (Pathfast, Chemical Medience Corporation, Tokyo, Japan), Procalcitonin (PCT) and

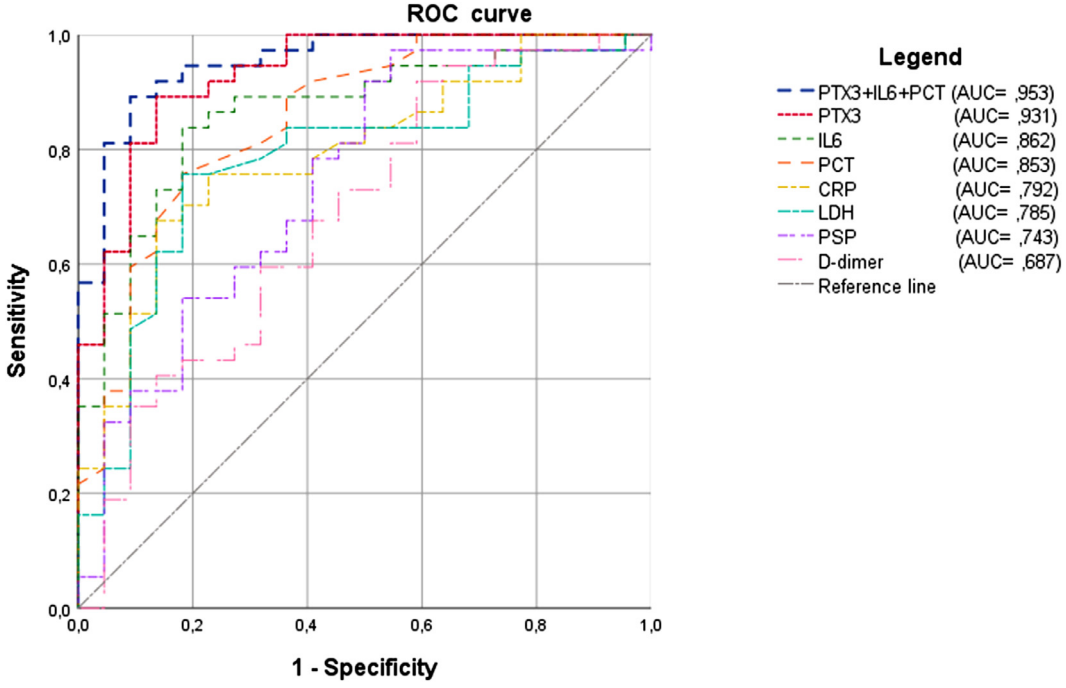

Fig. 1. ROC curve for serum level of PTX3, IL-6, PCT, LDH, PSP, D-dimer, PCT, and combination of PTX3 with IL-6 and PCT, detected at admission in relation to mortality in SARS-CoV-2 patients. (AUC: area under curve).

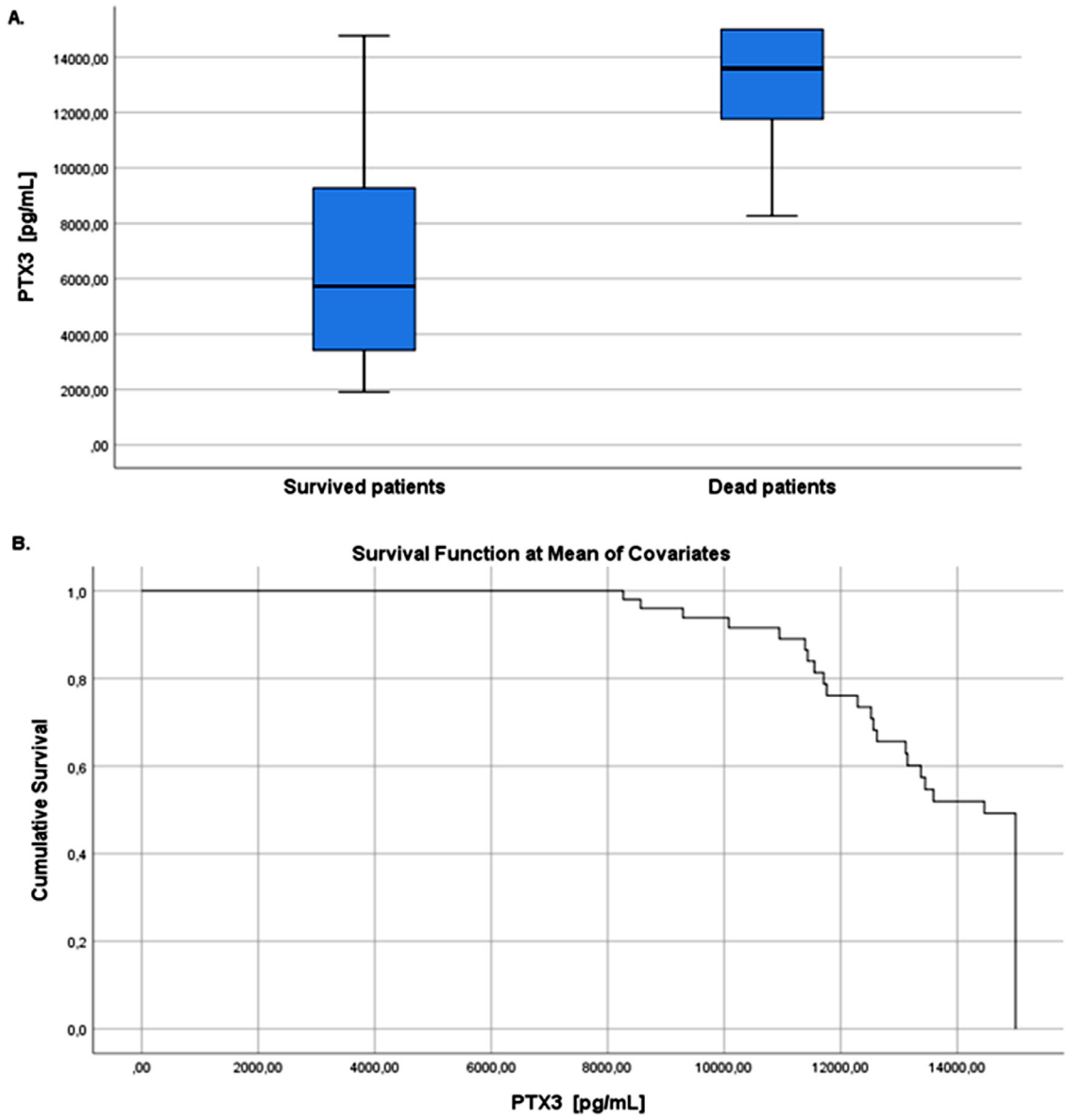

Fig. 2. Relationship between pentraxin 3 values and mortality (A), and survival analysis based on PTX3 values (B).

Interleukin-6 (IL-6) (Cobas 8000 system, Roche Diagnostics, GmbH, Mannheim, Germany), in line with other studies.[8,9] In particular 85% of patients showed CRP >10 mg/L, 73% D-dimer >500 ug/L, 66% LDH >241 U/L, 88% PSP >250 pg/mL, 26% PCT >0.5 ng/ml, and 82% IL-6 >7 pg/mL. PTX3 was measured at the admission of patients in emergency covid room and values higher than the cut-off suggested by the manufacturer (2000 pg/mL) were observed in patients who died (median; IQR =13,589; 11,734–15,000) as well as in patients who survived (median; IQR =5729; 3362–9470). According to ROC curve analysis of all biomarkers considered in our study, the AUC of PTX3 values in predicting the mortality was 0.93 (95% CI: 0.86–0.99) reaching a sensitivity of 89% and a specificity of 92% at the threshold level of 10,792 (Fig. 1). The AUC resulting from the combination of PTX3, IL-6 and PCT was significantly higher than that of PTX3 alone (0.95, 95% CI: 0.90–1.0). Fig. 2 shows the median values of PTX3 between patients who died and those who survived ($p < 0.001$). Moreover, PTX3 correlated (Spearman test) with some inflammation biochemical parameters commonly evaluated in SARS-CoV-2 patients, in particular with IL-6 ($r = 0.69$, $p < 0.001$), PCT ($r = 0.52$, $p < 0.001$), PSP ($r = 0.52$, $p < 0.001$), LDH ($r = 0.62$, $p < 0.001$), CRP ($r = 0.59$, $p < 0.001$), and D-dimer ($r = 0.43$, $p < 0.001$). Furthermore, a multivariate logistic regression analysis, using all considered variables, confirmed the independent prognostic role of PTX3 with an OR = 1.001 (95% CI: 1.000–1.001, $p = 0.005$). Taken together, data obtained from our preliminary study suggest a potential prognostic role of PTX3 in SARS-CoV-2 patients, with higher levels associated with poor outcome. Moreover, we observed that the combination of PTX3 with IL-6 and PCT associated with COVID-19 disease progression improves the accuracy of prognosis prediction. As such, PTX3, peaking within 6 to 8 hours of the inflammatory stimulus, might have important implications for the clinical management of patients with COVID-19 allowing to identify, at admission, the patients headed for adverse outcomes. Our study presents some limitations, namely the limited number of patients. Then, PTX3 concentrations should be assessed during the hospitalization period to better estimate the prognostic role of this biomarker. If further studies will confirm our preliminary findings, the manufacturer could be encouraged to improve the current diagnostic method in order to reduce the analytic turnaround time (TAT) according to clinical needs. Interest-

ingly, PTX3 is not only present in blood samples but can also be found in other biofluids, including pleural fluid.[10] It is hence possible to hypothesize that PTX3 concentration measurement in bronchoalveolar lavage fluid correlates with disease severity in SARS-CoV-2 patients presenting frequent pulmonary complications such as acute lung injury.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Ethical approval

The study has been cleared by the local Ethical Committee (AOU Policlinico Consorziale of Bari; No. 6388 COVID19 DOM - protocol number 0034687/12-05-2020).

Declaration of Competing Interest

Authors state no conflict of interest.

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