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

Short durations of corticosteroids for hospitalised COVID-19 patients are associated with a high readmission rate

Dear Editor,

Introduction

Outcomes for patients with COVID-19 infection have been widely reported for the initial peak of the pandemic.[1] However, there is a lack of data describing outcomes and characteristics of readmitted patients in the resurgent peak, after corticosteroids became standard of care.

Based on data and protocols from randomised controlled trials, most international treatment guidelines recommend 6 mg dexamethasone daily (or equivalent) for up to 10 days in those hospitalised with severe COVID-19 but stopping on discharge.[2–6]

The UK's second COVID-19 wave peaked on 9/1/2021.[7] Here we describe the characteristics of patients admitted, discharged and readmitted, due to COVID-19, to our hospital, during this second wave. We explored the relationship between clinical and biochemical variables, treatment received during a patient's first admission, and readmission risk, in relation to corticosteroid use.

Methods

We reviewed patients admitted from the community to University College Hospital (UCH) with COVID-19 as their primary diagnosis between 1st-31st December 2020. Re-attendance and readmission data were collected for patients who re-presented within 10 days following discharge from their first admission.

Data were retrospectively collected, including patient demographics, clinical data on first admission and readmission, steroid treatment and any treatment received on discharge from the first admission.

In the primary analysis, appropriate corticosteroid dosage was defined as receiving 6 mg dexamethasone daily. Statistical analysis was conducted in Stata ver. 12.1 (StataCorp). Independent data were compared using Mann-Whitney *U* test or *t*-test. Paired data were compared by Wilcoxon signed-rank and proportions by χ^2 test.

We fitted a logistic regression model to assess relationships between demographic and clinical factors and readmission risk. We conducted a sensitivity analysis considering anyone receiving a dose equivalent to 75% of 6 mg dexamethasone daily as having received steroids, using the outcome of readmission or re-attendance.

The study met the NHS definition of a quality improvement project with the departmental governance lead and did not require ethical approval.

Results

271 patients were admitted to UCH with COVID-19 in December. 25 patients were transferred from external hospitals or had nosocomially-acquired COVID-19 and 50 patients died during their first admission or remained an inpatient throughout the data collection period and were excluded from subsequent analysis. 196 patients were included in the analysis.

Median age was 58 years (IQR 47–71); 48% female; 133/196 (67.9%) had ≥1 comorbidity (as defined by the ISARIC 4C score),[8] 32 (16.3%) had diabetes mellitus. Median length of stay was 4 days (IQR 2–8). 125/196 (63.8%) required oxygen of whom 30 (15.3%) required respiratory support. 124/196 (63.3%) received corticosteroids on their first admission for a median of 5 days (IQR 3–8). All patients had acceptable peripheral oxygen saturations (SpO_2) at discharge(≥92% on air or within their target range). 10/196 (5.1%) were discharged with corticosteroids. 53/196 (27.0%) were followed up in a virtual clinic post-discharge.

26/196 (13.3%) patients re-attended UCH due to COVID-19, a median of 3 days (IQR 2–5) following discharge. Of these, 20 (10.2%) were readmitted. Median CRP (mg/L) rose significantly in those readmitted from 43.2 (IQR 29.4–71.6) on discharge to 91.8 (IQR 37.3–139.6) on readmission ($p = 0.021$). 17/20 (85%) required oxygen and corticosteroids on readmission of whom 6 (30%) required respiratory support.

The 11/20 patients receiving steroids during their first admission, subsequently readmitted, had a shorter initial admission (median 2 days [IQR 1–3] vs 5 days [3–9] $p = 0.005$), received shorter courses of steroids (median 2 days [IQR 1–3] vs 5 days [3–8] $p<0.001$) and were discharged earlier in their illness course (median day 8 [IQR 6–11] vs day 13 [IQR 9–18], $p = 0.005$) than those that were not readmitted. There was no difference in SpO_2 on air at discharge (95% IQR [94%–96%] for both) or in remdesivir use (27.2% vs 33.6%, $p = 0.669$). Data for patients receiving inpatient corticosteroids on their first admission were quartiled based on their duration of steroids. In the first quartile, (1–3 days) readmission rates were highest at 25% (Fig. 1 and Table 1). In an exploratory logistic regression analysis, only treatment with dexamethasone significantly reduced odds of readmission (OR 0.77 per day of dexamethasone 95% CI 0.61–0.92, $p = 0.012$). Results were similar in the sensitivity analysis considering both equivalent doses of other steroids and both re-attendance and readmission to hospital (supplementary data).

Discussion

To our knowledge this is the first study to evaluate readmission rate in the recent COVID-19 wave, in the context of corticosteroid use.

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Table 1
Patients admitted from the community who were discharged alive from their first admission. Excluding ITU transfers and nosocomial transmissions. Comparing characteristics of those receiving different steroid course durations by quartile ($n = 196$).

Characteristics	*Number of days of dexamethasone received as inpatient on 1st admission*				
	Did not receive ($n = 72$)	1st Quartile1–3 ($n = 40$)	2nd Quartile4–5 ($n = 26$)	3rd Quartile6–8 ($n = 32$)	4th Quartile≥9 ($n = 26$)
Number readmitted (%)	9 (12.5)	10 (25.0)	0 (0.0)	0 (0.0)	1 (3.8)
Median age (IQR) - years	61 (49–78)	51 (41–65)	56 (40–66)	64 (48–70)	55 (49–67)
Sex					
Males (%)	37 (51.4)	23 (57.5)	12 (46.2)	15 (46.9)	15 (57.7)
Females (%)	35 (48.6)	17 (42.5)	14 (53.8)	17 (53.1)	11 (42.3)
Ethnicity (%)					
White	27 (37.5)	19 (47.5)	10 (38.5)	10 (31.3)	9 (34.6)
Black	11 (15.3)	8 (20.0)	1 (3.8)	5 (15.6)	5 (19.2)
South Asian	7 (9.7)	1 (2.5)	3 (11.5)	3 (9.4)	3 (11.5)
Other Asian	7 (9.7)	0 (0.0)	3 (11.5)	1 (3.1)	2 (7.7)
Unknown	20 (27.8)	12 (30.0)	9 (34.6)	13 (40.6)	7 (26.9)
Median day of illness on admission (IQR)	4 (2–8)	7 (4–9)	8 (5–10)	8 (5–10)	8 (6–9)
Median length of admission (IQR) - days	2 (1–5)	2 (2–3)	4 (4–5)	7 (6–8)	10 (9–13)
Number of comorbidities (%)					
0	22 (30.6)	15 (37.5)	11 (42.3)	8 (25.0)	7 (26.9)
1	19 (26.4)	8 (20.0)	7 (26.9)	11 (34.4)	11 (42.3)
>2	31 (43.1)	17 (42.5)	8 (30.8)	13 (40.6)	8 (30.8)
Number with infiltrates of Chest X-ray (%)	34 (47.2)	34 (85.0)	23 (88.5)	31 (96.9)	26 (100.0)
Number of asymptomatic COVID (%)	18 (25.0)	0 (0.0)	0 (0.0)	1 (3.1)	0 (0.0)
Median admission sats (IQR) -%	97 (95–98)	93 (91–97)	92 (90–95)	92 (88–95)	92 (88–94)
Median CRP on admission (IQR) – mg/L	24.0 (8.0–63.4)	41.2 (24.8–77.5)	68.0 (51.8–102.8)	102.1 (59.8–168.9)	98 (73–164)
Median Urea on 1st admission (IQR) – mmol/L	4.5 (3.6–6.9)	4.2 (3.5–5.9)	4.5 (3.5–5.6)	4.4 (3.3–6.3)	6.0 (4.8–7.3)
Median lymphocytes on 1st admission (IQR) – $x10^9$/L	1.01 (0.80–1.47)	1.06 (0.79–1.32)	0.87 (0.63–1.23)	0.87 (0.69–1.31)	1.00 (0.73–1.10)
Median peak oxygen requirement (IQR) -%	21 (21–21)	28 (24–33)	32 (32–36)	40 (35–53)	60 (40–64)
Number of patients by peak respiratory support (%)					
Hospitalised, no oxygen	61 (84.7)	8 (20.0)	1 (3.8)	1 (3.1)	0 (0.0)
Oxygen by mask or nasal prongs	10 (13.9)	31 (77.5)	24 (92.3)	20 (62.5)	9 (34.6)
NIV or high-flow oxygen	1 (1.4)	0 (0.0)	1 (3.8)	11 (34.4)	16 (61.5)
Intubation and Mechanical Ventilation	0 (0.0)	1 (2.5)	0 (0.0)	0 (0.0)	1 (3.8)
Ventilation and additional organ support (Pressors, RRT, ECMO)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Number receiving remdesivir (%)	0 (0.0)	6 (15.0)	11 (42.3)	11 (34.4)	13 (50.0)
Median CRP on discharge from 1st admission (IQR) – mg/L	22.5 (4.6–53.9)	28.0 (16.3–62.2)	19.9 (13.2–41.4)	16.8 (10.2–31.1)	12 (5.3–40.6)
% Sats drop on exercise at discharge from 1st admission (%)					
0–1	21 (29.2)	14 (35.0)	10 (38.5)	10 (31.3)	6 (23.1)
2–3	2 (2.8)	8 (20.0)	5 (19.2)	5 (15.6)	3 (11.5)
≥4	5 (6.9)	3 (7.5)	7 (26.9)	4 (12.5)	11 (42.3)
Unknown	44 (61.1)	15 (37.5)	4 (15.4)	13 (40.6)	6 (23.1)
Number discharged with steroids on 1st admission (%)	2 (2.8)	6 (15.0)	1 (3.8)	0 (0.0)	1 (3.8)
Number discharged with thromboprophylaxis on 1st admission (%)	6 (8.3)	3 (7.5)	3 (11.5)	7 (21.9)	7 (26.9)
Number discharged with saturations probe on 1st admission (%)	8 (11.1)	10 (25.0)	6 (23.1)	3 (9.4)	6 (23.1)
Median ISARIC 4C mortality% (IQR)	11.7 (4.8–19.2)	7.8 (2.3–19.2)	11.7 (4.8–19.2)	19.2 (11.7–26.9)	14.4 (7.8–26.9)
Median ISARIC 4C deterioration%(IQR)	18.4 (12.3–24.4)	29.4 (20.1–44.9)	46.7 (26.6–59.7)	56.4 (35.9–65.7)	57.4 (40.3–65.4)
Number receiving telephone clinic follow up on 1st admission (%)	19 (26.4)	16 (40.0)	12 (46.2)	3 (9.4)	3 (11.5)

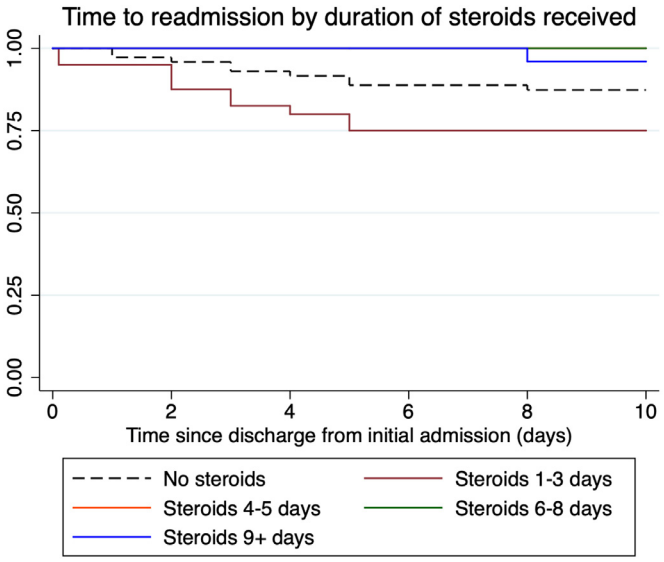

Fig. 1. Survival plot showing the probability of being out of hospital in the 10 days following discharge by duration of corticosteroids received during initial admission. The line showing readmission in the group receiving steroids for 4–5 days is not visible as masked by the line for steroids for 6–8 days – i.e. there were no readmissions for this group.

Despite the majority meeting safe discharge criteria, the readmission rate is significant, but concordant with rates from the first wave in similar hospitals[9]. Readmitted patients presented to and were discharged from hospital earlier in their COVID-19 illness than patients who were not readmitted. They returned to hospital after a short time after reaching their illness peak, displaying a proinflammatory phenotype as evidenced by their rising CRP. Significant oxygen requirements were observed and an appreciable proportion of patients required respiratory support.

Shorter courses of steroids on first admission increased risk of being readmitted to hospital with COVID-19. Those who received 1–3 days of steroids experienced quick clinical improvement and were discharged from hospital and corticosteroids were stopped at discharge. 25% of this subgroup were readmitted.

Our data suggest that short courses of corticosteroids may not be sufficient for patients requiring hospital admission with severe COVID-19. As patients are readmitted with evidence of ongoing inflammation, it is biologically plausible that increasing corticosteroid duration would reduce the chance of deterioration post-discharge. Many hospitals have now instigated virtual follow up with daily calls. It is therefore reasonable to consider continuing a course of corticosteroids after hospital discharge as treatment can be given within these frameworks to monitor side-effects of steroids. UK national guidelines now recognise that patients may be discharged to a virtual ward where continuation of steroids may be appropriate. Our data support this.

Despite the limitations of small sample size and retrospective data collection, our data demonstrate a high readmission rate amongst patients with COVID-19 who received shorter courses of steroids. Further research is required to establish the optimal duration of steroids and how to identify patients who require ongoing steroids at discharge.

Declaration of Competing Interest

No conflicts of interests declared by an author.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.03.002.

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Lack of significant association between dyslipidemia and COVID-19 mortality

Dear Editor,

Recently, Aung et al. reported that distribution frequency of angiotensin converting enzyme (ACE) insertion/insertion (II) genotype had a significant impact on coronavirus disease 2019 (COVID-19) mortality.[1] Dyslipidemia is one of the most comorbidities among COVID-19 patients, however, the conclusions from published articles on the relationship between dyslipidemia and COVID-19 mortality are still controversial. For instance, several studies found that there was a significant relationship between dyslipidemia and an increased risk for mortality among COVID-19 patients,[2–4] while other studies reported that dyslipidemia was not significantly associated with COVID-19 mortality.[5,6] Therefore, there is an urgent need to address the relationship between dyslipidemia and COVID-19 mortality by a quantitative meta-analysis. It has been reported that demographical characteristics (age and gender) and certain comorbidities (diabetes mellitus, cardiovascular disease, hypertension, chronic kidney disease and autoimmune diseases, etc.) are well-known modulators that affect the clinical outcomes of COVID-19 patients,[7–9] suggesting that these factors might modulate the relationship between dyslipidemia and COVID-19 mortality. Thus, in this current meta-analysis, risk factors-adjusted effect estimates rather than crude effect estimates were utilized to calculate the pooled effect sizes.

We did this systematic meta-analysis in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). All potentially eligible articles published between January 1, 2020 and February 26, 2021 were identify in the online databases (PubMed, Web of Science and EMBASE) with the following keywords: “SARS-CoV-2” or “severe acute respiratory syndrome coronavirus 2” or “COVID-19” or “coronavirus disease 2019” or “2019-nCoV” or "2019 novel coronavirus" and “dyslipidemia” or “hyperlipidemia” or “low-density lipoprotein” or “high-density lipoprotein” or “triglycerides” or “total cholesterol”. Reference lists of eligible articles were also searched to look for additional studies. The exposure group was defined as COVID-19 patients with dyslipidemia and the control group was defined as COVID-19 patients without dyslipidemia. The outcome of interest was mortality, which was defined as mortality, death, died, non-survivor, fatality or deceased. All peer-reviewed articles published in English language reporting the risk factors-adjusted effect estimate on the relationship between dyslipidemia and COVID-19 mortality were eligibly selected. Accordingly, we excluded preprints, case reports, review papers, corrections, comments, animal studies and in vitro studies, studies reporting crude effect estimate, studies without sufficient data and studies reporting clinical outcomes as severe/critical illness, intensive care unit admission, invasive mechanical ventilation/intubation or composite outcomes rather than mortality. Essential information including first author, number of COVID-19 patients, gender distribution, age (mean and standard deviation (SD) or median and interquartile range (IQR)), study design, region/country, clinical outcomes, adjusted effect estimates and adjusted variables was extracted from each included study (Table 1).

We utilized Stata (version 12.1) for all statistical analyses. The pooled effect estimate and its 95% confidence interval (CI) were computed using a random-effects model. Inter-study heterogeneity was investigated using the cochrane Q test and I^2 statistic, $P < 0.1$ or $I^2 > 50\%$ shows a statistically significant heterogeneity. The statistical stability of the overall effects was assessed using leave-one-out sensitivity analysis. The risk of publication bias was evaluated using Begg’s test. Subgroup analyses were carried out by sample size, age, male percentage, study design and effect estimate. Two-tailed P-value < 0.05 was considered statistically significant.

Initial search yielded 2608 articles. After screening eligible articles according to inclusion and exclusion criteria, a total of twenty-seven studies composing of 146,364 cases were enrolled into this quantitative meta-analysis. Among the included studies, twenty-four studies were retrospective, one was prospective, one was longitudinal cohort study and one was nationwide cohort study. The sample sizes across the eligible studies ranged from 98 to 35,302. There were sixteen odds ratio (OR)-reported studies, nine hazard ratio (HR)-reported studies, one risk ratio (RR)-reported study and one relative hazard (RH)-reported study.

The results of our pooled analysis are presented in Fig. 1A, which indicates that there was no significant relationship between dyslipidemia and COVID-19 morality (pooled effect size = 1.05, 95% CI [0.99–1.12], $P = 0.100$; $I^2 = 52.6\%$, random-effects model). Sensitivity analysis by deleting each study one by one demonstrated that our results were stable (Fig. 1B). When we limited dyslipidemia to hyperlipidemia, there was no significant relationship between hyperlipidemia and COVID-19 mortality (pooled effect size = 1.03, 95% CI [0.95–1.12]). We still observed no significant relationship between dyslipidemia and COVID-19 mortality in the subgroup analyses by age (pooled effect size = 1.08, 95% CI [0.99–1.18] for $<$ 65 years old and pooled effect size = 1.02, 95% CI [0.93–1.12] for $\geq$ 65 years old), male percentage (pooled effect size = 1.04, 95% CI [0.96–1.13] for $<$ 55% and pooled effect size = 1.08, 95% CI [0.97–1.20] for $\geq$ 55%), study design (pooled effect size = 1.06, 95% CI [0.99–1.14] for retrospective study), sample size (pooled effect size = 1.09, 95% CI [0.96–1.24] for $<$ 1500 cases and pooled effect size = 1.04, 95% CI [0.96–1.14] for $\geq$ 1500 cases), and effect estimates (OR = 1.08, 95% CI [0.98–1.20] and HR = 1.02, 95% CI [0.92–1.13]). Begg’s test indicated that there was no obvious publication bias ($P = 0.505$).

This meta-analysis has several limitations that need to be mentioned:[1] most of the included studies were from USA, which lim-

Table 1
Characteristics of the included studies.

Author	Country	Cases (n)	Male (%)	Age (years)[§]	Study design	Adjusted-effect (95% CI)	Adjusted variables	Clinical outcomes
Hashemi et al. (PMID: 32585065)	USA	363	55.4%	63.4 ± 16.5	Retrospective study	OR: 0.91 (0.46–1.81)	Chronic liver disease, age, obesity, male, cardiac diseases, hypertension, diabetes, pulmonary disorders	Death
Pettit et al. (PMID: 32589784)	USA	238	47.5%	58.5 ± 17	Retrospective study	OR: 1.7 (0.4–7.0)	Obesity, age, gender, hypertension, diabetes, pulmonary disease, cardiovascular disease, kidney disease, cancer, stroke, venous thromboembolism	Mortality
Grasselli et al. (PMID: 32667669)	Italy	3988	79.9%	63 (56–69)	Retrospective study	HR: 1.25 (1.02–1.52)	Age, men, respiratory support, hypertension, heart disease, type 2 diabetes, malignancy, chronic obstructive pulmonary disease, angiotensin-converting enzyme inhibitor therapy, angiotensin receptor blocker therapy, statin, diuretic, positive end-expiratory pressure at admission, fraction of inspired oxygen at admission, arterial partial pressure of oxygen/fraction of inspired oxygen at admission	Mortality
Tartof et al. (PMID: 32783686)	USA	6916	45%	49.1 ± 16.6	Retrospective study	RR: 1.47 (1.02–2.11)	Body mass index, age, sex, race/ethnicity, smoking, metastatic tumor/cancer, myocardial infarction, other immune condition, organ transplant, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, renal disease, hypertension, asthma, diabetes mellitus status, time	Death
Czernichow et al. (PMID: 32815621)	France	5795	65.4%	59±14	Retrospective study	OR: 0.97 (0.74–1.27)	Body mass index, age, diabetes, hypertension, sleep apnea, chronic kidney disease, heart failure, malignancies, history of smoking, sex	Mortality
Nimkar et al. (PMID: 32838205)	USA	327	55.7%	71 (59–82)	Retrospective study	OR: 1.4 (0.8–2.2)	Race, chronic kidney disease in addition to six essential covariates (age, sex, race, hypertension, diabetes, cardiac disease)	Mortality
Giorgi-Rossi et al. (PMID: 32853230)	Italy	2653	50.1%	72±24	Prospective study	HR: 1.4 (0.9–2.2)	Age, sex	Death
Esme et al. (PMID: 32871002)	Turkey	16,942	49%	71.2 ± 8.5	Retrospective study	OR: 0.77 (0.64–0.93)	Gender, hypertension, diabetes mellitus, chronic obstructive pulmonary disease, coronary artery disease, atrial fibrillation, chronic kidney disease, dementia, depression, malnutrition	Mortality
Yan et al. (PMID: 32949175)	China	1103	48.6%	63 (51–71)	Retrospective study	HR: 1.91 (0.46–7.99)	Age, male, diabetes, hypertension, chronic obstructive pulmonary disease, chronic heart diseases, chronic kidney diseases, chronic liver diseases, cerebrovascular diseases, tumor, C-reactive protein, d-dimer	Mortality
Ioannou et al. (PMID: 32965502)	USA	10,131	91%	63.6 ± 16.2	Longitudinal corhot study	HR: 0.96 (0.83–1.11)	All sociodemographic characteristics, comorbid conditions, symptoms	Mortality
Ghany et al. (PMID: 33024960)	USA	400	40%	72±8	Retrospective study	RH: 0.99 (0.99–1.00)	Age, gender, charlson score	Death
Graziani et al. (PMID: 33053774)	Spain	14,339	49%	66±15	Retrospective study	OR: 1.03 (0.81–1.31)	Chronic obstructive pulmonary disease, sex, age, heart failure, high blood pressure, stroke, arrythmia, ischemic heart disease, diabetes, sleep apnea, pulmonary thromboembolism, smoking	Death
An et al. (PMID: 33127965)	Korea	10,237	39.9%	44.97±19.79	Nationwide cohort study	HR: 0.89 (0.66–1.20)	Age, sex, income level, residence, household type, disability, symptom, infection route	Death
Zhang et al. (PMID: 33122929)	China	98	59.2%	63.9 ± 1.4	Retrospective study	OR: 2.94 (1.22–7.12)	Age, gender, lymphocyte count, glycated hemoglobin, hypersensitive C-reactive protein, N-terminal brain natriuretic propeptide, creatinine	Mortality
Shah et al. (PMID: 33169090)	USA	487	56.1%	68±17	Retrospective study	OR: 1.36 (0.83–2.21)	Age, gender, patient admitted from home, hypertension, cardiomyopathy, atrial fibrillation, chronic obstructive pulmonary disease, cerebrovascular accident, diabetes mellitus, acute kidney injury, initial chest x-ray/computed tomography findings, dyspnea in emergency department noted as positive	Mortality
Tomasoni et al. (PMID: 33179839)	Italy	692	69.5%	67.4 ± 13.2	Retrospective study	HR: 0.82 (0.47–1.44)	Age, sex, heart failure, hypertension, atrial fibrillation, coronary artery disease, chronic obstructive pulmonary disease, chronic kidney disease, oxygen saturation, arterial partial pressure of oxygen/fraction of inspired oxygen, hemoglobin, lymphocytes count, estimated glomerular filtration rate, C-reactive protein on admission, troponin	Death
Loffi et al. (PMID: 33229434)	Italy	1252	63.7%	64.7 ± 15.5	Retrospective study	HR: 0.94 (0.63–1.41)	Sex, left ventricular ejection fraction < 35%, cerebrovascular disease, atrial fibrillation, diabetes mellitus, hypertension, coronary artery disease, chronic kidney disease, age	Death

(*continued on next page*)

Table 1 (*continued*)

Author	Country	Cases (n)	Male (%)	Age (years)[§]	Study design	Adjusted-effect (95% CI)	Adjusted variables	Clinical outcomes
Rossi et al. (PMID: 33222020)	Italy	590	67.6%	76.2 (68.2–82.6)	Retrospective study	HR: 1.108 (0.859–1.431)	Age, gender, temperature, arterial partial pressure of oxygen / fraction of inspired oxygen, lactate dehydrogenase, C-reactive protein, white blood cell count, lymphocytes rate, cardiovascular disease, diabetes, atrial fibrillation, chronic obstructive pulmonary disease, chronic kidney disease, stroke, malignancy, 3 or more comorbidities, angiotensin-converting enzyme inhibitor, angiotensin receptor blockers, calcium-channel blockers, alpha blockers, diuretics, beta blockers	Mortality
Rosenthal et al. (PMID: 33301018)	USA	35,302	53.4%	63.6 ± 17.7	Retrospective study	OR: 1.11 (1.03–1.19)	Age, sex, race, payer type, admission point of origin, hospital region, hospital beds, hospital teaching status, statin, vitamin C, zinc, angiotensin-converting enzyme inhibitor, b blocker, calcium channel blocker, hydroxychloroquine and azithromycin use, sepsis, acute kidney failure, hypokalemia, hyperkalemia, hyponatremia, acidosis, acute liver damage, neurological disorder, myocardial infarction, congestive heart failure, cerebrovascular disease, chronic pulmonary disease, dementia, diabetes, any malignant neoplasm, metastatic solid tumor, hemiplegia, acquired immunodeficiency syndrome, hypertension	Mortality
Ozyilmaz et al. (PMID: 33322097)	Turkey	105	72.4%	45 (20–87)	Retrospective study	OR: 4.060 (0.011–1555.792)	Troponin I, C-reactive protein, lymphocyte count, shortness of breath, hypertension, diabetes mellitus, coronary artery disease	Mortality
Lohia et al. (PMID: 33453090)	USA	1871	51.6%	66 (54–75)	Retrospective study	OR: 0.97 (0.76–1.23)	Age, sex, race, smoking, body mass index, insurance and comorbidities which include coronary artery disease, congestive heart failure, chronic obstructive pulmonary disease, asthma, chronic kidney disease, end-stage renal disease on dialysis, any malignancy, any liver disease, history of previous stroke, hypertension, diabetes	Mortality
Gupta et al. (PMID: 33461499)	USA	473	45.5%	70 (61–80)	Retrospective study	OR: 1.28 (0.75–2.19)	Race, age, sex, coronary artery disease, diabetes, hypertension, chronic obstructive pulmonary disease/asthma, autoimmune diseases history of cancer, immunocompromised, congestive heart failure, chronic kidney disease with dialysis, chronic kidney disease without dialysis, end-stage renal disease with dialysis	Mortality
Mayer et al. (PMID: 33496668)	Spain	23,844	42.3%	49.93±19.4	Retrospective study	OR: 1.19 (1.03–1.39)	Age, sex	Death
Muhammad et al. (PMID: 33538998)	USA	200	60.5%	58.9 ± 15.1	Retrospective study	OR: 2.12 (0.94–4.77)	Age, hypertension, coronary artery disease, chronic kidney disease, history of stroke, oxygen saturation, creatinine, blood urea nitrogen, creatine phosphokinase, troponin, procalcitonin, lactic acid, lactate dehydrogenase, C-reactive protein, initial d-dimer, ferritin, highest d-dimer	Mortality
Yoshida et al. (PMID: 33546750)	USA	776	47.3%	60.5 ± 16.1	Retrospective study	OR: 0.95 (0.53–1.71)	Age, sex, hospital site, the charlson comorbidity index	Death
Girardin et al. (PMID: 33550849)	USA	4446	58.1%	62±18	Retrospective study	HR: 0.92 (0.79–1.06)	Age, ethnic minority, male sex, low income, smoking, obesity, chronic obstructive pulmonary disease, asthma, sleep apnea, hypertension, diabetes, peripheral artery disease, coronary artery disease, autoimmune disease, cancer	Mortality
Wargny et al. (PMID: 33599800)	France	2796	63.7%	67.9 ± 13.2	Retrospective study	OR: 1.15 (0.95–1.40)	Age	Death

Note:

[§] The values are presented as mean ± standard deviation or median (interquartile range, IQR); USA, the United States of America; CI, confidence interval; OR, odds ratio; RR, risk ratio; RH, relative hazard; HR, hazard ratio.

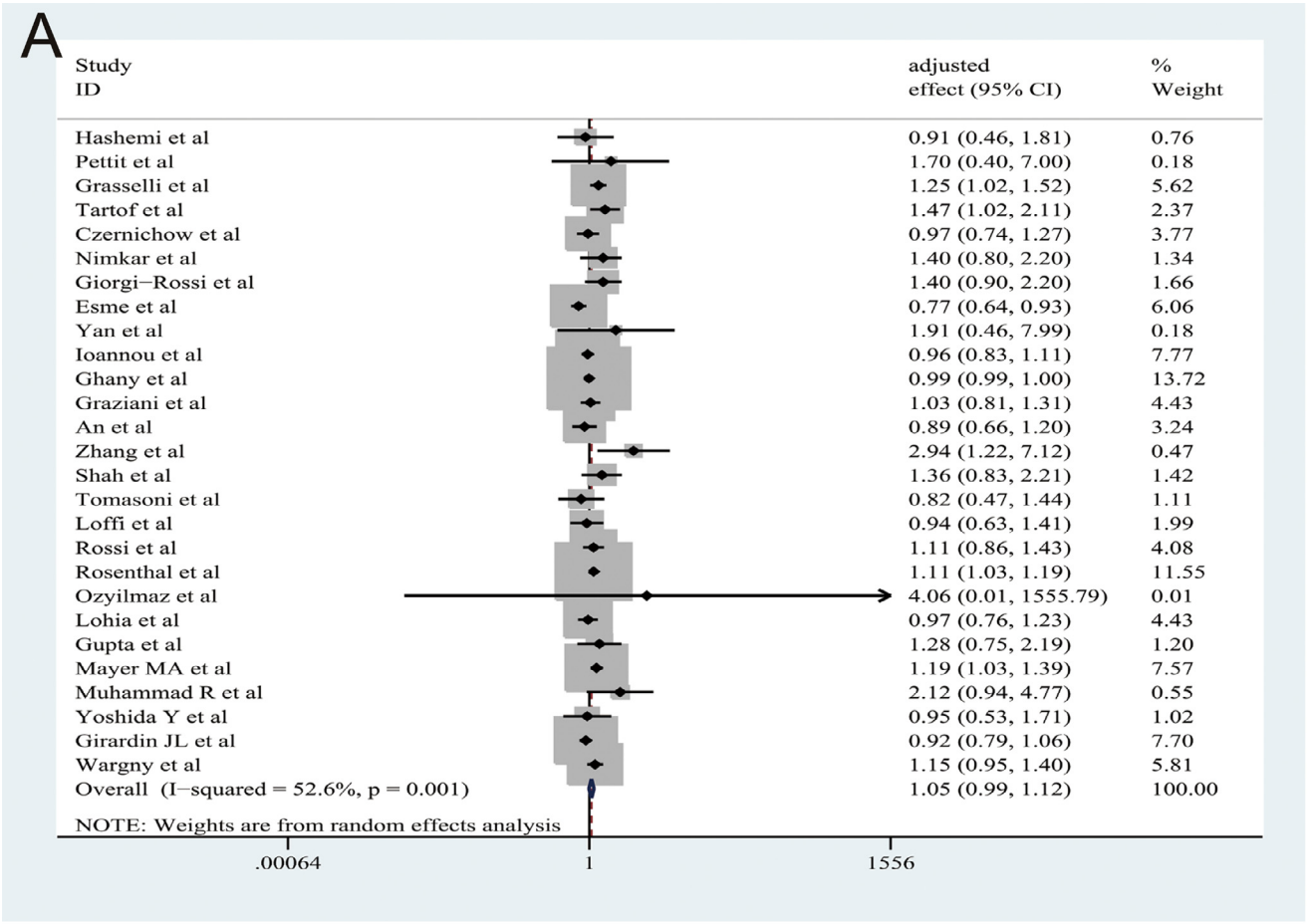

Fig. 1. (A) The forest plots demonstrated that dyslipedemia was not significantly associated with the risk of mortality among patients with coronavirus disease 2019 (COVID-19) on the basis of twenty-seven studies with 146,364 cases; (B) Sensitivity analysis by omitting individual study one by one indicated that the results were stable and robust.

its its wider applicability of the present findings;[2] the majority of studies were retrospective, thus further well-designed studies with more prospective researches are required to verify our results;[3] although the pooled effect estimate was calculated on the basis of adjusted effects, the adjusted variables are not completely consistent across the included studies;[4] only one included study explicitly states the specific type of dyslipidemia as total cholesterol, additional studies does not explicitly states the specific type of dyslipidemia such as abnormal levels of low-density lipoprotein, high-density lipoprotein, triglycerides and total cholesterol. Further studies should focus on the relationship between specific type of dyslipidemia and COVID-19 mortality when more data are available;[5] the detailed information on medications for patients with pre-existing dyslipidemia is not available presently, thus we could not address the effects of medications on the relationship between dyslipidemia and COVID-19 mortality.

In conclusion, our current study based on adjusted effect sizes demonstrated that dyslipidemia was not significantly associated with COVID-19 mortality. Further well-designed studies with large sample sizes are warranted to confirm our findings.

Declaration of Competing Interest

The authors declare that they have no any potential conflict of interest regarding this submitted manuscript.

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Author contributions

Haiyan Yang and Yadong Wang designed the study. Hongjie Hou and Jie Xu performed literature search. Hongjie Hou and Haiyan Yang performed data extraction. Xuan Liang, Haiyan Yang, Hongjie Hou and Jie Xu performed statistical analyses. Haiyan Yang, Hongjie Hou and Yadong Wang wrote and reviewed the manuscript. All the authors approved the final version of the manuscript.

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Disproportionate impact of SARS-CoV-2 on ethnic minority and frontline healthcare workers: A cross-sectional seroprevalence survey at a North London hospital

Dear Editor,

We read with interest the study by de Lusignan et al., who found that, among 1970,314 UK primary care patients aged ≥ 45 years, being male, increasing age, chronic disease, Black ethnicity and deprivation were associated with excess mortality during the first wave of the COVID-19 pandemic[1]. These findings highlight the unequal burden of COVID-19 across society and reflect our patient and staff experience at North Middlesex University Hospital (NMUH)[2]. NMUH is located in a socioeconomically and ethnically diverse area of London and, early in the pandemic, was identified as the second most COVID-19 pressured NHS trust in the UK[3]. During the first wave, 24 out of 26 wards were converted to COVID-19 care, intensive care capacity was doubled, and many non-acute medical services were moved offsite. Many healthcare workers (HCW) were redeployed to the frontline where they faced a potent combination of occupational and sociodemographic factors influencing COVID-19 risk.

Between 4th June and 3rd July 2020, voluntary SARS-CoV-2 antibody testing was offered to the NMUH workforce. Staff were invited to complete an online questionnaire detailing comorbidities, occupational and sociodemographic factors. Responses were anonymously linked to antibody results using occupational

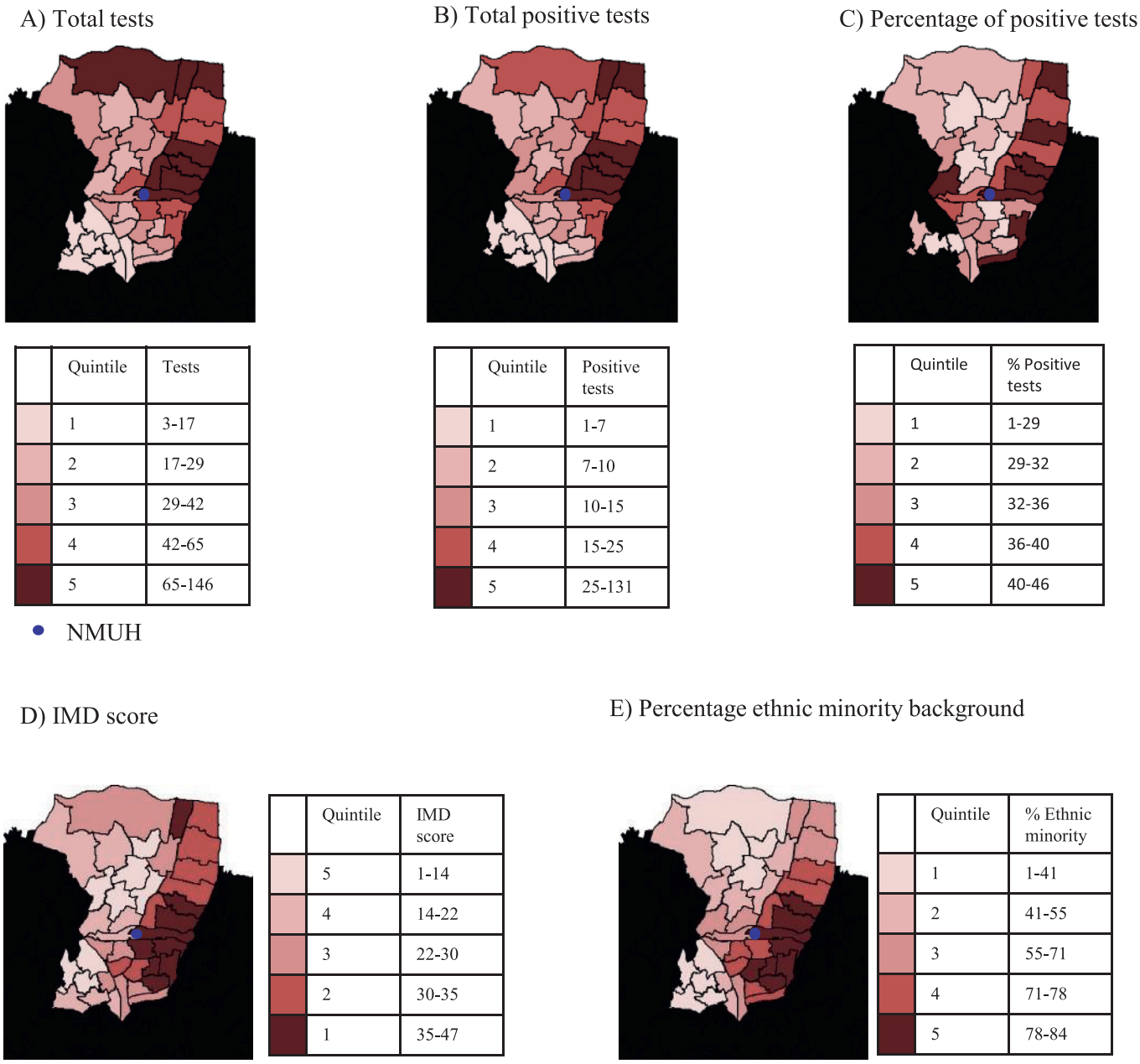

Fig. 1. (A) Total number of SARS-CoV-2 antibody tests at ward level (wards with ≥ 3 tests included to maintain anonymity). (B) Total positive SARS-CoV-2 antibody tests at ward level (wards with ≥ 3 tests included to maintain anonymity). (C) Percentage of positive SARS-CoV-2 antibody tests by ward. (D) Index of Multiple Deprivation (IMD) score (2019) by ward. Quintile 1 (most deprived), Quintile 5 (least deprived). (E) Percentage of residents from an ethnic minority background by ward (Census data, 2011). Data from Public Health England, https://www.localhealth.org.uk/. In all figures, wards were divided into even quintiles and then coloured by quintile. The values contained within each quintile are included in the quintile legends. Maps generated using the Greater London Authority mapping template, https://data.london.gov.uk/dataset/excel-mapping-template-for-london-boroughs-and-wards.

health numbers. Multivariable logistic regression was used to identify factors associated with seropositivity. Forward stepwise selection was used to determine which variables to retain in the model and checked against backward elimination. Base demographics of age, sex and ethnicity were always retained in the model. Variables tested for inclusion were underlying risk group, location of residence, Index of Multiple Deprivation (IMD) quintile, job banding, job role, workplace setting, patient interaction, HCW in household, and individual sites of work: emergency department (ED), endoscopy, estates, human resources and finance, intensive care, maternity, medicine, non-clinical, oncology, outpatient department, pathology, paediatrics, pharmacy, radiology, surgery, senior management, theatres, therapies. Statistical analyses were conducted using Stata v.14.2. Total serology tests, positive tests and positivity rates were plotted according to postcode, alongside IMD score and ethnicity using Microsoft Excel. This evaluation was conducted for service improvement and did not require ethical approval according to the NHS Health Research Authority algorithm.

Of 3945 invited staff, 3285 were tested and completed the survey. Overall seropositivity was 35.7% (1173/3285), median age was 41 (IQR 31, 51) years and 72% (2369/3285) were female; White British/Irish HCW represented 23% (764/3285), while 70% (2293/3285) were from ethnic minority backgrounds, most commonly Black African (738/3285, 24%) and the Indian Subcontinent

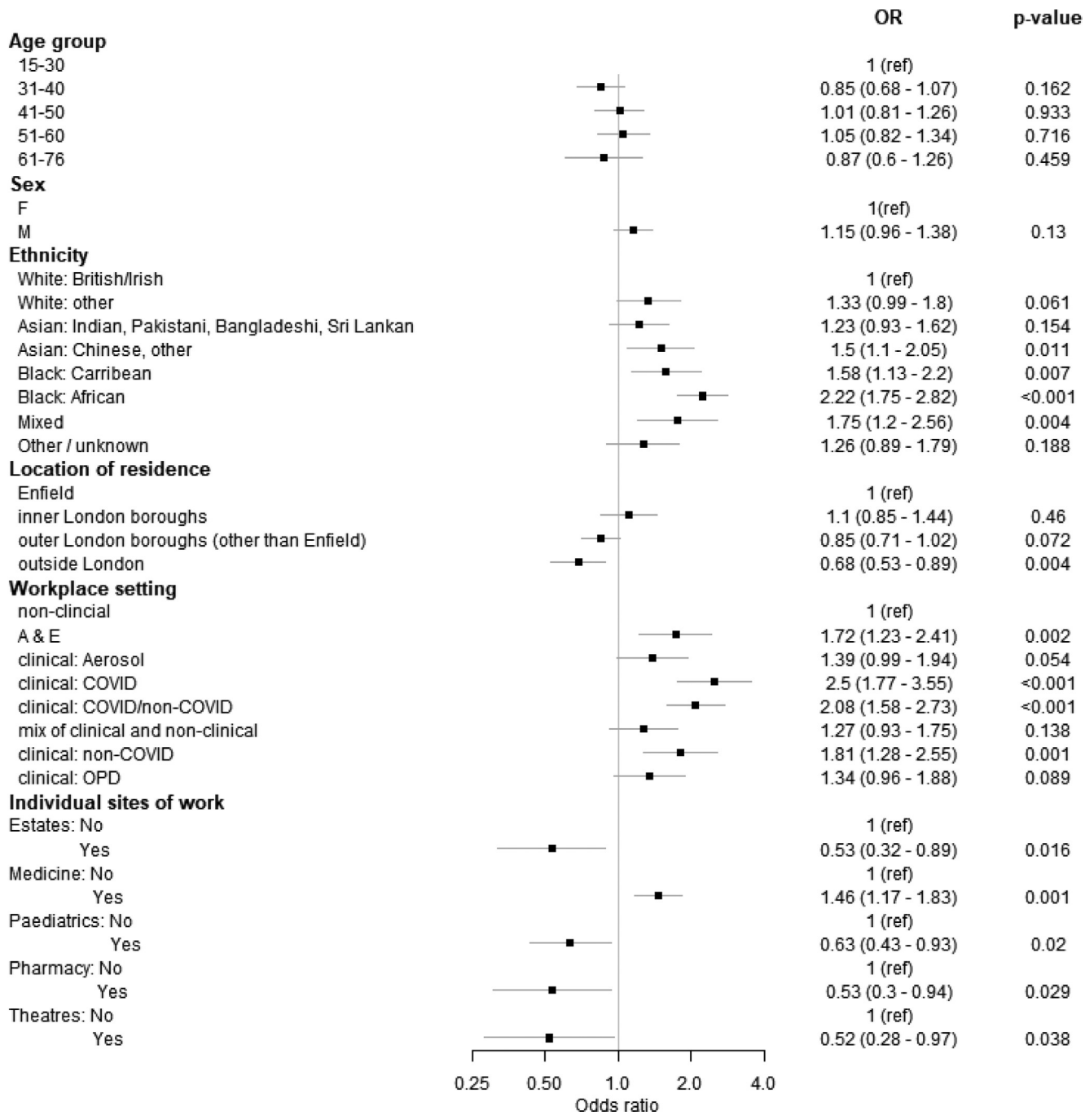

Fig. 2. Forest plot: multivariable logistic regression model for antibody status according to sociodemographic and occupational factors, following variable reduction. For individual sites of work: Yes – works in that site, No- does not work in that site. Odds ratio, OR. Outpatient department, OPD. A&E – Accident and Emergency (Emergency department). Clinical: Aerosol – refers to inpatient wards where aerosol-generating procedures (AGP) are carried out. Clinical: COVID, Clinical: COVID/non-COVID (mixed COVID), Clinical: non-COVID – refer to inpatient wards with no AGP.

(Indian, Pakistani, Bangladeshi, Sri Lankan; 484/3285, 15%). Overall, 79% (2585/3285) reported no comorbidities, two-thirds lived within the most deprived quintiles (IMD 1, 1021/3285, 31%; IMD 2, 1024/3285, 31%). In total, 31% (1029/3285) were nursing and midwifery staff, followed by administrative (577/3285, 18%) and medical (497/3285, 15%) staff. Most worked in clinical areas (2796/3285, 85%) with patient contact (2562/3285, 78%). A third were in the lowest two NHS job bands (1177/3285, 36%).

Half the staff tested (1692/3285, 52%) lived in Enfield and Haringey Boroughs, adjacent to NMUH. Staff seropositivity rates were highest to the East of Enfield and Haringey, corresponding to the most deprived wards with greatest ethnic diversity (Fig. 1).

In a multivariable logistic regression model for seropositivity, ethnicity and location of residence were the sociodemographic factors reaching significance for inclusion (Fig. 2). All ethnicities had increased odds of seropositivity compared with White British/Irish staff. Black African staff were at greatest risk (Odds Ratio 2.22, 95% Confidence Interval 1.75–2.82, $p < 0.001$), followed by mixed ethnicity (OR 1.75, 95% CI 1.2–2.56, $p = 0.004$), Black Caribbean (OR 1.58, 95% CI 1.13–2.2, $p = 0.007$), Asian Chinese/Other (OR 1.5, 95% CI 1.1–2.05, $p = 0.01$). Staff who identified as White Other and from

the Indian subcontinent also had increased odds of seropositivity, but this did not reach statistical significance.

In the same model, workplace setting and certain individual sites of work were the only occupational factors reaching significance for inclusion (Fig. 2). All clinical staff had increased odds of seropositivity compared to non-clinical staff. The greatest risk was in COVID-19 wards not performing aerosol generating procedures (AGP) (OR 2.5, 95% CI 1.77–3.55, $p < 0.001$), mixed-COVID-19 (OR 2.08 95% CI 1.58–2.73, $p < 0.001$), non-COVID-19 wards (OR 1.81, 95% CI 1.28–2.55, $p = 0.001$) and ED (OR 1.72, 95% CI 1.23–2.41, $p = 0.002$). Staff working in AGP areas had increased odds of seropositivity (OR 1.39, 95% CI 0.99–1.94, $p = 0.054$), but this was of borderline significance, while outpatient areas (OR 1.34, 95% CI 0.96–1.88, $p = 0.089$) was not statistically significant. Medical department staff had increased odds of seropositivity (OR 1.46, 95% CI 1.17–1.83, $p = 0.001$) compared to other sites of work.

We found that staff at highest risk worked in non-AGP COVID-19 wards and were from minority ethnic groups, and the highest seropositivity rates mapped to the most deprived local wards. Workforce ethnic diversity and locality was striking; the majority of staff lived in adjacent boroughs and 70% were from minority ethnic backgrounds, compared to 22% in the wider NHS[4]. Similar occupational risk factors have been reported in other HCW seroprevalence surveys, but their influence in combination with sociodemographic factors on COVID-19 risk in HCWs has not been fully described[5, 6]. One large American HCW seroprevalence study found that community and demographic factors, in particular Black ethnicity and contact with a suspected COVID-19 case, were more predictive of seropositivity than occupational factors. Similarly, we found staff seropositivity geographically mirrored COVID-19 cases among our inpatient population, mapping to local ethnically diverse and deprived areas[2]. The Health Service Journal reported a disproportionate number of NHS staff deaths among ethnic minorities[7]. Concerningly, British Medical Association surveys have found ethnic minority doctors feel less protected from COVID-19 at work than their White colleagues[8]. Furthermore, ethnic minorities are currently under-represented in national HCW surveillance studies[9]. Recent data suggest that there is significantly lower COVID-19 vaccine uptake among HCWs from minority ethnic groups and living in more deprived neighbourhoods, thus exacerbating these disparities[9,10].

Further work is needed to understand the interplay of occupational and sociodemographic risk factors facing HCWs. Inequalities must be urgently addressed in order to better protect NHS staff during the ongoing pandemic.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Use of lateral flow devices allows rapid triage of patients with SARS-CoV-2 on admission to hospital

Dear Editor,

We read with interest the case that Fowler et al. make for robust near patient testing in the coronavirus disease (Covid-19) pan-

Table 1
Sensitivity of Lateral flow device (LFD) compared with Polymerase Chain Reaction (PCR), depending on Cycle threshold (Ct) value (mean Ct of all detected targets).

		All PCR			Mean Ct across detected PCR targets				
		Positive	Negative	Invalid	<15	15–19.9	20–24.9	25–29.9	≥30
LFD result	Positive	133	0	1	59	47	23	4	0
	Negative	80	572	15	12	15	31	19	3
	Invalid	1	1	0	0	0	1	0	0
Cumulative sensitivity (95% CI)					83% (72–91)	80% (72–86)	69% (62–76)	63% (56–70)	62% (56–69)

demic to identify contagious cases.[1] The winter peak of Covid-19 in England has seen the highest number of Covid-19 cases and hospital admissions to date, with over 3000 admissions daily, and a peak of 34,015 inpatients with Covid-19.[1] Patient triage and cohorting are crucial to reducing nosocomial Covid-19,[2] but paucisymptomatic or pre-symptomatic cases limit clinical case detecting,[3] and screening with molecular diagnostics introduces delay.[4] We piloted the use of point of care antigenic testing for SARS-CoV-2 in patients admitted to hospital for rapid case detection in a period of high disease prevalence.

Between December 23, 2020 and January 30 2021, patients admitted to Oxford University Hospitals NHS Foundation Trust for emergency care were tested for SARS-CoV-2 using both lateral flow device (LFD) and real-time reverse transcription Polymerase chain reaction (PCR) testing. Swabs of the nose and throat were collected by health care workers. LFD testing was performed in the admitting department by staff, using the Innova LFD. Swabs for PCR were transferred to the clinical laboratory in viral transport medium (VTM) and tested by multiplex PCR (Thermo-Fisher TaqPath). 803 patients who had both tests performed with a maximum of 1 day between tests were included for analysis. 732/803 (91%) of patients tested had both tests on the same day. Clinical notes of patients testing positive for SARS-CoV-2 by PCR were reviewed and note made of the reported presence of symptoms of possible Covid-19 (cough, dyspnoea, fever, aguesia or anosmia) as well as admission temperature and oxygen saturation, and previous detection of SARS-CoV-2 by PCR.

Considering PCR results as the reference standard, LFDs showed high specificity (Table 1). Of 573 PCR-negative patients, 572 had a negative LFD, and 1 an invalid LFD result, i.e., specificity excluding the invalid result was 100% (exact binomial 95%CI 99.4–100%). Similarly the positive predictive value was high, among 133 patients with a positive LFD results, 133/134 (99.2%, 95%CI 95.9–99.8%) were PCR-positive, with one indeterminate PCR result in a patient testing PCR-positive 5 days later; none were PCR-negative. LFDs also had low rates of invalid results, 2/803 (0.2%).

Lateral flow testing showed modest sensitivity, and performed better in those with higher viral loads. Among all 214 SARS-CoV-2 PCR-positive patients, 133 tested positive by LFD, i.e. sensitivity was 62.4% (95% CI 55.6–69.0%), and the negative likelihood ratio was 0.38 (0.32–0.45). 80 patients were LFD-negative, PCR-positive. LFD-negative, PCR-positive individuals had lower viral loads, i.e. higher mean cycle threshold (Ct) values for the detected PCR targets (median 24, IQR 19–27), compared with LFD-positive, PCR-positive patients (median 16, IQR 13–20) (Fig. 1, Kruskal–Wallis $p < 0.001$). Sensitivity was greatest in those patients with a mean Ct <20 (78.5%, 95% CI 71.9–85.1%) (Table 1).

On a retrospective review of patient notes, we identified at least 11/133 (8%) of LFD positive patients had no Covid-19 symptoms recorded, presenting without cough, dyspnoea, fever, anosmia, ageusia or hypoxia. Furthermore, among LFD-negative PCR-positive patients, 28/80 (35.0%) had a pre-admission SARS-CoV-2 PCR-positive swab, so 161/214 (75.2%) of patients with SARS-CoV-2 detectable by PCR could be identified by either previous results

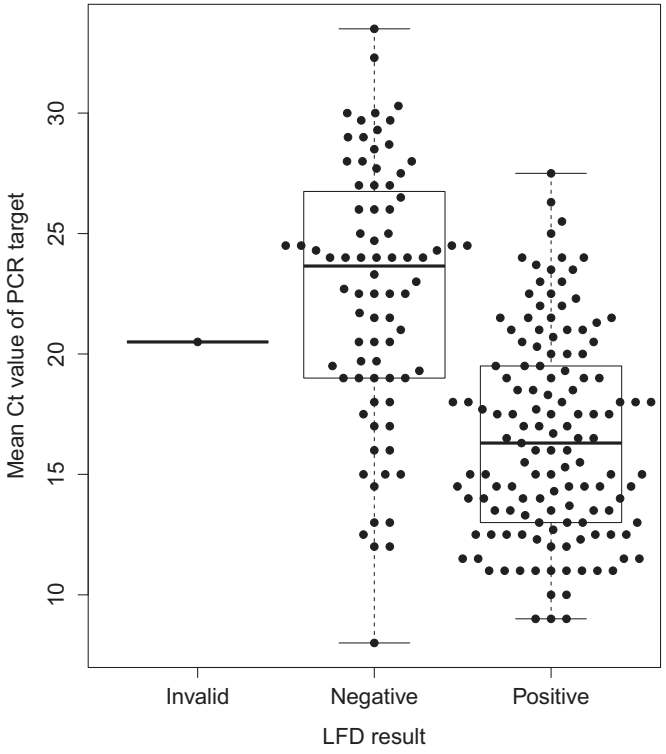

Fig. 1. Lateral flow device (LFD) results and Mean Cycle threshold (Ct) value of Polymerase Chain Reaction (PCR) target detection in 214 patients with SARS-CoV-2 detected. The median (central line), inter-quartile range (box) and range (whiskers) of Ct values are shown.

or LFD at admission. The absence of either previous PCR positive swab or a positive LFD at admission had a negative likelihood ratio 0.24 (95% CI 0.19–0.31).

Case identification is critical in reducing nosocomial transmission of SARS-CoV2.[2] While Ct values are not a direct measure of infectivity, they do correlate with RNA load and culture positivity and infectious dose.[5,6] Thus, LFD-positive patients, with higher viral loads, are most likely to represent those patients with the highest infectious risk in the healthcare environment. Additionally, in this cohort, LFDs provided incremental case detection above clinical assessment in asymptomatic adults. LFDs provide a rapid and incremental benefit to clinical triage for case finding.

The excellent specificity seen here corresponds with findings from other evaluation of LFDs,[7] as well local experience in testing asymptomatic healthcare workers, where LFDs showed a false positive rate of 0.03% compared with PCR.[8] This means a positive LFD can safely be used to triage patients to Covid-19 cohort areas for patients with confirmed infection without exposing these patients to risk of nosocomial acquisition. While a negative result cannot be used in isolation to triage a patient to a COVID-19 free area of the hospital, it does allow earlier identification of positive

cases, thus relieving pressure on cohort areas for patients with unconfirmed infection status, which are often the most challenging areas in which to prevent nosocomial transmission.

Despite imperfect sensitivity, when a known COVID diagnosis was taken in account, LFDs in this population had a negative likelihood ratio of 0.24. Therefore in patients where there is a low clinical suspicion of COVID-19, a negative LFD does provide further evidence against infectious SARS-CoV-2 infection, which can also play a role in triage decisions. The sensitivity of LFDs in the emergency hospital setting is lower than that reported in previous evaluations,[7] potentially reflecting the challenges of performing LFDs in emergency department settings and the later stage of infection in patients admitted to hospital compared to those attending symptomatic community testing. We did not monitor if all tests were read after the correct time interval, nor were photographs taken of devices at reading to allow for quality assurance. Therefore, reported performance could potentially be improved.

We conclude that LFDs provide a rapid and useful case detection in an acute setting, and are thus a helpful infection control tool.

Declaration of Competing Interest

DWE declares lecture fees from Gilead outside the submitted work. No other authors have a conflict to declare

Ethical statement

Patient screening and case reviews were undertaken as part of routine infection control in the hospital.

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BCY is an NIHR Clinical Lecturer. DWE is a Robertson Foundation Fellow.

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A retrospective multicenter analysis of candidaemia among COVID-19 patients during the first UK pandemic wave

Dear editor

It is with great interest that we read the recent article by De Francesco et al.[1], who reported on chlamydia pneumoniae and mycoplasma pneumoniae co-infection in patients with COVID-19. Here, we report our experience with candidaemia co-infection with COVID-19. An increased incidence of candidaemia has been noted in patients with COVID-19 and although patient characteristics, investigations and antifungal therapies have been described,[2] to our knowledge, compliance with candidaemia management bundles has not.[3]

Here, we present a retrospective review of candidaemias in adult patients (>17 years) with PCR proven COVID-19 between 1st March 2020–31st May 2020 across six acute London hospitals. All yeasts isolated from blood cultures were identified by matrix assisted laser desorption/ionisation-time-of-flight (MALDI-TOF) mass spectroscopy (Bruker Daltonik GmbH, Bremen, Germany). Antifungal susceptibility testing was carried out using broth micro-dilution in accordance with EUCAST guidelines.[4] An episode of candidaemia was defined as blood culture growth of any *Candida* species.

Eleven patients with concurrent candidaemia and PCR-proven COVID-19 were identified during the study period; ten were male (90.9%), mean age 62 (33–77 years). Underlying comorbidities were predominantly cardiovascular (10/11). Two patients were immunosuppressed (see Table 1), but neutropenia was not identified.

Ten patients (90.9%) were admitted to an intensive care unit (ICU) prior to their candidaemia diagnosis. Of the ICU patients ($n = 10$), all were intubated and ventilated, had an intravascular and urinary catheter and received inotropes. The non-ICU patient also had a urinary catheter. Nine (90%) of the ICU patients received haemofiltration. None of the patients in our cohort received total parenteral nutrition.

All eleven patients received broad-spectrum antibacterials. One patient received prior antifungal treatment in hospital with topical clotrimazole and oral terbinafine for a tinea infection.

The average number of days from PCR-proven COVID-19 to candidaemia was 14.8 days and from ICU admission to candidaemia, 15.5 days (range 6–24 days). Seven out of eleven candidaemias (63.6%) were *C.albicans*, two (18.2%) *C.parapsilosis*, one (9.1%) *C. glabrata* and one (9.1%) *C.dubliniensis*. All isolates were fluconazole susceptible, except one (*Candida glabrata*), which showed intermediate susceptibility, although the patient was successfully treated with azole therapy through dose-optimisation.

An echinocandin was commenced for ten patients, as per local guidelines, pending susceptibility testing. One patient died prior to blood culture positivity and treatment. Four out of ten (40%) patients were switched to fluconazole to complete treatment. In

Table 1
Characteristics of patients with concurrent COVID-19 and candidaemia.

Demographics	N=11
Age, years	62 (33-77)
Sex, male	10 (90.9%)
Past medical history	
Type 2 diabetes	8 (72.7 %)
Hypercholesterolaemia	8 (72.7%)
Hypertension	3 (27.3%)
Ischaemic Heart Disease	1 (9.1%)
Permanent Pacemaker	1 (9.1%)
Myaesthenia Gravis	1 (9.1%)
Previous solid organ malignancy	2 (18.2%)
Gastro-oesophageal Reflux Disease	1 (9.1%)
Benign prostatic hypertrophy	1 (9.1%)
Hypopituitarism	1 (9.1%)
Risk factors for candidaemia	
Immunosuppression	2 (18.2%) [a]
Broad spectrum antimicrobials	11 (100%)
Neutropenic	0 (0%)
Intensive care (ICU) admission	10 (90.9%)
Intravenous catheter	10 (90.9%)
• Average catheter day	6.3 (3-9)
• Number of patients where catheter day unknown	4 (40%)
Ventilated	10 (90.9%)
Inotropic support	10 (90.9%)
Haemofiltration	9 (81.8)
Urinary catheter	11 (100%)
Total parenteral nutrition	0 (0%)
Candida colonisation	
• Yes	4
• No	5
• Unknown	2
Clinical course	
Days to candidaemia since COVID-19 diagnosis	14.8 (7-24)
Days to candidaemia since ICU admission	15.5 (6-24)
Repeat blood cultures taken at 48 hours	6 (54.5%)
Days to candidaemia clearance	2.3 (1-3)
Non-albicans candidaemia	4 (36.4%)
Beta-D-glucan performed	6 (54.5%)
• positive	3 (50%) [b]
• negative	3 (50%)
Galactomannan performed	5 (45.5%)
• positive	0 (0%)
• negative	5 (100%)
Intravascular catheter removed	9 (90%)
• culture confirmation of same Candida spp.	1 (10%)
Echocardiogram performed	8 (72.7%)
• positive	0 (0%)
• negative	8 (100%)
Fundoscopy peformed	1 (9.1%)
• positive	0 (0%)
• negative	1 (100%)
Death at 30 days	6 (54.5%)

Values are reported as mean and range or frequency (%)

[a] One patient received 9mg prednisolone once daily plus 500mg mycophenolate mofetil twice daily for myasthenia gravis A second patient received hydrocortisone 10mg/5mg/5mg for hypopituitarism.

[b] Positive results included values of 256 pg/ml, 154 pg/ml and 110 pg/ml

line with recommended practice[3] six out of eleven patients (54.5%) had repeat blood cultures within 48 h of treatment, eight (72.7%) patients had an echocardiogram, but only one (9.1%) had fundoscopy. Serum (1-3)-β-D-glucan(BDG) testing was performed in 54.4% (6/11) of patients; three were positive(see Table 1).

Intravascular catheters were removed for nine out of ten patients (90%), the last patient dying prior to candidaemia notification. Seven out of nine patients had line tips sent for culture; two were positive for yeasts. One line tip confirmed an identical *Candida* spp., and hence constituted a line infection, but no further identification was available for the second.

Four patients had prior colonization with yeasts; one with the same species as their candidaemia, no further identification was available for the remaining three. Five patients were not colonized and two had an unknown status following transfer from other secondary care providers, developing candidaemia shortly after transfer.

In concordance with Mastrangelo et al.,[1] there was a high 30-day mortality of 54.4% (6/11) in our patient cohort. The four surviving patients (36.6%) were discharged; average total length of stay 58 days (range 31–78 days). One patient was stepped down after nine weeks in ICU but remained an inpatient until the end of our study period.

Given the high mortality rate, it is important to identify and address modifiable risk factors in an attempt to prevent the occurrence of candidaemia. Firstly, all our patients received broad-spectrum antibacterials, a recognized risk factor for candidaemia.[5,6] A recent study from Hughes et al.[7] demonstrated a low frequency (3.2%) of early bacterial co-infection in patients hospitalized with COVID-19, suggesting early broad-spectrum antibacterials may not be warranted. Hence, antimicrobial stewardship initiatives to review unnecessary antibacterial use remain important.

Secondly, intravascular catheters are a well-recognised risk factor for candidaemia[5] and over 90% of our patients had these. The incidence of candidaemia observed warrants further consideration, and whilst not compared to pre-COVID-19 incidence,[2] may potentially reflect pandemic unique challenges. Examples include increased ICU capacity, redeployment of less-experienced staff to ICU, challenges to aseptic technique with personal protective equipment (PPE), and patients requiring re-positioning to improve oxygenation, thus increasing possibility of line displacement/contamination. Improved aseptic intravascular catheter training focusing on PPE may be beneficial.

In addition, although we were unable to identify urinary catheters as a source in our cohort, they are a recognized risk factor for candidaemia[6] and all patients in our cohort had these.

One patient died prior to candidaemia notification. Time to blood culture positivity may be delayed, particularly for non-albicans candidaemias,[8] and delay in treatment is known to increase mortality,[9] therefore, non-culture-based diagnostics such as galactomannan antigen and BDG should be combine with clinical data to aid diagnosis.[10] 54.4% ($n=6$) of the patients were tested for BDG, and of those, 50% ($n=3$) were positive. Although not possible to demonstrate in this patient cohort, an early positive BDG may herald invasive fungal infection, enabling timely initiation of empirical antifungal therapy.

Guidelines for management of candidaemia recommend a care bundle, including repeat blood cultures at 48 h, echocardiogram, and fundoscopy to identify disseminated infection. In our cohort, only 54.5% (6/11) of patients had repeat blood cultures within 48 h, 72.7% (8/11) an echocardiogram and only 9.1% (1/11) fundoscopy. COVID-19 infection control concerns, patient positioning and PPE, with resultant challenges to ophthalmic examination, may account for the poor fundoscopy compliance, adding further weight to the need for COVID-19 specific practical training.

To conclude, during the ongoing COVID-19 pandemic it remains important to consider modifiable risk factors for candidaemia, non-culture based diagnositics to aid early diagnosis, as well as adherence to established treatment bundles.

Ethics

Ethical approval was not required for this service evaluation and audit of practice.

Authors' contributions

SD, AR and NM designed the study methodology. SD, TE and XG collated the data. SD drafted the initial manuscript with all authors contributing significantly to revising this for submission. All authors agreed on the final version for submission to the journal.

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Declaration of Competing Interest

EC has been paid for consultancy fees by bioMerieux.

SH reports personal fees from Pfizer and Shionogi.

DAJ holds share options in Pulmocide Ltd and has received research grants from Pulmocide Ltd, Gilead Sciences, Astellas and Pfizer. He has received speaker and consultancy fees from AstraZeneca, Pfizer, Gilead, and Astellas.

LSPM has consulted for DNAelectronics (2015–18), Dairy Crest (2017–2018), Umovis Lab (2020), bioMerieux (2013–2020), received speaker fees from Profile Pharma (2018) and Pfizer (2018–2020), received research grants from the National Institute for Health Research (2013–2020), CW+ Charity (2018–2020), and Leo Pharma (2016), and received educational support from Eumedica (2016–2018).

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Decrease in norovirus infections in Germany following COVID-19 containment measures

Dear editor,

We read with interest the recent systematic review by Fricke et al., showing that the number and positivity rate of influenza cases have decreased in result of non-pharmaceutic interventions targeted at the COVID-19 pandemic.[1] Similarly, a report in the United States had shown that the incidences of acute otitis media and streptococcal pharyngitis decreased, while gonorrhea increased during quarantine.[2] These studies show that COVID-19 containment measures and the overall behavioral changes in the communities are likely to have an effect in the transmission and/or reporting of other infections. We here show the results of Norovirus (NoV) surveillance data in Germany, and describe the effect of the containment measures taken in the context of the COVID-19 pandemic on the number and rate of NoV-positive tests in Germany.

NoV is the leading cause of acute gastroenteritis (AGE) globally across all age groups, causing an estimated 18% of all diarrheal disease cases worldwide,[3] and over 200,000 deaths every year.[4] In Germany, NoV is notifiable to the Robert Koch Institute, which has registered nearly 100,000 cases of NoV notified infections every year since 2010 (https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2020/24/Art_01.html). NoV hospitalizations account for 11–16% of all AGE hospitalizations, and they show a seasonal distribution with a peak from December-March each year.[5] NoV diagnosis is related to reimbursement rates for gastroenteritis hospitalization in Germany, providing a strong incentive to test for NoV in the hospital setting. Taking advantage of the routine testing in one of the largest commercial laboratories, a surveillance study was designed to provide up-to-date evidence on the occurrence of NoV across all ages, circulating genotypes and co-infections in Germany.

The 2020 COVID-19 pandemic has triggered the implementation of different containment measures across the globe. Germany reported the first cases in late January[6] and responded by implementing community mitigation and mobility restriction measures since the first COVID-related deaths were reported in March.[7] From mid-March until early May, schools and bars were closed, borders with neighboring countries were controlled, travel was restricted, and general social distancing measures were adopted. With the mandatory use of masks in place since the end of April, reopening was gradual, but several limitations were still in place by the date this article was submitted.[7]

This study is based on a larger prospective, laboratory-based surveillance study on NoV infection. The surveillance relies upon results from all clinical specimens tested for NoV and other enteropathogens submitted to the Limbach Laboratory (MVZ Dr. Limbach & Kollegen GbR, Heidelberg MVZ), from patients of all ages and all genders. The Limbach Laboratory tests samples referred by

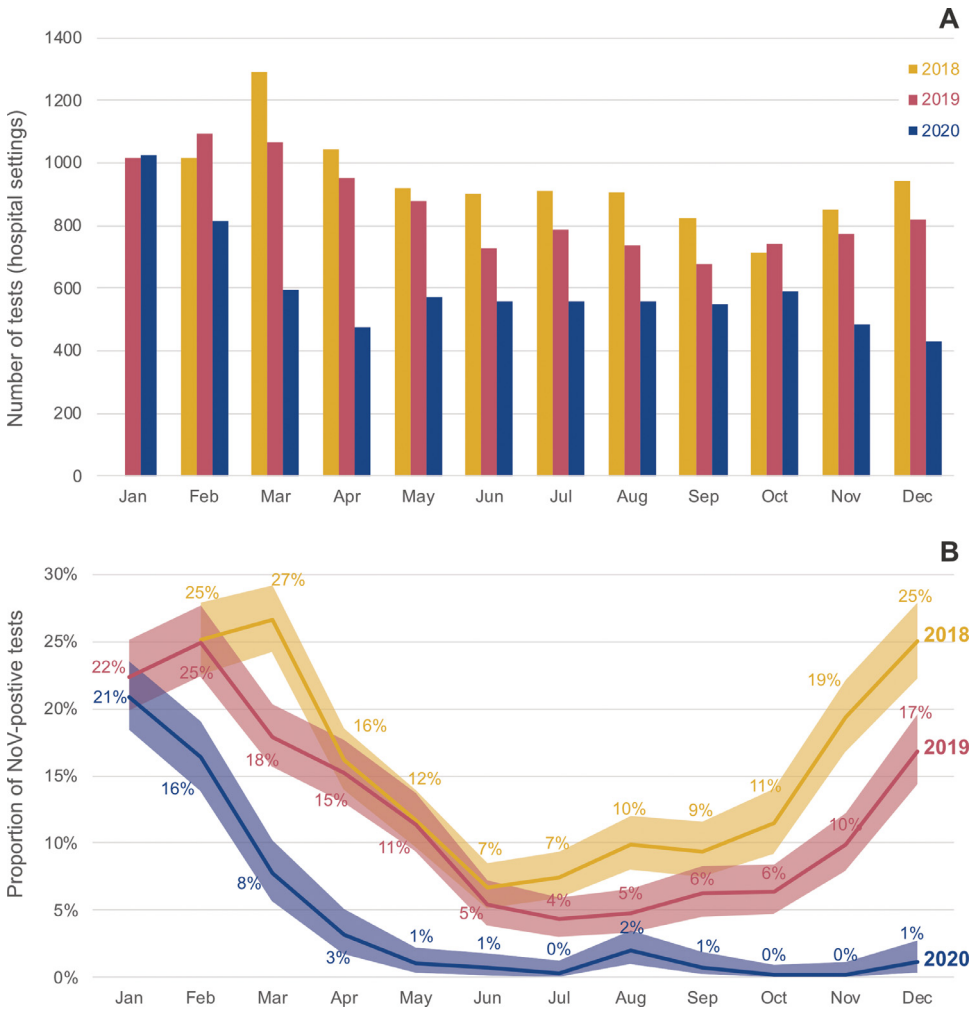

Fig. 1. Monthly distribution of total tests performed (A) and proportion of NoV-positive samples (B) among hospitalized patients, from February 2018 to December 2020. The shaded areas represent 95% confidence intervals.

hospitals across all of Germany. These samples can originate from all age groups, all types of inpatients across various departments as well as outpatients whose specimens are sent to the laboratory for testing. Here we report data from the start of the surveillance (February 2018) to December 2020. Data collected includes aggregated totals of samples tested for NoV and PCR testing results. Data is provided per setting (inpatients or outpatients) and per age group, although we only report totals for all ages. To analyze trends in NoV gastroenteritis, we summarized monthly positivity rates of NoV per setting. The proportions of positives were estimated with exact 95% confidence intervals using R v.4.0.2.[8]

From February 2018 to December 2020, 31,765 specimens were tested for NoV and other enteropathogens. Most specimens (27,795, 87.5%) were collected from hospitalized patients (reasons for hospitalization are unknown). Of all specimens tested, 3970 (12.5%) yielded positive PCR results for NoV. The overall percentage of NoV-positive specimens was similar in the hospital (12.4%) and outpatient (12.8%) settings.

Fig. 1 shows the monthly distribution of stool samples tested for NoV, and the NoV-positive proportion among hospitalized patients. The distribution among outpatients was similar, but with larger confidence intervals due to the smaller sample size (data not shown). As expected, a strong seasonal effect is visible in the proportion of NoV-positive specimens in the years 2018 and 2019, with the proportion of NoV positives increasing from November/December until March. In these two years, the lowest proportion of NoV-positive tests were observed in July (4.3%) and August (4.8%) 2019 (Fig. 1). The overall number of tests performed in the years 2018 and 2019 shows a weaker seasonal pattern, with the number of tests never below 600 per month.

In 2020, the percentage of NoV-positive specimens decreased sharply after January, reaching near 0% as of May and continuing around 0% thereafter. The total number of samples tested for NoV also decreased from February to May, but never went below 400 per month.

The surveillance data suggest a significant impact of the COVID-19 control measures on the NoV positivity rates among stool samples from patients hospitalized in Germany after January 2020. As previously reported for Germany,[5] the number of NoV hospitalizations typically starts to increase in November-December, until the peak is reached in January-March. In the 2019–2020 season, the peak in January was followed by a steep decrease in the number of tests and proportion of NoV-positives. The months of February to May 2020 have registered a significantly lower proportion of NoV-positive specimens than in previous years, until they almost disappeared from May onwards.

Starting in March 2020, Germany has adopted several measures to contain the COVID pandemic, including closure of schools, bars and large events. At the same time, the population adopted preventive behaviors such as social distancing measures and the use of hand sanitizer. It is safe to assume that these measures could result in a decrease of other infections. NoV is highly contagious via the fecal-oral route, through contaminated hands or by consumption of contaminated food and water, giving rise to frequent outbreaks in institutions or restaurants.[9] The sharp decrease in the proportion of NoV-positive cases observed in 2020 is likely related to the closure of schools, restaurants and other institutions, as well as of other containment measures. Behavioral changes preceding the containment measures, which only became effective in March, may have caused the decrease in the NoV positivity rates to start already in February, triggered by reports from Italy or Spain. In fact, the number of cases exploded in Italy from February 22nd. On March 10th, when Germany reported its first two COVID-related deaths, Italy already counted 464 deaths and over 9000 cases, and Spain had over 2000 reported cases.[6]

Our study has some limitations. We do not possess clinical information on the patients whose samples were tested. Though this is unlikely, we cannot exclude that patients infected with norovirus presented less frequently to medical care than other AGE patients in 2020. Our analyses are ecological in nature and we can also not exclude that there is a natural decline of NoV circulation, independent of the COVID-19 control measures. However, since NoV is known to be transmitted primarily via person-to-person contacts, it is fair to assume the control measures have played a major role in this decline. Finally, it is unknown whether NoV incidence will return to pre-COVID-19 values once behavioral restrictions are relaxed.

This study shows that NoV infections have become less frequent in Germany since the beginning of the COVID-19 epidemic in Europe. Whereas this is a positive observation from a public health perspective, it also has a significant impact on NoV vaccine development programs. Several NoV vaccines are under development, with one vaccine entering Phase II trials.[10] The COVID-19 pandemic hinders the conduct of these trials by the logistical challenges in enrolling and following up subjects, and Phase III trials are unlikely to show efficiency due to the current low NoV positivity rates. It is unknown how NoV infections will evolve once containment measures are loosened.

Declaration of Competing Interest

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Early Lopinavir/ritonavir does not reduce mortality in COVID-19 patients: Results of a large multicenter study ☆

Dear Editor,

The COVID-19 pandemic, an unprecedented event for current generations of physicians, has stricken hard on society.[1] There is a significant lack of effective drugs for stopping viral replication. Lopinavir/ritonavir (LPV/r) is a well-known combination used in patients with HIV which was included in the arsenal against SARS-CoV-2 early in the pandemic.[2] Its use in COVID-19 was based on inconsistent results from experimental and clinical research that was mostly done while investigating other β-coronaviruses (SARS and MERS).

A number of randomized clinical trials have observed no benefit of LPV/r beyond the standard of care.[3–5] However, voices have been raised against interpreting these results as grounds from definitively ruling out LPV/r since some of these studies lacked statistical power, reported encouraging outcomes in secondary endpoints, and included patients with a prolonged period of symptoms before initiation of treatment.[6,7] Indeed, there may be a subpopulation of COVID-19 patients – notably those early in the course of the infection – for whom LPV/r may improve their prognosis. In a recent report, Klement-Frutos et al. describe a favorable outcome of patient with COVID-19 after beginning of LPV/r on day 9 of symptoms.[8] Therefore, we aimed to assess the efficacy of LPV/r in a large, multicenter cohort of patients, with special interest in those who received treatment soon after the onset of symptoms.

This work belongs to the SEMI-COVID-19 Registry, which is an ongoing, nationwide, retrospective, anonymized cohort of consecutive adult patients hospitalized in Spain for microbiologically confirmed COVID-19.[9] The Registry was approved by the Ethics Committees of the participating centers, and included data on over 300 variables. The primary endpoint was raw-in hospital mortality at 30 days from admission. Patients were considered to have been treated with LPV/r if they had received at least one dose of the drug. Common dosage of LPV/r was 400/100 mg bid.

In order to mitigate the effects of possible confounding variables in a non-randomized assessment of treatment with LPV/r, propensity score (PS) was performed. The propensity of receiving LPV/r was estimated using a logistic regression model that included confounding variables which could have affected treatment choice or outcomes as independent variables. The nearest neighbor method with a caliper of 0.1 as used in PS matching and standardized mean differences (SMD) were calculated to evaluate adequacy of propensity matching. Both conditional logit and mixed effects logistic regressions were performed. Furthermore, univariate and multivariate logistic regression models were fitted in order to estimate the treatment effect using all data, as a sensitivity analysis. Multiple imputation was used to handle missing data and model estimates and standard errors were calculated using Rubin's rules.[10] Statistical analyses were performed using R software (v.3.6.2).

As of June 1, 2020, the Registry included 9,594 cases, of which 8,553 met the inclusion criteria (Suppl. Fig. 1). Fifty-seven percent were men, median age was 69 years (IQR 56–79), and half of subjects (50.2%) had high blood pressure. Patients were admitted after a median time since symptoms onset of 7 days (IQR 4–9), with median SaO_2/FiO_2 ratio of 376 (IQR 300–452), C-reactive protein of 58 mg/L (IQR 19–123), and lymphocytes of 940 cells/µL.

LPV/r was administered to 5,396 patients (63%) after a median of 0 days since admission (IQR 0–1). Table 1 shows that LPV/r was more likely to be prescribed to patients who presented with more severe clinical condition, including presence of fever, cough, radiological infiltrates (91.7% vs 80.4% $p<0.001$) and a lower SaO_2/FiO_2 ratio. On the other hand, LPV/r was less frequent among at-risk subjects in whom toxicity may be more likely: elderly patients presenting with altered mental status, dementia, or other debilitating baseline conditions, as well as patients on immunosuppressive drugs and pregnant women.

Overall, 1,509 patients died (17.6%). The univariate parameters predicting mortality is shown in Table 2. A PS allowed for comparing two cohorts with similar values on the parameters associated with the prescription of LPV/r (Table 1). Most parameters were adequately matched according to SMD, although some variables had SMD values >0.02 (Suppl. Table 1): In this matched cohort, the adjusted odds ratio (aOR) for mortality for the use of LPV/r was 0.932 (95CI 0.799–1.087; $p>0.05$) according to both conditional and mixed effects logistic models.

Of the 6,099 patients who were admitted to hospital within 8 days since onset of symptoms (median time to admission since onset of symptoms 5 days [IQR 3–7]), LPV/r was prescribed to 3,377 (55%). Variables associated with the use of LPV/r were similar to those observed in the cohort as a whole (Suppl. Table 2). In a propensity score matching carried out on this subset of patients, early use of LPV/r was not associated with a lower mortality (conditional logistic regression: aOR 1.110 (95CI 0.944–1.300; $p=0.245$); mixed effects logistic regression: aOR 1.105 (95CI 0.944–1.300; $p=0.272$)).

Consistent with previous studies, our analysis found no overall benefit to the use of LPV/r.[3–5] We have focused on patients who received the antiviral at an earlier stage in the hope of finding greater activity. Indeed, in other viral diseases, the administration of antiviral drugs must be done as soon as possible in order to have a clinically significant activity.[11] Of note, patients included in Rao's clinical trial had a median duration of symptoms of 13 days (IQR 11–16) [4], and those recruited in the RECOVERY trial presented after 8 days of disease (IQR 4–12).[3] In our sub-analysis, the median duration was 5 days (IQR 3–7), thus allowing us to perform a evaluation on patients who were indeed at a very early

☆ A complete list of the SEMI-COVID-19 Network members is provided in the Appendix.

Table 1
– Baseline characteristics and clinical presentation of all patients according to the administration of LPV/r.

	Unmatched data ($n = 8,553$)			Matched data ($n = 5,068$)		
	No LPV/r ($n = 3,157$)	LPV/r ($n = 5,396$)	p	No LPV/r ($n = 2,534$)	LPV/r ($n = 2,534$)	p
Baseline features						
Age (years)†	74.9 [60.4;85.2]	66.2 [54.3;75.8]	<0.001	70.8 [57.2;81.5]	69.5 [56.6;78.7]	<0.001
Sex (female)	1511 (47.9%)	2155 (39.9%)	<0.001	1124 (44.4%)	1083 (42.7%)	0.257
Pregnancy	22 (0.70%)	15 (0.28%)	0.008	18 (0.71%)	13 (0.51%)	0.471
Race (Caucasian)	2850 (91.2%)	4647 (87.9%)	<0.001	2268 (89.5%)	2279 (89.9%)	0.644
Charlson Comorbidity Index	1.00 [0.00;2.00]	0.00 [0.00;1.00]	<0.001	1.00 [0.00;2.00]	1 [0.00;2.00]	0.626
High blood pressure	1817 (57.7%)	2480 (46.1%)	<0.001	1357 (53.6%)	1288 (50.8%)	0.056
Immunosuppressive therapy	257 (8.14%)	291 (5.39%)	<0.001	205 (8.09%)	186 (7.34%)	0.040
Dementia	628 (20.0%)	199 (3.70%)	<0.001	171 (6.75%)	179 (7.06%)	0.009
Clinical presentation						
Duration of symptoms (days) †	6.00 [3.00;9.00]	7.00 [4.00;9.00]	<0.001	6.00 [3.00;9.00]	7.00 [4.00;9.00]	0.337
Cough	2170 (69.2%)	4281 (79.6%)	<0.001	1892 (74.7%)	1918 (75.7%)	0.678
Dyspnea	1787 (56.9%)	3108 (57.9%)	0.370	1439 (56.8%)	1440 (56.8%)	1.000
Altered mental status	574 (18.4%)	351 (6.58%)	<0.001	262 (10.3%)	249 (9.83%)	0.576
Temperature	36.9 [36.3;37.6]	37.1 [36.4;37.9]	<0.001	36.9 [36.3;37.7]	37.0 [36.4;37.8]	0.013
Heart rate (beats/min) †	86.0 [75.0;98.0]	88.0 [77.0;100]	<0.001	86.5 [76.0;99.0]	87.0 [76.0;99.0]	0.816
Respiratory rate > 20 breaths/min	958 (31.2%)	1558 (29.7%)	0.155	728 (28.7%)	735 (29.0%)	0.852
Laboratory						
SaO_2/FiO_2 †	387 [304;452]	372 [297;452]	<0.001	392 [307;457]	381 [304;452]	0.008
Lymphocytes (cells/μL) †	990 [700;1330]	910 [700;1260]	0.080	1000 [700;1320]	940 [700;1300]	0.042
C-reactive protein (mg/L) †	55.0 [17.0;120]	59.9 [19.8;125]	0.002	55.7 [17.1;120]	55.9 [18.0;119]	0.391
Creatinine (mg/dL) †	0.93 [0.75;1.23]	0.89 [0.73;1.10]	<0.001	0.90 [0.74;1.17]	0.90 [0.74;1.14]	0.718
Other treatments						
Interferon-β	52 (1.65%)	1073 (20.1%)	<0.001	52 (2.05%)	78 (3.08%)	0.049
Hydroxychloroquine	2299 (72.8%)	4893 (90.7%)	<0.001	2084 (82.2%)	2133 (84.2%)	0.071
Remdesivir	17 (0.54%)	26 (0.48%)	0.842	12 (0.47%)	12 (0.47%)	1.000
Mortality	688 (22.8%)	821 (15.7%)	<0.001	431 (17.0%)	406 (16.0%)	0.364

† Continuous variables expressed as median and [interquartile range]. LPV/r: lopinavir/ritonavir.

Table 2
– Univariate analysis of mortality in all patients and those with duration of symptoms of less than 8 days.

	All ($n = 8,553$)		Early cohort ($n = 6,099$)	
	OR (95CI)	P	OR (95CI)	p
Sex (female)	0.783 (0.698–0.879)	<0.001	0.775 (0.681–0.882)	<0.001
Age (per year)	1.090 (1.084–1.096)	<0.001	1.082 (1.076–1.089)	<0.001
Race (Caucasian)	3.267 (2.499–4.272)	<0.001	3.715 (2.734–5.047)	<0.001
High blood pressure	2.833 (2.509–3.200)	<0.001	2.700 (2.354–3.097)	<0.001
Dementia	4.323 (3.715–5.031)	<0.001	3.704 (3.137–4.374)	<0.001
Immunosuppressive treatment	1.747 (1.431–2.134)	<0.001	1.656 (1.328–2.066)	<0.001
Charlson Comorbidity Index (per point)	1.334 (1.296–1.373)	<0.001	1.307 (1.265–1.349)	<0.001
Duration of symptoms (per day)	0.930 (0.918–0.943)	<0.001	0.907 (0.886–0.928)	<0.001
Cough	0.652 (0.576–0738)	<0.001	0.703 (0.612–0.806)	<0.001
Temperature (per °C)	1.088 (1.026–1.154)	0.005	1.069 (1.000–1.141)	0.049
Dyspnea	1.865 (1.653–2.104)	<0.001	1.828 (1.597–2.091)	<0.001
Confusion	5.341 (4.620–6.174)	<0.001	4.433 (3.774–5.208)	<0.001
Respiratory rate > 20 breaths/min	3.396 (3.020–3.819)	<0.001	3.239 (2.838–3.696)	<0.001
SaO_2/FiO_2 (per unit)	0.993 (0.992–0.993)	<0.001	0.993 (0.992–0.993)	<0.001
Lymphocytes (per cells x 10^3/μL)	1.000 (1.000–1.000)	0.424	1.000 (1.000–1.000)	0.470
C-reactive protein (per mg/L)	1.005 (1.005–1.006)	<0.001	1.006 (1.005–1.006)	<0.001
Creatinine (per mg/dL)	1.789 (1.651–1.938)	<0.001	1.647 (1.515–1.792)	<0.001
Treatment with Hydroxychloroquine	0.447 (0.391–0.512)	<0.001	0.483 (0.416–0.562)	<0.001
Treatment with Interferon-β	2.094 (1.813–2.420)	<0.001	2.098 (1.787–2.463)	<0.001
Treatment with Remdesivir	1.096 (0.507–2.366)	0.816	0.871 (0.360–2.109)	0.760
Treatment with LPV/r	0.651 (0.581–0.729)	<0.001	0.705 (0.620–0.801)	<0.001

LPV/r: lopinavir/ritonavir. OR: odds ratio. 95CI: 95% confidence interval.

stage of disease. However, results were again disappointing, and add another nail in the coffin of LPV/r when considering its use for COVID-19.

Our study has some limitations. First, it has the biases inherent to retrospective observational studies. Also, despite the fact that the number of patients included allowed us to perform PS matching, which may have reasonably controlled for many of these biases, the balance of some parameters was not perfect according to SMD values. Still, as a multicenter study involving a large number of hospitals, it has the strength of being rooted in real-life practice, far from strict inclusion and exclusion criteria of clinical trials. Second, we have used mortality as a primary endpoint, as others have done, but we cannot rule out any benefits of LPV/r that would have emerged had we analyzed softer outcomes, such as time to improvement or disease duration, as suggested by the report of Klement-Frutos et al.[8] Finally, our analysis has used data from COVID-19 first wave in Spain, when efficacy of corticosteroids or other drugs was not yet proved. Thus, our analysis is not adjusted for these treatments. However, these therapies, at least during the first wave of the pandemic, have usually been reserved for severe patients, and thus may be surrogate predictors of unfavorable progress.

In conclusion, we have analyzed a large, multicenter cohort of patients with COVID-19 and have not found any benefits to administering LPV/r, even when it was administered within the first 8 days of symptoms. Our results discourage its use in SARS-CoV-2 infection.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.02.011.

Appendix

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Appendix

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Oral corticoid, aspirin, anticoagulant, colchicine, and furosemide to improve the outcome of hospitalized COVID-19 patients - the COCAA-COLA cohort study

Dear Editor,

Corticosteroids mitigate 28-day all-cause mortality in coronavirus disease-2019 (COVID-19) patients requiring oxygen or mechanical ventilation (meta-analysis summary odds ratio (OR), 0.66; 95%-confidence interval (95%IC), [0.53–0.82]; $P < 0.001$); however, mortality remains high (32.7%).[1] In a previous observational cohort study, we established that an early 4-day treatment combining corticosteroid (prednisolone dose equivalent, 1.25 mg/kg/24 h) and furosemide (80 mg/day) was effective in reducing the need for mechanical ventilation and overall mortality (OR, 0.35 [0.11–1.01]; $P = 0.04$) in non-critically ill COVID-19 patients.[2]

The GRECCO-19 randomized trial suggested a benefit of colchicine in preventing clinical deterioration in hospitalized non-critically ill COVID-19 patients.[3] Similarly, an observational cohort study reported that salicylate treatment was associated with reduction in intensive care unit (ICU) and mechanical ventilation requirements in hospitalized COVID-19 patients, although in-hospital death was not significantly modified.[4] Moreover, prophylactic or intermediate-dose anticoagulation was highly recommended in hospitalized COVID-19 patients who are at high-risk of venous thromboembolic events (VTE).[5] Specifically, direct oral anticoagulant use was shown to be associated with improved outcome.[6]

Table 1
Characteristics of the COVID-19 patients treated or not treated with the five-drug regimen combining prednisone, furosemide, salicylate, colchicine and direct anti-Xa inhibitor. Data are presented as percentages or medians [percentiles 25th-75th]. Comparisons were performed using Mann-Whitney or Fisher exact tests, as appropriate.

	Patients not receiving the five-drug regimen (*N* = 40)	**Patients receiving the five-drug regimen (*N* = 28)**	***P***
Demographics and past medical history			
Age (year)	64 [49–73]	68 [62–78]	0.06
Male gender, N (%)	33 (83)	20 (71)	0.37
Body-mass index (kg/m²)	28 [25–31]	26 [24–28]	0.13
Hypertension, N (%)	16 (40)	15 (54)	0.32
Diabetes mellitus, N (%)	15 (38)	15 (54)	0.22
Cardiovascular disease, N (%)	11 (28)	9 (32)	0.78
Chronic lung disease, N (%)	1 (3)	1 (4)	1
Clinical and biological parameters on admission			
Symptom duration (day)	8 [4–11]	8 [7–10]	0.99
4C Mortality Score	9 [6–12]	10 [9–12]	0.08
SpO_2 at room air (%)	92 [91–96]	94 [91–95]	0.65
PaO_2 at room air (mmHg)	63 [58–72]	65 [58–74]	0.72
Crazy paving area on CT-scan (%)	50 [25–50]	50 [25–50]	0.75
Proximal/segmental pulmonary embolism diagnosed on CT-scan, N (%)	4 (10)	1 (4)	0.64
C-reactive protein (mg/L)	97 [60–165]	86 [61–126]	0.68
Procalcitonin (µg/L)	0,14 [0.06–0.25]	0,11 [0.07–0.22]	0.73
White blood cells (G/L)	7.0 [4.9–9.5]	6.3 [4.8–7.4]	0.28
Lymphocytes (G/L)	1.0 [0.7–1.2]	0.9 [0.7–1.2]	0.88
Brain natriuretic peptide (ng/L)	19 [10–52]	38 [13–111]	0.12
Brain natriuretic peptide ≥ 100 ng/L	8 (20)	8 (29)	0.56
Troponin Ic high-sensitivity (ng/mL)	9 [4–20]	9 [4–31]	0.90
D-dimer (ng/mL)	935 [578–1402]	870 [528–1575]	0.92
Serum creatinine (µmol/L)	85 [71–105]	86 [68–111]	0.81
Estimated Glomerular filtration (mL/min)	78 [59–94]	74 [49–91]	0.47
Additional treatments			
Prophylactic/therapeutic anticoagulant, N (%)	40 (100)	28 (100)	1
Therapeutic anticoagulant, N (%)	8 (20)	9 (32)	0.27
Aspirin, N (%)	8 (20)	28 (100)	< 0.0001
Colchicine, N (%)	0 (0)	28 (100)	< 0.0001
Furosemide, N (%)	4 (10)	28 (100)	< 0.0001
Antibiotics, N (%)	29 (73)	17 (61)	0.43
Outcomes			
Invasive or non-invasive mechanical ventilation, high-flow oxygen therapy or 28-day death, N (%)	18 (45)	2 (7)	0.0009
Maximal oxygen flow (L/min)	6 [3–11]	3 [2–4]	0.002
High-flow oxygen therapy, N (%)	5 (13)	1 (4)	0.38
Non-invasive mechanical ventilation, N (%)	5 (13)	0 (0)	0.07
Invasive mechanical Ventilation, N (%)	6 (15)	1 (4)	0.21
28-day death, N (%)	2 (5)	0 (0)	0.5
Length of hospital stay (days)	7 [4–9]	7 [6–9]	0.28

Based on the data discussed above and the pathophysiology of COVID-19 and its complications, i.e. thrombosis, inflammation and congestion, we hypothesized that a five-drug regimen consisting in a 5-day course of 1 mg/kg/day prednisone, 80 mg/day furosemide, 75 mg/day salicylate, colchicine (1 mg loading dose followed by 0.5 mg one hour later then 0.5 mg every 8 h as recommended to treat acute gout)[7] and direct anti-Xa inhibitor such as rivaroxaban or apixaban would optimally mitigate COVID-19-attributed mortality. To address the effectiveness of this five-drug regimen, we designed an observational cohort study (COrtiCoid-Aspirin-Anticoagulant-Colchicine-LAsix®, the COCAA-COLA study) including all successive non-critically ill COVID-19 patients requiring >1 L/min-oxygen and admitted to our ward between 2020/01/09 and 2020/11/30 (during the second wave in France). Patients who did not receive this regimen were treated with dexamethasone (6 mg once daily for up to 10 days)[8] and low-molecular weight heparin (control group). All patients received standard of care, i.e. oxygen with flow adapted to oximetry, proton pump inhibitor, antibiotics, insulin, potassium supplementation and loperamide if needed. No antiviral or additional immunomodulatory therapy was used due to the absence of clearly demonstrated benefit. Systematic chest computed tomography angiography was performed on admission if not contra-indicated. Anticoagulants (direct anti-Xa inhibitor in the five-drug regimen-treated patients or low-molecular weight heparin in the others) were administered at prophylactic dose with the exception of patients exhibiting VTE or plasma D-dimer ≥5000 ng/mL (a threshold predicting increased VTE risk in COVID-19 patients)[9] who were administered anticoagulants at therapeutic dose. Usual monitoring including pulse oximetry, electrocardiogram, finger blood sugar and daily routine chemical tests was provided.

The primary composite endpoint was requirement of high-flow oxygen therapy, non-invasive or invasive mechanical ventilation (corresponding to care escalation from ward to ICU) or 28-day mortality. The 4C Mortality Score, a risk stratification score for hospitalized COVID-19 patients, was used to predict in-hospital mortality.[10] Data were expressed as median [25th-75th percentiles] or percentages. Univariate comparisons were performed using Mann-Whitney or Fisher exact tests, as appropriate. A multivariate logistic regression model was tested with the five-drug regimen as explanatory variable and adjustment for independent covariates (gender, age, body-mass index and comorbidities) to explain the outcome. Odds ratios (OR) and their 95%CI were determined. Stratified categorical data were compared using Cochran-Mantel-Haenszel tests. *P*-values≤0.05 were considered significant. Analyses were preformed using the R4.0 environment.

We included sixty-eight patients (age, 66years [54–75]; male/female sex-ratio, 3.5; body-mass index, 27 kg/m² [24–30]; hypertension, 46%; diabetes mellitus, 44%; cardiovascular disease, 29%; chronic lung disease, 3%). Twenty-eight patients (41%) re-

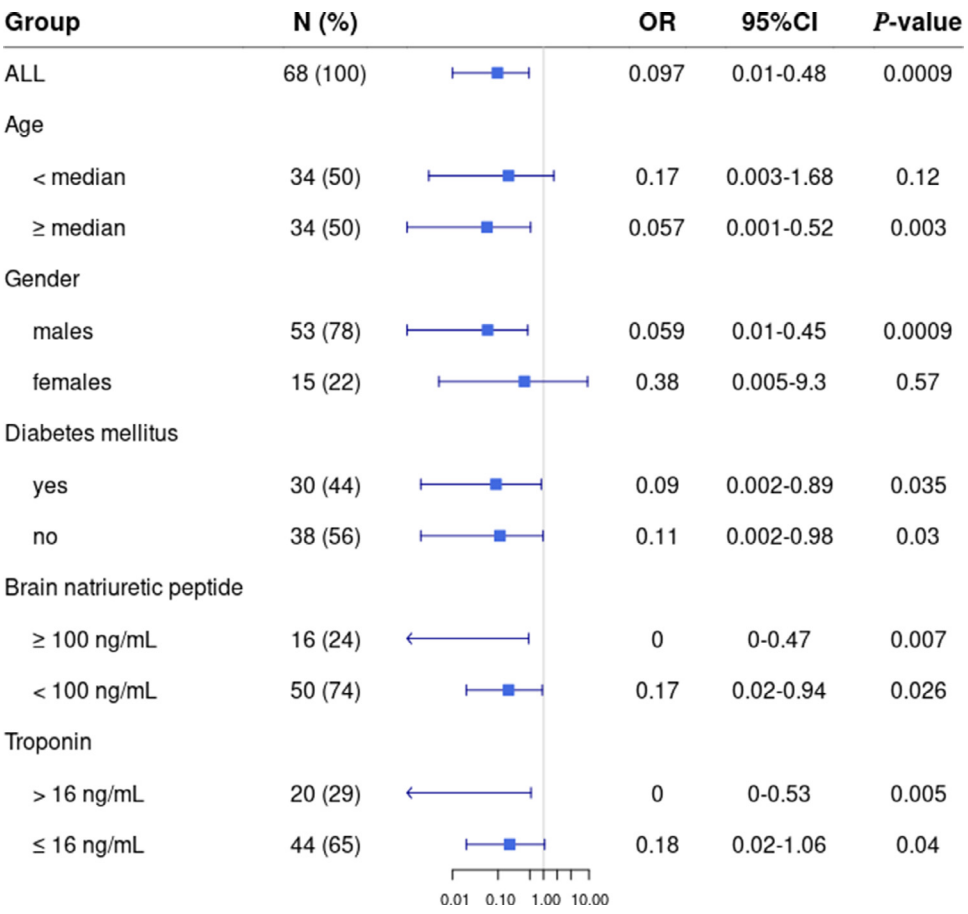

Fig. 1. Impact of the prednisone/furosemide/colchicine/salicylate/direct anti-Xa inhibitor regimen in the different patient subgroups defined according to age (using the median value, 66.5 years, as threshold), gender, presence of diabetes mellitus, serum brain natriuretic peptide (BNP; threshold at 100 ng/mL) and troponin levels (threshold at 16 ng/mL). Odds ratio (OR) and their 95%-confidence intervals were determined . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ceived the five drug-therapy regimen whereas forty (59%) were included in the control group. Based on the 4C Mortality Score (10 [8–12]), predicted mortality on admission was ~30%. No significant differences were observed between the groups regarding the clinical and biological characteristics and the predicted mortality (Table 1). Noteworthy, 4/40 control patients (10%) at risk of cardiogenic pulmonary edema (serum brain natriuretic peptide (BNP) ≥100 ng/mL) received furosemide.

Among patients receiving the five-drug regimen, the incidence of primary composite endpoint was lower than in the control group (OR=0.097 [0.001–0.48], $P=0.0009$). Multivariate analysis confirmed the significant effect of the five-drug regimen on outcome after adjustment for independent covariates, including age, body-mass index, 4C Mortality Score, high serum BNP level and high white blood cell count (OR=0.043 [0.0053–0.21], $P=0.0005$). The model was significant compared to a model without the five-drug regimen ($P < 0.00001$). Additionally, patient subgroups were analyzed following stratification by age (using the median value as threshold), gender and risk factors including diabetes, elevated BNP (threshold, 100 ng/ml) and troponin levels (threshold, 16 ng/mL; Fig. 1). Remarkably, the five-drug regimen was associated with a significant reduction in primary composite endpoint in males only. Additionally, there was a stronger and more significant protective effect of our regimen in patients with elevated-BNP (OR=0.0 [0.0–0.47], $P=0.007$) than in low-BNP patients (OR=0.17 [0.02–0.94], $P=0.03$). Thus, the primary composite endpoint was improved in elevated- versus low-BNP patients ($P=0.0003$). We observed no remarkable adverse effects attributed to the five-drug regimen except mild colchicine-related diarrhea (21%) resolved with loperamide.

The GRECCO-19 trial showed improved time to clinical deterioration in hospitalized COVID-19 patients receiving colchicine; however, the benefit relied on a narrow margin of clinical significance.[3] By adding colchicine to the recommended corticosteroid and anticoagulant, together with aspirin and furosemide, we succeeded in improving the outcome. The five drugs included in our regimen were given orally for a short course, paving the way for an outpatient treatment. Interestingly, the recent COLCORONA trial conducted in non-hospitalized COVID-19 patients supported colchicine-related benefit in reducing hospitalizations, need for mechanical ventilation and mortality.[11]

Colchicine dose regimen differed between the three studies with higher cumulative colchicine doses in the GRECCO-19 (22 mg) and COLCORONA trials (16.5 mg) compared to ours (8 mg). Using the same primary composite endpoint, our five-drug regimen significantly improved prognosis in comparison to the corticosteroid/furosemide combination of our previous study[2] ($P=0.0001$).

In conclusion, our data highlight the benefit and safety of an early short-course oral regimen combining prednisone/colchicine/salicylate/direct anti-Xa inhibitor/furosemide to reduce the risk of high flow oxygen need, mechanical ventilation requirement or 28-day mortality in hospitalized non-critically ill COVID-19 patients. Our preliminary observational findings should be confirmed in larger cohorts.

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

J.-P.K. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Consent for publication

All the authors agree to publish.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Low prevalence of post-COVID-19 syndrome in patients with asthma

Dear Editor,

A significant number of patients suffering from COVID-19 infection refers illness-related symptoms several weeks or months after the acute episode. The so-called post-COVID syndrome or long COVID syndrome includes persistent symptoms that could be the result of residual inflammation, organ damage, non-specific effects from the hospitalization or prolonged ventilation, social isolation or impact on pre-existing health conditions.[1–3] Very recently, Moreno-Perez et al[2] published in *Journal of Infection* a large prospective series of 277 patients (66% of them hospitalized) in a mediterranean population the post-COVID syndrome at about 3 months after diagnosis was detected in 141 individuals (51%), 59% of hospitalized patients and 37% of outpatients.[2] Several series with different follow-up periods have reported the incidence of persistent symptoms ranging from about one third in outpatients and up to 90% in hospitalized patients.[1–6] However, our clinical experience led us to suspect that patients with asthma had post-covid syndrome less frequently. In fact, in patients with preexisting asthma, a lower COVID-19 susceptibility has been reported.[7,8] It is possible that this lower susceptibility also affects the long-term persistence of disease symptoms. For this reason, we evaluated the persistence of symptoms attributed to COVID-19 three months after infection in a series of patients with asthma under follow-up in our department.

In the period between March 3 and December 11, 2020, a total of 2995 patients over 14 years of age tested positive for SARS-CoV-2 (determined by RT-PCR technique) in our health department, 77 of them with asthma (2.6%). Three of these 77 patients were excluded for this study (two of them due to death and one due to lack of follow-up). A total of 74 patients with asthma were periodically surveyed by telephone about their clinical evolution. Symptoms referred at three months were recorded for evaluation. The study was approved by the local institutional ethics board, that authorized the study without written individual patient consent statement due to the characteristics of the disease. Severity of asthma was established according to the prescribed therapy following international GINA recommendations (https://ginasthma.org/): mild (step 1 and 2 of GINA), moderate (step 3 and 4) or severe (step 5). Asthma was classified as allergic, eosinophilic and non-T2. The allergic group included patients with elevated IgE, a positive prick test to pneumo-allergens or seasonal asthma associated with rhinitis. Eosinophilic patients included those who were non-allergic with a blood eosinophil count of more than 300 per millilitre. Patients who did not meet these criteria were classified as non-T2 phenotype.

Of the 74 patients with asthma, 42 were females (57%). The median age was 49 years (interquartile range 34–60). Asthma was mild in 17 (23%) patients, moderate in 52 (70%) and severe in five (7%) (four receiving omalizumab and one benralizumab). Twenty-five patients with asthma (34%) were asymptomatic during SARS-CoV-2 infection, 34 (46%) developed symptoms but did not require hospital admission, and 15 (20%) were hospitalised. Forty-six patients were classified as having allergic asthma, seven as eosinophilic asthma, and 21 as non-T2 asthma. Admitted patients were five allergic, three eosinophil and seven non-T2 phenotypes.

Seven of the 74 (9.5%) patients with asthma infected by SARS-CoV-2 that were followed-up reported post-COVID syndrome at 3 months; none of the 25 asymptomatic patients, 3 of the 34 patients (8.8%) with COVID-19 that not required hospitalization (2 fatigue and one hyposmia), and 4 (27%) of hospitalized patients (cough 2 of them, and dyspnoea and fatigue one patient each symptom). If we only consider symptomatic patients at diagnosis, the prevalence of post-COVID syndrome was 14% (7 of 49). All but one of the 7 patients with post-COVID syndrome were receiving inhaled corticosteroids. Only one patient with post-COVID syndrome was classified as non-T phenotype asthma. Two patients with post-COVID syndrome had mild asthma, 4 moderate and 1 severe.

In our experience, patients with preexisting asthma have a lower prevalence of post-COVID syndrome than that reported among the totality of COVID-19 patients. In addition to the mentioned series by Moreno-Perez et al[2] other experiences with general COVID-19 population reported consistent results. In a series of 110 patients hospitalised with COVID-19, at 8–12 weeks postadmission most (74%) had persistent symptoms (notably breathlessness and excessive fatigue),[4] almost three times as many of our hospitalized asthmatic patients. (27%). In another series with 177 patients with less severe disease (6% asymptomatic, 85% with mild disease and 9% requiring hospitalization) with a median follow-up of 5.6 months after illness onset, one third reported at least one symptom (8.8% in our moderate cases), the most common, fatigue.[6]

The reason for this lower prevalence of post-COVID syndrome in asthma could theoretically be related to immune characteristics of the patients or to treatment. In fact, in-vitro studies have shown that inhaled glucocorticoids reduce the replication of SARS-CoV-2 in airway epithelial.[9] However, almost all our symptomatic patients were receiving this drug.

Our study has several limitations. Patients, of one single centre, were followed up by telephone and no face-to-face interview was conducted. In addition, it is possible that the patients did not report mild symptoms or psychological disturbances. This implies that it is an exploratory study that needs to be confirmed.

In conclusion, we have found that our patients with asthma have a low prevalence of persistent symptoms at three months of onset of COVID-19. It seems of interest to confirm this finding in other centres and with larger samples and to analyse its possible causes.

Declaration of Competing Interest

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Incidence of delayed asymptomatic COVID-19 recurrences in a 6-month longitudinal study

Dear Editor,

It has recently been suggested that prior SARS-CoV-2 infection is associated with protection against symptomatic reinfection [1,2]. The role of protective immunity after COVID-19 has been assessed in population-based and cohort studies, where symptomatic recurrences with positive SARS-CoV-2 RT-PCR results were investigated [2,3], usually lacking genomic sequencing to confirm reinfection. However, limited data are available to date about the frequency of long-term asymptomatic reinfections and/or recurrences. Because of their confirmed transmission risk [4], asymptomatic infections also have significant epidemiologic implications in terms of public health control. To answer this question, longitudinal studies with sequential sampling following SARS-CoV-2 infection would be required, ideally including sequencing of viral genomes to discern between reinfection and disease recurrence. Recently, the Centers for Disease Control (CDC) have proposed an investigation protocol for identifying cases with a high index of suspicion for reinfection [5], that prioritizes new detection of SARS-CoV-2 RNA $\geq$90 days since first infection, whether or not symptoms are present, availability of paired respiratory specimens with a RT-PCR cycle threshold (Ct) value $<$33, and genomic sequencing to confirm reinfection. An acknowledged limitation of the protocol consists in the exclusion of asymptomatic or mildly symptomatic individuals who never seek testing for SARS-CoV-2. We conducted a prospective study in a cohort of patients hospitalized for microbiologically-confirmed COVID-19 in the first wave, who were longitudinally followed-up during a 6-month period with sequential nasopharyngeal and blood sampling. We evaluated the incidence of late reinfections and recurrences, both symptomatic and asymptomatic, and validated the CDC predictive criteria to identify late reinfections occurring in our cohort by genomic sequencing of the suspected cases. Blood and nasopharyngeal samples were obtained during hospital stay and at 1, 2 and 6 months after patients' discharge for measuring antibody levels and SARS-CoV-2 RNA. IgG antibody plasma levels against the SARS-CoV-2 internal nucleocapsid protein (N-IgG) and the spike protein (S-IgG) (Anti-SARS-CoV-2 IgG ELISA, Euroimmun, Lubeck, Germany) were tested, and SARS-CoV-2 RNA was detected by RT-PCR (AllplexTM 2019-nCoV Assay, Seegene, Seoul, Korea) which targeted the E, RdRP, and N genes. Genome sequencing of SARS-CoV-2 was performed on nasopharyngeal samples following ARTIC amplicon sequencing protocol for MinIon version V3- Phylogenetic analysis was done using webserver Nextstrain (https://nextstrain.org/), with the SARS-CoV-2 database Nextclade (https://clades.nextstrain.org/).

146 patients admitted for COVID-19 were followed-up. Median age was 64 years, 88 (60.3%) were male, and 72.6% had coexisting comorbid diseases. SARS-CoV-2 shedding lasted a median (Q1-Q3) of 13 (2.2–33.8) days, median (Q1-Q3) time from illness onset to seropositivity was 12 (8–15) days, and peak S-IgG was 5.9 (0.3–7.1) absorbance/cut-off (S/CO) and peak N-IgG 4.1 (0.3–4.9) S/CO. At 1 month after discharge, 40/146 (27%) subjects tested positive for SARS-CoV-2 RNA; 15/127 (11.8%) at 2 months, and 5/134 (3.7%) at 6 months. We analyzed the 5 patients with positive RT-PCR occurring more than 90 days since first COVID-19 diagnosis (Table 1). Median (range) time from diagnosis to new detection of SARS-CoV-2 RNA was 183 (167–204) days. Cases included 3 men, with ages ranging from 44 to 73 years, and 3 of them had subjacent comorbidity. Two patients were readmitted to hospital at re-positivity, and 3 patients remained asymptomatic. Only one patient had a Ct$<$33, and in the other four patients the Cts ranged from 33 to 38. Genomic sequencing was performed in 4 individuals with available paired samples. In the three patients with Ct$\geq$33, all of them asymptomatic, the same clade 20B was detected. In two of them, the clade showed the same hallmark single nucleotide variants. In the third patient, the follow-up sample showed two new mutations, a K374R substitution in the N gene and an A222V substitution in the S gene, probably reflecting adaptive viral changes associated to persistent infection. Genomic sequencing of the symptomatic patient with a Ct of 18 showed phylogenetically distinct genomic sequences; the first sample was member of the clade 20A, and the most recent sample was member of the clade 20B. The 3 patients with asymptomatic recurrence and the symptomatic patient with no sequencing data showed detectable antibody levels at the time of SARS-CoV-2 RNA re-positivity, ranging from 3.01 to 6.01 S/CO for S-IgG and 2.6 to 2.46 S/CO for N-IgG. The patient with symptomatic reinfection had no detectable antibody levels at the time of re-positivity.

Our results show that late asymptomatic RT-PCR re-positivity does occur after COVID-19, even 6 months later, and does not necessarily represent new infection, despite the prolonged time interval elapsed and the negativity of subsequent RT-PCR tests since the first diagnosis. Although asymptomatic and symptomatic SARS-CoV-2 re-positivity had been reported, median time to recurrence was usually lower, around 1–2 months [6,7]. We found that the CDC criteria showed to satisfactorily predict reinfection, since none of patients not meeting the proposed criteria showed to be reinfected after genomic sequencing testing, while reinfection was actually confirmed in the suspected case according to criteria. Unfortunately, paired samples were not available for sequencing the viral genomes of one of the patients, who had a symptomatic re-positivity with a Ct value of 36. This patient would not have been classified as a case of suspected reinfection by CDC criteria. Interestingly, confirmed recurrences were accompanied by coexisting detectable antibody levels, as it also occurred with the symptomatic suspected recurrence, while antibodies were not present in the patient with reinfection. These findings reinforce the protective role of antibodies against reinfection. Peak antibody levels after the first SARS-CoV-2 infection in patients with recurrence did not differ from average values observed in the cohort, and S-IgG levels at the time of recurrence were within the range of peak levels. Whether this could have contributed to the absence of symptoms in 3 of the 4 patients is unknown. Immune dysfunction has been

Table 1
Characteristics of patients with late SARS-CoV-2 recurrence or reinfection.

Patient	#1	#2	#3	#4	#5
Sex	Female	Male	Female	Male	Male
Age	44	54	64	73	52
Comorbidity	No	No	Breast cancer in remission	Mental retardation, epilepsy	Refractory hypertension, heart disease, CKD, obesity
Highest WHO severity score at 1st infection	4	4	3	4	3
Therapy for 1st infection	HCQ, AZIT, LPV/r	HCQ, AZIT, LPV/r, steroid bolus	HCQ, AZIT, LPV/r, interferon	HCQ, AZIT	None
Hospital stay at 1st infection, days	10	13	10	11	5
Time to recurrence, days	167	183	181	204	240
Symptoms at recurrence	No	No	No	Yes*	Yes
No. of negative RT-PCR tests before recurrence	6	2	1	0	1
Cycle threshold at recurrence	33	34	38	36	18
Peak S-IgG, S/CO	4.14	13.8	7.24	75.10[&]	NA
Peak N-IgG, S/CO	9.51	4.89	4.53	NA	NA
S-IgG at recurrence, S/CO	3.04	5.62	6.01	NA	Undetectable
N-IgG at recurrence, S/CO	2.61	1.92	2.46	NA	Undetectable
Genomic sequencing	Same clade 20A	Same clade 20A	Same clade 20A	NA	Different clades, 20A and 20B

*The patient was readmitted to hospital.
HCQ, hydroxychloroquine; AZIT, azithromycin; LPV/r, lopinavir/ritonavir; S/CO, absorbance/cut-off; CKD, chronic kidney disease; NA, not available.
[&] Antibodies were detected with Liaison® SARS-CoV-2 S1/2 IgG Diasorin (Saluggia, Italia); positive cut-off ≥15.0 AU/ml.

implicated among the factors potentially contributing to reactivation of latent persistent virus after COVID-19 [2]. Despite the adequate antibody levels, additional immune deficits, such an insufficient cellular immune response to SARS-CoV-2, might have had a role in the delayed RT-PCR re-positivity.

Our study provides long-term data about the natural history of COVID-19. Asymptomatic recurrences are detected up to six months after COVID-19. The CDC criteria are helpful to distinguish between disease recurrence and reinfection.

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Rapid whole-genome sequencing to inform COVID-19 outbreak response in Vietnam

Dear Editor,

The emergence of new SARS-CoV-2 variants, especially those of concerns, and their rapid dispersal emphasize the importance of active surveillance for SARS-CoV-2 variants worldwide.[1,2]

In the morning of 28th January 2021, after 55 days without SARS-CoV-2 community transmission in Vietnam, two PCR-confirmed cases of SARS-CoV-2 infection were reported. They came from two neighboring provinces, Hai Duong (HD) and Quang Ninh (QN), in the north of Vietnam.[3] By the end of the day, 88 cases had been confirmed in these two provinces.

On the 28th of January 2021, a 28-year old man (patient 1) presented to a local district hospital in Ho Chi Minh city (HCMC) in southern Vietnam. He had just flown back from HD, where he had attended a relative's wedding party on 18th January. One of his relatives in HD tested positive for SARS-CoV-2 on the 28th January. As per the control measures in Vietnam,[4] a nasopharyngeal throat swab (NTS) was obtained from patient 1 and tested positive for SARS-CoV-2 by RT-PCR[5] on 29th January. Because the strain of the virus responsible for the outbreak in the north was unknown, we whole genome sequenced SARS-CoV-2 directly from the NTS of patient 1 using the ARTIC protocol,[6] and obtained a complete genome on 31st January. Lineage analysis using Pangolin[7] returned B.1.1.7, representing the first report of B.1.1.7 from a case of locally-acquired infection in Vietnam.[3] Contact tracing identified a total of 162 close contacts of patient 1, but none were positive for SARS-CoV-2 by RT-PCR on 4th February.

The rapid expansion of the outbreak in the north, possibly caused by variant B.1.1.7, raised concerns about a nationwide outbreak (Supplementary Figure 1A). This prompted HCMC to conduct enhanced surveillance for SARS-CoV-2, primarily focusing on high-risk groups, including those working at Tan Son Nhat (TSN) international and domestic airport in HCMC. Subsequently, a baggage handler (patient 2) working at the airport and his brother (not working at the airport) were found positive for SARS-CoV-2 on 6th February. The next day, four co-workers (patients 3–6) of patient 2 also tested positive for SARS-CoV-2, while all contacts of patient 2′s brother were negative. At this time, the outbreak in the north had expanded to another 10 provinces/cities (Supplementary Figure 1B).

To dissect the epidemiological picture of the ongoing outbreak, we whole genome sequenced SARS-CoV-2 from the NTS of patients 2–6 using the ARTIC protocol. We obtained 3 complete genomes (1 from patient 2 on 8th February, and 2 from patients 3 and 4 on 10th February). All were assigned to sub-lineage A.23.1 (Pangolin).

After the detection of these six confirmed cases, contact tracing and testing detected 30 additional PCR confirmed cases, totaling 36 infected cases, including 9 members of TSN airport staff in total.[3] The remaining cases were contacts of these 9 individuals (data not shown). Two additional SARS-CoV-2 whole genomes were successfully obtained from a brother of patient 6 and one of the airport staff; all belonged to A.23.1.

The 5 obtained whole-genome sequences of sub-lineage A.23.1 clustered tightly on a phylogenetic tree, and were closely related to A.23.1 strains collected from other countries (Fig. 1). Our findings suggest the TSN airport-associated cluster was caused by a single

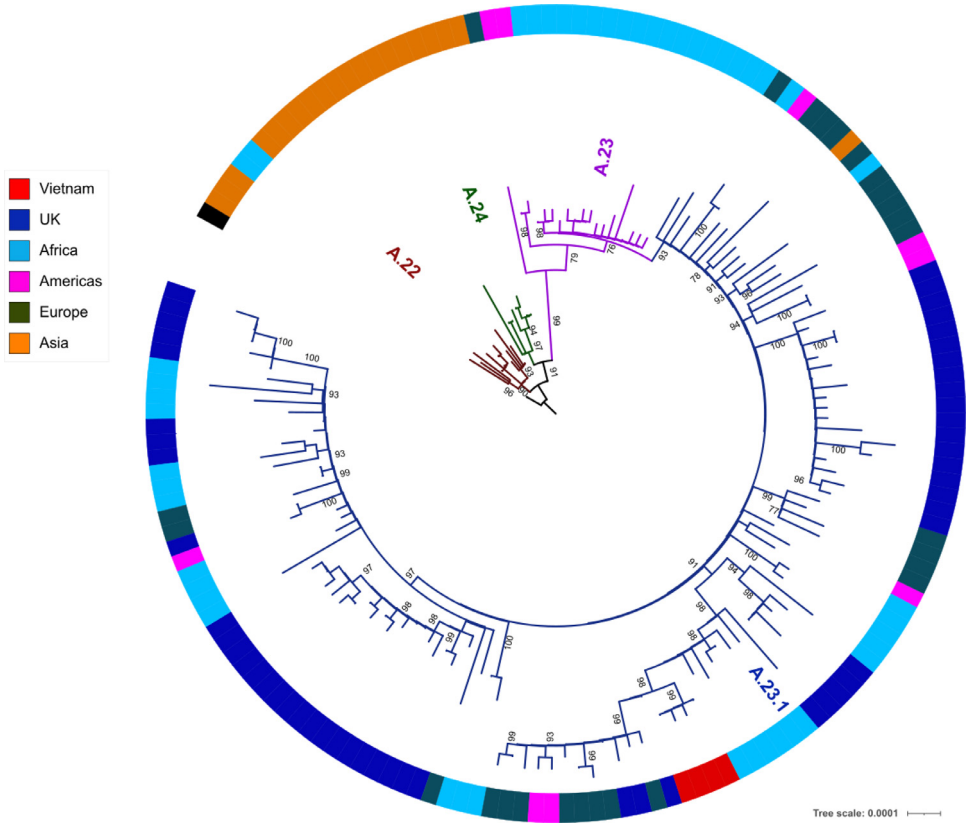

Fig. 1. Maximum likelihood phylogenetic tree showing the relatedness between the Vietnamese SARS-CoV-2 sub-lineage A.23.1 (in red) obtained from the present study and representatives of global A.23.1 strains (B). Continents or countries from where A.23.1 has been documented are color-coded. Africa includes Rwanda, Uganda and Ghana. Europe covers non-UK countries, including Switzerland, Belgium and Denmark. Asia includes United Arab Emirates. Americas includes Canada and United States. Similar to A.23.1 strains identified elsewhere, all the Vietnamese strains carried the four defining single nucleotide polymorphisms (F157L, V367F, Q613H and P681R) in the spike protein.

Table 1
Demographics of the study participants and contact details between RT-PCRconfirmed cases of the TSN airport associated cluster.

Patient	Age	Gender	Geographic locations	Occupation	Lineage determinatio n	Symptomatic*
1	28	Male	Hai Duong	Not available	B.1.1.7	Yes
2	28	Male	HCMC	Baggage handler	A.23.1	No
3	28	Male	HCMC	Baggage handler, a co-worker of patient 2	A.23.1	Yes
4	30	Male	HCMC	Baggage handler, a co-worker of patient 2	A.23.1	No
5	41	Male	HCMC	Baggage handler, a co-worker of patient 2	NA	No
6	30	Male	HCMC	A co-worker of patient 2	NA	No
7	23	Male	HCMC	A brother of patient 6	A.23.1	No
8	29	Male	HCMC	Baggage handler, a co-worker of patient 2	A.23.1	No
9	25	Male	HCMC	Baggage handler, a co-worker of patient 2	NA	No

NA: not available, sequencing attempts were not successful. *Mild respiratory symptoms without requirement of oxygen supplement. HCMC: Ho Chi Minh City.

introduction of A.23.1 into the airport, although the origin of the infection remains unknown. As of the 17th March HCMC had gone 35 days without any new community transmissions.[3]

Nine PCR-confirmed cases, including the 5 patients from whom a complete genome of A.23.1 was obtained, consented to have their clinical features reported.[8] Two had mild symptoms and 7 were asymptomatic (Table 1 and Supplementary Figure 2).

Since its first detection in Rwanda in October 2020, as of 19th March 2021, A.23.1 has been reported in 23countries worldwide.[9] Notably, recently, A.23.1 has emerged and become a predominant sub-lineage circulating in Kampala, Uganda.[2] Viruses of A.23.1 carry four defining mutations in spike protein (F157L, V367F, Q613H and P681R). Of these, Q613H is predicted to be biologically equivalent to the D614G, which emerged in early 2020, and has been shown to increase the transmisibility. As a consequence, A.23.1 is now listed as one of the five variants (B.1.1.7, P1, B.1.351, and B.1.525) to be tracked globally.[9]

The turn-around time from RT-PCR diagnosis to SARS-CoV-2 lineage determination by whole-genome sequencing was between 1.5–3 days. This was achievable because of pre-existing sequencing infrastructure and expertise, and helped by the low prevalence of SARS-CoV-2 in Vietnam. The sequencing findings were critical to informing rapid public health responses in HCMC. Indeed, the detection of the B.1.1.7 variant in the north led to enhanced surveillance in the south and the detection of the TSN airport cluster, which may otherwise have gone unnoticed.

Active surveillance for SARS-CoV-2 variants has been applied in developed countries since the beginning of the pandemic.[10] It is now one of the top priorities of the WHO. However, success stories from a resource-constrained setting like Vietnam remain uncommon. Thus, enhancing the sequencing capacity in these recognized hotspots of pathogen emergence is of vital importance for both the global COVID-19 research agenda and the control of future emerging infections.

In summary, while our findings have expanded the geographic distributions of B.1.1.7 and A.23.1 variants, the data emphasize the importance of active surveillance for SARS-CoV-2 worldwide. The sequencing capacity in low- and middle-income countries must be strengthened to address the challenges of the ongoing COVID-19 pandemic and future emerging infections.

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Supplementary materials

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Discovery of a SARS-CoV-2 variant from the P.1 lineage harboring K417T/E484K/N501Y mutations in Kofu, Japan

Dear Editor,

In this Journal, Tang and colleagues recently commented on the introduction of the South African SARS-CoV-2 variant 501Y.V2 into the United Kingdom[1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants are not always related to increased threat to human health because the virus acquires genomic diversity during the course of its life cycle[2]. However, some of these mutations have been shown to be associated with attenuation of the neutralizing activity of antibodies[3]. During the ongoing evolution of SARS-CoV-2, newly emerging lineages are likely to be circulating in the human population and genomic surveillance will be important for evaluating the emergence, spread, vaccine efficacy, and transmissibility of these lineages.

Currently, an emergent D614G mutation in the spike glycoprotein of SARS-CoV-2 is prevalent globally[4]. More recently, new emerging lineages with spike protein mutations have been discovered in the United Kingdom (B.1.1.7 lineage, 20I/501Y.V1, also named VOC 202,012/01)[5], South Africa (B.1.351 lineage, 20H/501Y.V2)[6], and Brazil (P.1 lineage, 20J/501Y.V3)[7,8]. All of these lineages have a N501Y mutation in the receptor binding domain (RBD), which directly binds to the angiotensin converting enzyme 2 (ACE2) receptor of the host cell, contributing to increased transmissibility. Both B.1.351 and P.1 lineages also have additional K417N/T and E484K mutations. K417N/T, E484K, and N501Y confer reduced neutralizing activity of convalescent and mRNA vaccine-elicited serum[3].

To determine the genomic characteristics of the SARS-CoV-2 variant identified in the Kofu, Japan, we started whole genome sequencing analysis using the Ion Torrent Genexus System (Thermo Fisher Scientific) on January 8, 2021. By February 15, 2021, a total of 136 samples from COVID-19-positive patients were obtained in our hospital from which 70 were subjected to analysis[9,10]. The sequence data were subjected to phylogenetic analysis using Nextclade, identifying five types of clades: 19A ($n=1$), 20A ($n=3$), 20B ($n=59$), 20C ($n=6$), and 20J/501Y.V3 ($n=1$) (Fig. 1). The SARS-CoV-2 strain from the Diamond Princess cruise ship patient was classified into clade 19A (Fig. 1), and four patients admitted at the end of March 2020 were classified into 20A ($n=3$) and 20B ($n=1$). For the patients admitted from September 2020–January 2021, 64 patients were classified into 20B clade ($n=58$) and 20C ($n=6$) (Fig. 1). The newly confirmed patient was classified as 20J/501Y.V3 (P.1 lineage) on February 10, 2021 (Accession No. EPI_ISL_978917).

This patient was a 46-year-old man who entered our hospital in early February 2021 with a fever at 38.9 °C and with a history of staying in Brazil. RT-qPCR indicated a high viral load (7.1 $\log_{10}$/μL) and low cycle threshold (Ct) value of 15. The patient had displayed mild symptoms upon returning to Japan 4 days earlier and was admitted to another hospital. However, he was declared negative for SARS-CoV-2 during quarantine.

Sequencing analysis revealed that the SARS-CoV-2 variant 20J/501Y.V3 (P.1 lineage) had 37 mutations, including 22 missense, 10 synonymous, three intergenic, one frameshift, and one in-frameshift mutation. In the spike protein, we observed 12 missense mutations (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I and V1176F). These mutations matched perfectly with the mutations in the P.1 variant previously discovered in Brazil[7] (Fig. 2A). In the RBD of the spike protein, three mutations (K417T, E484K and N501Y) were identified. These results indicated that we had identified a variant related to 20J/501Y.V3 (P.1 lineage) in Japan.

To examine the global prevalence of 20J/501Y.V3 (P.1 lineage), we referred to sequence data deposited in GISAID (https://www.gisaid.org/). By February 14, 2021, 121 sequence data were available, 119 of which were derived from patients. Variant 20J/501Y.V3 (P.1 lineage) was first discovered in a sample collected from Manaus, Amazonas, Brazil on December 4, 2020, and has subsequently been identified from numerous other samples (Fig 2B). Almost all 20J/501Y.V3 (P.1 lineage) samples sequenced have 33–40 mutations compared with original strain reported from Wuhan, China; our identified strain has 37 mutations (Fig. 2C). Of the 119 patients

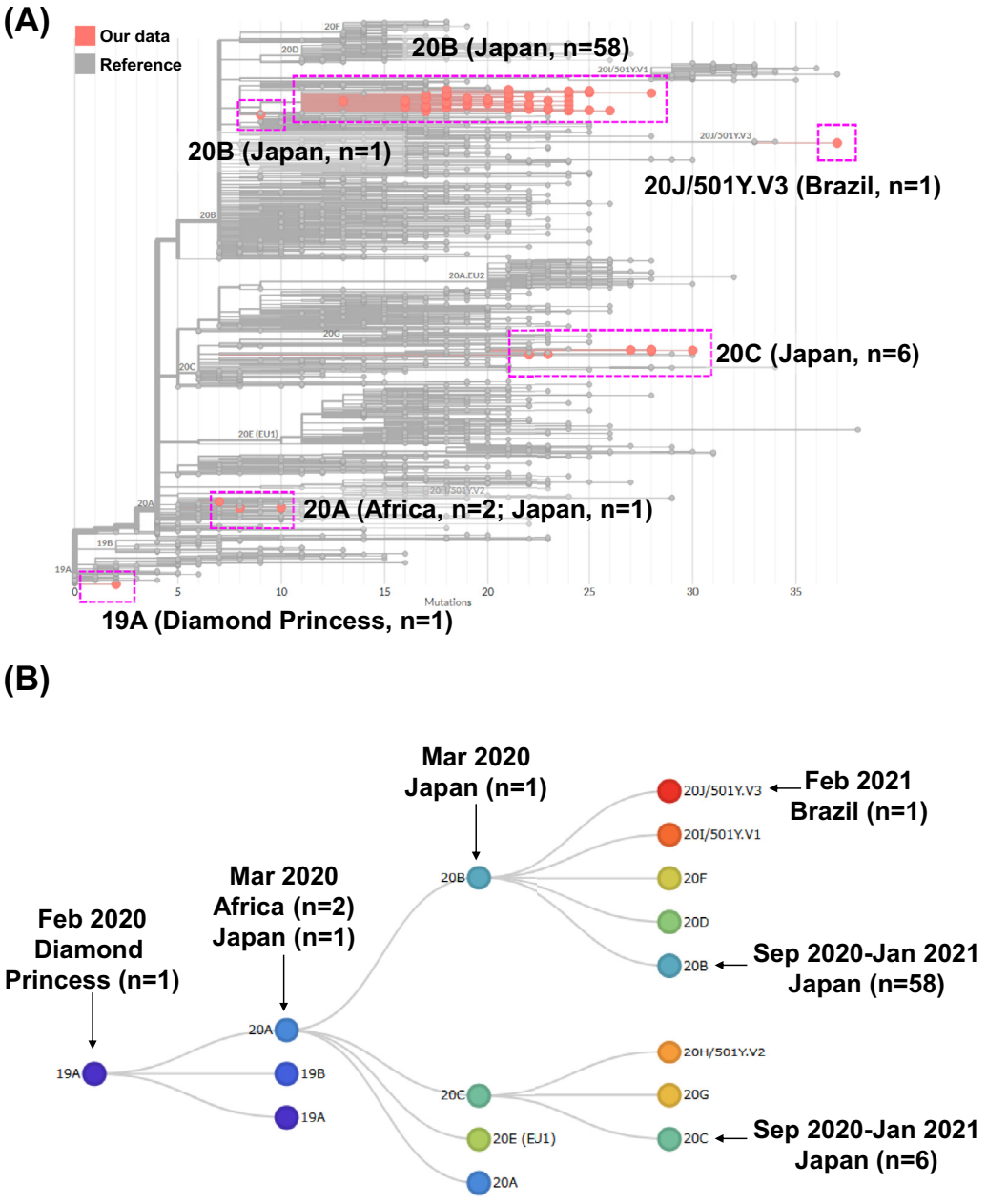

Fig. 1. Phylogenetic tree analysis of SARS-CoV-2. (**A**) Sequencing data were uploaded to Nextclade (https://clades.nextstrain.org/) for phylogenetic analysis. The boxed regions show the sequencing data obtained in this analysis. (**B**) Evolution of SARS-CoV-2 clades over time. Schematic tree from Nextstrain showing clade evolution in Japan since February 2020. The arrows indicate the clades into which the 70 patients identified in our analysis were classified.

infected with this variant, 82 (68.9%) were identified in Brazil, five (4.2%) in Japan, 20 (16.8%) in Europe, and three (2.5%) in the USA (Supplemental Table 1), suggesting global prevalence.

In summary, we have confirmed the emergence of five clades over time. Consecutive analysis identified SARS-CoV-2 variant 20J/501Y.V3 (P.1 lineage) in a patient and detected mutations that are identical to those of the original P.1 variant discovered in Brazil[7]. This is the first report of 20J/501Y.V3 (P.1 lineage) in Kofu, Japan.

Declaration of Competing Interest

None.

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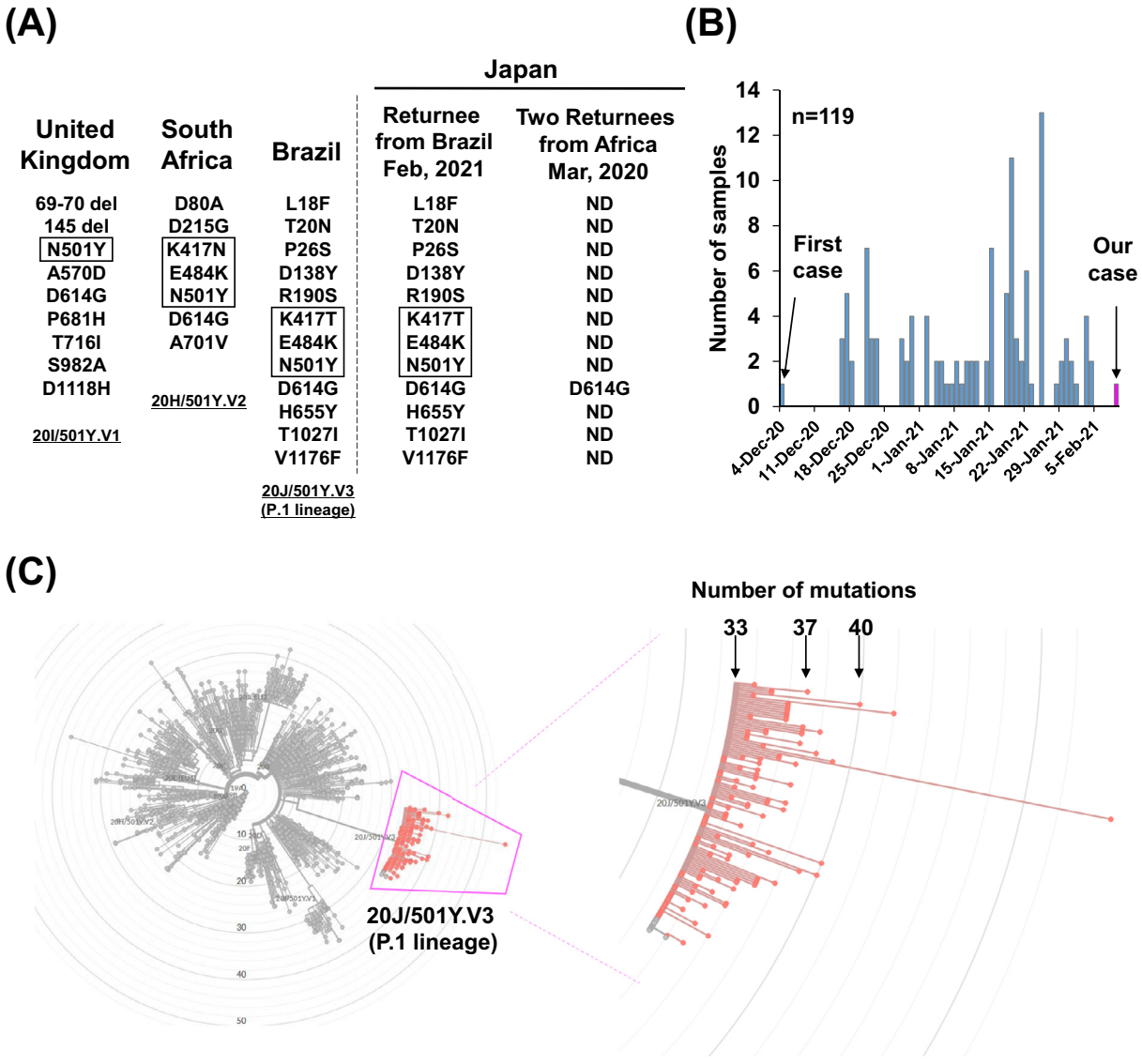

Fig. 2. SARS-CoV-2 spike protein mutation in emerging lineages and global distribution. (**A**) Mutations in the SARS-CoV-2 spike protein. (Left) Amino acid changes identified in the emerging strains reported in the United Kingdom, South Africa, and Brazil. (Right) Results of the current analysis: the patient who returned from Brazil on February 2021 had the same mutation as 20J/501Y.V3 (P.1 lineage); the two patients who returned from Africa on March 2020 had only a D614G mutation in the spike protein. The mutations highlighted by boxes indicate those in the receptor binding domain. ND, not detected. **(B)** The number of 20J/501Y.V3 (P.1 lineage) strains deposited in GISAID by February 14, 2021. The first case was identified on December 4, 2020 and our case on February 10, 2021. **(C)** A total of 119 sequencing data were analyzed on Nextclade. (Left) Radial phylogenetic tree showing the location of 20J/501Y.V3 (P.1 lineage). (Right) Magnified view of boxed area showing the P.1 lineage. The total numbers of mutations denoted are with respect to the SARS-CoV-2 strain from Wuhan, China.

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Supplementary materials

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Comparison of the role of neutrophil extracellular traps between patients admitted to the intensive care unit with influenza A and B virus infection

Dear Editor,

In contrast to influenza A (H1N1), influenza B infections are discounted given their limited host range, low rate of antigenic drift, low incidence, and milder disease severity than influenza A.[1] However, influenza B infection appeared to be similar to influenza A in clinical presentation,[2] and pediatric influenza B-associated mortality is greater than that of influenza A.[3] Additionally, oseltamivir has been reported to be less effective at reducing the viral response and duration of fever in outpatients with influenza B compared to those with influenza A;[4] however, high-dose oseltamivir might be more effective.[5] Despite these disparate reports, comprehensive studies comparing the characteristics and pathogenesis of influenza infections caused by A and B viruses are still lacking, particularly among severe cases who are admitted to the intensive care unit (ICU).

Neutrophils are an important component of the exaggerated inflammatory response in influenza infection;[6] however, little is known about neutrophil extracellular traps (NETs), which are expelled by the nuclear components of the cells.[7] We have recently reported the pathogenic role of NETs, and plasma NETs might be regarded as a sensitive biomarker for severe influenza A infection.[8,9] Therefore, it is reasonable to investigate the role of NETs in influenza B and to determine whether there are differences in the roles of NETs between influenza A and B viruses.

We included 30 influenza A and 10 influenza B virus-related ICU admissions of China–Japan Friendship Hospital from 2017 through 2018. Informed consent was obtained from all patients, and our study was approved by the Ethics Committee of the China–Japan Friendship Hospital (No. 2,001,814). Table 1 was the patients' clinical characteristics.

The levels of NETs and inflammatory mediators in blood and bronchoalveolar lavage fluid (BALF) were quantified as described in our previous study.[8] Comparisons of plasma NETs showed no significant difference in these two groups (Fig. 1A). However, the NETs burden of deaths in the influenza A group was higher than that in the influenza B group ($p = 0.0193$) (Fig. 1A). Additionally, the influenza A group with multiple organ dysfunction syndrome (MODS) was not significantly higher than the corresponding influenza B patients (Fig. 1A). Intriguingly, the NET level was specifically higher in the BALF of patients with influenza B compared to those with influenza A ($p = 0.0019$) (Fig. 1B). Consistent differences were also found between the pulmonary NET levels of deaths in the two groups ($p = 0.0050$) (Fig. 1B). Furthermore, a higher level of pulmonary NETs was observed in the influenza B patients with MODS than in the influenza A cases ($p = 0.0280$) (Fig. 1B).

As for the inflammatory mediators, the concentrations of circulating interleukin (IL)−7, IL-18, and interferon (IFN)-γ were higher in the influenza A group than in the influenza B group (Fig. 1C). In contrast to influenza A patients, pulmonary mediators, including IFN-γ, IL-1β, chemokine ligand (CCL) 3, CCL4, and fibroblast growth factor-2 were markedly elevated (Fig. 1C–D). However, levels of IL-2, monocyte chemotactic protein-1, interferon-inducible protein-10, stem cell factor, and vascular endothelial growth factor-D in BALF were lower in the influenza B group than in the influenza A group (Fig. 1D–E).

In this study, we compared the roles of NETs between severe influenza A and B patients. With indistinguishable plasma NET levels compared with influenza A patients, the pulmonary NET levels were significantly increased in influenza B patients. This finding suggests that the enhanced pulmonary NETs have pathogenic roles in influenza B infection but not in influenza A infection.

Although patients with influenza B have a relatively higher oxygen index, they are not distinguishable by clinical features from patients with influenza A, which is consistent with the previous studies.[2] To date, several studies have provided evidence for the role of NETs as a sensitive biomarker for severe influenza A infection.[9] Consequently, we further determined the levels of NETs in the lung and plasma from patients with severe influenza B and influenza A infection. The NET levels in BALF were increased in influenza B patients, although the plasma NET burden was similar, highlighting that pulmonary NET production in influenza B might induce more severe lung damage than influenza A infection. In fact, the generation of NETs systemically correlates with influenza A viral pathogenesis.[8] Hence, the pulmonary, rather than the circulating NETs seem to be pathogenic in influenza B infection but not in influenza A infection.

Patients with influenza A infection demonstrated an intense immune response, as evidenced by increased circulating inflammatory mediators.[10] In our findings, IL-7, IL-18, and IFN-γ in plasma indicated significant differences between the two groups, and followed the same trend as the plasma NET levels, suggesting a close correlation between the NET burden and the inflammatory response in circulation. As for the neutrophil chemoattractants, the circulating IL-18 was dramatically elevated in the influenza A group, indicating a pathogenic role of plasma NETs in patients with

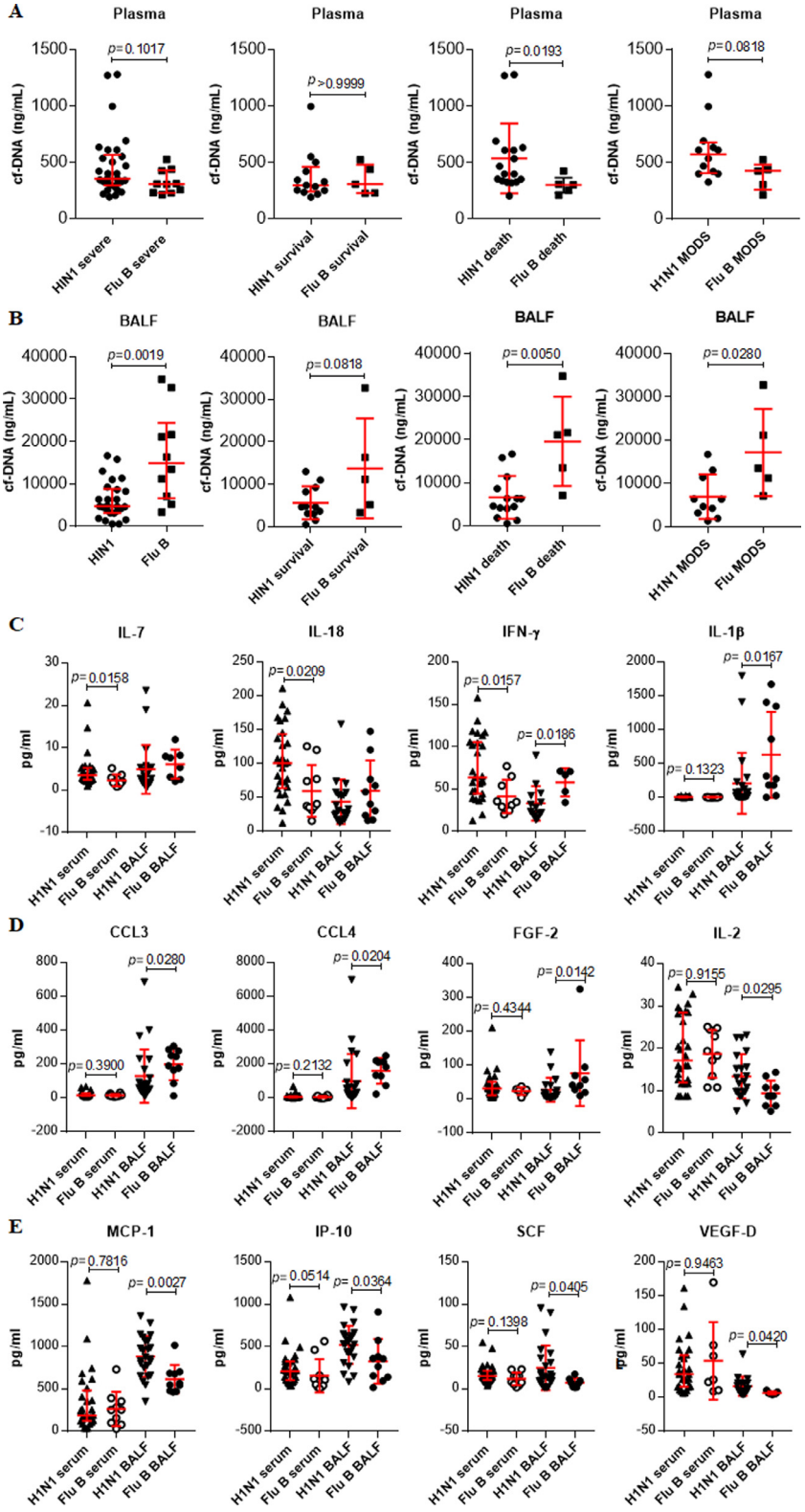

Fig. 1. NET levels and Inflammatory mediator profiles in plasma and BALF. (A) Plasma NET levels in influenza A and B groups. H1N1 severe, $n=30$; Flu B severe, $n=10$. H1N1 survival group, $n=13$; Flu B survival group, $n=5$. H1N1 death group, $n=17$; Flu B death group, $n=5$. H1N1 MODS group, $n=12$; Flu B MODS group, $n=5$. (B) Patients with influenza B had increased BALF levels of NETs when compared with patients with influenza A. Influenza A group, survived, $n=12$; died, $n=14$; MODS, $n=10$; influenza B group, survived, $n=5$, died, $n=5$; MODS, $n=5$. (C-E) Inflammatory mediator levels in plasma and BALF. Data are presented as means ± standard deviation (SD), or median (interquartile range, IQR). Comparisons were conducted using X^2 test, Student's *t*-test, or Mann–Whitney U test.

NET: Neutrophil extracellular trap, BALF: Bronchoalveolar lavage fluid, MODS: Multiple organ dysfunction syndrome, IL-7: Interleukin-7, IL-18: Interleukin-18, IFN-γ: Interferon-γ, IL-1β: Interleukin-1β, CCL3: Chemokine ligand 3, CCL4: Chemokine ligand 4, FGF-2: Fibroblast growth factor-2, IL-2: Interleukin-2, MCP-1: Monocyte chemotactic protein-1, IP-10: Interferon-inducible protein-10, SCF: Stem cell factor, VEGF-D: Vascular endothelial growth factor D.

Table 1
Summary of the patients' clinical characteristics.

Variables	Influenza A ($n = 30$)	Influenza B ($n = 10$)	p value
Age (year), mean ± SD	52.37 ± 15.31	51.30 ± 20.19	0.861
Male sex, n (%)	17 (56.7)	3 (30)	0.144
Health condition			
Respiratory disease, n (%)	2 (6.7)	1 (10)	1.000
Cardiovascular, n (%)	11 (36.7)	3 (30)	1.000
Diabetes, n (%)	14 (46.7)	4 (40)	1.000
Obesity (BMI > 28), n (%)	5 (16.7)	0	0.408
Laboratory parameters			
White blood cells (10^9/L)	10.23 (4.26, 13.36)	14.28 (8.15, 23.73)	0.102
Neutrophil count (10^9/L)	8.52 (3.53, 11.93)	12.84 (6.72, 22.77)	0.117
Lymphocyte counts (10^9/L)	0.64 (0.43, 1.11)	0.70 (0.48, 0.90)	0.9632
Positive culture of pathogenic microorganism, n (%)			
[a]Bacteria, n (%)	19 (63.3)	1 (10)	0.003
[b]Fungi, n (%)	6 (20)	1 (10)	0.810
Bacteria and fungi, n (%)	5 (16.7)	6 (60)	0.025
Medicine, n (%)			
Antiviral treatment	30 (100)	10 (100)	–
Antibiotics treatment	30 (100)	10 (100)	–
Glucocorticoid treatment	5 (16.7)	3 (30)	0.648
Disease severity			
Mechanical Ventilation, n (%)	22 (73.3)	9 (90)	0.512
ECMO, n (%)	14 (46.7)	2 (20)	0.264
MODS, n (%)	12 (40)	5 (50)	0.853
Oxygen index (mmHg), median (IQR)	122 (80.5, 198.5)	196 (144.25, 251.75)	0.0178
SOFA score, median (IQR)	6 (3.75, 8.75)	7 (3.75, 10)	0.7988
APACHE II score, median (IQR)	14.5 (11, 19.25)	21 (14, 24.25)	0.1369
Outcomes			
ICU duration days, median (IQR)	13.5 (9, 29.5)	14.5 (6.5, 30.5)	1.000
Death, n (%)	17 (56.7)	5 (50)	1.000
Fever date prior to diagnosis, mean ± SD	8.8 ± 3.21	9.9 ± 6.05	0.593
Fever date prior to ICU admission, (days, mean ± SD)	8.3 ± 2.72	12.2 ± 8.97	0.207

Data are presented as the mean ± standard deviation (SD) or median (interquartile range [IQR]). Comparisons were performed using X^2 test, Student's *t*-test, or Mann–Whitney U test.

[a] Bacteria include Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Corynebacterium striatum, methicillin-sensitive Staphylococcus aureus, Stenotrophomonas maltophilia, Burkholderia cepacia, and Ralstonia mannitolilytica.

[b] Fungi include Candida albicans and Aspergillus.BMI: Body mass index, ICU: Intensive care unit, ECMO: Extracorporeal membrane oxygenation, MODS: Multiple organ dysfunction syndrome, SOFA: Sequential organ failure assessment, APACHE: Acute physiology and chronic health evaluation.

severe influenza A. However, with a significantly increased BALF NET burden in influenza B patients, the IL-18 level was not elevated in the influenza B group, suggesting that other neutrophil chemoattractants in the lung might be potential NET-related mediators associated with influenza B infection. Moreover, the inflammatory mediator levels in BALF showed a similar trend with pulmonary NETs, suggesting a correlation between the pulmonary NETs and the robustness of the local pulmonary inflammation. Collectively, the pathogenic role of NETs in BALF is likely attributable to their roles as inducers of inflammation, which ultimately leads to lung damage following influenza B infection.

Limitations of our study include the small sample size, the single-center experience, and failure identification of the lineage of influenza B, which might have distinct pathogenicity.

In conclusion, in contrast to influenza A, in which circulating NETs are predictors of poor outcomes, influenza B infection could induce an enhanced production of pulmonary NETs, which may play a pathogenic role. Thus, targeting pulmonary NETs might be an innovative therapeutic approach for influenza B infection.

Declaration of Competing Interest

None.

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Author contributions

NNZ and FZS were responsible for the experimental design. LLZ and YHT collected the patient samples and analyzed the data. LLZ and YZ conducted the experiments and performed statistical analyses. NNZ and LLZ performed the experiments, analyzed the data, and wrote the manuscript. HZ and QYZ assisted with designing the experiments and interpreting the data. FZS supervised the process and revised the manuscript. All authors have approved the manuscript for submission.

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Heterogeneity of contact patterns with Ebola virus disease cases

Dear Editor,

In a recent paper, Majra et al. underlined the major role of superspreading events (SSEs) in SARS-Cov-2 transmission.[1] Heterogeneity in transmission, clustering, characterized by a small number of persons (superspreaders) responsible for the majority of the events, is a common feature of outbreaks, in particular at the early and late stages.

During the 2013–2016 Ebola outbreak in Guinea we quantified the exposures of contact persons to Ebola virus disease (EVD) patients and explored the consequences of the contacts pattern in terms of contact tracing and modeling.[2] After consent, a questionnaire detailing every exposure to EVD cases, including funerals, was passed and the number of exposures per contact person was summed. A high-risk exposure was defined as a close contact with a symptomatic EVD case or contact with body fluids or participating in a burial ritual.

Between May 2016 and September 2017, 1721 participants were enrolled in four locations (Conakry, Forécariah, Macenta, N'Zérékoré) (51.4% males, age range = [7–88 years], median age 21 years IQR [16–32]). They had made a total of 3074 contacts (exposures) with EVD cases (range = 1 to 17 exposures per person; median = 1; [IQR 1–2]). Overall, the frequency distribution of the cumulative number of exposures showed an overdispersed, aggregated, distribution: 84.3% of the participants ($n = 1451/1721$) made at most two exposures to an EVD case. They were only 1.2% to report ten exposures or more. Aggregation was less pronounced in rural than in urban setting: the proportion of participants reporting less than three contacts was respectively 94.1% (and conversely 5.9% reporting three or more contacts) and 78.6% (21.4%) ($p < 10^{-3}$).

Only 15.7% of the participants, at risk of being infected, concentrated three or more exposures with a large difference between the urban and rural settings (21.4% *vs* 5.9%). This clustering was also observed in terms of high-risk exposures with 86.2% of the participants reporting at most two high-risk exposures with again a marked difference between rural and urban settings (94.1% *vs* 81.6%, $p < 10^{-3}$). We fitted a negative binomial regression model using GAMLSS R-package with a zero-truncated distribution, people without contact being not included, by design.[3]

The median number of exposures by person surveyed did not differ by rural or urban setting while the variance of the distribution was 26 times larger in urban setting than in rural one (Table 1 and Fig. 1). This regression confirms the observed high degree of overdispersion of the contacts in an urban setting.

The aggregated distribution of the cumulative number of exposures means that a small number of the participants made many exposures and that the majority of the contact persons were exposed only once or twice. The median number of contacts did not differ but the associated variance was dramatically larger in urban setting. These trends are obviously driven by the population density and social closeness in large cities.

Although our study concerns contact persons who did not develop the disease after exposition, and may not be representative of the whole exposed population to Ebola virus, this clustering in exposure has implications for backwards contact tracing by targeting the surveillance toward the core of contact persons who made the greatest number of exposures. Indeed, another report on contacts from Kindia and Forecariah in Guinea suggested to stratify contacts-persons to focus on those most at risk.[4] Risk level assessment should include not only the closeness of exposures but also their number.

Table 1
Parameters of the fitted distributions of the number of exposures per contact person to Ebola virus disease cases by rural or urban setting, Guinea, 2014–2016.

Setting	Mean nb of exposures (95% CI)	SD	*p*-value*(SD)
Rural (Macenta-N'zérékoré)	1.266 (1.220 - 1.313)	1.319	0.00045
Urban (Conakry-Forécariah)	2.094 (1.958 - 2.230)	6.880	10^{-4}

* significantly rejecting homogeneous mixing.

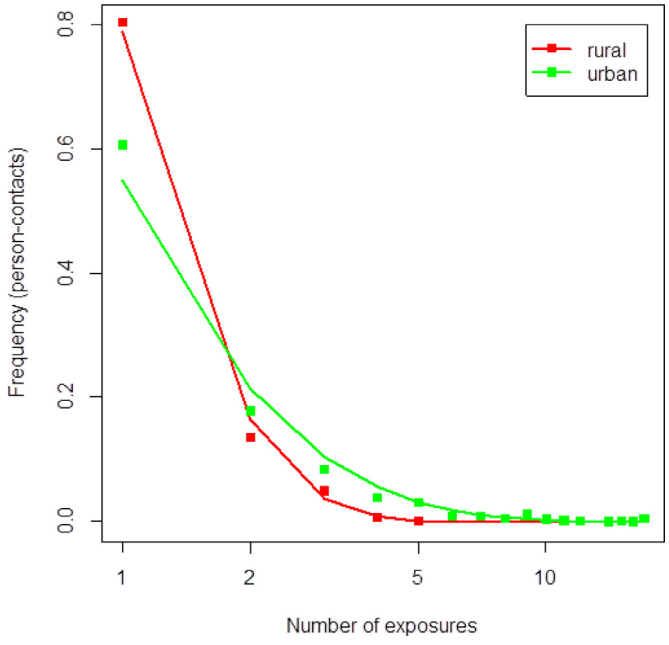

Fig. 1. Observed values (square) and fitted distribution (line) of the number of exposures per contact person to Ebola virus disease cases by rural or urban setting, Guinea, 2014–2016.

While these contact patterns do not concern the infected population, the EVD source cases potentially transmitting the infection, it provides an additional evidence of the heterogeneity in contact rate, with a high degree of clustering following a power-law, close to the 80/20 of the Pareto principle, frequently observed in life sciences, human behavior and infectious diseases.[5]

The main limitation of our study relies on the retrospective and declarative nature of the data and the likely recall bias. Recalling the number of exposures was however robust in our study since the network of contact persons was initially identified by the survivors regularly followed by our research staff. In addition, we showed in a previous study that the seropositivity against Ebola virus among participants was correlated with the declaration of high-risk contacts.[2]

Taking into account sources of heterogeneity in models of transmission, beyond basic compartmental models assuming homogeneous mixing of the population, in which everyone shares the same epidemiological profile, and no stochastic effects, could substantially affect the modeling of the transmission dynamics and the elimination threshold to achieve herd immunity.[6] Living settings as well as age-dependent incidence, infectiousness, genetic features, human behaviors, occupation or spatial patterns are all a source of heterogeneity.[7] Our findings argue to account at least for urban/rural heterogeneity and an unobserved heterogeneity, representing other sources of individual variability, in modeling transmission. Host heterogeneity is best incorporated in network-based models but SIR compartmental models as well could account for heterogeneity.[8] Surveys informing matrix of social contacts, "who might infect who", are still largely lacking, including in sub-Saharan Africa, limiting our ability to account for the heterogeneity in modeling transmission. However, mobile phone identification and social networks activities provide means to approach contact behavior.[9]

In the case of the current SARS-CoV-2 pandemic, screening and communication strategies targeting potential superspreaders, such as connected people whose occupation implies a high frequency of contact, and SSEs, could be a cost-effective strategy when *Re* is close to 1, decreasing, at the late stage of an outbreak. In sub-Saharan Africa where the indirect effects of general restrictions on the fragile economies, health system, immunization coverage, access to foods, are at the forefront, this targeted strategies could save more lives than a blanket strategy.[10]

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Increased incidence of listeriosis among pregnant women belonging to ethnic minorities in England

Dear Editor,

A recent paper in this journal by Rose et al. described the increasing hospitalizations due to infectious intestinal disease among vulnerable population groups in the UK, including those from ethnic minorities or unemployed.[1] Pregnancy related listeriosis is a severe illness for the unborn and newly delivered infant. Ethnic minorities may have higher incidence as in New Zealand and the USA.[2,3] In England, guidance on food consumption during pregnancy to avoid listeriosis is available through universal maternity care and NHS website.[4] The majority of cases of listeriosis occur amongst non-pregnant individuals.[5] Risk food consumption amongst pregnant women has not been recently investigated.[6]

We characterized listeriosis among pregnant women from the national *Listeria* surveillance database in England between 2005 and 2020 based on sampling dates. Population birth and deprivation (Index of Multiple Deprivation) postcode data was derived from Office for National Statistics, ONS.[7] A case of pregnancy associated listeriosis was defined microbiologically confirmed *Listeria monocytogenes* infection in a mother or her undelivered or newly delivered infant. Responses were classified into two nonnationality based ethnicity categories as British versus "ethnic minority" (other white or any other ethnicities). Of British population, 78.7% are white British, 6.2% other white, 8.0% Asian, 3.5% any Black, 3.7% others. The standard proportions of exposures were calculated with odds ratios and adjusted for deprivation (Stata, v15/16). All culture confirmed L. *monocytogenes* were tested by whole genome sequence analysis since December 2015 to 2020, and a subset of 49 retrospectively for 2008–2015.[8] L. *monocytogenes* clonal complexes (CC) were designated as of the Institute Pasteur International MLST database for L. *monocytogenes*.[9]

Of all 382 pregnancy related listeriosis cases reported in England between 2005 and 2020, 62.3% (236/379) were associated with mothers from ethnic minorities. The median number of cases reported annually was 24.5 (range 16–33), with a relative increase amongst mothers from ethnic minorities (p-value <0.0001), Fig. 1. Between March 2020 and January 2021, we observed 3 Covid-19 co-infections among listeriosis cases within 5 days of diagnosis, none were pregnant women. Travel outside UK was reported by 13.4% (40/298) of the cases with a median travel duration of 8.5 days. Of those 40 cases with travel outside UK; 80% (32/40) travelled to Europe, median travel duration was 8.5 days. Clinical presentation was typical for listeriosis, Supplementary table. Presentation of the three most common clonal complexes (CC1, CC2, and CC6) of L. *monocytogenes* did not differ, however, numbers were small.

Food exposures with significantly lower odds of illness among ethnic minorities versus British cases between 2005 and 2020 comprised: pork meat (OR 0.34, *p*-value <0.0001), poultry meat (OR 0.43, *p*-value 0.0012), sandwiches (OR 0.34, *p*-value 0.0001). Consuming foods in catering establishments (restaurants, cafes etc.) had somewhat lower odds for cases of for ethnic minorities (OR 0.48, *p*-value <0.060). Powdered or other unspecified milk products (e.g. almond/coconut milk, evaporation milk etc.) (OR 3.33, *p*-value 0.0045), dill (OR 10.01, *p*-value <0.0001), radish (OR 4.42, *p*-value 0.0010), carrots (OR 2.40, *p*-value 0.0017), parsley (OR 2.40, *p*-value 0.017), and consuming Kosher/Halal foods (OR 11.86, *p*-value <0.0001) had higher odds between ethnic minorities versus British cases (Supplementary table). Mothers from ethnic minorities reported eating equally vegetables (OR 1.31, *p*-value 0.51), and salads (OR 0.86, *p*-value 0.70) than British. Ethnic minority cases stored loose meat (mean 1.57 days) longer than British cases since purchase (mean 1.30 days, *t*-test *p*-value=0.093). Ethnic supermarkets selling non-British foods (OR 14.32, *p*-value <0.0001), or shopping at Supermarket K (OR 4.29, *p*-value <0.0001) were more common amongst the cases of ethnic minorities. Multivariate model indicated dill (OR 6.95, *p*-value 0.0006), carrots (OR 1.91, *p*-value 0.034), radish (OR 2.55, *p*-value 0.066), and powdered or other unspecified milk (OR 3.28, *p*-value 0.013) in addition to adjustment factor of index of multiple deprivation (OR 0.999918, *p*-value <0.0001) as most significantly different between ethnic minority mothers versus British.

Pregnancy associated listeriosis has been reported as more common amongst ethnic groups in England and Wales, 2001–2008[10] and this study reports an increasing trend among ethnic minorities in England. The current surveillance is directed towards foods previously associated with listeriosis and mainstream foods consumed by British populations. We aim to restructure the questionnaire to include both increasing vegetarian and ethnic foods, food preparation practices at home, and collect additionally loyalty card information. Herbs, and other fresh produce may be min-

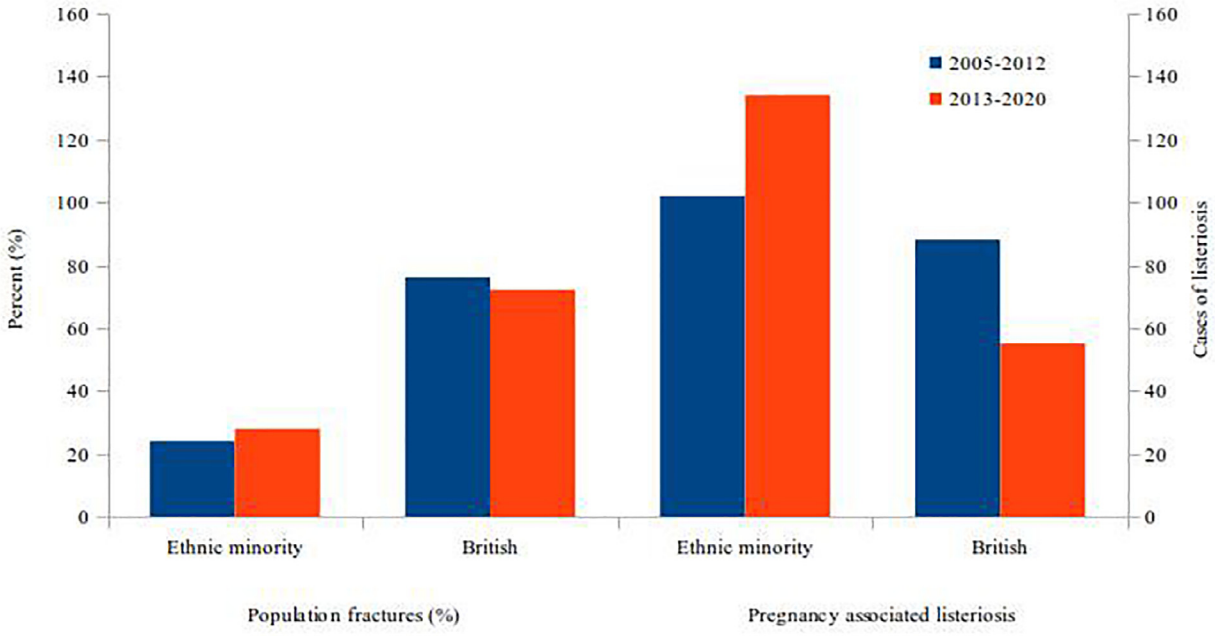

Fig. 1. Newborn population fractions in the UK and pregnancy associated listeriosis among ethnic minority and British cases in England for 2005–2012 and 2013–2020.

imally cooked and the association with listeriosis is being increasingly recognised[10].

In summary, the current dietary advice from the NHS for the UK pregnant woman[3] recommends avoiding eating unpasteurised dairy products including milk, soft cheeses (brie, camembert), chilled ready-to-eat foods like prepacked sandwiches and pâté. Targeted health education including safe food preparation practises at home among ethnic minority mothers is urgently needed not typically eating currently highlighted listeria risk foods.

Declaration of Competing Interest

We declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.03.024.

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