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.

Letters to the Editor

Low-dose corticosteroid therapy does not delay viral clearance in patients with COVID-19

Dear Editor,

Since December 2019, Corona Virus Disease 2019 (COVID-19) cases have occurred in Wuhan, Hubei.[1] The epidemic spread rapidly to other regions outside of China and had a great impact on public health and the economy. To the best of our knowledge, no specific antiviral treatment for COVID-19 has been confirmed so far. A recent study demonstrated that cytokine storm syndrome is associated with the severity of COVID-19.[2] Due to their anti-inflammatory and immunoregulatory properties, corticosteroids have been widely used in China for the treatment of patients with COVID-19, especially in cases with secondary acute respiratory distress syndrome (ARDS). Furthermore, China's National Health Commission has released a Diagnosis and Treatment Scheme for Pneumonitis with COVID-19 Infection.[3] According to this, systemic corticosteroid therapy (methylprednisolone, <1-2 mg per kg of body weight, 3-5 days) is recommended as adjuvant therapy.[3] The release of this guideline immediately caused controversy regarding whether patients with COVID-19 could benefit from corticosteroid therapy, since this has been associated with a delay in viral clearance. The present study investigated the effect of low-dose corticosteroid therapy on SARS-CoV-2 clearance time.

The present study collected clinical data of 78 patients with confirmed COVID-19 who were admitted to the Infectious Diseases Branch of Anhui Provincial Hospital between January 22, 2020 and March 1, 2020. A total of 55 and 23 cases were diagnosed with general and severe COVID-19, respectively. All patients received standard treatment, including antiviral therapy, oxygen therapy, antibacterial drugs and symptomatic therapies. Furthermore, patients in the severe group received the necessary supportive treatment. Corticosteroids were administrated to a subset of patients according to severity and the individual opinion of clinicians. More severe patients were treated with corticosteroids, leading to inconsistent baseline data (i.e., age, comorbidities and laboratory findings) between patients receiving corticosteroids and those who did not. Therefore, the 78 patients were divided into a general and a severe group, and separate data analysis was conducted for each group. Table 1 shows the demographic characteristics, comorbidities, laboratory test results and antiviral treatment of patients with or without corticosteroid treatment in the general and severe groups. In total, there were 25 patients who received corticosteroids, while the remaining patients did not receive corticosteroids (Table 1). Oral methylprednisolone [median hydrocortisone-equivalent dose, 237.5 mg/day (IQR, 206.3-300.0 mg/day)] was administered to 9 patients in the general group for a median duration of 7 days (IQR, 5.5-8.0 days), while intravenous methylprednisolone [median hydrocortisone-equivalent dose, 250.0

mg/day (IQR, 250.0-250.0 mg/day)] was administered to 16 patients in the severe group for a median duration of 4.5 days (IQR, 3.5-5.8 days). The 9 patients in the general group received a higher total dose of corticosteroids and had a longer duration of corticosteroids treatment, since all of them were hospitalized at the early stage of the epidemic and treated by the same medical team. Starting at 3 days after admission, ~2–3 throat swabs or sputum samples were routinely collected once per week from all patients for reverse transcription-polymerase chain reaction (RT-PCR) testing to assess viral clearance. If the RT-PCR test result was negative, the test was repeated the next day to avoid false-negative results. Briefly, an independent sample t-test was conducted to compare virus clearance time between the corticosteroid group and the non-corticosteroid group, and there was no significant difference identified in both patients in the general group (17.6 ± 4.9 vs. 18.7 ± 7.7 days; $P = 0.667$) and patients in the severe group (18.8 ± 5.3 vs. 18.3 ± 4.2 days; $P = 0.84$).

There has been controversy regarding whether corticosteroid use may delay viral clearance in patients with viral pneumonia for a long time. Initially, this phenomenon was observed in studies investigating severe acute respiratory syndrome and Middle East respiratory syndrome coronavirus (MERS).[4,5] However, the dose of corticosteroids may have a significant impact on the results. An observational study by Cao on influenza A (H7N9) viral pneumonia has demonstrated that high doses of corticosteroids (>150 mg/d methylprednisolone) are associated with increased risks of mortality and delayed viral clearance, while there was no difference between patients in the low-dose group (25–150 mg/d methylprednisolone) and controls.[6] In the present study, all 25 patients were treated with low-dose corticosteroids, and the conclusions were similar to those of Cao.

In another retrospective observational study reporting on patients with MERS who were critically-ill, nearly half of the enrolled patients received low-dose corticosteroids [median hydrocortisone-equivalent dose, 300.0 mg/day (IQR, 200.0-400.0 mg/day)].[5] When compared with the cases who were not treated with corticosteroids, the authors concluded that the administration of corticosteroids was associated with delayed viral clearance. Unlike in the study reporting on MERS, the patients enrolled in the present study exhibited an improved health status. Even in patients in the severe group, the SOFA score on the first day of admission was much lower than that of patients with MERS [2.0 (IQR, 0-2.8) vs. 9.0 (IQR, 6.0-12.0)].[5] Therefore, these two studies involved patients with different severities of illness; however, whether corticosteroids exerted greater effects in critically-ill patients requires further investigation. Furthermore, the study reporting on patients with MERS included RT-PCR data from 14 intensive care units, and the nucleic acid test results were not protocolized and varied among centers.[5] In the present study, all patients were from a single center, and all swab samples were tested using a unified

Table 1
Demographics and baseline characteristics of patients infected with COVID-19 according to illness severity and corticosteroid use.

Variable	General group ($n = 55$)			Severe group ($n = 23$)		
	Corticosteroids ($n = 9$)	No Corticosteroids ($n = 46$)	P-value	Corticosteroids ($n = 16$)	No Corticosteroids ($n = 7$)	P-value
Age (years), mean ± SD	40.2 ± 12.6	39.9±15.5	0.959	60.6±13.6	54.3±15.4	0.33
Male sex, n (%)	5 (55.6)	22 (47.8)	0.952	12 (75)	5 (71.4)	>0.99
Days from onset of symptoms to hospitalization, median (Q1, Q3)	7 (4, 7.5)	5 (3, 7)	0.305	6.39±3.9	8.3±3.6	0.429
SOFA score, median (Q1, Q3)	/	/	/	2.0 (0, 2.8)	2.0 (1.0, 3.0)	0.702
ARDS, n (%)	0	0	/	8 (50)	1 (14.3)	0.176
Comorbidities						
Hypertension, n (%)	1 (11.1)	4 (8.7)	>0.99	8 (50)	2 (28.6)	0.405
Diabetes, n (%)	1 (11.1)	3 (6.5)	0.522	4 (25)	0	0.273
Coronary heart disease, n (%)	0	1 (2.2)	>0.99	2 (12.5)	0	>0.99
Cerebrovascular disease, n (%)	0	0	/	2 (12.5)	1 (14.3)	>0.99
Chronic kidney disease, n (%)	0	2 (4.3)	>0.99	1 (6.3)	0	>0.99
Chronic liver disease, n (%)	2 (22.2)	1 (2.2)	0.066	0	0	/
Malignant tumor, n (%)	0	1 (2.2)	>0.99	0	0	/
Immunosuppressive, n (%)	0	1 (2.2)	>0.99	0	0	/
WBC count (x10^9/L), median (Q1, Q3)	4.6 (4.0, 5.5)	4.9 (3.9, 6.0)		6.5 (5.5, 10.6)	5.4 (4.8, 11.9)	0.789
Lymphocyte count (x10^9/L), median (Q1, Q3)	0.89 (0.84, 1.38)	1.33 (1.16, 1.79)	0.062	0.62 (0.37, 0.98)	1.14 (0.73, 1.36)	0.082
CRP (mg•/L), median (Q1, Q3)	22.1 (8.9, 36.4)	4.2 (0.7, 16.6)	0.091	47.8 (30.0, 102.3)	23.5 (8.5, 72.0)	0.124
PCT <0.5 ng/mL, n (%)	9 (100)	46 (100)	/	15 (93.8)	7 (100)	>0.99
PT (s), median (Q1, Q3)	14 (13.6, 15.3)	14.4 (13.4, 16.2)	0.793	14.3 (13.0, 15.0)	14.1 (13.7, 14.6)	0.894
APTT (s), median (Q1, Q3)	38.7 (35.3, 42.0)	37.1 (34.6, 42.9)	0.649	35 (30.2, 39.5)	36.7 (27.4, 38.8)	0.462
D-Dimer (µg/ml), median (Q1, Q3)	0.25 (0.2, 0.29)	0.18 (0.08, 0.25)	0.09	0.45 (0.25, 0.62)	0.22 (0.19, 0.63)	0.452
Troponin I (µg/L), median (Q1, Q3)	0.08 (0.07, 0.11)	0.08 (0.06, 0.19)	0.90	0.31 (0.09, 0.56)	0.12 (0.1, 0.44)	0.72
Total bilirubin (µmol/L), median (Q1, Q3)	14.5 (12.6, 23.3)	14.9 (11.3, 19.3)	0.838	17.1 (15.3, 21.4)	17.1 (11.1, 19.4)	0.452
Albumin (g/L), median (Q1, Q3)	44.2 (41.0, 45.0)	44.9 (41.3, 47.7)	0.847	35.2 (32.9, 37.4)	39.3 (36.8, 42.2)	0.022
Creatinine (µmol/L), median (Q1, Q3)	66 (58, 71)	71 (60, 81)	0.052	74.0 (54.0, 82.0)	68 (59, 76)	0.82
Antiviral therapy						
Lopinavir/Ritonavir only, n (%)	7 (77.8)	37 (80.4)	0.785	4 (25.0)	3 (42.9)	0.626
Lopinavir/Ritonavir+IFN-α inhalation, n (%)	2 (22.2)	9 (19.6)		12 (75.0)	4 (57.1)	
TCM, n (%)	3 (33.3)	19 (41.3)	0.941	16 (100)	6 (85.7)	0.304
Methylprednisolone						
Oral, n (%)	9 (100)	/	/	0	/	/
Intravenous, n (%)	0	/	/	16 (100)	/	/
Duration of corticosteroid treatment (days), median (Q1, Q3)	7 (5.5, 8.0)	/	/	4.5 (3.0, 5.8)	/	/
Total dose of methylprednisolone (mg), median (Q1, Q3)	280 (220, 360)	/	/	160 (120, 240)	/	/
Dose of methylprednisolone per day (mg), median (Q1, Q3)	38 (33, 48)	/	/	40 (40, 40)	/	/
Time to SARS-CoV-2 RNA clearance (days), mean ± SD	17.6±4.9	18.7±7.7	0.667	18.8±5.3	18.3±4.2	0.84

WBC white blood cell, CRP C-reaction protein, PCT Procalcitonin, TCM Traditional Chinese Medicine, PT prothrombin time, APTT Activated partial thromboplastin time

approach at the Chinese Center for Disease Control to avoid measurement bias.

In fact, a similar study analyzing data from 72 patients with COVID-19 was conducted at the First Affiliated Hospital of Zhejiang University, and the conclusions were consistent with the results of the present study.[7] However, these two retrospective studies have unavoidable limitations, such as small sample size, poor controllability of the data and bias in the process of data collection. In conclusion, low-dose corticosteroid therapy may not delay viral clearance in patients with COVID-19; however, this still needs to be confirmed by well-designed and large-scale RCTs with a longer follow-up duration.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Ethics approval was obtained from Anhui Provincial Hospital Institutional Review Board (ethical approval no. 2020-P-008).

Consent for publication

All the authors agree to publish.

References

1. Chen J., Qi T.K., Liu L., Ling Y., Qian Z.P., Li T., et al. Clinical progression of patients with COVID-19 in Shanghai, China. J Infect 2020 PMID: 32171869.
2. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;**395**(10223):497–506 PMID: 31986264.
3. National Health Commission of the People's Republic of China. The 5th trial version of Diagnosis and Treatment Scheme for Pneumonitis with 2019-nCoV Infection (In Chinese). [cited 2020 Mar 20]. Available from: http://www.nhc.gov.cn/yzygj/s7653p/202002/d4b895337e19445f8d728fcaf1e3e13a.shtml (2020).
4. Lee N., Allen Chan K.C., Hui D.S., Ng E.K., Wu A., Chiu R.W., et al. Effects of early corticosteroid treatment on plasma SARS-associated Coronavirus RNA concentrations in adult patients. J Clin Virol 2004;**31**(4):304–9 PMID: 15494274.

5. Arabi Y.M., Mandourah Y., Al-Hameed F., Sindi A.A., Al Mekhlafi G.A., Hussein M.A., et al. Corticosteroid Therapy for Critically Ill Patients with the Middle East Respiratory Syndrome. *Am J Respir Crit Care Med* 2018;**197**(6):757–67 PMID: 29161116.

6. Cao B., Gao H., Zhou B., Deng X., Hu C., Deng C., et al. Adjuvant corticosteroid treatment in adults with influenza A (H7N9) viral pneumonia. *Crit Care Med* 2016;**44**(6):e318–28 PMID: 26934144.

7. Ni Q., Ding C., Li Y.T., Zhao H., Liu J., Zhang X., et al. Retrospective study of low-to-moderate dose glucocorticoids on viral clearance in patients with novel coronavirus pneumonia. *Chin J Clin Infect Dis* 2020;**13**. 2020-02-28[cited 2020 Mar 20]. Available from http://rs.yiigle.com/yufabiao/1182773.htm .

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Clinical and laboratory-derived parameters of 119 hospitalized patients with coronavirus disease 2019 in Xiangyang, Hubei Province, China

Dear Editor,

The newly emergent Coronavirus disease 2019 (COVID-19) causes severe viral pneumonia in humans and poses a serious threat to public health worldwide, with cases reported from all 6 permanently inhabited continents. Effective clinical management, based on comprehensive laboratory findings, is critical for improving the survival rates of COVID-19 patients. By now, clinical and epidemiological characteristics of COVID-19 in cities outside of Wuhan, such as Beijing[1] and Wenzhou[2] are described. However, it is currently unknown whether there are any markers that can be informative of mild vs. severe disease. The objective of this study is to describe the comprehensive clinical characteristics of confirmed patients with COVID-19 and explore the potential markers correlating with prognosis.

We collected data from 119 hospitalized, symptomatic patients confirmed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) with throat swab specimens in Xiangyang, Hubei Province, between January and February 2020. The severe cases in this study refer to the patients who had enrolled to the intensive care unit (ICU) and received a treatment for more than 3 days, whereas the other confirmed cases were distributed to the mild group. As a control, we collected the laboratory results of 20 healthy subjects (normal cases) examined by the same laboratory department during early December 2019, when COVID-19 was not yet prevalent in Xiangyang. The epidemiological, clinical, laboratory and disease outcome data were obtained from data collection forms and electronic medical records. Information was collected on the date of illness onset, visits to clinical facilities, and hospital admissions. The date of disease onset was defined as the day when the symptom was first noticed. Laboratory tests were conducted at admission, including a complete blood count and serum biochemistry.

As shown in Table 1, we found that 85% (101 cases) of the patients were infected by another COVID-19 patient, 46% (55 cases) of the patients were categorized as collective cases, and 30% (36 cases) of patients were also diagnosed with a pre-existing medical condition. After hospital admission, 16.8 % (20 cases) of these patients progressed to severe disease, 4.2% (5 cases) of the patients had complications such as respiratory failure and distress, and 2.5% (3) patients succumbed to COVID-19. Fever was the most common symptom (86%, 102 cases), followed by fatigue (75%, 89 cases) and dry cough (63%, 75 cases). Headache and diarrhea were also reported among 14% (17 cases) and 12% (14 cases) cases, respectively.

Laboratory findings showed that decreased lymphocyte counts (Fig. 1A), as well as elevated levels D-dimer (Fig. 1B), may be early markers contributing to disease severity. Decreased albumin (Fig. 1C) and elevated CK (Fig. 1D) levels among severe patients indicate liver damage and shown as indicators of prognosis. Increased lactate dehydrogenase (LDH, Fig. 1E) and α-hydroxybutyrate dehydrogenase (HBDH, Fig. 1F) levels, indicative of heart damage, were detected in COVID-19 patients. Kidney damage in the COVID-19 patients was evidenced by urinary occult blood, increased C1q (Fig. 1G) and β2-MG (Fig. 1H) in COVID-19 patients.

In the study, we analyzed 119 cases of COVID-19 patients from a local hospital, in which 101 people had no residence or travel history to Wuhan, meaning most of the subjects in this study are non-first-generation cases. While some studies for clinical examinations have been published, many were not comprehensive and the studies took place in Wuhan.[3–6] Especially in the early stages of the outbreak, due to the overwhelmed medical system and lack of adequate medical resources and staff in Wuhan, clinical studies and laboratory examination results may not be reflective of the true nature of COVID-19 in patients. Indeed, this is reflected in the case fatality rates inside (4%) and outside of Wuhan (2.5%, according to our study). While other studies suggest that men are more susceptible to SARS-CoV-2 infection, there were no significant differences in susceptibility to the virus between men and women in our study, even though women had more mild disease cases. The results in this study support the suggestion that there are no significant differences in the levels of ACE2 (the re-

Table 1
Personal and clinical characteristics of patients with COVID-19 ($n = 119$).

Characteristics	No. (%)		
	All patients ($n = 119$)	Mild disease ($n = 99$)	Severe disease ($n = 20$)
Median (IQR) age (Y)	49 (38-61)	45 (34-57)	67.5 (60-77)
Age groups (Y):			
≤ 18	7 (6)	7 (7)	0 (0)
19-40	35 (30)	35 (35)	0 (0)
41-65	55 (46)	46 (46)	9 (45)
≥ 66	22 (18)	11 (11)	11 (55)
Gender			
Female	63 (53)	55 (56)	8 (40)
Male	56 (47)	44 (44)	12 (60)
Co-morbidities	36 (30)	18 (18)	17 (85)
Hypertension	23 (19)	10 (10)	13 (65)
Diabetes	12 (10)	7 (7)	5 (25)
Cardiovascular disease	7 (6)	3 (3)	4 (20)
Renal diseases	2 (2)	1 (1)	1 (5)
Liver disease	2 (2)	2 (2)	0 (0)
Travel history to Wuhan			
Yes	18 (15)	15 (15)	3 (15)
No	101 (85)	84 (85)	17 (85)
Cluster cases	55 (46)	47 (47)	8 (40)
Signs and symptoms			
Fever	102 (86)	86 (86)	16 (80)
Fatigue	89 (75)	75 (75)	14 (70)
Dry cough	75 (63)	63 (53)	13 (65)
Expectoration	22 (18)	16 (16)	6 (30)
Headache	17 (14)	15 (15)	2 (10)
Diarrhea	14 (12)	11 (11)	3 (15)
Pharyngalgia	11 (9)	10 (10)	1 (5)
Palpitation	6 (5)	4 (4)	2 (10)
Nausea and vomiting	4 (3)	3 (3)	1 (5)
Rhinobyon	3 (3)	2 (2)	1 (5)
Routine urinalysis			
Urine protein	21 (18)	21 (18)	0 (0)
Urinary occult blood	14 (12)	14 (12)	0 (0)
Symptom onset to hospital admission, median (IQR), days	5 (3-7)	5(3–7)	5 (4-9)
Symptom onset to laboratory confirmation via qRT-PCR, median (IQR), days	6 (4-9)	6 (4-8)	7 (5-11)
Symptom onset to negative detection via qRT-PCR, median (IQR), days	21 (18-24)	19 (15-21)	25 (23-27)

Abbreviations: IQR, interquartile range; Y, year.
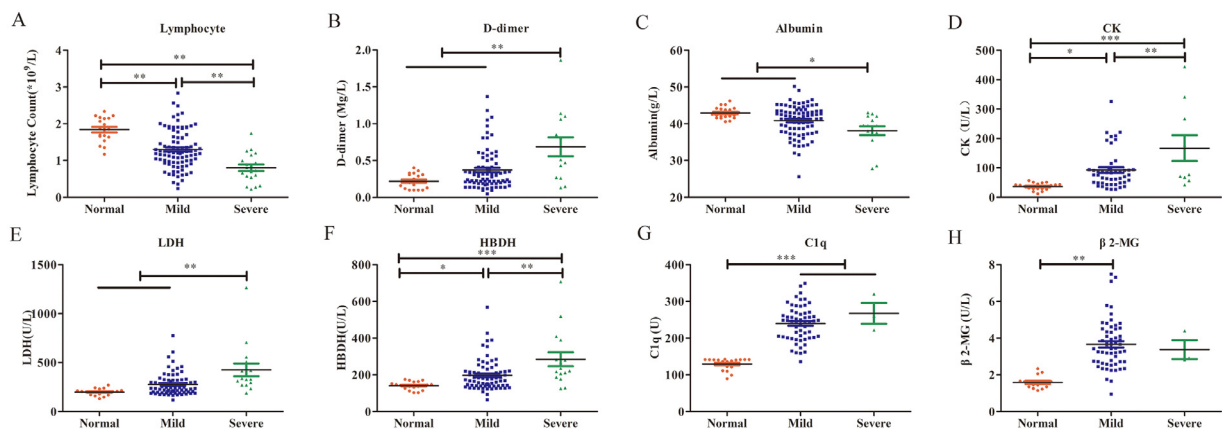
Fig. 1. Routine blood examination of severe, mild COVID-19 patients, and healthy people upon admission. (A) Lymphocyte counts and (B) D-dimer concentrations are shown as indicators of coagulation function, (C) Albumin and (D) Creatinine kinase (CK) levels/activities are shown as indicators of liver function, (E) Lactate dehydrogenase (LDH) and (F) -hydroxybutyrate dehydrogenase (HBDH) as indicators of myocardial damage, (G) Complement component 1q (C1q) and (H) 2-microglobulin (2-MG) as indicators for kidney damage. *P<0.05, **P<0.01 and ***P<0.001.

ceptor for SARS-CoV-2) expression between genders. As ACE2 is more highly expressed in elderly people, they theoretically would account for a higher percentage of the COVID-19 patients in this study.

Biomarkers that serve as reliable prognostic indicators predicting progression to mild vs. severe disease are urgently needed to enhance the quality of clinical care. In this study, we explored the possibility of identifying markers from a routine comprehensive laboratory examination. Consistent with other studies, we found that decreased lymphocyte counts and increased D-dimer concentrations might be an indication of a negative prognosis and enhanced disease severity. In addition, we provide several newly discovered bio-markers: decreased albumin as well as elevated CK, LDH and HBDH levels serve as indicators of a negative prognosis for COVID-19; urinary occult blood, increased C1q and β2-MG were observed in COVID-19 patients, indicated kidney damage.

The damage of SARS-CoV-2 to various major tissues and organs of the body during COVID-19 is an important area of investigation. In this study, we found that this virus can cause damage to the liver, heart and kidney, in which abnormal renal indicators may be caused by immunopathological damage. These findings are consistent with recent studies that the virus can damage multiple major organs including liver[7,8] kidney and heart[8]. The exact mechanism of this viral or immune-induced damage should be investigated in future studies.

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References

1. Tian S., Hu N., Lou J., Chen K., Kang X., Xiang Z., et al. Characteristics of COVID-19 infection in Beijing. *J Infect* 2020 pii: S0163-4453(20)30101-8. doi:10.1016/j.jinf.2020.02.018.
2. Yang W., Cao Q., Qin L., Wang X., Cheng Z., Pan A., et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): A multi-center study in Wenzhou city, Zhejiang, China. *J Infect* 2020 pii: S0163-4453(20)30099-2. doi:10.1016/j.jinf.2020.02.016.
3. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;**395**(10223):497–506. doi:10.1016/S0140-6736(20)30183-5.
4. Wang D., Hu B., Hu C., Zhu F., Liu X., Zhang J., et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020. doi:10.1001/jama.2020.1585.
5. Chen H., Guo J., Wang C., Luo F., Yu X., Zhang W., et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet* 2020. doi:10.1016/s0140-6736(20)30360-3.
6. Kui L., Fang Y.Y., Deng Y., Liu W., Wang M.F., Ma J.P., et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl)* 2020. doi:10.1097/CM9.0000000000000744.
7. Chen N Z.M., Dong X., Qu J., Gong F., Han Y., Qiu Y., Wang J., Liu Y., Wei Y., Xia J., Yu T., Zhang X., Zhang L. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020;**395**(10223):507–13. doi:10.1016/S0140-6736(20)30211-7.
8. Zhang L., Shen F.M., Chen F., Lin Z.. Origin and evolution of the 2019 novel coronavirus. *Clin Infect Dis* 2020 pii: ciaa112. doi:10.1093/cid/ciaa112.

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A cluster of pneumonia associated with the SARS-Cov-2 outside of Wuhan related to a house-warming banquet

Dear Editor,

We read the recent published review by Han and colleagues in this journal with great interest, which described the epidemiology, clinical features and treatment of Corona Virus Disease 2019 (COVID-19) and mentioned that people who were asymptomatic or in incubation period can be the sources of infection.[1]

An ongoing epidemic caused by a new coronavirus, which was formally named as SARS-Cov-2 by Coronavirus research group (CSG) of the International Committee, started in late 2019 in Wuhan, Hubei province, China and had become a worldwide public health concern.[2] A total of 81300 cases were confirmed in China so far (March 20, 2020) and increasingly more cases were confirmed abroad, especially in Europe. On Jan 30, 2020, the WHO Emergency Committee declared the outbreak as a global health emergency. Furthermore, it still remains unclear about the origin, transmission mode and incubation period of the SARS-Cov-2. Previous research has demonstrated the person-to-person transmission of the novel Coronavirus.[3]

Here, we report the clinical features of a cluster of seven patients suffered from pneumonia associated with the SARS-Cov-2 in Lishui City of Zhejiang Province, China. Epidemiological, demographic, clinical, laboratory, radiological and treatment data were collected through detailed interviews with each patient and electronic medical records. Laboratory confirmation of the virus was performed using RT-PCR by Lishui Center for Disease Control and Prevention (CDC). Case definitions of confirmed COVID-19 are in accordance with the interim guidance from a rapid advice guideline.[4]

The first enrolled patient (Patient A) returned to Lishui from Wenzhou on Jan 18, 2020 and participated a house-warming banquet. Two days later, he suffered with fever, chill, cough and generalized weakness, and was hospitalized on Jan 23, later diagnosed as COVID-19 on Jan 25. Patient B, father of patient A, presented with cough and fever on Jan 21 and was diagnosed on Jan 27. On the same day, patient C was diagnosed as COVID-19 with primary symptoms of cough and sputum. Tracking the medical history of
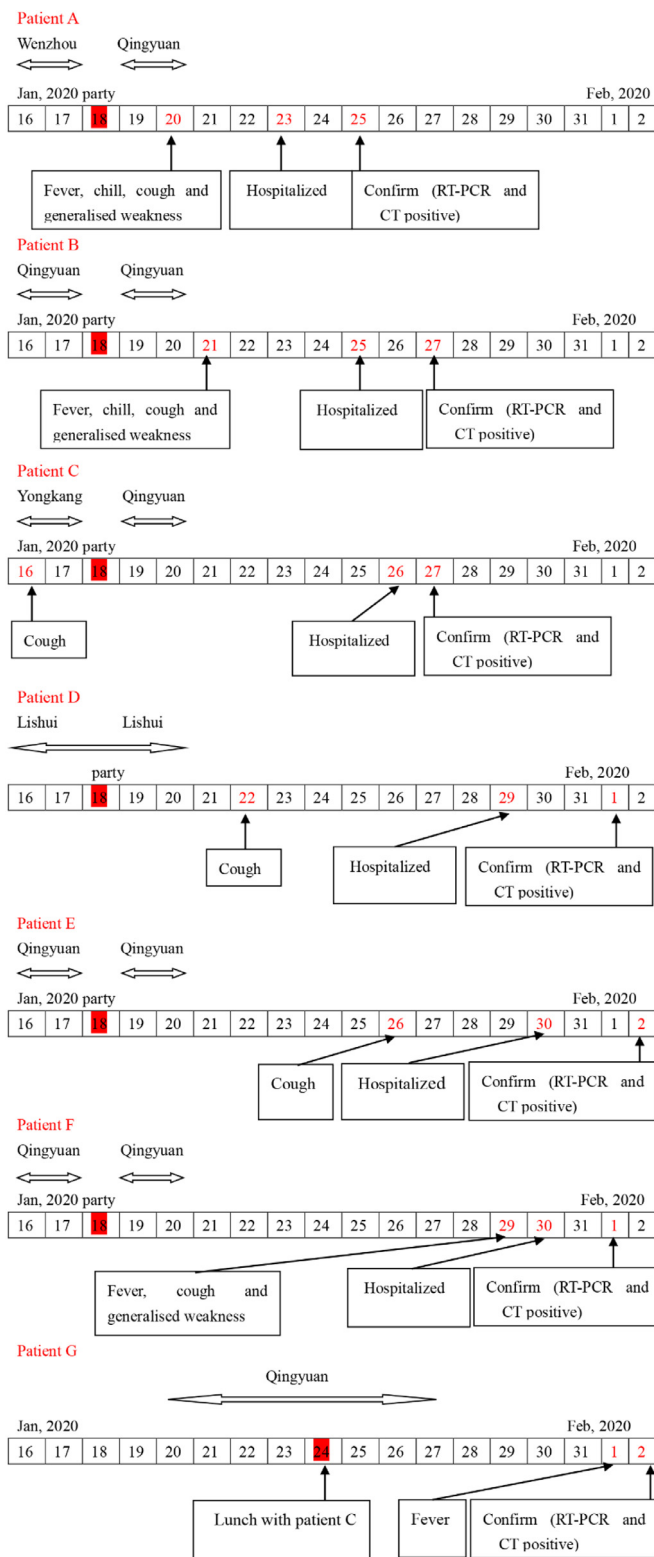
Fig. 1. Timeline of symptom onset of the cluster patients related to a house-warming banquet.

patient C, he began to cough on Jan 16 (two days before the banquet) when he was still in Yongkang, a city adjacent to Lishui. Patient D developed a bad cough on Jan 22 and patient E presented with similar symptom on Jan 26. They were diagnosed on Feb 1 and Feb 2, respectively. Patient F was hospitalized on Jan 30 with fever, chill, cough and generalized weakness and diagnosed as COVID-19 on Feb 1. All the above six patients attended the same house-warming banquet. Oddly enough, patient G, who did not at-

tend the same party, had fever on Feb 1 and was soon diagnosed on Feb 2. Tracing the history of epidemiology, she had dinner with patient C on Jan 24. It is worth noting that the cluster of seven patients did not go to Wuhan or Hubei province in the preceding 14 days, and they denied any exposure with wild animals, confirmed COVID-19 patients or febrile patients (Fig. 1).

Two possible scenarios of transmission exist. The first and most likely scenario is that patient A acquired the infection from an asymptomatic patient in Wenzhou (a city with most cases in Zhejiang province) before Jan 18, 2020. This period overlapped with the time period during the announcement of the first case of COVID-19 in Wenzhou (symptom onset on Jan 4, diagnosed on Jan 21) according to the Zhejiang Provincial Health Committee (http://www.zjwjw.gov.cn/col/col1659205/index.html). The other five patients (B∼F) attended the same house-warming banquet and had lunch together in a crowded, noisy hotel restaurant with patient A. Even more, patient B and C had taken the same car with patient A from Lishui back to Qingyuan. As a result, all the five patients were in close contact with patient A, who most likely had been infected before. Then patient G was infected because she was exposed to patient C. The second scenario is that patient C acquired the infection in Yongkang, because he had begun coughing two days before the banquet. As described in the first scenario, other patients were all in close contact with him and got infected. Though the median incubation varies from 3∼6.4 days according to the previous studies,[5,6] it is sometimes difficult to calculate the incubation period accurately such as patient A and patient C. Regardless of how the virus transmitted among the seven patients, it further indicated that the SARS-Cov-2 infection could cause clustering onset.[7] Quarantine and keeping social distance paly pivotal roles in the prevention of local outbreak of COVID-19.[8] The exact transmission mode still needs to be further studied.

The seven patients were aged 23–72 years old. Except patient G, the remaining 6 patients were all males. All patients had no chronic disease except patient C, who had mania years ago. On admission, cough (7/7), generalised weakness (7/7), sore throat (7/7), fever (4/7) and insomnia (4/7) were the main symptoms. The range of incubation period from exposure to symptoms was 3∼11 days (Table 1).

Regarding the inflammatory indicators, leucocytes, neutrophils, lymphocytes and procalcitonin (PCT) of all the patients were normal and C-reactive protein (CRP) were slightly increased in three patients on admission. Liver function was mildly abnormal in one patient and no patient developed renal dysfunction. Abnormalities in chest CT images were detected among all patients, among which 5 patients showed bilateral pneumonia while only 2 patients showed unilateral pneumonia. All patients showed multiple mottling and ground-glass opacities. Compared with COVID-19 patients initially found in Wuhan, all patients in this study had mild to moderate symptoms. No complications such as acute respiratory distress syndrome, renal dysfunction, myocardial injury and secondary bacterial infection occurred. Symptoms and abnormalities in chest CT scan of all the 7 patients disappeared or significantly improved after antiviral and supportive treatments.

In conclusion, this study further indicates that the SARS-Cov-2 infection can cause clustering onset, person-to-person transmission and intercity spread, and it is more likely to happen in crowded places. We urgently need to pay attention to the scale of transmission from asymptomatic or mildly symptomatic patients during the early phase of infection.

Ethics approval

The study has been approved by the research ethics committee of Lishui Peoples' Hospital (LLW-FO-403). Written informed consent has been waived in light of the urgent need to collect clinical data.

Table 1
Summary of clinical features of the cluster patients infected with SARS-Cov-2 (on admission).

Index	Patient A	Patient B	Patient C	Patient D	Patient E	Patient F	Patient G
Age(Year)	45	72	51	36	51	23	47
Sex	Male	Male	Male	Male	Male	Male	Female
Occupation	Business man	Farmer	Farmer	Farmer	Farmer	Civil servant	Farmer
Smoke	Y	Y	N	Y	Y	Y	N
Alcohol drinking habit	Y	Y	N	Y	Y	Y	N
Chronic medical illness	N	N	Mania	N	N	N	N
Interval between Admission to hospital and Symptom onset (days)	3	4	Uncertain	7	4	1	0.5
Incubation period (days)	Uncertain	3	Uncertain	4	8	11	8
Presenting symptoms and sign							
Fever	Y	Y	N	N	N	Y	Y
Chill	Y	Y	N	N	N	N	N
Cough	Y	Y	Y	Y	Y	Y	Y
Nasal congestion	Y	N	N	N	Y	N	Y
Rhinorrhoea	Y	N	N	N	Y	N	Y
Sneezing	Y	N	N	N	Y	N	Y
Dyspnea	Y	N	N	N	N	N	N
Sore throat	Y	Y	Y	Y	Y	Y	Y
Chest pain	N	N	N	N	N	N	N
Generalized weakness	Y	Y	Y	Y	Y	Y	Y
Diarrhoea	Y	Y	Y	Y	N	N	N
Nausea and(or) Vomiting	Y	N	Y	N	N	N	N
Headache	N	N	N	N	N	N	Y
Insomnia	Y	Y	Y	N	N	N	Y
Body temperature (°C)	39	38	36.3	36.8	37	38.4	37.5
Oximetry saturation (%)	98	95	98	99	99	99	98

N: no; Y: yes.

Declaration of Competing Interest

None

Reference

1. Han Q., Lin Q., Jin S., You L.. Coronavirus 2019-nCoV: A brief perspective from the front line. *J Infect* 2020. doi:10.1016/j.jinf.2020.02.010.
2. Zhu N., Zhang D., Wang W., Li X., Yang B., Song J., et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *Engl J Med* 2020. doi:10.1056/NEJMoa2001017.
3. Chan J.F.-W., Yuan S., Kok K.-H., To K.K.-W., Chu H., Yang J., et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;**395**:514–23. doi:10.1016/S0140-6736(20)30154-9.
4. Jin Y.H., Cai L., Cheng Z.S., Cheng H., Deng T., Fan Y.P., et al. A rapid advice guideline for the diagnosis and treatment of 2019 novel coronavirus (2019-nCoV) infected pneumonia (standard version). *Mil Med Res* 2020;**7**:4. doi:10.1186/s40779-020-0233-6.
5. Guan W.J., Ni Z.Y., Hu Y., Liang W.H., Ou C.Q., He J.X., et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *Engl J Med* 2020. doi:10.1056/NEJMoa2002032.
6. Xu X.W., Wu X.X., Jiang X.G., Xu K.J., Ying L.J., Ma C.L., et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. *BMJ* 2020;**368**:m606. doi:10.1136/bmj.m606.
7. Chen N., Zhou M., Dong X., Qu J., Gong F., Han Y., et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* 2020;**395**:507–13. doi:10.1016/S0140-6736(20)30211-7.
8. Wilder-Smith A., Freedman D.O.. Isolation, quarantine, social distancing and community containment: pivotal role for old-style public health measures in the novel coronavirus (2019-nCoV) outbreak. *J Travel Med* 2020. doi:10.1093/jtm/taaa020.

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Clinical and CT imaging features of 2019 novel coronavirus disease (COVID-19)

Dear Editor,

Tang JW, et al. and colleagues have written to this Journal describing the emergence of 2019 novel coronavirus disease (COVID-19).[1] We have had an opportunity to examine in detail the chest computed tomography (CT) findings in cases with microbiologically confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, to familiarize radiologists and clinicians with the imaging manifestations of this new outbreak. Meanwhile, we also studied the clinical characteristics of the cases, combined with CT manifestations, to provide more clues for the correct diagnosis of the disease.

Fourteen patients (P1–P14) aged from 10 to 75 years were referred to the fever clinic of our hospital, 7 of them (P8–P14) were from Hui-Ya branch of our hospital. P9 and P14 had diabetes and other patients had no underlying diseases. Among the 14 cases, 10 of them had a history of exposure to Wuhan or Hubei, while, P6 and P7 had no clear epidemiological history. P3/P4, P10/P11 were family clustering disease. All the patients performed oropharyngeal swabs test and confirmed as COVID-19. Common respiratory viruses, mycoplasma and chlamydia were negative. For patients' venous blood tests at disease onset, as given in (Table 1), we found that leucocytes and lymphocytes were slightly decreased or

Table 1
Clinical characteristics of the 14 patients infected with 2019-nCoV.

Patient	Gender	Age, years	Epidemiology	2019-nCoV-RNA test results of oropharyngeal swabs	Chief complaint	CRP, mg/L, 0.00–10.00	WBC#, x10^9/L, 4.00–10.00	NEUT#, x10^9/L, 1.80–6.40	LY#, x10^9/L, 1.00–3.30	EO#, x10^9/L, 0.05–0.50	PCT, ng/mL, 0.00–0.05
P1	F	27	Went to Wuhan 17 days ago	The second test was positive	Fever (<38°)	5.78	3.77	2.55	0.98	0.01	NA
P2	F	32	Went to Wuhan 12 days ago	Two tests were positive	Fever for 1 day (<38°)	1.47	3.40	2.22	0.80	0.01	NA
P3	F	56	Went to Hubei 1 day ago	The first test was positive	Fatigue and fever for 1 day (<38°)	6.40	5.69	3.45	1.42	0.20	0.04
P4	F	32	Family cluster with P3	The second test was positive	Fever for 1 hour (<38°)	1.60	6.87	5.30	1.01	0.12	0.04
P5	F	63	Went to Wuhan 2 days ago	Two tests were positive	Fever (<38°)	2.85	4.08	3.13	0.55	0.01	NA
P6	F	54	No clear history related to Wuhan	The first test was positive	Fever (40°C)	63.79	5.89	4.15	1.31	0.01	NA
P7	F	49	No clear history related to Wuhan	The first test was positive	Fever	10.36	3.76	2.56	0.94	0.00	NA
P8	F	35	Went to Wuhan 14 days ago	The first test was positive, the second test was negative	Fever for half day (37.5°C)	6.50	3.25	1.93	1.10	0.00	0.027
P9	F	63	Went to Wuhan 7 days ago	The fourth test was positive	Fever for 2 days (37.1°C)	88.20	5.92	5.20	0.55	0.00	0.208
P10	M	41	Contacted with person from Wuhan 25 days ago	The first test was positive	Fever for 2 days (37.8°C)	15.90	6.13	3.43	1.99	0.00	0.028
P11	F	10	Family cluster with P10	Two tests were positive	Asymptomatic (37.1°C)	0.00	6.92	5.11	1.59	0.00	0.020
P12	F	66	Went to Wuhan 4 days ago	The first test was positive	Dizziness, vomiturition, fever (38.9°C) and myalgia for more than 1 day	25.80	6.84	4.99	1.56	0.00	0.065
P13	F	54	Went to Wuhan 3 days ago	The first test was positive	Chills and headache for 3 days, fever (37.6°C) for 1 day	9.70	3.38	2.34	0.61	0.01	0.050
P14	M	75	From Wuhan	The first test was positive	Cough and fever 1 week (38.9°C)	78.70	5.36	4.28	0.93	0.00	0.105

Abbreviations: F, female; M, male; CRP, C-reactive protein; WBC, white blood cells; NEUT, neutrophil; LY, lymphocyte; EO, eosnophils; #: cell count; NA, not applicable. Note: The normal reference value range is listed behind the blood test index.

Table 2
Imaging characteristics during the first visit.

| Imaging characteristics	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11
Lobar location											
RUL						√		√	√	√	
RML	√	√							√	√	
RLL	√	√			√	√	√	√	√	√	√
LUL			√					√	√	√	
LLL				√		√	√	√	√	√	
Distribution											
Subpleural	√	√			√	√	√	√	√		√
Random or diffuse			√	√						√	
Morphology											
Patchy-like	√	√			√	√		√	√		
Nodular-like			√	√							√
Both							√			√	
Contour											
Clear			√			√					√
Blurry	√	√		√	√		√	√	√	√	
Attenuation											
GGO only	√		√	√	√				√		
Mixed GGO and consolidation		√				√	√	√		√	√
Consolidation only											
Other signs											
Reticulation						√					
crazy paving	√							√	√	√	
Cavitation											
Bronchiectasis						√					
Pleural effusion											
Lymphadenopathy											

Abbreviations: RUL-right upper lobe, RML-right middle lobe; RLL-right lower lobe, LUL-left upper lobe, LLL-left lower lobe; GGO, ground glass opacity. "P#" represents one patient. Note: Check mark (√) indicate the appearance of the corresponding sign.
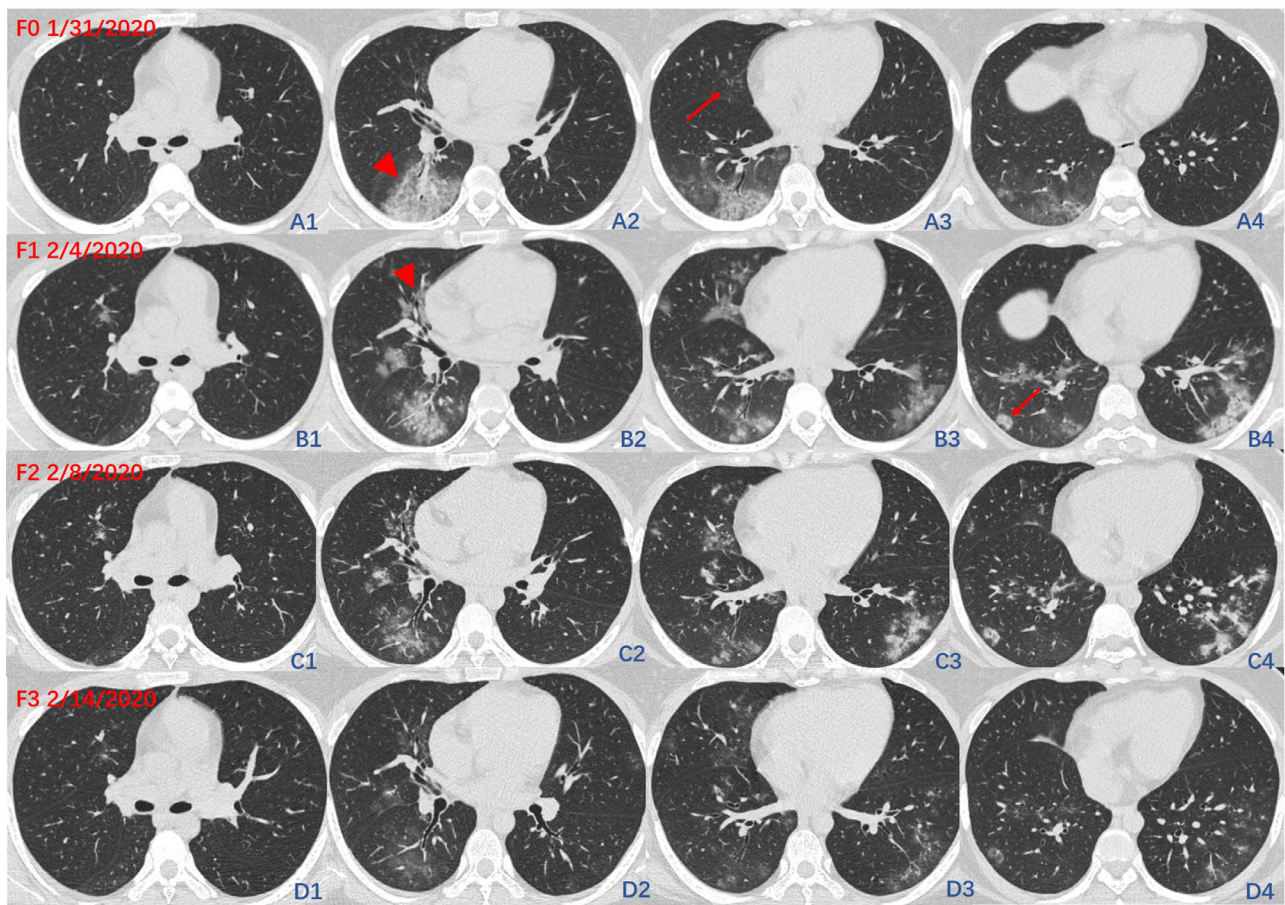
Fig. 1. The initial CT images (F0) and three times of follow-up CT images (F1–F3) of P1. F0 showed patchy-like pure GGO located in the subpleural regions of the right middle lobe (F0, A3, arrow) and the right lower lobe, accompanied by crazy paving sign (F0, A2, arrowhead). Follow-up 1(F1, B1–B4): CT images showed diseases progression. The lesions manifested as coexisted nodular-like (F1, B4, arrow) and patchy-like lesions as well as peribronchial (F1, B2, arrowhead), central and subpleural distribution. The lesions are migratory manifested as the absorption of the primary lesions and the emergence of new lesions. CT images of Follow-up 2 (F2, C1–C4) and Follow-up 3 (F3, D1–D4) showed lesion absorption.
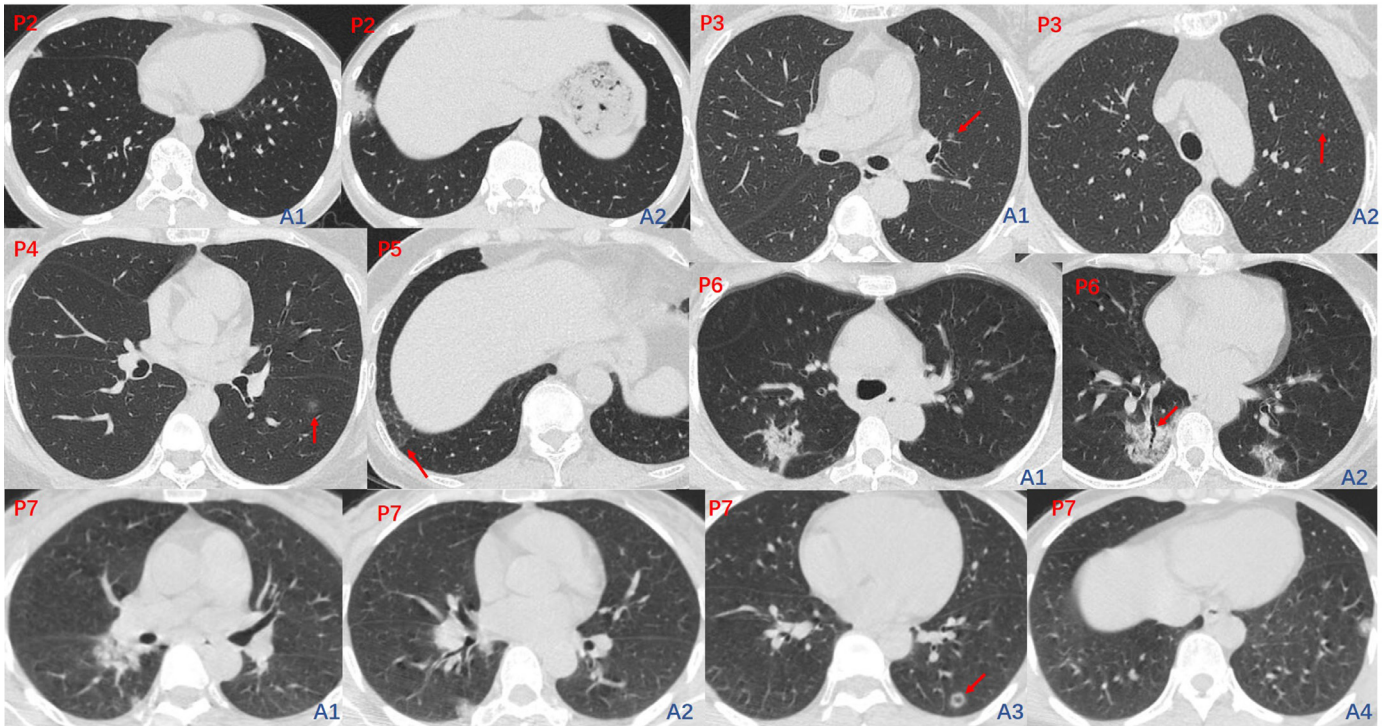
Fig. 2. The initial CT images of P2-P7. CT images of P2 (Fig. 2, P2, A1-A2), P5 (Fig. 2, P5), P6 (Fig. 2, P6, A1-A2) and P7 (Fig. 2, P7, A1–A4) showed subpleural lesions, a nodular-like lesion with pseudocavitary sign (Fig. 2, P7, A3, arrow) and mild bronchiectasis (Fig. 2, P6, A2, arrow) were also observed within the lesion. CT images of P3 (Fig. 2, P3, A1-A2) and P4 (Fig. 2, P4) showed round nodular-like GGO lesions (P3 and P4, arrow) located in the central area of the lung.
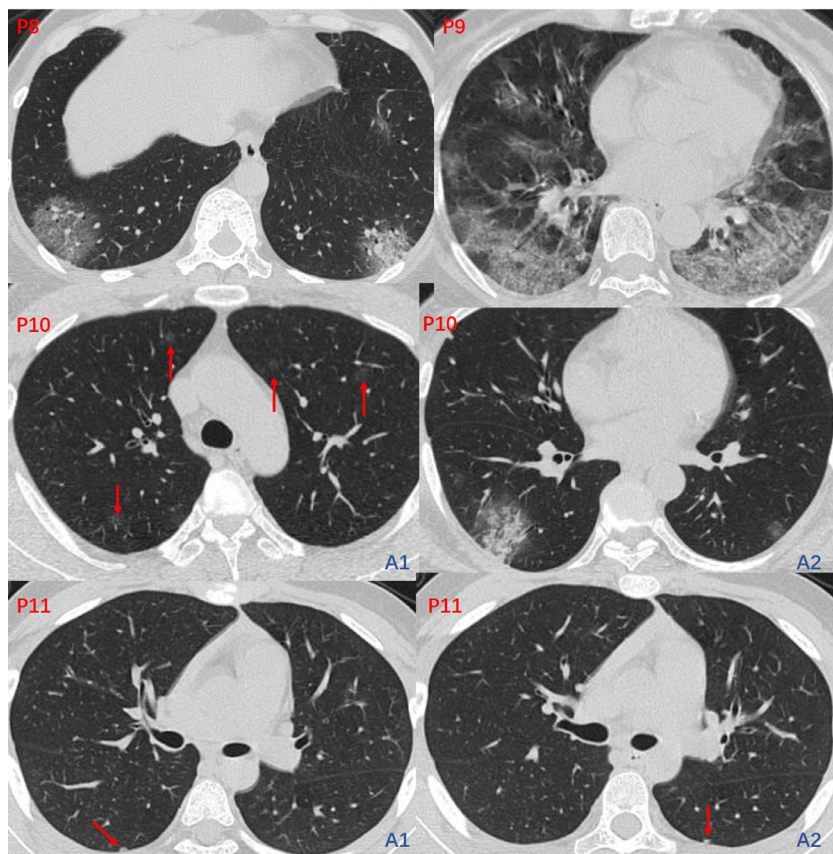
Fig. 3. The initial CT images of P8-P11. CT images of P8 (Fig. 3, P8) and P9 (Fig. 3, P9) showed bilateral subpleural lesions with crazy paving sign. CT images of P10 (Fig. 3, P10, A1-A2) showed bilateral multiple lesions, some of them were pure GGO located in the central region of the lung. CT images of P11 (Fig. 3, P11, A1-A2) showed bilateral subpleural small nodular-like lesions.

normal, eosinophil count were slightly decreased in 12 cases and normal in 2 cases. Neutrophil counts were normal for all the patients, and CRP was increased in 6 cases. PCT was normal or slightly elevated.

On admission, 11 patients (P1–P11) underwent high resolution chest CT examination and their manifestations were shown in (Table 2). P1 (Fig. 1, F0, A1–A4): CT images showed patchy-like pure ground glass opacity (GGO) involving subpleural regions of the right middle lobe (Fig. 1, F0, A3, arrow) and the right lower lobe. The slightly thickened interlobular septa within the lesion makes it appear the crazy paving sign (Fig. 1, F0, A2, arrowhead). P2 (Fig. 2, P2, A1-A2): CT images showed mixed GGO and consolidation that appeared at subpleural area of the right middle lobe and the right lower lobe. The lesion presented as patchy-like morphology. P3 (Fig. 2, P3, A1-A2): CT images showed two well circumscribed, round nodular-like GGO lesions (Fig. 2, P3, arrow) located in the central area of the left upper lobe. P4 (Fig. 2, P4): CT images showed a small nodular-like pure GGO (Fig. 2, P4, arrow) located in the central area of the left lower lobe. P5 (Fig. 2, P5): CT images showed a slight of irregular pure GGO (Fig. 2, P5, arrow) located in the subpleural region of the right lower lobe. P6 (Fig. 2, P6, A1-A2): CT images showed bilateral multi-focal mixed GGO and consolidation appeared at subpleural area of lung. Mild bronchiectasis (Fig. 2, P6, A2, arrow) can also be observed within the lesion. P7 (Fig. 2, P7, A1–A4): CT images showed bilateral subpleural lesions, among which the lesion in the left lower lobe was nodular-like with pseudocavitary sign (Fig. 2, P7, A3, arrow). P8 (Fig. 3, P8) and P9 (Fig. 3, P9): CT images showed bilateral subpleural lesions with crazy paving sign. P10 (Fig. 3, P10, A1-A2): CT images showed bilateral multiple lesions, some of them were pure GGO located in the central region of the lung. P11 (Fig. 3, P11, A1-A2): CT images showed bilateral subpleural small nodular-like lesions.

P1 had three follow-up CTs (Fig. 1, F1–F3). The time interval between initial chest CT and follow-up were 4, 8, 14 days. Follow-up 1(Fig. 1, F1, B1–B4): CT images showed diseases progression. The lesions showed diversified morphology and distribution, appearing as coexisted nodular-like (Fig. 1, F1, B4, arrow) and patchy-like lesions as well as peribronchial (Fig. 1, F1, B2, arrowhead), central and subpleural distribution. CT images of F1 showed the that lesions were migratory manifested as the absorption of the primary lesions and the emergence of new lesions. CT images of Follow-up 2 (Fig. 1, F2, C1-C4) and Follow-up 3 (Fig. 1, F3, D1–D4) showed the diseases were obviously absorbed.

In the current study, we investigated the detailed information including clinical features and CT imaging characteristics of 6 patients with COVID-19. Our research has some new findings on the basis of previous study: (1) The decrease of eosinophil count may be helpful for the early diagnosis of the disease. Nevertheless, till now, there is no study refer to blood tests[2,3] mentioned eosinophil, which is worthy of further study. (2) Our CT study found that COVID-19 has a variety of manifestations. In the early stage of the disease, the lesion can manifest as round nodular-like GGO in the central area of the lung lobe, which is different from the common imaging manifestations that are patchy-like lesion in subpleural region.[4–6] (3) The follow-up CT images showed the lesions are migratory manifested as the absorption of the primary lesions and the emergence of new lesions, which had not been reported yet. (4) The false negative rate of oropharyngeal swabs seems high. As we know that, oropharyngeal swabs are the recommended upper respiratory tract specimen types for SARS-CoV-2 diagnostic testing,[7,8] so a new detection technique should be developed as soon as possible.

Declaration of Competing Interest

The authors declare that they have no competing interests.

References

1. Tang J.W., Tambyah P.A., Hui D.S.C.. Emergence of a novel coronavirus causing respiratory illness from Wuhan, China. *J Infect* 2020 Jan 28. doi:10.1016/j.jinf.2020.01.014.
2. Huang C., Wang Y., Li X., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;**395**(10223). doi:10.1016/S0140-6736(20)30183-5.
3. Wang D., Hu B., Hu C., et al. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. *JAMA* 2020. doi:10.1001/jama.2020.1585.
4. Song F., Shi N., Shan F., et al. Emerging Coronavirus 2019-nCoV Pneumonia. *Radiology* 2020. doi:10.1148/radiol.2020200274.
5. Xie X., Zhong Z., Zhao W., et al. Chest CT for Typical 2019-nCoV Pneumonia: Relationship to Negative RT-PCR Testing. *Radiology* 2020. doi:10.1148/radiol.2020200343.
6. Pan Y., Guan H., Zhou S., et al. Initial CT findings and temporal changes in patients with the novel coronavirus pneumonia (2019-nCoV): a study of 63 patients in Wuhan, China. *Eur Radiol* 2020. doi:10.1007/s00330-020-06731-x.
7. Chan J.F., Yuan S., Kok K.H., et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 2020;**395**(10223). doi:10.1016/S0140-6736(20)30154-9.
8. To K.K., Tsang O.T., Chik-Yan Yip C., et al. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* 2020. doi:10.1093/cid/ciaa149.

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The role of phylogenetic analysis in clarifying the infection source of a COVID-19 patient

Dear Editor,

Previous reports indicated that the emergence of the novel coronavirus (SARS-CoV-2) infection (COVID-19) had raised global concern and was characterized as a pandemic event by the World Health Organization on March 11, 2020.[1-3] Till March 18, 2020, it has spread to 146 countries, including Taiwan.[4] People in Taiwan and mainland China travel frequently, which put Taiwan at a great risk of acquiring an epidemic of COVID-19. Taiwan has been on constant alert and react rapidly to epidemics change from China ever since the severe acute respiratory syndrome (SARS) epidemic in 2003 and has done much effort on the containment of COVID-19 with success.[5] Till March 18, 2020, there are only 100 cases of COVID-19 noted in Taiwan, including 79 imported cases and 21 cases belonging to seven occasions of limited local transmission (six family clusters and two transmissions in social societies).[4]

To contain the epidemics of COVID-19, prevention from both import and export of contagious people is an essential intervention. It is also important to clearly clarify the infection source in

Table 1
Summary of SARS-CoV-2 alignment.

MN908947 position	MN	A1a	A1	A2a	A2	A3	A5	B1	B2	B4-1	B4-2	NTU01	NTU02	NTU03	CDC02	CDC03	CDC04	CGMH1	CDS	CDS position	Codon	Amino acid
187	A	A	A	G	A	n/a	A	A	A	A	A	A	A	A	A	A	A	A	5'UTR	n/a	n/a	n/a
241	C	C	C	T	T	n/a	C	C	C	C	C	C	C	T	C	C	C	C	5'UTR	n/a	n/a	n/a
1397	G	G	G	G	G	A	G	G	G	G	G	G	G	G	G	G	G	G	orf1a	1132	GTA>ATA	378 V>I
2091	C	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	orf1a	1826	ACT>ATT	609 T>I
2113	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	orf1a	1848	ATC>ATT	616 I>I
3037	C	C	C	T	T	C	C	C	C	C	C	C	C	T	C	C	C	C	orf1a	2772	TTC>TTT	924 F>F
4402	T	T	T	T	T	T	T	T	T	T	C	T	T	T	T	T	T	T	orf1a	4137	CTT>CTC	1379 L>L
5062	G	G	G	G	G	G	G	G	T	G	G	G	G	G	G	G	G	G	orf1a	4797	TTG>TTT	1599 L>F
8782	C	C	C	C	C	C	C	T	T	T	T	T	C	C	C	T	C	C	orf1a	8517	AGC>AGT	2839 S>S
9034	A	A	A	A	A	A	A	A	A	A	A	A	G	A	A	A	A	A	orf1a	8769	AAA>AAG	2923 K>K
9430	C	C	C	C	C	C	C	A	C	C	C	C	C	C	C	C	C	C	orf1a	9165	ATC>ATA	3055 I>I
9491	C	C	C	C	C	C	C	C	C	C	C	C	T	C	C	C	C	C	orf1a	9226	CAT>TAT	3076 H>Y
11083	G	T	G	G	G	T	G	G	G	G	G	G	G	G	G	G	G	G	orf1a	10818	TTG>TTT	3606 L>F
13679	A	A	G	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	orf1b	212	TAC>TGC	71 Y>C
14408	C	C	C	T	C	C	C	C	C	C	C	C	C	T	C	C	C	C	orf1b	941	CCT>CTT	314 P>L
14805	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	orf1b	1338	TAC>TAT	446 Y>Y
15863	C	C	C	C	C	C	C	C	C	C	C	C	Y	C	C	C	C	C	orf1b	2396	GGA>GTA	799 G>V
16188	G	G	G	G	G	G	G	G	G	G	G	G	G	T	G	G	G	G	orf1b	2721	TGG>TGT	907 W>C
17247	T	C	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	orf1b	3780	CGT>CGC	1260 R>R
17373	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	orf1b	3906	GCC>GCT	1302 A>A
17747	C	C	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	orf1b	4280	CCT>CTT	1427 P>L
17858	A	A	A	A	A	A	A	G	A	A	A	A	A	A	A	A	A	A	orf1b	4391	TAT>TGT	1464 Y>C
18060	C	C	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	orf1b	4593	CTC>CTT	1531 L>L
18603	T	T	T	T	T	T	T	T	T	C	T	T	T	T	T	T	T	T	orf1b	5136	CAT>CAC	1712 H>H
18877	C	C	C	C	C	C	C	C	C	C	C	C	T	C	C	C	C	C	orf1b	5410	CTA>TTA	1804 L>L
18928	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	C	orf1b	5461	CCT>TCT	1821 P>S
18975	T	T	T	T	T	T	T	T	A	T	T	T	T	T	T	T	T	T	orf1b	5508	GTT>GTA	1836 V>V
19175	A	A	A	A	A	A	A	A	C	A	A	A	A	A	A	A	A	A	orf1b	5708	GAT>GCT	1903 D>A
21707	C	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	C	C	S	145	CAT>TAT	49 H>Y
23403	A	A	A	G	G	A	A	A	A	A	A	A	A	G	A	A	A	A	S	1841	GAT>GGT	614 D>G
24378	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	S	2816	TCT>TTT	939 S>F
25563	G	G	G	G	G	G	G	G	G	G	G	G	G	T	G	G	G	G	orf3a	171	CAG>CAT	57 Q>H
25964	A	A	A	A	A	A	A	A	A	A	A	A	G	A	A	A	A	A	orf3a	572	GAA>GGA	191 E>G
26144	G	T	G	G	G	G	G	G	G	G	G	G	T	G	G	G	G	G	orf3a	752	GGT>GTT	251 G>V
26211	G	G	G	G	G	G	G	G	G	G	G	G	K	G	G	G	G	G	orf3a	819	GTG>GTT	273 V>V
26894	C	T	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	M	372	CTC>CTT	124 L>L
27925	C	C	C	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	orf8	32	ACA>ATA	11 T>I
28144	T	T	T	T	T	T	T	C	C	C	C	C	T	T	T	C	T	T	orf8	251	TTA>TCA	84 L>S
28688	T	T	T	T	T	C	T	T	T	T	T	T	T	T	T	T	T	T	N	415	TTG>CTG	139 L>L
28878	G	G	G	G	G	G	G	G	G	G	A	G	G	G	G	G	G	G	N	605	AGT>AAT	202 S>N
29095	C	C	C	C	C	C	C	C	T	C	C	C	C	C	C	C	C	C	N	822	TTC>TTT	274 F>F
29374	G	G	G	G	G	A	G	G	G	G	G	G	G	G	G	G	G	G	N	1101	GAG>GAA	367 E>E
29742	G	G	G	G	G	n/a	G	G	G	G	A	n/a	G	G	G	G	G	G	3'UTR	n/a	n/a	n/a

SARS-CoV-2 genetic sequences, including NTU03, all 6 previously submitted Taiwan sequences, and representative sequences for clades A1, A1a, A2, A2a, A3, A5, B1, B2, and B4, were aligned and compared. MN908947 was used as the reference sequence, and orange shaded nucleotides indicated nucleotides different to the reference nucleotide, and gray shaded positions indicated lack of nucleotide information. The NTU01 and CDC03 are derived from the Case 3 patient, and CDC04 and CGMH1 are from the from the Case 4 patient. Sequence data were obtained from GISAID (https://www.gisaid.org/CoV2020/) . The GISAID accession number for each representative clade sequence: A1a, hCoV-19/Switzerland/1,000,477,102/2020|EPI_ISL_413,019; A1, hCoV-19/Singapore/11/2020|EPI_ISL_410,719; A2a, hCoV-19/Italy/UniSR1/2020|EPI_ISL_413,489; A2, hCoV-19/Germany/BavPat1/2020|EPI_ISL_406,862; A3, hCoV-19/Australia/NSW13/2020|EPI_ISL_413,599; A5, hCoV-19/USA/CA5/2020|EPI_ISL_408,010; B1, hCoV-19/USA/WA12-UW8/2020|EPI_ISL_413,563; B2, hCoV-19/USA/TX1/2020|EPI_ISL_411,956; B4-1, hCoV-19/South Korea/KUMC03/2020|EPI_ISL_413,513; B4-2, hCoV-19/USA/CA7/2020|EPI_ISL_411,954.
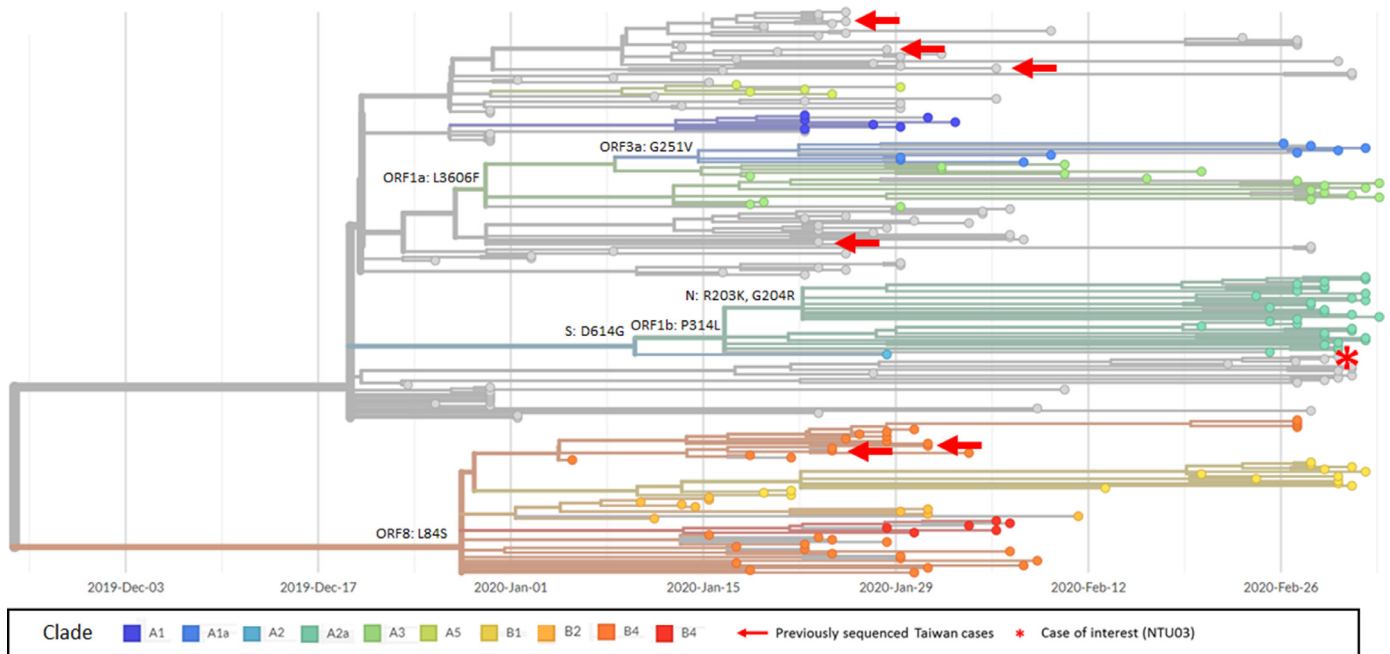
Fig. 1. Phylogenetic analysis of the full-length SARS-CoV-2 sequences. The phylogeny tree analysis was conducted to determine the clade of NTU03 (red asterisk) and its relationship to other viral sequences derived from case patients identified in Taiwan (red arrows). The phylogenetic tree was generated and modified for display purposes from Nextstrain (https://nextstrain.org/ncov),[9] which uses genetic sequences and metadata from GISAID (https://www.gisaid.org/CoV2020/) and sequence submission date for the horizontal axis.[10] The phylogenetic tree was generated at 2020/03/09 6PM (GMT+8) with a total of 240 viral genomes sampled (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

order to initiate an efficient and successful contact tracing for the SARS-CoV-2 infected patients, and thereafter people exposed to the contagious patients could be quarantined to avoid further disease spreading. Here we present a COVID-19 patient whose infection source could not be completely clarified initially, and later was illuminated by using the phylogenetic analysis of the isolated virus.

This 66-year-old Taiwanese woman was well before. She traveled to Dubai from January 29 to February 10, 2020, and Egypt from February 11 to February 21, 2020. When she stayed in Egypt, she ever participated an eight-day tourism on a Nile cruise boat. She returned to Taiwan via an international airline on February 21, 2020. She began to suffer from general malaise, myalgia, cold sweating, productive cough, and sore throat since February 18, 2020. She reported that there were another 16 persons in the same tourism group had similar symptoms at that time. Her symptoms persisted despite medication, prescribed on February 21 by a local medical doctor. On February. 26, the cough exacerbated, and she developed chest tightness, abdominal upset and vomiting. She visited the Department of Emergency of a teaching hospital in Taipei on February 28. No fever was noted during her disease course. In the context of her travel history and prominent respiratory symptoms, a nasopharyngeal swab was taken for test of SARS-CoV-2 by real-time reverse transcription-polymerase chain reaction (RT-PCR), and the result was positive. She was then transferred to a negative-pressure isolation room as a case of COVID-19.

The most interesting point of this patient is where she contracted her COVID-19. By history, she is more likely to contract SARS-CoV-2 infection while travelling abroad. However, despite that the median incubation period of COVID-19 was 5.1 days, it might be as long as more than three weeks in some extreme cases.[6,7] Therefore, an argument that she got the infection while she was in Taiwan couldn't be excluded completely.

To clarify this argument, more virologic studies were conducted. Virus whole genome sequencing was conducted for the SARS-CoV-2 isolate (NTU03) from her throat swab collected on March 2, 2020. The derived NTU03 sequence was most similar to clade A2a with only 5 nucleotide differences, which included 2 synonymous

mutations (Orf 1b/5410 CTA>TTA, and orf 3a/819 GTG>GTT), 2 nonsynonymous mutations (Orf 1b/799 *G*>*V*, and orf 3a/57Q>*H*), and a mutation within 5′UTR (Table 1). An average of 12 nucleotide differences were observed between NTU03 and sequences of other clades, whereas an average of 10 nucleotide differences were observed with other previous viruses isolated from Taiwan. The phylogenetic analysis also reveals that the NTU03 belongs to clade A2a (Fig. 1).

Based on the results of whole genome sequencing and phylogenetic analysis, NTU03 belongs to clade A2a, in which all other of the reported case patients were currently either from Europe or travelled to Europe recently according to the information provided by the laboratories who submitted the clade A2a sequences to the Global Initiative on Sharing All Influenza Data (GISAID). None of the previously submitted sequences of viruses isolated from Taiwan were assigned to clade A2a or A2.

With the limited transmission clusters in Taiwan and the fact that NTU03 exhibits at least 8 unique nucleotide difference compared to other previously reported viruses from Taiwan, we conclude that it is much more probable that the present patient was infected during her travelling abroad. To our best knowledge, in late February and early March, several foreign COVID-19 patients who had travel histories to Egypt were reported. Further phylogenetic analysis including viral sequences derived from the 45 confirmed SARS-CoV-2 infections on the quarantined Nile cruise boat will help to delineate the outbreak on Nile tourism boat and its impact on the COVID-19 epidemic.[8]

Declaration of Competing Interest

None to declare.

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References

1. Tang J.W., Tambyah P.A., Hui D.S.C.. Emergence of a novel coronavirus causing respiratory illness from Wuhan, China. *J Infect* 2020;**80**(3):350–71.
2. Zhang Z., Xiao K., Zhang X., Roy A., Shen Y.. Emergence of SARS-like coronavirus in China: an update. *J Infect* 2020 [Epub ahead of print]. doi:10.1016/j.jinf.2020.03.010.
3. World Health Organization [Internet]. Statement on the second meeting of the International Health Regulations (2005) Emergency Committee regarding the outbreak of novel coronavirus (2019-nCoV). [cited 2020 Mar 18]. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen.
4. Taiwan Centers for Disease Control. Situation of COVID-19 in Taiwan. [cited 2020 Mar 18]. Available from: https://sites.google.com/cdc.gov.tw/2019ncov/taiwan.
5. Wang C.J., Ng C.Y., Brook R.H.. Response to COVID-19 in Taiwan: big data analytics, new technology, and proactive testing. *JAMA* 2020 [Epub ahead of print]. doi:10.1001/jama.2020.3151.
6. Backer J.A., Klinkenberg D., Wallinga J.. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travelers from Wuhan, China. 20028 January 2020. *Euro Surveill* 2020;**25**(5):2000062.
7. Lauer S.A., Grantz K.H., Bi Q., Jones F.K., Zheng Q., Meredith H.R., et al. The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. *Ann Intern Med* 2020 [Epub ahead of print]. doi:10.7326/M20-0504.
8. The Washington Post. Egypt tires to reassure tourists as coronarivus spikes. [cited 2020 Mar 18]. Available from: https://www.washingtonpost.com/world/middle_east/egypt-tries-to-reassure-tourists-as-coronavirus-spikes/2020/03/08/ab1dfbf6-6160-11ea-8a8e-5c5336b32760_story.html.
9. Hadfield J., Megill C., Bell S.M., Huddleston J., Potter B., Callender C., et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* 2018;**34**(23):4121–3.
10. Sagulenko P., Puller V., Neher R.A.. TreeTime: maximum-likelihood phylodynamic analysis. *Virus Evol* 2018;**4**(1) vex042.

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Characteristics of deaths amongst health workers in China during the outbreak of COVID-19 infection

Dear Editor,

Since December, 2019, an outbreak of a novel coronavirus pneumonia (COVID-19) occurred in Wuhan (Hubei, China).[1] Recent papers in this journal also described the clinical and computed tomographic imaging features of novel coronavirus pneumonia caused by COVID-19.[2] During nearly two months of fighting against the epidemic, health workers were under great physiological and psychological pressure in China.[3] For example, due to wearing protective clothing, many health workers avoided drinking water and wore adult diapers for a long time, so that some of them fainted under hypoxia and hypoglycaemia.[4] Previous studies showed that stress could increase the risk of infection[5] as well as induce ventricular arrhythmia, and thus sudden cardiac death.[6] As a result, the medical staff in the front-line fighting against the novel coronary pneumonia were facing high risks of virus infection and sudden death. In 2003, more than 1,000 health workers were attacked by severe acute respiratory syndrome (SARS) and 124 deaths were observed in China. As of Mar 16, 2020, 24 health workers had died during the outbreak of COVID-19 infection in China.

We retrieved information on 24 cases of deceased health workers based on official reports from governmental institutes, as well as reports from news sites. Data available to the public included gender, age, cause of death, location city, date of disease onset, date of admission, date of death, and hospital levels they worked. We grouped cases into three groups based on the cause of death, which included COVID-19 infection, sudden death, and traffic accident groups. Mann-Whitney U test was applied to compare continuous variables because the data was non-normal distribution, and Fisher exact test was used for categorical variables because the data number was limited.

Thirteen (54.2%) cases died of COVID-19 infection, 8 (33.3%) suffered from sudden death including cardiac arrest, myocardial infarction, and other non-confirmed diseases, and 3 (12.5%) died in traffic accidents during work time or after work (Table 1). The basic information of all the deceased health workers was listed in Fig 1A. The median age was 50.5 years (IQR: 36.25-56.5), ranging from 26 to 69 years. A total of 72,314 patient record showed that 81% of dead cases were aged 60 years or older and 12.7% were aged 50 to 59 years.[7] The median age of deceased medical staff was obviously younger than that of the general population, because medical staff were mostly in employment who were younger than 60 years. Up to 83.3% of deceased medical workers were males and no sex differences existed among COVID-19 infection group, sudden death group, and traffic accident group.

Table 1

Demographics of deceased medical workers in China by Mar 16, 2020

Characteristic	Total(n=24)	COVID-19 infection(n=13, 54.2%)	Sudden death(n=8, 33.3%)	Traffic accident(n=3, 12.5%)	Z/χ2(P /Fisher P)
Age, Median (IQR) -yrs	50.5(36.25-56.5)	51(38.0-58.0)	50(36.25-56.5)	/	-0.399(0.690)
Male, No. (%)	20(83.3)	11(84.6)	7(87.5)	2(66.7)	1.180(0.579)
Hubei resident, No. (%)	11(45.8)	11(84.6)	0(0.0)	0(0.0)	17.293(0.000*)
Wuhan resident, No. (%)	9(37.5)	9(69.2)	0(0.0)	0(0.0)	11.684(0.001*)
Community hospital, No. (%)	11(45.8)	3(23.1)	5(62.5)	3(100.0)	6.644(0.022*)
Onset to admission, Median (IQR)-days	/	2(1-5.5) (n=9)	/	/	/
Admission to death, Median (IQR)-days	/	26(21.25-36.5) (n=12)	/	/	/
Onset to death, Median (IQR)-days	/	30.5(25-35.25) (n=10)	/	/	/
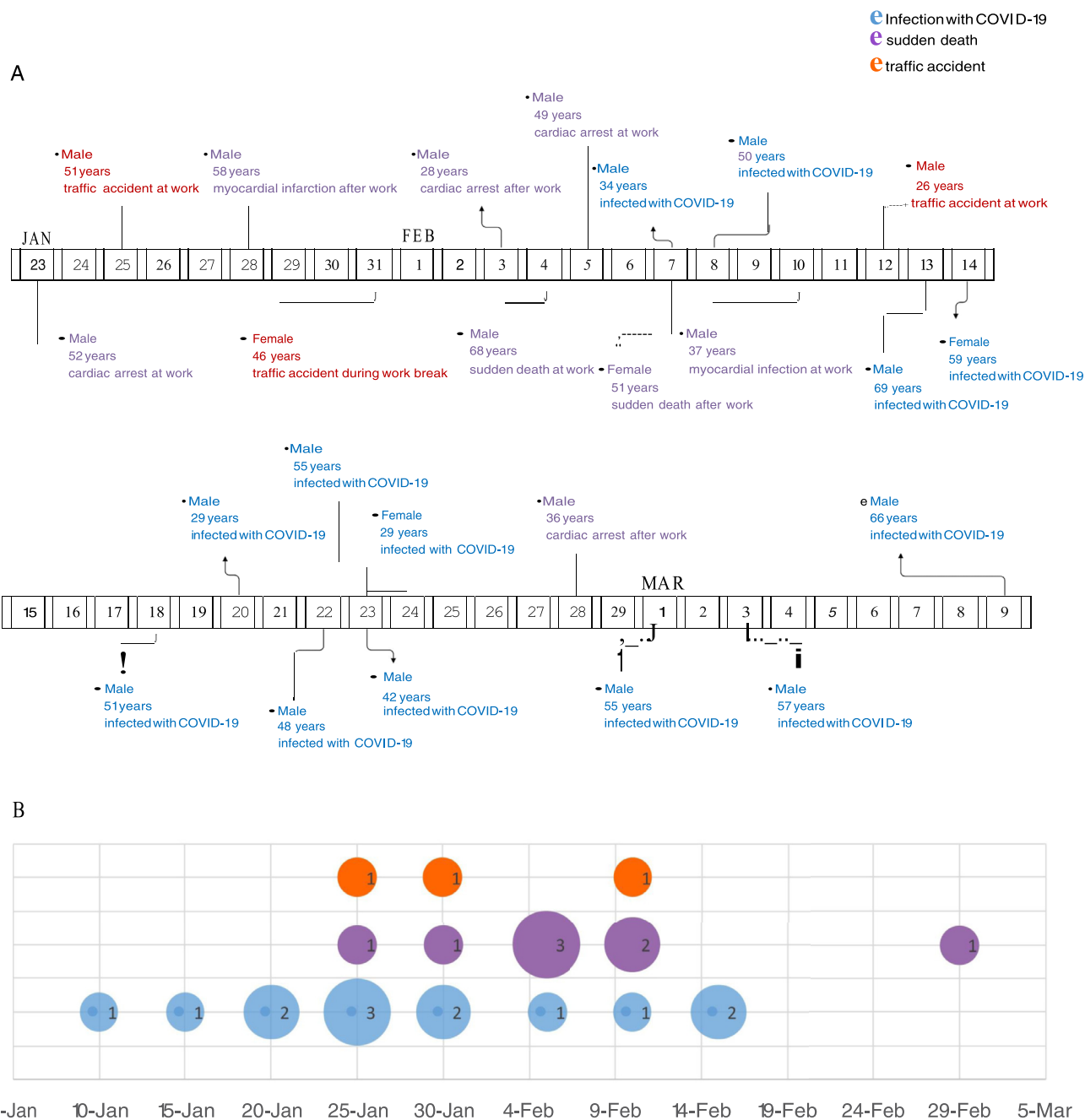
Fig. 1. A) Death date, demographics, and death cause of medical workers in China by Mar 16, 2020. Infection with COVID-19 marked with blue, sudden death marked with purple, and traffic death marked with red. B) The new number of deceased medical workers with confirmed COVID-19 infection at admission per 5 days (marked with blue), new number of medical workers with sudden death per 5 days (marked with purple), and new number of medical workers with traffic accident per 5 days (marked with red).

In the group of infection, 11 deceased cases (84.6%) were males. Zhang reported that the overall case fatality rate of male patients (rough estimate: 2.8%) was significantly higher than that of female patients (rough estimate: 1.7%).[7] In the group of sudden death, 7 cases (87.5%) were males. Previous study revealed that, at 45 years of age, lifetime risks for sudden cardiac death were 10.9% for men and 2.8% for women,[8] which was similar to the results in our study. The above data suggested that males had a higher risk of death due to COVID-19 infection and sudden death than females.

Transmission of COVID-19 occurred in the hospital setting. In the group of COVID-19 infection, there were more medical staff working in Hubei province (84.6%) and Wuhan city (69.2%), which was consistent with the result of 63% of infected medical staffs in Wuhan in a recent report.[9] Due to the severity of COVID-19 infection in Hubei, more nosocomial infections and deaths occurred in Hubei than other provinces. As of February 11, 2020, 3,019 cases have been observed among health workers, of whom there have been 1,716 confirmed cases. Among health workers infected, 14.8% of confirmed cases were classified as severe or critical, and 5 deaths were observed.[9] Among all the deceased medical staff with COVID-19 infection, the median of period from disease onset to hospital admission was 2 days (IQR: 1-5.5), and the median of period from admission to death was 26 days (IQR: 21.25-36.5) (Table 1). Based on the admission date of staff with COVID-19 infection and the death date of staff with sudden death, the new number of deceased health workers per 5 days was listed in Fig 2. Attacked infection mostly occurred on January and sudden death mainly happened from Jan 23 to Feb 10, 2020.

Furthermore, there were more health workers who worked in community hospitals suffering from sudden death or traffic accident. Sudden death due to huge work and lack of rest happened since Jan 23, 2020, when comprehensive measures for epidemic prevention and control were taken nationwide. Large-scale work including temperature measurements, door to door visit, medicine delivery, patients transfer, disinfection, etc., had been completed by community or village medical workers. Some village doctors even lived and ate in the village clinics. On Feb 22,2020, the Chinese government took a series of measures to protect and support health workers in the front line, such as improving the quality of life, strengthening personal protection, arranging rest in turns, and relieving mental stress. Afterwards, the incidence of accidental death decreased significantly.

In summary, there were more males in the fatality of health workers, more sudden death happening to community health workers, and more death due to COVID-19 infection occurring in Hubei health workers during the outbreak of COVID-19 in China.

Declaration of Competing Interest

None

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References

1. Chen Q., Liang M., Li Y., et al. Mental health care for medical staff in China during the COVID-19 outbreak. *The lancet Psychiatry* 2020.
2. YH X., JH D., WM A., et al. Clinical and computed tomographic imaging features of novel coronavirus pneumonia caused by SARS-CoV-2. *The Journal of infection* 2020.
3. Wu P., Fang Y., Guan Z., et al. The psychological impact of the SARS epidemic on hospital employees in China: exposure, risk perception, and altruistic acceptance of risk. *Canadian journal of psychiatry Revue canadienne de psychiatrie* 2009;**54**(5):302–11.
4. Zeng Y., Zhen Y.. Chinese medical staff request international medical assistance in fighting against COVID-19. *The Lancet Global health* 2020.
5. Calefi A.S., de Queiroz Nunes C.A., da Silva Fonseca J.G., Quinteiro-Filho W.M., Ferreira A.J.P., Palermo-Neto J.. Heat stress reduces Eimeria spp. infection and interferes with C. perfringens infection via activation of the hypothalamic-pituitary-adrenal axis. *Research in veterinary science* 2019;**123**:273–80.
6. Scorza F.A., Albuquerque R., Arida R.M., et al. What are the similarities between stress, sudden cardiac death in Gallus gallus and sudden unexpected death in people with epilepsy. *Arquivos de neuro-psiquiatria* 2010;**68**(5):788–90.
7. Novel Coronavirus Pneumonia Emergency Response Epidemiology T. [The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2020;**41**(2):145–51.
8. E M., A U.-E., K R., et al. Sudden cardiac arrest during sports activity in middle age. *Circulation* 2015;**131**(16):1384–91.
9. Z W., JM M.. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. *Jama* 2020.

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Recurrent PCR positivity after hospital discharge of people with coronavirus disease 2019 (COVID-19)

Dear Editor,

The outbreak of coronavirus disease 2019 (COVID-19) was reported by Tang and colleagues in late December 2019 in Wuhan, China, in this journal, with a series of respiratory infected by a novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] By March 3, 2020, 105,586 cases of COVID-19 pneumonia were laboratory-confirmed in over 100 countries worldwide.[2] Infection caused by SARS-CoV-2 can result in acute respiratory distress syndrome (ARDS), which is similar to the symptoms induced by the Middle East respiratory syndrome coronavirus.[3,4] With deeper understanding of the biological characteristic of SARS-CoV-2, great successful progress has been made in COVID-19 treatment. A total of 79,251 confirmed cases were reported in China by February 28, and 39,002 cases out of them have been cured and discharged from hospitals.[5]

During January 28 to March 13, 6 COVID-19 recurrence cases were found in Shangqiu, Henan Province, China (Fig. 1). Among the recurrence cases, one case (Case 1) had significant post-discharge clinical symptoms and discomfort for nine days, one case (Case 3)
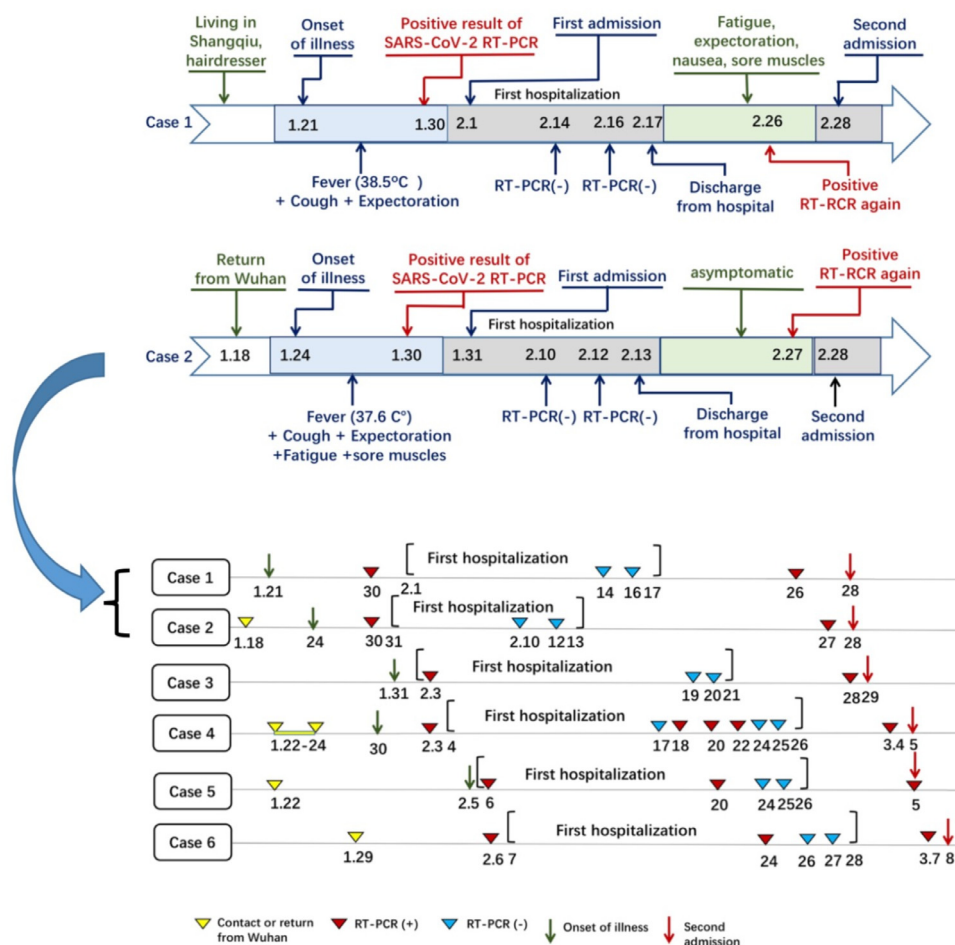
Fig. 1. Timeline of six recurrence cases.

had a mild cough, and 4 cases (Case 2, 4, 5, and 6) were asymptomatic with positive RT-PCR nucleic acid test.

Case 1, a 35-year old female living in Shangqiu, worked as a hairdresser, and her contact history with Wuhan-imported cases was not excluded. This patient experienced her first symptoms including fever (max temperature: 38.5 °C), cough, and expectoration (white sticky sputum) for 9 days before the first admission (February 1, 2020). After the SARS-CoV-2 test of RT-PCR assay was reported positive by CDC of Shangqiu City, on January 30, 2020, the patient was isolated for treatment in the hospital and was discharged on February 17, 2020, after a 16-day treatment, and received a 14-day quarantine at home. No contact history with other confirmed or possible COVID-19 patients was reviewed during the quarantine period. However, this female experienced repeated fatigue, expectoration, sore muscles, and nausea once again, with body temperature fluctuating in 36.5 ∼ 36.8 °C. Once again, the SARS-CoV-2 RT-PCR nucleic acid test was reported positive on February 26, 2020, and this woman was rehospitalized on February 28, 2020 (Fig. 1).

Case 2, a 56-year-old female travelling from Wuhan, arrived in Shangqiu on January 18, 2020, and was suffering from irregular fever, cough, expectoration, fatigue, and muscle soreness until January 25, 2020. This woman was laboratory confirmed as a COVID-19 patient on January 30, 2020 and received a 12-day treatment during February 1 ∼ 13, 2020. After that, this patient was discharged according to the criterion, including significantly improved manifestations in clinic and CT imaging, and two negative nucleic

acid testing with an interval of 24 hours. During subsequent isolation at home, the patient did not experience any symptoms or discomfort or contact history, however, the recurrence of a positive nucleic acid test was reported on February 28, 2020. This patient was readmitted to the hospital for another treatment on the same day. Case 2 also had a previous history of hypertension for 3 years (Fig. 1).

The median age of recurrence cases was 45.2 years (varying from 30 ∼ 56 years old) and all were female. Two cases had previous history, one with hypertension, and one with chronic bronchitis. All patients had no history of smoking. Of the 6 cases, one experienced significant symptoms during the relapse, one had occasional cough, and four cases were asymptomatic. Most frequent symptoms at their first admission were fever, cough, and expectoration.

Recurrence cases showed no significant difference ($P > 0.05$) with control cases in leukocyte, lymphocyte, neutrophil, platelet, and albumin counts (Table. 1). Most demonstrated lower albumin and abnormal coagulation indexes. The first admission showed abnormal coagulation function in five cases: 3 cases with higher blood platelet count (one was consistently above normal), 2 cases with prolonged prothrombin time, and one case with a transient D-dimer elevation (1319). At the second admission, 4 cases presented normal indexes of coagulation function while case 2 showed an elevated index of whole blood D-dimer (1033.46 ug/mL). Chest CT scanning was performed every 3∼4 days on average from the initial examination, and each case involved at least 5

Table 1
Levels of leukocyte, lymphocyte, neutrophil, platelet, and albumin between recurrence cases and cases without recurrence for more than two weeks.

	At first admission				Discharged from hospital			
	Recurrence ($N=6$)	Control ($N=29$)	t	p	Recurrence ($N=6$)	Control ($N=29$)	t	p
Leukocyte(109/L)	5.06 ± 1.95	5.92 ± 3.13	-0.646	0.523	5.68 ± 1.77	5.64 ±1.49	0.059	0.953
Lymphocyte (109/L)	1.23 ± 0.61	1.50 ±0.61	-0.972	0.338	1.69 ± 0.54	1.68 ±0.52	0.033	0.974
Neutrophil (109/L)	3.26 ±0.94	3.91 ±2.85	-0.547	0.588	3.41±1.37	3.41 ±1.15	0.002	0.999
Platelet(109/L)	201.00 ±88.56	221.66 ±95.60	-0.487	0.629	279.17 ±38.09	251.50 ±88.43	0.744	0.462
Albumin(g/L)	36.20 ±3.40	37.83 ±6.18	-0.621	0.539	36.08 ±3.40	37.25 ±5.62	-0.473	0.64

CT scans. CT manifestations of the 6 cases were characterized with patch-like ground-glass opacities (GGO) in bilateral lungs, and tend to have a gradually improved trend from admission to discharge, to relapse. Multiple antiviral treatments were applied, including recombinant human interferon α -1b / 2b antiviral therapy (5 million U, b.i.d., nebulization), oral lopinavir/ritonavir (100 mg, b.i.d., p.o.), and abidol (200 mg, t.i.d., p.o.), combined with traditional Chinese medicine treatments. Methylprednisolone was intravenously used in one case, for 1 day (40 mg, b.i.d.) during the first hospitalization.

Because the infectious period of COVID-19 infection was not completely clear and several recurrence cases occurred, we suggest that further consecutive observation and supervision for at least two weeks were needed for the people discharged from hospital. In addition, we may also combine other methods to detect this virus, such as the serologic examination of SARS-CoV-2-specific IgM antibody. Further, personalized treatment should be adopted in order to cure patients completely, thereby reducing the recurrence rate.

Declaration of Competing Interest

None.

Acknowledgment

References

1. Tang J.W., Tambyah P.A., Hui D.S.C.. Emergence of a novel coronavirus causing respiratory illness from Wuhan. China. *J Infect* 2020.
2. World Health Organization. Novel coronavirus (2019-nCoV). Situation Report—48 https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200308-sitrep-48-covid-19.pdf?sfvrsn=16f7ccef_4
3. Huang C., Li X., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;**395**(10223):497–506.
4. Graham R.L., Donaldson E.F., Baric R.S.. A decade after SARS: strategies for controlling emerging coronaviruses. *Nat Rev Microbiol* 2013;**11**(12):836–48.
5. Cumulative number of reported cases of 2019 novel coronavirus pneumonia. Beijing: National health commission of the People's Republic of China,2020[EB/OL]. [2020-02-29]. http://www.nhc.gov.cn/xcs/yqfkdt/202002/4ef8b5221b4d4740bda3145ac37e68ed.shtml

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Clinical features of critically ill patients with confirmed COVID-19

To the Editor,

We read with great interest the article by Wenjie Yang and colleagues,[1] accepted for publication in the *Journal of Infection*. The authors performed a retrospective multi-center cohort study and presented important data regarding the observation that most patients of 2019 novel coronavirus disease (COVID-19) from Wenzhou city, Zhejiang, exhibited mild infection. However, the information of critically ill patients, especially treated with extracorporeal membrane oxygenation (ECMO), was scare. No study to date has provided evidence that the clinical features of critically ill patients with confirmed COVID-19 from Zhejiang province. We performed a single-centered, retrospective, observational study to investigate the clinical characteristics and ventilation conditions of critically ill patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

From late January to February 23, 2020, 33 critically ill patients in the intensive care unit (ICU) of the First Affiliated Hospital of Zhejiang University who were diagnosed as COVID-19 in accordance with the diagnosis and treatment guidance published

Table 1
Demographics and baseline clinical features of 33 COVID-19 patients in the ICU

Parameters	All patients	With ECMO	Without ECMO	P value
Number	33	7	26	
Age, y	65.2 ± 16.6	67.0 ± 17.7	64.7 ± 16.6	0.75
Age groups (years)				
≤18	0 (0.0%)	0 (0.0%)	0 (0.0%)	
19-40	4 (12.1%)	1 (14.3%)	3 (11.5%)	
41-65	11 (33.3%)	3 (42.9%)	8 (30.8%)	
≥66	18 (54.5%)	3 (42.9%)	15 (57.7%)	
Sex				0.38
Men	22 (66.7%)	6 (85.7%)	16 (61.5%)	
Women	11 (33.3%)	1 (14.3%)	10 (38.5%)	
Chronic Comorbidities				
Hypertension	22 (66.7%)	4 (57.1%)	18 (69.2%)	0.66
Diabetes	6 (18.2%)	1 (14.3%)	5 (19.2%)	1.00
Cardiovascular diseases	6 (18.2%)	1 (14.3%)	5 (19.2%)	1.00
Chronic obstructive pulmonary disease	1 (3.0%)	1 (14.3%)	0 (0.0%)	0.21
Malignancy	1 (3.0%)	1 (14.3%)	0 (0.0%)	0.21
Renal diseases	2 (6.1%)	0 (0.0%)	2 (7.7%)	1.00
Liver diseases	3 (9.1%)	1 (14.3%)	2 (7.7%)	0.52
Onset of symptoms to hospital admission, median (IQR), d	7 (6-10)	10 (5-13)	7 (6.5-10)	0.53
Blood routine				
Leucocytes, $\times 10^9$/L	10.5 ± 5.8	6.5 ± 4.7	11.6 ± 5.7	0.039*
Increased	15 (45.5%)	1 (14.3%)	14 (53.8%)	
Decreased	3 (9.0%)	1 (14.3%)	2 (7.7%)	
Neutrophils, $\times 10^9$/L	9.1 ± 5.8	5.3 ± 4.2	10.1 ± 5.8	0.050
Increased	19 (57.6%)	1 (14.3%)	18 (69.2%)	
Lymphocytes, $\times 10^9$/L	0.5 (0.45-0.9)	0.4 (0.3-0.7)	0.6 (0.5-1.0)	0.004*
Decreased	22 (66.7%)	6 (85.7%)	16 (61.5%)	
Platelets count, $\times 10^9$/L	180.0 (139.0-196.0)	111.0 (99.0-142.0)	189.0 (169.0-201.5)	0.002*
Blood biochemistry				
ALT, U/L	20.0 (14.5-30.0)	30.5 (18.8-45.3)	17.0 (14.0-26.3)	0.034*
Increased	2 (6.0%)	1 (14.3%)	1 (3.8%)	
AST, U/L	25.0 (18.3-40.0)	38.5 (24.8-75.5)	23.5 (17.0-36.3)	0.049*
Increased	8 (24.2%)	3 (42.9%)	5 (19.2%)	
Total bilirubin, μmol/L	11.8 (8.0-18.5)	20.5 (11.8-36.6)	9.6 (7.4-14.7)	0.041*
Increased	4 (12.1%)	1 (14.3%)	3 (11.5%)	
Creatine kinase, U/L	24.0 ± 5.0	26.9 ± 4.9	23.1 ± 4.7	0.079
Increased	9 (30.3%)	4 (57.1%)	5 (19.2%)	
Infection-related biomarkers				
Procalcitonin, ng/mL	0.1 (0.05-0.32)	0.3 (0.1-0.7)	0.1 (0.04-0.2)	0.07
Increased	24 (72.7%)	7 (100.0%)	17 (65.4%)	
Interleukin-6, pg/mL	46.6 (20.5-90.2)	290.6 (96.0-446.5)	38.2 (16.5-74.9)	0.016*
Increased	25 (75.8%)	5 (71.4%)	20 (76.9%)	
hs-CRP, mg/L	45.5 (23.4-86.0)	50.0 (24.2-143.4)	40.0 (22.1-59.0)	0.53
Increased	30 (90.9%)	6 (85.7%)	24 (92.3%)	
Bilateral involvement of chest CT	32 (97.0%)	7 (100.0%)	25 (96.2%)	1.00

Data are presented as mean±SD, median (IQR), or counts (%).
Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; hs-CRP, high-sensitivity C-reactive protein; CT, computed tomograms.
P values denoted the comparison between ECMO treated cases and non-ECMO treated cases. *Significant $p<0.05$.

by the Chinese government were enrolled in the study.[2] We obtained patients′ demographics, epidemiology data, details of laboratory tests, treatments, and ECMO implantation.

The baseline epidemiological characteristics and clinical features of 33 studied patients as classified by with or without ECMO treatment were shown in Table 1. Most of the patients admitted to the ICU were older and had several common comorbid conditions, which demonstrated that age and comorbidities might be the indicators for severely ill one and poor prognosis. Of all patients, the mean age was 65.2±16.6 years, and most of the patients were aged 65 years and older. Of the seven patients who received ECMO, the mean age was 67.0±17.7 years. Twenty-two (66.7%) had underlying comorbidities, involving hypertension (66.7%), diabetes (18.2%), and cardiovascular diseases (18.2%). For the 7 patients who received ECMO, 5 (71.4%) patients had associated comorbidities, including hypertension (4 [57.1%]), cardiovascular diseases (1 [14.3%]), diabetes (1 [14.3%]), chronic obstructive pulmonary disease (1 [14.3%]), malignancy (1 [14.3%]), and liver diseases (1 [14.3%]). In our study, more than half of the critically infected patients were men (22 [66.7%]), especially in ECMO treated

patients (6 [85.7%]). Jing Li et al.[3] observed men probably had more complicated clinical conditions and worse in-hospital outcomes as compared to women in severe COVID-19 patients. The median time from onset of symptoms to hospital admission was 7 days (IQR 6-10 days) which was longer than Wenjie Yang and colleagues′ study.

In terms of baseline laboratory data of severely confirmed COVID-19 patients, three (9.0%) and 22 (66.7%) of 33 patients exhibited leucopenia and lymphopenia, respectively. Platelets levels on admission were lower in patients with ECMO treatment than non-ECMO patients. Also, a recent case report verified the counts of peripheral CD4 and CD8 T cells were both decreased in a 50-year-old man with SARS-CoV-2 infection through the technology of flow cytometric analysis.[4] Specifically, the levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) on admission were higher in ECMO treated patients (median AST 38.5U/L [IQR 24.8-75.5]; median ALT 30.5 U/L [IQR 18.8-45.3]) than non-ECMO treated patients (median AST 23.5 U/L [IQR 17.0-36.3], p=0.049; median ALT 17.0 U/L [IQR 14.0-26.3], p=0.034; Table 1). Besides, admission levels of total bilirubin were increased substan-

Table 2
Gas exchange after the commencement of ECMO

Parameters	Pre-ECMO	ECMO day 1	*P* value
FiO_2 (%)	70.0 ± 16.3	37.1 ± 12.9	0.011*
Peak inspiratory pressure, cmH_2O	22.0 ± 4.0	19.3± 6.9	0.299
PEEP, cmH_2O	7.0 ± 0.7	6.7 ± 2.0	0.356
PaO_2/FiO_2 ratio	106.8 ± 48.8	235.7± 120.7	0.042*
SPO_2 (%)	92.9 ± 5.6	94.4 ± 5.0	0.525
PaO_2, mmHg	72.4 ± 33.8	77.5 ± 24.7	0.785
$PaCO_2$, mmHg	46.4 ± 5.9	36.0 ± 1.8	0.005*
Lactate, mmol/L	4.2 ± 4.1	2.3 ± 0.5	0.276

Data are presented as mean±SD.
Abbreviations: ECMO, extracorporeal membrane oxygenation; FiO_2, fraction of in-spired oxygen; PEEP, positive end expiratory pressure.
P values denoted the comparison between pre-ECMO and the first day of ECMO.
*Significant $p<0.05$.

tially in ECMO treated patients. These abnormalities suggested that SARS-CoV-2 might be related to hepatic injury. However, almost all of the included patients received antivirus treatment, the drug in-duced liver injury could not be excluded. Huang *et al.* reported that increased level of AST was found in about 62% of the ICU patients in their study.[5] Therefore, damaged liver function is more common in serious COVID-19 patients. Up to now, there has been no suffi-cient evidence to clarify SARS-CoV-2 as the main reason of dam-aged liver function. Further studies should concentrate on the rea-sons of liver function damage in patients with COVID-19. The level of procalcitonin increased in more than 70% of included patients, and most of patients in our study received antibacterial and anti-fungal agents. One possible explanation for the results may be that many of the critically ill patients were associated with combined infection of bacterial or fungal.

ECMO has been increasingly being used as a rescue treatment for refractory hypoxemia in patients with severe acute respiratory distress syndrome.[6] The initial mode was veno-venous (VV) ECMO in the 7 patients. Initiation of ECMO was accompanied by a signifi-cant improvement in PaO_2/fraction of inspired oxygen (FiO_2) ratio, and significant decreases in $PaCO_2$, FiO_2 (Table 2). Research showed too high level of FiO_2 was related to increased production of reac-tive oxygen-derived free radicals which were noxious to the hu-mans health.[7]

In summary, our data indicated that SARS-CoV-2 infection might cause damage to the immune and liver function of COVID-19 patients. ECMO support was associated with improved venti-lation conditions in COVID-19 patients with refractory hypoxemia. The study may be helpful to providing evidence of the appropriate time to initiate ECMO for critically ill patients with COVID-19, and add further evidence for critically ill patients′ characteristics.

Declaration of Competing Interest

The authors of this study declared no conflict of interest.

Acknowledgments

References

1. Yang W.J., Cao Q.Q., Qin L., Wang, X.Y., Cheng Z.H., Pan A.S., et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus dis-ease (COVID-19): A multi-center study in Wenzhou city, Zhejiang, China. *J Infect* 2020;**80**(4):388–93. doi:10.1016/j.jinf.2020.02.016.
2. National Health Commission, National Administration of Traditional Chinese Medicine Guidelines for the diagnosis and treatment of novel coronavirus pneu-monia (trial version sixth). *Chin J Viral Dis* 2020;**10**:88–92 (in Chinese). doi:10.16505/j.2095-0136.2020.0016.
3. Li J., Zhang Y.H., Wang F., Liu B., Li H., Tang G.D., et al. Sex differences in clinical findings among patients with coronavirus disease 2019 (COVID-19) and severe condition. *medRxiv* 2020. doi:10.1101/2020.02.27.20027524.
4. Xu Z., Shi L., Wang Y.J., Zhang J.Y., Huang L., Zhang C., et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med* 2020;**8**(4):420–2. doi:10.1016/S2213-2600(20)30076-X.
5. Huang C.L., Wang Y.M., Li X.W., Ren L.L., Zhao J.P., Hu Y., et al. Clinical fea-tures of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;**395**(10223):497–506. doi:10.1016/S0140-6736(20)30183-5.
6. Peek G.J., Mugford M., Tiruvoipati R., Wilson A., Allen E., Thalanany M.M., et al. Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CE-SAR): a multicentre randomised controlled trial. *Lancet* 2009;**374**(9698):1351–63. doi:10.1016/S0140-6736(09)61069-2.
7. Helmerhorst H.J.F., Schultz M.J., van der Voort P.H.J., de Jonge E., J van West-erloo D.. Bench-to-bedside review: The effects of hyperoxia during critical ill-ness. *Crit Care* 2015;**19**(1):284 [published Online First: 2015/08/19]. doi:10.1186/s13054-015-0996-4.

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The index case of SARS-CoV-2 in Scotland

Dear Editor,

We read with interest Lillie and colleagues' letter on the first patients with COVID-19 in the UK.[1] Since its identification in De-cember 2019, SARS-CoV-2 has infected 125,048 persons globally

Table 1
Routine laboratory parameters.

Parameter	Normal	Day 1	Day 2	Day 3
Total white cell count (x10^9/L)	4–11	4.9	4.5	4.6
Neutrophil count (x10^9/L)	2–7.5	2.5	2.1	2.4
Lymphocyte count (x10^9/L)	1.5–4	1.6	1.8	1.3
Monocyte count (x10^9/L)	0.2–0.8	0.84	0.58	0.82
Platelet count (x10^9/L)	150–400	239	242	240
C-reactive protein (mg/L)	0–5	4	2	3

Renal and liver biochemistry values were normal.

with cases identified in 118 countries across all continents.[2] We report on the Scottish index case of SARS-CoV-2 infection, the virus causing COVID-19.

The patient, a 51-year-old male, contacted the Scottish tele-health service for advice on day +1 with a 24-hour history of fever and cough having returned to Scotland from northern Italy on day −2. His symptoms were in keeping with a "possible" case of COVID-19 according to the national case definition at the time. Community SARS-CoV-2 testing was arranged for day +2.

The patient had travelled to Italy on day −9 to watch a rugby match in Rome. He travelled with his partner and two friends in a private rental vehicle through Lombardy, Veneto and Tuscany, staying in private rental accommodation, before flying back to Scotland from Milan on day −2. He was not aware of any contact with cases of COVID-19.

On day 0 he developed fever, myalgia, malaise and sinusitis. This progressed to a cough productive of green sputum on day +1. His fever had subsided by day +2.

The patient remained in self isolation in the community whilst awaiting the results of SARS-CoV-2 PCR which was performed in the West of Scotland Specialist Virology Centre on a combined nose/throat swab. This sample tested positive for SARS-CoV-2 on day +3 with a threshold cycle (Ct) value of 36. An urgent teleconference was held between virology, public health, Scottish ambulance service and the receiving regional High Consequence Infectious Diseases (HCID) unit. The patient was subsequently transferred by Special Operations Response Team (SORT) ambulance to the HCID unit. The patient was escorted from the ambulance to a laminar flow room (with antechamber) on the first floor of the unit by medical staff in appropriate personal protective equipment (PPE). The patient was wearing a surgical face masque throughout transit. The ambulance staff returned to their base for doffing of PPE.

The patient was usually well and exercised regularly. He was a lifelong non-smoker. His medical history consisted only of hypertension. He had no known underlying lung disease. He took an ACE-inhibitor and self-medicated with paracetamol and non-steroidal anti-inflammatory drugs during his illness.

On admission, the patient was afebrile with a mild non-productive cough. He had no limitation in exercise tolerance during this acute illness and continued to exercise in his home whilst self-isolating. On assessment, lung fields were clear on auscultation and bilateral scleral injection was noted. Admission vital signs were: heart rate 79 beats per minute, blood pressure 171/105 mmHg, respiratory rate 18, pulse oximetry 96% on air and temperature 36.5. The results of routine laboratory parameters are shown in Table 1. By day three of admission the patient had lymphocytopenia, which was found in 80.4% of patients with laboratory-confirmed non-severe COVID-19 in mainland China.[3] Chest radiography was not clinically indicated. PCR testing for other respiratory viruses, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* on the throat swab was negative. Nose and throat samples for SARS-CoV-2 PCR were obtained daily to monitor viral shedding (Fig. 1). SARS-CoV-2 PCR on urine, faeces and EDTA blood was negative. The patient was
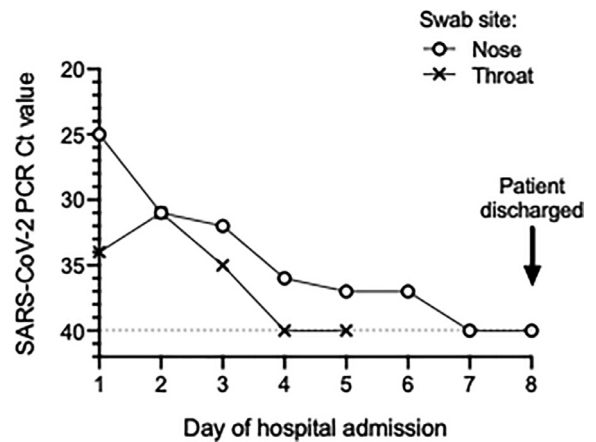
Fig. 1. SARS-CoV-2 PCR threshold cycle values during hospital admission
Note on day 2 a combined throat and nose swab was performed. Negative PCR is plotted as Ct value of 40 (dotted line).

clinically well throughout the admission; no supportive care was required. He was discharged after 8 days, following two sequential nose and throat swabs negative for SARS-CoV-2 by PCR. The initial viral sample was sequenced by the MRC-University of Glasgow Centre for Virus Research, mapped against the Wuhan-Hu-1 reference genome and aligned with a collection of global SARS-CoV-2 sequences, with the resulting analysis released open access. Phylogenetic analysis demonstrated that Scotland/CVR01/2020 belonged to a clade of isolates all acquired in northern Italy.[4]

Public health undertook contact tracing and testing and no further cases were identified

A large case series from China has reported that the majority of COVID-19 cases (81%) had mild disease only, with risk of severe disease and mortality higher in older patients with pre-existing medical conditions.[5] The patient described here also had a mild and self-limiting illness. Although asymptomatic and mild manifestations of previous epidemic-associated coronaviruses (SARS and MERS) occur,[6,7] these appear to be substantially more common in COVID-19. This poses a challenge for the early identification and isolation of cases, to limit transmission. In the "contain" phase of the UK COVID-19 response, early case identification was reliant on epidemiologic (not severity) criteria. The public health guidance at the time of this case was that travellers to Veneto and Lombardy were considered at risk. The patient had travelled to these areas but in private transport, staying in the area briefly and having limited interactions with residents. Therefore, whilst fulfilling 'at risk' criteria, from a practical perspective the risk seemed low.

The kinetics of SARS-CoV-2 viral load have not yet been well characterized in large numbers of patients. Prolonged nasopharyngeal viral shedding was reported in a series of 18 patients from Singapore, with a median of 12 days between first and last positive samples and an initial large decline in viral load followed by a slower decay of residual low-level virus.[8] Similar kinetics were observed in 17 patients from China, where results were stratified by site tested (nose/throat) suggesting quantitatively greater and more prolonged shedding from the nose.[9] The kinetics of shedding in this case match this pattern, with more prolonged nasal shedding (Figure).

National public health guidance on PPE requirements for managing cases of COVID-19 in infectious disease units stipulates use of disposable gowns, two pairs of disposable gloves, visor and FFP3 respirator.[10] These guidelines currently do not recommend use of specific footwear, so it is concerning that SARS-CoV-2 was detected by PCR from the shoe front of a healthcare worker caring for COVID-19 patients.[11] For this case we used disposable boot covers over plastic clogs.

As the SARS-CoV-2 outbreak continues, we are learning more about the clinical manifestations of the infection and the logistics of containing and managing cases. This case highlights the need to have a low index of suspicion for diagnosis of COVID-19 and for early isolation.

Declaration of Competing Interests

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.

Contributors

KH, CDR, SC, RS conceived of the correspondence. KH and CDR collected the data. KT contributed to the section on virological testing. All authors contributed to the writing of the final version of the article.

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References

1. Lillie P.J., Samson A., Li A., Adams K., Capstick R., Barlow G.D., et al. Novel coronavirus disease (Covid-19): the first two patients in the UK with person to person transmission. *J Infect* 2020 Published online ahead of print Feb 28. doi:10.1016/j.jinf.2020.02.020.
2. WHO. Coronavirus diseases 2019 (COVID-19): situation report - 52. Available from: https://www.who.int/docs/default-source/coronaviruse/20200312-sitrep-52-covid-19.pdf?sfvrsn=e2bfc9c0_2 [Accessed March 14 2020].
3. Guan W., Zheng-yi N., Hu Y., Liang W., Ou C., He J., et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med* 2020;**28** Published online February. doi:10.1056/NEJMoa2002032.
4. Robertson D.L. First report of COVID-19 in Scotland. Available from: http://virological.org/t/first-report-of-covid-19-in-scotland/412 [Accessed March 13 2020].
5. Wu Z., McGoogan J.M.. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in china: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA* 2020 Published online February 24,. doi:10.1001/jama.2020.2648.
6. Wilder-Smith A., Teleman M.D., Heng B.H., Earnest A., Ling A.E., Leo Y.S.. Asymptomatic SARS coronavirus infection among healthcare workers. *Emerg Infect Dis* Jul 2005;**11**(7):1142–5. doi:10.3201/eid1107.041165.
7. Arabi Y.M., Balkhy H.H., Hayden F.G., Bouchama A., Luke T., Baillie J.K., et al. Middle East respiratory syndrome. *N Engl J Med* 2017 Feb 9;**376**(6):584–94. doi:10.1056/NEJMsr1408795.
8. Young B.E., Ong S.W.X., Kalimuddin S., Low J.G., Tan S.Y., Loh J., et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA* 2020 Published online Mar 3. doi:10.1001/jama.2020.3204.
9. Zou L., Ruan F., Huang M., Liang L., Huang H., Hong Z., et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* 2020 Published online Feb 19. doi:10.1056/NEJMc2001737.
10. Health Protection Scotland. Infection prevention and control advice for acute care settings: acute respiratory illness from novel or emerging pathogens (Coronavirus (COVID-19)) *[Version 9.0]*. Published March 10 2020. Available from: https://hpspubsrepo.blob.core.windows.net/hps-website/nss/2526/documents/1_infection-control-acute-care.pdf [Accessed 13 March 2020].
11. Ong S.W.X., Tan Y.K., Chia P.Y., Lee T.H., Ng O.T., Wong M.S.Y., et al. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. *JAMA* 2020 Published online Mar 4. doi:10.1001/jama.2020.3227.

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The clinical characteristics of myocardial injury in severe and very severe patients with 2019 novel coronavirus disease

Dear Editor,

We read with interest the recent article published by Yang W et al.,[1] which described the clinical characteristics and imaging manifestations of hospitalized patients with confirmed COVID-19 infection in Wenzhou, Zhejiang, China. The 2019 Novel coronavirus disease (COVID-19) has drawn global intensive attention.[2–4] Previous studies suggest that severe COVID-19 may present with acute cardiac injury.[2–4] However, few have investigated the cardiac lesion markers and their correlation to disease severity. In this letter, we explored the cardiac lesion biomarkers in patients with severe and very severe COVID-19.

We enrolled 34 COVID-19 patients admitted to the West District of Union Hospital of Tongji Medical College from February 5th to February 13rd, 2020. COVID-19 was diagnosed upon admission based on the New Coronavirus Pneumonia Prevention and Control Program (4th edition).[5] Severe COVID-19 was defined as having either one of the flowing criteria: (1) Respiratory distress with respiratory rate more than 30 times/min; (2) Oxygen saturation ≤93% in resting state; (3) $PaO2/FiO2 \leq 300$ mmHg (1 mmHg = 0.133 kPa); and very severe either one of the flowing: (1) Respiratory failure in need of mechanical ventilation; (2) Shock; (3) Other organ dysfunction. Patients with medical history of cardiovascular disease were excluded. The study was approved by the ethics committee of the local hospital.

Demographic data and serum samples were collected upon admission. Laboratory confirmation of COVID-19 was done as recommended.[5] Laboratory test and cardiac lesion markers, including cardiac troponin I (cTnI), myoglobin (Myo), Creatine Kinase (CK), Creatine kinase–MB (CKMB), α-hydroxybutyrate dehydrogenase (HBDB), Lactate Dehydrogenase (LDH), and Aspartate Aminotransferase (AST), were tested by the laboratory department. Data were presented as percentages for categorical variables and median±IQR (Inter Quartile Range) for continuous variables.

Table 1

Baseline information and cardiac biomarkers in severe and very severe patients with COVID-19.

	Median(IQR)		P value	Reference
	Severe	Very Severe		
Number	26	8		
Sex(Male%)	46.15%	62.50%	ns	
age	63(58–69)	67(66–75)	ns	
CRE(μmol/L)	64.2(56.5–74.7)	82.6(69.6–98.6)	ns	57.0–111.0
AST(U/L)	32(25–45)	44(34–56)	ns	8–40
ALT(U/L)	34(27–67)	49(29–75)	ns	5–40
WBC count(*10^9/L)	5.93(4.77–7.45)	9.32(6.37–10.99)	ns	3.50–9.50
NEU%	78.20(71.10–84.70)	86.70(63.50–91.15)	ns	40.00–75.00
LYO%	14.30(11.90–18.90)	7.60(4.55–16.40)	ns	20.00–50.00
CRP(mg/L)	18.87(12.26–43.66)	73.00(36.57–116.95)	<0.05	0.00–8.00
Cardiac Biomarkers				
cTnI(ng/L)	4.8(2.5–8.4)	46.8(34.2–299.8)	<0.001	<26.2
Myo(ng/mL)	62.8(33.0–87.7)	101.75(59.4–212.4)	ns	<146.9
CK(U/L)	88(45–125)	199(77–285)	<0.05	24–194
CKMB(U/L)	10(17–13)	13(10–25)	ns	0–25
HBDB(U/L)	245(207–275)	453(347–547)	<0.01	72–182
AST(U/L)	32(25–45)	44(34–56)	ns	8–40
LDH(U/L)	287(246–331)	513(414–641)	<0.01	109–245

Abbreviations: IQR: inter quartile range; CRE: Creatine; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; WBC: White blood count; NEU%: Neutrophil percentage; LYO%: Lymphocyte percentage; CRP: C reactive protein; cTnI: cardiac troponin I; Myo: myoglobin; CK: Creatine Kinase; CKMB: Creatine kinase–MB; HBDB: α-hydroxybutyrate dehydrogenase; AST: Aspartate aminotransferase; LDH: Lactate Dehydrogenase.

Table 2

Comparison of the severe and very severe patients with normal or elevated cardiac biomarkes.

Group	Number	cTnI (ng/L)		CK (U/L)		HBDB (U/L)		LDH (U/L)	
		Normal	Elevated	Normal	Elevated	Normal	Elevated	Normal	Elevated
Severe	26	25	1	23	3	4	22	6	20
Very Severe	8	0	8	4	4	0	8	0	8
P value		<0.001		<0.05		ns		ns	

Abbreviations: cTnI: cardiac troponin I; CK: Creatine Kinase; HBDB: α-hydroxybutyrate dehydrogenase; LDH: Lactate Dehydrogenase.

Simple t-test and Mann-Whitney U test was used to compare continuous variables. Fisher's exact test was used to compare categorical variables.

We noted significantly increased cTnI, CK, HBDB and LDH levels in very severe group as compare to severe (Table 1). We then applied Fisher's exact test to determine the positive rate of cardiac lesion markers between severe and very severe patients. Increasingly, the percentage of very severe patients with elevated cTnI levels was markedly higher, with 8/8 patients exhibiting increased cTnI in very severe group, and only 1/26 patient in severe group (*P* value<0.001). In addition, the abnormal percentage of HBDB and LDH showed no significant difference between 2 groups (Table 2).

Recently, a number of studies have described the epidemiological and clinical characters of COVID-19.[2–4] A study of 41 patients with COVID-19 has suggested that 12% of the mild and severe cases combined showed increased hyper sensitivity troponin I, suggesting acute myocardial injury.[2] It is also reported that severe acute respiratory syndrome coronavirus (SARS-CoV)[6] and Middle East respiratory syndrome coronavirus (MERS-CoV)[7] have caused critical cardiac lesions. In the present study, we have focused on cardiac lesion biomarkers in severe and very severe patients with COVID-19. We have proved elevation of cTnI, CK, HBDB, and LDH in critical cases. It is important to notify that, in very severe group, 8/8 patients exhibit cTnI above reference level; while 1/26 in severe group. This suggests that elevated cTnI could be a potential indicator for critically ill patients. It is worth notifying that among the 8 critically ill patients enrolled, the kidney and liver function markers are not as significantly disturbed as the cardiac lesion markers, suggesting that most patients enrolled have not been suf-

fering from multiple organ dysfunction syndrome (MODS). Thus, the consistently high cTnI levels in very severe group point to the importance that the heart injury could be a distinct, or even lethal feature in very severe COVID-19. Protecting from myocardial injury could be of vital importance in clinical treatment for reducing the mortality rate. The study was limited by small sample size. And we haven't analyzed the echocardiography and MRI for the patients enrolled. Further analysis is needed to determine the etiology.

Declaration of Competing Interest

We declare no competing interests.

Author Contributions

BZ, XM and YW collected the clinical and laboratory data. JS processed statistical analysis. JS and BZ drafted the manuscript. XM and YW revised the final manuscript. XM and YW is responsible for all clinical and laboratory data.

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Additional contributions

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References

1. Yang W., Cao Q., Qin L., et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19):a multi-center study in Wenzhou city, Zhejiang, China. *J Infect* 2020.
2. Huang C., Wang Y., Li X., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020.
3. Chen N., Zhou M., Dong X., et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet* 2020.
4. Wang D., Hu B., Hu C., et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA* 2020.
5. National Health Commission of China. *New coronavirus pneumonia prevention and control program*. 4th edn; 2020. Jan 22 http://www.gov.cn/zhengce/zhengceku/2020-01/28/5472673/files/0f96c10cc09d4d36a6f9a9f0b42d972b.pdf (in Chinese).
6. Ksiazek T.G., Erdman D., Goldsmith C.S., et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;**348**(20):1953–66.
7. Cauchemez S., Fraser C., Van Kerkhove M.D., et al. Middle east respiratory syndrome coronavirus: quantification of the extent of the epidemic, surveillance biases, and transmissibility. *Lancet Infect Dis* 2014;**14**(1):50–6.

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Wuhan and Hubei COVID-19 mortality analysis reveals the critical role of timely supply of medical resources

Dear Editor,

The 2019 novel coronavirus diseases (COVID-19) outbreak caused by SARS-CoV-2 is on-going in China and has hit many countries.[1–3] As of 3 March 2020, there have been 80,270 confirmed cases and 2981 deaths in China, most of which are from the epicenter of the outbreak, Wuhan City, the capital of Hubei Province. New COVID-19 cases have been steadily declining in China and more than 60,000 patients have been recovered,[4] largely due to the effective implementation of comprehensive control measures in China.[5,6] Here we report that some of these measures, such as a dramatic and timely increase of medical supplies, may play a critical role such that the mortality and recovery rates of COVID-19 in Wuhan follow exponential decay and growth modes, respectively.

We collected data for analysis on the officially released cumulative numbers of confirmed, dead and recovered cases (from 23 Jan to 3 Mar 2020) in five geographic regions, i.e., mainland China, Hubei Province, outside Hubei (in China), Wuhan City and outside Wuhan (in Hubei). As of 3 Mar 2020, crude fatality ratios (CFRs) in the above regions are 0.027 ± 0.006, 0.035 ± 0.007, 0.005 ± 0.002, 0.045 ± 0.012 and 0.021 ± 0.008, respectively, in line with earlier reports.[5,6] While the mortality rates of COVID-19 outside Hubei and outside Wuhan appear constant over time, the mortality rates in Hubei and Wuhan decline continuously (Fig. 1(A)). Strikingly, the mortality rates in Hubei and Wuhan are well-fitted with the exponential decay mode (R^2 being 0.93 and 0.82, respectively; Fig. 1(A) and Table S1), and it is the same forth with that in China (R^2 being 0.86) but not with that outside Hubei and outside Wuhan (R^2 being 0.39 and 0.32, respectively). Remarkably, we found that the recovery rates of COVID-19 patients in the above regions were all well-fitted with the exponential growth mode (R^2 being 0.96, 0.95, 0.95, 0.88 and 0.95, respectively; Fig. 1(B) and Table S1). Such intriguing pattern for the COVID-19 mortality and recovery rates in Wuhan (or Hubei) somehow contradicts traditional epidemiological models wherein both are assumed as constants.[7]

The above unique pattern may reflect the fact that COVID-19 patients in Wuhan (or Hubei) have been treated more effectively day by day. Here we focused on two components essential for effective treatments, i.e., the supply of health workers and hospital beds. As a matter of fact, a great number (up to 42,000, as of 1 March 2020) of health workers have been aided by other provinces in China (Table S2) and they are working in different cities of Hubei (Table S3). This extraordinary aid keeps the ratio of the health workers to patients in Hubei at above 0.6 despite the number of remaining confirmed cases has ten-fold increased up to 50,000 on 18 Feb (Fig. 1(C)). Results also show that the number of acute care beds from more than 45 designated hospitals plus two newly built ones in Wuhan has been consecutively increasing up to 23,532 (as of Feb 24) under the government-directed re-allocation (Fig. 1(D)). This supply thus enabled the severe and critical patients to be treated timely and effectively.[6] More importantly, there have been over 10 temporary hospitals (named Fangcang hospitals) reconstructed from gymnasium and exhibition centers, which provide more than 26 000 makeshift beds for mild patients (Fig. 1(D)). These combinations guaranteed nearly 100% of COVID-19 patients to be treated in hospitals even if the number of remaining confirmed cases has ten-fold increased up to 38 000 on 18 Feb (Fig. 1(D)). In contrast, a lot of patients had to stay at home in the early stage of the outbreak in Wuhan due to the shortage of beds such that many transmissions in households occurred.[6]

Accordingly, the effective implementation of comprehensive control measures and infection-treatment practices is critical for combatting any new pathogens, not only interrupting the transmissions but also saving the patients. Timely supplied medical resources, including re-allocation of acute care beds, rapid construction of new hospitals and generous aid of health workers by other less-severe areas, apparently help the epicenter of the outbreak Hubei (Wuhan) to accomplish a unique and also encouraging outcome for life-saving such that the mortality and recovery rates of nearly 50,000 COVID-19 patients exponentially decays and grows, respectively. Other crucial factors contributing to this success may include the improved and optimized diagnosis and treatment strategies,[8] which are critical for saving severe and critical patients.[5,6,9] This speculation appears to be supported by the exponential growth of the COVID-19 recovery rate outside Hubei (Fig. 1(B)) where medical resources are relatively sufficient over time.[10] Collectively, the achievement made in Hubei (or Wuhan) may provide useful guidance for many countries to be better prepared for the potential pandemic[2] that may overwhelm local health care systems.
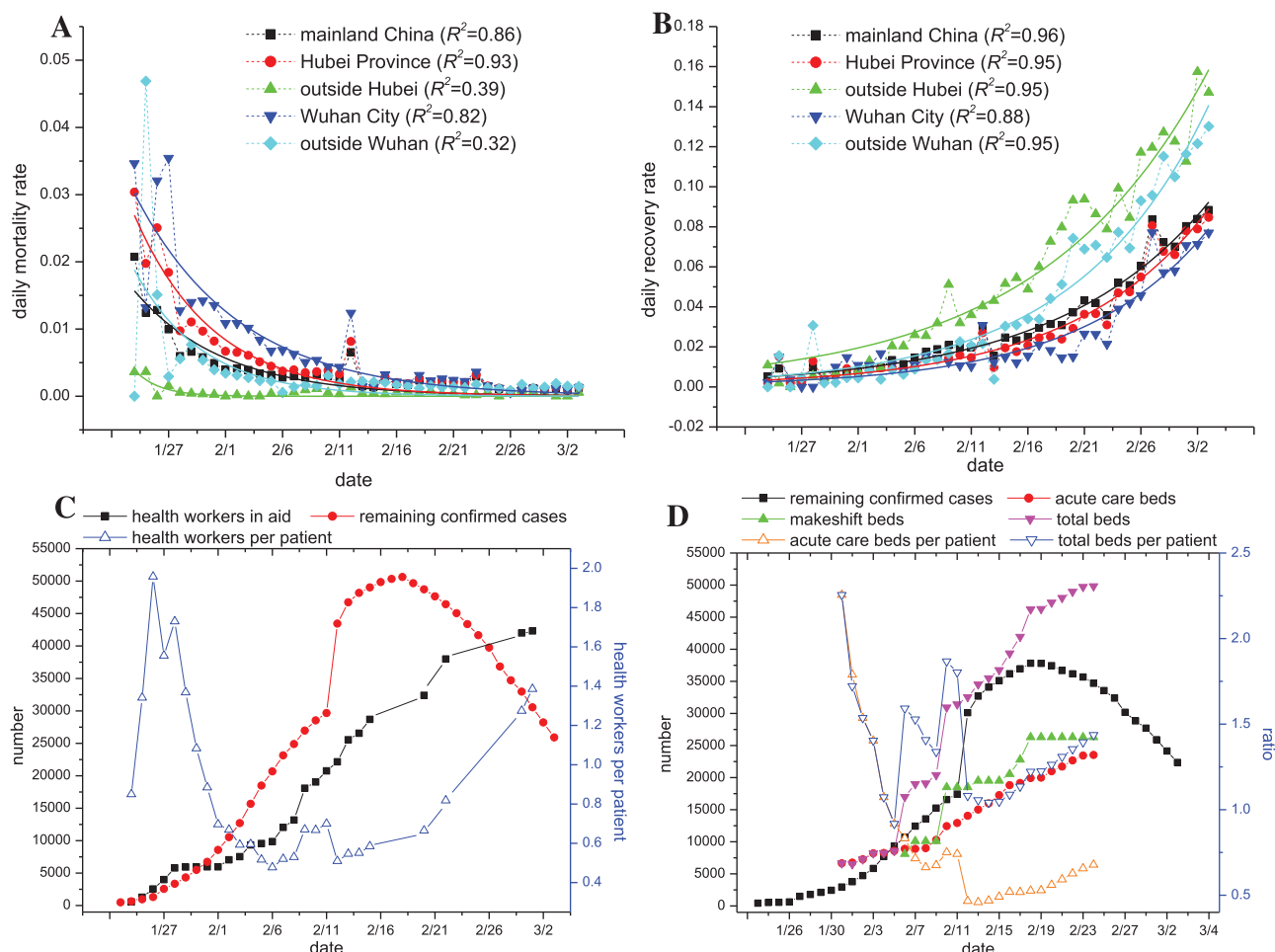
Fig. 1. Fitting the COVID-19 mortality and recovery rates with exponential decay and growth functions, respectively and timely supply of medical resources. (**A, B**) Mortality rate (panel A) and recovery rate (panel B) for COVID-19 in China over time and by location, with exponential decay- and growth-based regression analyses being performed, respectively (as shown by colored solid lines). Parameters from the regression analyses are shown in Table S1, with R^2 being shown here. (**C**) Numbers of the aided health workers in Hubei over time. Ratio of the aided health workers to patients was also plotted (note: most of the aided health workers are working in Wuhan; refer to Table S3). (**D**) Numbers of the remaining confirmed cases of COVID-19, and acute care beds, makeshift beds from Fangcang hospitals and total beds in Wuhan over time. Ratio of beds to patients was also plotted. Here the data of newly supplied beds in Hubei are not available and thus the data for Wuhan were analyzed.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.03.018.

References

1. Tang J.W., Tambyah P.A., Hui D.S.C.. Emergence of a novel coronavirus causing respiratory illness from Wuhan, China. *J Infect* 2020. doi:10.1016/j.jinf.2020.01.014.
2. WHO: Coronavirus disease (COVID-19) outbreak. Available from: https://www.who.int/emergencies/diseases/novel-coronavirus-2019. visited on 4 March, 2020.
3. Zhou P.. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020. doi:10.1038/s41586-020-2012-7.
4. The National Health Commission of China, real-time data of the COVID-19 outbreaks in China (in Chinese), http://www.nhc.gov.cn/xcs/yqfkdt/gzbd_index.shtml. accessed on 4 March, 2020.
5. The Novel Coronavirus Pneumonia Emergency Response Epidemiology Team. Vital surveillances: the epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19)—China, 2020, http://weekly.chinacdc.cn/en/article/id/e53946e2-c6c4-41e9-9a9b-fea8db1a8f51, in China CDC Weekly.
6. Report of the WHO – China Joint Mission on Coronavirus Disease 2019 (COVID-19), https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf. 2020.
7. Wang X.S., Wu J., Yang Y.. Richards model revisited: validation by and application to infection dynamics. *J Theor Biol* 2012;**313**:12–19.
8. The National Health Commission of China, coronavirus disease 2019 (COVID-19) diagnosis and treatment plan (in Chinese, 7th version). Available from: http://www.nhc.gov.cn/yzygj/s7653p/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml., visited on 5 March, 2020.
9. Yang X., et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med* 2020. doi:10.1016/S2213-2600(20)30079-5.
10. Ji Y., et al. Potential association between COVID-19 mortality and healthcare resource availability. *Lancet Glob Health* 2020. doi:10.1016/S2214-109X(20)30068-1.

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Comparisons of viral shedding time of SARS-CoV-2 of different samples in ICU and non-ICU patients

Dear Editor,

We read with interest the recent letter by Hao[1] that addressed the initial negative RT-PCR result in atypical patients. Since outbreak of unexplained pneumonia cases in Wuhan, China in December, 2019,[2] the coronavirus disease 2019 (COVID-19) has spread to more than 90 countries. By 7th March, the infection of SARS-CoV-2 has influenced 101,918 patients globally.[3] Recommended by World Health Organization (WHO),[4] a positive real-time reverse transcriptase polymerase chain-reaction (RT-PCR) result could confirm the diagnosis of suspected COVID-19 patients. However, there still lacks thoroughly research concerning viral shedding time among different samples in COVID-19 patients. Here we compared the viral shedding time of SARS-CoV-2 in different samples of intensive care unit (ICU) and non-ICU patients and analyzed their characteristics.

We analyzed a total of thirty-two COVID-19 patients admitted to Central Hospital of Xiangtan from January to February, 2020. Dynamic clinical samples including nasal swabs, blood, fecal, urine, saliva and tears were collected from each patient for surveillance. What is more, we recorded synchronous clinical and epidemiologic information with oral consent. Total RNA was extracted from clinical samples and we performed RT-PCR tests targeting SARS-CoV-2. The continuous variants were described by mean when they conform to Kolmogorov-Smirnov test. We did analysis of Chi-square tests and Mann-Whitney tests to compare differences among groups. $P < 0.05$ was considered significant. Statistical analyses and figures were conducted using the Stata 14 and GraphPad Prism 8.

From Table 1, the thirty-two patients include eight ICU and twenty-four non-ICU patients, their age ranged from 34 to 54 years old. A proportion of 43.8% (14/32) patients were from Wuhan and none come back from other cities of Hubei province. Three patients had not been to Hubei province but infected by people from Hubei. Nine of thirty-two patients were identified as family clustered infection. Seven patients had no clear contact history. For the enrolled patients, 40.6% (13/32) of them carried underlying diseases, of which the common diseases were hypertension (5 patients) and

Table 1
Clinical and epidemiologic characteristics of COVID-19 patients.

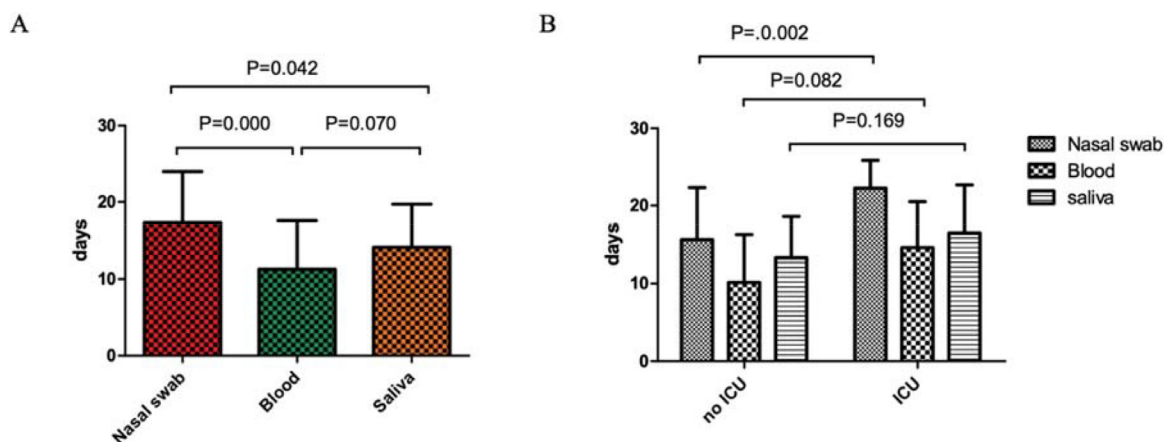|	Patients ($n = 32$)
Age-year	41(34–54)
Male/Female-no.	16/16
BMI	24.5 (22.6–26.5)
Epidemic-no. (%)	
From Hubei province	Wuhan 14(43.8%), other area of Hubei province 0(0.0%)
Not been to Hubei province, but infected by people from Hubei province	3(9.4%)
Without any clear contact history	7(21.9%)
Family cluster infection	9(28.1%)
Hospital-related transmission rate (Xiangtan City)	0(0.0%)
Underlying disease- no. (%)	
Diabetes	4(12.5)
Hypertension	5(15.6)
Cardiovascular diseases	1(3.1)
Liver disease	2(6.3)
Malignancy	0(0.0)
Others	1(3.1)
Initial symptom-no. (%)	
Fever	17 (53.1)
Cough	24 (75.0)
Fatigue	5 (15.6)
Headache	6 (18.8)
Diarrhea	3 (9.4)
Sore throat	7 (21.9)
Muscular soreness	6 (18.8)
Anhelation	10 (31.2)
No symptoms	4 (12.5)

diabetes (4 patients). The average onset days was five days and the most common initial symptom was cough (75. 0%, 24/32). Surprisingly, only 53.1% (17/32) patients presented with fever in the beginning. The other symptoms encompassed fatigue, headache, diarrhea, sore throat, muscular soreness and anhelation. Four patients were diagnosed as COVID-19 without symptoms.

As indicated by RT-PCR results, we obtained positive rate and viral shedding time of different samples in ICU and non-ICU patients. The positive rate of nasal swab in these patients was 100.0% (32/32) and three patients experienced negative result at the first tests. The positive rate of saliva (78.1%, 25/32) was significantly higher than that of tears (15.6%, 5/32) in enrolled patients ($p<0.001$). All the urine samples from thirty-two patients were negative. In the tests of blood, the positive rate was 87.5% (7/8) in ICU and 66.7% (16/24) in non-ICU patients respectively.

What is more, we performed consecutive analyses of nasal swab, blood and saliva in non-ICU and ICU groups. As shown in Fig. 1(A), the viral shedding (from positive to negative) time of SARS-CoV-2 of nasal swab was significantly longer than that of blood ($p = 0.000$) and saliva ($p = 0.05$). Though shorter time of viral shedding in blood samples than saliva was observed, no significant p value was detected ($p = 0.070$). The viral shedding time of SARS-CoV-2 of nasal swab was 15.67±6.68 days in non-ICU group and 22.25±3.62 days in ICU group. For blood, the viral shedding time was 10.17±6.13 and 14.63±5.88 days in non-ICU and ICU patients respectively. Saliva in non-ICU and ICU patients took 13.33±5.27 and 16.50±6.19 days separately to converse to negative. As shown in Fig. 1(B), the viral shedding time of SARS-CoV-2 of nasal swab in non-ICU patients was significantly shorter than ICU patients ($P = 0.02$). No obvious significance was indicated in blood and saliva samples between non-ICU and ICU groups.

RT-PCR was a widely used tool in diagnosing SARS-CoV-2 infection. Guided by WHO,[4] two consecutive negative RT-PCR result with at least two days apart should be achieved before discharge of patients. Several cases have been reported negative of RT-PCR tests before confirmation.[1] In our study, one patient experienced five continuous negative results before admission and then the sixth

Fig. 1. Comparisons of viral shedding time of SARS-CoV-2 in different samples and patients.
(A) Comparisons of viral shedding time of SARS-CoV-2 in different samples.
(B) Comparisons of viral shedding time of SARS-CoV-2 of different samples in non-ICU and ICU patients.

nasal swab on admission finally supported his diagnosis. Considering this situation, the molecular diagnosis should be made with other clinical samples or other etiologic tests including serological tests, CRISPR, or metagenomic sequencing. The viral shedding time of SARS-CoV-2 was reported around two weeks.[5] The longer time consumed to turn from positive to negative in nasal swabs than in blood and saliva samples was in accordance with the respiratory transmission characteristics of the disease, and indicated that a longer surveillance needed in respiratory samples for nucleic acid testing.

What is more, in our study, we categorized patients into two groups: ICU and non-ICU. In ICU patients, the viral shedding time of blood, nasal and saliva sample all exceeded two weeks, illustrating a relatively longer period than non-ICU patients. These differences of viral shedding time in ICU and non-ICU patients might be correlated with virus load, severity and invasive operations of patients.

In conclusion, our study originally illustrated that nasal swab samples consumed more time to turn negative than blood and saliva samples. What is more, viral shedding time of SARS-CoV-2 in ICU patients was longer than that of non-ICU patients, especially nasal swab samples. A combination tests of different samples might provide us with further information concerning the transmission characteristics of COVID-19 patients.

Declaration of Competing Interest

All authors report no potential conflict of interest.

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References

1. Hao W.. Clinical features of atypical 2019 novel coronavirus pneumonia with an initially negative RT-PCR assay. *J Infect* 2020 PubMed PMID: 32092387. Epub 2020/02/25.
2. Zhou P., Yang X.L., Wang X.G., Hu B., Zhang L., Zhang W., et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020 PubMed PMID: 32015507. Epub 2020/02/06.
3. Available from: https://ncov.dxy.cn/ncovh5/view/pneumonia?scene=2&clicktime=1579582186&enterid=1579582186&from=timeline&isappinstalled=0.
4. World Health Organization. Clinical management of severe acute respiratory infection when novel coronavirus (nCoV) infection is suspected: interim guidance. [cited 394 10196]. Available from: https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf.
5. To K.K., Tsang O.T., Chik-Yan Yip C, Chan K.H., Wu T.C., Chan J.M.C., et al. Consistent detection of 2019 novel coronavirus in saliva. *Clin Infect Dis* 2020 PubMed PMID: 32047895. Epub 2020/02/13.

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Profile of specific antibodies to SARS-CoV-2: The first report

Dear Editors,

A novel coronavirus (COVID-19) epidemic threatens the world.[1,2] Before this study, some studies reported cases of viral detection by RT-PCR at different timepoints throughout the disease course.[3,4] However, these reports monitored SARS-CoV-2 in the acute phase of infection. Currently no study reported the profile of specific antibodies to SARS-CoV-2 infection. Profile of specific antibodies in patients' blood can assist diagnosis and reflect the disease course. Here, we first studied the profile of IgM and IgG for SARS-CoV-2 from 34 COVID-19 patients.

A total of 34 hospitalized patients (admission date from Feb 1st to Feb 29th, 2020) with confirmed SARS-CoV-2 infection were included in this study. All enrolled patients were confirmed diagnosed of COVID-19 according to the diagnosis and treatment guideline for SARS-CoV-2 from Chinese National Health Committee (Version 5) and the interim guidance from Centers for Disease Control and Prevention.[5,6] Blood samples were obtained at different date after onset of symptoms to detect the specific antibodies to SARS-CoV-2. IgM and IgG were analyzed by chemiluminescent immunoassay according to the manufacturer's protocol (Shenzhen Yahuilong Biotechnology Co., Ltd). All data (test dates and results of IgM and IgG) were collected up to the final follow-up date (March 3rd, 2020).

Details of demographic characteristics and test dates and results of IgM and IgG were list in Table 1. Except for two patients (2 days and 3 days after symptoms onset), all included patients had IgM and IgG tests after 2 weeks from symptoms onset. We categorized patients by weeks according to the date of antibodies test after symptoms onset. In week 3 after symptoms onset, all patients were tested positive for IgM and IgG, with the mean value of 322.80AU/ml and 112.40AU/ml (Reference:<10AU/ml) respectively. In week 4, all the results were still positive for IgM and IgG. IgM declined while IgG continued to go up, with the mean value of 147.92AU/ml and 157.01AU/ml respectively. In week 5, however, all patients were positive for IgG, while 2 patients (16.7%) got negative results for IgM. IgM level kept going down to 78.03AU/ml and IgG continued up to 163.56AU/ml. At the end of observation (7 weeks), 2 patients (33.3%) got negative results for IgM, while all patients positive for IgG, with the mean value of 21.83AU/ml and 167.16AU/ml respectively. (Fig. 1)

Genomic studies have shown that SARS-CoV-2 shared around 80% identity sequencing with SARS-CoV, which caused a global epidemic with 8096 confirmed cases worldwide in 2002–2003.[7] Study of case series suggested the viral nucleic acid shedding pattern of patients infected with SARS-CoV-2 is different from SARS-CoV.[3] For SARS-CoV, studies revealed that IgM reached the highest point within 4 weeks and was not detectable on 3 months after onset of symptoms. IgG were persistently detectable up to 24 months.[8]

Our results suggested that the profile of specific antibodies to SARS-CoV-2 is similar to SARS-CoV. Detectable and continuous high level of IgM indicated the acute phase of infection. Furthermore, IgM last more than a month indicating the prolonged virus replication in SARS-CoV-2 infected patients. IgG responded later than IgM and persisted high in our study, indicating the humoral immune reaction to protect the body against SARS-CoV-2 virus.

The detection and profile of specific antibodies to SARS-CoV-2 will provide valuable information for rapid screening of suspects,

Table 1
Characteristics of demographic and specific antibodies in COVID-19 patients ($N = 34$).

Patients	Age	Gender	IgM AU/ml	IgG AU/ml	Test days, after onset	Week	Mean IgM AU/ml	SEM	Mean IgG AU/ml	SEM
Patient1	82	Female	3.56	2.48	2	1	2.24	1.33	1.96	0.53
Patient2	87	Female	0.91	1.43	3					
Patient3	59	Female	122.73	163.85	14	3	322.80	127.65	112.40	21.43
Patient4	72	Male	924	187.4	14					
Patient5	32	Female	111.78	110.17	16					
Patient6	63	Male	207.39	55.43	17					
Patient7	69	Male	401.19	78.71	17					
Patient8	64	Male	169.72	78.82	21					
Patient9	65	Female	104.77	187.94	22	4	147.92	94.50	157.01	12.40
Patient10	39	Male	16.63	152.88	23					
Patient11	52	Male	89.31	179.18	23					
Patient12	49	Male	41.55	128.75	24					
Patient13	35	Male	62.5	103.89	27					
Patient14	31	Male	11.26	193.83	28					
Patient15	58	Female	709.39	152.57	28					
Patient16	44	Male	36.57	190.85	30	5	78.03	33.55	163.56	15.95
Patient17	26	Female	14.37	277.91	30					
Patient18	57	Male	1.65	76.85	30					
Patient19	45	Male	35.44	165.27	30					
Patient20	62	Female	3.38	200.95	31					
Patient21	65	Male	119.35	155.29	32					
Patient22	83	Male	18.95	158.32	33					
Patient23	43	Male	48.88	73.64	33					
Patient24	53	Male	134.24	135.54	33					
Patient25	33	Female	54.39	162.01	33					
Patient26	64	Male	422.78	159.38	34					
Patient27	60	Female	46.34	206.7	34					
Patient28	73	Male	5.27	134.94	36	6–	21.83	5.72	167.16	12.24
Patient29	25	Female	15.11	179.23	36	7				
Patient30	71	Male	36.11	184.56	37					
Patient31	56	Female	34.07	121.08	37					
Patient32	54	Male	42.28	216.96	38					
Patient33	47	Male	11.2	176.73	38					
Patient34	54	Male	8.75	156.59	49					

Abbreviations: SEM, standard error of mean. *Reference of IgG and IgM are 10AU/ml.
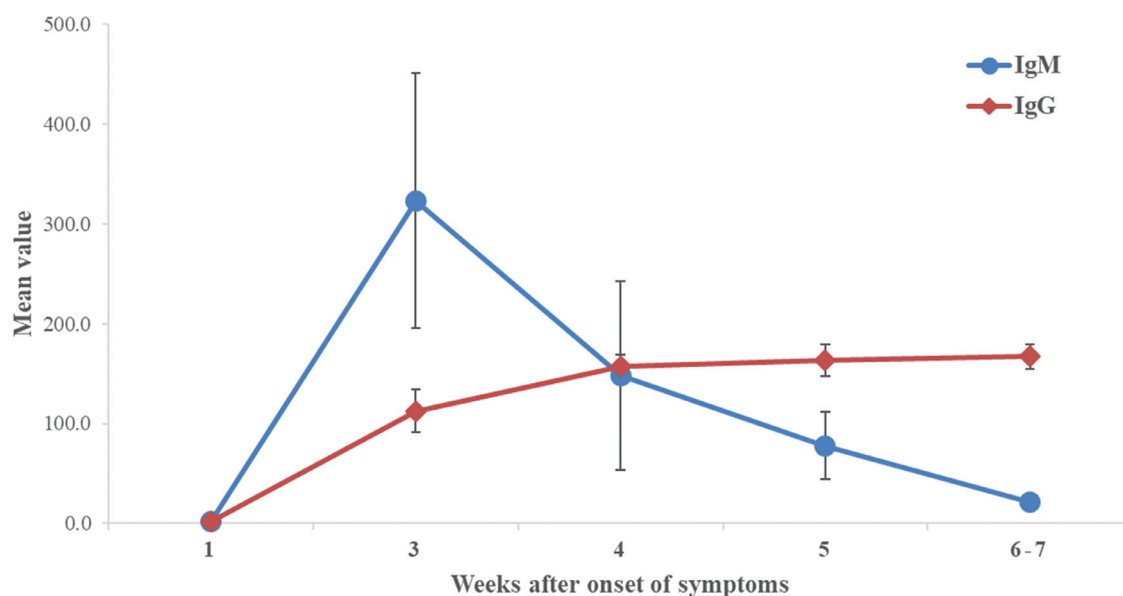
Fig. 1. Timeline of IgM and IgG Antibodies level to SARS-CoV-2 from the onset of symptoms.

assist diagnosis and evaluate the disease course. Furthermore, concentrated IgG antibody may be informative in vaccine development and treatment for SARS-CoV-2.

Declaration of Competing Interest

All authors declare that there are no conflicts of interest.

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Informed consent

Oral consent was obtained from patients involved before enrollment when data were collected retrospectively.

References

1. Tang J.W., TAMbyah P.A., Hui D.S.. Emergence of a novel coronavirus causing respiratory illness from Wuhan, China. *J. Infect.* 2020 https://doi.org/. doi:10.1016/j.jinf.2020.01.014.
2. Zunyou W., Jennifer M.G.. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in china: Summary of a report of 72,314 cases from the Chinese center for disease control and prevention. *JAMA* 2020 Published online February 24. doi:10.1001/jama.2020.2648.
3. Zou L, Ruan F., Huang M., et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl. J. Med.* 2020 Published online February 19. doi:10.1056/NEJMc2001737.
4. Pan Y., Zhang D.T., Yang P., et al. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis.* 2020. Published Online February 24https://doi.org/10.1016/S1473-3099(20)30113-4.
5. Interim infection prevention and control recommendations for patients with confirmed coronavirus disease 2019 (COVID-19) or persons under investigation for COVID-19 in healthcare settings. (Update: February 21, 2020). https://www.cdc.gov/coronavirus/2019-ncov/hcp/infection-control.html.
6. China National Health Commission Diagnosis and treatment of 2019-nCoV pneumonia in China; 2020. (Version 5) In Chinese. Published February 8Accessed March 3, 2020 http://www.nhc.gov.cn/yzygj/s7653p/202002/d4b895337e19445f8d728fcaf1e3e13a/files/ab6bec7f93e64e7f998d802991203cd6.pdf
7. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. Geneva: World Health Organization 2004 https://www.who.int/csr/sars/country/table2004_04_21/en/.
8. Mo H.Y., Zeng G.Q., Ren X.L., et al. Longitudinal profile of antibodies against SARS-coronavirus in SARS patients and their clinical significance. *Respirology* 2006;**11**:49–53.

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Community-acquired Group B streptococcal meningitis in adults

Dear Editor,

We were somewhat surprised that the recent review of community-acquired Group B streptococcal meningitis in adults by van Kassel et al.[1] contained no reference to recent work implicating foodborne transmission, particularly from raw or undercooked fish, as an important mode of acquisition of this condition in some parts of the world. The first evidence for this came from a large outbreak of invasive Group B streptococcal (GBS) infection, primarily bacteraemia, septic arthritis and meningitis, in Singapore in 2015[2]. Both epidemiological and molecular evidence suggested that these infections, caused by GBS of serotype III, subtype

4 (serotype III-4), multilocus sequence type (MLST) 283 (ST283), were associated with the consumption of raw, farmed freshwater fish.[3,4] GBS are well-known primary pathogens in fish.[5] More recently it has been shown that this lineage is actually widespread amongst both human isolates of GBS in SE Asia and fish isolates both from Asia and Brazil. GBS is now a not uncommon cause of meningitis in adults in countries such as the Lao People's Democratic Republic;[6] in addition, earlier reports had already noted an increase in the incidence of adult GBS meningitis in both Hong Kong and Singapore, including many cases subsequently confirmed as being caused by ST283.[7,8] It thus appears possible that an extensive but previously unrecognised outbreak of invasive GBS disease associated with the consumption of raw or undercooked farmed fish, a common practice in some regions, may have been going on for several decades.[8]

We feel that the failure to mention these observations, which include 60 cases of adult meningitis and could fundamentally change the way in which we view the epidemiology of adult GBS disease, was an unfortunate omission in a review of adult community-acquired GBS meningitis.

Yours sincerely

References

1. van Kassel M.N., van Haeringen K.J., Brouwer M.C., Bijlsma M.W., van de Beek D.. Community-acquired Group B streptococcal meningitis in adults. *J Infect* 2020;**80**:255–60.
2. Kalimuddin S., Chen S.L., Lim C.T.K., Koh T.H., Tan T.Y., Kam M., et al. 2015 Epidemic of severe *Streptococcus agalactiae* Sequence Type 283 infections in Singapore associated with the consumption of raw freshwater fish: a detailed analysis of clinical, epidemiological, and bacterial sequencing data. *Clin Infect Dis* 2017;**64**(suppl_2):S145–SS52.
3. Tan S., Lin Y., Foo K., Koh H.F., Tow C., Zhang Y., et al. Group B Streptococcus Serotype III Sequence Type 283 bacteremia associated with consumption of raw fish, Singapore. *Emerg Infect Dis* 2016;**22**:1970–3.
4. Rajendram P., Mar Kyaw W., Leo Y.S., Ho H., Chen W.K., Lin R., et al. Group B Streptococcus Sequence Type 283 disease linked to consumption of raw fish, Singapore. *Emerg Infect Dis* 2016;**22**:1974–7.
5. Chen S.L.. Genomic insights into the distribution and evolution of Group B streptococcus. *Front Microbiol* 2019;**10**:1447 doi: 10.3389/fmicb.2019.01447.
6. Dubot-Pérès A., Mayxay M., Phetsouvanh R., Lee S.J., Rattanavong S., Vongsouvath M., Davong V., Chansamouth V., Phommasone K., Moore C., Dittrich S., Lattana O., Sirisouk J., Phoumin P., Panyanivong P., Sengduangphachanh A., Sibounheuang B., Chanthongthip A., Simmalavong M., Sengdatka D., Seubsanith A., Keoluangkot V., Phimmasone P., Sisout K., Detleuxay K., Luangxay K., Phouangsouvanh I., Craig S.B., Tulsiani S.M., Burns M.-A., Dance D.A.B., Blacksell S.D., de Lamballerie X., Newton P.N.. Revisiting management of central nervous system infections in Southeast Asia. *Emerg Infect Dis* 2019;**25**:898–910.
7. Wilder-Smith E., Chow K.M., Kay R., Ip M., Tee N.. Group B streptococcal meningitis in adults: recent increase in Southeast Asia. *Aust N Z J Med* 2000;**30**(4):462–5.
8. Barkham T., Zadoks R.N., Azmai M.N.A., Baker S., Bich V.T.N., Chalker V., et al. One hypervirulent clone, sequence type 283, accounts for a large proportion of invasive *Streptococcus agalactiae* isolated from humans and diseased tilapia in Southeast Asia. *PLoS Negl Trop Dis* 2019;**13**(6):e0007421.

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Cerebrospinal fluid analysis from bilateral external ventricular drains in suspected nosocomial infection

To the Editor,

We read with interest the recently published systematic review on mass spectrometry-based proteomic techniques to identify cerebrospinal fluid biomarkers for diagnosing suspected central nervous system infections.[1] We have investigated the variability of CSF characteristics between drains in patients with bilateral external ventricular drains (EVDs) resulting in challenges in diagnosing an external ventricular catheter-associated infection (ECAI).

External ventricular cerebrospinal fluid (CSF) drains can be used for the monitoring of intracranial pressure, the temporary diversion of cerebrospinal fluid from an obstructed ventricular system or as part of the treatment approach for infected internal catheters.[2] Bilateral ventricular CSF drain insertion may be necessary in those with substantial subarachnoid hemorrhage potentially obstructing CSF flow in the foramen of Monro.[3,4] If infection is suspected in patients with an EVD, CSF is typically analyzed for leukocyte count, glucose and total protein concentration, and CSF cultures are performed.[2,5] No guidance is provided for patients with bilateral CSF drains on whether one or both drains should be sampled.[5] We performed a consecutive single-center prospective observational cohort study on the diagnostic accuracy of CSF parameters for ECAI. The objective of the current study was to analyze the variability of CSF characteristics between drains in patients with bilateral EVDs.

Adult patients admitted to the Intensive Care Unit of the Haaglanden Medical Center with an EVD were included. Exclusion criteria were expected death <24 h and infection at presentation. As part of standard care, CSF samples were obtained daily at the proximal stopcock of a closed external drainage- and monitoring system until the drain was removed. CSF was analyzed for leukocyte count, glucose- and total protein concentration. CSF Gram stain and culture were performed daily. Blood leukocyte and erythrocyte count were also collected when available. The study was approved by the medical ethical committee.

Patients were diagnosed with ECAI based on the Infectious Diseases Society of America (IDSA) guideline in which an infection is defined as 'single or multiple positive CSF cultures with CSF

Table 1
Median values of the lowest and highest drains, median difference- and median fold difference between drains per parameter.

Parameter	Median value lowest drain (IQR) [range]	Median value highest drain (IQR) [range]	Median difference (IQR) [range]	Median fold difference (IQR) [range]
Leukocyte count (x 10^6/L)	138 (26–451) [0–3080]	424 (69–1054) [0–10,600]	123 (16–477) [0–10,570]	1.9 (1.2–3.1) [1–388]
Percentage polymorphonuclear cells	62% (46–75%) [0–1]	75% (57–81%) [0–1]	7% (2–16) [0–73]	1.1 (1.0–1.3) [1–12]
Erythrocyte count (x 10^6/L)	43,000 (10,000–132,000) [0–678,000]	107,000 (28,000–453,000) [0–3050,000]	30,000 (7000–210,000) [0–3038,000]	1.9 (1.2–3.8) [1–254]
Leukocyte count corrected for blood admixture per 1000 erythrocytes (x 10^6/L)	56 (0–288) [−658–2525]	180 (18–693) [−161–7840]	77 (13–340) [0–7876]	Not applicable
Cell index	0.67 (0.37–1.32) [0–12.97]	1.0 (0.59–1.84) [0–16.13]	0.28 (0.12–0.83) [0–8.73]	1.5 (1.2–2.2) [1–8.5]
Glucose concentration (mmol/L)	3.8 (3.1–4.6) [0.3–6.7]	4.4 (3.7–5.1) [2.6–7.2]	0.4 (0.2–0.6) [0–6.8]	1.1 (1.0–1.2) [1–23.7]
Protein concentration (g/L)	0.7 (0.4–1.3) [0.1–23.5]	1.3 (0.6–2.1) [0.1–25.1]	0.27 (0.08–0.89) [0–23.8]	1.4 (1.2–2.0) [1–43]

pleocytosis and/or hypoglycorrhachia, or an increasing cell count, and clinical symptoms suspicious for ventriculitis or meningitis.'[5]

We analyzed the difference in CSF-results between CSF sampled simultaneously from both drains by using SPSS version 26. The median difference and median fold difference in leukocyte count, percentage polymorphonuclear cells, glucose- and total protein concentration between drains was calculated. Leukocyte count results were also corrected for blood admixture by subtracting 1 leukocyte per 1000 erythrocytes in CSF and by using the cell index.[6]

Between August 2014 and September 2017, nineteen patients received bilateral EVDs for treatment of hydrocephalus due to intraventricular hemorrhage ($n = 13$) and subarachnoid hemorrhage ($n = 6$). All patients had a relative bicaudate index >1. In two patients, CSF was sampled from only one drain and therefore, these patients were excluded from further analysis. The median age of the seventeen included patients was 59 years (range 32–85 years) and ten patients were female (59%). The median number of drainage days was fourteen days (range 1–35 days). The median days of bilateral drainage was nine days (range 1–31 days). Five patients died during admission (29%).

From a total of 201 days of bilateral drainage, CSF characteristics of both drains were available for 123 days (61%). During 114 measurement days, the median (uncorrected for erythrocytes) leukocyte count of the drain with the lowest and the highest values were 138×10^6/L (range 0–3080×10^6/L) and 424×10^6/L (range 0–$10,600 \times 10^6$/L) (Table 1). The median difference in leukocyte count between drains was 123×10^6/L (range 0–10,570) and the median fold-difference in uncorrected leukocyte count between drains was 1.9 (interquartile range (IQR) 1.2–3.1; range 1–388). The median fold-difference in uncorrected leukocyte counts between drains was over five on 14 out of 114 days (13%) and over ten on seven days (6%). The median difference in percentage uncorrected polymorphonuclear cells was 7% (IQR 2–16; range 0–73%) with a median fold-difference of 1.1 (IQR 1.0–1.3; range 1–12). In absolute numbers, the median difference in erythrocyte count was $30,000 \times 10^6$/L (IQR 7000–210,000; range 0–$3038,000 \times 10^6$/L). The median fold difference in the amount of blood admixture between drains was 1.9 (IQR 1.2–3.8; range 1–254). When the leukocyte count was corrected for blood admixture, differences between drain results persisted (Table 1).

The median difference in glucose concentration between drains was 0.4 mmol/L (range 0–6.8 mmol/L). The median fold difference was 1.1 (IQR 1.0–1.2; range 1–23.7). The median difference in total protein concentration between drains was 0.27 g/L (range

0–23.8 g/L). The median fold difference in total protein concentration was 1.4 (IQR 1.2–2.0; range 1–43).

Five patients developed ECAI (26%), of whom three developed the infection during bilateral drainage (Table 2). In these three patients, ten culture results were positive. In one patient simultaneously taken samples from both drains resulted in a positive CSF culture from one side and negative culture on the other. Gram staining in the culture positive patients was positive in 3 of 10 samples (30%).

Our data show that in patients with suspected ECAI and bilateral EVDs, CSF analysis and culture should be performed of both drains to increase the chance of identifying an external catheter-associated infection. CSF parameters between bilateral drains varied considerably in individual patients and yields of culture is increased by sampling both drains. CSF culture is considered the most important test for diagnosing an infection. The sensitivity of culture results for diagnosing ECAI is 80%.[7]

Bilateral drainage appears to increase the risk of infection with an infection rate of 26% in our cohort. The patients with unilateral EVDs in our cohort had an infection rate of 12% (10 out of 84 patients) which is more in line with the 10% commonly described in literature.[8] Previous studies described infection rates of respectively 39% (22 out of 57 patients) and 14% (2 out of 14 patients) in patients with bilateral drains.[4,9]

Our study has several limitations. First, CSF results were not available or not sampled from both sides for 39% of bilateral drainage days. Second, about 20% of all data on bilateral drain sampling were derived from one single patient with normal CSF skewing the data towards normal. Third, during this study daily sampling of CSF was performed to find early markers of infection. Routine CSF testing has been described to increase the risk of ECAI.[8]

Declaration of Competing Interest

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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Table 2
Culture results, CSF leukocyte count, erythrocyte count and CSF glucose concentration in bilateral drains in patients with an external catheter-associated infection.

Pt.	Day	Culture result		CSF leukocyte count (x10⁶/L)		Erythrocyte count (x10⁶/L)		CSF glucose conc. (mmol/L)	
		Drain 1	Drain 2	Drain 1	Drain 2	Drain 1	Drain 2	Drain 1	Drain 2
01	4	Negative	*Moraxella catarrhalis*	–	16	–	64.000	–	1.3
02	8	*Moraxella* sp.	*Moraxella* sp.	736	1020	53.000	223.000	3.6	3.6
	9	*Moraxella* sp., *S. epidermidis*	*Moraxella* sp., *S. epidermidis*	979	2510	172.000	202.000	2.8	2.6
03	12	No material	*S. epidermidis*	–	129	–	108.000	–	5.7
	14	*S. epidermidis*	*S. epidermidis*	–	–	230.000	855.000	5.9	5.6
	16	*S. epidermidis*	*S. epidermidis*	0	0	144.000	174.000	5.6	5.6

References

1. Bharucha T., Gangadharan B., Kumar A., de Lamballerie X., Newton P.N., Winterberg M., et al. Mass spectrometry-based proteomic techniques to identify cerebrospinal fluid biomarkers for diagnosing suspected central nervous system infections. A systematic review. *J Infect* 2019;**79**:407–18. doi:10.1016/j.jinf.2019.08.005.
2. van de Beek D., Drake J.M., Tunkel A.R.. Nosocomial bacterial meningitis. *N Engl J Med* 2010;**362**:146–54. doi:10.1056/NEJMra0804573.
3. Hinson H.E., Melnychuk E., Muschelli J., Hanley D.F., Awad I.A., Ziai W.C.. Drainage efficiency with dual versus single catheters in severe intraventricular hemorrhage. *Neurocrit Care* 2012;**16**:399–405. doi:10.1007/s12028-011-9569-9.
4. Staykov D., Huttner H.B., Lunkenheimer J., Volbers B., Struffert T., Doerfler F., et al. Single versus bilateral external ventricular drainage for intraventricular fibrinolysis in severe ventricular haemorrhage. *J Neurol Neurosurg Psychiatry* 2010;**81**:105–8. doi:10.1136/jnnp.2008.168427.
5. Tunkel A.R., Hasbun R., Bhimraj A., Byers K., Kaplan S.L., Scheld W.M., et al. Infectious diseases society of america's clinical practice guidelines for healthcare-associated ventriculitis and meningitis. *Clin Infect Dis* 2017;**64**:701–6. doi:10.1093/cid/ciw861.
6. Pfausler B., Beer R., Engelhardt K., Kemmler G., Mohsenipour I., Schmutzhard E.. Cell index- a new parameter for the early diagnosis of ventriculostomy (external ventricular drainage)-related ventriculitis in patients with intraventricular hemmorhage? *Acta Neurochir (Wien)* 2004;**146**:477–81. doi:10.1007/s00701-004-0258-8.
7. Dorresteijn K.R.I.S., Jellema K., van de Beek D., Brouwer M.C.. Factors and measures predicting external CSF drain-associated ventriculitis: a review and meta-analysis. *Neurology* 2019;**93**(22):964–72. doi:10.1212/WNL.0000000000008552.
8. Lozier A.P., Sciacca R.R., Romagnoli M.F., Connolly E.S. Jr.. Ventriculostomy-related infections: a critical review of the literature. *Neurosurgery* 2002;**51**:170–82. doi:10.1227/01.neu.0000316273.35833.7c.
9. Zheng W.J., Li L.M., Hu Z.H., Liao W., Lin Q.C., Zhu Y.H., et al. Bilateral external ventricular drains increase ventriculostomy-associated cerebrospinal fluid infection in low modified Graeb score intraventricular hemorrhage. *World Neurosurg* 2018;**116**:e550–5. doi:10.1016/j.wneu.2018.05.030.

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