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

Testing strategies for the control of COVID-19 in nursing homes: Universal or targeted screening?

Dear Editor,

We read with interest the outbreak investigation carried out in late March/early April 2020 in four nursing homes affected by COVID-19 outbreaks in central London.[1] During this study, 27 days after the first death and 21 days after the first resident tested positive, 126 (40%) of nursing home residents were found SARS-CoV-2 positive. A striking finding of this investigation was that 60% of SARS-CoV-2 positive residents were either asymptomatic or only had atypical symptoms for COVID-19 during the two weeks prior to testing. Asymptomatic nursing home staff (4%) were identified as potential source of viral transmission to the residents. Additionally, genomic analysis identified one cluster involving one staff member and two residents in the same home. This work highlights the need for evaluations of the accuracy of testing strategies to identify the reservoir of asymptomatic COVID-19 cases in nursing home (NH) and adjust the control measures. In light of our local experience, and in the context of a post-lockdown period with a low incidence of COVID-19 in the general population, we analysed the results of strategies adopted by 50 NH to assess the accuracy of testing professionals, residents or both for investigating the spread of COVID-19 around a positive case.

In France, a general lockdown was implemented from 03/17/2020 to 05/11/2020. Since 07/04/2020, the French national screening recommendations in NH rely on testing all professionals when a COVID-19 positive case is identified (resident or professional).[2] The screening of residents is recommended up to three suspected cases. Since 27/05/2020, the health authorities in the Vendée department, located in western France (Fig. 1), recommended to systematically screen all residents and professionals after the identification of a COVID-19 positive case in NH. Moreover, a universal RT-PCR screening was organized in voluntary NH to estimate the invisible reservoir of COVID-19 at the end of the lockdown.

In the Vendée department, a total of 71 COVID-19 positive residents and 49 professionals were identified from the 04/01/2020 to the 06/30/2020 in 23 (17%) of the 136 NH, with 8 clusters of three cases or more.[3] From the 17/04/2020 to 26/06/2020, 50 NH situated in the Vendée department tested a median number of 42 (IQR: 15–76) residents and 54 (35–73) professionals by nasopharyngeal sample and RT-PCR, totalizing 2003 residents and 2822 professionals. (Figure) Overall, 25 (1.25%) residents and 25 (0.88%) professionals were positive for COVID-19, among whom 19 (76%), and 22 (88%) asymptomatic, respectively. Among the 14 NH which tested both residents and professionals to investigate around a COVID-19 positive resident, 6 (42%) NH did not found any positive case, 4 (29%) found one to two cases, and 4 (29%) identified ≥3 cases. (Table 1) Among the 4 (8%) NH which tested residents and professionals to investigate the spread of COVID-19 around a positive professional, one (25%) NH did not found any positive case, two (50%) found one to two cases, and one (25%) NH identified ≥3 cases. All positive cases were asymptomatic. Among the 32 NH which performed a universal screening in absence of known COVID-19 positive case on the day of testing, two (6%) identified one positive resident.

These results suggest that 7/14 (50%) NH would have missed asymptomatic residents by testing professionals only to investigate around a positive resident. Half of NH would also miss cases by testing residents only. A quarter of NH would have missed one asymptomatic resident by testing all professionals only to investigate the spread around a positive professional. Three facilities would have missed from one to three asymptomatic professionals by testing residents only. Finally, 2/32 (6%) NH which performed a blinded universal testing without any known case would missed one case. In the literature, 40.7 to 57% of residents/staff tested positive for SARS-CoV-2 in high prevalence NH were asymptomatic on the day of testing.[4–6] Our results suggest a higher rate of asymptomatic persons (41/50, 82%) in a low incidence context. Symptom-based screening of NH residents might fail to identify all SARS-CoV-2 infections. Asymptomatic NH residents and professionals might contribute to SARS-CoV-2 transmission. In a post-lockdown context with an estimated mean daily incidence rate of 0.59 COVID-19 cases per 100.000 population, the universal screening in NH free of known positive case seems inefficient. In such context, supplies should be saved, and adverse effects (pain, complication of nasopharyngeal sampling, logistics, and costs) can be avoided. However, once a facility has confirmed a COVID-19 case, extensive testing should be performed for all professionals and residents, and all residents should be cared for using personal protective equipment.

Declarations

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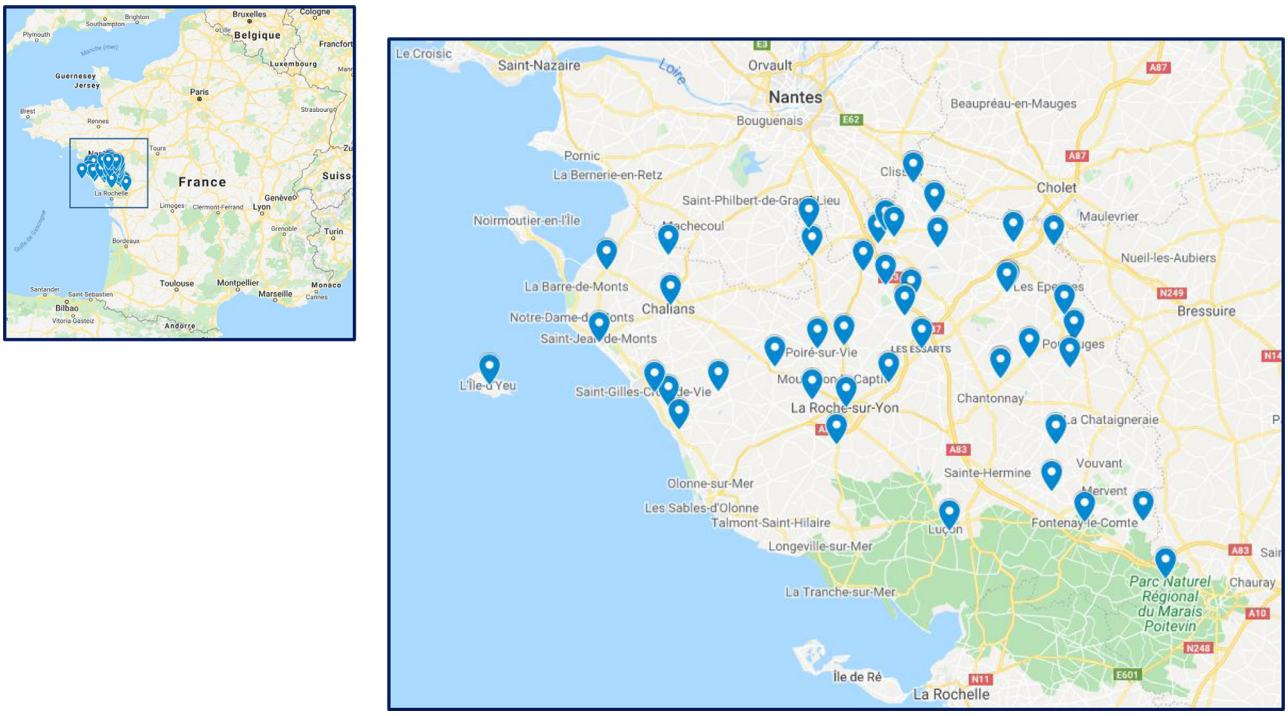

Fig. 1. Geographical distribution of the 50 participating nursing home in the Vendée department, France.

Table 1
Description of testing results performed to investigate the spread around a COVID-19 positive resident, professional, and in nursing homes free of case.

	Total	Residents	Professionals
Number of NH with screening results	50	35	48
Screening performed to investigate the spread around a COVID-19 positive resident			
Number of NH with screening results	14 (28%)	14 (40%)	14 (29%)
Number of NH without positive case identified	6 (42%)	7 (50%)	7 (50%)
Number of NH with 1 to 2 positive cases identified	4 (29%)	4 (29%)	4 (29%)
Number of NH with >3 positive cases identified	4 (29%)	3 (21%)	3 (21%)
Total number of participants tested	1538	754	784
Total number of positive cases identified	42 (2.7%)	22 (2.9%)	20 (2.5%)
Screening performed to investigate the spread around a COVID-19 positive professional			
Number of NH with screening results	4 (8%)	4 (11%)	4 (8.5%)
Number of NH without positive case identified	1 (25%)	3 (75%)	1 (25%)
Number of NH with 1–2 positive cases identified	2 (50%)	1 (25%)	2 (50%)
Number of NH with >3 positive cases identified	1 (25%)	0	1 (25%)
Total number of participants tested	410	238	172
Total number of positive cases identified	6 (1.4%)	5 (2.1%)	1 (0.6%)
Universal screening in NH free of COVID-19 confirmed case on the day of testing			
Number of NH with screening results	32 (62%)	17 (49%)	30 (62.5%)
Number of NH without positive case identified	30 (94%)	17 (88%)	30 (100%)
Number of NH with one positive case identified	2 (6%)	2 (12%)	0
Total number of participants tested	2897	1011	1886
Total number of positive cases identified	2 (0.07%)	2 (0.2%)	0

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Large-scale, molecular and serological SARS-CoV-2 screening of healthcare workers in a 4-site public hospital in Belgium after COVID-19 outbreak

Dear Editor,

We read with great interest the study of Chen Y et al., who analyzed, during the Chinese epidemic peak, the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among 105 healthcare workers (HCWs) exposed to COVID-19 patients.[1] They found 17.14% of seropositive asymptomatic or paucisymptomatic HCWs although their nasopharyngeal swab samples were SARS-CoV-2 RNA negative. Our purpose was to document at the end of the Belgium epidemic the seroprevalence of SARS-CoV-2 in HCWs exposed to COVID-19 at varying degrees and to compare these rates with those observed by other teams worldwide. Another objective was to highlight SARS-CoV-2 carriage in *a priori* healthy staff members to sensitize them to the need to respect individual protection measures and distancing to avoid patient contamination.

In Belgium, the COVID-19-outbreak peak was reached on 10 April 2020.[2] At the end of May, when the epidemic spread was greatly slowed down, our management decided to offer screening tests to all staff members ($n=3145$), regardless of their status and function. The campaign took place from May 25 to June 19, 2020 in the network of Iris hospitals South (HIS-IZZ, Brussels, Belgium), a 550-bed public hospital spread over 4 sites. Participation was voluntary and regardless of whether the HCW had already contracted the disease or not. A questionnaire was prepared focusing on the type of service the participant works in, the practice of medical procedures potentially at risk for SARS-CoV-2 infection, its status, function and perception of being infected or not. People with COVID-19 symptoms[3] were excluded from routine screening.

On the same day, all asymptomatic HCWs who agreed to participate benefited from both serological and RT-qPCR SARS-CoV-2 tests. The quantitative analysis of IgG antibodies directed against the S1 and S2 subunits of the virus spike protein was carried out using the LIAISON®SARS-CoV-2 IgG kit (DiaSorin, Saluggia, Italy). This CLIA method was extensively evaluated in our laboratory and showed 100% sensitivity two weeks after positive qRT-PCR diagnosis using an adapted cut-off.[4] Equivocal results were confirmed by a semi-quantitative ELISA method directed against the S1 subunit spike protein (Euroimmun Medizinische Labordiagnostika, Lübeck, Germany). HCWs with a previous COVID-19 documented history and a persistent positive RT-qPCR benefited from a viral culture. Statistical analyses were carried out using MedCalc version 10.4.0.0 (MedCalc Software, Ostend, Belgium). A P-value <0.05 is considered statistically significant.

During the study period, 1499 staff members participated (47.7%). Table 1 shows the participant characteristics. Among them, 215 workers (14.3%) reported having a function with no contact with patients while 1138 (75.9%) have had regular or occasional contact. This information was missing for 146 (9.7%). Among all workers, having had contact with patients, 838 had contacts with COVID patients: 205 worked in a COVID emergency department (of which 54.6% regularly), 399 worked in a COVID hospitalization unit (of which 54.9% regularly) and 234 worked in an intensive care unit (47.4% of them on a regular basis). Sixty-one performed bronchoscopies and 119 intubated patients (45.9% and 61.3% regularly). One hundred and eighty (12.0%) thought they were infected with SARS-CoV-2, 630 (42.0%) did not, 612 (40.8%) did not know and 77 (5.1%) did not answer.

A negative serology (<6.1 AU/mL) was observed in 1093 people, 206 were positive (>=15 AU/mL) and 200 were equivocal. Among the equivocal results re-tested with the second (ELISA) method, 11 were positive, 175 were negative and 14 remain undetermined. The overall seroprevalence reached 14.6% (217/1485). Seroprevalence was 53.9% in HCWs who thought they had the disease, 7.1% for those who did not and 9.6% for those who doubted.

The median values [95% IC] of the UA/mL were significantly higher in HCWs who thought they had become ill (16.2 [10.6–24.2]) compared to others (4.1 [3.9–4.2]) ($P<0.0001$, Mann-Whitney U test). No difference was found between people with regular patient contact and those with none ($P=0.0522$, Mann-Whitney U test). Seroprevalence was higher in HCWs in COVID units (COVID hospitalization units, COVID emergencies, intensive care) compared to the ones in non-COVID units: 13.5% versus 12.6% ($P=0.0007$, Chi-square). Fig. 1 shows the quantitative results of serology in 4 HCW groups with various exposure levels.

Among the 1499 samples sent for molecular diagnosis, 13 were positive, 1479 negative and 7 invalid. Amid the 13 HCWs, 5 had already a positive RT-qPCR result in the past. The median value of the delay between the first and the second RT-qPCR was 63 days (min-max: 50–67). Twelve people with positive RT-qPCR agreed to undergo a new sampling for viral-culture assessment. All came back negative meaning the RT-qPCR identified residual debris of viral RNA rather than living viruses. Among people with previously documented infection, 47 had positive serology, 5 had no antibodies and 2 were equivocal.

During the COVID-19 pandemic, several studies were conducted in HCWs, based on molecular and serological testing. Hunter et al. performed a massive PCR screening of UK healthcare personnel, which showed 14% positivity, but without serological documentation.[4] Korth et al. reported low seroprevalence (1.6%) in 316 German HCWs.[5] Our seroprevalence result of 14.6% is closer to that

Table 1
. Population characteristics.

Demography			
Males ($N = 414$)			
Age min		21.9	
Age max		71.9	
Age (median ; 95% CI)		47.45 ; 46.00–48.84	
Females ($N = 1085$)			
Age min		21.6	
Age max		72.7	
Age (median ; 95% CI)		43.90 ; 42.80–45.10	
Type of occupation	N		% positive serology
Nurse, Caregiver, Dietitian, Midwife, Occupational therapist, Psychologist, Social worker	588		19.2%
Medical doctor, Dentist, Physiotherapist, Logopedist	323		11.8%
Pharmacist, Administrative staff, IT	320		9.1%
Maintenance staff, Technical services	134		16.4%
Imaging technologist, Laboratory technologist	61		6.6%
Other	17		11.0%
Not specified	56		19.6%
COVID-19 exposure (patient exposed personnel only)	N		% positive serology
Workers at least once exposed to COVID-19 patients	550		13.5%
Workers never exposed to COVID-19 patients	588		12.6%
Status of the workers		N	
Employee		1178	
Self-employed		297	
Not specified		24	
Fields of occupation	regular	intermittent	% positive serology
COVID inpatient unit	219	180	24.3%
Intensive care unit	111	123	13.2%
COVID emergencies	112	93	14.6%
Non-COVID emergencies	98	86	18.5%
Non-COVID inpatient unit/Other services with patient contact	800	306	14.4%
Other services without patient contact	250	67	10.5%
Not specified	146		11.0%
Procedures at risk	regular	intermittent	% positive serology
Bronchoscopies	28	33	4.9%
Intubations	73	46	9.2%
Other*	174	39	17.4%

Legend: "Regular" means being assigned to a care unit or to perform a technical act and "intermittent" means providing voluntary or on-demand assistance to work in a care unit or to perform a technical act.

* Medical procedures potentially at risk of SARS-CoV-2 transmission: dental or stomatology care ($N = 19$); upper respiratory and digestive tract endoscopies (ENT fibroscopy, transesophageal ultrasound, gastroscopy) ($N = 40$); aerosol procedures (respiratory physiotherapy, aspirations, etc.) ($N = 89$); other procedures at risk (COVID smear, etc.) ($N = 52$).

reported by Chen et al.[1] To the best of our knowledge, seroprevalence in HCWs after the epidemic peak was never studied in as many participants. At the end of May, the Belgian Public Health Institute, Sciensano, assessed the seroprevalence of HCWs at 8.4% among 785 samples.[6] The difference between our results and those of Sciensano can be explained by the outbreak evolution which led to seroprevalence increase.

Unexpectedly, our screening campaign failed to identify a single new case of COVID-19 among the participants. People positive to RT-qPCR were not living-virus carriers. This confirm that molecular methods can give positive results at a distance from

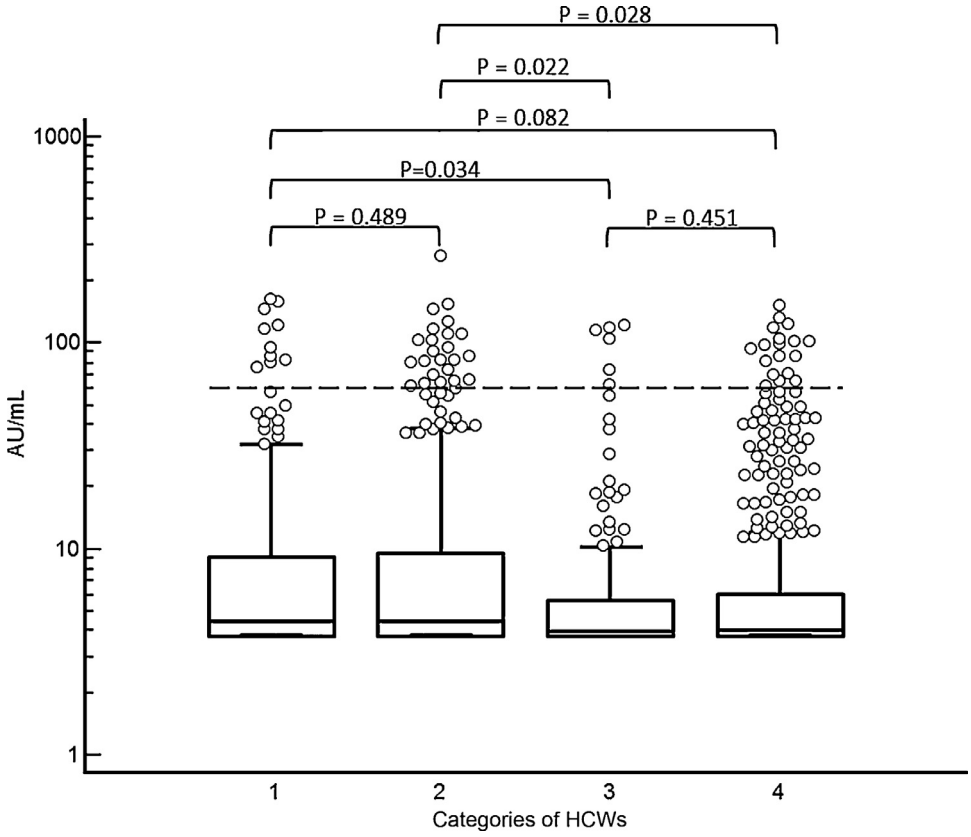

Fig. 1. Distribution of AU/mL in HCWs with varying degrees of exposure to SARS-CoV-2. 1: COVID units only; 2: Mixed COVID and non-COVID units; 3: No contact with patients; 4: Non-COVID units only. Dashline: cut-off of positivity.
A P-value <0.05 is significant.

a documented infection with an up to 67-day delay. Seroprevalence is higher than that documented by Sciensano during the epidemic peak and higher among HCWs who worked in COVID units. This shows that it is important to re-evaluate national seroprevalence in both the general population and HCWs at the end of the outbreak, especially as SARS-CoV-2 infection may be paucisymptomatic or asymptomatic and therefore infected people might ignore their status.

Ethical statement

The study design, the procedure of results communication, the information circular and the questionnaire have been submitted to and approved by our hospital's ethics committee (ethical agreement number: CEHIS/2020–19). An informed consent form has been requested from each participant, guaranteeing anonymity of the data and requesting permission to use them for statistical analysis. Out of respect for everyone's privacy, the participant was free to not answer to certain questions.

Declaration of Competing Interest

Authors state no conflict of interest.

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Comorbidities for fatal outcome among the COVID-19 patients: A hospital-based case-control study

Dear Editor,

Since the discovery of coronavirus disease 2019 (COVID-19), there have been numerous evidences supporting the pathogenic role of chronic comorbidities in the prognosis of infections, including the study by Galloway et al.,[1] however, with the extent of the risk remained controversial.[2–6] The existing univariate models do not adequately distinguish between risk due to age and that due to increased presence of co-morbidities in older patients, thus these assessments of real effect from comorbidities are inevitably confounded. Here by performing a retrospective multi-center study, we try to evaluate the adjusted effect of the common preexisting comorbidities on COVID-19 related death, based on which, the therapy effect of three widely used anti-hypertension drugs were assessed.

From 18 January to 24 February 2020, 1138 confirmed COVID-19 patients consisting of 920 survivals and 218 deaths from three designated hospitals for COVID-19 treatment in Hubei province were included for analysis (Table S1). The presence of comorbidities was reported in 49.12% (559/1138) of the total patients, with significantly higher frequency in the deceased than in the survivals (77.06% *vs.* 42.50%, $P < 0.001$; Table S1). As a whole, hypertension was the most prevalent comorbidity (32.95%), followed by diabetes mellitus (DM, 15.64%) and chronic heart diseases (CHD, 9.31%). The chronic obstructive pulmonary diseases (COPD), malignancy, cerebrovascular diseases (CVD), chronic kidney diseases (CKD), and chronic viral hepatitis (CVH) were less frequent, with prevalence ranging from 2.11% to 6.41%. By multivariate logistic regression model adjusting age, sex, and delay from symptom onset to hospital admission, six comorbidities showed significant associations with the disease outcome, with malignancy exhibiting the highest risk of death, followed by CKD, CVD, hypertension, CHD, and DM (Fig. 1A and Table S2).

An age-stratified analysis revealed the effect of comorbidities on death was reduced as the age increased. Among the patients ≤60 years, CVD had the highest effect on death, followed by hypertension. Among the patients aged 60–70 years, only malignancy and DM were related to fatal outcome. Among the patients aged >70 years, none of the eight comorbidities demonstrated the significant association with fatal outcome. The sex-stratified analysis disclosed that male patients presenting with any of the three comorbidities (hypertension, DM, or CVD) had an increased risk of developing fatal outcome, in contrast, female patients presenting any of the four comorbidities (CHD, COPD, malignancy, or CKD) had an increased risk of fatal outcome (Table S3).

For patients with isolated DM, four parameters displayed significantly higher abnormal levels than those without any comorbidity, i.e., fibrinogen, activated partial thromboplastin time, prothrombin time and IL-6 (Fig. 2A–D). For patients with isolated hypertension than those without, five laboratory indicators were deviated from normal value with greater extent, i.e., higher levels of D-dimer, fibrinogen degradation products, lactic dehydrogenase (LDH) and neutrophil percentage, and lower lymphocyte percentage (Fig. 2E–I).

Among the 1138 patients, 149 (13.09%) had two coexisting comorbidities (Table S1), with hypertension-DM most frequently observed (Table S4). Fifty-seven (5.01%) had three coexisting comorbidities, and 27 (2.37%) had four or more. The coexisting of multiple comorbidities had significantly increased the risk of death (Fig. 1B and Table S5). In the multivariate analysis, over 4-fold risk of death was observed in the patients with ≥3 comorbidities. Age-stratified analysis again revealed the effect of comorbidities on death was reduced as the age increased.

Forty-one (50.00%) of 82 fatal patients had taken calcium channel blocker (CCB) drugs, significantly lower than those among the survived (68.15%, 92/135; Table S6). The effect of CCB drugs on reducing fatal outcome was shown to be significant. The use of angiotensin receptor blockers (ARBs) or angiotensin converting enzyme inhibitors (ACEIs) was comparable between the fatal patients (24.39%, 20/82) and the survivals (24.44%, 33/135), showing no effect in reducing risk of death. Decreased levels of fibrin degradation product, D-dimer, C-reactive protein, IL-6 and LDH, less incidence of leukocytosis, and more rapid recovery of lymphocytes and neutrophils percentages were observed in the patients with CCB drugs treatment (Fig. 2J–Q). The patients who regularly received oral hypoglycemic agents or insulin treatment had over 70% reduced risk of death without significance (Table S6).

As is known, mechanisms that lead to hypertension, DM, and CVD were increasingly recognized to overlap with pathways that regulate immune function. Most importantly, older age is an important risk factor for these conditions and the effect of aging on immune function was equally important for COVID-19 severity. Therefore, the effect of age was mixed with those from the comorbidities, resulting in heterogenicity of effects among age groups. For those aged >70 years, none of the underlying condition played role in affecting the outcome any more. It's suggested that old age had exerted the strongest effect on death, that all effects from the comorbidities could be compromised when the patients are old enough.

DM was found to be a strong risk factor for adverse outcome, with its risky effect also observed in those aged 60–70 years, which was reported for the two earlier CoV infections, severe acute respiratory syndrome[7] and the Middle East respiratory syndrome.[8] In this study, inflammation-related biomarker such as IL-6, was elevated to a significantly higher level among the DM patients, indicating more intense induction of inflammatory storm. It is suggested accordingly that the anti-inflammatory drugs in treating diabetes-COVID-19 should be proposed. Moreover, the higher risk of diabetes-COVID-19 death could also be reduced by good glycaemic control, as displayed by the therapy effect of insulin.

RAAS blockade might decrease proinflammatory activity of Ang II, decreasing the risk of ARDS, myocarditis, or mortality in COVID-19.[9,10] We provided evidence that CCB drugs offered beneficial effect of reducing risk for fatal outcome in hypertension-COVID-19 patients, mostly mediated through enhancing the recovery of abnormal parameters and reducing host inflammatory re-

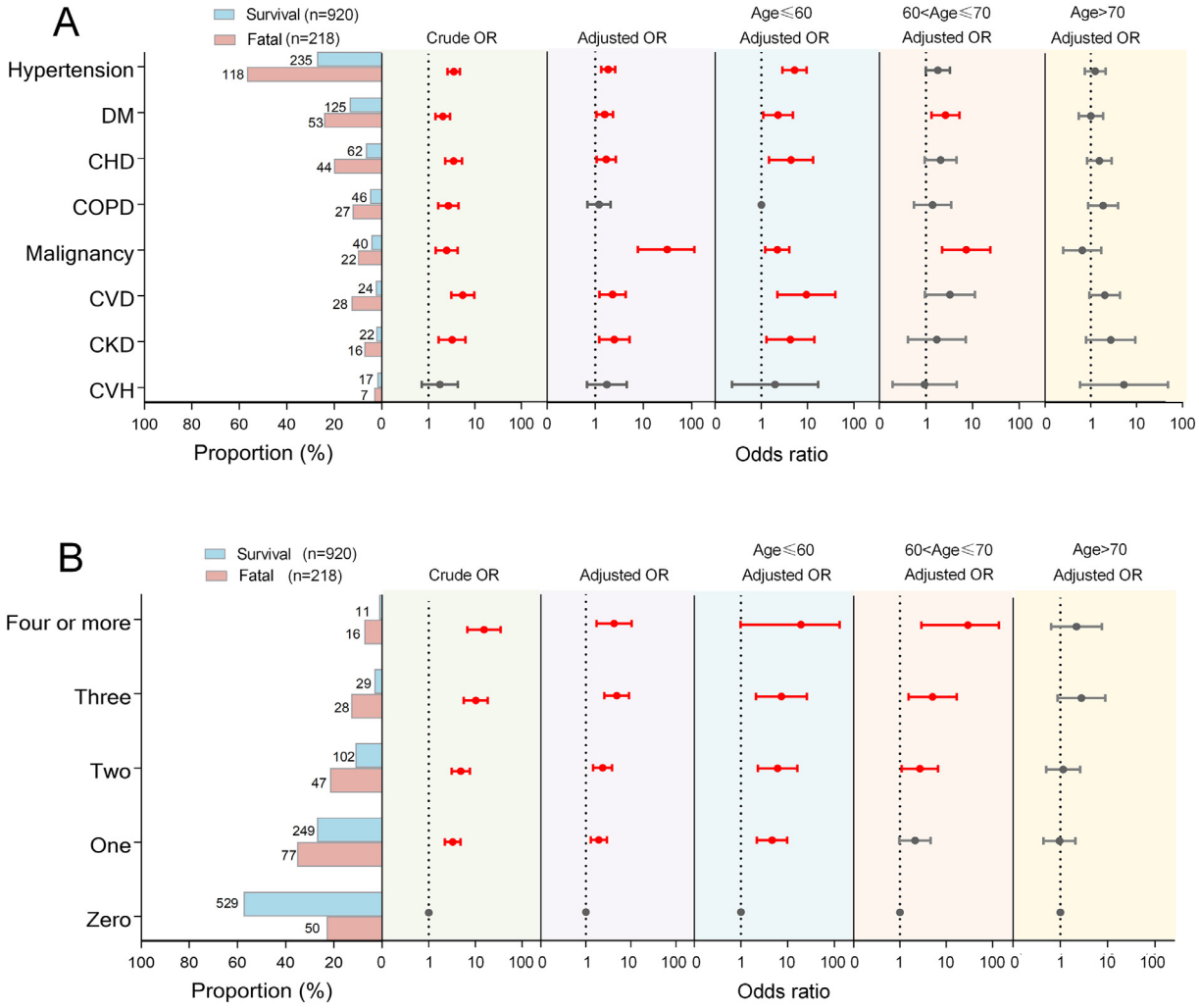

Fig. 1. Distribution of comorbidities and ORs for fatal outcome of the COVID-19 patients.
Association between each comorbidity and risk of fatal outcome was shown in Panel A, and association between multiple coexisting comorbidities and risk of fatal outcome was shown in Panel B. The numbers of multiple coexisting comorbidities are classified as zero, one, two, three, and four or more. The number of the COVID-19 patients is shown to the left of column. The dots are the odd ratios (ORs) and the error bars are the 95% confidence intervals; the red color represents $P < 0.05$ and the gray color represents $P \geq 0.05$. For the model with all the patients, the adjusted ORs are calculated with the use of multivariate logistic regression model by adjusting age, sex and the delay from symptom onset to hospital admission. For the models with the patients stratified by age, only sex and the delay from symptom onset to hospital admission are adjusted. Three age groups are stratified as ≤ 60 years, 60–70 years, and > 70 years. The dotted line indicates an OR of 1. DM, diabetes mellitus; CHD, chronic heart diseases; CVD, cerebrovascular diseases; CKD, chronic kidney diseases; COPD, chronic obstructive pulmonary diseases; CVH, chronic viral hepatitis.

sponse that had been proven to aggravate the disease severity. Hence the current study provided further pharmacoepidemiologic data supporting the effect of CCBs in treating SARS-CoV-2 infection combined with hypertension.

In conclusion, the comorbidities significantly affected the outcome of OCVID-19 but were age-dependent. The anti-hypertensive treatment, especially CCBs can offer beneficial effect in reducing the mortality of COVID-19.

Declaration of Competing Interest

Authors not named here have disclosed no conflicts of interest.

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IRB approval

The study was conducted in accordance with guidelines approved by the Ethics Committees from the hospitals (WDRY2020-K044, TJ-IRB20200102). The Research Ethics Committee waived the requirement informed consent before the study started because of the urgent need to collect epidemiological and clinical data. All the data were analyzed anonymously.

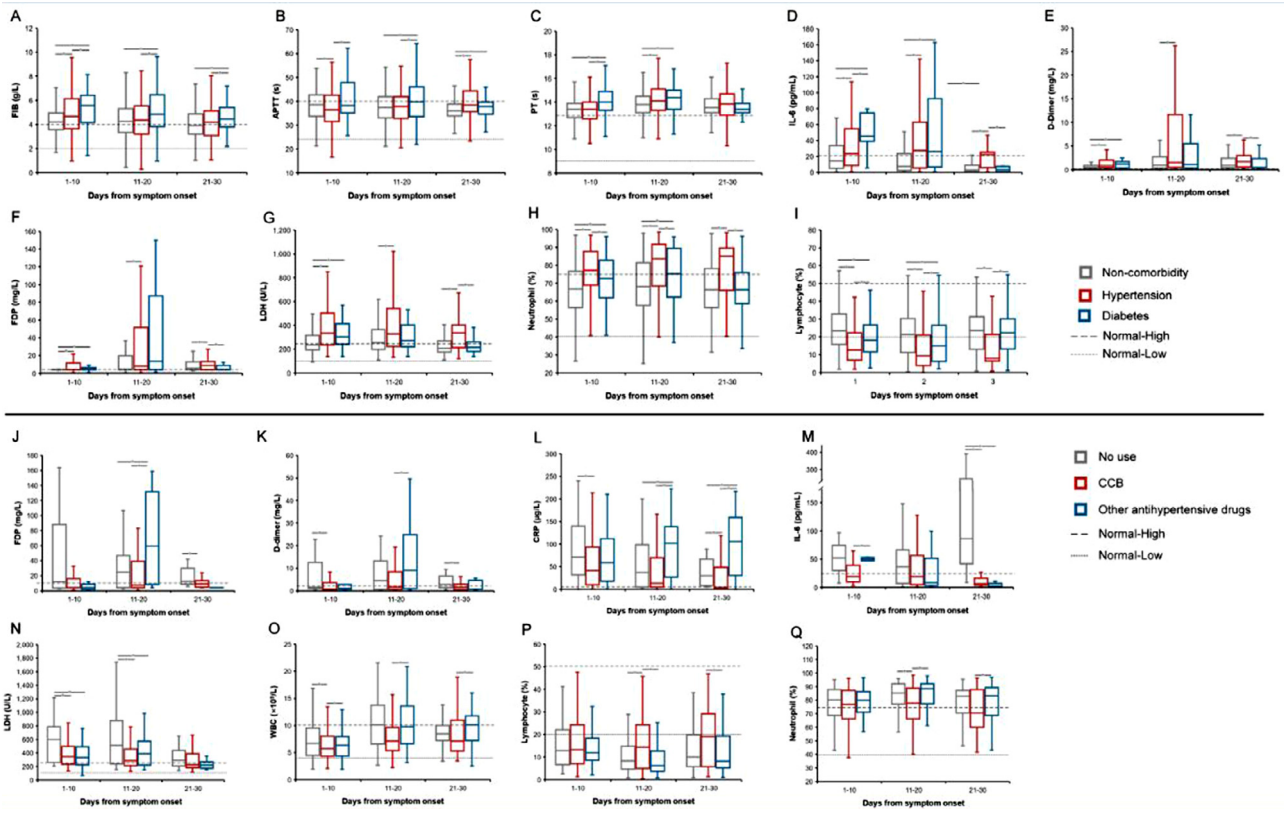

Fig. 2. Coagulation and inflammation-related biomarkers in the patients of COVID-19 and among the COVID-19 patients with hypertension under three different medications. The gray, red, and blue boxes represent the COVID-19 patients without any of the eight comorbidities, only with hypertension, and only with diabetes above the black line, respectively; the gray, red, and blue boxes represent no drug use, calcium channel blocker (CCB) drugs treatment, and other antihypertensive drugs treatment in the COVID-19 patients with hypertension under the black line, respectively. A, fibrinogen (FIB); B, activation of partial thrombin time (APTT); C, prothrombin time (PT); D, interleukin 6 (IL-6); E, D-Dimer; F, fibrinogen degradation products (FDP); G, lactate dehydrogenase (LDH); H, neutrophil percent; I, lymphocyte percent; J, fibrinogen degradation products (FDP); K, D-dimer; L, C reactive protein (CRP); M, interleukin 6 (IL-6); N, lactate dehydrogenase (LDH); O, white blood cell (WBC); P, lymphocyte percent; Q, neutrophil percent. The star (*) means $P<0.05$ between two groups. Three stages were classified based on the days from symptom onset: 1–10 days, 11–20 days and 21–30 days. Other antihypertensive drugs include angiotensin receptor blockers, angiotensin converting enzyme inhibitors, β-blockers, and thiazide.

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Role of the funder/sponsor

The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication

Access to data

After publication, the data will be made available to others on reasonable requests to the corresponding author. A proposal with detailed description of study objectives and statistical analysis plan will be needed for evaluation of the reasonability of requests. Additional materials might also be required during the process of evaluation. Deidentified participant data will be provided after approval from the corresponding author and Wuhan Tongji Hospital.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.07.026.

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Mobile health technology combats COVID-19 in China

To the Editor,

Recently, there have been some papers of coronavirus disease 2019 (COVID-19) reported in this journal, which focused on mobile health technologies.[1] The COVID-19 pandemic has precipitated a global crisis due to the continued absence of a vaccine or cure, affecting more than 200 countries and areas, with more than 10 million confirmed cases and more than 0.5 million confirmed deaths globally by 4 July 2020.[2] China is one of the countries that has contained COVID-19 propagation and almost stopped indigenous transmission. Mobile health, a comprehensive strategy that uses mobile apps, sensors, social media, and location tracking technologies to obtain medical data and to provide health services, played a major role in tackling COVID-19 epidemic.[3] We explore China's application of three mobile health technologies to replenishing traditional public-health and social approaches for mitigating and suppressing COVID-19, including (1) Internet hospital, (2) fact-check and information-release platform, and (3) infection risk score (Fig. 1).

Internet hospitals alleviate the unavailability, inaccessibility, and inequity of health services during the outbreak. Health services had been overwhelmed by the rapid and widespread person-to-person transmission, and could not be accessed due to strict quarantine, isolation, and lockdown policies. Internet hospitals provided telemedicine-based consultation for both COVID-19 and common chronic diseases, psychological counseling, and health education via smartphone apps and hospitals' official websites.[4] This is a possible solution to delivering routine health care without COVID-19 exposure risk and to avoiding unnecessary hospital consultations for patients with mild flu symptoms. During the epidemic, the health services delivery by Internet hospital increased 17 times over the same period last year.[5] From January 1 through April 30, 2020, a total of 146 Internet hospitals were launched, accounting for about 30% of the total.[6] Several innovations and collaborations empower Internet hospital to combat COVID-19. Artificial intelligence-aided online medical chatbots were employed to decrease physicians' workloads and to enhance capabilities to triage suspected patients. Blockchain companies and pharmacies were cooperated with Internet hospitals to deliver prescription drugs to patients' doorsteps. A large number of doctors, including medical students, newly registered at Internet hospitals, provided voluntary consultations and addressed public inquiries. The authorities issued regulations requiring that eligible "Internet+" medical service fees be covered in medical insurance payments.[7]

Fact-check and information-release platforms reduce the spread of misinformation. We're not just fighting an epidemic; we're fighting an infodemic.[8] Digital social networks play an unprecedented role in health information communication because of physical distancing and near-complete global lockdown. Users in the self-media era increasingly see influencers within their peer networks as trustworthy sources of information. However, this method lacks expertise and responsibilities related to information inspection and dissemination, thus fostering the spread of misinformation which destabilizes public trust and further imperil public health. The Chinese governments and health bodies cooperated with social media giants Sina, and Tencent to take action to eliminate misinformation, such as flagging, fact-checking, and even removing false or outdated information, and to provide trustworthy sources of ongoing updates for COVID-19 about transmission, diagnosis, treatment, and policies. The fact-check and information-release platforms leverage their efforts to flatten the curve of misinformation and to elevate facts over fiction so as to not incite panic amongst the public.

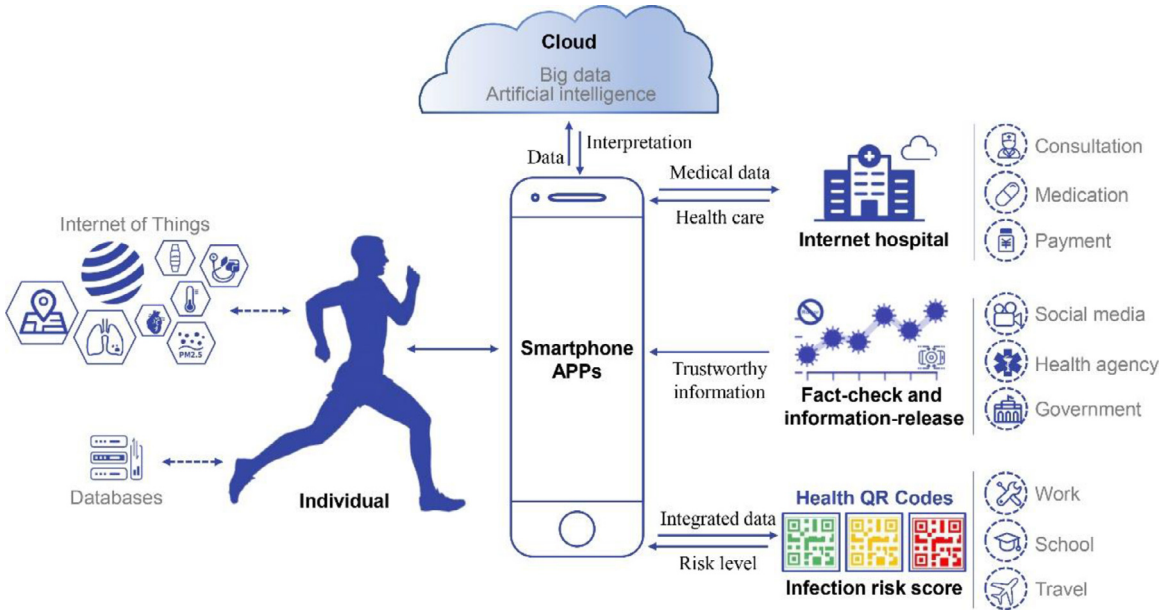

Fig. 1. Application of Mobile Health Technology in China during the COVID-19 epidemic and beyond. This figure presents a simplified view of the workflow of mobile health. The Internet of Things (IoT) communicates with the individual's smartphone via Bluetooth or Wi-Fi. The databases include household registration information, drug purchase records, medical records, travel history and other data. Once on the phone, those passive data combined with active data reported by individuals can be displayed in the app or can be sent up to the cloud storage. This cloud "backend" stores data and can apply artificial intelligence (AI) algorithms as well as big data analytic techniques to generate predictions, visualizations, or decision support. The cloud output can then be delivered back and displayed on a mobile phone app or website that is accessible to individuals and available to the relevant personnel after authorized. Internet hospitals provided telemedicine-based consultation and medication delivery services, and the cost can be reimbursed by medical insurance. The governments and health agencies partnered with social media companies to eliminate misinformation and to provide trustworthy information. The integration of active self-reported health status and passive background creates an infection risk score system, also known as health QR codes. Now citizens are required to hold a "green" code for entering public facilities, workplaces, schools, or traveling.

The infection risk scoring systems facilitate restoring the order of production and life. While the outbreak continues to ease, work and production resumption has been a key priority in China. The national government service platform and the local governments combined history of individuals' locations, medical information, and medication purchase records to create infection risk scoring systems, what is called the health QR codes, embedded in widely used apps such as Alipay and WeChat. The health QR codes are generally divided into three colors: green, yellow, and red. Yellow or red indicates that the resident has not passed the health check because of one of the following reasons with 14 days: fever clinic visits records, fever-reducing drugs purchase records, travel history in high-risk regions, close contacts of confirmed or suspected cases, or physical symptoms when filling in an application form.[9] Now citizens are required to have a "green" code when entering public facilities, workplaces, or traveling. Active voluntarily self-reported data on health status via daily one-minute surveys complement passive background-based health QR codes. Besides, coronavirus nucleic acid detection and paper certificates were used as supplementary measures for those uncertain residents. The use of the health QR codes have overcome the fragmentation of traditional data collection and greatly promoted the government's precise measures in epidemic prevention and control and resumption of work and production in an orderly fashion.

Although the mobile health technology-enabled approaches will definitely play an unprecedented role during and after the COVID-19 pandemic, we still need to pay attention to their potential limitations, including intergenerational differences in smart devices ownership and the digital divide in media access. Digital refugees are groups that are far from digital technologies due to economic, social, and cultural reasons. Mobile health is based on smartphones, but nearly 40% of Chinese are not mobile Internet users, including two categories: owning a mobile phone but not surfing the Internet, and not owning a mobile phone. In the process of digitization, we must consider that these digital refugees, such as the elderly, provide them with alternative solutions so that they will not be left by mobile health.

Declaration of Competing Interest

All authors declare that there are no conflicts of interest.

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Immunological detection of serum antibodies in pediatric medical workers exposed to varying levels of SARS-CoV-2

Dear Editor,

Since the initial reports of COVID-19 disease outbreak in Wuhan, China, it has continued to spread rapidly with cases identified in virtually all countries, worldwide.[1] The population is generally susceptible to SARS-CoV-2, including children and pregnant women, and medical staffs are a high-risk population for this disease. In this journal, Chen et al. have reported the high SARS-CoV-2 antibody prevalence among healthcare workers exposed to COVID-19 patients.[2] Here we would like to share our finding about the serum antibodies analyzed in a special group of pediatric medical workers exposed to varying levels of SARS-CoV-2 after Wuhan severe epidemic of COVID-19.

A preliminary study suggests children can be infected with SARS-CoV-2 like adults but are less likely to be symptomatic or develop severe symptoms.[3,4] The asymptomatic or mildly symptomatic children might transmit the disease.[5] Therefore they are tested for SARS-CoV-2 less often than adults, leading to an underestimate of the true numbers of children infected.[6] Laboratory tests play a pivotal role in the diagnosis and management of COVID-19; the current gold standard being real-time reverse transcription polymerase chain reaction (rRT-PCR) on respiratory tract specimens.[7] The measurement of specific COVID-19 antibodies (both IgG and IgM) should serve as an additional, non-invasive tool for disease detection and management, especially in patients who present late, with a low viral load. Due to the high infection rate of medical workers and the uncertainty of child-to-person transmission, we chose a special group of pediatric medical workers as the research subjects to investigate their infection status with SARS-CoV-2 and analyze possible causes. This study also helps clarify the potential of different immunological techniques for antibody detection as an auxiliary diagnosis of COVID-19.

On March 19–20, 2020, pediatric medical workers ($n = 325$) in one hospital but not the designated hospital for COVID-19 in Wuhan were recruited. They were divided into three groups depends on their level of contact with confirmed and/or suspected COVID-19 cases during the outbreak: i. close contact group (contact with confirmed and/or suspected cases of COVID-19), ii. non-close contact group (contact only with non-COVID-19 patients), and iii. non-contact group (no contact with any patients). Three different immunological detection methods were used to measure SARS-CoV-2 serum antibodies: colloidal gold-based detection, enzyme-linked immunosorbent assay (ELISA), and dual-target immunofluorescence assay (DTFA) (details in the Supplementary methods). The overall positive rate for SARS-CoV-2 IgG and IgM antibodies in the pediatric medical workers was 43.08 and 5.85%, respectively. For the close contact, non-close contact, and non-contact groups, respectively, the DTFA positive rates for IgG were 41.36, 14.68, and 12.50% ($p < 0.05$), and the ELISA positive rates for IgG were 34.55, 10.91, and 4.17% ($p < 0.05$) and 8.38, 0.91, and 0% for IgM ($p < 0.05$). Colloidal gold detection results were negative for IgG and only two participants tested positive for IgM, both in the close contact group (Table 1). It suggests the colloidal gold detection kit used in this research is not sensitive enough to be useful in accurate antibody detection, whereas the DTFA and ELISA positive rate performed similarly.

We further conducted a multivariate logistic regression analysis using antibody results as the independent variables to investigative the relationship of positive serum antibody results, with the performance of aerosol procedures, exposure levels to COVID-19 cases, clinical symptoms (including fever, cough, headache, stuffy nose, runny nose, sneezing, pharyngalgia, diarrhea, fatigue, etc.), chest CT imaging changes, and age of participant (Table 2). The results showed that participants who had performed an aerosol procedure had a 2.70-fold higher risk of testing positive, and with each additional level of exposure to COVID-19, the risk of testing positive for antibodies increased 5.26-fold. None of the antibody positive participants contained neutralizing antibodies in their serum maybe cause of the low viral load exposure.

After one more month at the end of April, 70 of the 325 participants who had a positive result using any of the above test methods volunteered to participate in a retest of IgG/IgM for SARS-CoV-2. Positive results were observed for 33 of the 70 (47.14%) participants when they were first tested for rN-IgG and rRBD-IgG by DTFA, and 30 of the 33 (90.91%) participants became negative or weakly positive by the same detection method one month later. Meanwhile, 47 of the 70 (67.14%) participants tested positive when they were first retested for rN-IgG by ELISA; 41 of the 47 (87.23%) became negative or weakly positive when similarly tested again one month later. Eleven of the 70 (15.71%) participants tested positive when they were first tested for rRBD-IgM by ELISA, 4 of the 11 (36.36%) became negative or weakly positive one month later. Although we cannot clearly track antibody kinetics for asymptomatic infections, we can observe that the majority of participants with positive IgG antibodies had a significant decline in antibody levels after one month. That means the SARS-CoV-2 antibodies diminish to near undetectable levels within two months.

This research revealed that pediatric medical workers are a high-risk group for infection by SARS-CoV-2, and the higher the exposure levels to COVID-19 patients and aerosol production, the greater chance of being infected. Meanwhile, we found that pediatric workers had lower levels of IgG antibodies than patients with COVID-19.[2] Children-to-person transmission is almost inevitable, but pediatric medical workers often have no or only mild clinical symptoms and also cannot produce enough antibodies to neutralize the virus. The antibody protection that healthcare workers obtained after infection by SARS-CoV-2 in this study, could not be maintained for a long time and no NAbs were detected to provide them with sufficient protection.

Table 1
Test results of serum antibodies in pediatric medical workers exposed to different levels of SARS-CoV-2.

			DTFA			ELISA				Colloidal Gold Detection	
	Total positive rate of IgG%	Total positive rate of IgM (%)	rN-IgG	rRBD-IgG	Total positive rate of IgG (%)	rN-IgG	rN-IgG positive rate (%)	rRBD-IgM	rRBD-IgM positive rate (%)	IgG positiverate (%)	IgM positive rate (%)
All participants	43.08 (140/325)	5.85 (19/325)	1076.52±1153.14	738.42±988.99	30.25 (98/324)	0.13±0.16	24.31 (79/325)	0.06±0.04	5.23 (17/325)	0.00 (0/325)	0.62 (2/325)
Close contact group	58.12 (111/191)	9.42 (18/191)	1308.98±1323.92	958.49±1188.43	41.36 (79/191)	0.16±0.20	34.55 (66/191)	0.06±0.05	8.38 (16/191)	0.00 (0/191)	1.05 (2/191)
Non-close contact group	22.73 (25/110)	0.91 (1/110)	784.02±791.23	434.45±463.47	14.68 (16/109)	0.08±0.06	10.91 (12/110)	0.06±0.04	0.91 (1/110)	0.00 (0/110)	0.00 (0/110)
Non-contact group	16.67 (4/24)	0.00 (0/24)	587.58±362.93	385.63±282.21	12.50 (3/24)	0.07±0.04	4.17 (1/24)	0.05±0.02	0.00 (0/24)	0.00 (0/24)	0.00 (0/24)
F or χ^2	43.02	10.80	10.28	12.35	27.29	9.95	26.93	1.36	9.28	–	1.41
p	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.26	0.01	–	0.49

Table 2
Multivariate logistic regression analysis of positive antibody tests.

		Variables in the equation							
		B	SE	Wald	df	Sig.	OR	95% Confidence interval of OR	
								Lower	Upper
Step 1	Aerosol operation	.992	.297	11.118	1	.001	2.696	1.505	4.828
	Exposure levels	1.660	.298	31.142	1	.000	5.262	2.937	9.428
	Clinical symptoms	.109	.281	.150	1	.698	1.115	.643	1.935
	Chest CT imaging changes	.492	1.028	.229	1	.632	1.636	.218	12.267
	Age	.015	.017	.754	1	.385	1.015	.982	1.048
	Constant	−5.846	.871	45.015	1	.000	.003		

B: Regression coefficients; SE: Standard error; Wald: Chi-square value; df: Degrees of freedom; Sig: Significance; OD: Odds Ratio.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.07.023.

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Changes in the hospital admission profile of COVID-19 positive patients at a central London trust

Dear Editor,

There is currently no WHO approved method for counting and coding deaths attributable to COVID-19. Since the start of the pandemic, Public Health England have counted all deaths from individuals who have tested positive for SARS-CoV-2 at any time as being attributable to COVID-19, which has resulted in recent debate.[1] Experiences such as these highlight the ongoing challenges of coding and modelling the COVID-19 pandemic as we move past the initial peak and societal restrictions are relaxed.

Here we describe the changing profile of hospital admissions and readmissions, and its potential impact on modelling. In the UK, both the incidence and mortality burden associated with COVID-19 peaked in April 2020 and have subsequently declined.[2] NHS trusts are now facing new challenges as the UK transitions to the next stage of the pandemic. In particular, there is a pressing need to reopen non-COVID-19 clinical services safely. As national incidence falls, a clear understanding of the profile of newly admitted SARS-CoV-2 positive patients over time would help hospital trusts focus resources and plan for re-introduction of services.

Imperial College Healthcare NHS Trust (ICHT) is a large central London trust which, by 7th July, had successfully discharged 1283 COVID-19 patients, with 427 deaths. At ICHT, reported COVID-19 admission numbers returned as part of National Health Service situation reporting remained high despite anecdotal clinical experience that numbers of acute admissions were falling. This – and other possible anomalies in national level data available to one of the authors (SJB) – led to an urgent public health request to examine ICHT data in detail. This was approved by the Trust's Chief Clinical Information Officer and Caldicott Guardian.

We speculated that patients with historic, or incidental, infections were being included in daily admission figures by automated data feeds, with a consequent potential impact on modelling and service planning. We compiled a list of patients who had been reported under 'Number of confirmed COVID-19 patients admitted with COVID-19 in last 24 h (total)' in daily situation reports over a 6-week period following the peak of hospital admission (8th April to 19th May 2020). This included patients who were admitted to hospital with a historic laboratory diagnosis of SARS-CoV-2 or were diagnosed within 24 h of their hospital admission. Electronic records were reviewed for date of relevant admission and initial PCR diagnosis. We examined clinical notes to determine the reason for admission, which was categorised into six groups: acute COVID-19 infection; a complication from previous COVID-19 infection; incidental SARS-CoV-2 diagnosis with unrelated presentation; previous COVID-19 diagnosis not related to current admission; transfer into trust with pre-existing COVID-19 diagnosis; and no evidence of clinical or PCR diagnosis of COVID-19. Categorisation was determined based on presenting symptoms, imaging, blood results, and the medical notes. Fourteen miscategorised patients without clinical or laboratory evidence of COVID-19 were removed from the analysis.

A total of 319 admissions comprising 314 individual patients were included in the analysis (Fig. 1). We observed a drop in reported weekly COVID-19 admissions from weeks 1 to 3, followed by a stabilisation in weeks 4 to 6. Notably, we observed a clear change in admission profile over time. For the week of 13th-19th May, the interval between diagnosis and admission was 37 days (18.5–49.3) days (median, IQR), compared with 0 (0–6) days for 8th-14th April. This was due to a fall in the numbers of true acute COVID-19 disease. However, admission figures appeared to be sustained by patients miscoded as acute COVID-19. These included patients with a historic diagnosis of COVID-19 readmitted with an unrelated condition; those admitted with complications of COVID-19 (e.g. venous thromboembolism, deconditioning); repatriations or inter-hospital transfers; or asymptomatic patients with an incidental finding of SARS-CoV-2 on naso-pharyngeal screening.

The majority of readmissions with complications had ongoing respiratory symptoms in the absence of proven venous thromboembolism (16/31; 52%). This is similar to a recent letter in this Journal looking at readmissions at a London trust[3] and suggests that patients were being discharged optimistically from their primary admission. Deconditioning in the elderly was the second commonest complication (8/31; 26%) and suggests that clinical and social care post-discharge was not able to meet expectations. Interestingly, the number of incidental new diagnoses persisted beyond the drop in acute admissions. This is likely due to an increase in screening of asymptomatic patients, or a reflection of prolonged PCR positivity following infection.

We were concerned that some patients diagnosed over 24 h after their admission would be missed. Indeed, on examination of additional trust data, 31 patients over the 6-week period were diagnosed beyond 24 h and would not have appeared in our dataset. We expect most of these unaccounted patients to be acute diagnoses in April, which would amplify our findings rather than contradict them.

These findings raise two key points. Firstly, the potential impact on epidemiology and disease modelling. Miscategorisation of COVID-19 admission profiles may cause an overestimate of acute disease and estimates of community transmission. The daily report studied here was created by semi-automated data processing. As the epidemic proceeded, the original logic underpinning the design of data systems became detached from subsequent requirements and expectations as central data requests developed and strategies around testing evolved. Trusts with higher levels of digital functioning may be more vulnerable to unidentified errors in coding, compared with smaller or less digitally advanced institutions. Policymakers must repeatedly ensure clarity in definitions and guidance documents and trusts must regularly review data collection routines.

Secondly, these data are relevant to planning reconfigured services, suggesting that even as acute COVID-19 admissions fall, there will be a constant flow of patients with previous diagnoses. Trusts must urgently develop clear pathways for readmissions and incidental diagnoses as non-COVID-19 services are being reintroduced nationwide. Key to this will be research on length of infectivity,

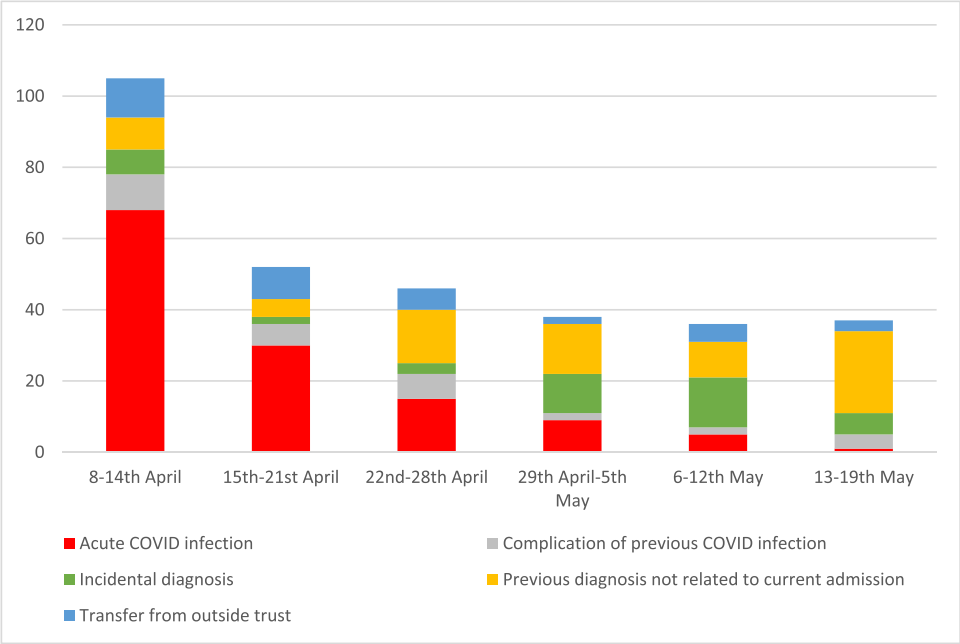

Fig. 1. Number of patients in each diagnostic category for weekly COVID-19 admissions to ICHT from 8th April to 19th May 2020.

given most patients with a previous diagnosis of COVID are now presenting over a month following their initial diagnosis, and the risk of re-infection.

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Declaration of Competing Interest

None.

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Insufficient evidence to advise a single dose of doxycycline to prevent Lyme disease from an *Ixodes* tick bite.

Dear Editor,

Harms and colleagues advised a single dose of doxycycline to prevent Lyme disease from a *Ixodes ricinus* tick bite.[1] Using a modified-intention-to-treat analysis, the authors that reported a single 200 mg dose of doxycycline resulted in a 67% relative risk reduction for the subjects in the treatment arm.

However, due to issues of trial design and execution, the actual benefit is difficult to determine. A 52% loss of subjects following randomization is high. The study employed an open-label design and allowed subjects in the prophylaxis group who developed an erythema migrans lesion within 72 h of the bite to cross over to the no treatment group in order to allow for full antibiotic treatment. The use of a modified-intent-to-treat analysis instead of an intent to treat protocol also weakens the investigators' conclusion.

For the record, the trial design in the tick bite prophylaxis study by Nadelman and colleagues did not allow the investigators to draw any conclusions regarding the prevention of Lyme disease.[2] It that study, the primary end-point was prevention of an EM rash at the bite site. Three patients with symptoms of early Lyme disease and evidence of seroconversion were not identified as disease positives because they lacked an EM at the bite site. The six-week

observation was too short to determine whether later manifestations of the illness were prevented

The International Lyme and Associated Diseases Society (ILADS) opposed a single dose of doxycycline to prevent Lyme disease from a tick bite in their guidelines.[3] When antibiotic prophylaxis therapy is offered for a blacklegged tick bite, ILADS advises a three-week treatment course of doxycycline.

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The determination of release from isolation of COVID-19 patients requires ultra-high sensitivity nucleic acid test technology

Dear Editor,

The prevention and control of SARS-CoV-2 has entered a critical period. Recent one paper in this journal also discussed weather qualitative RT-PCR be used to determine release from isolation of COVID-19 patients [1]. This issue is really important. Since the outbreak of COVID-19 worldwide, discontinuation of isolation has been presenting a dilemma of COVID-19, despite of the test-based strategy or the symptom-based strategy [1]. The reason for the confusion is that nucleic acid testing presents false negative based on qPCR technology, because of its low sensitivity [2–4]. There are several factors for false negative, including sample collection, preservation, transportation, virus inactivation, nucleic acid extraction and technical sensitivity, amongst which technical sensitivity and precise sampling are the most important quality control measures to eliminate false negative.

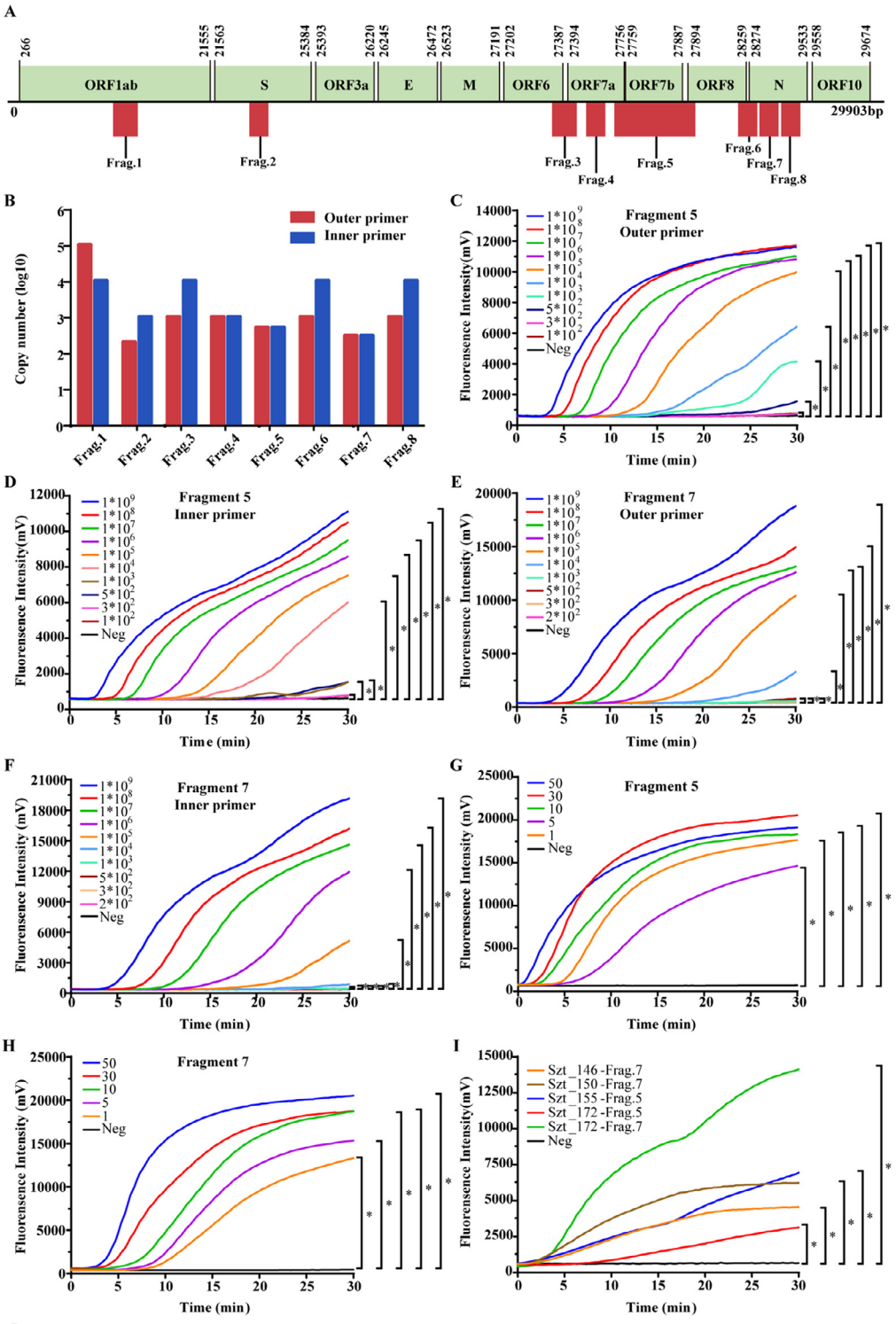
It is well known that SARS-CoV-2 nucleic acid test is the main diagnostic method of COVID-19. Recombinase polymerase amplification (RPA) is a new technology for testing nucleic acid with some advantages of simple operation, fast speed and low cost based on isothermal amplification. In our study, we developed an improved strategy, termed as nestRPA (nest recombinase polymerase amplification), which could greatly improve the sensitivity of nucleic acid detection for SARS-CoV-2 than RPA or qPCR.

Firstly, we designed eight sets of primers and probes for RPA on the conservation regions of SARS-CoV-2 genes, in which some fragments were designed to span multiple gene regions (Fig. 1A) which is one of the important technical tips. Through the two rounds of primer screening, we found that the limit of detection (LOD) of 16 pairs of primers and 8 probes is quite different (Fig. 1B), in which Fragment 1 against ORF1 gene had the worst amplification efficiency. And Fragment 5 and 7 had the smallest LOD value, 300 and 500 copies/uL (Fig. 1C to 1F), respectively.

As far as we know, we firstly proposed the concept of nestRPA. The basic principles of nestRPA are as follows: in nestRPA, the first amplification fragment of target gene is amplified by outer primers, then a second fragment of target gene completely within the first amplification fragment is amplified by inner primers. In order to eliminate the influence of the fluorescence signal of enzymes, fluorescent probe is not included in first RPA reaction which is another important technical tips. And in the second RPA reaction, fluorescent probe will be added into reaction system. Using nestRPA technology, we found that positive plasmid containing SARS-CoV-2 with the concentration of 1 copy/ul could also be stably detected by Fragment 5 and Fragment 7 within 1–10 min (Fig. 1G and 1H), suggesting that nestRPA technology indeed performed very well for the detection of SARS-CoV-2 nucleic acid.

In order to promote the clinical application of nestRPA technology, we firstly collected 14 samples from 14 patients diagnosed as COVID-19, all of which SARS-CoV-2 nucleic acid were positive using qPCR. The results of nestRPA assay showed that SARS-CoV-2 nucleic acid of these samples were 100% (14/14) positive. And then one positive sample (Szt_P_002) with the lowest Cq-value was selected to test the sensitivity of nestRPA technology. We found the detection result of Szt_P_002 sample was still positive after 11 times of 10-fold serial dilution by nestRPA assay, whilst after the fourth times of the same dilution fold, the result by qPCR test has been negative. Secondly, 101 samples from 73 patients diagnosed as COVID-19 were collected, all of which had negative results using qPCR, whilst 32.67% (33/101) of the samples were identified as by nestRPA assay. Furthermore, we collected 25 samples from 8 re-positive patients who repeatedly hospitalized suffering from COVID-19. Our results showed that 96.00% (24/25) of the samples tested positive by nestRPA whilst only 24.00% (6/25) of the samples were confirmed as positive by qPCR. These six samples with positive results by qPCR also had positive results by nestRPA. Our detection results were basically consistent with the clinical diagnosis results. Moreover, to explore whether there were asymptomatic patients with SARS-CoV-2 nucleic acid positive in healthy population, we collected 32 nasal swabs samples from those returning to work, all of which the SARS-CoV-2 nucleic acid detection results were negative using qPCR. However, we found 12.50% (4/32) of the samples were positive using Fragment 5 and/or Fragment 7 by nestRPA (Fig. 1I), which was consistent with those reported by other researchers [5]. Our results suggested that the ultra-sensitive nucleic acid detection technique has important implications for early identification of those asymptomatic carriers infected with SARS-CoV-2. Of course, in order to avoid false positive results, the target sequence of positive amplification products was 100% detected by high-throughput sequencing. In summary, 36.18% (55/152) of the samples with qPCR negative results were identified as positive by nestRPA technology in 172 clinical samples from 127 patients, which indicated the analytical sensitivity of nestRPA assay is much better than that of qPCR (Fig. 1J).

In addition, many experts of COVID-19 prevention and treatment clearly pointed out that the inaccurate sample collection was also one of the important reasons for the false negative result of SARS-CoV-2 nucleic acid [6–8]. The most commonly sites used as sampling are oropharynx and nasopharynx. The sample collectors should fix the tongue with a spatula, and the sampling swab is



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Result	nestRPA Positive	nestRPA Negative	Total
qPCR Positive	20 (11.63%)	0 (0.00%)	20 (11.63%)
qPCR Negative	55 (31.98%)	97 (56.39%)	152 (88.37%)
Total	75 (43.61%)	97 (56.39%)	172 (100.00%)
Pearson Chi-Square, p < 0.01			

Fig. 1. Nucleic acid detection results using nestRPA. (A) The distribution of target fragments on SARS-CoV-2 genome. (B) The LOD of optimum primer pairs from different gene regions. (C) The sensitivity of outer primers for Fragment 5. (D) The sensitivity of inner primers for Fragment 5. (E) The sensitivity of outer primers for Fragment 7. (F) The sensitivity of inner primers for Fragment 7. (G) The sensitivity of nestRPA for Fragment 5. (H) The sensitivity of nestRPA for Fragment 7. (I) The five positive results of four people returning to work by nestRPA. (J) Statistics of nucleic acid detection results by nestRPA and qPCR assays for SARS-CoV-2. "*", the statistical difference of fluorescence intensity difference between test sample and blank control serves as the criterion for judging the positive ($p < 0.05$) of per reaction.

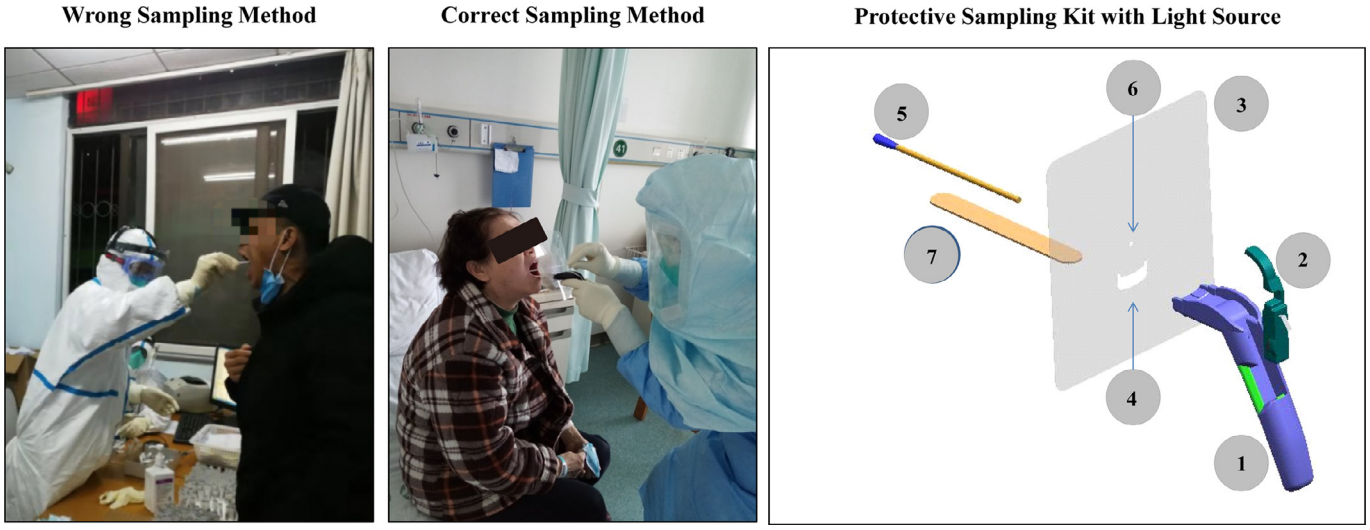

Fig. 2. Comparison of clinical sampling method and a protective sampling kit with light source. (Left) Wrong sampling method; (Middle) Correct sampling method; (Right) protective sampling kit with light source. This device is a protective oral-nasopharyngeal sampling set with built-in light source, including 7 components: (1) LED inspection lamp handle; (2) LED inspection light; (3) Disposable use of anti-droplet baffle; (4) U-shaped slot; (5) Sterile swab; (6) Sampling hole; (7) Sterile tongue spatula.

used to scrape the cells from tonsil recess and lateral wall when sampling from the oropharynx [9]. However, the sample collectors were often fear of contagion with SARS-CoV-2. Under great infection pressure, inaccurate sampling sites and inadequate sample volume will lead to false negative test results. Therefore, it is helpful to reducing the false negative through strict and normative operation of precise sampling with well protection for sample collectors (Fig. 2).

Except for the technical sensitivity and precise sampling, we also need to pay more attention for the quality control of sample preservation and transportation, virus inactivation, nucleic acid extraction [10]. If all the links in the detection of SARS-CoV-2 nucleic acid could be strictly administrated, false negative could be completely eliminated, and the discontinuation of isolation will no longer be a dilemma for us.

Author contributions

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Jian Huang was responsible for study concept and design. Zheng Zhang and Xinchun Chen were responsible for specimens sampling. Wanqiu Huang and Dachuan Lin were responsible for the experiment and statistical analysis. Wanqiu Huang, Dachuan Lin, Cuini Wang, Chaohui Bao and Zhaoqi Zhang were responsible for the analysis of data. Wanqiu Huang and Jian Huang were responsible for drafting the manuscript.

Declaration of Competing Interest

No authors declared any potential conflicts of interest.

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Letter in response to article in journal of infection: "Cardiovascular complications in COVID-19: A systematic review and meta-analysis"

To the Editor,

We read with great interest the systematic review and meta-analysis by Kunutsor and Laukkanen, which was recently published in the Journal of Infection and attempted to investigate the cardiovascular complications of coronavirus disease 2019 (COVID-19).[1] The authors investigated and reported the pooled incidence for cardiac arrhythmia, heart failure, cardiomyopathy, disseminated intravascular coagulation, cardiac arrest, acute coronary syndrome, stroke, and among these pulmonary embolism (PE) and venous thromboembolism (VTE). The authors report an extensive search that included three databases (MEDLINE, Embase, Cochrane Library) with the last search date reported as of May 27th, 2020. The proposed search term combinations included "Pulmonary Embolism" and "Venous Thromboembolism", however, the authors identified only one study reporting on VTE and PE.

There are several limitations regarding the systematic review and meta-analysis of PE and VTE complications. First, the authors should have clarified how they defined VTE in their study since VTE, by definition, includes both PE and deep vein thrombosis (DVT). Second, the study by Klok et al. has been updated with 65 PE events and a total of 75 thrombotic events.[2] Third, to our knowledge, there are at least nine studies published before May 27th, 2020 that we identified on a similar search on May 26th, 2020 and that report extractable data on the incidence of PE and DVT and that the authors failed to include.[2–10] Cui et al. (published on April 9th, 2020) reported a lower extremity DVT incidence of 25% ($n = 20/81$).[3] Tavazzi et al. (published on April 22nd, 2020) reported a DVT incidence of 14.8% ($n = 8/54$) in mechanically ventilated patients admitted in an intensive care unit (ICU), despite being on anticoagulant prophylaxis.[4] Leonard-Lorant et al. (published on April 23rd, 2020) investigated the results of pulmonary computed tomography scans in 106 patients diagnosed with COVID-19 and reported a PE incidence of 30% ($n = 32/106$).[5] Middeldorp et al. (published May 5th, 2020) investigated the incidence of VTE (DVT, PE, other venous thromboses) in hospitalized patients with COVID-19 treated with standard of care anticoagulant prophylaxis and reported a cumulative incidence of 16%, 33%, and 42% on days 7, 14, and 21, respectively.[6] Poissy et al. (published on April 24th, 2020) recruited 107 patients admitted in the ICU and identified 22 cases of PE with a 20.4% cumulative incidence on day 15 after ICU admission.[7] Thomas et al. (published on April 25th, 2020) reported a cumulative VTE incidence of 27% in 63 COVID-19 patients in the ICU.[8] In a multicenter prospective study, Helms et al. (published on May 4th, 2020) included COVID-19 patients admitted in four ICUs in two French centers and documented a PE incidence of 25% ($n = 25/99$).[9] Last but not least, Ren et al. (published on May 15th, 2020) reported a lower extremity DVT incidence of 85.4% ($n = 41/48$).[10]

Based on the abundance of studies reporting on VTE incidence, the findings of this systematic review and meta-analysis should be considered with caution. The standard thromboprophylaxis doses seem inadequate to prevent VTE in critical or severe COVID-19, contributing to an unacceptably high rate of thromboembolic events. Ongoing prospective randomized trials (NCT04401293, NCT04359277) are already enrolling patients and will hopefully elucidate the role of higher heparin doses for the prevention of thromboembolic events in COVID-19. Future, well-designed systematic reviews that include the constantly increasing literature on VTE will provide further insights into the actual incidence of COVID-19 associated DVT and PE.

Declaration of Competing Interest

The authors state that they have no competing interests.

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Older age is associated with sustained detection of SARS-CoV-2 in nasopharyngeal swab samples

Dear Editor,

In this Journal, Iwasaki and colleagues compared the quality of PCR from saliva and nasopharyngeal swabs as a diagnostic measure of SARS-CoV-2 infection[1]. Currently, the standard for diagnosis of Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) infection is a positive result based on a polymerase chain reaction (PCR) test from nasopharyngeal swab samples. PCR test is also used as a guide for patient discharge from designated hospitals and medical institutions. We recently experienced a case of a 97-year-old female who was diagnosed with coronavirus disease 2019 (COVID-19). Although her clinical symptoms and radiological findings resolved within a few days, PCR results from nasopharyngeal swab samples remained positive for 50 days after the onset. This case prompted us to conduct a retrospective study of the association of age the duration of positive PCR testing. We specifically hypothesized that old age could be a risk for prolonged duration of positive PCR results from nasopharyngeal swab samples.

This study was approved by the ethics committees of National Hospital Organization Hokkaido Medical Center and Hokkaido University Hospital. Nasopharyngeal swab sample was collected and quantitative real-time reverse transcription–PCR (RT-PCR) was conducted as described before[1]. In a RT-PCR assay, cycle threshold (Ct) value is defined as the number of cycles required for the fluorescent signal to cross a baseline threshold. The test results of SARS-CoV-2 were reported as negative in tests in which Ct>45).

We analyzed the records of 66 patients who were diagnosed with COVID-19 between March 1, 2020 and April 30, 2020 at National Hospital Organization, Hokkaido Medical Center. In this hospital, when respiratory physicians (T.H. and M.A.) recognized the peak-out of the COVID-19 infection, PCR tests were performed every day on nasopharyngeal swab samples from each patient. This enabled us to determine the precise day that each COVID-19 patient tested "negative" by PCR. We defined "negative PCR" as confirmed negative results over two sequential days. Characteristics of the 66 patients diagnosed with mild COVID-19 are presented in Table 1. Forty-two subjects were mild cases, who did not require supplemental oxygen treatment. Eighteen subjects were moderate cases who needed oxygen treatment. Six subjects were severe cases who needed ventilator or/and ECMO.

We found that older age was significantly associated with prolonged positive PCR tests ($P=0.0053$; Fig. 1A). This relationship remained unchanged when the findings were adjusted for the potential impact of severity of the disease (mild, moderate, severe) and the used of medication ($P=0.026$). When we analyzed only mild cases of COVID-19 in order to exclude the influence of disease severity, the result remained significant ($P=0.036$; Fig. 1B).

At the time of this writing, Hokkaido, most northern island in Japan, has controlled the second wave of COVID-19 and the rate of new cases continues to decrease. However, as per the statements of and guidance from the World Health Organization (WHO)[2], patients are discharged only upon confirmed negative PCR tests of nasopharyngeal swabs taken on two sequential days. This results in prolonged hospital stays as patients remain in beds in the designated infectious disease units designed for acute and ongoing care.

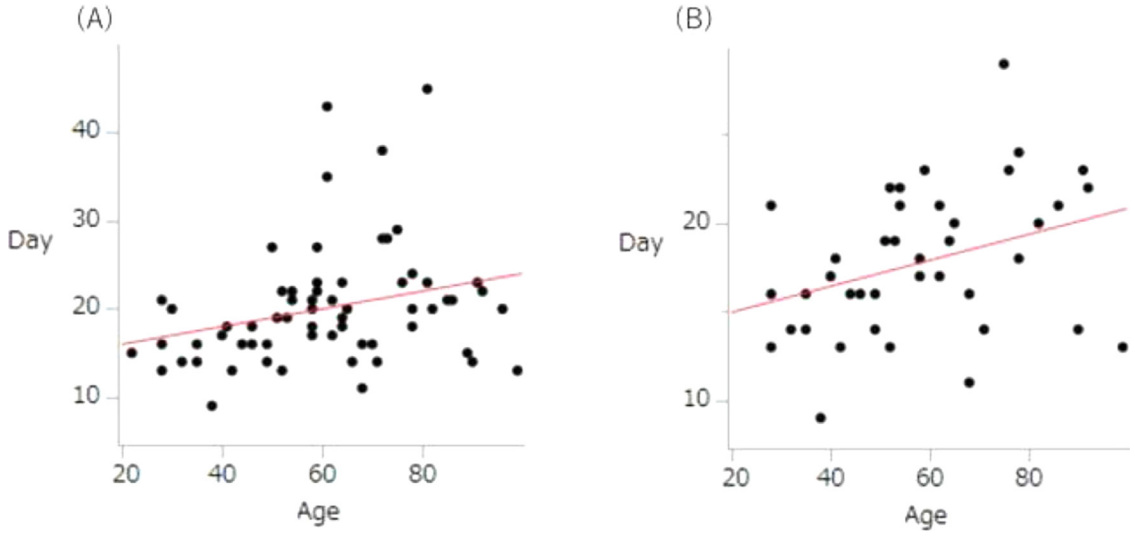

Fig. 1. The association of patient age with prolonged duration of positive PCR results among patients diagnosed with COVID-19 at National Hospital Organization, Hokkaido Medical Center.

Table 1
Clinical characteristic of subjects diagnosed as COVID-19 in National Hospital Organization Hokkaido Medical Center.

	Total ($n=66$)	Mild ($n=42$)	Moderate ($n=18$)	Severe ($n=6$)	p value*
Male, N (%)	38 (57.6)	23 (54.8)	12 (66.7)	3 (7.9)	0.6421
Age (yr)	61 (22–99)	56 (28–99)	71 (30–96)	59 (22–61)	0.0553
LDH (U/L)	260.5 (127–555)	249.5 (127–452)	330.5 (165–555)	314 (192–452)	0.2927
D-dimer (μg/mL)	0.75 (0.05–20.3)	0.6 (0.05–5)	1.8 (0.4–11.3)	3.25 (0.2–20.3)	0.0076
CRP (mg/dL)	3.495 (0.02–25.82)	2.1 (0.02–17.68)	5.82 (0.28–24.49)	5.54 (1.02–25.82)	0.0122
Ferritin (ng/mL)	293.3 (12.5–1760.3)	268.9 (12.5–1760.3)	402.9 (75.1–156.9)	606.3 (225.5–837.9)	0.1452
HbA1c (%)	5.9 (5.1–11.1)	5.9 (5.1–11.1)	6.15 (5.4–8.0)	6.85 (5.7–9.9)	0.173
WBC (/μL)	4.9 (1.9–14.8)	4.8 (1.9–9.1)	4.8 (2.6–11.6)	6.95 (3.5–14.8)	0.2178
Bas (%)	0.2 (0–3)	0.2 (0–3)	0.15 (0–0.5)	0.2 (0–0.4)	0.7529
Eos (%)	0.05 (0–4.5)	0.05 (0–3.9)	0.1 (0–2.1)	0.1 (0–4.5)	0.9102
Lym (%)	20.85 (5.7–58.0)	24.7 (8.8–58)	15.4 (6–27)	11.2 (5.7–20.7)	0.0009
Mon (%)	6.1 (1.5–15.0)	6.4 (2–11.9)	5.4 (1.5–15)	6.05 (1.9–8)	0.2372
Neu (%)	71.8 (34.0–92.5)	68.65 (34–88)	76.85 (60–92.5)	80.7 (68.8–92)	0.0028
Medication (for COVID-19)	36 (54.5)	17 (40.5)	14 (77.8)	5 (54.6)	0.0097
Cyclesonide, N (%)	32 (48.5)	16 (38.1)	12 (66.7)	4 (66.7)	0.0824
Favipiravir, N (%)	21 (31.8)	5 (11.9)	12 (66.7)	4 (66.7)	<0.0001
Ritonavir/Lopiravir, N (%)	8 (12.1)	2 (4.8)	2 (11.1)	4 (66.7)	<0.0001
Camostat, N (%)	3 (4.6)	1 (2.4)	2 (11.1)	0 (0)	0.2826
Predonisolone, N (%)	2 (3.0)	0 (0)	2 (11.1)	0 (0)	0.0639
Duration of positive PCR (day)	19 (9–45)	17.5 (9–29)	21 (14–45)	24.5 (15–43)	0.0043

Continuous data were presented as medians and interquartile ranges (IQR). * Comparisons among three groups (mild, moderate, and severe).

In our analysis, older age is significantly associated with prolonged duration of positive PCR tests from nasopharyngeal swab samples, irrespective of the disease severity and the used of medication (Fig. 1). The reasons underlying these observations remain unclear. Of note, we recently reported that quality of PCR from saliva as a diagnostic measure of SARS-CoV-2 infection was equivalent to that of the samples from nasopharyngeal swabs[1]. We also found that findings from PCR tests reverted from positive to negative much more quickly when using saliva than nasopharyngeal samples[1]. We speculate that in older individuals, cell turnover is less robust and as such, clearance of virus from the nasopharynx is prolonged; these factors may lead to positive PCR tests that persist after acute disease has resolved.

One group from Taiwan has already discussed the possibility that COVID-19 may no longer be contagious at two weeks after the onset of symptoms[3]. It is possible the positive PCR tests reflect the presence of that inactive virions remaining within nose. In a linked study, Zheng and colleagues evaluated viral loads in respiratory samples, stool, serum, and urine using PCR: they found that more than half the respiratory samples remained positive for SARS-CoV-2 as did a full one third of the stool samples at the end of the four week trial period[4]. As such, we propose that there should be a change in the strategy currently in use for determining time of discharge to one that relies on other clinical tests or/and patients' condition, which could be helpful to inhibit the development of patients' flail and dementia and to reduce the burden of ongoing and potentially unnecessary prolonged hospitalization.

In summary, we demonstrated that old age is significantly associated with prolonged duration of positive PCR results from nasopharyngeal swab samples; this is the case regardless of disease severity. Further studies will be needed in order to clarify how long these patients are actually contagious.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Variability but not admission or trends in NEWS2 score predicts clinical outcome in elderly hospitalised patients with COVID-19

Dear Editor,

In a recent article in the *Journal*, Bruno and colleagues present short-term outcomes in elderly patients with severe COVID-19 disease admitted to a single Italian Infectious Disease unit.[1] The study found that elderly patients are at increased risk of adverse outcomes due to high number of comorbidities and emphasises the need to improve clinical management in these patients. In particular, elderly patients who are likely to deteriorate will need to be rapidly identified.[2] Existing prognostic models for COVID-19 based on clinical, laboratory and radiological variables are at high risk of bias.[3] In the UK, the National Early Warning Score (NEWS) and its updated version NEWS2 – an *a priori* weighted composition of the patient's observations - is used routinely to monitor patients in hospital and identify early those who may deteriorate.[4]

Compared to other early warning scores, the NEWS Score has a greater ability to discriminate patients at risk of cardiac arrest, unanticipated intensive care unit admission or death.[5] Currently, guidance from the National Institute of Clinical Excellence (NICE) supports the use of the NEWS2 score to risk assess patients with COVID-19 in the community, who may require hospitalisation.[6]

In a recent rapid review, Greenhalgh and colleagues do not recommend using the NEWS2 Score alone for risk assessment of patients with COVID-19 in the community.[7] Blood pressure and oxygen saturation measurements are difficult to take remotely. The score also does not include age or comorbidities, strong independent predictors of survival in patients with COVID-19. The value of the NEWS2 Score in predicting outcome in patients admitted to hospital with COVDI-19 remains uncertain.

We therefore undertook, as part of service evaluation, a prospective pilot assessment of patients with confirmed COVID-19 admitted to a tertiary infectious diseases unit, in the first month of the pandemic reaching the UK.

We studied all patients who had a clinical outcome (either discharged from hospital or died) between 12th March and 2nd April 2020. Clinical (presenting symptoms, comorbidity and the NEWS2 Score throughout hospital stay), laboratory (routine blood tests) and radiological (chest x-ray reports) findings on admission were collated. Our main aim was to examine the utility of the NEWS2 Score in predicting the clinical deterioration of hospitalised COVID-19 patients. Continuous data are expressed as a median (25th - 75th percentiles) and categorical data are expressed as n (%). Independent *t*-tests and Mann-Whitney U tests were used to compare two continuous variables for normally and

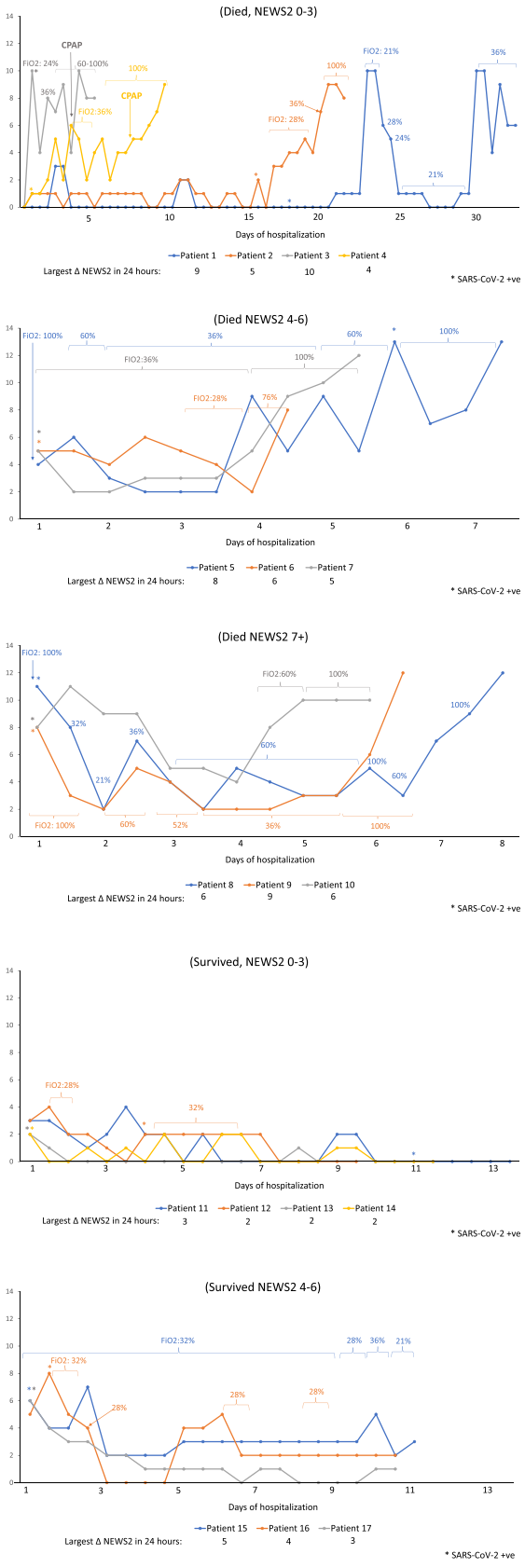

Fig. 1. NEWS2 scores of all patients with COVID-19, stratified by admission NEWS2 score.

Table 1
Baseline characteristics compared those who died vs those who survived.

	Died (N=10)	Survived (N=7)	P value (Died vs Survived)
Demographics			
Age	86 (83-88)	83 (82-88)	0.59
Sex (male), N (%)	7 (70)	2 (29)	0.09
Ethnicity, N (%)			0.68
Caucasian	8 (80)	6 (86)	
Asian Indian	1 (10)	1 (14)	
Black	1 (10)	0	
Number of comorbidities, N (%)			
0	0	1 (14)	0.46
1-2	3 (30)	2 (29)	
3+	7 (70)	4 (57)	
Ischaemic heart disease, N (%)	1 (10)	0	0.39
Peripheral vascular disease, N (%)	1 (10)	2 (29)	0.32
Stroke, N (%)	2 (20)	1 (14)	0.76
Hypertension, N (%)	6 (60)	1 (14)	0.06
Atrial fibrillation, N (%)	1 (10)	2 (29)	0.32
Heart failure, N (%)	1 (10)	4 (57)	0.04
Diabetes, N (%)	2 (20)	1 (14)	0.76
Dementia, N (%)	2 (20)	1 (14)	0.76
Chronic kidney disease, N (%)	1 (10)	0	0.39
Cancer, N (%)	4 (40)	1 (14)	0.25
COPD, N (%)	2 (20)	3 (43)	0.31
Obstructive sleep apnoea, N (%)	2 (20)	0	0.21
Clinical frailty scale	6 (5-7)	6 (4-7)	0.65
Observations on admission			
NEWS2	5 (0-8)	3 (2-6)	0.92
Respiratory rate (breaths/min)	21 (18-28)	24 (20-28)	0.69
O2 saturation (%)	96 (92-97)	96 (93-98)	0.96
FiO2 (%)	28 (21-100)	21 (21-21)	0.09
Systolic blood pressure (mmHg)	129 (124-133)	146 (141-157)	**0.03**
Diastolic blood pressure (mmHg)	74 (58-84)	81 (66-98)	0.26
Heart rate (beats per minute)	81 (74-95)	84 (79-86)	0.53
Temperature (degree celcius)	37.5 (36.8-38.5)	37.4 (36.8-38.1)	0.70
Presenting complaint			
Fever, N (%)	8 (80)	2 (29)	**0.03**
Cough, N (%)	3 (30)	5 (71)	0.09
Breathlessness, N (%)	5 (50)	4 (57)	0.77
Diarrhoea, N (%)	0	3 (43)	**0.02**
Confusion, N (%)	2 (20)	2 (29)	0.68
Falls, N (%)	1 (10)	0	0.39
Blood tests on admission			
White cell count ($x10^9$/L)	8.5 (3.7-15.9)	7.3 (2.3-9.1)	0.38
Neutrophils ($x10^9$/L)	6.8 (2.8-13.6)	5.1 (1.8-8.0)	0.33
Lymphocyte ($x10^9$/L)	0.99 (0.63-1.48)	0.63 (0.31-1.07)	0.14
Haemoglobin (g/L)	125 (113-142)	116 (102-119)	0.22
Platelets ($x10^9$/L)	173 (114-260)	206 (135-261)	0.38
C-reactive protein (mg/L)	65 (33-156)	31 (9-110)	0.24
Sodium (mmol/L)	133 (131-137)	136 (131-138)	0.46
Potassium (mmol/L)	4.7 (4.2-5.1)	3.9 (3.4-4.4)	**0.03**
Urea (mmol/L)	9.2 (7.0-15.9)	7.2 (3.0-10.5)	0.28
Creatinine (µmol/L)	122 (87-168)	91 (57-119)	0.12
Chest radiographs (CXR - findings on admission)			
Total number during admission	2 (1-3)	1 (1-1)	0.12
Clear	3 (30)	4 (57)	**0.007**
Unilateral consolidation	0	3 (43)	
Bilateral consolidation	7 (70)	0	
Ct Value (nasopharyngeal swab)	22.51 (17.96-27.46)	25.12 (18.54-31.04)	0.40
Treatment			
Oxygen therapy, N (%)	10 (100)	3 (43)	**0.006**
Nasal canulae, N (%)	9 (90)	3 (43)	**0.04**
Non-rebreathe mask, N (%)	9 (90)	0	**<0.001**
High-flow oxygen, N (%)	3 (30)	0	0.11
Non-invasive ventilation, N (%)	2 (20)	0	0.21
Length of stay (days)	6 (4-13)	9 (7-10)	0.13

non-normally distributed data. The chi-squared test was used to compare proportions between groups.

Overall, 17 patients with COVID-19 had an outcome by 2nd April 2020. The median age of our cohort was 85 years (IQR 83-88 years); 53% were male and 82% were Caucasian. All patients who were unsuitable for escalation to intensive care and received ward-based care as per NICE rapid guidance for COVID-19.[5] The majority of patients died (N=10, 59%). Compared to patients who survived, those who died were more likely to be male, with bilateral consolidation on chest radiographs on admission. Admission SARS-CoV-2 quantitative PCR Ct values on nasopharyngeal swab did not seem to relate to survival. All patients who died required some form of oxygen therapy, ranging from nasal canulae to non-invasive ventilation through continuous positive airway pressure. Less than half

of those who survived required oxygen therapy, all of which were delivered via nasal canulae.

Fig. 1 shows the trend in the National Early Warning Score2 (NEWS2) throughout hospitalisation, stratified by severity of NEWS2 on admission and clinical outcome. First, we found that the initial NEWS2 score did not predict mortality. For example, four out of the ten patients (40%) who died presented with a NEWS2 score of 0-3 while three out of seven patients (43%) who survived presented with a NEW2 Score of 5 or above. Secondly, there was no significant difference in the admission NEWS2 score and its components, between patients who died and those who survived, apart from systolic blood pressure. (Table 1). Thirdly, examining the NEWS2 scores over time, patients who died had a higher variability in their scores compared to those who survived. Seven out of ten patients (70%) who died had a maximum daily change in NEWS2 score of over 5, while none of those who survived had such dramatic fluctuations. (Fig. 1)

In our small pilot of elderly patients admitted to hospital with COVID-19, admission NEWS2 scores did not seem to be useful in predicting clinical outcomes. For some patients, death occurred regardless of admission NEWS scores and without a prior deteriorating trend. Originally, the NEWS score was developed using data from 35585 acute hospital admissions, most of whom would have had an underlying diagnosis of sepsis.[8] Sepsis is a clinical syndrome caused by overwhelming systemic bacterial infection. Clinical deterioration is seen within days in hospital. However, COVID-19 is caused by SARS-CoV-2, a coronavirus which predominately appears to affect the respiratory system as an initial viral pneumonitis. In China, a fifth of all COVID-19 inpatients rapidly became critically ill with hypoxia and respiratory failure.[9] The weighting of the NEWS2 score does not account for the degree of supplemental oxygen (FiO2) a patient may require, thus limiting its utility to identify early deterioration in patients with COVID-19. In our cohort, patient 8 had a NEWS2 score of 2 on day 2 and 3 despite requiring a large increase in FiO2 (from room air to 60%). A more sensitive early warning score for COVID-19 needs to be urgently developed and validated.

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Improved sensitivity using a dual target, E and RdRp assay for the diagnosis of SARS-CoV-2 infection: Experience at a large NHS Foundation Trust in the UK

Dear Editor,

We read with interest the letter from Hao *et al* highlighting the issues regarding the sensitivity of real time reverse-transcriptase polymerase chain reaction (RT-PCR) testing of upper respiratory tract samples for COVID19 disease [1]. Extensive RT-PCR testing by has been key to clinical decision-making, epidemiological analysis and policy development during the current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. The majority of RT-PCR assays target the RNA-dependent RNA polymerase (RdRp), envelope protein (E) or nucleocapsid protein (N) genes [2]. However, initial testing algorithms and expert opinion from the European Centre for Disease Prevention and Control (ECDC) advised that E gene amplification in isolation should be treated cautiously, due to concerns of non-specificity and issues related to contamination of reagents [3]. Early experience at Sheffield Teaching Hospitals NHS Foundation Trust (UK) on serially sampled patients with confirmed SARS-CoV-2 infection suggested that E gene detection persists beyond RdRp detection, and may offer enhanced diagnostic sensitivity. Therefore we explored the significance of E gene de-

tection in relation to RdRp, and in the absence of RdRp detection in a retrospective evaluation of SARS-CoV-2 RT-PCR testing.

A total of 12,015 clinical samples (combined nose/throat swabs or lower respiratory tract samples) were tested for SARS-CoV-2 as part of routine clinical diagnostics between 2nd March 2020 and 5th April 2020 at Sheffield Teaching Hospitals NHS Foundation Trust. Samples were extracted on the MagnaPure96 platform (Roche Diagnostics Ltd, Burgess Hill, UK). SARS-CoV-2 RNA was detected on 6µl of extract using a dual target (E gene and the RdRp gene) in-house PCR on ABI Thermal Cycler (Applied Biosystems, Foster City, United States) (supplementary material) [4]. The assay was modified to a multiplex single-well assay with the addition of PCR primers to detect a housekeeping gene, Ribonuclease P (RNAse P), which acts as an internal control and to assess sample quality.

Of the samples tested, 2,593 samples (21.6%) were positive with amplification curves for one or both target genes. Amongst positive results, we found E gene amplification alone to be common (n= 319, 12.3%), although the majority were positive for both RdRp and E gene targets (n = 2273, 87.7 %) and only 1 sample (<0.1 %) had RdRp gene amplification alone.

From the E-only positive group (n=319), 69 (21.6%) samples had low level amplification in the E gene (cycle threshold (CT) ≥35) and were investigated further. Within this subset, the majority (n=59, 85.5%) were considered to be true positives because they were either a) confirmed by an alternative assay (n=48) or b) a preceding or subsequent sample was positive for both E and RdRp (n=11) (Table 1). The alternative assay employed was a modified version of the Centers for Disease Control and Prevention (CDC) assay targeting the N gene (Micropathology Ltd, Coventry, UK) in most cases (n=47) or an alternative RdRp assay (n=1) [7]. Six samples (8.7 %) could not be confirmed in an alternative assay which had either high CT values for the E gene (n=4, CT values ≥39.0) or had good amplification curves not reaching the threshold (n=2). To further confirm the presence of SARS-CoV-2 RNA in samples with E gene only amplification, 11 samples were selected and successfully underwent whole genome sequencing (supplementary material). Analysis of the RdRp primer or probe binding sites in these samples did not reveal any mismatches to explain the lack of RdRp RT-PCR positivity (supplementary material).

We further explored the relationship between E gene detection and RdRp gene detection. Amongst samples with both RdRp gene and E gene amplification (n= 2273), we found that CT values for the E gene target were significantly lower than the CT values for RdRP, with a mean difference of 5.8 (Paired t test, p-value < 2.2e-16, 95% CI 5.79-5.92) (supplementary material). In a subset of samples where symptom onset was available (145 samples from 128

Table 1
Summary of samples with low level E gene amplification alone (CT ≥ 35). CT, cycle threshold; E, envelope gene.

	n	%
Sent for confirmation at reference laboratory[ϕ]	**54**	**78.26**
Confirmed by alternative assay	48	(88.89)
Not confirmed	6	(11.11)
Repeat clinical sample positive	**5**	**7.25**
Previous clinical samples positive[ψ]	**6**	**8.70**
Resulted without further testing[ǂ]	**4**	**5.80**
Total	69	

[ϕ] Most samples (n=53) were tested at Micropathology Ltd (Coventry) using a SARS-CoV-2 N gene assay using a modified CDC assay [6]. The other sample confirmed positive at PHE Colindale using an alternative SARS-CoV-2 RdRp assay.

[Ψ] As part of the High Consequences Infectious Diseases network, Sheffield received some of the first positive patients in the United Kingdom, who had daily swabs taken. E gene amplification appeared to persist in this cohort after the RdRp became negative.

[ǂ] Four results were authorised without further testing due to high pre-test probability e.g. compatible symptoms with a confirmed household exposure to SARS-CoV-2.

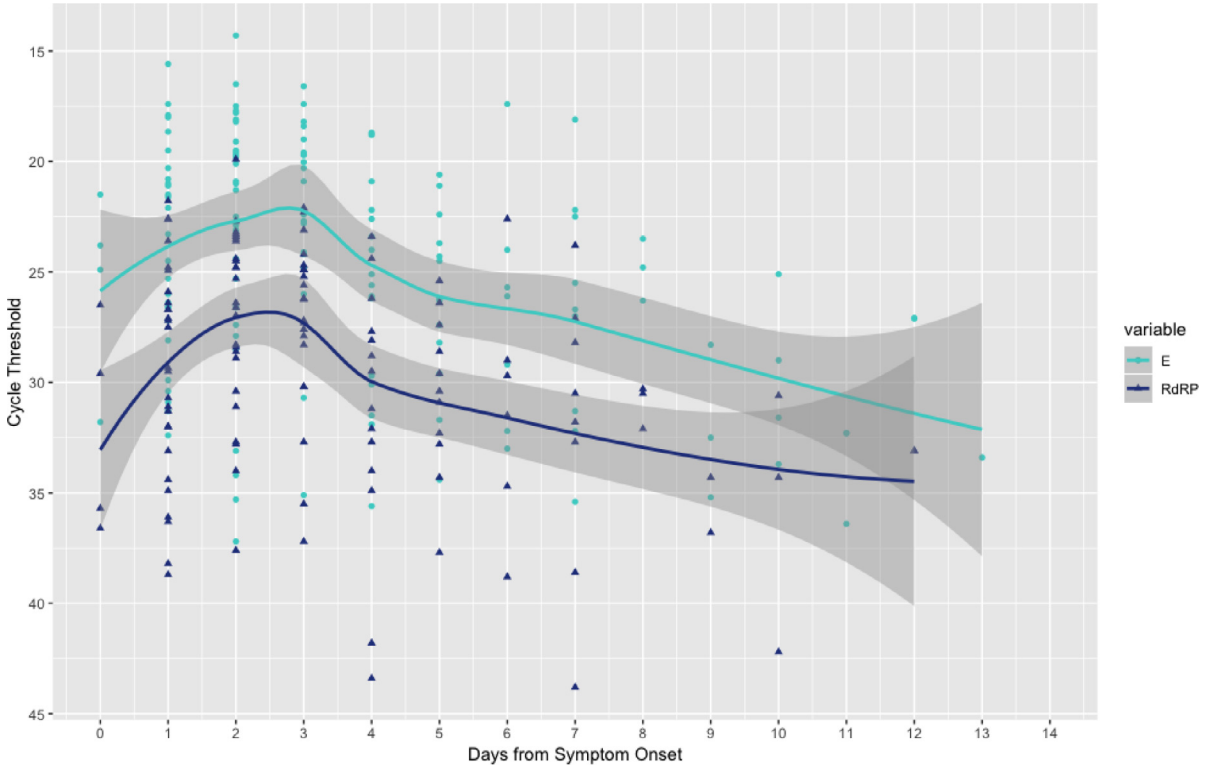

Fig. 1. E and RdRp gene cycle threshold results in relation to symptom onset. E and RdRp amplification results plotted against days of symptom onset in 145 samples from 128 patients. Lowest CT values were seen around day 3 of symptoms, with mean RdRp CT higher at a given day compared to E gene CT value. The lines represent the smoothed conditional mean with 95% confidence intervals in the grey bars. E, envelope gene; RdRp, RNA-dependent RNA-polymerase.

patients), it was clear that the CT values for both RdRp and E gene were lowest around 48 – 72 hours following symptom onset (Fig. 1). At each stage of infection, the median CT values for RdRp were higher than those for the E gene.

By using the E gene target in addition to the RdRp gene target we observed a significantly increased diagnostic pick up (11.9%). In one patient, E gene amplification was detected for three days beyond RdRp amplification, indicating a possible widening of the diagnostic window. Our findings confirm that clinical samples with E only amplification should not be dismissed as non-specific results. Not only were we able to obtain whole genome sequences for SARS-CoV-2 from a subset of this group, we also found that 85% of E only samples with high CT values were confirmed by a second assay targeting the N gene or an alternative RdRp only assay.

The enhanced sensitivity seen for the E gene in our dual target E-RdRP assay is yet to be explained. We observed a mean difference of over five CT values when comparing E gene to RdRp values, which may suggest the possibility of higher copy numbers of E gene being present in the primary or extracted sample. Due to the unique transcription strategy of coronaviruses, genes towards the 3' end of the genome would be present in higher copy numbers during active viral replication, which could explain these findings [5]. It is also possible that PCR optimised conditions in a multiplex system favours E gene amplification, however we found no significant loss of RdRp detection when comparing single and multiplex systems during validation, with observed CT rises averaging 1-2 cycles (data not shown). In addition, we found no evidence of primer or probe mismatches in the RdRp region.

We believe dual target testing, using the E gene as a second target, will help improve both diagnostic sensitivity and the appropriate clinical response to this pandemic. We urge testing laboratories to carefully consider the use of the E gene as a target in order to optimise SARS-CoV-2 diagnostics, including strategies to confirm samples with E gene only amplification as we have described.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.05.061.

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COVID-19 Patients with Recent Influenza A/B Infection: A Retrospective Study

Dear editor,

We read with the interest the recent paper by Chen *et al.* who described the clinical progression of 249 patients with coronavirus disease 2019 (COVID-19).[1] As the author mentioned, some factors, such as age and CD4 T cell counts, would be associated with intensive care units (ICU) admission. In addition, the application of host-directed therapy and early control of viral replication might be crucial for improving the prognosis of COVID-19. We are interested in investigating the potential risk factors associated with the progression and prognosis of COVID-19. To date, cases of severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) and influenza A co-infection have been reported in COVID-19 patients.[2-4] We suspected that the recent infection with influenza among COVID-19 patients might affect disease prognosis and progression to some extent. The high specificity and the sensitivity of Immunoglobulin M (IgM) assays suggest that IgM is a reliable biomarker for the surveillance of recent influenza infection.[5,6] Here, we reported that recent infection of influenza A/B and produce specific IgM in COVID-19 might be a common phenomenon, and influenza IgM status could be the significant factor associated with clinical outcomes and prognosis of COVID-19.

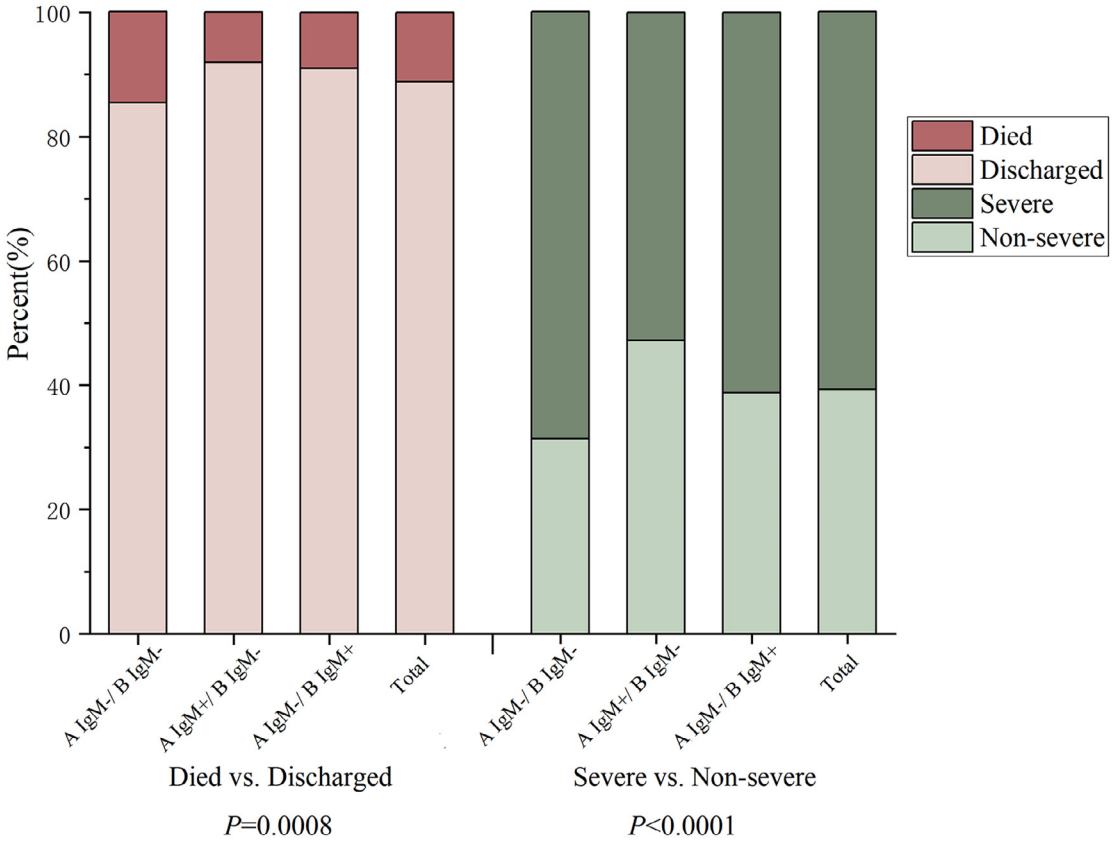

Figure 1. The clinical outcomes and the rate of severe illness among different influenza A/B IgM status groups. Abbreviations: A IgM: influenza A IgM; B IgM: influenza B IgM.

For this retrospective study, the 1386 COVID-19 patients were hospitalized between 18 January and 26 April 2020 at Tongji Hospital in Wuhan, China. All patients were pathogen-confirmed COVID-19 cases and accepted serological influenza A/B IgM antibody tests upon admission. SARS-CoV-2 infection was confirmed by reverse transcriptase polymerase chain reaction assay (RT-PCR), and the methods were consistent with other studies.[7] The influenza A/B IgM antibody tests were conducted by indirect immunofluorescence assay (IIFA) of specific IgM antibodies (EUROIMMUN, FI 2821-17M, Germany). All operations were carried out according to the provided instructions.

The patients analyzed in this study were not vaccinated against influenza A/B at the time of admission. In our study, severe COVID19 cases were defined as oxygen saturation of 94% or less while breathing ambient air or needing oxygen support, consistent with the report of Ohmagari *et al.* [8] The Ethical Committee of Tongji Hospital approved the study. Informed consent was not obtained because this retrospective study was analyzed anonymously.

We performed a retrospective analysis on 1386 confirmed COVID-19 patients with influenza A/B IgM antibody test results. In total, 88.8% (1231/1386) of patients survived and 11.2% (155/1386) of patients died. More than half of patients (60.8%, 842/1386) were identified as severe cases, and 39.3% (544 of 1386) were classified as non-severe. According to patients' specific IgM status, influenza A IgM positive (A IgM^+) or negative (A IgM^-) and influenza B IgM positive (B IgM^+) or negative (B IgM^-), the patients were divided into three categories: A IgM^-/ B IgM^- group (47.6%, 660/1386), A IgM^+/ B IgM^- group (47.5%, 659/1386), and A IgM^-/ B IgM^+ group (4.8%, 67/1386). The A IgM^+/ B IgM^+ group was not included as we identified no such cases. In Figure 1, in terms of the clinical outcome, the mortality rates of the A IgM^+/ B IgM^- group and A IgM^-/ B IgM^+ group were lower than that of the A IgM^-/ B IgM^- group. Figure 1 also indicates that the A IgM^-/B IgM^- group had the highest rate of severe cases among the three groups. Statistically significant differences existed across the different groups when considering mortality ($P = 0.0008$) and severe illness rates ($P < 0.0001$).

To further explore the relationship between the influenza A/B IgM status and clinical outcome and illness severity among the COVID-19 patients, we established univariate analysis and multivariate analysis models (Table 1). For the univariate analysis, we found that sex, age, and comorbidities were significant cofactors among mortality and severe illness. The A IgM^+/ B IgM^- group has showed lower risk of mortality (OR =0.514, 95%CI: 0.360–0.732) and severe illness (OR =0.511, 95% CI:0.408–0.640). For multivariate analysis, after adjustment for cofactors, patients in the A IgM^+/ B IgM^- group were less likely to die than patients in the A IgM^-/ B IgM^- group (OR = 0.671, 95% CI: 0.463–0.973). However, the mortality rate of the A IgM^-/ B IgM^+ group was not statistically different from that of the A IgM^-/ B IgM^- group according to the adjusted model (OR = 0.903, 95% CI: 0.359–2.272). Furthermore, our analysis also indicated that a similar trend was also observed in severe/non-severe analysis. The A IgM^+/B IgM^- group had a lower rate of severe illness than the A IgM^-/B IgM^- group (OR = 0.601, 95% CI: 0.476–0.760), whereas no such difference was found for the A IgM^-/B IgM^+ group (OR = 0.968, 95% CI: 0.563–1.665).

Table 1
Univariate and multivariate analysis of risk factors of Death vs. Discharged or Severe vs.Non-severe.[a]

Variables	[n(%)]				Death vs. Discharged [OR (95%CI)]		Severe vs. Non-severe [OR (95%CI)]	
	Died (n=155)	Discharged (n=1231)	Non-severe (n=544)	Severe (n=842)	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
Sex								
Male	104 (14.9)	596 (85.1)	251 (35.9)	449 (64.1)	REF	REF	REF	REF
Female	51 (7.4)	635 (92.6)	293 (57.3)	393 (42.7)	**0.460 (0.323-0.655)**	**0.458 (0.316-0.662)**	**0.750 (0.604-0.931)**	**0.753 (0.599-0.946)**
Age (mean [SD])	69.6 (12.2)	57.1 (15.7)	53.5 (16.4)	61.7 (14.6)	**1.069 (1.054-1.085)**	**1.067 (1.050-1.083)**	**1.035 (1.027-1.042)**	**1.035 (1.027-1.043)**
Comorbidities								
No	64 (8.3)	708 (91.7)	324 (42.0)	448 (58.0)	REF	REF	REF	REF
Yes	91 (14.8)	523 (85.2)	220 (35.8)	394 (64.2)	**1.925 (1.371-2.702)**	1.154 (0.803-1.658)	**1.295 (1.041-1.611)**	0.863 (0.677-1.100)
Influenza A/B IgM status groups								
A IgM^-/ B IgM^-	96 (14.6)	564 (85.5)	207 (31.4)	453 (68.6)	REF	REF	REF	REF
A IgM^+/ B IgM^-	53 (8.0)	606 (92.0)	311 (47.2)	348 (52.8)	**0.514 (0.360-0.732)**	**0.671 (0.463-0.973)**	**0.511 (0.408-0.640)**	**0.601 (0.476-0.760)**
A IgM^-/ B IgM^+	6 (9.0)	61 (91.0)	26 (38.8)	41 (61.2)	0.578 (0.243-1.374)	0.903 (0.359-2.272)	0.721 (0.429-1.210)	0.968 (0.563-1.665)

[a] The statistically significant differences are shown in bold.Abbreviations: A IgM, influenza A IgM; B IgM, influenza B IgM; OR, odds ratio; 95%CI, 95% confidence interval; IQR, interquartile range.

In the analysis, older age, male gender, and comorbidities were more prone to poor outcomes and progression, which were consistent with previous studies.[1,9] Therefore, in the multivariate analysis, we adjusted these cofactors. We found that COVID-19 patients positive for influenza A IgM had a lower risk of mortality and severe illness compared with those showing negative A/B IgM status. However, these trends were not significant differences between A IgM^-/ B IgM^+ group and A IgM^-/ B IgM^- group.

The reason for better prognosis and clinical outcome in influenza A IgM^+ COVID-19 patients is likely complicated, but could be due to potential interactions between influenza A and SARS-Cov-2, or because IgM^+ is a marker of patient functional immune status. However, the second hypothesis cannot fully explain why these protective effects were not observed among influenza B IgM^+ COVID-19 patients. Due to the suddenness of the COVID-19 pandemic outbreak, more studies are needed to confirm these findings.

In summary, our results showed that recent influenza A/B infection in confirmed COVID-19 patients might be a common phenomenon. Moreover, we also observed that COVID-19 patients positive for influenza A IgM showed a lower risk of mortality and severe illness compared with those showing negative A/B IgM status. In contrast, this trend was not observed in influenza B IgM^+ patients.

Author Contributions

Jia Liu, Ping Wu, Wanrong Lu designed and conceived the study; Ping Wu, Wanrong Lu performed the statistical analysis and drafted the article; Liang He, Yifan Meng, Peng Wu, Wencheng Ding, Ke Ma contributed to data collection; Jia Liu made critical revisions to the manuscript. All authors revised and commented on the article and approved the final version before submission.

Declaration of Competing Interest

All authors have declared there is no competing interest exists.

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Markers of liver injury and clinical outcomes in COVID-19 patients: A systematic review and meta-analysis

To the Editor,

Since January 2020 when it was first isolated in China, coronavirus disease 2019 (COVID-19) has spread throughout the world and caused substantial morbidity and mortality.[1] Despite the rapidly growing knowledge base on the clinical course of the disease, no therapeutic agents have been proven to be effective for COVID-19. Further clarification of the clinical course of the disease could help in the development of effective treatment strategies. Wang and colleagues in their recent elegant study to investigate characteristics and prognostic factors in 339 elderly patients with COVID-19, observed a high proportion of severe and critical cases as well as high fatality rates.[2] Common complications included bacterial infection, acute respiratory distress syndrome as well as liver enzyme abnormalities. In their analyses to explore prognostic factors for fatal outcomes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (known markers of liver injury) were not found to be independently associated with the risk of mortality. Though it has been reported that liver injury is more prevalent in severe cases of COVID-19,[3, 4] whether circulating levels of markers of liver injury at admission could predict clinical outcomes in COVID-19 patients is uncertain. In this context, we aimed to determine the nature of the relationships of admission levels of five main markers of liver injury (ALT, AST, gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) and total bilirubin) with the risk of clinical outcomes in patients with COVID-19 using a systematic meta-analysis.

We conducted this review using PRISMA and MOOSE guidelines (**Supplementary Materials 1-2**) and in accordance with a registered protocol in the PROSPERO International prospective register of systematic reviews (CRD42020183672). MEDLINE, Embase, and The Cochrane library were searched from 2019 to 17 May 2020 for published studies reporting on relationships between admission levels of markers of liver injury (GGT, ALT, AST, ALP and total bilirubin) and clinical outcomes in patients with COVID-19. The detailed search strategy has been reported in **Supplementary Material 3**. Outcomes were categorised into severe illness and mortality. Mean differences (95% CIs) for comparing mean levels of circulating markers across outcomes and relative risks (RRs) (95% confidence intervals, CIs) for associations between markers and outcomes were used as summary measures across studies.[5] The inverse variance-weighted method was used to effect estimates using random-effects models to minimize the effect of heterogeneity. STATA release MP 16 (StataCorp LP, College Station, TX, USA) was used for all statistical analyses.

Sixteen retrospective cohort studies comprising 10,540 COVID-19 patients were eligible (**Table 1; Supplementary Materials 4-5**). All studies were based in China. The average age at baseline ranged from approximately 38 to 71 years. Comparing elevated vs low levels of ALT and AST respectively, the RRs (95% CIs) of severe illness were 1.03 (0.23-2.15) and 2.09 (0.44-9.9) respectively. Pooled analyses of 9 studies each showed significantly higher levels of ALT and AST in COVID-19 patients who developed severe illness compared to patients who did not deveop severe illness: mean differences (95% CIs) of 9.15 U/L (1.47, 16.82; p=0.02) and 12.60 U/L (8.43, 16.77; p<0.001) respectively (**Fig. 1A**)

In pooled results of two studies each, the RRs (95% CIs) of mortality associated with elevated ALT and AST were 3.35 (2.37-4.75) and 10.42 (7.05-15.40) respectively. In results from single studies, increased levels of ALP and total bilirubin were each associated with an increased risk of mortality (**Supplementary Material 6**). Admission levels of AST and total bilirubin were higher in those who died; whereas ALT levels were not significantly different in both groups: mean differences (95% CIs) of 17.13 U/L (11.25, 23.01; p<0.001); 4.21 μmol/l (3.97, 4.46; p<0.001) and 5.82 U/L (-2.57, 14.21; p=0.17) respectively. In single reports, levels of ALP and GGT were higher in those who died compared with survivors (**Fig. 1B**).

Taking the overall evidence together, the data supports a higher prevalence of elevated admission levels of markers of liver injury in severe or mortality due to COVID-19 disease, which suggests that patients with elevated levels of liver markers at baseline (during admission) had higher risks of developing worse outcomes in COVID-19. The likely explanation for the worse outcomes observed in patients with baseline elevated markers of liver injury (as seen in chronic liver disease) could be attributed to compromised immune status.[3, 4]

Irrespective of the fact that about 2-11% of patients with COVID-19 have liver comorbidities,[3] COVID-19 also causes liver injury. However, there is controversy regarding the causes of liver injury in COVID-19.[3, 4] Proposed explanations include (i) drug-induced liver injury; (ii) direct injury to the liver due to COVID-19 hepatitis [4]; (iii) COVID-19 induced myositis [4]; (iv) binding of SARS CoV-2 directly to angiotensin-converting enzyme 2

Table 1
Baseline characteristics of included studies of COVID-19 patients

Author, year ofpublication	Source of participants	Country	Date of datacollection	Mean/medianAge (yrs)	Male %	Total participants	No. of outcomes	Outcomes	NOSscore
Zhou, 2020	Jinyintan Hospital and Wuhan Pulmonary Hospital	China	Dec 2019 - Jan 2020	56.0	62.0	191	54	In-hospital mortality	5
Huang, 2020	Jin Yintan Hospital	China	Dec 2019 - Jan 2020	49.0	73.0	41	13	ICU care	4
Ruan, 2020	Jin Yin-tan Hospital and Tongji Hospital	China	NR	57.7	68.0	150	68	Mortality	4
Guan, 2020	National Health Commission	China	Dec 2019 - Jan 2020	47.0	58.1	1099	173 (67)	Severe disease (Composite outcome of ICU admission, the use of mechanical ventilation, or death)	4
Liu, 2020	3 tertiary hospitals in Wuhan	China	Dec 2019 - Jan 2020	38.0	50.0	78	11	Severe disease	5
Qian, 2020	5 hospitals in Zhejiang province	China	Jan - Feb 2020	50.0	40.7	91	9	Severe disease	4
Zheng, 2020	North Hospital of Changsha first Hospital	China	Jan - Feb 2020	45.0	49.7	161	30	Severe disease	4
Wang, 2020	Zhongnan Hospital of Wuhan University	China	Jan, 2020	56.0	54.3	138	36	ICU care	4
Wang, 2020b	Union Hospital in Wuhan	China	Jan - Feb 2020	42.0	46.0	69	14	SpO_2<90%	4
Wang, 2020c	Renmin Hospital of Wuhan University	China	Jan – Feb 2020	71.0	49.0	339	65	Mortality	4
Chen, 2020	Tongji Hospital in Wuhan	China	Jan - Feb 2020	62.0	62.0	274	113	Mortality	4
Chen, 2020b	National Health Commission	China	Dec 2019 - Jan 2020	NR	NR	1,590	50	Mortality	6
Cai, 2020	Third People's Hospital of Shenzhen	China	Jan - Feb 2020	47.0	47.5	417	91	Severe disease	6
Yang, 2020	Wuhan Jin Yin-tan hospital	China	Dec 2019 – Jan 2020	59.7	67.0	52	32	Mortality	4
Lei, 2020	10 hospitals in Hubei Province	China	Dec 2019 – Mar 2020	56.0	47.2	5,771	1,186	Severe disease	5
Xie, 2020	Jinyintan Hospital	China	Feb 2020	60.0	55.7	79	28	Severe disease	4

ICU, intensive care unit; NOS, Newcastle-Ottawa Scale; NR, not reported

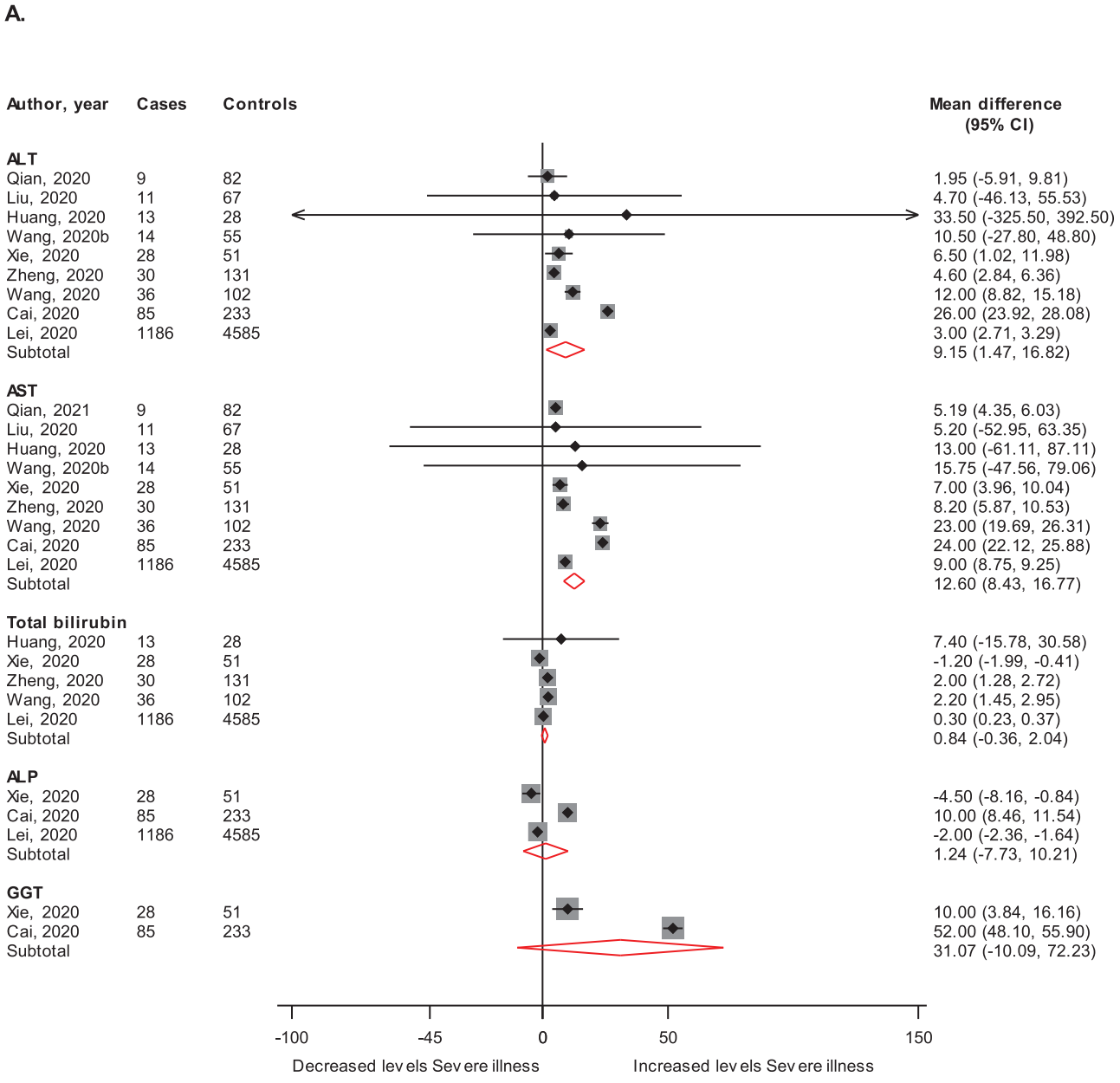

Fig. 1. Admission levels of markers of liver injury in (A) patients with or without severe COVID-19 illness and in (B) patients who died or survived ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CI, confidence interval (bars); GGT, gamma-glutamyltransferase

(ACE2) positive rich cholangiocytes and causing liver damage;[6] (v) hepatic congestion due to high levels of positive end expiratory pressure during mechanical ventilation; [4] and (vi) aggravation of liver injury by SARS CoV-2 in patients with pre-existing viral hepatitis.[7, 8] In the absence of robust association studies and formal risk prediction analyses, the overall evidence suggests that increased baseline levels of markers of liver injury could predict poor outcomes. The global prevalence of chronic liver disease remains high and continues to increase. Treatment options for COVID-19 are currently supportive; hence, there should be more intensive monitoring of levels of markers of liver injury during admission so that therapeutic approaches can be individually tailored.

There are several limitations which deserve mention. First, the heterogeneous reporting of severe illness outcomes prompted the use of composite measures. Second, the possibility of patient overlap as all 16 studies were reported from China; there have been concerns with duplicate reporting of study participants in articles.[9] Third, due to the limited sample sizes and low events, some studies were unable to assess risk ratios to quantify the relationships. Finally, though we extracted data on baseline (admission) levels of these markers, studies were not very specific regarding the exact time of blood sampling in relation to the disease status; hence, these results may have some biases.

In conclusion, elevated admission levels of markers of liver injury particularly the aminotransferases, may be associated with

B.

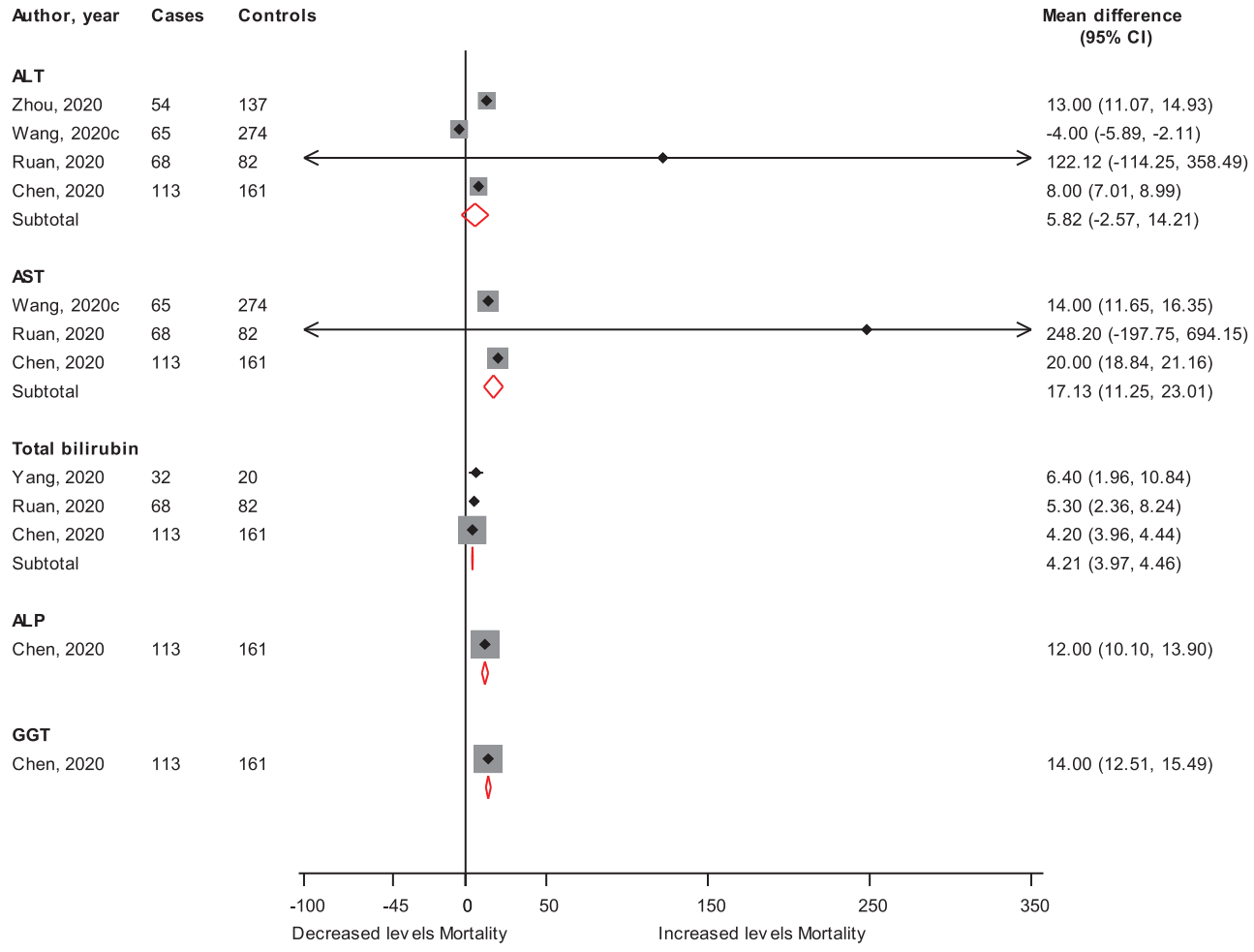

Fig. 1. (Continued)

progression to severe disease or death in COVID-19. Monitoring levels of these markers could assist in the optimum management of patients.

Declaration of Competing Interest

None.

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Supplementary materials

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Derivation and validation of a scoring system to assess pre-test probability of being COVID-19 positive

Dear Editor,

We read with interest the work by Bermejo-Martin et al. [1] underlining the role of lymphopenia as a predictive marker of severe COVID-19 pneumonia. In an epidemic setting biomarkers can be useful at both patient level and for adequate resources allocation.

The gold standard for COVID-19 diagnosis relies on SARS-CoV-2 RNA detection by reverse transcription polymerase chain reaction (RT-PCR) through nasal and oropharyngeal swabs (despite suboptimal sensitivity, with the proportion of false negative results ranging from 56 to 83% [2]). Through this observational, cross-sectional study from a large Italian teaching hospital, we aimed to derive a diagnostic score to rapidly identify the possibility of being affected by COVID-19 at hospital admission, thus limiting the use of second-level diagnostic tests or a second swab.

Randomly selected, adult (≥18 years-old) patients coming to first aid with symptoms consistent with COVID-19 were considered for this analysis. The diagnosis relied on the results of RT-PCR on nasopharyngeal/oropharyngeal swabs, as recommended by International guidelines [3,4]. For most patients with initial negative swab, testing was repeated at least after 24 hours to definitely exclude the diagnosis.

The derivation cohort consisted in patients arrived at first aid between the 1st and the 15th of March 2020 (corresponding to the beginning of the epidemic in our hospital). The validation cohort included patients who came to first aid between the 21st and the 15th of April (i.e. at least after 14 days since national lockdown measures were declared by Italian government). Factors associated with a positive swab for SARS-CoV-2 (at a p-value≤0.05) were identified through a backward step-wise logistic regression. The multivariable regression model was then transformed into a point-based rule, as described by Sullivan et al.[5]. The discriminatory power and calibration of the prediction rule in the derivation and validation cohorts were assessed by the area under the receiver-operator characteristic curve (ROC AUC) and the Hosmer-Lemeshow test, respectively.

Data from 194 patients were analysed, 103 (53.4%) of whom in the derivation set and 91 (46.6%) in the validation set.

Fifty persons (48.5%) in the derivation set tested positive for SARS-CoV-2. Patients with negative swabs had other bacterial or viral infections (35 cases, 66.0%), cardiovascular and gastroenteric diseases (2 cases each, 7.6%), neoplasia (4, 7.6%), other/unspecified conditions (10, 18.8%). The derivation cohort was mainly composed of men (58.3%), with 53 years of median age (interquartile range, IQR, 38-70). Forty-one patients (39.8%) had at least one comorbidity among cardiovascular disease, hypertension, diabetes, active cancer and COPD. Fifty-two patients reported a possible contact with a case of COVID-19. At admission, the most frequent symptoms were represented by fever (88.3% of cases), cough (57.3%) and dyspnoea (35.9%). Twenty-three of 99 (23.2%) patients with a chest-X ray had interstitial pneumonia.

Five variables independently predicted COVID-19 diagnosis: the presence of an epidemiological link (aOR 10.35, p=0.001), total white blood cell count (per 100 cells/μL more, aOR 0.96, p=0.001), a CRP level<5 mL/min (versus ≥5 mL/min, aOR 0.07, p=0.002), the presence of the triad fever, cough and dyspnoea (versus the absence, aOR 10.02, p=0.012), time since symptoms onset (per 1 day more, aOR 1.33, p=0.001).

The validation group (90 persons) included 30 persons (33.3%) with a positive swab. Alternative diagnosis in patients without COVID-19 were: other viral or bacterial infections (30 of 60, 50.0%), cardiovascular disease (6, 10.0%), gastroenteric disease (8, 13.3%), neoplasia (9, 15.0%), other/unspecified conditions (7, 11.7%).

Overall, the validation group was mainly composed by men (57.8%), with 73 years of median age. Sixty-three patients (70.0%) had at least one comorbidity. Most of them also presented with fever (75.6%), cough (37.8%) and dyspnoea (55.6%). Compared with patients in the validation group, COVID-19 positive patients in derivation group were younger (60 versus 70 years of median age), had less frequently interstitial pneumonia at chest X-ray (38.0% versus 66.7%), more frequently fever (98.0% versus 86.7%) and cough (68.0% versus 40.0%) at admission, and lower median platelets count (169 versus 229×10^5 cells/μL).

Starting from regression coefficient, a score system was built in the derivation set (see table 1).

In the derivation group the ROC AUC for this model was 0.89 (95% CI 0.83-0.96) indicating excellent discriminatory power. Result of the Hosmer-Lemeshow chi-squared testing ($\chi 2$ 13.50, p=0.334) also indicated good calibration. A cut-off of ≥-3 showed the highest negative predictive value (100%), whereas a cut-off of ≥5 exhibited the highest positive predictive value (100%).

When applied to the validation set, the predictive score demonstrated good predictive power, with a ROC AUC of 0.83 (95% CI 0.74-0.92), and a good calibration (Hosmer-Lemeshow $\chi 2$ 7.58, p=0.476). A score cut-off of ≥-3 exhibited the highest negative predictive value (100%) and a cut-off of ≥3 had the highest positive predictive value (72.7%). Fig. 1 compares the score ROC AUC in both derivation and validation sets.

As noticed by Bermejo-Martin et al. [1], biomarkers are urgently requested for correct categorization of patients with COVID-19. Several evidences showed that lymphopenia could have a prog-

Table 1
Estimated points corresponding to each risk factor category.

Risk factor	Categories	Reference value (W_{ij})	βi	Points= $\beta i(W_{ij} - W_{iREF})/B$
Epidemiological link (presence vs absence)			2.34	
	Absent	$0 = W_{iREF}$		0
	Present	1		3
Total white blood cell count (per cells/μL increase)	0-4,000	3,000	-0,00038	2
	>4,000-6,000	5,000		1
	>6,000-8,000	7,000= W_{iREF}		0
	>8,000-10,000	9,000		-1
	>10,000-12,000	11,000		-2
	>12,000-14,000	13,000		-3
	>14,000-16,000	15,000		-4
	>16,000	17,000		-4
C-reactive protein			-2.62	
	≥5 mL/L	$0 = W_{iREF}$		0
	<5 mL/L	1		-3
Fever, cough and dyspnoea at admission			2.31	
	Absence	$0 = W_{iREF}$		0
	Presence	1		3
Days since symptoms onset	0-3	1.5	0.29	-1
	>3-6	4.5 = Ref		0
	>6-9	7.5		1
	>9-12	10.5		2
	>12-15	13.5		3

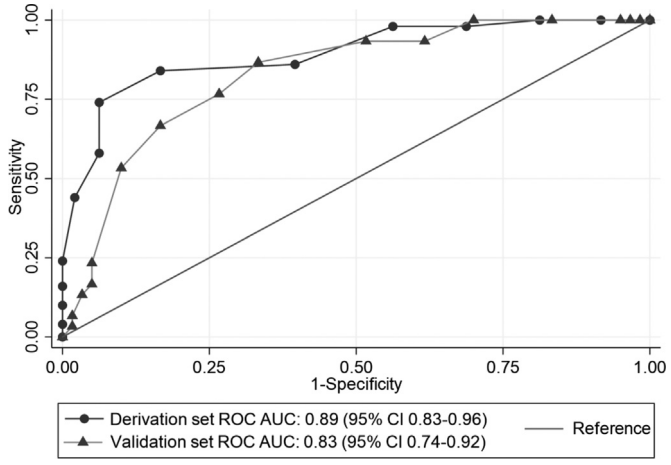

Fig. 1. Receiver-operator characteristic curves (ROC AUC) for the scoring system in the derivation and validation set.

nostic value in COVID-19 [1], in addition to its diagnostic value, already recognized in Chinese guidelines [6]. Interestingly, in our work total white cells count was more associated with a diagnosis of COVID-19 than lymphocytes count. This could be related with an overall low severity spectrum of disease shown by our patients (only a half of COVID-19 patients had interstitial pneumonia at admission). CRP, epidemiological link, clinical symptoms and time since symptoms onset were also associated with the pre-test probability of COVID-19. Particularly, the derived prediction-rule showed higher utility in ruling-out COVID-19 diagnosis: in the derivation and validation groups only 1 of 21 (4.8%) and 2 of 29 (6.9%) patients with a score of less than -1 tested positive for SARS-CoV-2. Despite its intrinsic limitations (retrospective data collection, derivation and validation cohorts belonging to the same hospital), our works represents to our knowledge the first attempt to measure the impact of an easily-available score for stratifying the risk of COVID-19. This could represent an important tool for assisting clinicians as well for driving hospital policies of infection control, particularly in resource-limited settings.

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This study was performed as part of our routine work.

Transparency declarations

The authors report no conflicts of interest relevant to this work.

Declaration of Competing Interest

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Follow-up study on pulmonary function and radiological changes in critically ill patients with COVID-19

Dear Editor,

The clinical manifestations of coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),[1] range from asymptomatic, mild pneumonia to acute respiratory distress syndrome (ARDS).[2] An epidemiological study with 72,314 patients has reported that around 4.7% of patients with COVID-19 developed ARDS, and the mortality rate in these patients was up to 49%.[3] Most patients surviving from ARDS caused by other coronaviruses like SARS-CoV and Middle East respiratory syndrome coronavirus suffered from impaired pulmonary function and radiological abnormalities.[4,5] Up till now, follow-up data regarding critically ill COVID-19 survivors is rare. To facilitate the understanding of the prognosis of these patients, we here present a follow-up study of two COVID-19 patients with severe ARDS up to 3 months after the illness onset.

Two patients with COVID-19 who developed severe ARDS in The Second People's Hospital of Fuyang (Anhui, China) were enrolled. Institutional review board approval and written informed consent were obtained.

Patient 1 was a 20-year old female working in Wuhan and returned to Fuyang to celebrate the Spring Festival with her family. She presented in the hospital with fever, dry cough, and deteriorated dyspnea for 10 days on 29 January 2020. On admission, her vital signs were unstable (temperature 39 °C, heart rate 112 beats per minute, respiratory rate 32 breaths per minute, blood pressure 104/65 mmHg) with obvious dyspnea (SpO2 89% under conventional oxygen therapy of 40% FiO2). Laboratory results indicated leukopenia (2.65×10^9/L), lymphopenia (0.45×10^9/L), elevated C-reactive protein (CRP) (102 mg/L), IL-6 (62 pg/ml) and D-dimer (1.06 mg/L). Arterial blood gas analysis (ABGA) reported impaired oxygenation of PaO2/FiO2 at 213 mmHg. The diagnostic RT-PCR on a nasopharyngeal swab specimen was positive for SARS-CoV-2 and a chest CT scan illustrated bilateral pneumonia (Fig. 1a). Lopinavir-ritonavir (500 mg, twice per day) was administrated immediately. Her condition progressed rapidly on illness day 12 and she required intubation and mechanic ventilation because of severe ARDS (PaO2/FiO2 92 mmHg). On illness day 17, both oxygenation (PaO2/FiO2 348 mmHg) and chest CT significantly improved (Fig. 1b), and she was then weaned from mechanical ventilation. She was discharged on illness day 21 with mild lung abnormalities on chest CT images (Fig. 1c). One month later after discharge, both chest CT scan (Fig. 1d) and pulmonary function test (forced vital capacity (FVC) of predicted 103.7%, FEV1/FVC 84.64%, carbon monoxide diffusing capacity (DLCO) of predicted 94%) indicated no-abnormalities.

Patient 2 was a 68-year old man who permanently resides in Fuyang. He visited the hospital because of fever (37.5 °C) and cough for one week on 4 February 2020. The nasopharyngeal swab was positive for SARS-CoV-2 by RT-PCR and a chest CT revealed bilateral ground-glass opacity (Fig. 2a). Laboratory results indicated leukopenia (3.33×109/L), lymphopenia (0.55×109/L), elevated CRP (116 mg/L), and IL-6 (357 pg/ml). His-condition was stable on admission, with oxygen saturation of 97% on ambient air. Lopinavir-ritonavir (500 mg, twice per day) was administrated. He reported the history of hypertension and diabetes for around 10 years, blood pressure and glucose were controlled well, no history of chronic respiratory diseases. On illness day 12, he was transferred to the intensive care unit and converted to invasive mechanical ventilation because of worsening oxygenation (PaO2/FiO2 84 mmHg) and progressing abnormalities on chest CT scan with extensive ground-glass opacities and partial consolidation on bilateral lungs (Fig. 2b). His-oxygenation improved slowly, but could not be weaned from mechanic ventilation, hence he received a tracheostomy on illness day 22. During this period, bronchoalveolar lavage culture reported *Escherichia coli* that was sensitive to carbapenems but resistant against third-generation cephalosporins. Therefore, imipenem was administrated. On illness day 30, oxygenation of the patient suddenly deteriorated, and a chest CT scan revealed right-sided pneumothorax (Fig. 2c). The patient then received thoracic drainage with a closed system which was removed 2 days later. On illness day 35, the oxygenation of the patient im-

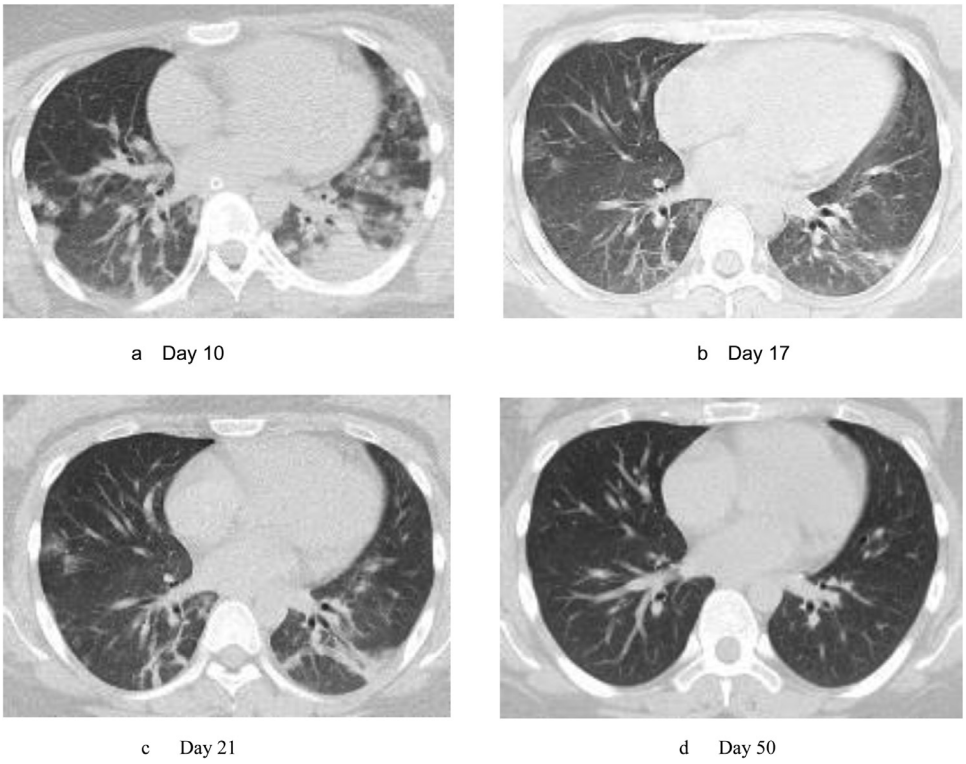

Fig. 1. Transverse serial CT scans from a 20-year-old woman with COVID-19. (a) On admission (Day 10 from onset of symptoms): multiple areas of consolidation in bilateral lungs with peripheral and basal distribution. (b) On the day weaned from mechanical ventilation (Day 17): opacifications being dissipated into ground-glass opacities and irregular linear opacities. (c) On the day discharged from hospital (Day 21): ground-glass opacities and consolidations with decreased extent. (d) One month after discharge (Day 50): no abnormalities presented.

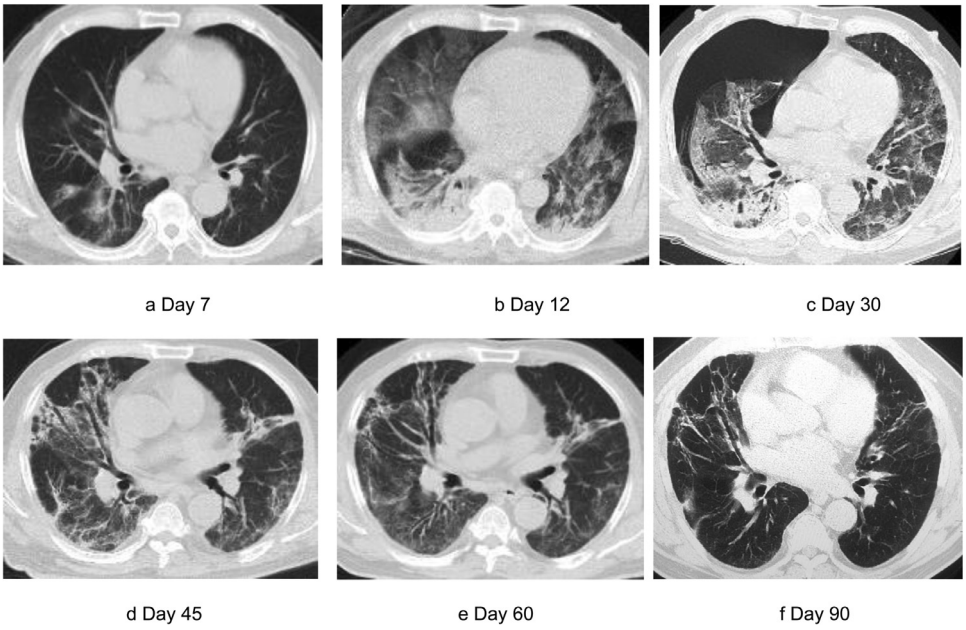

Fig. 2. Transverse serial CT scans from a 68-year-old man with COVID-19. (a) On admission (Day 7 from onset of symptoms): small air space consolidation in right lung, largely peripheral in location. (b) On the day received invasive mechanical ventilation (Day 12): Disease deteriorated with extensive ground-glass opacities, air space consolidation in bilateral lungs, mainly with peripheral and basal distribution. (c) On day 30, right pneumothorax has developed. (d) On the day discharged from hospital (Day 45): still had obvious abnormalities on bilateral lungs with gradually dissolved ground-glass opacities superimposed with irregular linear densities, partially presented as subpleural reticular opacities, cysts and bronchiectasis were also identified. (e) Two months follow-up scan (Day 60): Gradual resolution of bilateral ground-glass opacities and consolidation, with distortion of architectures and bronchiectasis. (f) Three months follow-up scan (Day 90): almost all ground-glass opacities dissolved, showing reticular densities, distortion of architectures and bronchiectasis in bilateral lungs with volume loss suggestive of fibrotic changes.

proved and he was successfully weaned from mechanic ventilation. He was discharged from hospital on illness day 45 with obvious abnormalities on the chest CT scan (Fig. 2d). Because of the impaired pulmonary function on discharge, he was transferred to a rehabilitation center. Two months after the onset of illness, he went to the hospital for the first follow-up visit. The pulmonary function test indicated restrictive lung function defect, with decreased FVC of predicted (62.3%) and DLCO of predicted (49.6%), but FEV1/FVC was at the normal range of 80.1%, which was consistent with the manifestations on chest CT images (Fig. 2e). On the second follow-up visit (3 months after illness onset), almost all ground-glass opacities were dissolved, but with obvious architectural distortion, bronchial dilatation and volume loss in bilateral lungs suggestive of fibrotic changes on chest CT (Fig. 2f). Lung ventilation was worse than that of the previous month, featured as the restrictive pulmonary disease with decreased FVC of predicted (54%), but diffusion capacity improved significantly, albeit it was still lower than that of the normal range (DLCO of predicted increased from 49.6% to 64.3%). The patient complained of shortness of breath and general weakness (6-minute walking distance was 200 m), and ABGA indicated low PaO2 (61.6 mmHg) on ambient air.

Discussion

Little is known about the sequelae of COVID-19. The two patients reported in this study showed distinct consequences. The young patient completely recovered with non-abnormality on both chest radiology and function tests, while the older patient manifested with obviously radiological changes and functional defects during the follow-up period. The results of the older patient in this study, suggest that a proportion of severe COVID-19 patients developed fibrosis. Similar fibrotic changes had been reported for SARS, which seem to have the ability of self-rehabilitation as gradual improvements were observed over time.[6,7] Nevertheless, the restrictive ventilatory defect and impaired diffusion capacity still affect the patient's physical abilities significantly in the early recovering stage, which suggests the importance of early rehabilitation.[8] The limitation of this study was the short-term follow-up of only two cases; therefore, to understand any long-term effects of COVID-19, long-term follow-up studies with large cohorts of patients are warranted.

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Declaration of Competing Interest

None.

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Early administration of ritonavir-boosted lopinavir could prevent severe COVID-19 ☆

To the Editor,

There is currently no specific treatment with demonstrated efficacy against the respiratory infection outbreak of severe acute

☆ On behalf of the « COVID SMIT PSL STUDY GROUP »

respiratory syndrome coronavirus 2 (SARS-CoV-2) disease (COVID-19) that affected more than 4000,000 persons and killed 300,000 around the world during the last 6 months (1,2). Like Peiris and al. suggested with the SARS-CoV1, we believe that an effective antiviral agent is needed to decrease the viral load and direct cytolytic damage during the first phase of infection, and in turn reduce the immunologic storm during the second phase with the risk of progression to acute respiratory distress syndrome (3). Among existing antiviral therapeutics tested, protease inhibitors seemed promising, and ritonavir-boosted lopinavir (LPV/r) has been shown to inhibit the replication of SARS-CoV-2 in vitro and in hospitalized patients (4–6).

Here we report the viral dynamics in multiple clinical samples in regards to pharmacological LPV/r levels during and after treatment in a SARS-CoV-2-infected patient. This first SARS-CoV-2 infection in a French resident was diagnosed in our department on January 29th 2020, six days after his exposure to a laboratory-confirmed case from Asia (7).

We performed monitoring of SARS-CoV-2 infection from day 2 (D2) after onset of symptoms in different sequential clinical samples by real-time RT-PCR targeting E gene (8). Viral loads were estimated with the cycle threshold (Ct) values: Ct > 50 was considered as negative. Detection of specific antibodies was performed on plasma specimens with the Abbott SARS-CoV-2 IgG assay. When chest CT-scan confirmed small areas of ground-glass opacities in both lower lungs on D9, the patient started ritonavir-boosted lopinavir (LPV/r) 400/100 mg BID until hospital discharge on D18. LPV plasma concentration (C_{min}) was measured by liquid chromatography tandem mass spectrometry method (LC-MS/MS); the limit of quantification (LOQ) was 15 ng/mL.

The outcome of the patient was good. He experienced the typical pattern of COVID-19 symptoms, such as sore throat, muscle pain, headaches and anosmia, then lung infection signs but did not develop severe pneumonia and never required supportive treatments with oxygen or immunomodulators. During the whole period of viral monitoring, SARS-CoV-2 RNA was detected not only in nasopharyngeal swab (NPS), but also in induced sputum, saliva, plasma, and stool (Fig. 1). However, SARS-CoV-2 RNA was never detected in urine. The whole genome sequence obtained from positive NPS sample is available in Global Initiative on Sharing All Influenza Data (GISAID) with the sequence number EPI_ISL_408,431. Between D2 and D4, high viral loads (Ct<30) were detected in NPS, induced sputum, saliva, and plasma. Viral load decreased gradually in NPS to become undetectable on D15, after 6 days of treatment. In plasma, after a rapid initial drop, a low-level rebound (Ct>35) occurred on D11 and D12, corresponding to a transient plateau in NPS. This phenomenon was observed between 2 and 3 days after the start of LPV/r treatment and despite expected LPV C_{min}. On D14, SARS-CoV-2 RNA was still detected at high level (Ct<30) in sputum, but at low level (Ct>35) in NPS, illustrating differential compartmentalization of SARS-CoV-2 in upper and lower respiratory tracts. SARS-CoV-2 RNA was detected once in stool sample on D23, after LPV/r removal. Further additional samples (i.e., NPS, saliva, plasma and stool) collected on D30 and D90 were negative for SARS-CoV-2. In terms of immunity, IgG seroconversion was evidenced on D16 (Fig. 1).

In a retrospective cohort study, 96 patients infected with SARS-CoV-2, the median duration of virus detection in NPS samples varied from 14 to 21 days according to disease severity (9). A recent study showed that SARS-CoV-2 RNA could not be detected in NPS from half of non-severe patients after 14 days of LPV/r treatment (10. However, in a randomized trial involving 199 patients, LPV/r treatment did not significantly improve clinical symptoms or survival, nor diminish throat viral RNA detectability in late-presenters patients with severe pneumonia (11). Interestingly, in a post-hoc analysis of the subgroup of patients treated less than 12 days after the onset of symptoms, clinical cure was obtained after 16 days in the LPV/r arm versus 17 days and the mortality rate was 19.0% versus 27.1%, without statistical significance, possibly due to the weak study power. In our study, the viral clearance in NPS of this single patient was obtained within 2 weeks, coinciding with the antibody response (D16). The rebound of viral load observed in NPS during treatment could be explained by the transient subtherapeutic levels of LPV/r between D13 and D15. The SARS-CoV-2 RT-PCR became positive in stool only after the end of treatment, addressing the question of longer treatment need for oro-fecal transmission prevention.

Our findings suggest that, if LPV/r treatment does not seem to constitute the treatment of choice for salvage therapy in patients with severe COVID-19, it could be effective in early presenting non-severe patients to decrease the SARS-CoV-2 load and to prevent the secondary immune-related severe evolution.

Ritonavir-boosted lopinavir (LPV/r) use in SARS-CoV-2′s infected patients should be better evaluated in a prospective controlled study including multisite drug dosages and pharmacokinetic/pharmacodynamic (PK/PD) study, and treatment should be given for at least 14 days to reduce long-term viral carriage and related transmission risks.

Ethics

The patient signed an informed consent accepting the extra tests performed for this study. The Infectious Diseases Department obtained the clearance from the Hospital's Ethic Committee to proceed with COVID-19 cohort follow-up.

Transparency declarations

All authors contributed towards the writing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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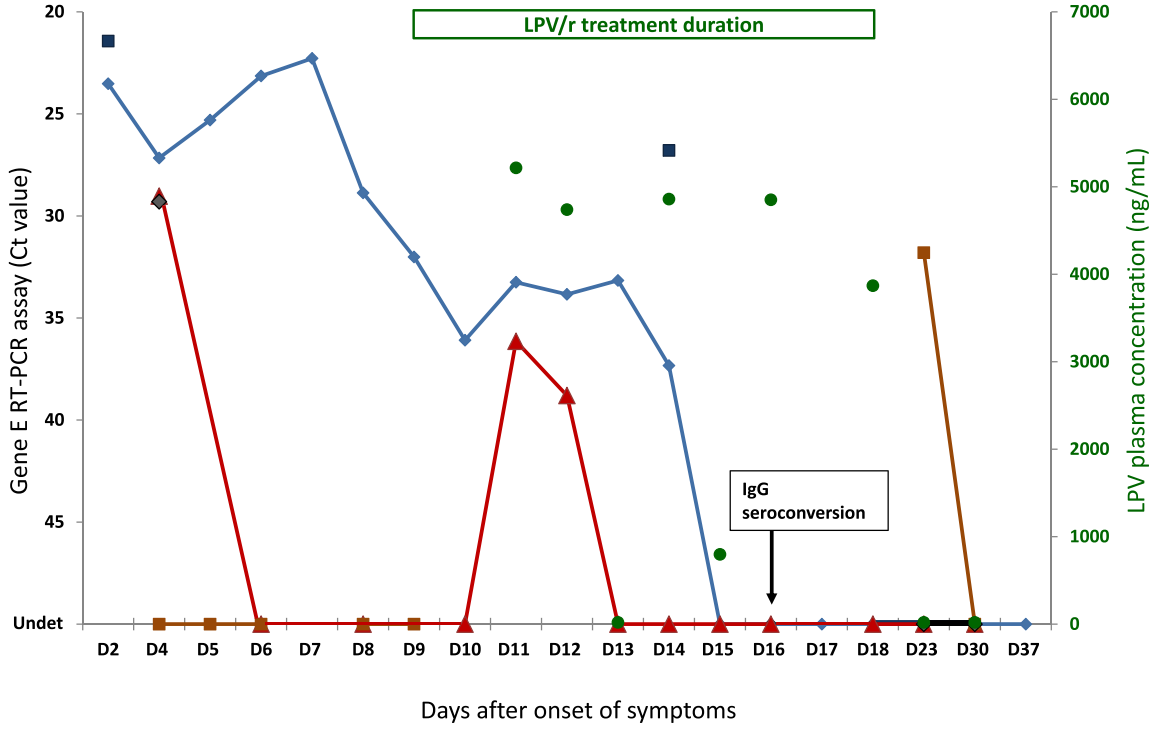

Fig. 1. Viral dynamics in multiple and sequential clinical samples and kinetics of lopinavir plasma concentrations in a patient with confirmed SARS-CoV-2 infection and treated with oral ritonavir boosted lopinavir. Real-time RT-PCR targeting viral E gene, presented by reverse Ct values on left vertical axis, was performed in serial different types of clinical samples collected from the patient: nasopharyngeal swab (♦), induced sputum (■), saliva (♦), plasma (▲), and stool (■). Lopinavir concentration (●), expressed in ng/mL on right vertical axis, was measured in sequential plasma samples by liquid chromatography tandem mass spectrometry method. Range of lopinavir minimal plasma concentrations: 4.660 ± 2.250 ng/mL Duration of ritonavir-boosted lopinavir (400/100 mg) treatment (D9 to D18) is indicated on the top of the graph. SARS-CoV-2 antibody response (IgG seroconversion) in indicated on the graph (D16). Undet: undetectable (Ct>50).

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Use of Xpert MTB/RIF in a low prevalence setting in the Southwest of England

Dear Editor,

We read with interest the recent article by Wang G and colleges describing the higher sensitivity of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF in the diagnosis of paucibacilliary TB.[1] We have performed a local study looking at the sensitivity and specificity of Xpert MTB/RIF in our local population. The use of rapid molecular methods in the diagnosis and prevention of transmission of tuberculosis is recommended by both NICE[2] and Public Health England,[3] but adoption of these recommendations around the country is variable. Variation may relate to confidence in using rapid molecular methods as much of the data to support use comes from areas with a high incidence of tuberculosis. In 2017, Parcell Benjamin et al.[4] demonstrated, in their cohort in a low prevalence area, that sensitivity of Xpert MTB/RIF (Cepheid) was 95.8%. Chaisson Lelia et al.[5] showed that using a combination of smears and one or two samples for rapid molecular testing reduced both isolation times and length of hospital stay.

We reviewed the use of Xpert MTB/RIF in a tertiary centre with a tuberculosis prevalence of 4.3 per 100,000 population (compared to national prevalence of 10.2 per 100,000). At the time of the review, molecular testing was limited to samples that were smear positive (excluding patients with a diagnosis of bronchiectasis, cystic fibrosis or a positive result in the past 3 months), or smear negative cases where the requesting clinician thought additional testing would be of value. The aim was to establish the sensitivity of Xpert MTB/RIF in a low prevalence setting in England. We performed a retrospective analysis of all samples processed using Xpert MTB/Rif since the initiation of testing in our laboratory. Over the two and half years a total of 126 samples were processed (Fig. 1). In the final analysis we included 80 specimens.

The sensitivity of Xpert MTB/RIF using culture as the gold standard was 95.1% (95% CI 0.839–0.987). In comparison the sensitivity of smears using culture as the gold standard was 82.9% (95% CI 0.687–0.915). This evaluation demonstrates that the use of Xpert MTB/RIF on a selected population is more sensitive than using smear. This could help time to treatment. Furthermore, the samples used for testing included sputum and non-sputum samples

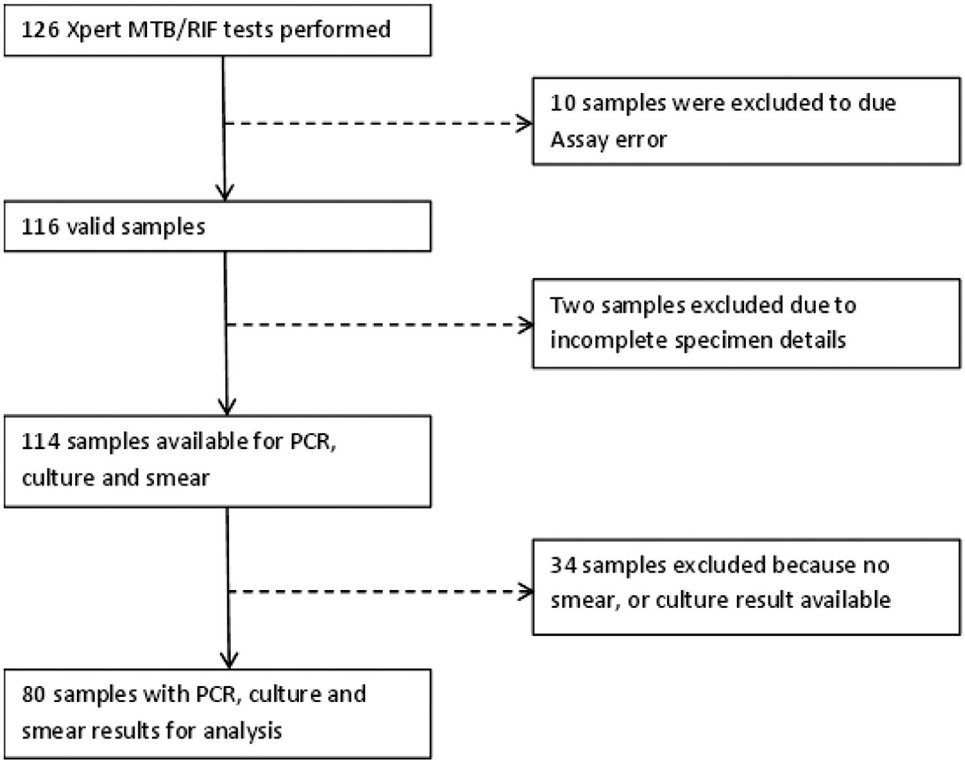

Fig. 1. Overview of Xpert TB/RIF and cultures performed.

Table 1
Comparison of Xpert MTB/Rif and smear against MTB culture based on sample type.

Specimen type	Total (%)	Culture positive for MTBC	Smear positive when Culture positive for MTBC (%)	PCR positive when culture positive for MTBC (%)
BAL	17 (21)	7	6 (86)	7 (100)
CSF	2 (3)	1	0	1 (100)
Fluid	2 (3)	1	1 (100)	1 (100)
Not listed	8 (10)	3	3 (100)	3 (100)
Pus	1 (1)	1	1 (100)	1 (100)
Sputum	45 (56)	26	23 (88)	25 (96)
Tissue	5 (6)	2	0	1 (50)

(Table 1). The current standard microbiological investigations[6] in the United Kingdom recommends that the Xpert MTB/RIF should be used in accordance to the manufacturer's instructions. This would preclude testing on all samples except sputum. There are many studies, and endorsements by the World Health Organisation[7,8] for the use of Xpert MTB/RIF on other sample types such as cerebrospinal fluid. A recent publication on the use of Xpert MTB/RIF for the diagnosis of bone and joint tuberculosis in the UK[9] demonstrated that, patients whose biopsy samples underwent molecular testing waited significantly less time to start treatment.

The negative predictive value of molecular testing also makes it a valuable tool for infection control. At present the smear is the investigation of choice used to judge the relative infectivity of a patient. We propose that to judge infectivity a smear could be performed on a sample that is positive by molecular testing. This would reduce the number of smears needed and therefore reduce the workload in many routine microbiology laboratories.

The current recommendation is that laboratories who perform less than 10 smears a week should not perform smear testing[10]. These laboratories could use Xpert MTB/RIF as the level of experience required for a smear is not required for a test. Furthermore, the recommendation to report smears within 24 h, six days a week is often not met due to the time taken to perform smears. The main concern with such a move is the positive predictive value of the test is influenced by the prevalence of tuberculosis in the population. Indiscriminate use could generate false positives. The results of our evaluation and the other limited data available from low prevalence settings demonstrate that where the clinical suspicion is high then the use of molecular testing may be more efficient than using smears as first line testing.

Yours sincerely,

Declaration of Competing Interest

None.

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