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

Concerning the OptiGene Direct LAMP assay, and it's use in at-risk groups and hospital staff

Dear Editor,

The COVID-19 pandemic has triggered an unprecedented demand for diagnostic tests. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is highly contagious in the pre-symptomatic period, when the viral load is high. In the effort to reduce transmission, for the first time in infection diagnostics history, testing is being aimed not only at symptomatic, but also at asymptomatic individuals, both in the health care setting and in the community.

Shortages in PCR reagents and platforms, related to the demand for large-scale testing, fuelled the effort for alternative diagnostic solutions. We have read with interest the Journal of Infection (JoI) article, regarding the laboratory based assay chosen by the British government, for SARS-CoV-2 mass testing (Operation Moonshot), a loop mediated isothermal amplification (LAMP), produced by OptiGene.[1,2] The testing of saliva by OptiGene direct ORF1ab LAMP (with no RNA extraction), in newly built laboratories under the management of acute NHS Trusts, is costing the taxpayer over 400 million British pounds and has resulted in the movement of key staff away from essential roles in acute NHS Trust diagnostic laboratories, at short notice.

The OptiGene assay on saliva samples is intended for the testing of asymptomatic NHS staff, to help prevent hospital acquired outbreaks. The assay however, failed to detect more than 50% of saliva positive cases in a pilot in Greater Manchester, when compared to a "gold standard" qPCR (polymerase chain reaction) assay.[2] In contrast, recently published data from the Department of Health and Social Care (DHSC) report 70% sensitivity for swabs and 79% for saliva for this assay.[2]

To understand the performance in more detail, 86 PCR positive nose and throat swabs, with a variable viral load, collected from patients and symptomatic or asymptomatic members of hospital staff, were tested in parallel by direct LAMP and in-house CDC N1/N2 qPCR (Limit of Detection (LOD) 156 digital copies/mL or 2•6 digital copies per reaction, Qnostics) at the Southampton Specialist Virology Centre. These samples were stored at 4 °C, prior to being frozen at −80 °C within 36 h of sample collection. The SARS-CoV-2 Molecular Q Panel (SCV2MQP) from Qnostics was used to quantify the qPCR results in digital copies/mL.

Consistent with results from the Manchester Regional Virus Laboratory, which evaluated freshly collected saliva samples from asymptomatic individuals, we have been unable to replicate the sensitivity (see Table 1) reported by the DHSC. For this reason, we would like to raise a number of points on which we seek clarity.

A sample of >10,000,000 ($>10^7$) digital copies/mL (dc/mL), collected from a symptomatic NHS staff member, swabbed on day one of symptoms, produced a negative result when tested by the OptiGene direct LAMP method. Similarly, a swab from an asymptomatic staff member, a target group for this assay, with 2,500,000 (2.5×10^6) dc/mL was missed. A false negative result, with a high viral load, is a patient safety risk, particularly if the sample belongs to an asymptomatic member of staff.

The sensitivity of a diagnostic test is assessed in comparison to a gold standard, in this case OptiGene direct LAMP compared to qPCR. However, the performance of individual PCR assays varies, depending on a number of factors, including the choice of viral RNA amplification targets, among many other technical variations, which impact on PCR efficiency. For this reason the comparison between assays should be based on quantitation standards and not on Ct values, as published in the evaluation.[2] Which criteria were used for "sufficient sensitivity" for an assay used to isolate infected NHS staff from patients?[2] The experiments relating to the Limit of Detection (LoD) of 10^3 copies/mL in the DHSC evaluation were flawed. The lack of difference in LoD between extracted and direct LAMP, with the quantitated NIBSC samples, is artificial and not seen in patient samples in the evaluation or in the Journal of Infection paper that states an LoD of 100,000–1,000,000 (10^5–10^6) dc/ml.[1,2]

Critically, the inclusion of an internal control, added before nucleic acid extraction, is a vital quality requirement for molecular diagnostic work.[3] Lack of amplification, due to a number of reasons, such as a LAMP platform failure, in individual reaction wells, or presence of inhibitory substances in the clinical sample, is flagged by the use of an internal control, thus preventing false negative results from being reported. The OptiGene direct LAMP method does not contain an internal control, despite being present in alternative SARS-CoV-2 LAMP assays.[4,5]

It has been asserted that nucleocapsid protein (N-) gene targets for qPCR are not valid for assessment of the performance of the OptiGene direct LAMP assay.[6] The reason provided is that N-gene qPCR detects subgenomic mRNA that may persist, and be detectable, beyond the period of viral replication, with an inability to differentiate between RNA from replicating virus and residual RNA after a resolved infection. It is important to stress that duration of detection of subgenomic mRNA, and its implications for infectiousness, are currently debated. Why is this being asserted to be scientific fact, along with the theory that OptiGene direct LAMP assay only detects infectious virus and those not detected are non-infectious, for which no evidence is provided and the evidence available indicates otherwise?[1,7,8]

The choice of sample type for use with the OptiGene, for asymptomatic screening for NHS staff is saliva, a heterogeneous sample, which suffers from a reduced sensitivity in comparison to the higher sensitivity of a nose and throat swab. Meta-analyses of saliva testing studies have consistently shown that the sensitivity is inferior to nose and throat swabs, at 83–85% when compared

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Table 1
Direct OptiGene LAMP (ORF 1ab) compared to N1/N2 CDC PCR (the Southampton Specialist Virology Centre in-house diagnostic assay).

SARS-CoV-2 viral load digital copies/mL (dc/mL)		CDC SARS-CoV-2 N1/N2 PCR samples	OptiGene direct LAMP	
			Number of positive samples	Positivity rate compared to PCR (%)
1000 – <10,000	10^3–10^4	18	0/18	0
10,000 – <100,000	10^4–10^5	20	0/20	0
100,000 – <1,000,000	10^5–10^6	17	6/17	35
1,000,000 – <10,000,000	10^6–10^7	25	18/25	72
>10,000,000	>10^7	6	5/6	83
Total		86	29/86	34

by qPCR.[9,10] The lower sensitivity of saliva, in addition to an assay with significantly inferior sensitivity, is of concern to us. Saliva may be acceptable for community mass testing, but not in the health care setting, where a missed positive sample can lead to an outbreak with significant consequences for patients.

We would be grateful for further data to enable the clinical virology and healthcare community, to understand the rational for prioritising the use of this insensitive assay, which lacks an internal control.

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Declaration of Competing Interest

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

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Critical evaluation of the methodology used by Wilson-Davies et al., (2020) entitled "Concerning the Optigene Direct LAMP assay, and it's use in at-risk groups and hospital staff"

Dear Editor-in-Chief,

As part of a multi-institution pilot project across various sites in England and Gibraltar, on the 1st of December, in conjunction with NHS Test and Trace and the Department of Health and Social Care (DHSC) we published the diagnostic sensitivity (DSe) and specificity (DSp) of the OptiGene Direct PLUS RT-LAMP assay (OptiGene Ltd) on both swabs and saliva[1].

We write with concern regarding the recent letter from Wilson-Davies et al. who have carried out an evaluation of the performance of the OptiGene Swab Direct RT-LAMP assay against the CDC N1/N2 SARS-CoV-2 RT-qPCR and have used that data to suggest that the OptiGene Direct Saliva PLUS RT-LAMP assay is significantly less sensitive than published[1].

Firstly, the authors cite Fowler et al.[2] as the publication of topic in the letter. This is not the assay format evaluated by the Test

Table 1
Analytical/Diagnostic sensitivity reported by Wilson-Davies et al., with the dc/ml adjusted for overestimation of actual viral genome copies.

SARS-CoV-2 Viral load estimated using CDC N1/N2 PCR		Number of positive samples	Adjusted dc/mL 100X overestimation	Adjusted dc/mL 1000X overestimation
1,000 - <10,000	10^3–10^4	0/18	10^1–10^2	10^{-1}–10^1
10,100 - <100,000	10^4–10^5	0/20	10^2–10^3	10^1–10^2
100,000 - <1,000,000	10^5–10^6	6/17	10^3–10^4	10^2–10^3
<1,000,000 - <10,000,000	10^6–10^7	18/25	10^4–10^5	10^3–10^4
>10,000,000	10^7–10^8	5/6	10^5–10^6	10^4–10^5

and Trace/DHSC pilot for wider application[1]. The Direct RT-LAMP assay described in the Fowler et al. publication[2] was for use on swabs and was superseded with a new sample preparation method (Direct PLUS RT-LAMP assay) to enable the Direct RT-LAMP assay to be performed on swabs and saliva. It was the Direct PLUS RT-LAMP assay that was in use at all the sites which participated in the studies reported in the NHS Test and Trace and the DHSC report[1].

Secondly, Wilson-Davies et al. state that the Direct PLUS RT-LAMP assay "failed to detect more than 50% of saliva positive cases in a pilot in Greater Manchester" and use the Guardian newspaper as their source of evidence[3]. The Guardian reported, out of context, a subset of data selectively extracted from within the Test and Trace/DHSC pilot[1]. Notably, the data generated by the Manchester Regional Virus Laboratory team, contrary to the statement by Wilson-Davies et al., verified the test performance, achieving 100% DSp and 100% DSe ($C_T \leq 25$), well within the confidence intervals reported in the government publication, which includes the Manchester pilot data[1]. The Manchester subset analysis included 15 positive samples, of which only six had a high-to-medium viral load ($C_T \leq 25$). The nine lower viral load samples ($C_T \geq 25$) artificially skewed the overall sensitivity of the test as the categories were not balanced (exhibiting the full dynamic range in equal proportions of high medium and low C_T values).

Thirdly, Wilson-Davies et al. state "consistent with results from the Manchester Regional Virus Laboratory, we have been unable to replicate the sensitivity". However, as we have stated, the Manchester Regional Virus Laboratory produced results that were consistent with the performance of the test within the range of detection for its intended use case[1], therefore Wilson-Davies et al. data is not consistent with the Manchester Regional Virus Laboratory data. In addition, the Manchester Regional Virus Laboratory evaluated the performance of the Direct PLUS RT-LAMP assay on saliva and not on swabs as used by Wilson-Davies et al. The performance of a test differs depending on the sample matrix (saliva or swab) being analysed (see Table 4 and paragraphs below for further explanation).

Fourthly, Wilson-Davies et al. have estimated genome copies (digital copies per millilitre: dc/mL) using the nucleocapsid (N) gene target. The Direct PLUS RT-LAMP assay targets the viral genomic RNA which is only present during active replication. Tests targeting regions of the viral genomic RNA which are not present also as subgenomic mRNAs (e.g., ORF1ab) are the most accurate approximates of actual number of viral genomes present. In contrast, N gene RNAs are present in abundance as subgenomic mRNAs and because of increased copy number, lead to overestimations of the number of actual viral genomes by ~100–1000 times[4]. In addition to this, the N gene has been reported by many to persist well beyond ORF1ab[4,5,6,7] and is therefore a poor indicator of active replication when present as the only target in the RT-qPCR. The swab samples listed in Table 1 therefore, could include samples which were N gene positive and ORF1ab negative. If the same genomic region is not used when comparing different chemistries, the DSe and DSp can be significantly miscalculated. Wilson-Davies et al. have previously used RT-qPCR assays including ORF1ab for outbreak management in NHS staff and therefore could have easily made more appropriate calculations and comparisons based on the use case of this test which is to identify individuals who are likely to be infectious[8]. Nevertheless, if the data by Wilson-Davies et al. is adjusted in line with the likely overestimation of genome copies calculated from using N gene[4] (Table 1), the analytical sensitivity is representative of the Direct PLUS RT-LAMP assay reported performance ($<10^3$)[1,2].

Table 2
Analytical sensitivity using UV inactivated SARS-CoV-2.

PFU/ml	PFU/Reaction	Replicate 1	Replicate 2	Replicate 3
100,000	500	Positive	Positive	Positive
10,000	50	Positive	Positive	Positive
1000	2	Positive	Positive	Positive
100	0.5	Positive	Negative	Positive
10	0.05	Negative	Negative	Negative
1	0.005	Negative	Negative	Negative

We have further independently explored the analytical sensitivity of the Direct PLUS RT-LAMP assay using UV treated virus as this causes much less fragmentation to RNA when compared to chemically and heat inactivated virus such as the NIBSC blinded panel[1]. The analytical sensitivity of the Direct PLUS RT-LAMP assay was determined to be between 100 and 1000 plaque forming units [PFU]/ml (0.5–2 PFU/reaction) (Table 2) which translates to 100–1000 copies of infections virus/ml given that one PFU represents progeny derived from one infectious virion. This sensitivity is consistent with the previous publications[1] and further emphasize the methodological problems in the conclusions drawn by Wilson-Davies et al. in their original Table 1.

It is also important to note that the swab samples used for analysis by Wilson-Davies et al. had not been tested fresh but had been stored at 4 °C for up to 36 h prior to being frozen at −80 °C. The Direct PLUS RT-LAMP assay has been validated for use on fresh, non-freeze thawed samples (saliva and swabs) and there is a dedicated sample collection standard operating procedure (SOP) to reflect this requirement. Wilson-Davies et al. did not collect and store their samples according to this SOP.

To understand the effect of freeze thaw on success rates and to inform the SOP, we analysed twenty positive saliva samples from asymptomatic individuals detected by the Direct PLUS RT-LAMP assay and confirmed by RT-qPCR when performed on freshly collected, non-freeze thawed samples. These were re-analysed in triplicate after the sample had been frozen for at least a week (e.g. had received one freeze thaw cycle) (Table 3). Only eight of the twenty samples remained positive in all replicates following freeze thaw. This highlights the considerable effect of freeze thawing on the performance of the Direct PLUS RT-LAMP assay due fragmentation of RNA (LAMP amplifies much larger fragments when compared to RT-qPCR and is therefore susceptible to sample degradation). Whilst this stability data was performed on saliva, we would anticipate the same effect on swabs since the process of degradation by freeze thaw is no different between sample types

Table 3
Effect of one freeze thaw on the ability to detect SARS-CoV-2 by Direct RT-LAMP assay.

	Fresh		One freeze thaw		
Sample	RT-qPCR	LAMP 1	LAMP 2A	LAMP2B	LAMP 2C
1	20.07	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
2	20.25	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
3	20.31	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
4	20.35	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
5	20.93	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
6	23.08	**POSITIVE**	NEGATIVE	**POSITIVE**	**POSITIVE**
7	23.33	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
8	23.61	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
9	23.93	**POSITIVE**	**POSITIVE**	**POSITIVE**	**POSITIVE**
10	24.08	**POSITIVE**	NEGATIVE	**POSITIVE**	**POSITIVE**
11	25.7	**POSITIVE**	NEGATIVE	**POSITIVE**	NEGATIVE
12	26.4	**POSITIVE**	NEGATIVE	NEGATIVE	**POSITIVE**
13	27.47	**POSITIVE**	NEGATIVE	NEGATIVE	NEGATIVE
14	27.49	**POSITIVE**	NEGATIVE	NEGATIVE	NEGATIVE
15	27.60	**POSITIVE**	**POSITIVE**	NEGATIVE	NEGATIVE
16	27.62	**POSITIVE**	NEGATIVE	NEGATIVE	NEGATIVE
17	28.14	**POSITIVE**	NEGATIVE	**POSITIVE**	**POSITIVE**
18	28.14	**POSITIVE**	NEGATIVE	**POSITIVE**	NEGATIVE
19	28.22	**POSITIVE**	**POSITIVE**	**POSITIVE**	NEGATIVE
20	28.89	**POSITIVE**	NEGATIVE	NEGATIVE	NEGATIVE

when considering LAMP based amplification. Given that all samples used in Table 1 of Wilson-Davies et al. were freeze thawed prior to analysis the data is not representative of samples which would be used in the designated use case of this assay. Storing samples at 4 °C was also more greatly affected than those stored at room temperature, which is also what Wilson-Davies et al. did prior to freezing. We also assume that the swabs used were nasopharyngeal/orophayngeal collected in viral transport media (VTM) which is an instructions for use (IFU) requirement for the CE market test. Any other diluent or type of swab (including anterior nasal swabs) would also impact the performance.

Wilson-Davies et al. state "A sample of >10,000,000 (>10^7) digital copies/mL (dc/mL), collected from a symptomatic NHS staff member, swabbed on day one of symptoms, produced a negative result when tested by the OptiGene Direct LAMP method. Similarly, a swab from an asymptomatic staff member, a target group for this assay, with 2500,000 (2.5×10^6) dc/mL was missed. A false negative result, with a high viral load, is a patient safety risk, particularly if the sample belongs to an asymptomatic member of staff". Taking the adjusted dc/ml from Table 1, these two samples would be: symptomatic: [10^4–10^6] and asymptomatic [10^3–10^5] which are well within the range of detection and also detected in other samples by this team. Explanations for the above result include: a) the effect of freeze thawing which would have no effect on RT-qPCR detection, but have potentially profound effects on RT-LAMP detection (as described above), or b) the technical performance of the operator. NEQAS/NIBSC verification for technical performance of this assay is a prerequisite to ensure consistent and expected performance before the assay is used on clinical samples. We have worked with NEQAS and NIBSC to construct a validation panel that all sites must utilise before running the Direct PLUS RT-LAMP assay and has been validated by multiple independent sites. This panel will be listed on the NEQAS/NIBSC website in due course. Unfortunately, Wilson-Davies et al. have to date not participated in this quality assurance programme and therefore cannot guarantee or substantiate the technical performance of the assay in their hands.

Fifthly, this laboratory had previously reported to two signatories on this letter (Fowler and Kidd, personal communication) an inability to reproduce sensitivities expected for the RNA RT-LAMP assay. A training visit was made to this laboratory in August 2020, and several critical observations were constructively made, the most significant of which was lack of mixing of the mastermix and the RNA sample prior to use. During that visit, a number of samples were provided by this group's laboratory and were run under the supervision of RT-LAMP assay experts. With proper mixing of mastermix and RNA, at this time, this laboratory produced the expected performance of the assay as agreed by the laboratory team at the time. No such training has ever been provided for the Direct PLUS RT-LAMP assay protocol. Critically the Direct PLUS RT-LAMP assay mix is much more viscous than the RNA RT-LAMP assay mix so if the same behaviours observed during the training visit for RNA RT-LAMP remained during the performance of the Direct PLUS RT-LAMP assay, performance of the assay will have been compromised.

Sixthly, when working on clinical samples there is a lysis and heat step which needs to be performed with no deviation from the IFU and dedicated bench card. This laboratory has not approached the UK quality assurance leads for LAMP testing for the bench card or IFU, which may explain a loss of performance due to technical error. As mentioned above the Direct PLUS RT-LAMP swab assay must also be performed using nasopharyngeal or oropharyngeal swabs collected into VTM.

Seventhly, Wilson-Davies et al. state "It has been asserted that nucleocapsid protein (N-) gene targets for qPCR are not valid for assessment of the performance of the OptiGene direct LAMP assay. The reason provided is that N-gene qPCR detects sub-genomic mRNA that may persist, and be detectable, beyond the period of viral replication, with an inability to differentiate between RNA from replicating virus and residual RNA after a resolved infection. It is important to stress that duration of detection of sub-genomic mRNA, and its implications for infectiousness, are currently debated". This statement of Wilson-Davies et al., is factually incorrect. The replication cycle of SARS-CoV-2 is well defined, fundamental virology and not a matter of debate[9]. Samples have been shown by multiple different authors to remain positive for N gene for several days/weeks/months after targets on the ORF1ab viral genomic RNA are no longer detected[4,5,6,7]. Given that Wilson-Davies et al., have used RT-qPCR assays containing the ORF1ab (which also includes N as a different target) for outbreak management in NHS staff, it is surprising that this team would choose to use the CDC N1/N2 RT-qPCR as a single gene mis-matched comparator which is likely to have overestimated actual viral genomic copies present in the sample.

Eighthly, Wilson-Davies et al. state "[saliva has] reduced sensitivity in comparison to the higher sensitivity of a nose and throat swabs" without presenting any new data on saliva-based testing. In raising this topic, Wilson-Davies et al. do not provide a balanced reference to an ongoing debate, which includes published reports demonstrating high RT-qPCR test concordance between appropriately collected paired saliva and nasopharyngeal samples[10,11].

To evaluate whether there was a difference in detection of SARS-CoV-2 in swabs compared to saliva a panel of 26 paired samples (52 samples) provided blinded by the Milton Keynes Lighthouse laboratory were analysed by an RT-qPCR (including ORF1ab) and Direct PLUS RT-LAMP on both saliva and swabs. The saliva and swabs had been collected from the same individual (Table 4). Eight samples were detected only in swabs or saliva with the remaining 10 samples detected in both swabs and saliva. Fifteen samples had viral loads (lower C_Ts) which were higher in saliva when compared to the paired swab. Ten samples had viral loads (lower C_Ts) which were higher in swabs when compared to the paired saliva and one sample had comparable viral loads in both the saliva and swab. This is consistent with the publication of Hansen et al.[12]., who reported that no single specimen type detected all SARS-CoV-2 infections, but that nasopharyngeal swabs (n = 80) and saliva (n = 81) were comparable and superior when compared to that of anterior

Table 4
Performance of Direct RT-LAMP on blinded and paired saliva and swab samples from lighthouse laboratory samples.

	Swab		Saliva	
	Direct RT-LAMP	**RT-qPCR ORF1ab**	**Direct RT-LAMP**	**RT-qPCR ORF1ab**
1	**POSITIVE**	24.7	NEGATIVE	25.38
2	**POSITIVE**	21.72	NEGATIVE	26.14
3	**POSITIVE**	21.52	NEGATIVE	25.29
4	NEGATIVE	27.87	**POSITIVE**	25.33
5	NEGATIVE	30.42	**POSITIVE**	25.35
6	NEGATIVE	30.33	**POSITIVE**	25.2
7	**POSITIVE**	26.59	NEGATIVE	29.16
8	**POSITIVE**	26.51	NEGATIVE	30.17
9	NEGATIVE	30.39	**POSITIVE**	26.48
10	**POSITIVE**	28.49	**POSITIVE**	25.96
11	**POSITIVE**	22.72	**POSITIVE**	19.15
12	**POSITIVE**	22.49	**POSITIVE**	19.02
13	**POSITIVE**	22.67	**POSITIVE**	24.1
14	**POSITIVE**	22.63	**POSITIVE**	24.18
15	**POSITIVE**	25.67	**POSITIVE**	15.64
16	**POSITIVE**	24.87	**POSITIVE**	15.3
17	**POSITIVE**	21.51	NEGATIVE	26.64
18	**POSITIVE**	21.24	NEGATIVE	24.09
19	NEGATIVE	31.25	**POSITIVE**	27.89
20	**POSITIVE**	21.86	**POSITIVE**	22.04
21	**POSITIVE**	21.65	**POSITIVE**	21.93
22	NEGATIVE	29.34	**POSITIVE**	24.07
23	NEGATIVE	29.43	**POSITIVE**	23.87
24	**POSITIVE**	30.93	**POSITIVE**	24.42
25	NEGATIVE	30.53	**POSITIVE**	23.5
26	**POSITIVE**	29.21	NEGATIVE	28.79

nasal swabs (n = 70). It is inaccurate and misleading of Wilson-Davies et al. to therefore claim that saliva is inferior to swabs. In fact in a recent preprint, viral load in saliva but not nasopharyngeal swabs has been shown to be a dynamic unifying correlate of COVID-19 disease presentation, severity and mortality[13].

Ninthly, Wilson-Davies et al. state "Critically, the inclusion of an internal control, added before nucleic acid extraction, is a vital quality requirement for molecular diagnostic work". The Medicines and Healthcare Products Regulatory Agency (MHRA) target product profile (TPP) for SARS-CoV-2 molecular assays says that an internal control is desirable, but the availability of one as a separate inclusion is acceptable[14]. OptiGene have a range of human RNA controls compatible with RT-LAMP assays which sites can choose to use if desired.

Finally, the Direct PLUS RT-LAMP assay requires fresh reagents that have not been allowed to deteriorate as per the IFU for this assay. Wilson-Davies et al. have never ordered reagents from OptiGene so it remains unclear where their reagents were sourced from. This raises significant concern about the provenance, storage conditions, shelf-life and the version & exact type of RT-LAMP reagents used in this study which may account for the apparent reduced performance reported in the letter from Wilson-Davies et al.

In conclusion, we have highlighted a series of methodological concerns with the laboratory evaluation carried out at Wilson-Davies laboratory. Novel diagnostic technologies, such as RT-LAMP require different ways of working compared to conventional virology technologies (i.e., RT-qPCR) and if precise attention to detail is not paid to the IFU this may lead to methodological errors such as in this dataset. The use case for this test is to identify asymptomatic/presymptomatic/symptomatic infectious individuals as a surveillance or case finding tool and not as a diagnostic test. Without this type of assay the UK will likely fall short of providing the diagnostics capacity it so desperately needs.

Dr Veronica Fowler
Signed on behalf and with agreement of all authors

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Home-based SARS-CoV-2 lateral flow antigen testing in hospital workers

Dear Editor,

We read with interest the recent paper from Fowler *et al.* on the use of a reverse transcriptase loop mediated isothermal amplification (RT-LAMP) assay for rapid detection of SARS-CoV-2 infection[1] and the subsequent recommendation of its suitability for asymptomatic staff testing.[2] Lateral flow device (LFD) antigen tests have also been proposed as rapid point-of-care diagnostics for SARS-CoV-2 infection and have the advantage over the RT-LAMP assay in that they can be self-delivered by healthcare workers at home with immediate results. One LFD, Innova, had a specificity of 99.7% and sensitivity of 77% in a large national evaluation, with >90% of high viral load infections (cycle threshold, Ct ≤25.5) detected.[3] However, sensitivity can be lower when LFDs are used by the general public and some have claimed these tests are not fit for purpose.[4] In England from November 2020, twice weekly LFD testing has been offered to hospital workers. We present our experience at Oxford University Hospitals of deploying self-administered home-based LFD testing of anterior nasal swabs for our staff.

Between 23 November 2020 and 09 January 2021, 8657 healthcare workers and support staff registered and collected a box of 25 Innova LFD tests (Fig. 1A). 46,503 test results were reported using an online form by 7567 asymptomatic individuals: median (IQR)[range] 6 (3–9) [1–23] tests/individual. 45,710 (98.3%) tests were negative, 465 (1.0%) invalid and 328 (0.7%; from 295 individuals) positive. 28 (9%) positive LFD results were in recently PCR-positive staff tested a median (IQR) [range] 3.5 (1-6) [1-13] days earlier. Of the remainder, 169 (52%) positive LFD results were followed by confirmatory PCR (Thermo Fisher TaqPath) at our hospital within 3 days (Fig. 1B), 161 were PCR-positive and 8 PCR-negative (positive predictive value, 189/197, 96% [95%CI 92-98%], false positive rate 8/27930, 0.03% [95%CI 0.01–0.06%], accounting for incomplete confirmatory testing by reducing the denominator proportionally to the percentage of confirmatory tests undertaken).

In contrast, more positive results were detected in staff asymptomatic screening using PCR of combined nasal/oropharyngeal swabs[5] over the same time period, 127/8329 (1.5%). Viral loads, assessed using Ct values, were higher in those with positive LFD antigen results versus other PCR-positive asymptomatic staff, median (IQR) Ct 14 (12–18) versus 30 (22-33) ($p<0.001$; Fig. 1C).

1398/7567 (18.5%) staff reporting LFD results had not attended asymptomatic screening for ≥90 days, another 1128 (14.9%) never had. Of 295 staff who tested LFD positive, 116 (39%) had not attended for asymptomatic screening in the previous 30 days including 54 (18%) not in the last 90 days and another 40 (14%) had never attended. Staff reported the nasal swab used for LFD testing was preferable to the combined nose and throat swab taken for PCR, found testing kits easy to use, and the process acceptable even when done regularly.

Use of LFDs identified asymptomatic SARS-CoV-2 infections that would not otherwise have been detected. This enabled interventions to reduce staff-to-staff and staff-to-patient transmission, which were focused on staff with high viral loads, i.e. potentially those most infectious. Although individuals with low viral loads may be missed, with regular serial testing at least twice per week, those with early infection and rising viral loads are likely to be identified. Determining whether on-going PCR-based screening is required in addition to LFDs will require better estimates of the relative infectiousness of individuals by viral load. Kit failure rates and false positive results in this mixed population of healthcare workers and support staff were sufficiently low to support widespread use of LFDs in asymptomatic populations.

Ethics statement

Deidentified data were obtained from the Infections in Oxfordshire Research Database which has generic Research Ethics Committee, Health Research Authority and Confidentiality Advisory Group approvals (19/SC/0403, 19/CAG/0144).

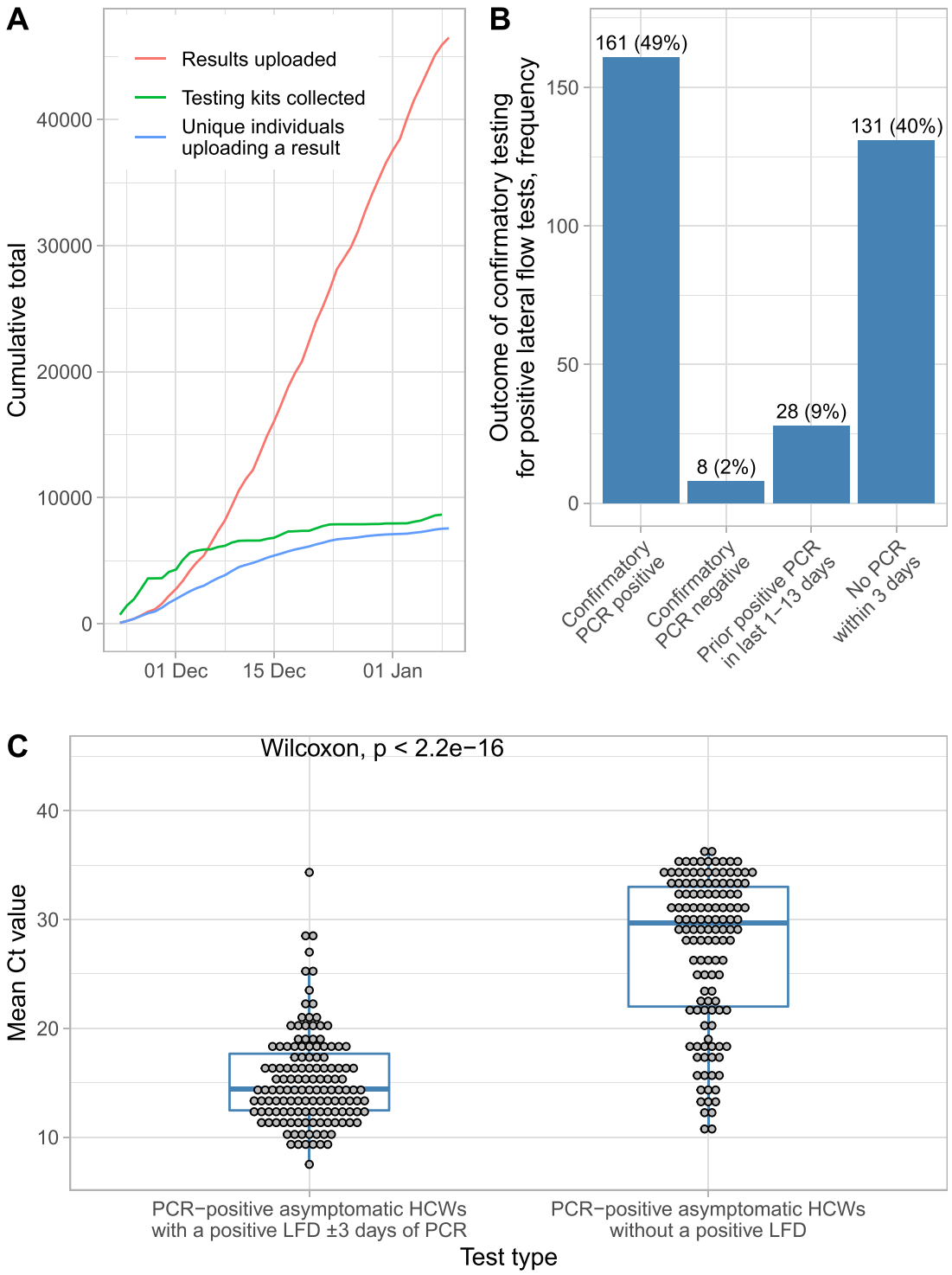

Fig. 1. Lateral flow device collections and result submission (panel A) and confirmatory results within 3 days (panel B) and associated Ct values (panel C). Results are presented for 23 November 2020 to 09 January 2021 inclusive. PCR tests were performed using the Thermo Fisher TaqPath assay (targeting S and N genes, and ORF1ab, available mean cycle threshold (Ct) values for detected targets are shown). Panel C shows the PCR results from asymptomatic staff obtained within ±3 days of a positive LFD device, and PCR results from asymptomatic staff without a positive LFD. The box plots indicate median and interquartile ranges.

Declaration of Competing Interests

DWE declares lecture fees from Gilead, outside the submitted work. No other author has a conflict of interest to declare.

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The usefulness of NEWS2 at day 7 of hospitalization in predicting COVID-19 evolution and as an early endpoint in therapeutic trials

Dear Editor,

We read with interest in your journal the paper by Sze et al. who reports that National Early Warning Score 2 (NEWS2) in elderly patient is not useful to predict outcome during COVID-19.[1]

However, NEWS2 has been described as a robust tool to predict outcome during sepsis[2] and seems to predict evolution of COVID-19 upon admission to the hospital in other recently studies.[3–5]

Considering the evolution of the epidemic, there is a need for reliable tools to predict some evolution in particular with regard to hospitalized patients, in order to discharge more quickly the patients from medical wards to home.

Moreover, more than 2000 clinical trials have been registered regarding COVID-19.[6] Most of them use an endpoint defined 28 days after inclusion or hospitalization, as recommended by the World Health Organization.[7] This relatively long time needed to conduct these trials may lead to uncontrolled prescription of drugs with unproved efficacy. Thus, early endpoints predictive of long-term evolution may not only reduce the risk of lost to follow-up and protocol deviations, but could also allow knowing the results of the trials faster.

We evaluate if NEWS2 could predict outcome of COVID-19 at day 28 and look for the earliest time point of assessment which could be used as a strong surrogate marker of day 28 evolution.

All adult patients who were directly hospitalized in our department for a confirmed COVID-19 between the 27th of February and the 30th of April 2020 (during the first peak of the epidemic in France) were retrospectively included. For each patient, epidemiological, demographic, clinical, biological, radiological, therapeutic data, and outcomes were collected from medical records. NEWS2 was calculated at admission, at day (D) 7 and D14 of hospitalization. With the total score, patients were classified into 3 groups of risk: low risk from 0 to 4; medium risk from 5 to 6 and high risk above 7.

The occurrence of an unfavourable event (defined as death or transfer to ICU) during hospitalization was the primary outcome.

The diagnostic accuracy of the NEWS2 to predict unfavourable outcome at D28 was evaluated thanks to the area under receiver operating characteristic curve (AUROC) for:

- NEWS2 at admission,
- NEWS2 at D7 after excluding from the analysis patients with unfavourable outcome within the first seven days of hospitalization.
- NEWS2 evolution at D7 (delta NEWS) after excluding from the analysis patients with unfavourable outcome within the first seven days of hospitalization.

Sensitivity and specificity, negative predictive value (NPV), positive predictive value (PPV) and positive and negative likelihood ratio (LR+ and LR-) were calculated for the best corresponding cut-off.

Overall, 222 patients were included. Characteristic of patient, NEWS2 value and NEWS2 class at admission, D7 and D14, are represented in Table 1

An unfavourable event was observed in 64 patients (29%), 52 during the first week and, 12 in the second week of hospitalization.

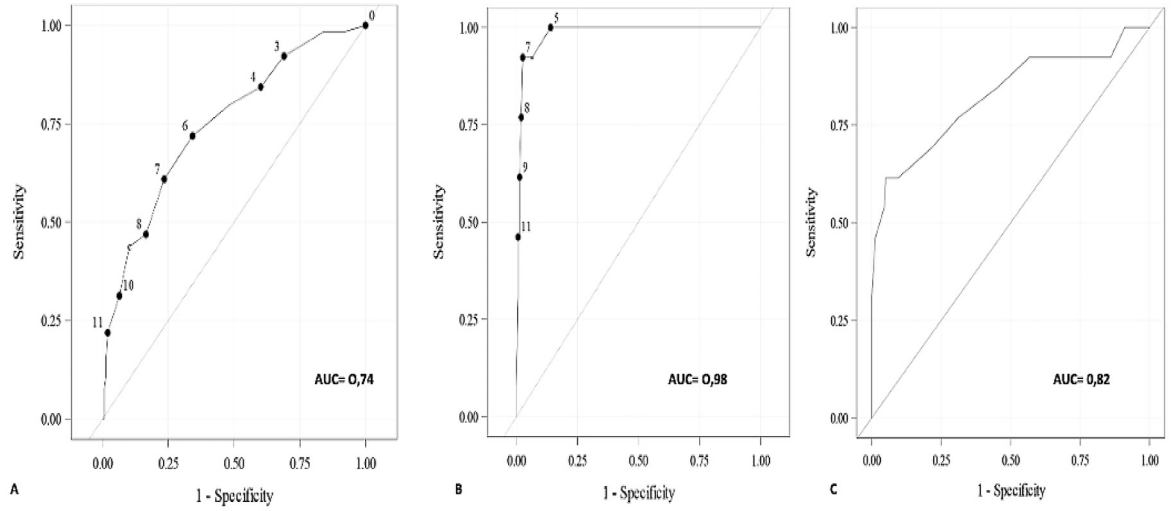
For the 170 patients who did not present with any unfavourable event within the first week of hospitalization, 154 (90%) had a NEWS2$<$7 at D7.

ROC curves of NEWS2 at admission and at D7 to predict unfavourable events within the first 28 days of hospitalization are shown in Fig. 1. At D0, AUROC curve was 0.74, and the best cut-off of NEWS2 was 6. At D7, AUROC curve was 0.98, with a best cut-off of 7. For this latter cut-off, sensitivity, specificity, PPV, NPV, LR+ and LR- were respectively of 0.92, 0.97, 0.75, 0.99, 2.5 and 0.08. The AUROC curve of NEWS2 evolution at D7 (Delta NEWS) was 0.82, significantly lower than the area under the curve of NEWS2 at D7 (0.82 vs 0.98, $p<0.005$).

Herein, we showed in our study that a NEWS2 $\geq$ 6 at admission predicts unfavourable outcome with 76% sensitivity and 64% specificity, these results being close to those reported in other studies.[3,4] Compared to other scores specifically developed for COVID-19,[8,9] NEWS2 is an easily and rapidly applicable score. Furthermore, its performance reported in the literature seems to be su-

Table 1
Characteristics, NEWS2 value and NEWS2 class of patients hospitalized for COVID-19.

	Overall ($n = 222$)
Age, mean ±SD	70.1 (±17.1)
Sex	
Male, n (%)	123 (55)
Serious COVID-19 risk factors, n (%)	176 (79)
Age > 75 y.o, n (%)	90 (41)
Diabetes mellitus, n (%)	51 (23)
Cardiovascular diseases, n (%)	133 (60)
Chronic respiratory diseases, n (%)	30 (14)
BMI < 16 kg/m², n (%)	1 (0)
BMI ≥ 30 kg/m², n (%)	34 (16)
Pregnancy, n (%)	2 (1)
Immunodepression, n (%)	19 (9)
Cirrhosis, n (%)	1 (0)
Chronic kidney failure, n (%)	18 (8)
Other past medical history	
Current smoker, n (%)	8 (4)
Chronic alcoholism, n (%)	13 (8)
Depression, n (%)	32 (14)
Dementia, n (%)	48 (22)
Charlson's score, median (IQR)	4.0 (2.0–6.0)
Charlson's score without age, median (IQR)	1.0 (0.0–2.0)
Duration between first symptoms and admission at hospital, median (IQR)	6.0 (3.0–9.0)
Clinical signs before admission	
Fever, n (%)	169 (76)
Cough, n (%)	146 (66)
Dyspnea, n (%)	137 (62)
Flu syndrome, n (%)	82 (37)
Digestive disorders, n (%)	66 (30)
Confusion, n (%)	37 (17)
Anosmia, n (%)	13 (6)
Dygueusia, n (%)	17 (8)
Biological data at admission	
Lymphocytes, G/L, median (IQR)	0.9 (0.6–1.4)
Fibrinogen, g/L, median (IQR)	6.0 (5.3–6.8)
C-reactive protein, mg/L, median (IQR)	83.7 (37.7–127.0)
Albumin, g/L, median (IQR)	26.0 (23.0–30.0)
Prealbumin, g/L, median (IQR)	0.1 (0.1–0.1)
Creatinine, µmol/L, median (IQR)	76.0 (60.0–100.0)
Urea, mmol/L, median (IQR)	6.6 (4.8–10.4)
Alanine aminotransferase, UI/L, median (IQR)	34.0 (23.0–49.5)
Thoracic CT-scan	
Non evocative of COVID-19, n (%)	17 (10)
Minimal, n (%)	19 (12)
Moderate, n (%)	53 (32)
Extent, n (%)	48 (29)
Severe, n (%)	22 (13)
Critical, n (%)	5 (3)
Specific treatment studied for COVID, n (%)	15 (7)
Hydroxychloroquine, n (%)	6(40)
Remdesivir, n (%)	4(27)
Lopinavir/ritonavir/Interferon Beta, n (%)	3(20)
Lopinavir/Ritonavir, n (%)	2(13)
Corticoids, n (%)	7 (3)
Antibiotic therapy, n (%)	174 (78)
NEWS2 at admission	**Overall ($n = 222$)**
Median (min, max)	5.0 (0.0, 17.0)
Class 1 of NEWS2, n (%)	87 (39)
Class 2 of NEWS2, n (%)	50 (23)
Class 3 of NEWS2, n (%)	84 (38)
NEWS2 at D7	**Overall ($n = 170$)**
Median (min, max)	0.0 (0.0, 13.0)
Class 1 of NEWS2, n (%)	134 (79)
Class 2 of NEWS2, n (%)	18 (11)
Class 3 of NEWS2, n (%)	17 (10)
NEWS2 at D14	**Overall ($n = 158$)**
Median (min, max)	0.0 (0.0, 15.0)
Class 1 of NEWS2, n (%)	151 (95)
Class 2 of NEWS2, n (%)	6 (4)
Class 3 of NEWS2, n (%)	1 (1)



	Sensitivity	Specificity	PPV	PPN	LR+	LR-
NEWS2≥6 at admission to predict unfavorable event at D28	0.72	0.66	0.46	0.84	1,6	0,37
NEWS2≥ 7 at D7 to predict unfavorable event at D28	0.92	0.97	0.75	0.99	30,7	0.08

Fig. 1. Receiver Operating Characteristic (ROC) curve and performance value for the best cut off for: A: NEWS2 at admission using unfavourable event within the 28 first days as the gold standard B: NEWS2 at D7 using unfavourable event within the 28 first days as the gold standard and after excluding from the analysis patients with unfavourable outcomes within the first seven days of hospitalization. C: NEWS2 evolution at D7 (Delta NEWS) using unfavourable event within the 28 first days as the gold standard and after excluding from the analysis patients with unfavourable outcomes within the first seven days of hospitalization.

perior to other usual scores like the quick Sequential Organ Failure Assessment and the Systemic Inflammatory Response Syndrome.[4,5]

However, we observed that these performances of NEWS2 at admission are weaker than those at D7, with high sensitivity and specificity rates by using a threshold of 7. This indicates a close to 0 probability of unfavourable event for patients who did not present with any unfavourable event within the seven first days of hospitalization and who have a NEWS2<7 at this time. In our cohort, this latter threshold concerned 90% of the patients.

The assessment at NEWS2 at D7 could thus be useful in two ways.

First, the patients with a NEWS2<7 at this time could be confidently, safely, and more quickly discharged from medical wards towards follow-up care facilities, thus reducing the burden of hospital occupation and improving the turnover of the patients in a highly epidemic context.

Second, a NEWS2<7 at D7 combined with the lack of unfavourable outcome during the first week could be used as an important judgement criterion in therapeutic trials, as they were observed in many patients, and associated with a nearly non-existent likelihood of subsequent unfavourable evolution. It may thus reduce the time of participation in therapeutic trials, as well as the risk of loss to follow-up. The fact that no specific antiviral therapy with significant efficacy was used in our study strengthened the potential usefulness of such a surrogate marker in further trials on drugs with genuine clinical efficacy.

Last, the main characteristics of our cohort are close to those previously published,[10] making our results likely to be observed in other settings as well. In addition, even though it could be advocated that the delay between contamination and hospitalization could impact on the observed results, the median delay between the onset of symptoms and hospitalization in our study (6 days) also correlates with that reported in other studies, as well as the median time elapsed from admission to unfavourable event (within the first two weeks).[10]

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Sex differences in mortality in the intensive care unit patients with severe COVID-19

Dear Sir,

The available evidence suggests that mortality from coronavirus disease 2019 (COVID-19) in males is higher than in females.[1] In a recent cross-sectional study, de Lusignan et al. evaluated the absolute excess risk (AER) of mortality and excess mortality rate (EMR) in the UK from a COVID-19 sentinel surveillance network in people aged 45 years and above.[2] The AER in mortality was calculated by comparing mortality for weeks 2–20 this year with mortality data from the Office for National Statistics (ONS) from 2018 for the same weeks. The absolute excess mortality was approximately 2 deaths per 100 person years in the first wave of COVID-19, whereas the EMR for male gender, compared with female, was 1.4 (95% confidence interval [CI] 1.35–1.44, $p<0.00$).

We investigated the sex-related differences in the occurrence of comorbidities and mortality rates in a nationwide study in 1522 consecutive patients with severe SARS-CoV-2 pneumonia who were admitted to intensive care unit (ICU) for respiratory support. Medical records of patients were submitted by the COVID-19 hospitals located in 70 regions across Russia to the Federal Center at the Sechenov University (Moscow) that provided advice on critical care of patients. In 995 patients (65.4%), diagnosis of SARS-CoV-2 pneumonia was confirmed by polymerase chain reaction (PCR), whereas in the other cases SARS-CoV-2 pneumonia was defined as severe acute respiratory infection with typical CT findings[3] and no other obvious etiology. Most patients were older than 40 years of age and had various comorbidities. Among 1522 patients enrolled in this study, 995 patients (65.4%) died, and 527 patients (34.6%) recovered. Most patients (93.2%) died from progressive respiratory failure.

In the total cohort, the requirement for mechanical ventilation and mortality rates were similar in males and females (Table 1). However, female patients were older and had a higher occurrence of various chronic illnesses, that is, arterial hypertension, obesity and type 2 diabetes, that impair prognosis in patients with COVID-19.[4,5] Coronary artery disease (CAD) and chronic obstructive pulmonary disease (COPD) were more frequent in males than in females, although their occurrence was lower compared to that of the other significant comorbidities. The mortality rates increased with age both in males and females. In patients aged 50 years or younger, the mortality rates were similar in males and females (odds ratio [OR] 0.975; 95% CI 0.596–1.596; $p=0.92$) despite a higher requirement for mechanical ventilation in male patients. In the other age groups, the mortality rates were significantly higher in males than in females (51–60 years: OR 1.796; 95% CI 1.192–2.705; $p=0.005$; 61–70 years: OR 1.952; 95% CI 1.290–2.952; $p=0.002$; ≥71 years: OR 2.006; 95% CI 1.109–3.629; $p=0.021$), whereas the requirement for mechanical ventilation did not differ between sexes. In all age groups, the occurrence of arterial hypertension, type 2 diabetes and obesity was higher in females than in males, although these differences reached statistical significance only in a proportion of cases (Table 1). On the contrary, CAD occurred significantly more frequently in males aged 51–60 and 61–70 years than in females of similar age, whereas the frequency of COPD was increased in males aged 61–70 and ≥70 years.

In summary, the mortality rate in the ICU patients with severe SARS-CoV-2 pneumonia was higher in males aged >50 years than in females of similar age. Our findings are in line with de Lusignan et al. data, who reported a higher EMR in males during the COVID-19 pandemic in the English population. The differences between mortality rates in males and females cannot be explained by comorbidities, given the divergent trends in the occurrence of chronic illnesses that may worsen survival in COVID-19 patients.

Table 1
Demographic and clinical characteristics and the mortality rates in the different age groups of the ICU patients with severe SARS-CoV-2 pneumonia requiring respiratory support

Parameters	All			Age ≤50 years		Age 51-60 years		Age 61-70 years		Age ≥71 years	
	All(n=1522)	Males(n=864)	Females(n=658)	Males(n=226)	Females(n=88)	Males(n=237)	Females(n=156)	Males(n=230)	Females(n=194)	Males(n=171)	Females(n=220)
Age, years; Me (IQR)	62 (53; 71)	59 (50; 68)[d]	64 (56; 74)	–	–	–	–	–	–	–	–
Death, n (%)	995 (65.4)	582 (67.4)	413 (62.8)	109 (48.2)	43 (48.9)	148 (62.4)[b]	75 (48.1)	172 (74.8)[b]	117 (60.3)	153 (89.5)[a]	178 (80.9)
Oxygen supplementation, n (%)	199 (13.1)	102 (11.8)	97 (14.7)	32 (14.2)[a]	22 (25.0)	34 (14.3)	28 (17.9)	25 (10.9)	24 (12.4)	11 (6.4)	23 (10.5)
Non-invasive ventilation, n (%)	95 (6,2)	53 (6.1)	42 (6.4)	19 (8.4)	9 (10.2)	18 (7.6)	9 (5.8)	10 (4.3)	12 (6.2)	6 (3.5)	12 (5.5)
Mechanical ventilation, n (%)	944 (62,0)	709 (82.1)	519 (78.9)	175 (77.4)[a]	57 (64.8)	185 (78.1)	119 (76.3)	195 (84.8)	158 (81.4)	154 (90.1)	185 (84.1)
ECMO, n (%)	7 (0,5)	1 (0.1)[a]	6 (0.9)	1 (0.4)	3 (3.4)	0	2 (1.3)	0	0	0	1 (0.5)
CVD, n (%)	976 (64.1)	508 (58.8)[d]	468 (71.7)	52 (23.0)	28 (31.8)	131 (55.3)	97 (62.2)	175 (76.1)	149 (76.8)	150 (87.7)	194 (88.2)
Hypertension, n (%)	905 (59,5)	452 (52.3)[d]	453 (68.8)	49 (21.7)	27 (30.7)	121 (51.1)	94 (60.3)	153 (66.5)	143 (73.7)	129 (75.4)[b]	189 (85.9)
CAD, n (%)	234 (15.4)	151 (17.5)[b]	83 (12.6)	7 (3.1)	2 (2.3)	26 (11.0)[a]	7 (4.5)	65 (28.3)[d]	24 (12.4)	53 (31.0)	50 (22.7)
Stroke, n (%)	113 (7.4)	61 (7.1)	52 (7.9)	2 (0.9)	0	9 (3.8)	8 (5.1)	26 (11.3)	12 (6.2)	24 (14.0)	32 (14.5)
AF, n (%)	161 (10,6)	98 (11.3)	63 (9.6)	3 (1.3)	1 (1.1)	13 (5.5)	5 (3.2)	32 (13.9)	17 (8.8)	50 (29.2)[a]	40 (18.2)
Diabetes mellitus, n (%)	406 (26,7)	191 (22.1)[d]	215 (32.7)	24 (10.6)	15 (17.0)	57 (24.1)	45 (28.8)	65 (28.3)[a]	76 (39.2)	45 (26.3)[a]	79 (35.9)
Obesity, n (%)	396 (26,0)	183 (21.2)[d]	213 (32.4)	51 (22.6)	32 (36.4)	61 (25.7)[b]	60 (38.5)	51 (22.2)[d]	74 (38.1)	20 (11.7)[a]	47 (21.4)
Asthma, n (%)	35 (2,3)	13 (1.5)[a]	22 (3.3)	5 (2.2)	3 (3.4)	1 (0.4)	4 (2.6)	3 (1.3)	9 (4.6)	4 (2.3)	6 (2.7)
COPD, n (%)	78 (5,1)	58 (6.7)[c]	20 (3.0)	10 (4.4)	1 (1.1)	9 (3.8)	4 (2.6)	22 (9.6)[a]	8 (4.1)	17 (9.9)[b]	7 (3.2)

[a] p<0.05.
[b] $p \leq 0.01$.
[c] $p \leq 0.001$.
[d] $p \leq 0.0001$; characteristics were compared using a Pearson chi-square test for categorical variables and Mann-Whitney U test for continuous variables; Me(IQR) = median (interquartile range); ECMO = extracorporeal membrane oxygenation; CVD = cardiovascular disease; CAD = coronary artery disease (a history of myocardial infarction or interventions on the coronary arteries); AF = atrial fibrillation/Obesity was defined as body mass index ≥30 kg/m^2; COPD = chronic obstructive pulmonary disease.

Al-Lami et al. suggested that low levels of testosterone, as can occur in normally aging men, may account for more severe lung damage since testosterone deficiency has been linked with autoimmune disease and increases in inflammatory markers.[6] Moreover, anti-inflammatory effects of estrogens may protect females from progression of SARS-CoV-2 induced lung disease. Our data indirectly support that sex steroid hormones underlie sex-related differences in COVID-19 mortality.

Declaration of Competing Interest

The authors report no competing interests.

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Excess pneumonia and influenza death as herald wave of COVID-19 in England and Wales, United Kingdom

Dear Editor,

We read with interest Gosce et al's modelling on COVID-19 in London, United Kingdom (UK),[1] concerning approaches to lift the lockdown. Their work utilized notified cases, deaths, contacts, and mobility data. The authors pointed out "the recent observed increase in all-cause mortality during lockdown, which is in part due to individuals dying from other causes warrants investigation." In this work, we analyse the impact of COVID-19 on all-cause mortality and pneumonia and influenza (P&I) deaths in England and Wales (E&W), UK with the aim to show that these data could embed useful information. Compared with confirmed COVID-19 cases, the reported COVID-19 deaths may be relatively more reliable as an

index since deceased patients are more likely to have been hospitalized, and thus more likely to have been tested and ascertained than the general infected population[2,3]. All-cause deaths were also used to reconstruct the course of the pandemic, often through implementing the well-known Serfling's method[4]. Weinberger et al. (2020) found that the all-cause deaths are 28% higher than the reported number of COVID-19 deaths from March 1 to May 30, 2020, in the United States[5]. For comparison, we found that the excess all-cause mortality is 23.4% higher than the reported number of COVID-19 deaths over the same period in the United Kingdom.

However, the impact of COVID-19 on P&I deaths is complex. On the one hand, both COVID-19 and influenza may contribute to the mortality risk of pneumonia at the populational scale. On the other hand, pneumonia deaths, as one of the major clinical outcomes of COVID-19, due to unascertained COVID-19 may be recorded as an unknown type of pneumonia especially during the early stages of the outbreak.

In this work, we analysed the P&I mortality, all-cause mortality, and COVID-19 mortality in E&W, UK.[6] The reason we chose E&W, UK as an example is largely due to the availability of the data (COVID-19 deaths, P&I deaths and all-cause mortality). However, we only have daily COVID-19 cases and weekly influenza positives for the whole UK. Since the E&W population accounts for 89% of the UK population, it is reasonable to assume COVID-19 cases and influenza positives in UK are good proxy for that of E&W.

The first two cases of COVID-19 were reported in the UK on February 1, 2020, and the number of reported cases has been increasing since then. Up until August 6, 2020, 307,188 confirmed COVID-19 cases were reported in the UK, including 46,364 deaths due to COVID-19, with a raw case fatality rate of 15%. These are shown as timeseries in Fig. 1A. In the same figure we also show the weekly laboratory confirmation of influenza. Seasonal influenza and COVID-19 could compete for patients, deaths, and medical resources. However seasonal influenza should have a smaller basic reproductive number than that of COVID-19, and thus is more sensitive to social distancing. According to the weekly influenza positives timeseries in Fig. 1A, across the UK the number of influenza positives in the first half year was significantly lower overall than previous years, except for a brief peak in January 2020. Weekly influenza positives fell rapidly to a low level when social distancing was in place in the UK. Fig. 1A shows data for UK, but we suppose the same trends would be apparent for E&W.

We first noticed that the P&I mortality in the first half year of 2020 was unusually aligned with the previous five-year baseline average in E&W, UK, as seen in Fig. 1B. In this figure we have in fact plotted the P&I mortality after applying a small constant vertical shift (amounting to 550 deaths per week) to help emphasise the correspondence. The alignment is astounding. We plotted the differences in alignment as a histogram by subtracting the actual P&I mortality of this year from the baseline. The histogram shows that there is a peak difference of 550 deaths (Fig. 1C).

In the period week-11 to week-24 in 2020, some 59,138 excess all-cause deaths were reported in E&W, UK. Over the same period, there were 48,218 laboratory confirmed COVID-19-associated deaths (Fig. 1D). If we denote the true number of COVID-19 related deaths as A, we have $A > 48{,}218$ presumably[7–9]. The excess all-cause mortality may be denoted as $A - B$, where B is the reduced death of other causes due to the city lockdown (for instance traffic accident deaths, improved air conditioning or suppression of influenza). While we can easily calculate excess mortality $A - B$ from the data, we can never find A without knowledge of B.

Interestingly, but unsurprisingly, the time series of excess all-cause deaths is similar to that of COVID-19 deaths (Fig. 1D). There is a seemingly irrational phenomenon in the time series of excess all-cause mortality in Fig. 1D which reached negative values in the first few months of 2020. Harrison et al. (2020) suggested this may have resulted from the public having learnt about the severity of COVID-19 elsewhere and having taken a series of precautionary measures which may have led to fewer deaths from other diseases before the outbreak.[10] These negative values are a manifestation of the aforementioned B value. Fig. 1A also shows that influenza epidemics were more severe in 2018 and 2019 than in 2020 at the beginning of the year, and thus possibly another reason for the lower number of overall excess deaths.

More surprisingly, excess P&I deaths showed an almost perfect exponential increasing trend from week 6 until its peak at week 14 and then quickly fell back to a normal level (i.e., zero excess; Fig. 1D). This pattern seen in the P&I excess deaths indicates the arrival time of COVID-19 and its spreading. We crudely estimated the basic reproductive number to be approximately R_0=1.66 (95% confidence interval: 1.58–1.74) (see Supplementary Materials).

The excess P&I deaths in Fig. 1D, could be seen as a "herald-wave" of COVID-19 in that it has the appearances of an outbreak that peaks several weeks in advance of COVID-19. Before and after the "herald wave", the P&I deaths matched the previous five years average astonishingly well. Upon consideration, the "herald wave" is most likely an identification and coding issue, namely, when a new disease emerges, there will be a transition from "coding the new disease related deaths with the closest existing code" to "coding them with the newly created specific code". But importantly, this timeseries analysis illuminates the importance of quality real-time disease surveillance to detect anomalies, and to predict major disease outbreaks that might otherwise go undetected for significant time periods. Also our observation may provide a signal for commencing enhanced population protection.[1]

Ethical approval and consent to participate

The ethical approval and individual consents were exempted as the aggregated data were used in this study.

Availability of data and materials

All data used are from public domain.

Authors' contributions

All authors conceived and conducted the research and wrote the draft. All authors critically revised the manuscript, and all authors approved the submission.

Declaration of Competing Interest

DH was supported by an Alibaba (China) Co. Ltd. Collaborative Research project. Other authors declare no conflict of interest.

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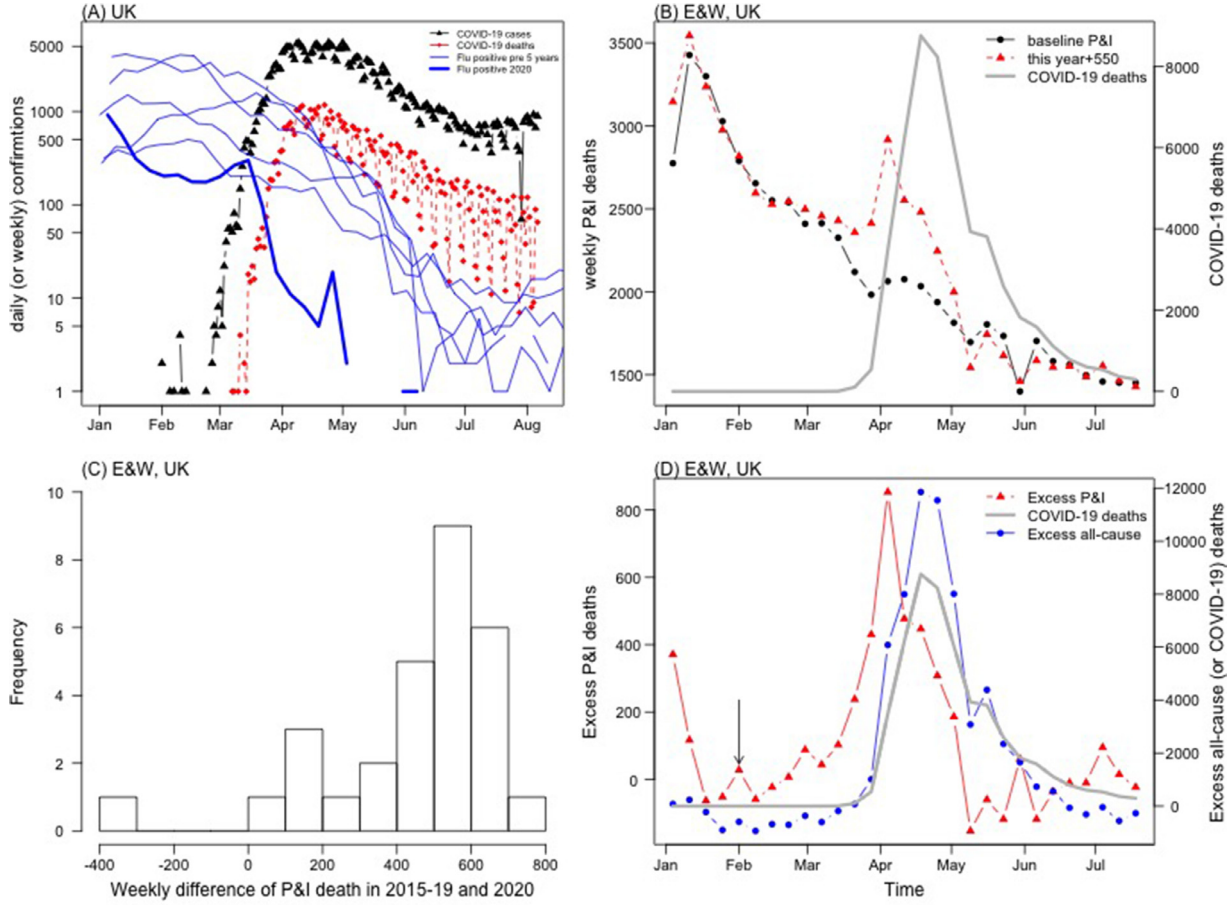

Fig. 1. Time series plots of daily confirmed COVID-19 cases (in black), COVID-19 deaths (in red), and influenza positive cases in 2015–2019 (in light blue) and in 2020 (in dark blue) in the UK (panel A). The comparison between COVID-19 and P&I deaths in E&W, UK (panel B). Frequency distribution of the difference between weekly P&I deaths in 2020 and the baseline of previous five years (panel C). The comparison amongst excess P&I, COVID-19 and excess all-cause deaths in E&W, UK (panel D). In panel D, the arrows indicate the time that the first two confirmed case of COVID-19 were reported. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.09.019.

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Decline in invasive pneumococcal disease during COVID-19 pandemic in Taiwan

Dear Editor,

We read with great interest Lim et al's report,[1] which showed a decreased incidence of pneumococcal disease in Singapore during the first 27 weeks in 2020 in the time of COVID-19. Although the collateral benefit of controlling COVID-19 for other common respiratory infectious diseases, such as influenza and tuberculosis in Taiwan have been demonstrated,[2,3] the impact of the infection control and policy to prevent COVID-19 outbreak on pneumococcal disease remained unclear. Therefore, this study was conducted to determine whether the incidence of pneumococcal disease in Taiwan would be decreasing as Lim et al's study in Singapore.

In Taiwan, invasive pneumococcal disease (IPD) is a notifiable disease for which reporting is mandatory for all clinicians. Therefore, this study can obtain the case number of patients with IPD used data from open data website of Taiwan's CDC.[4] To compare the case number of IPD during the same period each year, we extracted the monthly cases between January and August from 2015 to 2020.

First, a total of 162 IPD cases were reported during the first 8 months in 2020, By contrast, the accumulative case number within the 8 months ranged from 282 in 2019 to 400 in 2016, which were much higher than that in 2020 (Fig. 1A). Second, in 2020, the case number of IPD was highest in January ($n = 62$) and gradually decreased with time, which was lowest in May ($n = 7$) (Fig. 1B).

In this study, we found the similar phenomena that IPD was decreasing during COVID-19 in Taiwan, like Lim et al's findings in Singapore.[1] The possible explanation could be the strict performance of infection control and policy during COVID-19 pandemic. Since the first case of COVID-19 was identified in January, a total of 498 COVID-19 cases were reported till now and caused seven deaths. To prevent the outbreak of COVID-19, Taiwan authority immediately practiced many infection control measures, particularly mask wearing, hand hygiene and avoid visiting crowd area.[5] Most of these interventions can help prevent the transmission of SARS-CoV-2 via respiratory droplets and may also provide additional benefit in the controlling other respiratory infectious diseases, such as pneumococcal disease, which was demonstrated here.

Although many confounding factors, such as vaccine strategy or under-report of IPD during COVID-19 pandemic were not evaluated in this study, our findings was consistent with Singapore's study[1] suggest that strictly performance of infection control and policy not only mitigate the threaten of COVID-19 but also reduce the burden of other respiratory infections disease – invasive pneumococcal diseases.

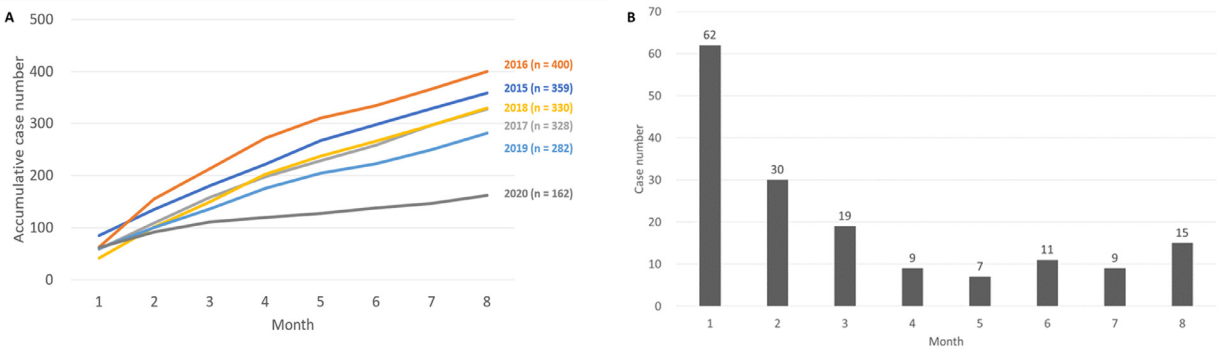

Fig. 1. (A) Accumulative case number from 2015 to 2020, (B) Monthly case number in 2020.

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Kinetics of SARS-CoV-2 specific antibodies (IgM, IgA, IgG) in non-hospitalized patients four months following infection

Dear Editor,

In a recent publication in this journal Yin et al. described the longitudinal anti-SARS-CoV-2 antibody profile and neutralization activity in a single COVID-19 patient.[1] Here we report results that confirm and extend these observations.

Antibody responses of different immunoglobulin classes (IgM, IgA and IgG) against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) have been detected in most infected individuals 1–3 weeks after onset of symptoms.[2,3] Until now, studies on antibody persistence are limited, although such data would be crucial to understand the possible role of humoral immunity in protection against re-infection as well as for immunization strategies.

Recently, we demonstrated anti-SARS-CoV-2-antibody kinetics in a well-characterized cohort of 20 mild to moderately diseased office-based physicians with PCR-confirmed infection, during the first four weeks after symptom onset.[3] Here, we present data on longitudinal profiles of different antibody immunoglobulin classes (IgM, IgA, IgG) using tests with different antigens as well as neutralizing titers in the same cohort four months after symptom onset.

Informed consent was obtained from all study participants. Serum samples, collected three to four weeks after symptom onset were retested in parallel with samples acquired four months post onset of symptoms. We quantitatively assessed IgM antibodies against SARS-CoV-2 nucleocapsid protein (NCP), IgA antibodies against structural protein S1 and IgG antibodies against S1 and NCP using commercial enzyme linked immunosorbent assay (ELISA) kits (EUROIMMUN Medizinische Labordiagnostik AG, Lübeck, Deutschland). Additionally, we performed the Wantai anti-SARS-CoV-2-IgM antibody ELISA (Beijing Wantai Biological Pharmacy Ent, Beijing, China) using the receptor-binding-domain (RBD) as antigen. For all tests the manufacturers' protocols and cutoff values were used. The neutralization assay was performed as described previously.[4] Statistical analyses were performed using GrapPadPrism version 8.0.

As shown in Fig. 1, at week three to four after disease onset, 85% (17/20) of the participants tested positive for RBD-specific IgM, 55% (11/20) for NCP-specific IgM, 100% (20/20) for S1-specific IgA, 95% (19/20) for S1-specific IgG and 85% (17/20) for NCP-specific IgG.

Four months after symptom onset, we observed a decline of RBD-specific IgM and NCP-specific IgM antibody positivity rate to 70% (14/20) and to 5% (1/20), respectively. The detection rate also decreased for S1-specific IgA antibodies to 90% (18/20) and NCP-specific IgG to 60% (12/20), while it remained relatively stable for S1-specific IgG (95%; 19/20).

The median percentage of decrease in antibody concentration was 77.68% for RBD-specific IgM, 80.65% for NCP-specific IgM levels, 68.06% for IgA, 15.52% for S1-specific IgG and 32.11% for NCP-specific IgG. Three patients even showed a slight increase in S1-specific IgG levels.

Neutralizing antibody titers slightly declined but were still detectable in all but two individuals (Fig. 1F). In both the presence of antibodies was confirmed by a total antibody ELISA (Wantai, Beijing, China). One individual initially displayed low levels and only a slight increase in S1-specific IgG antibodies. The other patient only showed IgA antibodies.

With regard to isotype switching of virus-specific B-cells from IgM to IgG antibody production (causing a decline of circulating IgM) and considering that IgA antibodies peak early after the infection, such a decline is not surprising.[5] Our findings are also in accordance with observations made for the first SARS virus, where IgG antibodies persisted in most infected individuals even within 2 years post-infection.[6]

Long and colleagues recently reported a decrease of SARS-CoV-2-specific-IgG antibody levels directed against recombinant antigens containing NCP and an S-peptide in a cohort of 37 symptomatic and 37 asymptomatic patients, and more asymptomatic (40%) than symptomatic individuals (12.9%) tested IgG-seronegative within three months after symptom onset. The decline in neutralizing antibody titers was only moderate and most importantly, still showed positive titers in all individuals.[2]

In accordance with these findings, 40% and 5% of the moderately ill individuals from our cohort respectively tested negative for NCP- and S1-specific IgG antibodies four months after the infection. Furthermore, neutralizing titers declined only slightly and were still detectable in all but two individuals. Although a false-positive PCR result cannot be completely ruled out, the possibility has to be acknowledged that certain individuals only produce very low levels of (neutralizing) antibodies although they clear the infection.

Importantly, our data and those of Long et al.[2] and Yin et al.[1] indicate that kinetics and observed decreases of antibody concentrations are influenced by differences in test sensitivities, especially in relation to the antigenic spectrum antibody responses

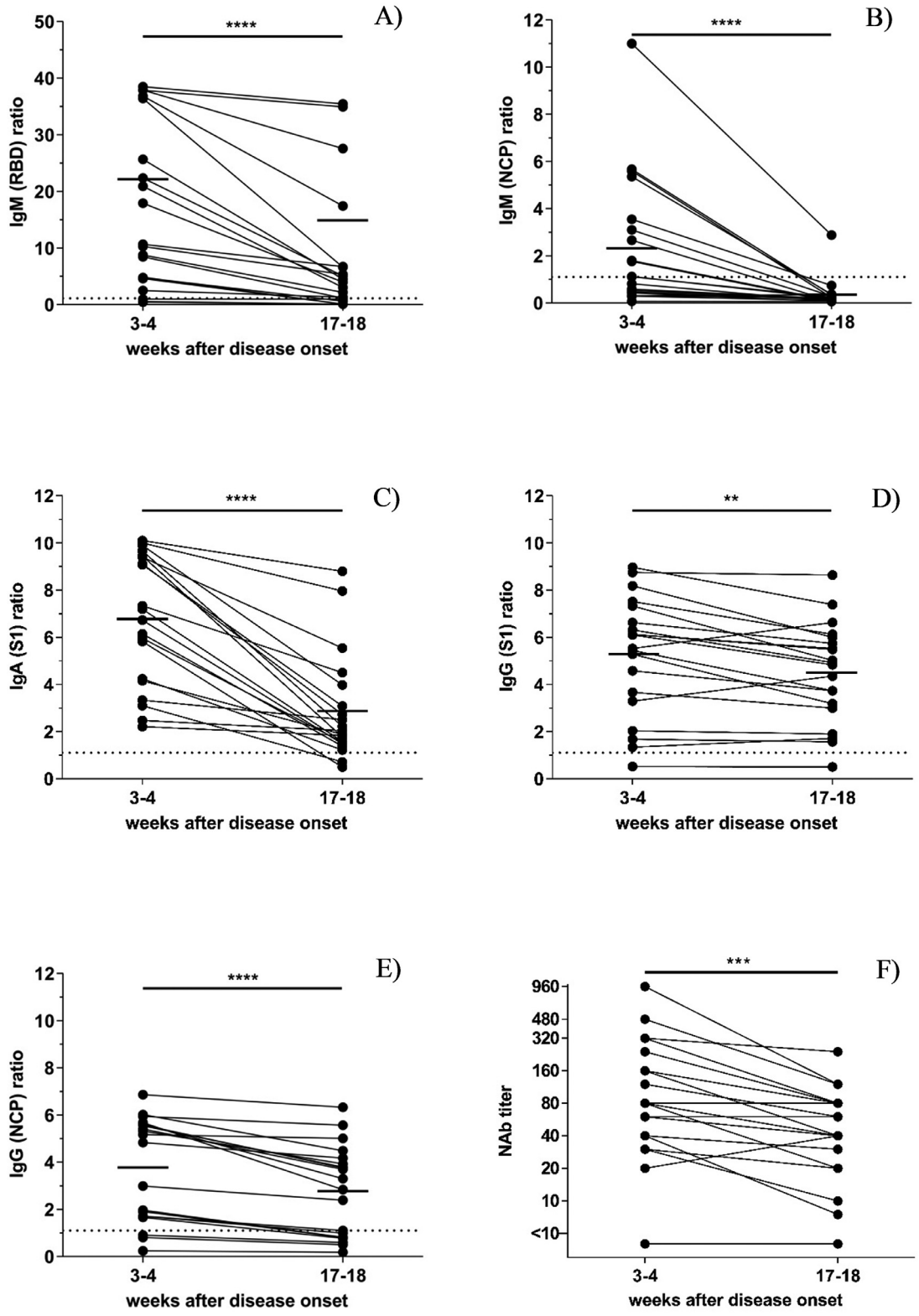

Fig. 1. A-F. Longitudinal profile of SARS-CoV2- IgM, IgA and IgG antibodies against different antigens in 20 patients with a PCR confirmed SARS-CoV2 infection among two time points (week three to four and week 17–18 after disease onset) (A) Receptor-Binding Domain (RBD)-specific IgM (B) Nucleocapsid protein (NCP)-specific IgM (C) S1-specific IgA (D) S1-specific IgG (E) NCP-specific IgG (F) Neutralizing antibody (Nab) titers. The significance was calculated with the Wilcoxon matched-pairs signed rank test using GraphPadPrism version 8.0. The median is shown as a continuous line, the cut-off of the assay as dotted line. *, $p<0.05$; **, $p<0.01$; ***$p<0.001$; ****$p<0.0001$.

are directed to. Furthermore, the final evaluation of the longevity of SARS-CoV-2-specifc antibody responses requires studies with longer follow-up periods.

In summary, our observations indicate that RBD- and NCP-specific IgM as well as S1-specific IgA levels significantly decrease within four months after disease onset, with RBD-specific IgM and S1-specific IgA being still detectable at this time point. Furthermore, we demonstrate a stronger decrease for NCP- than for S1-specific IgG antibodies and neutralizing titers, indicating that the observed durability of SARS-CoV-2 antibody responses strongly depends on the tests used for their assessment. Thus, a single SARS-CoV-2 antibody test should neither be used to exclude or confirm a previous infection nor to extrapolate on the immune status of an individual.

Authors' contributions

DO, AE, LW and JM designed the study. All authors analyzed the data. DO wrote the first draft, LW and JM revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of Competing Interest

We declare no competing interests.

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Regular mass screening for SARS-CoV-2 infection in care homes already affected by COVID-19 outbreaks: Implications of false positive test results

Dear Editor,

We recently reported in the *Journal of Infection* high rates of symptomatic and asymptomatic SARS-CoV-2 infections associated with high fatality in residents across four London care homes experiencing a COVID-19 outbreak during the peak of the COVID-19 pandemic in England.[1] Similar findings were reported in other London care homes.[2] By 11 May 2020, 44% of all London care homes had experienced a COVID-19 outbreak,[3] leading to the national implementation of widespread screening of care home staff every week and of residents every 28 days to identify and isolate infected individuals and limit the spread of SARS-CoV-2 in care homes.[4] In July 2020, Public Health England (PHE) was informed of two asymptomatic staff and one asymptomatic resident in the same care home who tested positive for SARS-CoV-2 through national screening (Table 1). This care home had been part of the initial outbreak investigation in April 2020 and all three individuals were known to have SARS-CoV-2 antibodies.[5] We, therefore, undertook additional investigations to assess whether these were re-infections or false positive results and discuss the implications of our findings for residents, staff and care homes in general.

Following our initial COVID-19 outbreak investigations in April 2020,[3] the four London care homes implemented strict lockdown procedures, with closure to new admissions and cessation of family visits. To prevent re-introduction of SARS-CoV-2 into the care homes, we implemented a local intervention to collect weekly nasal swabs from all residents and staff for four consecutive weeks in mid-May. No new infections were identified. SARS-CoV-2 antibody testing after the 4-week swabbing using the Abbott SARS-CoV-2 IgG assay[6] found two-thirds of residents and staff to be seropositive,[5] consistent with other care home investigations in London.[7] Since July 2020, the four London care homes have been participating in national screening for SARS-CoV-2, whereby swabs for residents and staff are ordered online and sent to one of several national testing centres.[4] Only qualitative results with no RT-PCR cycle threshold (Ct) values or other parameters are reported back to the care home.

Between 22–25 July 2020, three asymptomatic individuals – a care worker, a resident and an office staff member – in one of the London care homes under investigation tested positive for SARS-CoV-2 RNA as part of national screening. These tests were performed in two different national testing centres and on different days. The three individuals had all had a history of COVID-19 like symptoms during March-April 2020, had subsequently repeatedly tested negative for SARS-CoV-2 RNA during the weekly local nasal swab screening during May-June 2020 and were seropositive for SARS-CoV-2 antibodies in June 2020 (Table 1).

The care home immediately re-instituted lockdown procedures. Following the new positive SARS-CoV-2 RT-PCR result, all three individuals were re-tested at the PHE national reference laboratory within 24 h and were RT-PCR negative with detectable SARS-CoV-2 antibodies. Additionally, all residents and staff in the care home – including the three individuals – were re-tested for SARS-CoV-2 RNA as part of the outbreak management and were all negative.

Table 1
. Tests performed in two staff members and a resident who tested positive in the national SARS-CoV-2 infection screening programme for care homes in England.

Staff or resident	Floor in care home	Previous illness	Confirmed COVID-19?	SARS-CoV-2 Swab Results	SARS-CoV-2 IgG antibody Results	New outbreak: date swab result*	Date results available	New outbreak: date repeat swab result	Repeat SARS-CoV-2 antibody test
Staff	1st floor	Yes (March 2020)	No	Negative (04 June)	Positive 2.91 (04 June)	Positive (18 July)	22 July	Negative (24 July)	Positive 1.3 (24 July) Positive 1.1 (24 August)
Resident	Ground	Yes (April 2020l)	Yes	Positive (16 April) Positive (13 May) Negative (28 May) Negative (05 June)	Positive (25 June)	Positive (21 July)	23 July	Negative (24 July)	Positive (24 July) Positive (24 August)
Staff	office	Yes (March 2020)	No	Negative (15 May) Negative (22 May) Negative (01 June) Negative (05 June)	Positive 5.93 (05 June)	Positive (22 July)	25 July	Negative (25 July)	Positive 5.35 (25 July) Positive 5.40 (24 August)

subsequent investigations identified that only one of three RT-PCR targets was positive at the limit of detection of the assay (Ct 34) at the national testing centre.

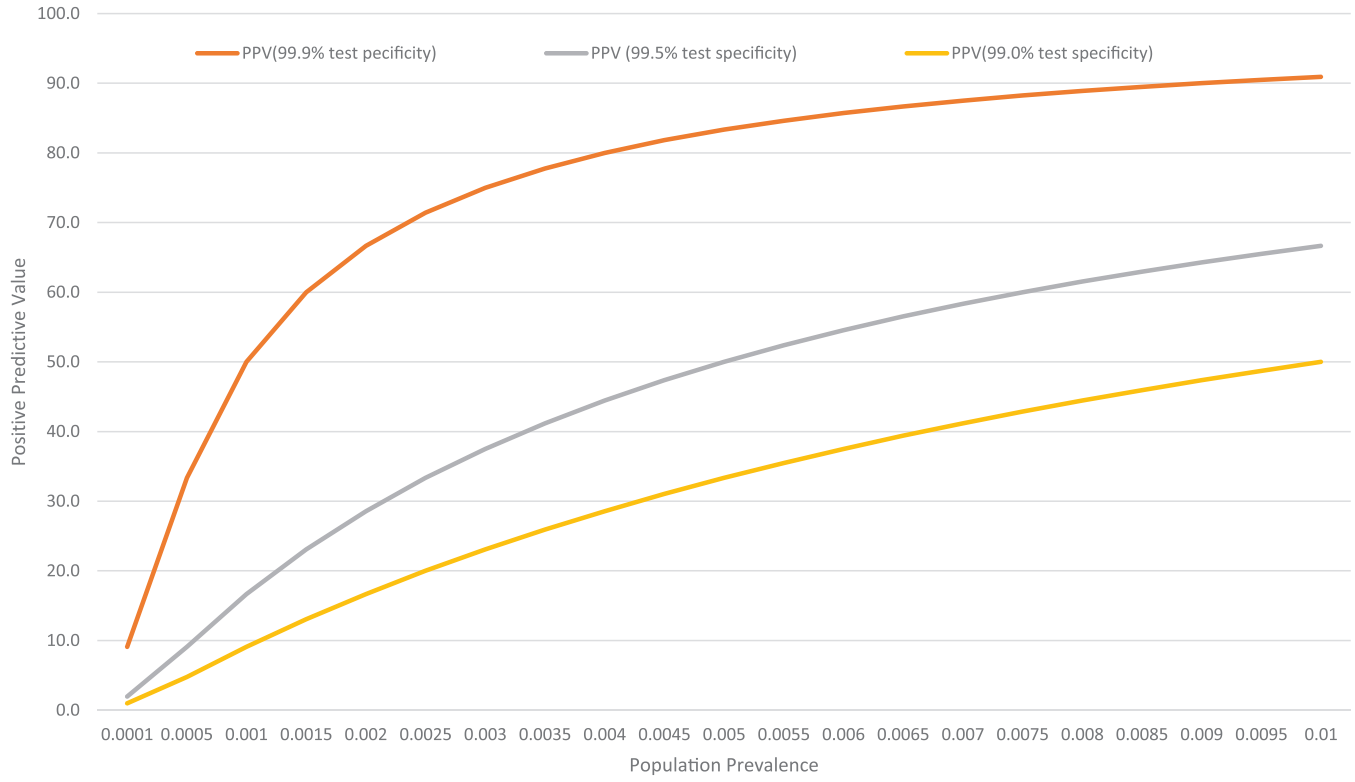

Fig. 1. Positive predictive value (PPV) of testing based on population prevalence and specificity of the test. PPV falls rapidly when prevalence falls below 1% even with the most specific test assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Four weeks later, repeat testing in the two staff showed no rise in SARS-CoV-2 antibodies. The resident was also antibody positive 4 weeks later but the test was performed in a different laboratory which did not report quantitative results.

The protective role of SARS-CoV-2 antibodies against re-infection and disease remains to be established, but there is increasing evidence showing that those with neutralising antibodies are unlikely to be infected with live virus,[8] which in turn reduces their risk of infecting others. Despite the large numbers of ongoing COVID-19 outbreaks in England,[9] these four London care homes did not have any additional cases prior to the national screening programme. The reporting of three positive results in a single care home was, therefore, unexpected and prompted additional investigations, which included repeat swabs that were all negative, and blood sampling which confirmed their seropositivity at the time of re-testing. The lack of an antibody rise four weeks later confirmed that these detections were not new infections and, therefore, false positive screening tests.

Further work needs to be undertaken to assess the value of repeated mass swab testing in care homes during periods of low community prevalence,[10] particularly if SARS-CoV-2 positivity rates fall below 1%, when the likelihood of false positive results increases exponentially even with RT-PCR assays that have very high specificity (Fig. 1). This can have a significant impact on care homes, in terms of unnecessary isolation of vulnerable residents and loss of workforce leading to suboptimal care provision.[11] Declaration of an outbreak places additional constraints on the care home, including closures to external visitors and new residents.[4] Repeated unnecessary interventions are also likely to be detrimental to the long-term mitigation strategy in care homes, have significant resource implications and impact on the wellbeing of residents and staff. In addition, there is the danger of behavioural fa-

tigue so that, when strict infection control measures are required in a genuine outbreak, recommended measures may not be adhered to.

This problem of false positivity has recently been recognised, with new national guidance published on how to interpret low level RT-PCR positive samples, including a recommendation to retest all samples testing that are positive at the level of detection of the assay before undertaking wider public health action.[12] It is hoped that this recommendation will reduce the number of similar closures of care homes or other institutions exposed to mass testing as a result of non-reproducible positive SARS-CoV-2 RT-PCR results.

In conclusion, in care homes that have already experienced a COVID-19 outbreak, up to two-thirds of staff and surviving residents develop neutralising antibodies which is likely to reduce the risk of new infections and, consequently, further outbreaks. Whilst community SARS-CoV-2 prevalence is low, rather than repeated mass swabbing, there is an opportunity to assess a role for wider testing for SARS-CoV-2 antibodies to assess past exposure accompanied with early and rapid testing for SARS-CoV-2 RNA as needed. Any positive result could then initiate wider testing for SARS-CoV-2 RNA in the care home, include re-testing the index case, and a more nuanced risk assessment of the likelihood of a true outbreak.

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

Conflicts of interest: none

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A possible role for GRP78 in cross vaccination against COVID-19

Dear Editor,

We previously reported in this Journal [1] that cell-surface Glucose Regulated Protein 78 (CS-GRP78), also termed heat shock protein A5 (HSPA5), could be a possible route for SARS-CoV-2 internalization. We predicted the binding site on the spike protein of SARS-CoV-2 that can recognize CS-GRP78. A recent communication by Braun and colleagues reported that the spike glycoprotein of the

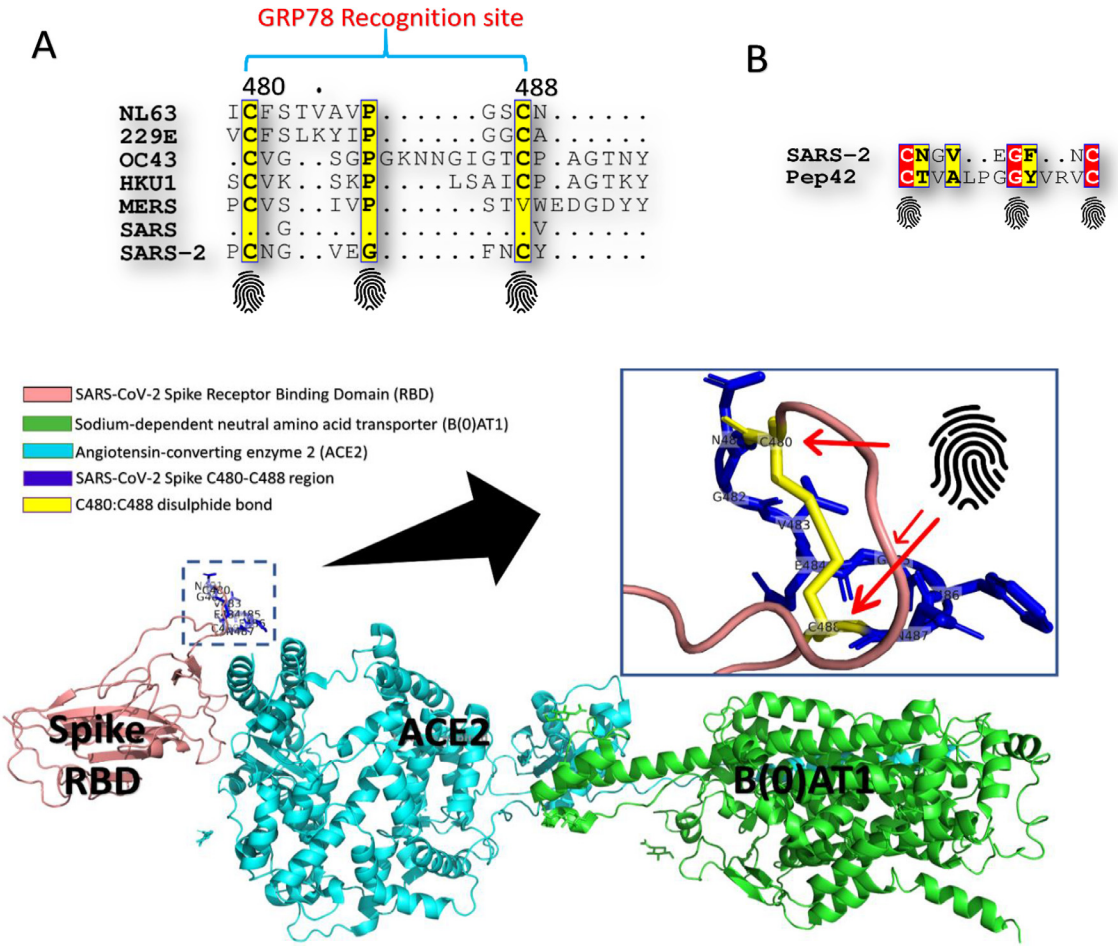

Fig. 1. Coronavirus spike protein and GRP78 recognition site. (A) Part of the multiple sequence alignment for the spike glycoproteins of the seven reported human coronavirus strains (NL63, 229E, OC43, HKU1, MERS-CoV, SARS-CoV, and SARS-CoV-2). Yellow columns are the conserved residues among the seven HCoVs. GRP78 recognition site (C480-C488 in SARS-CoV-2) is marked at the top. (B) Pairwise sequence alignment between Pep42 and SARS-CoV-2 S (C480-C488 region). Red and yellow residues are identical and similar residues, respectively. Fingerprint residues are marked (bottom). (C) The structure of spike protein RBD (rose) bound to ACE2 (cyan) and B(0)AT1 (green) (PDB ID: 6M17) while the GRP78 recognition site (C480-C488 in spike protein RBD) is depicted in the blue sticks in the enlarged panel. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SARS-CoV-2 bears many conserved motifs to the previously determined human coronavirus strains such as HKU1, 229E, NL63, OC43, MERS-CoV, and SARS-CoV.[2] However, we would like to emphasize that using a simple bioinformatics approach can suggest a possible role of the GRP78 in T cell cross immunization against COVID-19. Based on the findings, we can conclude that mild human coronaviruses can be useful as a vaccination against COVID-19.

For SARS-CoV-2, the binding site to CS-GRP78 was predicted to be the nine residues CNGVEGFNC (C480-C488 region of the spike glycoprotein) in the S1 C-terminal domain (the receptor-binding domain (RBD)).[1] This region (C480-C488) of the spike glycoprotein represents the best fit for the 13-residue Pep42 cyclic peptide (CTVALPGGYVRVC), which was reported earlier to be selectively associated with CS-GRP78 in cancer cells.[3]

Fig. 1A shows part of the multiple sequence alignment (MSA) of the seven human coronavirus strains (NL63, 229E, OC43, HKU1, MERS-CoV, SARS-CoV, and SARS-CoV-2). The most conserved residues in SARS-CoV-2 compared to other strains are C480, G485, and C488 (yellow columns). These three residues are fingerprints for CS-GRP78 recognition as they resemble the C1, G8, and C13 in the Pep42 cyclic peptide (red columns in Fig. 1B). These findings are in good agreement with the work of Braun and coworkers,[1] where the GRP78 recognition site that we are reporting here lies in the most conserved S1 C-terminal RBD of the spike glycoprotein.[2] The three fingerprint residues are found in the four mild HCoV strains (NL63, 229E, OC43, and HKU1) and the SARS-CoV-2.

The recognition of the CS-GRP78 by the peptide Pep42 is previously reported to be restricted for the cyclic form (the first and last CYS residues form a disulfide bond).[4,5] Interestingly, the solved structure of the SARS-CoV-2 S (PDB ID: 6M17) shows that the C480-C488 region is cyclic (a disulfide bond is formed between the residues C480 and C488). Fig. 1C shows the solved structure of the SARS-CoV-2 spike protein RBD (rose cartoon) in conjunction with the human receptor Angiotensin-Converting Enzyme 2 (ACE2) (cyan cartoon) and Sodium-dependent neutral amino acid transporter (B(0)AT1) (green cartoon). The enlarged panel shows the CS-GRP78 recognition site (C480-C488) in blue sticks with the yellow sticks representing the two CYS residues with the formed disulfide bond. The fingerprint residues (C480, G485, and C488) are marked with red arrows. As reflected in Fig. 1C, the GRP78 recognition site is surface exposed and protrude apart from the ACE2 binding site.

HADDOCK webserver is utilized to dock the GRP78 into spike protein RBD, and the docking complex is superimposed with the solved structure (PDB ID: 6M17) in Fig. 2A. GRP78 can bind to the C480-C488 region of the spike protein RBD with an excellent HAD-

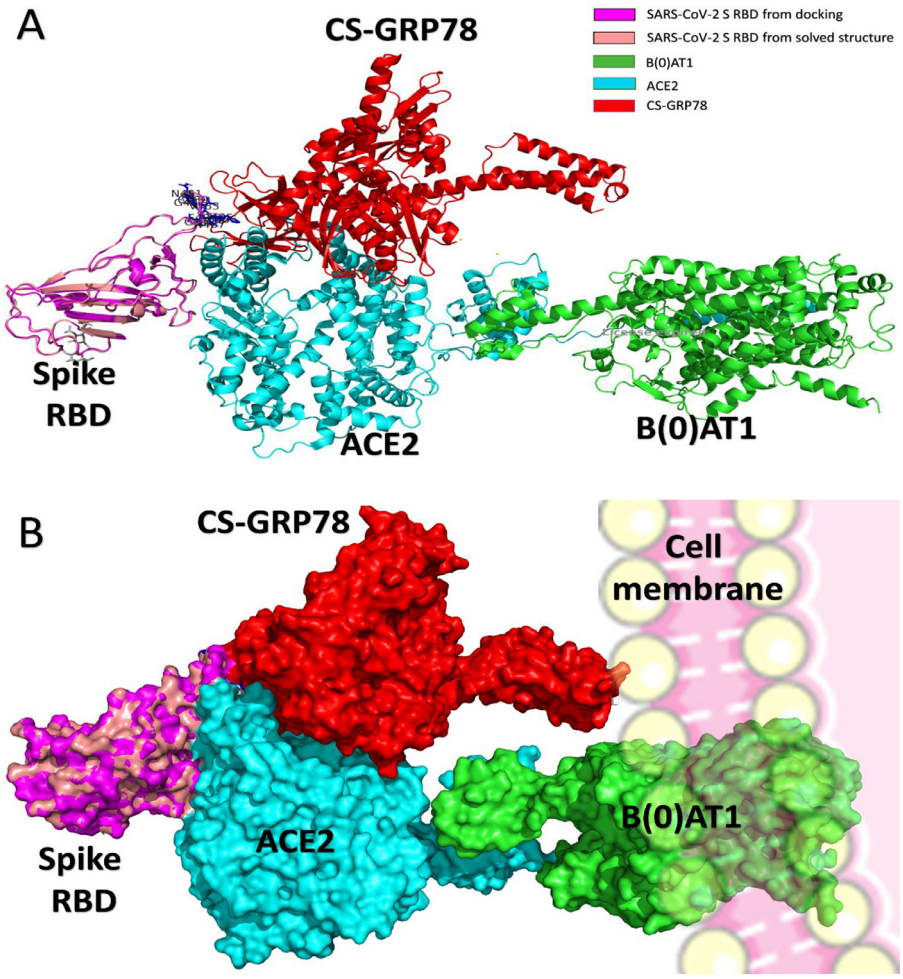

Fig. 2. The docked complex of GRP78 (red) and S RBD (magenta) superimposed to the solved structure (PDB ID: 6M17) containing spike protein RBD (rose), ACE2 (cyan), and B(0)AT1 (green). (A) shows the cartoon representation, while (B) indicates the surface representation. The C480-C488 are labeled with its one-letter code and shown in blue. The membrane is depicted in (B) to show how the CS-GRP78 would look like when binding the spike protein RBD. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DOCK score (-77 ± 3.1). The substrate-binding domain β (SBDβ) was reported to be the possible binding site to SARS-CoV-2 S RBD.[1] Molecular Dynamics Simulation (MDS) (NAMD software) for the SARS-CoV-2 spike protein combined with molecular docking (HADDOCK) revealed the existence of more than four interactions (H-bonds or hydrophobic contacts) between GRP78 and C480-C488 of the SARS-CoV-2 spike protein. At least two hydrophobic contacts are formed in all the docking experiments (seven replicas are used during 100 ns MDS). This is in support of the previous reports about GRP78 recognition of the hydrophobic patches in the unfolded proteins.[6,7]

As shown in the surface representation of the complex of spike protein RBD (rose), GRP78 (red), ACE2 (cyan), and B(0)AT1 (green) in Fig. 2B, the CS-GRP78 to be associate with spike protein RDB and ACE2, should also be associated with a membrane-bound protein to carry it. It was reported that CS-GRP78 was associated with murine tumor cell DnaJ-like protein 1 (MTJ-1) and that this association was essential for GRP78 membrane localization and its binding to the activated α2-macroglobulin.[8,9] MTJ-1 may be the GRP78 carrying transmembrane protein that could be of therapeutic potential against COVID-19 and other viral infections.

Finally, the human coronaviruses NL63, 229E, OC43, and HKU1, had mildly impacted human beings (characterized by mild flu-like symptoms). People previously infected with these strains of human coronaviruses may develop immunity against SARS-CoV-2. The milder human coronaviruses could be used as a vaccine against COVID-19 as they share the same CS-GRP78 recognition region on their spikes.

Author Contributions

A.E. drafted the manuscript and draw figures, I.I. Did the MDS calculations, A.I. drafted the document, and the hypothesis, W.E. revised the manuscript and supervision.

Declaration of Competing Interest

The authors declare that there is no competing interest in this work.

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The implementation of an active surveillance integrating information technology and drive-through coronavirus testing station for suspected COVID-19 cases

To the editor,

We read with great interest the study by Chen et al., which showed high seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) among 105 healthcare workers exposed to four laboratory confirmed coronavirus disease 2019 (COVID-19).[1] This study indicated the possible nosocomial spread of COVID-19.[1] As for August 29, 2020, SARS-CoV-2 causing has involved more than 24 million patients and COVID-19 has been associated with a case fatality rate of 3.4%.[2] Although COVID-19 can present as asymptomatic or mild pneumonia, a significant portion of patients presenting with severe pneumonia or acute respiratory distress syndrome required hospitalization.[3] Because severe cases can carry high viral load and SARS-CoV-2 owns a great ability to infect human, nosocomial transmission of COVID-19 is a serious concern. In addition to appropriate personal protective equipment (PPE), early detection and prompt isolation of patients with SARS-CoV-2 infection is essential to prevent COVID-19 nosocomial spread.

Recently, Lin et al. used information technology to build an inpatient COVID-19 alarm system in active surveillance of suspected COVID-19 cases among inpatients with nonresolving pneumonia after treatment.[4] However, caregivers and HCWs – another population within the hospital at risk of acquiring SARS-CoV-2 infection from nosocomial transmission was missed in this study.[4] By contrast, a cross-sectional study in Singapore reported that a multi-pronged approach including risk-based PPE, staff fever and sickness surveillance, and enhanced medical surveillance of unwell staff was effective in prevent HCW from SARS-CoV-2 infection.[5] To effectively prevent inpatients, caregivers and HCWs from nosocomial spread of SARS-CoV-2 within hospital, we built a "quarantine map" to help clinicians to early detect suspected COVID-19 cases among them using information technology.

Chi Mei medical center is a 1284-bed tertiary care hospital which have 2 buildings and one campus. Three confirmed COVID-19 patients were hospitalized between March and May. Since 2020/02/21, the hospital implemented an active surveillance of suspected COVID-19 cases using information technology. First, all inpatients was screened using information technology system and aimed to identify the patients met the at least two of three criteria: 1) fever, defined as temperature$\geq$ 38 °C; 2) cough, defined as use of antitussive agents; 3) pneumonia, denied as key words of pneumonia pattern in the chest x-ray report. When inpatients had more than 2 of 3 criteria meet, COVID-19 case manager would review the chart. If the patient did not receive PCR test for SARS-CoV-2, the case manager would send a message to the primary care team to remind them to perform the diagnostic test. Otherwise, the primary care team could consult rapid response team infection specialist (R.R.T) to evaluate the patient. All patients would be followed up daily until the patient had receiving test for SARS-CoV-2 or had been assessed by R.R.T. Although inpatients hospitalized in intensive care unit and pediatric patients were excluded because they often had an infection source when admission, the primary care team in these two unit could decide when to perform PCR test. In addition, the HCWs and caregivers should report the body temperature and the symptom of infection then integrate to the surveillance system daily. Once they have fever or infection symptom, they should stop working until the associated symptom resolved for at least 24 h. Any cluster of fever and respiratory symptoms screened among the three groups in the same or neighboring room cared by the same nursing staff within a station by quarantine map was sent for SARS-CoV-2 PCR test immediately (Fig. 1).

To improve the process of collecting clinical specimen for SARS-CoV-2 tests, 2 strategies were developed -"drive-through coronavirus testing station" and "duty resident". Initially, when inpatient needed to receive PCR tests for SARS-CoV-2, he should be transferred to negative pressure ward. A doctor in charge did oropharyngeal or nasopharyngeal swab to collect the specimen. Then the patient should be isolated in the negative pressure ward until the PCR test was negative. Drive-through coronavirus testing station was set up for collecting specimen since March 17, 2020, and inpatients were allowed to return to their wards immediately after receiving the examination. In addition, 36 medical residents were assigned as duty resident, who was responsible to collect the clinical specimen form inpatients of internal medicine department since March 27, 2020, and every shift was daily.

Between March 2, 2020 and May 3, 2020, 948 cases were detected by quarantine map using information technology. After excluding 699 cases who had received PCR test or infection specialist consultation through chart review by infection case manager, 249 cases remained in remind list (Fig. 2). Among them, 93 cases (37%) were in internal medicine department, 55 cases (22%) in surgical department, 63 cases (26%) in pediatrics department, 33 cases (13%) in ICU and 5 (2%) in the other department. After excluding cases in pediatrics department, ICU and the other department, 91 cases (61%) was tested by PCR for SARS-CoV-2. 52 cases (35%) was furtherly consulted by the infection specialist and 15 cases (10%) was cases discharged before tested or consulted among 148 cases in list. Ten inpatients received PCR test after R.R.T 2nd consultation. In the meanwhile, 16 HCWs and 2 caregivers had reported fever and associated respiratory symptoms during the study period. The

Fig. 1. Quarantine map.

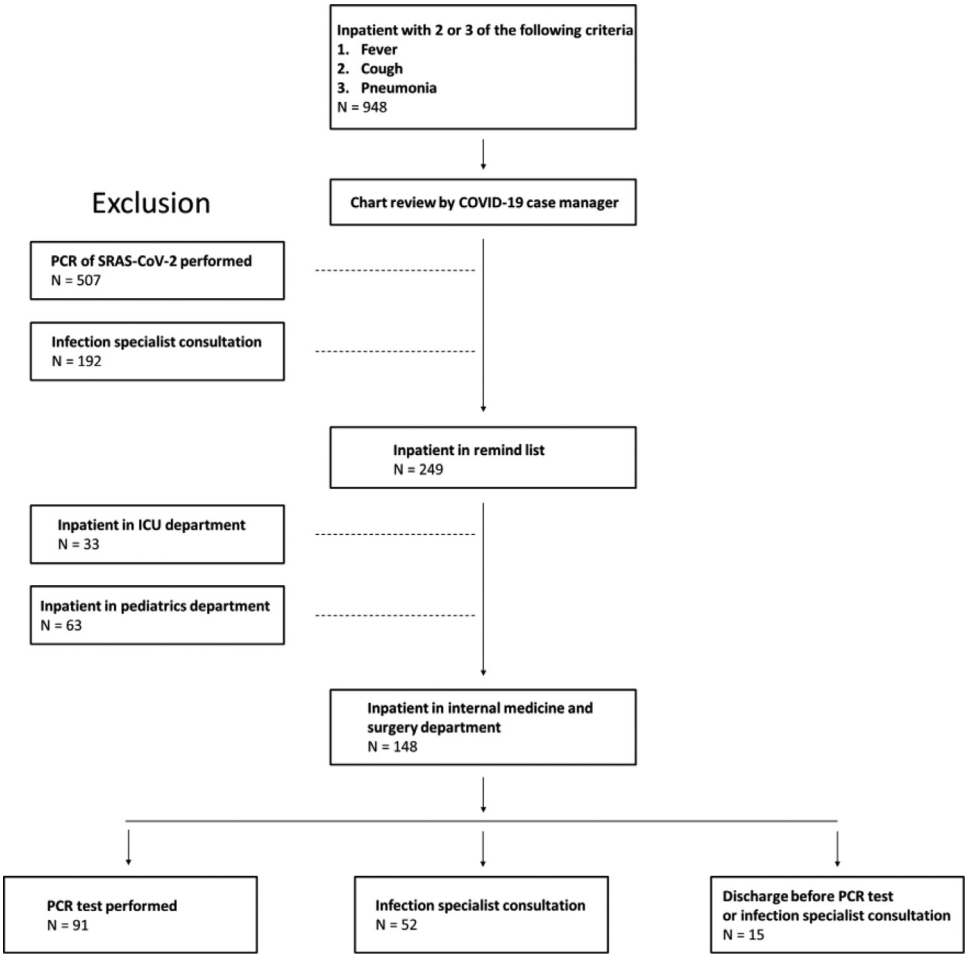

Fig. 2. Study algorithm.

reasons when the cases did not receive PCR test included infection disease other than COVID-19 (urinary tract infection), fever with non-infection disease (autoimmune or hematology disease), lung infiltration on chest x-ray with non-infection disease (pulmonary edema, lung tumor), or suggestion by infection specialist. There was significant change of testing rate after setting "drive-through coronavirus testing station" and a duty internal medicine resident. The weekly testing rate was 48.1% (13/27) initially, and increased to 84.8% (56/66) for internal medicine department. By contrast, the rate was 62.5% (5/8) initially, and decreased to 36.1% (17/47) for surgical department inpatients, which was still low.

Overall, the PCR test of 91 cases were negative for SARS-CoV-2, and no any cluster or outbreak was screened by quarantine map. By the quarantine map, early surveillance and detection of occult

SARS-CoV-2 cases among inpatients, caregivers and RCWs before nosocomial outbreak is extremely important in such a pandemic situation.

Conflict of interest

None.

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Initial MEWS score to predict ICU admission or transfer of hospitalized patients with COVID-19: A retrospective study

Dear Editor,

Early warning scores (EWS) were introduced in early 2001 to identify patients at risk of deterioration in a busy clinical environment as a track-and-trigger system where an increasing score produced an escalated response.[1] EWS have demonstrated better capability in identifying deteriorating patients leading to improved clinical outcomes.[2] Furthermore, an EWS at admission can be used to determine in-hospital mortality and intensive care unit (ICU) transfer.[2–4] Our academic medical center developed a modified early warning score (MEWS) system in 2015 and it was rolled out hospital-wide the following year. Since serious adverse events in hospitalized patients are often preceded by signs of clinical deterioration, we believed MEWS scores could be used to predict events, such as cardiopulmonary arrest. As the coronavirus pandemic continues, could MEWS provide any usefulness in predicting ICU level care among hospitalized COVID-19 patients?

A retrospective study was conducted at the University of Toledo Medical Center (Toledo, OH) on COVID-19 positive patients hospitalized from late March to the end of May 2020. All patients were confirmed for COVID-19 by real-time reverse transcription polymerase chain reaction. Patient demographics, biometrics, and comorbidities were gathered from the electronic medical record. The highest level of hospital disposition was used to create the medical floor and ICU groups. The MEWS scores, which are calculated hourly using the criteria in Fig. 1, were retrieved from an electronic database. Initial MEWS scores were considered either at admission or prior to ICU transfer. Sequential organ failure assessment (SOFA) scores were manually calculated and included as a point of comparison.[5] As a validated scoring system, SOFA scores are used to determine a patient's prognosis and have been considered for use in hospitalized COVID-19 patients.[6]

Overall, 142 COVID-19 positive patients were identified in the hospital during the study period. The baseline characteristics of each group are represented in Table 1. Ninety-eight patients were admitted to the medical floor, while 14 of those patients were subsequently transferred to the ICU. The median (interquartile range [IQR]) initial MEWS score of the 14 ICU transfer patients at admission was 3.5 (5.0), while the median score at ICU transfer was 7.0 (2.0). The median change in score was 3.5 (4.3). The comparative SOFA values were 3.0 (2.5), 7.0 (3.5), and 3.0 (5.3), respectively. Forty-four patients were directly admitted to the ICU.

According to the largest Youden's index, the optimal cutoff value for predicting ICU admission or transfer was a MEWS score of 5. The area under the curve of the receiver operating characteristic (AUC) was 0.935 (95% confidence interval [CI], 0.892–0.979). With regards to SOFA, the score was also 5 with an AUC of 0.924 (95% CI, 0.874−0.973). When the MEWS and SOFA optimal cutoff values are used as predictors of ICU admission or transfer, there is no difference in the two models as assessed by DeLong's test ($Z = -1.061$, p-value $= 0.289$).

The study suggests that MEWS could be used as a surrogate measure alongside other parameters to determine ICU admission or transfer when the SOFA score is unavailable. A downside to using the SOFA score at admission is the requirement for lab values, such as arterial blood gas, creatinine, platelets, and bilirubin for score accuracy. Most of the time these values are absent early in a patient's hospital course. Among COVID-19 patients, MEWS may have another slight advantage over SOFA considering the latter does not take into consideration temperature, respiratory rate, oxygen therapy, and oxygen saturation. We questioned whether these nuances in the MEWS scoring system would be able to detect COVID-19 patients at risk for clinical deterioration especially in the context of 'silent hypoxia'[7]. For example, based on the available information as presented in Tobin et al., these patients would have quite low SOFA scores. Even quick SOFA scoring would not sufficiently capture silent hypoxia considering most patients do not present with altered mental status or tachypnea.[8] In comparison, their MEWS scores would extend well beyond our threshold of 5 considering fever is a prominent feature of COVID-19, use of supplemental oxygen, and oxygen saturation below 90%.

There are several limitations to these study findings, which should be addressed. First, the study suffers from a small sample size from a single institution and should be replicated with a larger cohort of patients. Secondly, the scoring systems mentioned in this study have been developed for different reasons other than

Early Warning Score = Sum of All Points

Points	3	2	1	0	1	2	3
SBP	≤ 90	91-100	101-110	111-219			≥ 220
Temp (°F)	≤ 95		95.1-96.8	96.9-100.4	100.5-102.2	≥ 102.3	
HR	≤ 40		41-50	51-90	91-110	111-130	≥ 131
RR	≤ 8		9-11	12-20		21-24	≥ 25
O_2 Sat	≤ 91 %	92-93 %	94-95 %	≥ 96 %			
FiO2				Room Air		Suppl O_2	
Alertness				Awake, alert			Altered*

* Altered = stuporous, lethargic, obtunded, unresponsive, or comatose

Fig. 1. MEWS scoring system.

Table 1
Baseline characteristics of hospitalized COVID-19 patients.

Variable	Medical floor $N = 84$	ICU $N = 58$	p-value
Mean initial MEWS score, (SD)	1.8 (1.5)	6.3 (2.3)	0.000
Mean initial SOFA score, (SD)	2.3 (1.8)	7.8 (3.2)	0.000
Mean age, years (SD)	61.4 (16.1)	66.2 (14.1)	0.067
Sex, male (%)	47 (56.0)	29 (50.0)	0.598
Race (%)			0.511
White or Caucasian	33 (39.3)	27 (46.6)	
Black or African-American	50 (59.5)	31 (53.4)	
Unknown	1 (1.2)	0 (0.0)	
Mean body mass index, kg/m^2 (SD)	32.0 (8.3)	32.2 (9.4)	0.898
Comorbidities			
Diabetes mellitus (%)	34 (40.5)	32 (55.2)	0.120
Hypertension (%)	54 (64.3)	35 (60.3)	0.764
Coronary artery disease (%)	12 (14.3)	12 (20.7)	0.439
Congestive heart failure (%)	7 (8.3)	11 (19.0)	0.106
Chronic obstructive pulmonary disease (%)	8 (9.5)	12 (20.7)	0.102
Chronic kidney disease (%)	15 (17.9)	13 (22.4)	0.648
Outcomes			
Median length of stay, days [IQR]	6.0 [7.0]	14.0 [10.0]	0.000
In-hospital mortality (%)	5 (6.0)	28 (48.3)	0.000

Definitions of Abbreviations.
SBP: systolic blood pressure; HR: heart rate; RR: respiratory rate; FiO_2: fraction of inspired oxygen; ICU: intensive care unit; MEWS: modified early warning system; SOFA: sequential organ failure assessment; SD: standard deviation; IQR: interquartile range.

predicting hospital disposition. Thirdly, we did not explore other options, such as scoring trends or using the highest score in the preceding 24-h period in our prediction model.

Considering MEWS is a less burdensome scoring system, hospitals should consider adopting a method to calculate MEWS scores on admission with a plan to periodically monitor their patients for increasing scores. Additionally, an optimal cutoff value can aid in decisions to either admit or transfer patients with known or suspected COVID-19 to higher levels of care.

Declarations

Ethics approvals and consent to participate.

The study was approved by the University of Toledo Biomedical Institutional Review Board.

Consent for publication

Not applicable.

Availability of data and materials

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

Author's contributions

WB conceived and designed the study. MR and JM participated in the acquisition of data. WB analyzed the data. WB drafted the manuscript, and WB, NA, BH, and RA revised the manuscript. All authors read and approved the final manuscript. WB is guarantor of this work and had full access to all the data in the study and takes responsibility for its integrity and the accuracy of the data analysis.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Viral transmission in asymptomatic cases of SARS-CoV-2 infection

Dear Editor,

Numerous reports have suggested transmission from asymptomatic SARS-Cov2 patients[1] in the community,[2] within the family[3] or in public places.[4] Most of these reports highlighted the role of pre symptomatic carriers for transmission.[5] Also, the viral load in mild and asymptomatic patients seems similar to that found in symptomatic patients.[6]

Asymptomatic patients are sources for transmission[7]; however the main transmission routes remain undefined.

In this prospective work, we wanted to evaluate the daily viral spreading and environmental contamination around patients infected by SARS-CoV-2 and without any respiratory symptoms.

Between April 10, 2020 and May 10, 2020, patients hospitalized in our Rehabilitation Care ward, tested positive for COVID-19, without any respiratory symptoms at diagnosis and having given their prior consent were included. The exclusion criteria included any patient with influenza-like illness or any respiratory symptoms with or without fever in the last month prior to inclusion.

Daily and over a period of 8 consecutive days, we sampled early in the morning and before any cleaning 3 surfaces (mobile phone, doorbell, and patient over bed table) and we collected the surgical masks (175 mm x 95 mm, 3 layers, model PLANETYPE) worn 30 min by the patients during the clinical examination (patients were prompted to talk and answer questions.). In case of symptoms onset we stopped the follow-up.

The swabbing of the surfaces was carried out by the same operator using a pre-impregnated e-swab (reference ESWABR1,COPAN)..

Each mask was cut over an area of 5 cm^2 facing the patient's mouth/nose. The internal face was collected and then vortexed for 30 s at maximum speed with 3 ml of Phosphate-Buffered-Saline (PBS). The process was performed by the same operator for all samples. The analysis was then carried out from 200 µL of the recovered sample.

RNA extraction was performed on a NucliSENS® easyMAG® (Biomerieux, Marcy-l'Etoile,France) device, following strictly the manufacturer's recommendations. RT-PCR was performed on a ABI QuantStudio 7 (Thermofisher) device, using the commercial RealStar® SARS-CoV-2 RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) test. Briefly, 10 µL of RNA is added to the 20 µL RT-PCR mix. Two targets are detected, one specific for betacoronavirus, and one specific for the SARS-COV-2 strains. Internal Control was added in the lysis buffer to validate both extraction and amplification steps.

Ten consecutive patients (P1 to P10) were included in the study period (Table 1). The median threshold cycle (Ct) value of the RT-PCR performed at diagnosis was 16 (13–33). Three patients were transferred within 2 days of completion. Only one patient (P9) became symptomatic with coughing at day 5 after inclusion. The most frequent contaminated samples were the phone and over bed table in 44% of cases whereas masks and ring bell were respectively contaminated in 38% and 28% of cases. Except for one patient (P8), the viral spreading through the mask was high in the first few days after diagnosis and decreased in the following days (Fig. 1). Also, environment of 1 asymptomatic patient (P8) was contaminated for a longer period of time compared to the other.

The median Ct thresholds for different positive samples was respectively 34, 34, 31.5 36 for masks, phone ring bell and over bed table (Fig. 1).

Our work on a small number of patients suggests despite a high viral load at diagnosis, an overall low viral spreading and environmental contamination, during a period of 8-days of follow up. Asymptomatic patients seems to contaminate more frequently the surfaces compared to their masks in view of the fact that sampled masks were rarely contaminated beyond the third day of follow up. Also, the thresholds cycles obtained from different samples suggest the presence of low viral concentrations. Unlike previous studies, we tried to focus on strictly asymptomatic patients. Indeed, except one patient (P9) all remained asymptomatic during the follow-up.

According to recent data, there is more evidence of transmission from individuals in the incubation period that could occur as early as five days before the onset of symptoms.[8] However, recent study suggests a high risk of transmission from asymptomatic in-

Table 1
Patients characteristics.

	Age (years)	Gender	Date of admission	Date of diagnosis	Respiratory signs within the last 21 days	Sample condition	Clinical signs at diagnosis	Ct threshold on respiratory samples	Clinical signs during the 3 weeks of follow up	% of days with at least one positive environmental sample	Nb of days with at least one positive mask sample
P-1	64	Female	20/03/2020	14/04/2020	No	Case contact	No	19	No	12.5%	1
P-2	61	Female	03/12/2019	15/04/2020	No	Case contact	No	16	No	50%	2
P-3	46	Male	11/02/2020	14/04/2020	No	Case contact	No	17	No	12.5%	1
P-4	68	Female	06/04/2020	14/04/2020	No	Case contact	No	33	No	0%	0
P-5	66	Female	29/01/2020	14/04/2020	No	Case contact	No	16	No		
P-6	48	Female	27/12/2018	15/04/2020	No	Case contact	Headache	14	No		
P-7	75	Female	15/01/2020	15/04/2020	No	Case contact	No	13	No		
P-8	73	Male	01/04/2020	21/04/2020	No	Case contact	No	19	No	100%	6
P-9	61	Male	07/02/2020	24/04/2020	No	Case contact	No	13	Cough and diarrhea (Day-5)	100%	0
P-10	81	Female	09/04/2020	29/04/2020	No	Case contact	No	13	No	14%	3

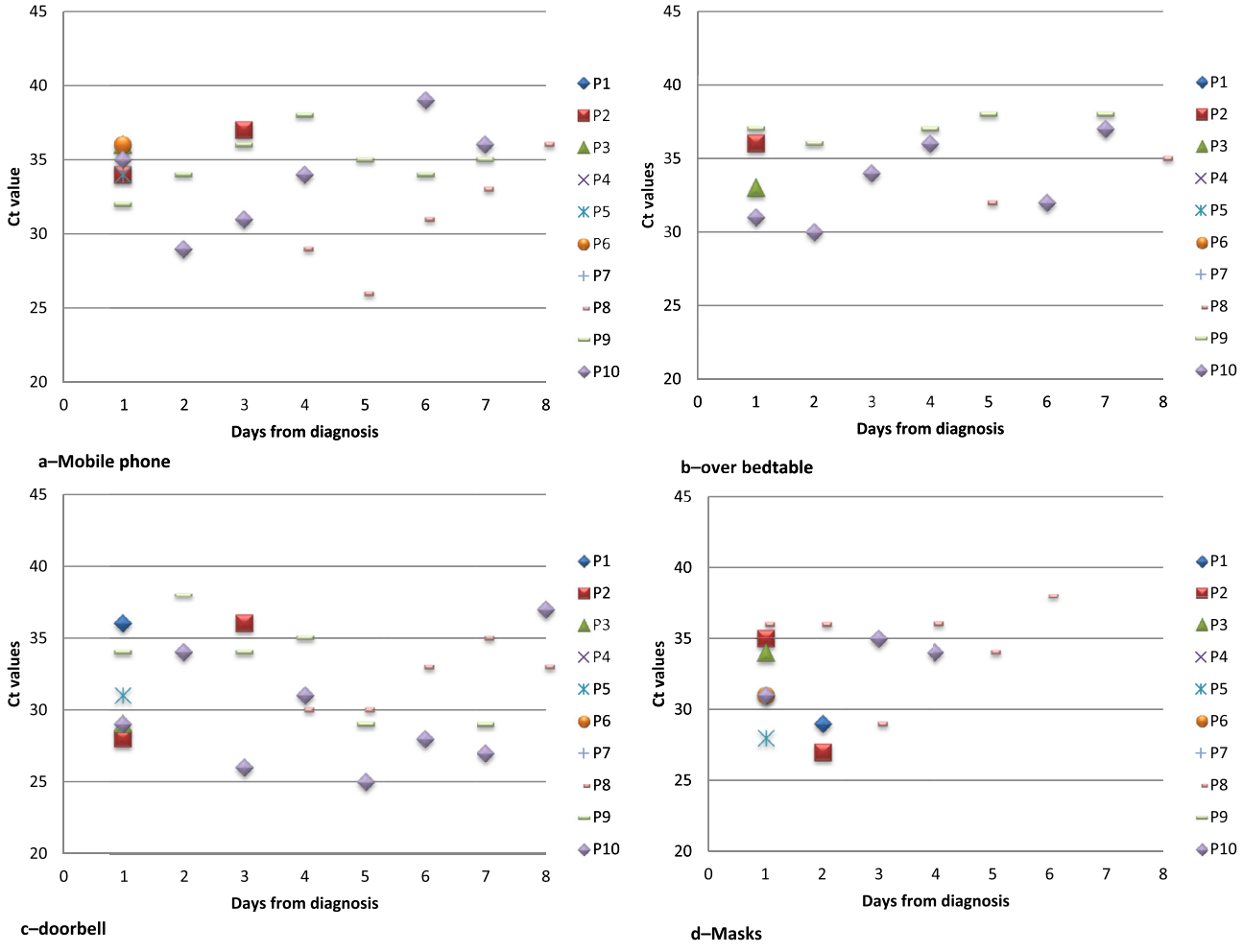

Fig. 1. CT threshold for different environmental samples during the 8 days of monitoring.

dividuals in the community and nursing homes[9] Also rare are the reports that suggests transmission from asymptomatic patients in the hospital setting. Indeed, asymptomatic carrier state can be further divided into asymptomatic infection and pre-symptomatic infection after extended follow-up based on the nature of disease progression. In this context, we could consider our (P9) patient as a pre symptomatic one. To our knowledge, the transmissibility of asymptomatic cases has not been quantified and the main transmission routes remain as yet undetermined. Recent authors concluded that the relative transmissibility from asymptomatic case could be significantly smaller than that of symptomatic cases.[10]

Knowing whether asymptomatic patients are at risk of transmission in the hospital setting is of major importance, as far as prevention measures are concerned. Indeed, as up to 18% of patients infected with SARS-CoV-2 are asymptomatic[4] and the diagnosis cannot be ruled out on the basis of either PCR or CT scan results or both.[11] It is therefore crucial to address the issue of the risk of transmission in the hospital setting from asymptomatic patients. Regarding our quantitative and qualitative results, it suggest a higher risk related to environmental contamination.

Our study has several limitations. Firstly, we cannot exclude that our included patients were infected before the inclusion date, however they were enrolled first day after a contact with an recent known case index. Secondly, our results are based on RT-PCR tests that only imply the potential infectivity and we were unable to analyze the live virus isolation. Thirdly, it would have been important to study the daily throat viral load in parallel to ensure that there was no correlation with environmental contamination. Finally, our study is monocentric with a low number of cases which may limit the extent of the message.

In conclusion, data on strictly asymptomatic carriers acting as potential viral transmitters in the hospital are scarce. We need larger studies involving a greater number of patients in order to better define the transmission routes in this specific population.

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High rates of SARS-CoV-2 seropositivity in nursing home residents

To the editor,

Nursing home residents have high morbidity and mortality due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[1] Spread of the virus within nursing homes has been estimated in point prevalence surveys using real time reverse transcriptase polymerase-chain reaction (RT-PCR) and naso/oropharyngeal swabs. These surveys have revealed high rates of SARS-CoV-2 infection in residents, which is frequently asymptomatic or presents with atypical symptoms.[2,3] However, the true incidence of infection and the extent of possible future protection from re-infection in this group is unclear.

During March-April 2020 we investigated outbreaks in four UK nursing homes where 40% of 394 residents tested positive on RT-PCR, of whom 43% had no identifiable symptoms in the preceding two week period.[3] The first COVID-19 case was confirmed on 25 March, with the final new case on 17 April. Part of the initial control strategy was to implement RT-PCR testing for all residents, with re-testing one week later in those testing negative; this was completed on 23 April. Ongoing infection prevention and control included strict training and adherence to personal protective equipment (PPE) wearing, and weekly resident RT-PCR testing from mid-May. Testing was carried out with the aim of detecting new infections in staff or residents early, so that they could be immediately isolated for 14 days.

The sensitivity of RT-PCR is imperfect and ascertainment is highly dependent on the timing of testing in relation to the onset of infection. This limits the development of appropriate strategies for preventing further outbreaks. Serological assessment provides additional retrospective information to assess the extent of a COVID-19 outbreak in institutional settings.

Here we report SARS-CoV-2 seroprevalence in the same four nursing homes using assays for IgG antibodies.[4] Testing was performed as part of an outbreak investigation with Public Health England and verbal consent obtained from residents (or their relative/friend as appropriate) who had a RT-PCR result available. Serum samples were collected in June 2020 and analysed using the Abbott Architect nucleocapsid IgG assay. Samples with binding ratios near to the cut-off were confirmed on an in-house receptor

Table 1
Rt-PCR status during COVID-19 outbreak and subsequent SARS-CoV-2 IgG serology

	RT-PCR status		
	Positive	Negative	***Total***
Antibody status			
Positive, N (%)	87 (92.6%)	86 (58.5%)	***173 (71.8%)***
Negative , N (%)	7 (7.44%)	61 (41.5%)	***68 (28.2%)***
Total , N (%)	***94 (100%)***	***147 (100%)***	***241 (100%)***

binding domain double antigen bridging assay to determine final status.

Seventy two percent of nursing home residents (95% CI 66 – 77) were anti-SARS-CoV-2 IgG antibody positive, representing 173 of 241 residents available and consenting to testing. Of residents who had previously tested positive by RT-PCR, 93% had developed antibodies (95% CI 85 – 96; 87 of 94) and 59% of those who were previously RT-PCR negative were antibody positive (95% CI 50 – 66, 86 of 147; see Table 1). 35% of antibody positive residents (95% CI 29 – 43, 62 of 173) had been asymptomatic in the two-week ascertainment window prior to PCR testing during the outbreak. Seropositivity was not associated with the presence of comorbidities ($\chi 2$ P=0.81).

These results demonstrate that COVID-19 infection was considerably more widespread within the nursing homes studied (72% of residents) than estimated by serial point prevalence surveys using oropharyngeal and nasal swabs during the acute outbreak (40%). The estimate is also far in excess of data from the UK Office for National Statistics, who have estimated a 20% infection rate in care homes with at least one COVID-19 case, albeit based on RT-PCR testing results.[5] In contrast, the 72% figure is comparable to that found in a PHE investigation in a mixed group of residential and nursing homes (the 'Easter Six'). This work, pending peer-reviewed publication, found 151 of 186 (81.2%) care home residents were seropositive and that these antibodies were neutralising in 89% of cases.[6]

The discrepancy between RT-PCR and serological results likely reflects the delay in initiating point prevalence RT-PCR surveys at the start of the outbreaks we studied. Clinicians first suspected an outbreak on 13 March but widespread RT-PCR testing was not available until mid-April 2020. Given the high rates of atypical or asymptomatic infection in this population, it is likely that a substantial proportion of patients had already been infected and lost PCR-positivity prior to our first RT-PCR survey. In addition, the sensitivity of RT-PCR testing in nursing homes may be limited as some patients have difficulty co-operating with the swabbing procedure.

A key question is whether the presence of SARS-CoV-2 antibodies directed at neucleoprotein are indicative of protection against re-infection. Early evidence suggests that this may be the case. Of the Easter six care home residents 89% with IgG seropositivity to neucloeoprotein antigen also had neutralising antibodies,[6] and a preprint from Addetia et al. suggests that neutralizing antibodies correlate with protection from SARS-1 CoV-2 in humans during a fishing vessel outbreak with high attack rate.[7] With this caveat, our findings of high antibody prevalence are reassuring. Residents and staff who were previously exposed and antibody positive may be protected against re-infection and contribute to herd immunity, protecting antibody-negative residents through a reduction in virus introduction or transmission.

In summary, we provide the first description of SARS-CoV-2 antibody prevalence in a large, high-dependency nursing home population which experienced a COVID-19 outbreak during the peak of the epidemic in April 2020. The results indicate that spread within the home was more extensive than previously estimated using a combination of classic symptoms and positive PCR tests. In advance of possible further waves of infection, there is an urgent need to determine whether seropositivity to the nucleocapsid or alternative viral antigen is an indicator of clinically meaningful protection from reinfection in the nursing home population.

Table 1. SARS-CoV-2 RT PCR results in nursing home residents taken using oro/naso-pharngeal swabbing during outbreaks in April 2020, and SARS-CoV-2 nucleocapsid IgG serology in these individuals in June 2020.

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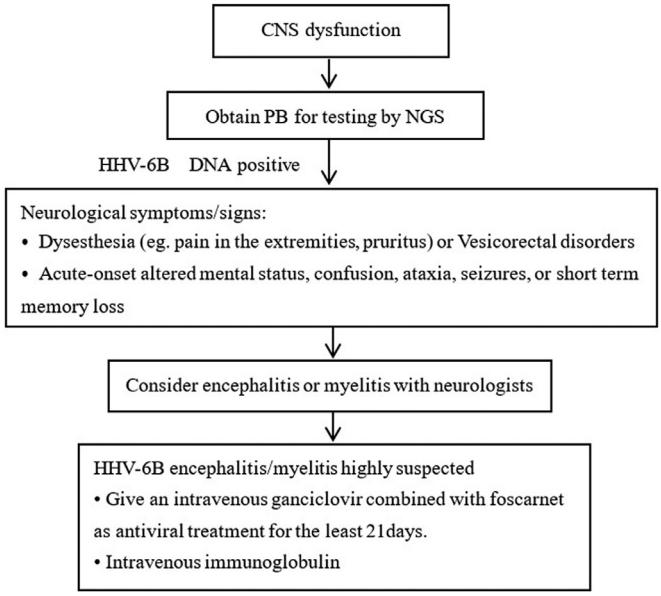

Fig. 1. Flowchart of the management of CNS dysfunction. CNS: central nervous system; PB: peripheral blood; NGS: next-generation sequencing.

Can we distinguishing HHV-6B encephalitis/myelitis in the early phase of cord blood transplantation by next-generation sequencing of peripheral blood?

Dear Editor,

Most studies emphasized cerebrospinal fluid (CSF) analysis was the main contributor to the human herpes virus-6 (HHV-6) B encephalitis/myelitis diagnosis.[1] Thus, little is known identifying HHV-6 encephalitis/myelitis without CSF samples. Here, we present the management of 17 highly suspected cases of HHV-6B encephalitis/myelitis post-cord blood transplantation (CBT) by next-generation sequencing (NGS) in peripheral blood (PB) samples. We report the characteristics and a favorable outcome by an early intervention. Transplant physicians should recognize neurological symptoms in early post transplantation as possible signs of HHV-6B encephalitis/myelitis, and should be prompted to decide on immediate intervention for early posttransplant neurological symptoms in combination with the detection of HHV-6B DNA in the PB by NGS.

From July 2017 until July 2019, thirty-eight patients (age range 1–60 years; male, 43.8%) among 389 allogeneic CBT recipients presented with neurological symptoms within 100 days after CBT were recruited. The patients obtain PB for testing by NGS. Informed consent was obtained in all cases in accordance with the Declaration of Helsinki. Patients received the combination of cyclosporine A (CsA) and mycophenolate mofetil (MMF) dual-agent prophylaxis for GVHD, and an intravenous acyclovir as antiviral prophylaxis. Next generation sequencing was performed at the Wuhan BGI Clinical Laboratory, the peripheral blood specimens were collected according standard procedures. The detected reads number was calculated after normalizing the total sequencing reads number to 20 M.

In our series, HHV-6 DNA was detected in 17 blood samples. Primary diseases included acute myeloid leukemia (AML) ($n=7$), acute lymphoblastic leukemia (ALL) ($n=6$), aplastic anemia (AA) ($n=3$), and paroxysmal nocturnal hemoglobinuria (PNH) ($n=1$). One patient received a second allogeneic transplantation. The typical features of 7 patients are a similar array of symptoms notable for acute-onset altered mental status, paresthesia, confusion, ataxia, seizures, or short term memory loss. Ten patients show dysesthesia (eg. pain in the extremities, pruritus), vesicorectal disorders, or superficial dysesthesia at the segmental levels of the spinal cord. The median onset of engraftment timing were 19.8 (range from +11 to +37) days after cord blood infusion, which CNS dysfunction were 28.4 days. A total of 23 episodes of CNS disorders were documented, 3 episodes were documented before neutrophil engraftment, while 20 episodes were after engraftment. Prior to the onset of CNS dysfunction, methylprednisolone ($\geq$1 mg/kg) immunosuppressive treatments had commenced for 14 patients against pre-engraftment syndrome (PES) or graft versus host disease (GVHD).

We highly suspected that these patients developed HHV-6B encephalitis/myelitis, and an intravenous ganciclovir combined with foscarnet were given as antiviral treatment for the least 21days, and intravenous immunoglobulin. Only one patient died of HHV-6B encephalitis, 3 patients showed improvement but died because of pulmonary infection, 1 patients died of disease progression, 2 was left with lingering neurological compromise, and the last 10 appeared to make a full recovery. The flowchart are presented in Fig. 1.

Twelve of 17 (70.6%) blood samples had at least 2 (range, 2–8 sites) pathogens. CMV was the most commonly identified mixed-infection pathogens ($n=7$), followed by gram- positive bacteria ($n=6$), gram-negative bacteria ($n=5$), and HSV-1 ($n=2$). Median number of reads of HHV6B were 1420 (range, 7 to 10,586) in PB, while HHV-6A virus was 8 (range, 3 to 27). The etiology type were listed in Table 1.

Our study presented the use of NGS as an adjunct for the diagnosis of HHV-6B encephalitis/myelitis. NGS has the potential to identify any pathogens in PB sample thus assisting with precise diagnosis and avoiding multiple conventional tests. In our study, the diagnosis of HHV-6B encephalitis/myelitis was based on the presence of neurological symptoms/signs consistent with encephalitis or myelitis in combination with the detection of HHV-6B DNA in the PB by NGS. Previous studies provided supporting evidence that HHV-6 DNA in blood is an important condition for diagnosing HHV-6 encephalitis/myelitis.[2–4] PCR assay was used in previous studies, which has been the gold standard of HHV-6B virus

Table 1
The etiology type of blood.

	Viruses (reads)					Fungus (reads)		Bacteria	
	HHV-6B	HHV-6A	CMV	BK	HSV-1	Candida	aspergillus	G+	G-
Sample 1	284	–	1	–	–	–	–	–	–
Sample 2	10,586	27	204	–	–	–	–	+	+
Sample 3	13	–	–	–	–	–	–	+	+
Sample 4	1680	3	–	–	48	–	–	–	–
Sample 5	3410	3	683	–	–	12	15	–	–
Sample 6	18	–	–	–	–	–	–	–	–
Sample 7	203	–	–	–	–	–	–	–	–
Sample 8	987	–	–	8	–	–	–	–	–
Sample 9	1000	–	–	–	–	–	–	–	–
Sample10	52	–	–	–	–	–	–	+	+
Sample 11	141	–	102	–	–	–	–	–	–
Sample 12	40	–	12	1	–	–	–	–	–
Sample 13	3548	3	166	–	–	–	–	+	+
Sample 14	1944	3	–	–	–	–	–	–	–
Sample 15	8	–	104	–	–	–	–	+	+
Sample 16	225	–	–	–	–	–	–	–	–
Sample 17	7	–	–	–	52	–	–	+	–

Abbreviation: HHV-6, human herpes virus-6; CMV, cytomegalovirus; BK, BK polyomavirus; HSV-1, human herpes virus-1; G+, gram positive bacteria; G-, gram negative bacteria; +, positive; –, negative.

replication diagnosis and is usually recommended for diagnosis of HHV-6B infection.[5–7]

However, NGS given the difficulty distinguishing between reinfection (exogenous) and reactivation of latent virus (endogenous). When detected HHV-6B DNA in PB, donor- or recipient-derived chromosomally integrated HHV-6 (CIHHV-6) since latently-integrated viral DNA cannot be distinguished from replicating virus DNA. On the other hand, there was HSV-1 detected in 2 samples as very aggressive virus for CNS. So, the diagnostic specificity is high but not 100%. Five PB samples were also detected of HHV-6A DNA, which number of reads were much lower than for HHV-6B. The chromosomally integrated (CI) HHV-6A was highly suspected, which can be confirmed by evidence of one copy of viral DNA/cellular genome, or viral DNA in hair follicles/nails, or by FISH demonstrating HHV-6 integrated into a human chromosome.

Transplant physicians should be prompted to decide on immediate intervention for early posttransplant neurological symptoms in combination with the detection of HHV-6B DNA in the PB by NGS.

Ethics approval and consent to participate

Ethical approval to undertake this study was examined from the Research Ethics Committee. A written informed consent for publication was obtained from the patient.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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COVID-19 patients display distinct SARS-CoV-2 specific T-cell responses according to disease severity

To the editor,

SARS-CoV-2 virus induces symptoms of variable severity; some patients only have mild illness whereas other rapidly become critically ill progressing to an acute respiratory distress syndrome (ARDS). This critical state of the disease supports the immediate relevance for the development of protective therapeutics against SARS-CoV-2 but requires fundamental knowledge concerning adaptive immune responses induced by the virus. Therefore we have read with interest the recent findings published by Bo and colleagues describing in the early stage of the disease a positive correlation between T-cells decrease and COVID-19 severity.[1] However, Thijsen and colleagues demonstrated the presence of specific T-cell responses against S and N proteins few days post onset symptoms in patients with severe pneumonia hospitalized in intensive care unit (ICU).[2] Although the existence of SARS-CoV-2 specific T-cells has been described,[2,3] the frequency and the intensity of SARS-CoV-2 specific T-cell responses among mild illness and severe pneumonia convalescent COVID-19 patients remains to be investigated.

In this prospective study, 60 patients who had COVID-19 were enrolled in a two cohorts study that were entitled mild illness ($n=30$) and severe pneumonia ($n=30$) at least 21 days after the first symptoms of COVID-19 (38 days [21–53]; Table 1). The aim was to describe adaptive immune responses in COVID-19 convalescent patients according to their disease severity. All patients were considered as cured at the time of inclusion. Patients' characteristics are detailed in Table 1. The mean age was 54.1 +/- 17.4, and 30 patients (50.0%) were male. Patients with severe pneumonia had more diabetes (20.0% vs 0.0%, $P<0.001$), hypertension (47.0% vs 6.7%, $P<0.001$), higher body mass index (24.1 vs 20.5, $P<0.001$), they were older (68.3 vs 39.8, $P<0.001$) and more frequently male (77.0% vs 23.0%) $P<0,001$) as previously described.[4] Interestingly, patients with severe pneumonia had also more fever (86.7% vs 43.3%, $P<0.001$), dyspnea (86.7% vs 6.7% $P<0.001$) and less myalgia (16.7% vs 60.0%, $P<0.001$). Among patients who presented severe pneumonia, 23 (76.7%) were hospitalized in ICU for acute respiratory distress related to COVID-19. No patient died during the study period. The identification of SARS-CoV-2 specific T-cell responses was performed using *ex vivo* ELISpot assays that measure IFN_γ produced by activated T-cells (Fig. 1A). Median intensities for CoV-N T-cells responses were higher ($P=0.006$) and the one of CoV-S ($P=0.056$) and CoV-M ($P=0.29$) tends to be increased in patients with mild illness compared to patients with severe pneumonia (73.0 IFN_γ spot/3×10^5 cells [IQR: 39.0–595.0] vs 33.5 IFN_γ spot/3×10^5 cells [IQR: 20.5–75.2]; 108.5 IFN_γ spot/3×10^5 cells [IQR: 46.0–669.0] vs 50.0 IFN_γ spot/3×10^5 cells [IQR: 27.4–100.1] and 150.5 IFN_γ spot/3×10^5 cells [IQR: 43.0–591.8] vs 66.5 IFN_γ spot/3×10^5 cells [IQR: 94.0–178.0] for CoV-N, CoV-S and CoV-M respectively; Fig. 1B). Additionally, T-cells' specificity was analyzed based on IFN_γ secretion using flow cytometry in thirteen patients from both cohorts ($n=6$ mild illness patients and $n=7$ severe pneumonia patients). Specific T-cell responses against S, M and N proteins were mediated by CD8 and CD4T-cells in both cohorts (data not shown). The presence of SARS-CoV-2 specific T-cells in healthy donors (33% for CoV-S and M and 14% for CoV-N) might be explained by the sequence homology between structural proteins from various coronavirus suggesting the existence of cross reactive memory T-cells.[5] Indeed, high degrees of similarities between coronaviruses and SARS-CoV-2 concerning S, M and N structural proteins have been recently described.[6,7]

Beyond specific cellular responses, typical humoral responses to acute viral infection are wildly induced in COVID-19 patients.[8] Zhao et al. showed that the seroconversion rate and antibody levels increased rapidly during the first two weeks with a cumulative seropositive rate of 50.0% on the 11th-day and 100% on the 39th-day. High titers of IgG antibodies detected by Enzyme immunoassays have been shown to positively correlate with neutralizing antibodies. [8,9] In this study, the median serology index of severe pneumonia patients was equal to 7.19 S/CO [IQR: 6.1–8.28] and was significantly higher than the one of mild illness patients that was equal 4.92 S/CO [IQR: 2.52–7.32], ($P<0.001$) (**Table 1**). A heatmap was then generated, using the online Morpheus software (https://software.broadinstitute.org/morpheus/), to correlate all immunological parameters investigated (serology index and T-cell immune responses to SARS-CoV-S, M and N proteins) (Fig. 1C). We observed that all patients with severe pneumonia had a positive serology index and most of them had at least one specific cellular response for SARS-CoV-2 proteins (28 out of 30). In contrast, patients with mild illness had less specific cellular responses (20 out of 29) than severe pneumonia patients ($P=0.0211$) (Fig. 1D). Among mild illness patients, three had a negative serology index (Fig. 1C). Specific T-cell responses for S, M and N proteins were simultaneously shown for 70.0% of severe pneumonia patients while only for 37.9% of mild illness patients ($P=0.0191$) (Fig. 1E). Of note, levels of T-cell responses were not influenced by previous exposition to a specific COVID-19 treatment (Lopinavir/ritonavir; Interferon-Beta-1A; Hydroxychloroquine and Remdesivir) (data not shown). We notice that despite a lower intensity of response in terms of INF_γ secretion (Fig. 1B), patients with severe pneumonia had frequencies of responses clearly distinct from the one of mild illness patients.

Another immunological issue is the potential impact of SARS-CoV-2 virus mediated infection on pre-existing memory T-cell repertoire against common viruses. To address this issue, we concomitantly measured in all the COVID-19 patients the reactivity against common viruses (Cytomegalo-, Epstein-Barr and Fluvirus: CEF) using IFN_γ ELISpot assay. Median intensities of CEF specific T-cell responses in healthy donors, mild illness and severe pneumonia patients were similar in terms of intensities (respectively 144.0 IFN_γ spot/3×10^5 cells [IQR: 48.8–665.9], 88.2 IFN_γ spot/3×10^5 cells [IQR: 42.8–218.6] and 116.0 IFN_γ spot/3×10^5 cells [IQR: 67.5–179.0]) and frequencies (respectively 83.0%, 76.0% and 77.0%) (Fig. 1F).

The present study shows the existence of both specific SARS-CoV-2 cellular and humoral responses depending on COVID-19 severity. The presence of potent adaptive immunity even in patients who underwent severe pneumonia sustain the rationale for the development of protective therapeutics against SARS-CoV-2 virus.

Author contributions

Designing research studies: MK, LV, CB, SL, VW, SPF, GC, CC and KB conducting experiments: LS, AB, EO, QL, MD, LM, LB acquiring data: LS, AB, EO, GE analyzing data: LS, MK, QL, KB providing reagents: QL, LV, CB, CC, ALC, SL and writing the manuscript: MK, LS, CB, KB.

Declaration of Competing Interest

Authors declare no competing financial interests.

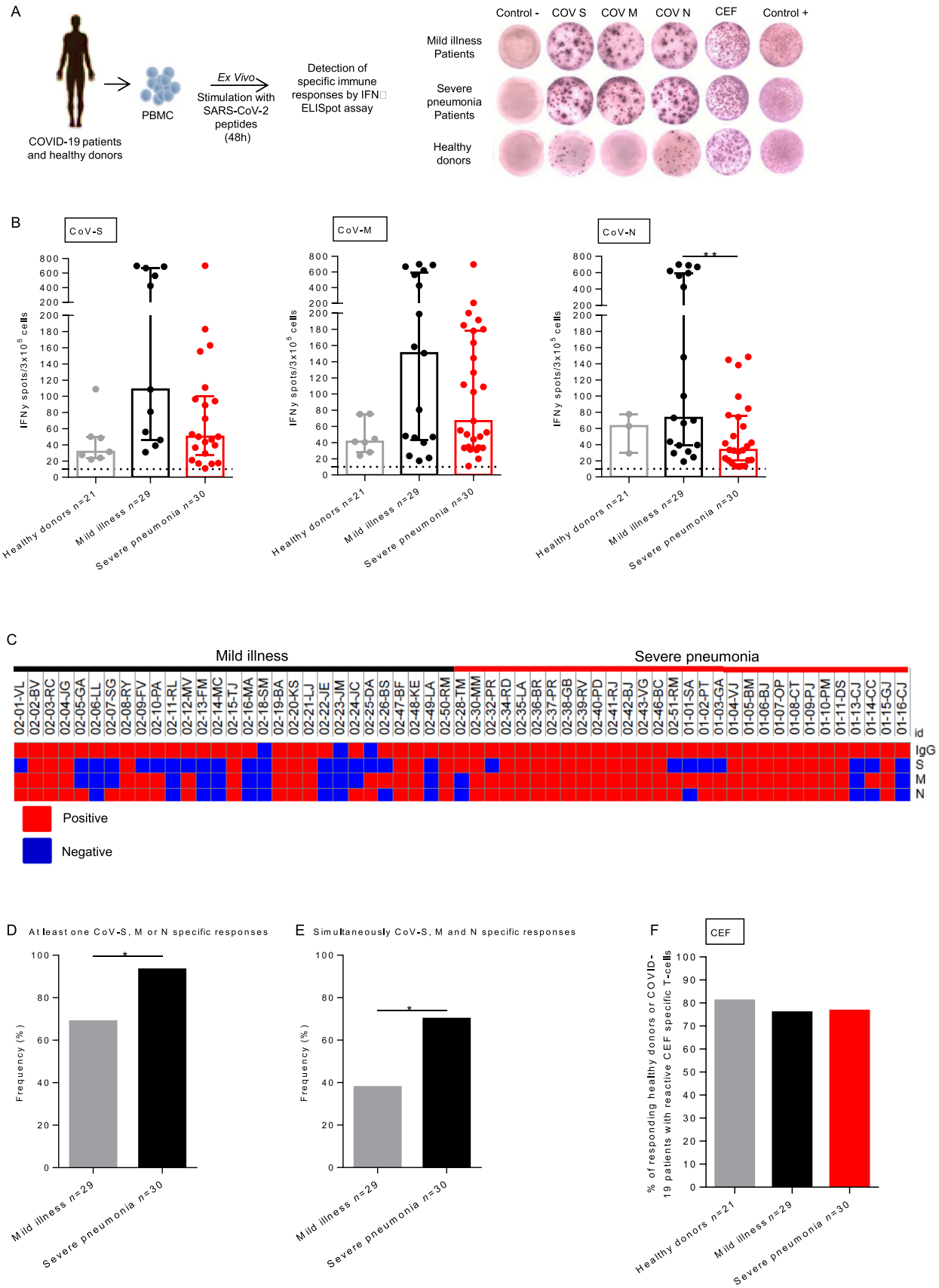

Fig. 1. SARS-CoV-2 specific T-cell responses were increased in mild illness compared to severe pneumonia COVID-19 patients. **A.** PBMC from 21 healthy donors and COVID-19 patients with mild illness ($n = 29$) or severe pneumonia ($n = 30$) were analyzed for SARS-CoV-2 and antiviral-specific T-cell responses by IFN_{γ} ELISpot assay. **B.** Intensity of positive SARS-CoV-2 specific immune responses in healthy donors and COVID-19 patients. Mann Whitney test, $^{**}P > 0.001$. Median with interquartile range were indicated. **C.** Heatmap showing the positivity or the negativity of the serology index, T-cell immune responses to SARS-CoV-S, M and N proteins for each patients included in the study (online Morpheus software). **D.** Frequency of patients with a specific T-cell response for at least one SARS-CoV-2 proteins (CoV-S, CoV-M or CoV-N) ($P = 0.0211$). **E.** Frequency of mild illness and severe pneumonia patients with specific T-cell responses for simultaneously CoV-S, CoV-M and CoV-N proteins ($P = 0.0191$). **F.** Frequency (%) of positive antiviral memory CD8T-cell responses for healthy donors, mild illness and severe pneumonia COVID-19 convalescent patients. Healthy donors were represented by light gray points. COVID-19 convalescent patients were respectively represented by black point (mild illness) and red points (severe pneumonia). PBMC: Peripheral Blood Mononuclear Cells.

Table 1
Characteristics of patients with COVID-19. Comparison between severe pneumonia and mild disease.

Variables	Total ($n=60$)	Mild disease ($n=30$)	Severe pneumonia ($n=30$)	Univariate P value
Male sex	30 (50)	7 (23.3)	23 (76.7)	**<0.001**
Mean age ±SD	54.1 (±17.4)	39.8 (±11.7)	68.3 (±7.59)	**<0.001**
Body mass index, mean ±SD	22.3 (±4.23)	20.5 (±3.72)	24.1 (±3.99)	**<0.001**
Comorbidities, n (%)				
Hypertension	16 (26.7)	2 (6.7)	14 (46.7)	**<0.001**
Chronic pulmonary disease	8 (13.3)	1 (3.3)	7 (23.3)	0.052
Diabetes melitus	6 (10)	0 (0)	6 (20)	**0.024**
Coronary heart disease	1 (1.7)	0 (0)	1 (3.3)	1
Chronic renal insufficiency	1 (1.7)	0 (0)	1 (3.3)	1
McCabe score				
Non fatal, n (%)	39 (65)	28 (93.3)	11 (36.7)	**<0.001**
Ultimately fatal, n (%)	19 (31.7)	2 (6.7)	17 (56.7)	
Rapidly fatal, n (%)	2 (3.3)	0 (0)	2 (6.7)	
Clinical characteristics, n (%)				
Fever	39 (65)	13 (43.3)	26 (86.7)	**<0.001**
Myalgia or arthralgias	23 (38.3)	18 (60)	5 (16.7)	**<0.001**
Fatigue	44 (73.3)	24 (80)	20 (66.7)	0.24
Headache	23 (38.3)	15 (50)	8 (26.7)	**0.063**
Diarrhea	14 (23.3)	3 (10)	11 (36.7)	**0.015**
Dyspnea	28 (46.7)	2 (6.7)	26 (86.7)	**<0.001**
Sputum production	12 (20)	8 (26.7)	4 (13.3)	0.2
Cough	40 (66.7)	18 (60)	22 (73.3)	0.27
Hospitalisation, n (%)	30 (50)	0	30 (100)	–
Length of stay, dy mean ±SD	26±15		26±15	–
Transfer in ICU, n (%)	23 (38.3)	0	23 (76.7)	**–**
Length of stay, dy mean ±SD	19±12		19±12	–
ARDS, n (%)	28 (46.7)		28 (93.3)	
Non specific therapy, n (%)				
Oxygenotherapy	30 (50)	0	30 (100)	**–**
Mechanical ventilation	20 (33.3)		20 (66.7)	–
Neuromuscular blocking agent	20 (33.3)		20 (66.7)	–
Prone positionning	17 (28.3)		17 (56.7)	–
Inhaled nitric oxide	2 (3)		2 (6.7)	–
Vasopressors	11 (18.3)		11 (36.7)	–
Specific therapy, n (%)	28 (46.7)	0	28 (93.3)	**–**
Corticosteroids	10 (16.7)		10 (33.3)	–
Interferon	7 (11.7)		7 (23.3)	–
Lopinavir/ritonavir	9 (15)		9 (30)	–
Hydroxychloroquine	12 (20)		12 (40)	–
Remdesivir	4 (6.7)		4 (13.3)	–
Laboratory findings, moy (±SD)				
Delay first symptom/blood sample, dy med [min-max]	38 [21–53]	39 [27–51]	38 [21–45]	**0.019**
Lymphocyte count (G/L), mean (±SD)	2.10 (±0.761)	2.29 (±0.565)	1.91 (±0.884)	**<0.01**
Serology				
Positive IgG serology, n (%)	57 (95)	27 (90)	30 (100)	0.24
Index, mean ±SD	6.05 (±2.18)	4.92 (±2.40)	7.19 (±1.09)	**<0.001**
T-cell responses*, n (%)				
CEF positivity	45/59 (76.3)	22/29 (75.9)	23/30 (76.7)	0.94
COV M positivity	44/59 (74.6)	17/29 (58.6)	27/30 (90)	**<0.01**
COV N positivity	43/59 (72.9)	19/29 (65.5)	25/30 (83.3)	0.14
COV S positivity	33/59 (55.9)	11/29 (37.9)	22/30 (73.3)	**<0.01**

* ELISpot was not performed for one patient, ARDS = Acute Respiratory distress syndrome.

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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.2020.08.036.

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Characterizing COVID-19 severity, epidemiology and SARS-CoV-2 genotypes in a regional business hub of China

To the Editor:

We read with great interest the article of Sijia Tian et al.[1] on "Characteristics of COVID-19 infection in Beijing", which informed the ratio of COVID-19 clinical outcome and epidemiological characteristics in Beijing. Clinical outcome-based surveillance is con-

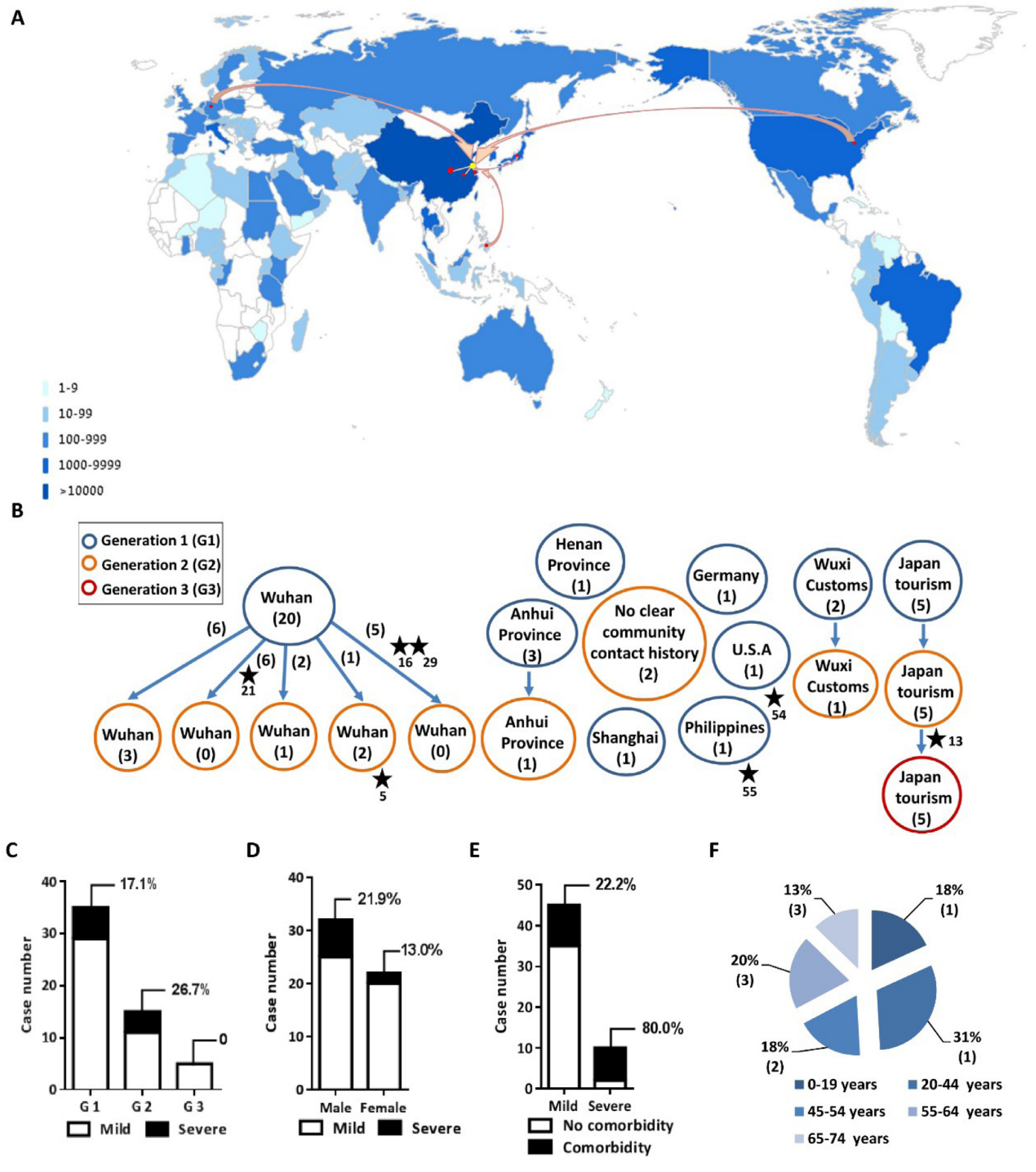

Fig. 1. Fifty-five SARS-CoV-2 infected cases and migration map. A. Migration world map of SARS-CoV-2 cases to Wuxi city, Jiangsu Province, China (from January to March 2020). B. Geographic migration areas of the SARS-CoV-2 epidemic in Wuxi. Generation 1 was imported cases, and generation 2 and generation 3 cases were found in Wuxi. The number of cases is shown in brackets, while ⋆ and the number beside it represents the specimen number of sequencing-positive specimen. These positive samples were registered in NCBI GenBank and accession numbers obtained. C and D. Comparison of mild and severe illness case numbers and percentages (mark above the columns) through epidemic generation and sex groups. E. Composition comparison with or without comorbid conditions in severe and mild illness cases. Percentages of severe cases or cases with comorbidities are marked above the columns. F. Composition percentage of each age group is marked on the pie chart. The number of severe illnesses is marked in brackets below each age composition percentage.

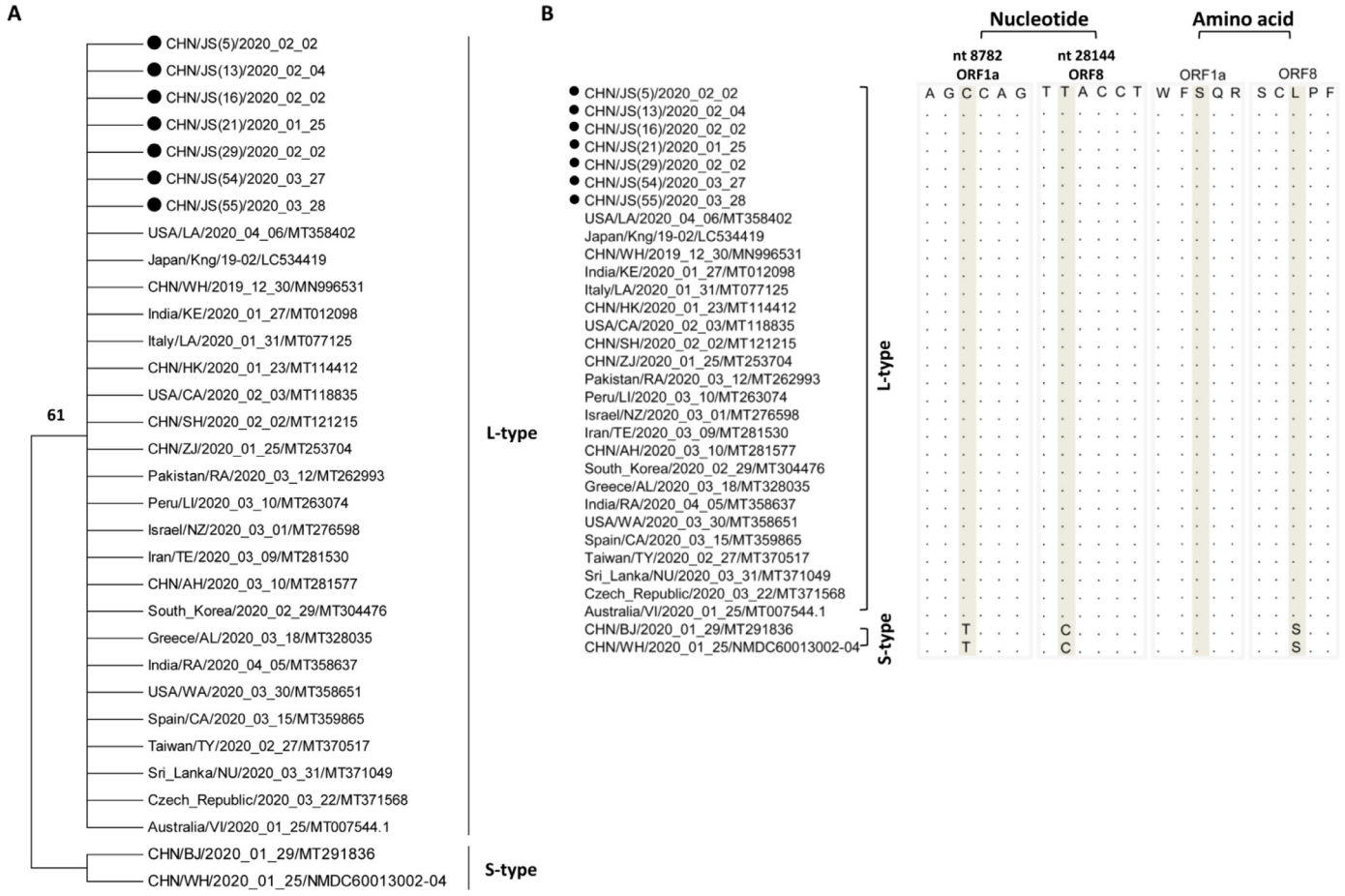

Fig. 2. Maximum likelihood method (MLM) analysis and haplotype analysis of SARS-CoV-2. A. Phylogenetic analysis of SARS-CoV-2. Analyses of the mutations at key sites in the genome and the phylogenetic tree of the Wuxi strains. PCR primers for SARS-CoV-2 ORF1a and ORF8 were designed referring to the reference strains downloaded from the Coronavirus Global Shared Database website (CGSD, http://nmdc.cn/coronavirus) using Primer Premier 5.0 software, and the primer sequences are as follows: 5′-AAT AAT TGG TTG AAG CAG C-3′ (ORF1a sense) and 5′-TCT ATA AGT TTT GAT GGT-3′ (ORF1a antisense), and 5′-CTT ATT ATC TTT TGG TTC TCC-3′ (ORF8 sense) and 5′-GGG GTC CAT TAT CAG ACA TTT T-3′ (ORF8 antisense), yielding 378 bp and 465 bp DNA products, respectively. Genome sequences were extracted and aligned using BioEdit 7.02 software. Phylogenetic trees for targeted genomes were constructed using Molecular Evolutionary Genetics Analysis (MEGA, *version* 6.06) by the maximum likelihood method (MLM) with 1000 replicates. A black solid circle (•) was plotted before Wuxi strains. The region of the country was expressed in abbreviated form. Each sequence was marked with the accession number and the collection date in the figure. Bootstrap values above 50 are shown. B. Haplotype analysis of SARS-CoV-2. Alignment analysis of nucleotide and amino acid sequences was performed with MEGA 6.06. Nucleotide and amino acid sequences of Wuxi strains and reference strains are shown as single letters or dots when they are the same.

ducive to make rational assessment of medical cost, while epidemiology and genotypes of SARS-CoV-2 circulating locally and globally are important for prevention and vaccine development. China authority agencies have established an open and real-time updated online database of SARS-CoV-2 sequences (http://nmdc.cn/coronavirus) as an information resource for scientists and clinicians.[2] However, it remains challenging to control and prevent this virus infection since information is rapidly evolving, and there are still many gaps that are not yet fully understood as to how this animal virus crossed species boundaries to infect humans.[3,4] Based on epidemiological analyses and prediction, this highly contagious virus has a high probability of causing a recurrent epidemic in the 2020/2021 winter.[5] Therefore, to better control future outbreaks, it is important to understand SARS-CoV-2 transmission and pathogenesis by characterizing the clinical and epidemiological features of COVID-19 and the SARS-CoV-2 strains circulating locally and globally.

In this retrospective study, we investigated COVID-19 disease severity, epidemiology and genotypes of SARS-CoV-2 in a regional business hub, Wuxi of China. Posterior oropharyngeal mucosal specimens of suspected patients were collected. We studied fifty-five COVID-19 patients (Ethics No. 2020–010–1) admitted to the hospital between January 25 and March 31, 2020. The clinical results showed that most cases were mild illness (45/55, 81.8%) with the rest being severe (10/55, 18.2%), and were mainly imported (Generation one cases (G1) = 35, 63.6%) and occurred mainly in January and February of 2020 after the implement of monitoring and quarantine measures. The cases in March were significantly decreased, and most source cases were from neighboring provinces (Wuhan, Henan, Anhui, and Shanghai) and foreign countries (USA, Germany, Japan, and Philippines) (Fig. 1A and 1B). High incidence of severe illness was found in G1 (6/35, 17.1%) and G2 group (4/15, 26.7%). Nearly 32.7% (18/55) cases (G2 plus G3 groups) were attributable to household transmission, and 3.6% (2/55) G2 had no clear community contact history (Fig. 1B and 1C). Among the total cases, 58.2% (32/55) were male, and men (7/32, 21.9%) also had a higher proportion of severe cases than females (3/23, 13.0%) (Fig. 1C). The main infection group was 20–44 years old (17/55, 30.9%). The proportion of severe cases with comorbidities (8/10, 80%) was ~4-fold greater than that of mild cases (10/45, 22.2%) (Fig. 1E), while 60% (6/10) severe cases occurred in over 55 years old (Fig. 1F). The average age (53.4 ± 6.6) of severely ill patients was significantly higher than all patients' average age (41.6 ± 2.6) ($p = 0.038$).

Concerning SARS-CoV-2 transmission and pathogenesis, current knowledge is limited. Coronaviruses are prone to mutations because they are single-stranded RNA viruses.[6] It is probable that these viruses would undergo mutations over time that could substantially change their features.[6,7] SARS-CoV-2 genomes of different pandemic locations inside[6,7] and outside of China[8] have become available. It is crucial to monitor protein mutations in viruses, which refers to changes in the sequence of amino acids of SARS-CoV-2 genome. According to the changes in the genomic base and amino acid sequences, the mutation time point of a virus can be traced.[9] If the amount of data is sufficient, the development can be traced from the perspective of space and time.[2] Such information, in combination with the clinical outcomes of infected individuals, may help design prevention and treatment strategies.

There are several standards for the classification of SARS-CoV-2 epidemic strains at home and abroad.[7,8] We adopted Tang's analysis and classification method which was developed to analyze 103 available whole-genome sequences from the early stage of the SARS-CoV-2 outbreak. [7] According to Tang's analysis, S-type and L-type subtypes were prevalent at the early period of the SARS-CoV-2 outbreak, and the L-type dominated the epidemic in Wuhan in the early period (~70%), but the frequency subsequently decreased since early January 2020.[7] Tang's study has been confirmed by Forster et al.,[8] in which S-type corresponds to A-type, and L-type corresponds to B-type. This nomenclature is now being used by GISAID. We subsequently sequenced partial ORF1a and full ORF8 of seven SARS-CoV-2 Wuxi strains, which covered the highest frequency of genomic variation in ORF1a (nt 8782) and ORF8 (nt 28,144), respectively. Finally, the regions of partial ORF1a (nt 8573–8938, 366 bp) and full ORF8 (nt 27,881–28,246, 465 bp) were identified. Given the need for a specific segment comparison with the global reference genes, we registered Wuxi strains (CHN/JS(5)/2020_02_02, CHN/JS(13)/2020_02_04, CHN/JS(16)/2020_02_02, CHN/JS(21)/2020_01_25, CHN/JS(29)/2020_02_02, CHN/JS(54)/2020_03_27 and CHN/JS(55)/2020_03_28) in GenBank and obtained accession numbers: ORF1a, MT415833 ~ MT415839; ORF8, MT415840 ~ MT415846, and downloaded the reference gene sequences from the GISAID (https://www.gisaid.org/), BLAST (NCBI, https://www.ncbi.nlm.nih.gov/) and CGSD websites.

Through analysis of SARS-CoV-2 epidemic strains inside and outside Wuxi city, including the first imported COVID-19 case from Wuhan city in late January 2020 (strain name: CHN/JS(21)/2020_01_25, Fig. 2A), 26 local cases directly related to Wuhan, and CHN/JS(13)/2020_02_04 strain related to severe outcome (other six strains were related to mild outcome), we performed multiple alignment analyses to identify the subtype of Wuxi prevalent strains. Similar to that observed in most foreign countries, the genotype of Wuxi strains from patients was L-type subtype regardless of severe or mild outcome, and this subtype could be found in many foreign countries as of April 2020 (Fig. 2A). We also found that the S-type was an epidemic strain in Beijing (BJ, January 29, 2020) and Wuhan (WH, January 25, 2020). Homology comparison of the analyzed sequences indicated that the nucleotide identity was 99.7% between the S- and L-type subtype reference strains. The nucleotide and amino acid mutations of SARS-CoV-2 Wuxi strains that appeared in ORF1a (nt 8782, T8517C) were synonymous, whereas the mutation that appeared in ORF8 (nt 28,144, C251T, S84L) was nonsynonymous, compared with S-type subtype reference sequences (Fig. 2B).

In conclusion, our findings have revealed the clinical features of COVID-19 and the genotypes of SARS-CoV-2 in a regional business hub during the lockdown period, providing a key reference for implementing such study locally and globally to understand SARS-CoV-2 transmission and pathogenesis.

Declaration of Competing Interest

The authors declare no competing interest.

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Serum cholinesterase associated with COVID-19 pneumonia severity and mortality

Dear Editor,

Kunutsor and Laukkanen have written to this journal regarding elevated admission levels of markers of liver injury (alanine aminotransferase and aspartate aminotransferase, gamma-glutamyltransferase, alkaline phosphatase and total bilirubin) may be associated with progression to severe disease or death in COVID-19.[1] On the other hand, serum cholinesterase plays an important role in the inflammatory response and may be associated with prognosis in sepsis.[2–4] We focused on the similarities between severe COVID-19 pneumonia and sepsis.

We examined associations between cholinesterase levels on admission and the severity, and mortality of patients with COVID-19 pneumonia, as well as the interaction between cholinesterase and the previously reported factors of severity and mortality. We included patients who had tested positive for severe acute respiratory syndrome coronavirus 2 from February to May 2020 at Yokohama City University Hospital and Yokohama City University Medical Center. Ultimately, 26 patients were included in the study. Outcomes were aggravation of symptoms and in-hospital death.

The clinical characteristics of the patients grouped by severity are shown in Table 1. There was no significant difference in patient characteristics between the groups. Supplementary Materials 1 shows the time course of cholinesterase and other factors in critically ill patients with good outcome and death. In critically ill patients with favorable outcome, cholinesterase, lymphocytes, albumin, and PaO_2/FiO_2 ratio decreased but C-reactive protein increased toward the peak of inflammation. Later, C-reactive protein decreased with improvement in inflammation, but there was a tendency for cholinesterase, lymphocytes, albumin, and PaO_2/FiO_2 ratio to increase. In contrast, in the severely ill patient who died, C-reactive protein poorly decreased, and cholinesterase, lymphocytes, albumin, and PaO_2/FiO_2 ratio were not elevated.

Fig. 1A and Supplementary Materials 2 show the association between severity and cholinesterase levels in COVID-19 patients. Cholinesterase levels on admission were significantly lower in the severe group than in the mild-to-moderate group (326 vs. 218 IU/L, $p = 0.006$). The optimal cut-off value for cholinesterase on severe cases was 301 U/L using the ROC curve, sensitivity was 93.3, and specificity was 63.6. The area under the ROC curve (AUC) in this case was 0.81. The positive and negative likelihood ratios were 2.6 and 0.1, respectively. In addition, ROC curves of C-reactive protein, albumin, lymphocytes, D-dimer, and PaO_2/FiO_2 ratio were prepared, and their AUCs were determined to be 0.94, 0.87, 0.73, 0.61, and 0.94, respectively. Fig. 1B and Supplementary Materials 2 show the association between mortality and cholinesterase levels in COVID-19 patients. Cholinesterase levels on admission were significantly lower in the death group than in the survival group (274 vs. 187.5 IU/L, $p = 0.028$). The optimal cutoff value for cholinesterase on mortality was 190 U/L using the ROC curve, sensitivity was 66.7, and specificity was 95.0. The AUC in this case was 0.79. The positive and negative likelihood ratios were 12.7 and 0.4, respectively. ROC curves of C-reactive protein, albumin, lymphocytes, D-dimer, and PaO_2/FiO_2 ratio were prepared, and their AUCs were 0.84, 0.77, 0.66, 0.70 and 0.79, respectively. Supplementary Materials 3A and 3B show analyses of the interaction between cholinesterase and the previously established factors of severity and mortality.

Our results demonstrate that the potential of cholinesterase levels and their interactions were significantly associated with severity and mortality in COVID-19 pneumonia patients. Cholinesterase is an enzyme produced in the liver that hydrolyzes cholinesters, and measured as a liver function test. Cholinesterase levels are high in patients with nephrotic syndrome, diabetes, hyperthyroidism, fatty liver, dyslipidemia, and obesity and low in patients with liver cirrhosis, hepatitis, malignant tumor, malnutrition, sepsis, and organophosphate poisoning.[5] Although the mechanism underlying cholinesterase reduction in sepsis has not yet been determined, it is thought to be affected by acute-phase infections and inflammatory processes.[6] It has been hypothesized that cholinesterase synthesis decreases owing to hepatic dysfunction with disease progression, capillary permeability enhancement, dilution with fluid challenges, cholinesterase catabolism enhancement, and cholinesterase inhibition by inflammatory mediators (cytokines).[7]

Levels of "positive" acute-phase proteins such as C-reactive protein, amyloid A, and ferritin generally increase in patients with inflammatory diseases. In contrast, levels of "negative" acute-phase proteins such as albumin, prealbumin, and transferrin decrease in response to inflammation and increase during the recovery period.[2,8] Cholinesterase and lymphocytes behave similar to the "negative" acute-phase proteins in response to inflammation.[9,10] Even in patients with COVID-19 pneumonia, amyloid A, which is classified as a "positive" acute-phase protein; albumin, which is classified as a "negative" acute-phase protein; and lymphocytes showing similar reactions as those of "negative" acute-phase proteins against inflammation have been suggested to be related to severity.[4]

Our study suggests that cholinesterase, which responds similar to the "negative" acute-phase proteins in response to inflammation, is reduced even in the acute phase of severe COVID-19 pneumonia. Following the changes in cholinesterase over time, we found that it decreased with deterioration of the condition and increased with improvement. Cholinesterase level on admission is suggested to be an independent predictor of severity and mortality for COVID-19 pneumonia. Cholinesterase levels on admission were significantly lower in the severe group than in the mild-to-moderate group, and they were also significantly lower in the death group than in the survival group. Cholinesterase was comparable to other markers, such as C-reactive protein, PaO_2/FiO_2 ratio, albumin, lymphocytes, and D-dimer regarding associations with the severity and mortality of COVID-19 pneumonia.

Limitations of this study include individual variances in cholinesterase, limited sample size, and potential bias owing to the confounding factors due to the retrospective nature of the study. Multiple factors may have been involved and multivariate analysis might have yielded more detailed results. Finally, owing to the limited number of facilities and regions, close attention should be paid to the generalization of the results.

In conclusion, cholinesterase may reflect the disease state of COVID-19 pneumonia, suggesting that a patient's cholinesterase level on admission may be useful as one of predictors of severity and prognosis. It has potential to be used as an indicator of

Table 1
Clinical characteristics grouped by severity.

		Mild-to-moderate cases* ($n=11$) Median (interquartile range)/frequency (%)		Severe cases** ($n=15$) Median (interquartile range)/frequency (%)		p-value
Age		70	(49–75)	69	(61–77)	0.878
Male		7	(64)	14	(93)	0.128
Nationality – no. (%)						
	Japan	8		12		
	United States	1		2		
	China	0		1		
	Canada	1		0		
	Republic of the Philippines	1		0		
Past history – no. (%)						
	Diabetes	2		8		
	Hypertension	4		5		
	Chronic kidney disease	0		3		
	Ischemiac heart disease	0		2		
	Asthma	1		1		
	Dyslipidemia	1		0		
	Anything	2		2		
Oxygen-support therapy – no. (%)						
	Oxygen support	7	(64)	15	(100)	
	Mechanical ventilation	0		13	(87)	
	Extracorporeal membrane oxygenation	0		4	(27)	
Treatment – no. (%)						
	Antibaiotics	8	(73)	15	(100)	
	Ciclesonide	5	(45)	12	(80)	
	Lopinavir/Ritonavir	3	(27)	11	(73)	
	Steroid	3	(27)	3	(20)	
	Favipiravir	1	(9)	5	(33)	
	Peramivir	1	(9)	2	(13)	
	Remdesivir	0	(0)	2	(13)	
	Nafamostat	0	(0)	1	(7)	
Median laboratory values (IQR)						
	ChE (U/L)	326	(228–394)	218	(185–279)	0.006
	CRP (mg/dL)	2.23	(1.04–4.28)	14.63	(7.04–18.00)	<0.001
	WBC (/μL)	7100	(5200–9300)	7100	(5900–11,200)	0.574
	Lymphocytes (%)	16.9	(7.6–22.3)	7.0	(5.8–10.8)	0.047
	Alb (g/dL)	3.8	(3.4–4.1)	3.1	(2.7–3.3)	0.001
	D-dimer (μg/dL)	1.2	(0.6–5.1)	2.1	(0.9–5.0)	0.384
	AST (U/L)	29	(24–39)	56	(38–94)	0.025
	ALT (U/L)	20	(16–56)	30	(18–46)	0.467
	P/F ratio (mmHg)	308	(300–380)	154	(113–209)	<0.001
	Death	0	(0)	6	(40)	0.051

* The mild-to-moderate group was defined based on the need for oxygen inhalation or no oxygen inhalation.
** The severe group was defined as having a respiratory condition requiring ventilator management (PaO_2/FiO_2 ratio <200 mmHg to respiratory rate >30/min).

severity or death and for recommending therapeutic interventions including intensive care during early stages of the disease.

Declaration of Competing Interest

None.

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Supplementary materials

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Previous cardiovascular surgery significantly increases the risk of developing critical illness in patients with COVID-19

To the editor,

We read with great interest the article by Dr. Galloway JB and colleagues recently published in the *Journal of Infection* entitled "A clinical risk score to identify patients with COVID-19 at high risk of critical care admission or death: An observational cohort study".[1] Early identification of patients with high-risk of poor prognosis may facilitate the provision of timely supportive treatment in advance and reduce the mortality of patients. In this study, the authors identified several comorbidities as risk factors of worse outcomes of COVID-19 patients, including diabetes, hypertension, and chronic lung disease. However, little is known about the impact of previous surgery on COVID-19. Herein, we evaluated whether COVID-19 patients with previous surgery are at high-risk of critical illness.

We conducted a multicenter study focusing on the clinical characteristics of COVID-19 patients with previous surgery in six designated hospitals in the Hubei and Guangdong provinces, China. COVID-19 was diagnosed according to the WHO interim guidance. 461 patients with COVID-19 that hospitalized from January 1 to March 31, 2020 were enrolled. We collected demographics, comorbidities, laboratory variables, and chest CT images from medical records. We defined the severity of COVID-19 according to the newest COVID-19 guidelines of China[2] and the guidelines of American Thoracic Society for community-acquired pneumonia.[3] Critical illness is defined as meeting at least one of the following criteria: respiratory failure requiring mechanical ventilation, shock, intensive care unit (ICU) admission, or death. According to surgical sites, previous surgeries were categorized into cardiovascular surgery, skeletal surgery, urogenital surgery, head and neck surgery, gastrointestinal surgery and others. Baseline features were compared between patients with and without previous surgery. To identify risk factors for critical illness, baseline variables with $p < 0.10$ in univariable analysis were entered into multivariate logistic regression. Time from diagnosis of COVID-19 to death was explored using Kaplan-Meier survival analysis. Considering in-hospital death is competing risk of ICU admission, time from diagnosis of COVID-19 to ICU admission was analyzed by a competing-risk model. Our institutional ethics review board approved the study and waived the need for informed consent.

In total, 47 (10.2%) COVID-19 patients with previous surgery. Gastrointestinal surgery was the most frequent surgery type (19/47, 40.4%), followed by cardiovascular surgery (11/47, 23.4%), urogenital surgery (10/47, 21.3%), skeletal surgery (7/47, 14.9%), and head and neck surgery (6/47, 12.8%). COVID-19 patients with previous surgery had obviously worse outcomes ($p < 0.001$). Compared with patients who had no previous surgery, patients had previous surgery were older ($p < 0.001$), had higher rate of hypertension ($p = 0.039$), coronary heart disease ($p = 0.002$), diabetes ($p = 0.017$), and chronic lung disease ($p = 0.020$), lower lymphocyte ($p = 0.001$) and albumin ($p = 0.022$), and higher aspartate aminotransferase

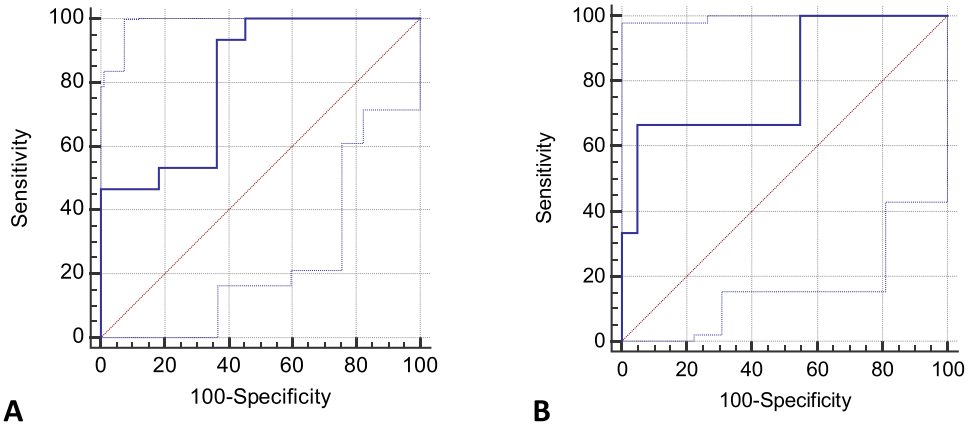

Fig. 1. Prediction of severity (A) and mortality (B) based on cholinesterase level on admission.

Table 1
Identification of risk factors of critical illness in COVID-19 patients using univariable and multivariable logistic regression.

	Univariable OR (95% CI)	*P* value	Multivariable OR (95% CI)	*P* value
Age (years)	1.075 (1.054, 1.096)	<0.001	1.053 (1.022, 1.085)	0.001
Male	1.728 (1.031, 2.896)	0.038	1.178 (0.493, 2.814)	0.713
Comorbidities				
Hypertension	3.650 (2.133, 6.244)	<0.001	1.966 (0.831, 4.648)	0.124
Coronary heart disease	2.992 (1.271, 7.043)	0.012	0.382 (0.096, 1.526)	0.173
Diabetes	3.804 (1.981, 7.302)	<0.001	1.205 (0.404, 3.590)	0.738
Chronic liver diseases	1.054 (0.296, 3.759)	0.935		
Chronic lung diseases	1.970 (0.839, 4.626)	0.119		
Types of previous surgery				
Gastrointestinal surgery	2.372 (0.873, 6.446)	0.090	1.287 (0.297, 5.567)	0.736
Head and neck surgery	5.067 (1.003, 25.586)	0.050	5.785 (0.735, 45.534)	0.095
Urogenital surgery	2.149 (0.543, 8.498)	0.276		
Skeletal surgery	6.847 (1.501, 31.228)	0.013	1.946 (0.205, 18.438)	0.562
Cardiovascular surgery	9.342 (2.665, 32.747)	<0.001	11.998 (2.068, 69.612)	0.006
Others	–	0.999		
Laboratory findings				
WBC ($\times 10^9$/L)	1.223 (1.122, 1.334)	<0.001	1.015 (0.458, 2.252)	0.971
Neutrophil ($\times 10^9$/L)	1.305 (1.190, 1.432)	<0.001	1.136 (0.505, 2.552)	0.758
Lymphocyte ($\times 10^9$/L)	0.129 (0.067, 0.248)	<0.001	0.982 (0.288, 3.342)	0.977
LDH (U/L)	1.010 (1.007, 1.012)	<0.001	1.009 (1.005, 1.013)	<0.001
Hemoglobin (g/L)	1.002 (0.989, 1.015)	0.812		
Platelet (g/L)	0.994 (0.990, 0.997)	0.001	0.994 (0.988, 1.000)	0.051
Albumin (g/L)	0.827 (0.783, 0.873)	<0.001	1.039 (0.929, 1.161)	0.507
AST (U/L)	1.029 (1.017, 1.041)	<0.001	1.002 (0.984, 1.020)	0.851
ALT (U/L)	1.004 (0.996, 1.011)	0.344		
DBIL (μmol/L)	1.176 (1.078, 1.284)	<0.001	1.151 (1.004, 1.319)	0.043
IBIL (μmol/L)	0.932 (0.873, 0.994)	0.032	0.893 (0.771, 1.035)	0.133
TBIL (μmol/L)	1.016 (0.986, 1.046)	0.299		
APTT (s)	1.017 (0.979, 1.058)	0.381		
PT (s)	1.038 (0.996, 1.081)	0.080	1.038 (1.000, 1.077)	0.053
D-dimer (μg/ml)	1.002 (0.999, 1.004)	0.285		
Creatinine (μmol/L)	1.023 (1.012, 1.034)	<0.001	1.006 (0.994, 1.018)	0.325
hs-CRP (mg/L)	1.013 (1.007, 1.019)	<0.001	0.991 (0.981, 1.000)	0.062
Procalcitonin (ng/ml)	1.124 (1.036, 1.220)	0.005	1.043 (0.929, 1.173)	0.475
Urea nitrogen (mmol/L)	1.316 (1.206, 1.435)	<0.001	1.191 (1.056, 1.343)	0.005
FBG (mmol/L)	0.953 (0.887, 1.024)	0.188		
CT score	0.953 (0.887, 1.024)	0.188		

Abbreviations: OR, odds ratio; CI: confidence interval; WBC, white blood cells; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBIL, Total Bilirubin; DBIL, Direct Bilirubin; IBIL, indirect bilirubin; APTT, activated partial thromboplastin time; PT, prothrombin time; hs-CRP, high-sensitivity C-reactive protein; FBG, fasting blood glucose. A semiquantitative CT scoring system was designed to assess the involvement degree or area of pneumonia for each lung lobe (total 5 lung lobes): 0 for 0% involvement; 1 for 1–25% involvement; 2 for 26–50% involvement; 3 for 51–75% involvement; 4 for 76–100% involvement. A CT score (range, 0–20) was assigned by summarizing for the total scores of five lobes. CT images were reviewed independently by two radiologists with >10 years of experience.

($p = 0.013$) and high-sensitivity C-reactive protein ($p = 0.011$) (Supplementary Table 1). Among various surgery types, only previous cardiovascular surgery was an independent risk factors of developing critical illness (odds ratio [OR] = 12.0, 95% CI: 2.1–69.6, $p = 0.006$) (Table 1). After adjusting for age, sex, and other comorbidities, the OR was 11.6 (95% CI: 2.7–49.6, $p = 0.001$). Patients with previous cardiovascular surgery had significantly worse survival ($p = 0.001$) and higher cumulative incidence of ICU admission ($p < 0.001$) than those patients without previous cardiovascular surgery (Supplementary Fig. 1).

To our best of knowledge, this is the first study that showed that patients with previous cardiovascular surgery instead of other surgeries significantly increase the risk of developing critical illness among patients with COVID-19.

Close attention should be paid to this population. High-level monitoring and aggressive treatment may be necessary to improve the outcomes of these patients.

Declaration of Competing Interest

None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.08.012.

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Setting the criteria for SARS-CoV-2 reinfection – six possible cases

Dear Editor,

We read with interest the article by Batisse et al.[1] describing 11 possible cases of symptomatic severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) reinfection. However, the intervening period of 'clinical cure' was not confirmed by a negative SARS-CoV-2 PCR test result. Similarly, Lafaie et al.[2] reported three elderly patients who were infected with SARS-CoV-2 who recovered 'clinically' then were readmitted with 'new' coronavirus disease 2019 (COVID-19) symptoms, again with no SARS-CoV-2 PCR negative result between the two COVID-19 episodes. This SARS-CoV-2 reinfection phenomenon is indeed one of the many ongoing debates during the present COVID-19 pandemic and it is still unclear to what extent this is due to true reinfection, or possible persistent low level infection.[3,4]

Part of the problem is that there is no well-defined, consensus definition or criteria for deciding what constitutes true SARS-CoV-2 reinfection. Here we present 6 cases of hospitalised patients or staff with likely SARS-CoV-2 reinfection based on objective laboratory-based criteria and rationales.

From our diagnostic laboratory database, we searched for a pattern of SARS-CoV-2 POS-NEG-POS polymerase chain reaction (PCR) results in any COVID-19 case, then extracted and tabulated the details of the clinical episodes for each patient fitting these initial search criteria.

The SARS-CoV-2 PCR assay that we were using during this time was the commercial AusDiagnostics SARS-CoV-2 PCR kit (AusDiagnostics UK Ltd., Chesham, England) with a manufacturer's stated sensitivity and specificity of 97.7% (90.8–100%) and 99.4% (97.4–100%), respectively. The SARS-CoV-2 IgG antibody testing was performed using the commercial DiaSorin Liaison SARS-CoV-2 S1/S2 IgG assay (Diasorin Ltd., Kent, England) with a stated manufacturer's sensitivity and specificity of 97% (86.8%–99.5%) and 98.5% (97.6%–99.1%), respectively.

Patients or staff meeting the following criteria were included in this possible SARS-CoV-2 reinfection cohort:

- an initial SARS-CoV-2 PCR-confirmed acute coronavirus disease 2019 (COVID-19) illness
- followed by clinical recovery and discharge with at least one negative SARS-CoV-2 PCR result
- followed by a confirmed SARS-CoV-2 PCR positive result (with or without symptoms) at least 28 days after the previous SARS-COV-2 PCR result

These criteria were based on the findings that in most COVID-19 cases, viral shedding reaches a minimum by day 28 after an initial acute SARS-CoV-2 infection.[5] It is still unclear how protective SARS-CoV-2 IgG antibodies are in the convalescent period, and how long any such protection may last. Such antibodies start to rise 5–10 days post-onset of infection, peaking by days 12–15,[6] and will contain a proportion of neutralising antibodies,[7] making any SARS-CoV-2 reinfection very unlikely within this period. Hence, we assumed that SARS-CoV-2 reinfection cannot occur within the first 28 days post-illness onset. As patients move beyond 28 days post-illness onset, SARS-CoV-2 IgG antibodies gradually wane,[8] increasing the possibility that SARS-CoV reinfection may occur.

We identified 6 patients or staff that fit the criteria above (Table 1, Fig. 1). All 6 cases had at least one SARS-CoV-2 IgG antibody test (Abbott Architect SARS-CoV-2 IgG assay; Abbott, Maidenhead, UK). Cases 24 and 26 had two SARS-CoV-2 IgG antibody tests, all of which were positive.

For Case 24, these IgG tests were positive on days 88 and 92, with the first of these testing positive the day after the second positive PCR swab result. This indicates that the SARS-CoV-2 PCR

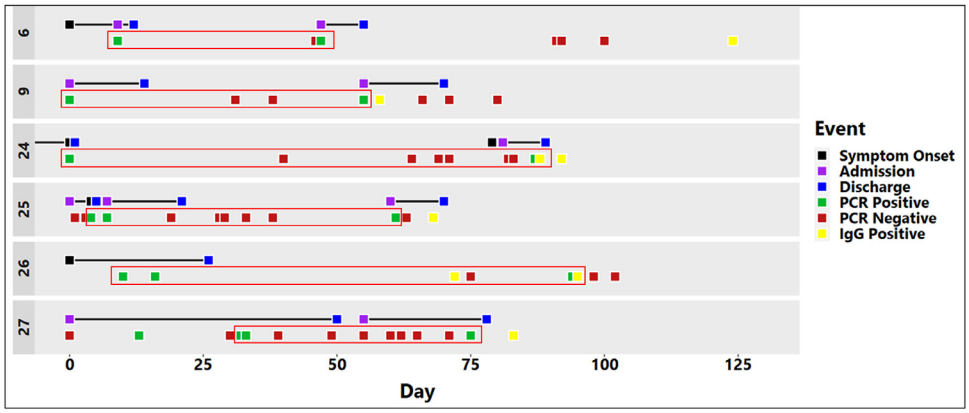

Fig. 1. Timeline of events for each patient. As the specific date of illness onset was unclear for several patients the first SARS-CoV-2 PCR positive result was used as time zero (Day 0). The image shows all the testing that was performed for each patient. The specific interval over which reinfection is likely to have occurred (SARS-CoV-2 PCR POS-POS) in each case is highlighted using red boxes.

Table 1
Characteristics and timeline of possible COVID-19 reinfection cases.

Case no.	Age(yrs)	Sex	Ethnicity	Past medical history	1st COVID-19 episode/ PCR POS result (Day 0)	Treatment	Recovery/ discharge date (days since Day 0)	Onset of second episode (days since Day 0)	Symptoms	SARS-CoV-2 IgG (Day tested)
6	49	F	Asian	Obesity, T1DM, epilepsy, previous sepsis due to obstructive pyelonephritis.	Fever, cough, myalgia, diarrhoea	IV AB, CS	3	38	Fever, low BP, treated for sepsis due to obstructive ureteric calculus but CT chest shows evidence of COVID19 and PCR positive.	Positive (Day 72)
9	93	M	White British	IgG multiple myeloma, AF, CCF, HTN, cognitive impairment	Lethargy, reduced appetite, diarrhoea	IV and PO AB	14	55	Cough, fever, dyspnoea	Positive (Day 58)
24	82	M	White British	AF, CCF with PPM/ICD, AAA, T2DM, lung cancer, AS	Cough, fever, sore throat, dyspnoea, new oxygen demand, haemoptysis	PO AB	1	87	Fever, cough, dyspnoea	Positive (Days 88, 92)
25	86	F	White British	IHD, HTN, HF, hypothyroidism, OA, PMR	Unresponsive episode, low BM	IV AB	17	57	Asymptomatic, admitted with fall and sternal fracture. Routine swab on admission.	Positive (Day 62)
26	62	F	White British	Healthcare staff	Cough, fever, dyspnoea	Home care	16	84	Asymptomatic, routine staff screening	Positive (Days 62, 85)
27	83	M	White British	T2DM, 1st degree AV block, bronchitis, GORD	Dyspnoea, new oxygen requirement	IV AB, O2	18	43	Asymptomatic, routine swab prior to discharge.	Positive (Day 51)

AAA: abdominal aortic aneurysm, AB: antibiotics, AF: atrial fibrillation, AS: aortic stenosis, AV: atrioventricular, CCF: congestive cardiac failure, CS: corticosteroids, GORD: gastro-oesophageal reflux disease, HF: heart failure, HTN: hypertension, ICD: implantable cardioversion device, IV: intravenous, OA: osteoarthritis, O2: oxygen, PMR: polymyalgia rheumatica, PO: oral, T1DM: type 1 diabetes mellitus, T2DM: type 2 diabetes mellitus.

positive swab was taken in the presence of SARS-CoV-2 IgG antibodies, as these typically take 5–10 days to appear.[6] This apparent SARS-CoV-2 reinfection in Case 24 was symptomatic. Similarly, for Case 26, the SARS-CoV-2 IgG was positive on samples taken on days 62 and 85, again indicating that the second positive PCR result (on day 84) must have occurred in the presence of SARS-CoV-2 IgG antibodies. Reinfection in Case 26 was asymptomatic. This was a staff member whose testing had been conducted as part of the routine screening for staff who worked on immunosuppressed patient wards. The same argument can be applied to Cases 9 (symptomatic reinfection), 25 (asymptomatic reinfection) and 27 (asymptomatic reinfection), where the second SARS-CoV-2 PCR and SARS-CoV IgG positive results occurred within 5–10 days of each other (Table 1, Fig. 1). We did not culture the second COVID-19 episode SARS-CoV-2 PCR positive swabs to check for virus viability. Batisse et al.,[1] however, did find viable SARS-CoV-2 in one out of two patient samples tested during their second COVID-19 episodes.

Thus while SARS-CoV-2 reinfection is a possibility for any of these 6 cases, we are the most confident of Cases 24 and 26 being true cases of SARS-CoV-2 reinfection, as they exhibited the largest interval (87 and 84 days, respectively) between their two COVID-19 episodes. Also, their two positive SARS-CoV-2 IgG antibody tests showed that antibodies were present after the first and persisted through to the second COVID-19 episode, and were therefore less likely to be a false positive finding.

Reinfection with the four known human seasonal coronavirus infections has been described, even in the presence of pre-existing coronavirus antibodies, and is not unusual.[9] However, 'reactivated', 'relapsed' or 'latent' infection seems less likely and is not yet described for the family of coronaviruses.[10]

Our SARS-CoV-2 reinfection criteria are not perfect and will inevitably be refined as new findings accumulate. Yet our small case series here indicates that symptomatic and asymptomatic SARS-CoV-2 reinfection can occur in the presence of SARS-CoV-2 IgG antibodies. Further studies are needed to determine to what extent SARS-CoV-2 shedding and transmission occur during symptomatic and asymptomatic reinfection.

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