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

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Letters to the Editor

The dilemma of COVID-19 recurrence after clinical recovery

Dear Editor,

We read with interested the recent article published by Batisse et al. (1) regarding the possible recurrences of COVID-19 symptoms after recovery and their discussion on the possible hypothesis of reactivation or reinfection.

In this specific context, the duration of immunization after clinical recovery is still unknown and this could be of particular concern regarding the management and spread of infection. According to the WHO's guidelines on clinical management, a patient can be discharged from hospital after two consecutive negative real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) tests at nasopharyngeal swabs at least 24 hours apart in a clinically recovered patient (https://ecdc.europa.eu/en/publications-data/covid-19-guidance-discharge-and-ending-isolation). Nevertheless, some recent reports described patients with recurrent RT-PCR tested positive again after clinical recovery (2-5), but these reports usually included a small number of patients followed-up for a limited period of time (6-7).

We collected clinical data of COVID-19 positive patients who had cured and discharged from two hospitals of ASST Rhodense, in Milan Province, Northern Italy, from March 9th to June 30th 2020. We considered patients with a positive RT-PCR test for COVID-19 on nasopharyngeal swab who were subsequently discharged when symptoms disappeared and two negative nasopharyngeal swabs repeated after 24-48 hours from each other were obtained. After discharging, patients were followed-up in designated medical wards or in a designated nursing home, where nasopharyngeal swabs were periodically collected (usually every week or anytime the patients developed clinical symptoms). We included in the analysis all the patients with a recurrence of COVID-19 infection, defined as a new positive nasopharyngeal swab after two negative tests.

A total of 1146 patients were hospitalized and then discharged for COVID-19 in our hospitals during the time-frame considered. Among these, 125 (10.9%) had a recurrence of COVID-19 infection. Table 1 summarized the clinical and demographic characteristics of this population; mean age was 65,7 years (95% CI 26-95) and most of patients were primarily hospitalized for interstitial pneumonia (n=103, 82.4%). The mean time to clinical recovery and two negative nasopharyngeal swabs was 27.7 days (95% CI 11-51); after that, the mean time to recurrence was 19.9 days (95% CI 3-43). Recurrence of COVID-19 infection was mainly diagnosed by chance during follow-up surveillance (n=96, 76.8%), whereas 29 patients (23.2%) developed clinical symptoms (fever in 16, malaise/fatigue in 9 and respiratory failure in 4 patients). After a mean time of 14.8 days (95% CI 6-36), 102 subjects (81.6%) had two additional negative nasopharyngeal swabs and were considered clinically recovered for the second time. During follow-up, 11 patients (8.8%) died and 12 (9.6%) were still positive when database was closed. Patients who died were older than others (mean age 86.4 years, 95% CI 77-92) and 8 of them (72.7%) had clinical symptoms at the time of recurrence (4 fever and 4 respiratory failure). The mean time from recurrence of COVID-19 infection to death was 8 days (95% CI 5-11).

Table 1
Main demographic and clinical characteristics of a cohort of 125 subjects with recurrent COVID-19 infection.

Female, n (%)	64 (51.2)
Age, years old (mean, 95% CI)	65.7 (26-95)
Hospitalized for interstitial pneumonia, n (%)	103 (82.4)
Time to first clinical recovery, days (mean, 95% CI)	27.7 (11-51)
Time to recurrence, days (mean, 95% CI)	19.9 (3-43)
Time to second clinical recovery, (n=102), days (mean, 95% CI)	14.8 (6-36)

Currently, there is a certain possibility of RT-PCR rendering false negative results due to sampling procedures, sources of samples and the sensitivity/specificity of the nucleic acid test kit (8). At the moment, it is impossible to discriminate if the positive nasopharyngeal swab results are due to real recurrence of COVID-19 infection or intermittent shedding of RNA fragments, especially in asymptomatic subjects. It is therefore possible that recurrences should be persistent infections in which nasopharyngeal swab resulted falsely negative at discharge. Alternatively, it cannot be excluded that truly negative discharged patients suffered reactivation or were re-infected with another COVID-19 strain, especially in elderly or in subjects with comorbidities (5). In our cohort, a certain amount of patients (23.2%) with RT-PCR recurrences developed new clinical symptoms, considering this interpretation plausible. To our knowledge, no studies have been conducted to investigate the contagiousness of patients with recurrence of viral RNA shedding. If these patients were contagious, they could represent a potential source of infections for the community.

At our knowledge, this is the largest cohort of subjects with recurrent COVID-19 infection. Our data confirmed that more than 10% of patients clinically recovered from COVID-19 infection had re-positive RT-PCR at nasopharyngeal swab during post-discharge follow-up (6-7); most of these subjects were asymptomatic at the time of recurrence.

In conclusion, our data confirm that recurrence of COVID-19 infection is a fairly frequent phenomenon. Little is known on how to manage these patients and how this will impact the evolution of the pandemic in the future.

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References

1. Batisse D, Benech N, Botelho-Nevers E, Bouiller K, Collarino R, Conrad A, et al. Clinical recurrences of COVID-19 symptoms after recovery: viral relapse, reinfection or inflammatory rebound? *J Infect* 2020 Jun 30 S0163-4453(20)30454-0.
2. Ye G, Pan Z, Pan Y, Deng Q, Chen L, Li J, et al. Clinical characteristics of severe acute respiratory syndrome coronavirus 2 reactivation. *J Infect* 2020;**80**(5):e14–17.
3. Yuan J, Kou S, Liang Y, Zeng J, Pan Y, Liu L. PCR assays turned positive in 25 discharged COVID-19 patients. *Clin Infect Dis* 2020 Apr 8. doi:10.1093/cid/ciaa398.
4. Jiang M, Li Y, Han M, Wang Z, Zhang Y, Du X. Recurrent PCR positivity after hospital discharge of people with coronavirus disease 2019 (COVID-19). *J Infect* 2020;**81**(1):147–78. doi:10.1016/j.jinf.2020.03.024.
5. Bongiovanni M, Basile F.. Re-infection by COVID-19: a real threat for the future management of pandemia. *Infect Dis* 2020;**52**(8):581–2. doi:10.1080/23744235.2020.1769177.
6. Zhu H, Fu L, Jin Y, Shao J, Zhang S, Zheng N, et al. Clinical features of COVID-19 convalescent patients with re-positive nucleic acid detection. *J Clin Lab Anal* 2020; June 7. doi:10.1002/jcla.23392.
7. Wu J, Liu X, Liu J, Liao H, Long S, Zhou N, et al. Coronavirus Disease 2019 test results after clinical recovery and hospital discharge among patients in China. *JAMA Netw Open* 2020 May 1;**3**(5). doi:10.1001/jamanetworkopen.2020.9759.
8. Xie C, Lu J, Wu D, Zhang L, zhao H, Rao B, et al. False negative rate of COVID-19 is eliminated by using nasal swab test. *Travel Medicine and Infectious Disease* 2020. https://doi.org/10.1016/j.tmaid.2020.101668.

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Chronic conventional disease-modifying anti-rheumatic drugs masking severe SARS-CoV-2 manifestations in an elderly rheumatic patient

Dear Editor,

In early 2020 a new beta-corona virus (SARS-CoV-2) spread all over the world, and with a high incidence in Europe, especially in Italy [1,2]. SARS-CoV-2 infection may lead to a wide range of clinical presentations, from an asymptomatic form to a severe acute respiratory syndrome [3]. The symptoms more frequently observed were fever, chills, myalgia or fatigue, followed by a dry cough and dyspnea 3-7 days later. The age and the presence of chronic comorbidities (hypertension, cardiovascular disease, diabetes, chronic lung, kidney or cerebrovascular disease or malignancy) have been considered as the major risk factors for acute respiratory distress syndrome (ARDS) and the need for intensive care in COVID-19 patients [4]. ARDS is an immunopathologic event with hyper-activity of the systemic inflammatory response that induces cytokine storm, that increase pro-inflammatory cytokines like interferons, interleukins (IL), tumor necrosis factor and chemokines, suggesting the use of anti-inflammatory agents for SARS-CoV-2 pulmonary symptoms [5]. Few data are available on SARS-CoV-2 infection in rheumatological patients chronically treated with immunosuppressive therapy.

We present a clinical case of an 82-year-old Caucasian woman with a history of rheumatoid arthritis (RA) and idiopathic arterial hypertension hospitalized for SARS-CoV-2 pneumonia. The diagnosis of RA was performed in 2007 for the appearance of rheumatoid factor and anti-citrullinated protein antibody-positive symmetrical polyarthritis, without signs of pulmonary or systemic disease. She had been under methotrexate, 10 mg/weekly (cumulative dose 6080 mg) and methylprednisolone (4 mg/day) treatment for two years, with a low disease activity status (DAS 28 $_{PCR}$ 2.9).

A week before admission, the patient had low grade fever (37.5°C) and a dry cough; she had stopped methylprednisolone and had started antibiotic treatment, without improvement; on 27 March, she underwent nasopharyngeal SARS-CoV-2 swab, which resulted positive, and was hospitalized on 30 March. Despite the absence of any pulmonary symptoms, a lung CT scan showed interstitial bilateral pneumonia (Fig. 1), and a thoracic ultrasound with lung ultrasound reaeration score (LUS) of four. Hydroxycloroquine, lopinavir/ritonavir, and low molecular weight heparin (LMWH, 4000 UI/die) were started. Two days later, although afebrile, she presented dyspnea (respiratory rate-RR 32) with SpO2 of 93% in FiO2 21% and PaO2 / FiO2 309 mmHg and Oxygen therapy was started. High values of D-dimer and C-reaction protein were observed, a CT angiography excluded embolism, but showed a worsening of pneumonia (Fig. 1), and the LUS score was 10. Because of the persistence of signs of cytokine storm, without worsening in respiratory function, tocilizumab was administered (Fig. 2). The next day she worsened (PaO2 / FiO2 137.8 mmHg), so another dose of tocilizumab was administered, and methylprednisolone was started. A gradual clinical and biochemical improvement was observed (Fig. 2). On 12 and 14 April, nasopharyngeal-oropharyngeal swabs resulted negative. On 16 and 22 April, a LUS score of eight and two was observed, respectively, and the patient was discharged in good general condition.

Our clinical case teaches to pay particular attention in the management of COVID-19 infection in the rheumatological field: in the absence of fever during the entire hospitalization period and clinical signs of pulmonary failure, the patient developed severe pneumonia. In most of case, the COVID-19 is asymptomatic or oligosymptomatic; while in a low percentage of case the fever persist up to 14 days from the onset of symptoms with clinical and

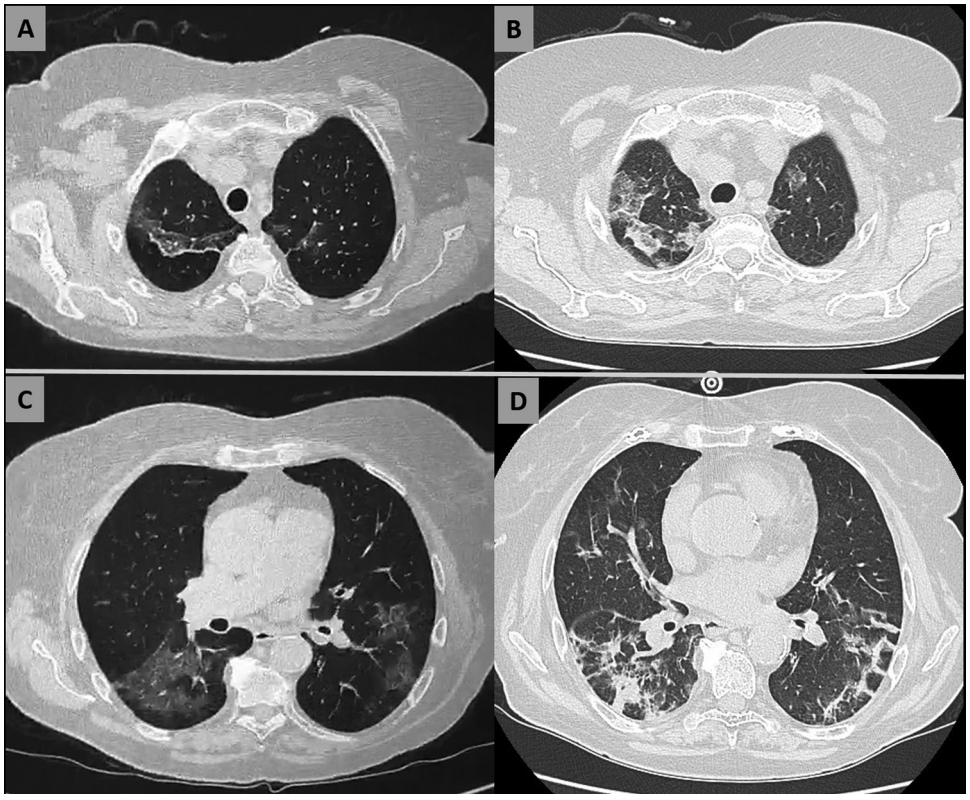

Fig. 1. Axial non-contrast CT scans of the upper chest in the lung in an 82-year-old woman with COVID-19 pneumonia.
A: Single sub-pleural thin band-like consolidation in the right upper lobe associated with ground-glass opacities (GGO). A small GGO is also seen in the left upper lobe, 7 days after the onset of symptoms.
B: Scan showed an increased extension of GGO in the right upper lobe associated with septal thickening (crazy paving) and posterior consolidations. Two smaller GGO are also visible in the left upper lobe, 10 days after the onset of symptoms.
C: Axial non-contrast CT image obtained below the carina showed bilateral multifocal GGO in both lower lobes with prevalence of the peripheral regions, 7 days after the onset of symptoms.
D: Scan showed a mixed pattern with parenchymal consolidations and parenchymal bands in both lower lobes with sub-pleural and posterior distribution. The perilobular bands of consolidation associated with thickening of the interlobular septa suggested the presence of organizing pneumonia, 10 days after the onset of symptoms.

radiological evidence of pneumonia from the day 7 and 14 and sometimes with a pulmonary failure. In the present case, in the absence of fever during the entire hospitalization and clinical signs of pulmonary failure, the patient developed a severe pneumonia. Thus, a close and continuous monitoring of PaO2 / FiO2, of biochemical signs of cytokine storm (D-dimer and CRP) and of imaging signs of pneumonia are needed to identify the initial signs of the respiratory failure.

We can hypothesize that by controlling the excessive activation of the immune system, chronic cDMARD treatment may mask the clinical presentation of COVID-19 with a silent development of severe acute pneumonia. In fact, although the immunological mechanism behind the risk of greater severity of COVID-19 infection is unknown, the coronavirus infection (SARS and MERS) may induce a cytokines storm especially in patients who developed fatal complications. Moreover, the pathological findings associated with acute respiratory distress syndrome in COVID-19 showed abundant interstitial mononuclear inflammatory infiltrate in the lungs, dominated by lymphocytes, once again implying that the immune hyperactivation mechanisms are at least partially accountable for COVID-19 severity [7-9].

Thus, close and continuous clinical, biochemical and imaging monitoring are needed to identify the initial signs of respiratory failure.

Our case shows a different course from that described by Mihan et al.[6], who reported a peculiar SARS-CoV-2 with mild symptoms in a 57-year-old woman with systemic sclerosis (SSc) with interstitial lung disease as main organ manifestation of SSc and chronically treated with tocilizumab (8 mg/kg body weight every 4 weeks iv). A month after the last infusion of tocilizumab, the patient developed a SARS-Cov-2 infection. However, her symptoms remained mild and she was monitored from home, resulting negative at the nasal swab after 14 days since symptoms had started [6]. The authors hypothesized that IL-6 blocking treatment given for chronic autoimmune diseases, such as rheumatoid disease, may even prevent the development of severe COVID-19. [6].

In our case, although the patient had negative prognostic factors (older age, chronic disease and arterial hypertension), the use of tocilizumab and corticosteroid was associated with the control of severe pneumonia, supporting the role of tocilizumab in controlling severe SARS-CoV-2-related life-threatening conditions.

Declaration of Competing Interest

All the authors of the manuscript declare they have no conflict of interest in connection with this paper.

Founding

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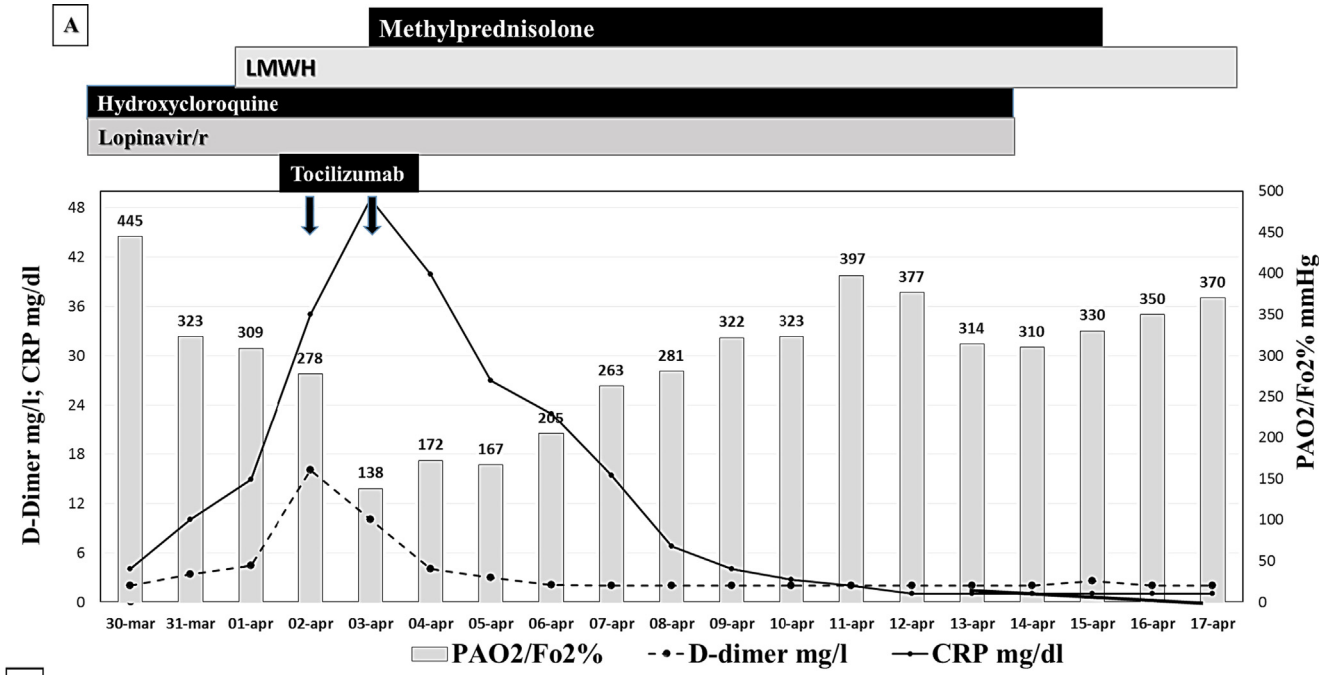

B

Day	March 30	March 31	April 1	April 2	April 3	April 4	April 5	April 6	April 7	April 8	April 10	April 11	April 13	April 15	April 17	April 22
Fever (maximal CT)	38	36.5	36.5	36	36	36	36	36	364	36	36	36.2	36	36	36.5	36
WBC, cells x 10^3		5.4	4.5		3.94	3.44		9.12	6.07		10.01	11.38	13.95	17.03	11.82	9.0
Lymphocyte, cells x 10^3		1.0	0.8		0.78	0.31		6.7	7.2		5.6	6	5.2	4.8	1.4	2.6
Ferritin			559					1398							324	350
Creatinine, mg/dl		0.81	0.83		1.51	1.8	2.26	2.84	2.44	1.81	1.21	1.11	1.37	0.9		0.8
eGFR		68			32	28.6	18	16	24	34	42	47	48		50.5	
LUS	4			10										8		2

Fig. 2. D-Dimer, CRP and PAO2/FO2% and therapy in a 82-year-old woman.

References

1. Ciotti M, Angeletti S, Minieri M S, et al. COVID-19 Outbreak: An Overview. *Chemotherapy* 2020:1–9. doi:10.1159/000507423.
2. World Health Organization main website. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200410-sitrep-81-covid-19.pdf?sfvrsn=ca96eb84_2
3. Wu Z, McGoogan JM.. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72314 Cases From the Chinese Center for Disease Control and Prevention. *JAMA* 2020. doi:10.1001/jama.2020.2648.
4. Rothan HA, Byrareddy SN.. Epidemiology and pathogenesis of the coronavirus epidemic (COVID-19*). J. Autoimmun* 2020;**109**:102433. doi:10.1016/j.jaut.2020.102433.
5. Gianfrancesco MA, Hyrich KL, Gossec L, et al. Rheumatic disease and COVID-19: initial data from the COVID-19 Global Rheumatology Alliance provider registries. *Lancet Rheumatol* 2020. doi:10.1016/S2665-9913(20)30095-3.
6. Mihai C, Dobrota R, Schröder M, et al. COVID-19 in a patient with systemic sclerosis treated with tocilizumab for SSc-ILD. *Ann Rheum Dis* 2020;**79**(5):668–9. doi:10.1136/annrheumdis-2020-217442.
7. Zhang C, Wu Z, Li JW, Zhao H, Wang GQ. The cytokine release syndrome (CRS) of severe COVID-19 and Interleukin-6 receptor (IL-6R) antagonist Tocilizumab may be the key to reduce the mortality. *Int J Antimicrob Agents* 2020:105954. doi:10.1016/j.ijantimicag.2020.105954.
8. Magro G.. SARS-CoV-2 and COVID-19: is interleukin-6 (IL-6) the 'culprit lesion' of ARDS onset? What is there besides Tocilizumab? SGP130Fc. *Cytokine X* 2020 May 14:100029. doi:10.1016/j.cytox.2020.100029.
9. Stebbing J, Phelan A, Griffin I, et al. COVID-19: combining antiviral and anti-inflammatory treatments. *Lancet Infect Dis* 2020;**20**(4):400–2. doi:10.1016/S1473-3099(20)30132-8.

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COVID-19 is more severe in patients with hypertension; ACEI/ARB treatment does not influence clinical severity and outcome

Dear Editor,

A number of pneumonia cases of unknown causes have emerged in Wuhan, Hubei, China since December 2019.[1] After sequencing analysis of samples from the lower respiratory tract, a coronavirus,[2] which was last named as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2),[3] was newly discovered. On February 11, 2020, the World Health Organization (WHO) announced a new name for the disease caused by 2019-nCoV: coronavirus disease 2019 (COVID-19).[4] With the arrival of the Spring Festival, an epidemic SARS-CoV-2 infection has spread rapidly. It has swept across China and all over the world, and became a major global health concern. Chinese scientists found that SARS-CoV-2, like the SARS virus in 2003, enters human cells by recognizing angiotensin-converting enzyme 2 (ACE2) protein, which is the key to the invasion of the "new coronavirus" into the body.[5] Decreased ACE2 expression is a cause of hypertension because ACE2 is identified as a major angiotensin 1-7 (Ang1-7)-forming enzyme.[6] Based on studies of COVID-19, we found that hypertension initially occurs in many complications in COVID-19 patients.[7] However, limited reports on COVID-19 patients with hypertension are available in literature. Whether patients with hypertension who undergo angiotensin-converting enzyme inhibitor (ACEI)/angiotensin receptor blocker (ARB) therapy are more likely to suffer SARS-CoV-2 infection and whether ACEI/ARB therapy would have an influence on the clinical outcomes of patients with COVID-19 are controversy.[8, 9] Moreover, the epidemiologic and clinical features of COVID-19 patients with hypertension are also not completely elucidated. Thus, in this study, we describe the demographic, epidemiologic, and clinical characteristics of COVID-19 patients with hypertension. And we also attempted to analyze whether ACEI/ARB treatment would have an influence on the clinical severity and outcomes of COVID-19 patients.

Altogether, 884 COVID-19 patients between January 17, 2020 and February 8, 2020, who confirmed with SARS-CoV-2 infection in Zhejiang Province, diagnosed as having COVID-19 according to WHO interim guidance[10] were enrolled in this study. Among various coexisting conditions, the proportion of patients with hypertension (149 patients, 16.86%) was higher than that of others. Compared with COVID-19 patients without hypertension, those patients with hypertension had a higher percentage of male sex (59.06% vs 49.93%, P=0.042), were older (57.00 years vs 43.00 years, P=0.000) and had a higher percentage of age ≥60 years (43.62% vs 13.88%, P=0.000). In this study, 723 patients were diagnosed to have a mild type; 123 patients, severe type; and 37 patients, critical type. Patients with hypertension had a lower rate of mild type (59.06% vs 86.39%, P=0.000), but had a higher rate of severe (26.17% vs 11.43%, P=0.001) and critical types (14.77% vs 2.04%, P=0.000) than patients without hypertension. Compared with patients without hypertension, patients with hypertension had a higher incidence of acute respiratory distress syndrome(ARDS) (24.16% vs 6.67%, P=0.000), were more likely to use glucocorticoids (31.54% vs 12.79%, P=0.000), antibiotic (50.33% vs 39.32%, P=0.013), and intravenous immune globulin therapy (21.48% vs 6.67%, P=0.000) and more likely to need mechanical ventilation (14.77% vs 2.04%, P=0.000) and intensive care unit (ICU) admission (16.11% vs 2.31%, P=0.000), extracorporeal membrane oxygenation (ECMO) (4.03% vs 0.82%, P=0.007) and continuous renal replacement therapy (CRRT) (2.01%vs 0.14%, P=0.016) therapy. The time intervals from illness onset to discharge and from admission to discharge in patients with hypertension (median 25.00 days and 20.00 days, respectively) were longer than those in patients without hypertension (median 22.00 days and 18.00 days, respectively) (P=0.000, P=0.002) (Table 1).

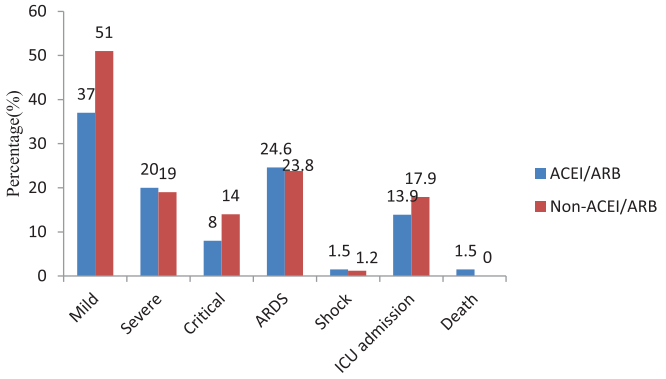

Fig. 1. Clinical type, complications and outcome of COVID-19 patients with hypertension of different anti-hypertensive drugs.

We found that the level of leukocyte count (median 5.40×10^9/L vs 4.70×10^9/L, P=0.000) and neutrophil count (median 3.60×10^9/L vs 2.90×10^9/L, P=0.000) was higher, but the level of lymphocyte count (median 1.00×10^9/L vs 1.20×10^9/L, P=0.000) was lower in patients with hypertension than in patients without hypertension. In terms of liver function, patients with hypertension had a lower level of albumin (median 40.34 vs 41.30, P=0.001), but a higher level of alanine aminotransferase (ALT)(median 25.00 vs 21.00, P=0.001), aspartate aminotransferase (AST) (median 28.00 vs 25.00, P=0.001), and total bilirubin (TB)(median 10.60 vs 9.50, P=0.010). As for renal function, patients with hypertension had a higher level of serum creatinine (Scr)(median 72.00 μmol/L vs 66.00μmol/L, P=0.000) and blood urea nitrogen (BUN) (median 4.30 mmol/L vs 3.77 mmol/L, P=0.000) . In addition, patients with hypertension had a higher level of creatine kinase (CK) (median 83.00 U/L vs 69 U/L, P=0.003), lactate dehydrogenase (LDH) (median 250.00 U/L vs 211 U/L, P=0.000), and C-reactive protein(CRP) (median 16.88 mg/L vs 7.80 mg/L, P=0.000), but lower level of serum potassium (median 3.80 mmol/L vs 3.85 mmol/L, P=0.031) and serum sodium (median 137.00 mmol/L vs 138.60 mmol/L, P=0.000). (Table 1)

Among the 149 patients with hypertension, most patients (102 patients) treated with CCB including 62 patients used CCB alone. 149 patients were divided into two groups according to whether or not ACEI or ARB included in the antihypertensive drug regimen, 65 patients in ACEI/ARB group and 84 patients in non-ACEI/ARB group. Compared with patients treated without ACEI/ARB, the clinical presentations and laboratory results between the two groups did not reach significant difference (all P>0.05). No difference was found in severe/critical cases between patients with and those without ACEI/ARB treatment (all P>0.05). There were no significant differences of the complication (Shock, ARDS), the treatments, including anti-coronavirus treatment, glucocorticoids treatment, antibiotic treatment, mechanical ventilation, ECMO, CRRT and so on (all P>0.05). In addition, no difference was found in the rate of death, admission to ICU, interval between illness onset to discharge and the time of hospitalization (all P>0.05). (Table 1, Fig. 1).

Table 1
Clinical characteristics of COVID-19 patients with and without hypertension

	With Hypertension (n=149)				Without Hypertension (n=735)	*P*-Value[#]
	Total (n=149)	ACEI/ARB (n=65)	Non-ACEI/ARB (n=84)	*P*-Value*		
Sex (male)	88 (59.06%)	40 (61.54%)	48 (57.14%)	0.588	367 (49.93%)	0.042
Age (years)	57.00 (49.50-66.00)	56.00 (48.00-64.00)	58.00 (52.00-67.00)	0.043	43.00 (34.00-54.00)	0.000
≥60 yr	65 (43.62%)	25 (38.46%)	40 (47.62%)	0.264	102 (13.88%)	0.000
Coexisting Condition						
Diabetes	30 (20.13%)	16 (24.62%)	14 (16.67%)	0.230	35 (4.76%)	0.000
Heart disease	7 (4.70%)	2 (3.08%)	5 (5.95%)	0.469	8 (1.09%)	0.006
COPD	2 (1.34%)	1 (1.54%)	1 (1.19%)	1.000	3 (0.41%)	0.200
Chronic liver disease	9 (6.04%)	5 (7.69%)	4 (4.76%)	0.691	26 (3.54%)	0.153
Chronic renal disease	6 (4.03%)	4 (6.15%)	2 (2.38%)	0.404	2 (0.27%)	0.000
Cancer	3 (2.01%)	0 (0.00%)	3 (3.57%)	0.257	6 (0.82%)	0.379
Clinical Type						
Mild Type	88 (59.06%)	37 (56.92%)	51 (60.71%)	0.641	635 (86.39%)	0.000
Severe Type	39 (26.17%)	20 (30.77%)	19 (22.62%)	0.262	84 (11.43%)	0.000
Critical Type	22 (14.77%)	8 (12.31%)	14 (16.67%)	0.457	15 (2.04%)	0.000
General symptoms						
Fever	127 (85.23%)	58 (89.23%)	69 (82.14%)	0.226	587 (79.86%)	0.129
Fatigue	32 (21.48%)	17 (26.15%)	15 (17.86%)	0.221	126 (17.14%)	0.208
headache	7 (4.70%)	4 (6.15%)	3 (3.57%)	0.699	74 (10.07%)	0.038
Muscle ache	22 (14.77%)	11 (16.92%)	11 (13.10%)	0.514	77 (10.48%)	0.130
Respiratory symptoms						
Nasal obstruction	3 (2.01%)	1 (1.54%)	2 (2.38%)	1.000	48 (6.53%)	0.031
Sore throat	20 (13.42%)	11 (16.92%)	9 (10.71%)	0.270	103 (14.01%)	0.849
Cough	100 (67.11%)	47 (72.31%)	53 (63.10%)	0.235	471 (64.08%)	0.480
Sputum production	56 (37.58%)	26 (40.00%)	30 (35.71%)	0.592	245 (33.33%)	0.318
Hemoptysis	7 (4.70%)	5 (7.69%)	2 (2.38%)	0.240	7 (0.95%)	0.003
Shortness of breath	21 (14.09%)	10 (15.38%)	11 (13.10%)	0.690	20 (2.72%)	0.000
Gastrointestinal symptoms						
Nausea and vomiting	7 (4.70%)	4 (6.15%)	3 (3.57%)	0.699	24 (3.27%)	0.386
Diarrhea	12 (8.05%)	7 (10.77%)	5 (5.95%)	0.284	59 (8.03%)	0.991
Complications						
Acute respiratory distress syndrome	36 (24.16%)	16 (24.62%)	20 (23.81%)	0.909	49 (6.67%)	0.000
Shock	2 (1.34%)	1 (1.54%)	1 (1.19%)	1.000	2 (0.27%)	0.134

(*continued on next page*)

Table 1 (*continued*)

	With Hypertension (n=149)				Without Hypertension (n=735)	*P*-Value#
	Total (n=149)	ACEI/ARB (n=65)	Non-ACEI/ARB (n=84)	*P*-Value*		
Treatment						
Glucocorticoids	47 (31.54%)	19 (29.23%)	28 (33.33%)	0.559	94 (12.79%)	0.000
Antibiotic treatment	75 (50.33%)	31 (47.69%)	44 (52.38%)	0.570	289 (39.32%)	0.013
Intravenous immune globulin therapy	32 (21.48%)	14 (21.54%)	18 (21.43%)	0.987	49 (6.67%)	0.000
Admission to intensive care unit	24 (16.11%)	9 (13.85%)	15 (17.86%)	0.509	17 (2.31%)	0.000
Mechanical ventilation	22 (14.77%)	8 (12.31%)	14 (16.67%)	0.457	15 (2.04%)	0.000
EMCO	6 (4.03%)	3 (4.62%)	3 (3.57%)	1.000	6 (0.82%)	0.007
CRRT	3 (2.01%)	1(1.54%)	2 (2.38%)	1.000	1 (0.14%)	0.016
Interval between illness onset to hospital outpatient (Days)	2.00(0.00-5.00)	2.00(1.00-4.00)	1.00(0.00-5.00)	0.688	2.00 (1.00-4.00)	0.931
Interval between illness onset to admission (Days)	4.00(1.00-7.00)	4.00(2.50-6.00)	4.00(1.00-7.00)	0.548	3.00 (1.00-6.00)	0.206
Interval between illness onset to confirmation (Days)	4.00(2.00-8.00)	4.00(2.50-7.00)	4.50(2.00-8.00)	0.782	4.00 (2.00-7.00)	0.576
Interval between illness onset to discharge (Days)	25.00(19.00-32.00)	26.00(18.25-32.00)	25.00(19.00-31.50)	0.955	22.00(17.00-28.00)	0.000
Interval between admission to discharge (Day)	20.00(14.00-27.00)	20.50(14.00-26.75)	20.00(14.50-27.00)	0.915	18.00(13.00-23.00)	0.002
Death	1 (0.67%)	1 (1.54%)	0 (0.00%)	0.436	0 (0.00%)	0.169
Laboratory detection						
Leucocytes ($\times 10^9$ /L; normal range 4-10)	5.40 (4.29-6.48)	5.30 (4.26-6.32)	5.45 (4.30-6.64)	0.749	4.70 (3.80-5.90)	0.000
Neutrophils ($\times 10^9$ /L; normal range 2-7)	3.60 (2.80-4.80)	3.43 (2.92-4.95)	3.65 (2.71-4.79)	0.928	2.90 (2.20-3.83)	0.000
Lymphocyte ($\times 10^9$ /L; normal range 0.8-4.0)	1.00 (0.71-1.37)	0.96 (0.70-1.40)	1.04 (0.77-1.33)	0.896	1.20 (0.90-1.60)	0.000
Platelets ($\times 10^9$ per L; normal range 83-303(Male), 101-320 (female)	171.00 (138.00-220.50)	166.00 (140.00-196.50)	179.00 (137.00-225.50)	0.336	183.00 (149.00-223.00)	0.095
INR(normal range 0.85-1.15)	1.02 (0.97-1.10)	1.02 (0.97-1.11)	1.02 (0.98-1.08)	0.871	1.01 (0.97-1.08)	0.190
Alb (g/L; normal range 40-55)	40.34 (36.73-42.60)	40.70 (37.2-42.89)	39.20 (36.20-42.40)	0.339	41.30 (38.50-43.80)	0.001
ALT (U/L; normal range 9-50 (Male), 7-40 (Female))	25.00 (18.20-39.00)	26.00 (19.00-41.00)	25.00 (17.00-32.75)	0.375	21.00 (15.00-33.00)	0.001
AST (U/L; normal range 15-40 (Male),13-35 (Female))	28.00 (21.50-40.00)	27.00 (19.95-40.10)	28.00 (22.00-39.75)	0.857	25.00 (19.00-32.00)	0.001
TB (umol/L; normal range 0-26 (Male), 0-21 (Female))	10.60 (7.70-14.95)	11.40 (7.50-16.95)	10.25 (7.73-14.40)	0.716	9.50 (6.90-13.10)	0.010
Scr (μmol/L; normal range: 57-97(Male), 41-73 (Female))	72.00 (58.00-85.00)	71.00 (57.00-85.25)	73.00 (58.30-85.00)	0.829	66.00 (55.00-76.43)	0.000
BUN (mmol/L; normal range 3.1-8.0 (Male), 2.6-7.5 (Female)	4.30(3.50-5.93)	4.20 (3.31-5.71)	4.33 (3.56-6.15)	0.277	3.77 (3.00-4.50)	0.000
CK (U/L; normal range 50-310 (Male), 40-200 (Female)	83.00 (53.5-130.50)	72.00 (49.50-131.00)	90.00 (55.00-129.75)	0.521	69.00 (47.00-105.00)	0.003
LDH (U/L; normal range 120-250)	250.00 (194.50-315.00)	244.00 (181.00-318.00)	253.00 (204.00-311.50)	0.332	211.00 (169.00-256.00)	0.000
Serum potassium (mmol/L; normal range 3.5-5.3)	3.80 (3.50-4.05)	3.84 (3.49-4.03)	3.79 (3.50-4.06)	0.576	3.85 (3.60-4.14)	0.031
Serum sodium (mmol/L; normal range 137-147)	137.00 (135.00-139.90)	137.00 (135.14-139.85)	137.15 (135.00-139.90)	0.942	138.60 (136.39-140.08)	0.000
CRP (mg/L; normal range 0-8)	16.88 (7.20-44.55)	21.00 (7.29-40.90)	15.53 (6.39-51.00)	0.556	7.80 (2.30-19.00)	0.000

Note 1: *, P value of comparison between ACEI/ARB and non- ACEI/ARB; #, P value of comparison between with and withour hypertention.

Note 2: Alb, Albumin; ALT, Alanine aminotransferase; AST: Aspartate aminotransferase; BUN, Blood urea nitrogen; CK, Creatine kinase; COPD, Chronic obstructive pulmonary disease; CRP, CRRT: Continuous renal replacement therapy;C-reactive protein; ECMO, Extracorporeal membrane oxygenation; INR: International normalized ratio; LDH: Lactate dehydrogenase; Scr, Serum creatinine; TB,Total bilirubin.

In summary, we reported the largest cases of COVID-19 patients with hypertension. This study showed that patients with hypertension might have more severe respiratory symptoms, more abnormality laboratory indication, and more proportion of severe/critical type of COVID-19. Moreover, they may need more antibiotic, hormone, and intravenous immune globulin therapy and intensive care unit admission and have a longer hospital stay. Treatment with ACEI/ARB have no influence on the severity and the clinical outcome of COVID-19 patients with hypertention.

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Potential conflicts of interest

None.

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References

1. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *Journal of medical virology* 2020 2020-Jan-29. PubMed PMID: MEDLINE:31994742.
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. *The New England journal of medicine* 2020 Feb 20;**382**(8):727–33 PubMed PMID: 31978945. Pubmed Central PMCID: 7092803.
3. Alexander E. Gorbalenya SCB, Ralph S. Baric, Raoul J. de Groot, Christian Drosten, Anastasia A. Gulyaeva, Bart L. Haagmans, Chris Lauber, Andrey M. Leontovich, Benjamin W. Neuman, Dmitry Penzar, Stanley Perlman, Leo L.M. Poon, Dmitry Samborskiy, Igor A. Sidorov, Isabel Sola, John Ziebuhr. Severe acute respiratory syndrome-related coronavirus: The species and its viruses - a statement of the Coronavirus Study Group. 2020.
4. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID-19): the epidemic and the challenges. *Int J Antimicrob Agents* 2020 Feb 17 105924. PubMed PMID: 32081636. eng.
5. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020 Feb 3 PubMed PMID: 32015507.
6. Kuba K, Imai Y, Penninger JM. Multiple functions of angiotensin-converting enzyme 2 and its relevance in cardiovascular diseases. *Circ J* 2013;**77**(2):301–8 PubMed PMID: 23328447.
7. 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 Feb 15;**395**(10223):497–506 PubMed PMID: 31986264. Pubmed Central PMCID: 7159299.
8. Zhang P, Zhu L, Cai J, Lei F, Qin JJ, Xie J, et al. Association of Inpatient Use of Angiotensin Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers with Mortality Among Patients With Hypertension Hospitalized With COVID-19. *Circulation research* 2020 Apr 17 PubMed PMID: 32302265.
9. Sunden-Cullberg J.. Chronic Use of Angiotensin-Converting Enzyme Inhibitors and Angiotensin II Receptor Blockers Is High Among Intensive Care Unit Patients With Non-COVID-19 Sepsis but Carry a Moderately Increased Risk of Death. *Hypertension (Dallas, Tex: 1979)* 2020 Apr 10 HYPERTENSIONAHA12015178. PubMed PMID: 32275190. Epub 2020/04/11. eng..
10. WHO. Clinical management of severe acute respiratory infection when Novel coronavirus (nCoV) infection is suspected: interim guidance. Jan 11, 2020. https://wwwwhoint/internal-publications-detail/clinical-management-of-severe-acute-respiratory-infection-when-novel-coronavirus-(ncov)-infection-is-suspected (accessed Jan 30,2020) 2020.

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Serum Ferritin is an independent risk factor for Acute Respiratory Distress Syndrome in COVID-19

Dear Editor,

Identifying the significant parameters for early progression toward worse prognosis is fundamental for the management of COVID-19 patients.

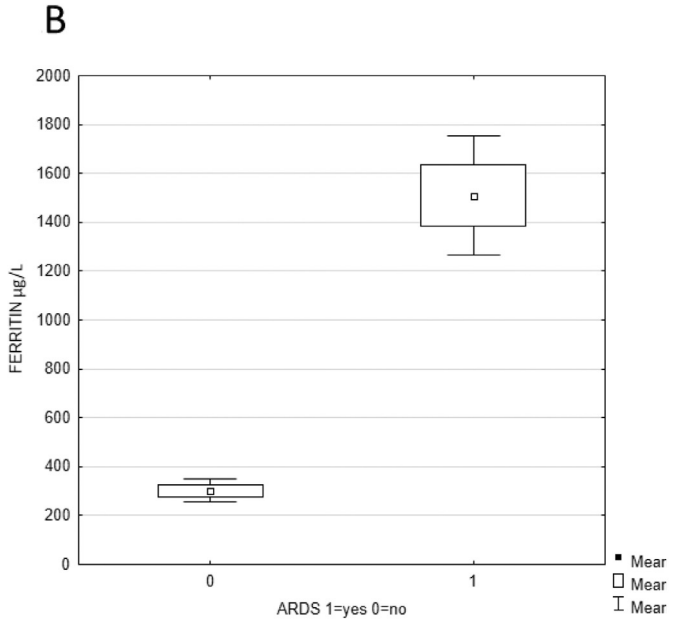
In this Journal, Zhi Lin and colleagues[1] recently reported that Chinese patients with severe Sars-CoV2 disease showed higher levels of serum ferritin than patients with not severe one, confirming data from other authors on Chinese[2,3] and Caucasian populations.[4,5]

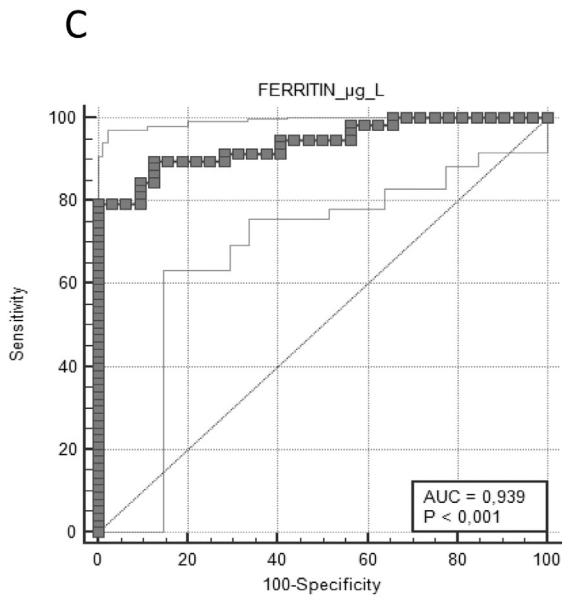
Here we aimed to establish the most suitable panel for routine prognostic serum laboratory testing in COVID-19 patients upon first admission to the Emergency Department.

We thus enrolled 141 patients (59 females and 82 males, aging 64,48 ± 16,58 years) diagnosed as COVID-19 by means of real-time polymerase chain reaction testing and admitted to the isolation ward of Emergency Department at Policlinico Umberto I Hospital

A

Variables [Mean(Std. Dev.)]	Groups	mild cases (81patients)	Severe cases (60 patients)	p value
SEX				
	male	37 (45,7%)	44 (73,35)	<0,001
	female	44(54,3%)	16 (26,7%)	<0,001
Age years		61,11(18,12)	69,16(12,92)	<0,005
FERRITIN µg/L		303(224)	1509(968)	<0,001
NLR		6,86(6,85)	10,88(9,84)	<0,005
D-D ug/dL		1672(1569)	2357(1581)	<0,05
LDH UI/L		286(135)	403(192)	<0,005
CRP mg/dL		6,70(8,29)	11,86(10,77)	<0,05
Albumin g/dL		3,28(0,52)	2,79(0,61)	<0,01
Fibrinogen mg/dL		465(138)	503(111)	ns
Platelet x10³/µL		251(110)	25181319	ns
Troponin µg/L		0,06 (0,12)	0,08(0,11)	ns
Pro-calcitonin µg/L		0,34(0,37)	0,81(0,71)	ns





Variable	Analysis of Variance SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
FERRITIN µg/L	50184923	1	50184923	59285842	139	426516,8	117,6622	0,000000

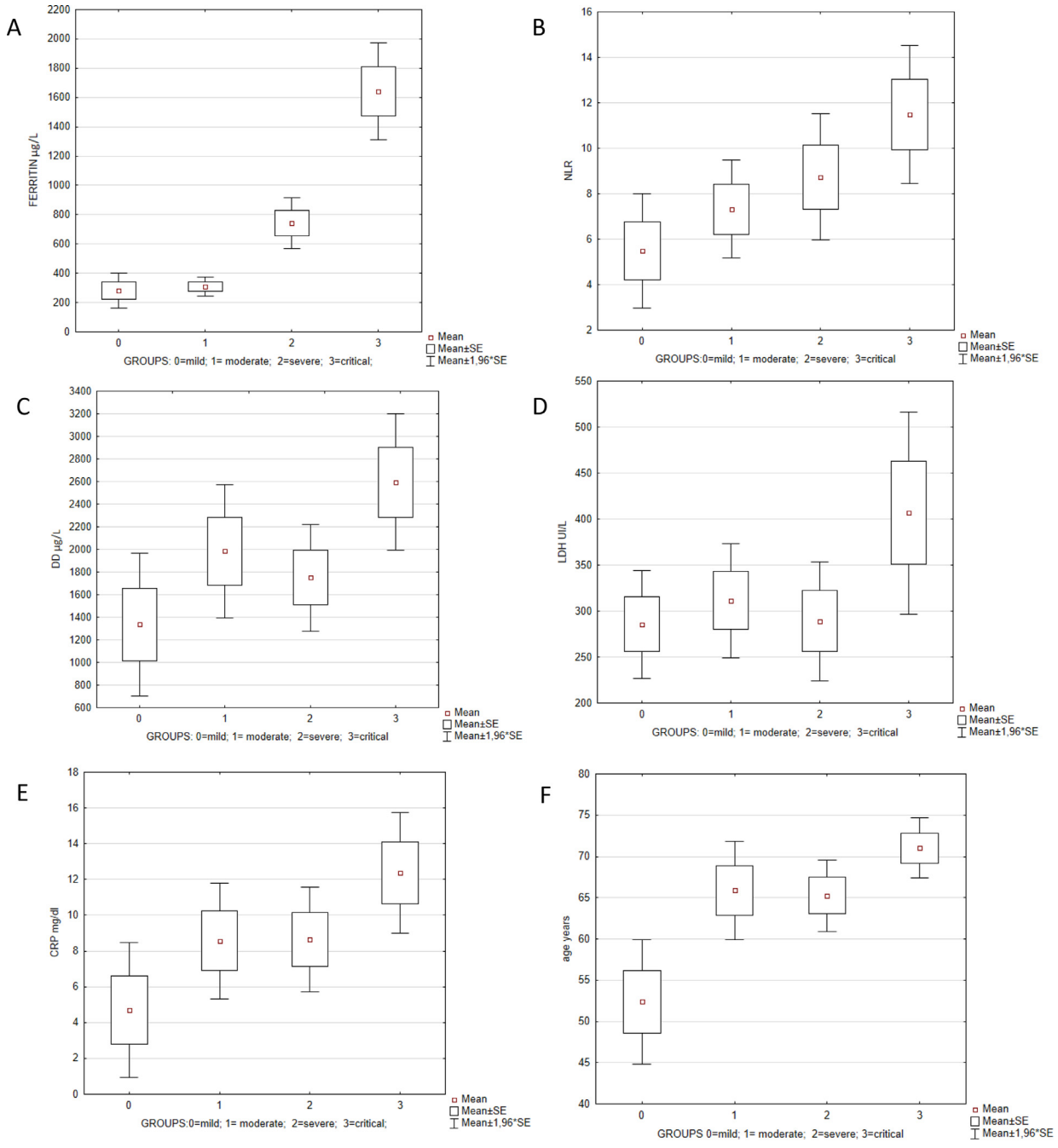
Fig. 1. 1A) Characteristics of the study population; 1B) Analysis of Variance of serum ferritin between severe (60) and non severe COVID-19 patients (81); 1C) ROC curve analysis of serum ferritin levels for the severity of COVID-19.

in Rome, Italy, between March 2020 and June 2020. Serum samples were collected from patients upon admission before starting any treatment and tested by Laboratory Department.

Of all patients included, 81 patients (57%) showed mild disease (control group) and 60 (43%) showed acute respiratory distress syndrome (ARDS) and systemic inflammation (severe group). Fig. 1A shows the differences in the baseline characteristics between severe and non-severe COVID-19 patients. The severe patients were older and more frequently males and showed significant higher levels of C Reactive Protein (CRP), D-Dimer (D-D), Lactate Dehydrogenase (LDH), Neutrophil to Lymphocyte ratio (NLR) and Ferritin.

Serum ferritin levels were positively correlated with severity of COVID-19 (Fig. 1B) and hyperferritinemia (ferritin level > 500 µg/L), was observed in all patients with severe disease on admission. Moreover, ROC curve analysis confirmed the excellent prognostic accuracies of serum Ferritin in discriminate patients with severe clinical conditions. (AUC 0.939, CI: 0,894 to 0,985 $p < 0.001$) (Fig. 1C).

The triaging of COVID-19 patients is based on a combination of clinical, laboratory and instrumental parameters, mainly represented by Computed Tomography (CT). Thus, based on the severity of pulmonary impairment in CT scan and respiratory failure in need of mechanical ventilation, patients were further divided in 4 groups according to the WHO guidelines updated in May 2020[6]: 29



G

	Groups					
Variables [Mean(Std. Dev.)]	**0 - mild** (29 patients)	**1 - moderate** (32 patients)	**2 - Severe** (38 patients)	**3 - Critical** (42 patients)	**All** (141 patients)	p value
FERRITIN µg/L	281(323)	308(190)	741(544)	1640(1094)	816(884)	<0,001
NLR	5,48(6,67)	7,32(6,03)	8,73(8,72)	11,47(10,03)	8,62(8,49)	<0,05
D-D ug/dL	1335(1583)	1983(1597)	1749(1369)	2594(1688)	1942(1602)	<0,05
LDH UI/L	285(143)	311(138)	289(124)	744(1366)	395(666)	ns
CRP mg/dL	4,69(9,79)	8,57(8,74)	8,63(8,60)	12,36(10,42)	8,90(9,73)	<0,05
AGE years	52,37(19,88)	65,87(17,16)	65,23(13,63)	71(11,60)	64,48(16,57)	<0,01

Fig. 2. 2A–F) Analysis of Variance – Categorized box and whisker plot of ferritin, NLR, DD, LDH, CRP and age according to COVID-19 severity; 2 G) Analysis of Variance and concentrations of ferritin, NLR, D-D, LDH, CRP and age according to COVID-19 severity.

patients with no CT alterations (Group 0-mild); 32 patients with changes in CT scan no oxygen (Group 1-moderate); 38 patients with CT scan plus oxygen (Group 2-severe) and 42 patients with CT abnormalities plus intensive care unit (ICU) admission (critical-Group 3).

Our data strongly confirm that increased levels of ferritin were directly related with the disease severity (Fig 2A). Particularly, not only severe group showed 2.6 times higher ferritin levels than the mild group, but patients who needed admission to the ICU showed 5.8 times higher ferritin compared to patients with mild COVID-19. Among all parameters considered, we also noted that the NLR was statistically correlated with the severity of disease. (Fig. 2B). Conversely, D-D, LDH and CRP increased only in the group of critical patients (group 3), being substantially stable in the other groups characterized by mild, moderate and severe disease (Fig. 2, panel C, D, E).

Multivariate logistic regression model adjusted for several disease-related risk factors at admission, including age, sex, NLR, D-D, LDH, ferritin and CRP, demonstrated that serum ferritin resulted as an independent predictor of disease severity in COVID-19 patients (OR = 1,0048, 95% CI, 1,0029 to 1,0083, $p < 0,001$.).

If patients were grouped according to the serum ferritin level with a cut off of 500 µg/ml derived from the HLH-2004[7] criterion, hyperferritinemia accounted for 48,22% (68/141) of patients and the hyperferritinemia group had a higher proportion of severe cases (77,94% vs 10,30%, $p < 0,001$) than patients without hyperferritinemia.

This is the first Italian report about the prognostic value of laboratory biomarkers considering 4 groups of mild, moderate, severe and critical patients with COVID-19. We clearly demonstrated that serum levels of ferritin progressively increased with the severity of disease and correlate with poor prognosis in COVID-19 patients.

Increased ferritin levels could be indicative of a strong inflammatory reaction in COVID-19 and recent studies suggest that increased levels of circulating ferritin levels play a critical role by contributing to the development of a cytokine storm[8,9] resembling macrophage activating syndrome.[10] Timely control of the cytokine storm in its early stage through immunomodulators and cytokine antagonists, as well as the reduction of lung inflammatory cell infiltration, is the key to improving the treatment success rate and reducing the mortality rate of patients with COVID-19. In this regard, ferritin evaluation could be an early, available and easy to use screening tool to assess the disease severity at the first admission in the emergency department. This test might be of crucial importance for the timely identification of patients at higher risk of an adverse outcome.

References

1. Lin Z., Long F., Yang Y.. Serum ferritin as an independent risk factor for severity in COVID-19 patients. *J Infect* 2020 Jun 24 Epub ahead of print. doi:10.1016/j.jinf.2020.06.053.
2. Wu C., Chen X., Cai Y., Xia J., Zhou X., Xu S., et al. Risk Factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 Pneumonia in Wuhan, China. *JAMA Intern Med* 2020.
3. Henry B.M., de Oliveira M., Benoit S., Plebani M., Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med* 2020.
4. Bhatraju P.K., Ghassemieh B.J., Nichols M., Kim R., Jerome K.R., Nalla A.K.. Covid-19 in critically ill patients in the Seattle region - case series. *N Engl J Med* 2020. doi:10.1056/NEJMoa2004500.
5. Xu X.W., Wu X.X., Jiang X.G., Xu K.J., Ying L.J., Ma C.L.. 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.
6. Clinical management of COVID-19 - interim guidance (May 2020)-WHO - https://reliefweb.int/sites/reliefweb.int/files/resources/2005_clinical_management_of_covid-19-v7.pdf
7. Henter J.I., Horne A., Aricó M., et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer* 2007;**48**(2):124–31. doi:10.1002/pbc.21039.
8. Kernan K.F., Carcillo JA. Hyperferritinemia and inflammation. *Int Immunol* 2017;**29**(9):401–9.
9. Wessling-Resnick M.. Crossing the iron gate: why and how transferrin receptors mediate viral entry. *Annu Rev Nutr* 2018;**38**:431–58.
10. Shoenfeld Y.. Corona (COVID-19) time musings: our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning. *Autoimmun Rev* 2020:102538.

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Serial simultaneously self-swabbed samples from multiple sites show similarly decreasing SARS-CoV-2 loads in COVID-19 cases of differing clinical severity

Dear Editor,

Detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is essential for diagnosis of Coronavirus disease (COVID-19). Nasopharyngeal swabs (NPS) are recommended by the World Health Organisation (WHO) as the current standard method to detect SARS-CoV-2 in suspected patients.[1] However, collection of an NPS or oropharyngeal swab (OPS) can be technically difficult, uncomfortable for patients, may induce sneezing or coughing, and expose those nearby to aerosolised SARS-CoV-2).[2]

We postulate that alternative easier-to-sample swabs such as those from the nose (NS) or cheek (CS) may be equally sensitive in acute COVID-19 cases, and can be effectively taken by patients themselves. In addition, longitudinal analysis of serial samples collected contemporaneously from multiple upper respiratory tract (URT) sites, from the same patient, has not been well described. Here, we performed a prospective longitudinal analysis of

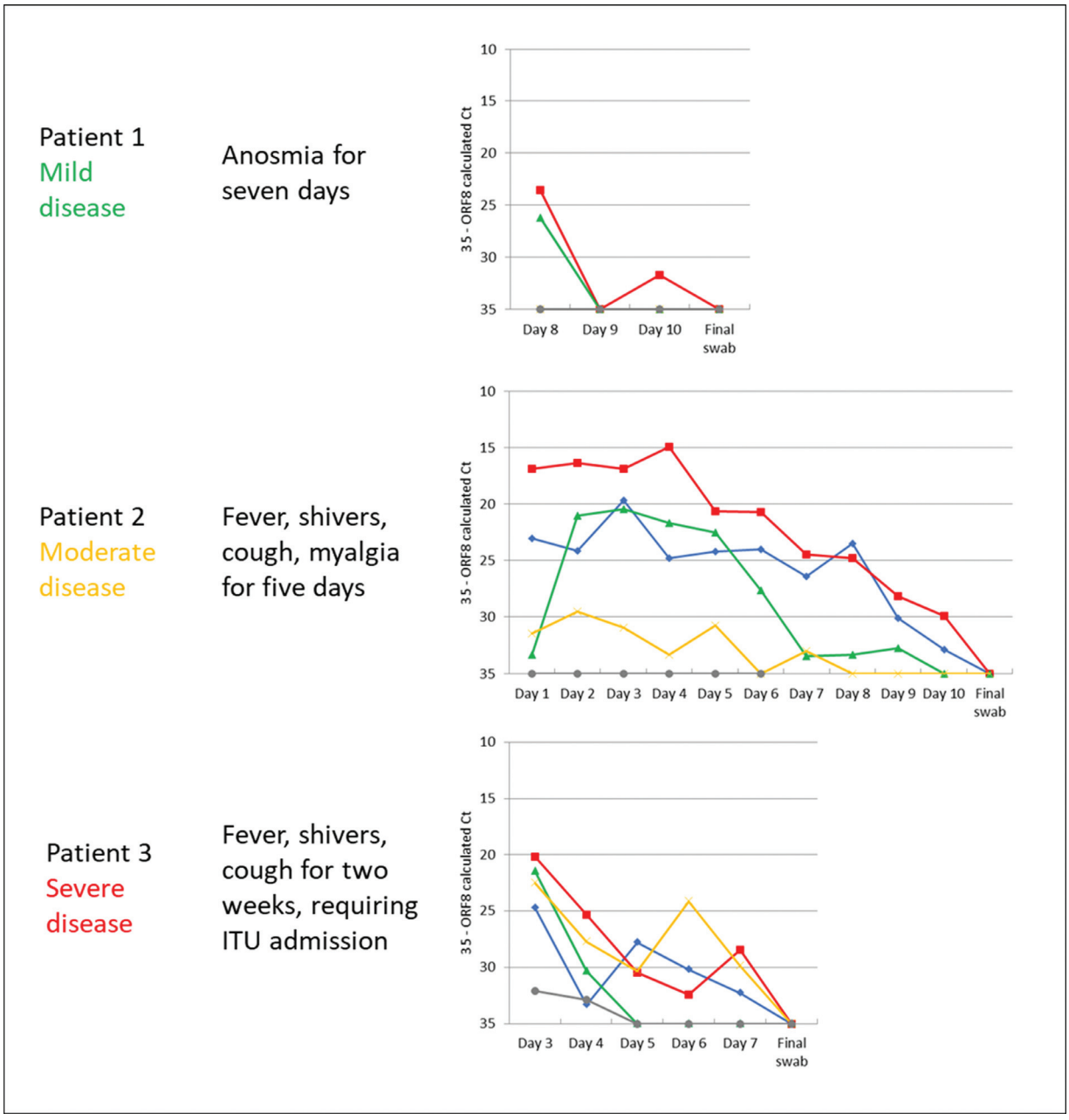

Fig. 1. Relative SARS-CoV-2 viral loads indicated by Aus Diagnostics ORF8 multiplex-tandem RT-PCR calculated cycle threshold (Ct) values (inverted y-axis) of Patient s 1–3 with differing severities of COVID-19. Samples which did not amplify have been given a nominal value of 35 (equivalent to the maximum calculated Ct for this assay). The 'final' (PCR negative) swab was taken 14 days post-illness onset for all Patients. Corresponding sample types according to sampling site (as discussed in the main text): Red squares: nasopharynx – nasopharyngeal swab (NPS); green triangles: nose – nasal swab (NS); blue diamonds: oropharynx – oropharyngeal swab (OPS); yellow crosses: cheek – cheek swab (CS); grey circles: conjunctiva – conjunctival swab (CJS). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

three healthcare worker volunteers with differing clinical severities of acute COVID-19.

Shortly after a confirmed diagnosis of COVID-19 using the local diagnostic AusDiagnostics (Ausdiagnostics UK Ltd., Chesham, England) SARS-CoV-2 PCR test,[3] each participant volunteered to provide serial self-collected swabs from the nasopharynx (NPS, 1 swab), just inside the soft part of the nose (NS, 1 swab), the oropharynx (OPS, 1 swab), inside the cheek (CS, 1 swab). In addition, we also decided to check for the presence of SARS-CoV-2 in the conjunctiva (CJS, 1 swab for both eyes). All of these swabs were taken at the same time-point, on a daily basis - or as frequently as was practical and tolerable - until all the SARS-CoV-2 viral loads became undetectable. Thus, each patient collected up to 5 separate swabs on a daily basis for this study.

All three participants (hereafter referred to individually as 'Patient' 1, 2, or 3) became symptomatic with confirmed COVID-19 during the week of 12th April 2020. Patient 1 had *mild* COVID-19, complaining of a 7-day history of anosmia only; Patient 2 had *moderate* COVID-19, with a 5-day history of fevers, shivers, dry cough and myalgia; Patient 3 had *severe* COVID-19, presenting with a 2-week history of fevers, shivers and a productive cough that required supplemental oxygen therapy, and eventual admission to the intensive care unit (ICU) during the second week, after which no further swabs were taken. Final follow-up swabs were performed by the participants on 5th May 2020, two weeks after symptom onset for all participants.

From 17th April to 5th May 2020, a total of 105 swabs were collected from three participants (Fig. 1). Patient 1 (*mild* COVID-19)

provided a total of four days of swabs (20 swabs) from Day 8 days post-symptom onset; Patient 2 (*moderate* COVID-19) provided a total of 11 days of swabs (55 swabs) from Day 1 post-symptom onset; Patient 3 (*severe* COVID-19) provided a total of 6 days of swabs (30 swabs) from Day 3 post-symptom onset. All three participants were PCR negative for SARS-CoV-2 on the final swabs taken on 5th May 2020.

Patient 1 (*mild* COVID-19) only had positive PCR results from the NPS and NS, with negative PCR results from the NS after day 8. Patient 2 had positive PCR results from all sites except for the conjunctiva, with the lowest cycle threshold (Ct) value (i.e. the highest viral load) in the NPS, followed by the OPS, NS and CS. Patient 3 had positive PCR values from all sites; with the lowest Ct value (highest viral load) in the NPS followed by NS, CS, OPS and CJS.

For all three participants, Ct values appeared to increase (i.e. the viral loads decreased) over time. For Patient 1 (*mild* COVID-19), the virus was only detected in the NPS and NS from Day 8 onwards, with some fluctuation in the detectability of the virus in the NPS. For Patient 2 (*moderate* COVID-19), the Ct values of the NPS, OPS and NS also increased over time, with some fluctuation in the detectability of the virus in the CS. For Patient 3 (*severe* COVID-19), the NPS, OPS and CS Ct values increased over time, demonstrating decreasing viral loads.

Our small longitudinal study cohort demonstrated several findings. Firstly, the most symptomatic case, Patient 3 was most likely to be viremic at multiple sites in the URT, as reported elsewhere.[4] Secondly, self-swabbing from these various URT sites is an effective and sensitive way to collect diagnostic samples, as found elsewhere.[5] Note that only one out of these three cases, Patient 3 who did not exhibit overt conjunctivitis, exhibited detectable virus from the conjunctiva within the first 5 days of illness. Patient 1′s first swab was only taken on Day 8 post-illness onset, so it is possible that any virus present earlier in conjunctival fluids may have been missed. However, this lower detection rate for conjunctival swabs (with or without overt conjunctivitis) is consistent with previous reports.[6] Thirdly, the relative SARS-CoV-2 viral loads from the URT decreased with time in the 1–2 weeks post-COVID-19 symptom onset, regardless of disease severity. This has been shown elsewhere,[7] though this is not always the case.[8]

From this small longitudinal cohort study on serially collected samples in acute COVID-19 cases of differing severity, we conclude that for symptomatic patients, it is difficult to obtain a 'false negative' result on NPS, OPS, NS or CS samples, if sampled early (within 5 days) post-symptom onset, even if the swab was 'poorly' taken. Despite a previous meta-analysis showing that sputum testing is possibly more sensitive for SARS-CoV-2 PCR testing,[9] other studies have shown that self-sampling from various URT sites performed satisfactorily for the diagnosis of acute COVID-19.[5] Sputum testing is not standard in many virology labs due to long-recognised problems related to its viscosity and risks of PCR inhibition,[10] and not all COVID-19 patients will have a productive cough.

Therefore, we further confirm that early (within 5 days of symptom onset), self-swabbed NPS, OPS, NS or CS samples for SARS-CoV-2 diagnostic testing in acute COVID-19 cases is a sensitive, practical approach, which reduces patient discomfort (as self-swabbing can be controlled) and minimises virus exposure to healthcare workers.

References

1. WHO. *Laboratory testing of 2019 novel coronavirus (2019-nCoV) in suspected human cases* 2020. https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117 (Accessed 1st December).
2. Marty F.M., Chen K., Verrill K.A. How to obtain a nasopharyngeal swab specimen. *N Engl J Med* 2020;**382**(22):e76. doi:10.1056/NEJMvcm2010260.
3. Bird P., Badhwar V., Fallon K., Kwok K.O., Tang J.W. High SARS-CoV-2 infection rates in respiratory staff nurses and correlation of COVID-19 symptom patterns with PCR positivity and relative viral loads. *J Infect* Sep 2020;**81**(3):452–82. doi:10.1016/j.jinf.2020.06.035.
4. Wölfel R., Corman V.M., Guggemos W., et al. Virological assessment of hospitalized patients with COVID-2019. *Nature* 2020;**581**(7809):465–9. doi:10.1038/s41586-020-2196-x.
5. Tu Y.P., Jennings R., Hart B., et al. Swabs collected by patients or health care workers for SARS-CoV-2 testing. *N Engl J Med* 2020;**383**(5):494–6. doi:10.1056/NEJMc2016321.
6. Güemes-Villahoz N., Burgos-Blasco B., Arribi-Vilela A., et al. Detecting SARS-CoV-2 RNA in conjunctival secretions: is it a valuable diagnostic method of COVID-19?. [published online ahead of print, 2020 Jun 24] *J Med Virol.* 2020 10.1002/jmv.26219. doi:10.1002/jmv.26219.
7. 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;**382**(12):1177–9. doi:10.1056/NEJMc2001737.
8. Arons M.M., Hatfield K.M., Reddy S.C., et al. Presymptomatic SARS-CoV-2 infections and transmission in a skilled nursing facility. *N Engl J Med* 2020;**382**(22):2081–90. doi:10.1056/NEJMoa2008457.
9. Mohammadi A., Esmaeilzadeh E., Li Y., Bosch R.J., Li J.Z. SARS-CoV-2 detection in different respiratory sites: a systematic review and meta-analysis. [published online ahead of print, 2020 Jul 18]. *EBioMedicine* 2020:102903. doi:10.1016/j.ebiom.2020.102903.
10. Jeong J.H., Kim K.H., Jeong S.H., Park J.W., Lee S.M., Seo Y.H. Comparison of sputum and nasopharyngeal swabs for detection of respiratory viruses. *J Med Virol* Dec 2014;**86**(12):2122–7. doi:10.1002/jmv.23937.

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Incidence of spread of clinically relevant SARS-CoV2 infection between children in a tertiary emergency department: An evaluation ☆

Dear Editor,

Grall and colleagues highlight that asymptomatic carriers are rarely potential viral transmitters in hospital.[1] Their study was in adults and to our knowledge spread in healthcare settings between children has not been reported. Leicester was the first area in the United Kingdom to undergo a localised lockdown with reports of

☆ Letter in response to Grall et al. https://doi.org/10.1016/j.jinf.2020.08.044

relatively high numbers of children and young people affected.[2] Our evaluation aimed to identify the number of clinically significant SARS-COV-2 paediatric patients (age <18 years) presenting to our Children's Emergency Department (CED) at the Leicester Royal Infirmary (LRI), to investigate the effectiveness of infection control measures. We determined clinically significant infection to be that which prompted parents or carers to bring their child to the CED and be admitted. We highlight that national guidance in England determines only admitted patients are swabbed for SARS-COV-2. Clinical information was gathered by retrospectively looking at the attendances in Nervecentre® (the University Hospitals of Leicester NHS Trust Electronic Patient Record system, version 6.02). The contact tracing feature on NerveCentre® allowed us to find all patients in the department at the same time as an index case.

The CED at LRI followed national public health guidance when implementing infection control measures. Policies ranged from adopting rigorous hand washing and provision of Personal Protective Equipment (PPE) for patient contacts, to the separation of the department into "red" (suspected COVID) and "blue" (non-suspected COVID) zones (criteria in Fig. 1).

Our evaluation was submitted to the University Hospitals of Leicester NHS Trust Audit and Improvement committee (Identifier 10786) and ratified as a service evaluation project. Between 17th March 2020 to 31st July 2020, we saw 10,777 children in the CED. During this period 22 samples tested positive for SARS-COVID-19 by Polymerase chain reaction (PCR) (AusDiagnostics® and Cepheid Genexpert(®) for children admitted from the CED. Nearly all of the patients came through the red zone; 21/22 (95.4%). A 6-year old patient, managed as bullous impetigo with a course of antibiotics, presented with no COVID suspected symptoms and was placed in the blue zone. There was no overlap between any SARS-CoV-2 positive patients with any other patients who subsequently tested positive in the department. Thus, no clinically relevant COVID19 cross infection was noted.

Out of the 22 included patients presenting to the CED, 20 (90.9%) patients were admitted. The two that were discharged from the CED had a sample sent from the CED due to an initial plan to be admitted, which was later changed due to improvement in clinical status (one an infant presenting with suspected fever and inflammatory parameters within normal range and the second due to the referred speciality decision to discharge. All the other 20 patients were eventually discharged from hospital.

Out of the 22 SARS-CoV-2 positive patients presenting to the children's ED, 2 patients were felt to have the novel paediatric Inflammatory Multisystem Syndrome temporally related to SARS-CoV-2 (PIMS-TS), both needing paediatric intensive care stay.

Red Zone Criteria
1. Fever ≥ 37.8^0 C
2. Fever in past 7 Days
3. Cough
4. Sore Throat
5. Respiratory Symptoms
6. Coryzal Symptoms
7. Member of household self-isolating at present

Fig. 1. Criteria for directing patients in into the Red zone from the sieve during data collection period.

Children presented with lower respiratory tract infection (LRTI) (3/22) suspected sepsis (4/22), and Bronchiolitis (2/22). A single patient presented with each diagnosis of infectious gastro-enteritis, seizures, meningo-encephalitis, bullous impetigo, and perforated appendix as the primary diagnosis.

The average length of stay in hospital of the 22 included patients was 120.68 h (range 5–545 h; SD 127.48)

Our evaluation was designed to understand the cross-infection rates amongst paediatric patients presenting to our hospital and ensure we were deploying robust methods of infection prevention. No child who was in the department at the same time as a child who was subsequently found to be positive returned to be admitted with SARS-CoV-2 infection themselves. Therefore, there was no evidence of clinically relevant SARS-CoV-2 infection between patients in our department. However, it is important to note that only patients in whom the decision to admit was made, were the SARS-CoV-2 tests done, therefore the actual incidence of SARS-CoV-2 in our department would be very difficult to obtain. Also, as we did not swab patients unless there was a decision to admit them our results should also not be taken to indicate that any child with SARS-CoV-2 needs admission.

The COVID19 pandemic has presented unprecedented challenges to the healthcare infrastructure. In particular, there is limited number of high quality data on paediatric cases,[3] largely due to infrequent symptomatic and severe infection in this population.[4]

In our Paediatric ED, we employed stringent methods of infection prevention. This included standard measures like hand washing, wearing of PPE (gloves, aprons and protective eye gear), and regular training (face-to-face and online) of staff. However, the measure that involved the biggest change in our way of working was a separation of the CED into "red" and "blue" areas based on the presence or absence of, respectively, fever, respiratory complaints (Fig. 1). This led to the creation of a standardised checklist to be used by staff employed in a "sieve", who would direct patients to different areas using the symptom checklist. This in turn meant a substantial change in the way we work requiring staff allocation amongst the two areas, and challenges to overall oversight of the ED by medical and nursing team leaders as there could potentially be complex patients in both areas at the same time. Given children don't appear to be causing clinically relevant spread within our CED, and the numbers of positive cases are low, we believe that the current measures to split the departments are effective, but also perhaps, unnecessary.

This could be crucial in managing future CED patient flow, especially during the winters when the other seasonal viral infections are likely to overburden the services.

Contributions

DR conceptualised the initial data and MP and SP collected and collated data. MP wrote a first draft to which all authors contributed to and agreed a final version.

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Declaration of Competing Interest

The authors report no competing interests.

References

1. Grall I., Alloui C., Tandiaoui-Lamboitte Y., et al. Viral transmission in asymptomatic cases of SARS-CoV-2 infection. *J Infect* 2020. online first https://doi.org/10.1016/j.jinf.2020.08.044 .

2. Rapid Investigation Team. Preliminary investigation into COVID-19 exceedances in Leicester. London: 2020.
3. Castagnoli R., Votto M., Licari A., et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. *JAMA Pediatr* 2020. doi:10.1001/jamapediatrics.2020.1467.
4. Han Q., Lin Q., Jin S., et al. Coronavirus 2019-nCoV: a brief perspective from the front line. *J Infect* 2020 online first. doi:10.1016/j.jinf.2020.02.010.

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Household transmission of COVID-19-a systematic review and meta-analysis

Dear Editor,

We read with interest the recent study of Wang et al.,[1] regarding the household transmission of coronavirus disease 2019 (COVID-19). In December 2019, an outbreak of COVID-19, caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-COV-2), became a global pandemic. Many epidemiologic studies have been carried out to characterize the epidemiology of COVID-19 and inform decisions about possible interventions. Of particular early interest was the frequency of transmission from confirmed cases to their close contacts. It had been reported that about 75–80% of clustered COVID-19 infections in China were within families,[2] suggesting high rates of intra-family transmission. Thus timely household studies can be highly informative for COVID-19 prevention. Here, we report a systematic review of household transmission studies of COVID-19 and try to assess the secondary attack rate of household COVID-19 transmission.

Studies containing data on household transmission of COVID-19 were retrieved from the electronic databases: PubMed, Embase, and a Chinese database, China National Knowledge Infrastructure (CNKI) on 1 July, 2020. All titles identified by the search strategy were independently screened by 2 authors (H.L. and X.X.). Eligible articles reported a household transmission for COVID-19 or sufficient data to determine a secondary attack rate, and must have reported data based on >=10 households. Case reports with only one family involved were also excluded for analysis, because the household transmissions event collection was biased towards infection caused more serious illness. The bias could be reduced with more families involved. When multiple reports of the same dataset were identified, only the most comprehensive report of the study was included. Household secondary attack rate (SAR) was calculated as the number of identified cases divided by the number of household contacts. Further, the SAR was calculated separately for adults and children, using the age criterion of the study, further, the SAR by other contacts was are summarized, when data were available. SARs were calculated as the number of cases divided by the number of contacts, using the fisher's exact test for the 95% confidence interval (CI). We assessed statistical heterogeneity among studies using the I^2 index. All analyses were performed by the software SPSS. The meta-analysis was conducted in Review Manage 2020.

A total of 463, 402 and 212 titles were identified from PubMed, Embase and CNKI, respectively; and 24 articles were included in the mata-analysis. The characteristics of 24 included articles are summarized in Supplementary Table S1. 10 studies are retrospective cohort studies, one is a prospective study, and the other 13 are case ascertainment studies. Most studies (19/24) were conducted in China, with two in South Korea, two in the USA and one in Germany. All the studies were conducted between Jan 1 and March 31, 2020.

Reported SARs were substantially heterogeneous, ranging from 4.6% to 90.0% (I^2 = 96%), with pooled rate of 27% (95% CI: 21−32%) (Fig. 1). In the 10 retrospective cohort studies, the SARs ranged from 11.2% to 68.2%, with mean value 29.7%. In the 13 case ascertainment studies, the SARs ranged from 4.6% to 90.0%, with mean value 28.3%. There is no significantly difference between the SARs in the retrospective cohort and case ascertainment studies ($p = 0.93$).

In 6 of the 24 studies, SAR was stratified by age, yielding a range of SARs from 15.7% to 47.6% in adults and from 5.2% to 26.9% in children. The meta-analysis indicates the risk of household transmission in adults is about 3-times higher than that in children (odds ratio (OR)=3.67, 95% CI: 2.76–4.87, $p < 0.001$) (Fig. 2). In 10 studies, the SAR among other contacts (not household contacts) was also reported, and ranged from 0.1% to 28.8%. The meta-analysis indicates the risk of household transmission is about 10 times higher than that from other contacts (OR = 10.72, 95% CI: 5.70–20.17, $p < 0.001$) (Supplementary Fig. S4).

There were substantial heterogeneities in estimated SAR from the various studies, with point estimates of the SAR ranging from 4.6% to 90.0%. The intrinsic transmissibility is not thought to have various transmissibility in different regions, indeed, as a comparison, review studies reported 2009 pandemic influenza A(H1N1) in household SAR with H1N1 virus were also widely varying, from 3% to 38%.[3] In these studies, with 10 or more families involved, the highest SAR were observed in Wuhan, China, which also had the greatest number of COVID-19 confirmed cases in China. Generally, the data-based SAR estimated from literatures would be higher than the real SAR since data-based estimates might have included some untraced exposures from outside.

Household SAR were greater than SAR by other contacts (OR = 10.72, 95% CI: 5.70–20.17, $p < 0.001$), suggesting much higher rates of intra-family transmission of COVID-19. Several studies have reported that adults were more vulnerable to SARS-CoV-2.[4] In this study, we also found that within households, adults were about 4 times as susceptible to COVID-19 from a household member as children (OR = 3.67, 95% CI: 2.76–4.87, $p < 0.001$). In the study with the highest SAR (62.1%, 95% CI: 52.4−71.9%) among the 10 retrospective cohort studies, the mean age of the households (58.7 ± 16.0)[5] was also much higher than these in other studies with detailed age of the households. The age of the households might partly explain the varying SARs. However, because the age of

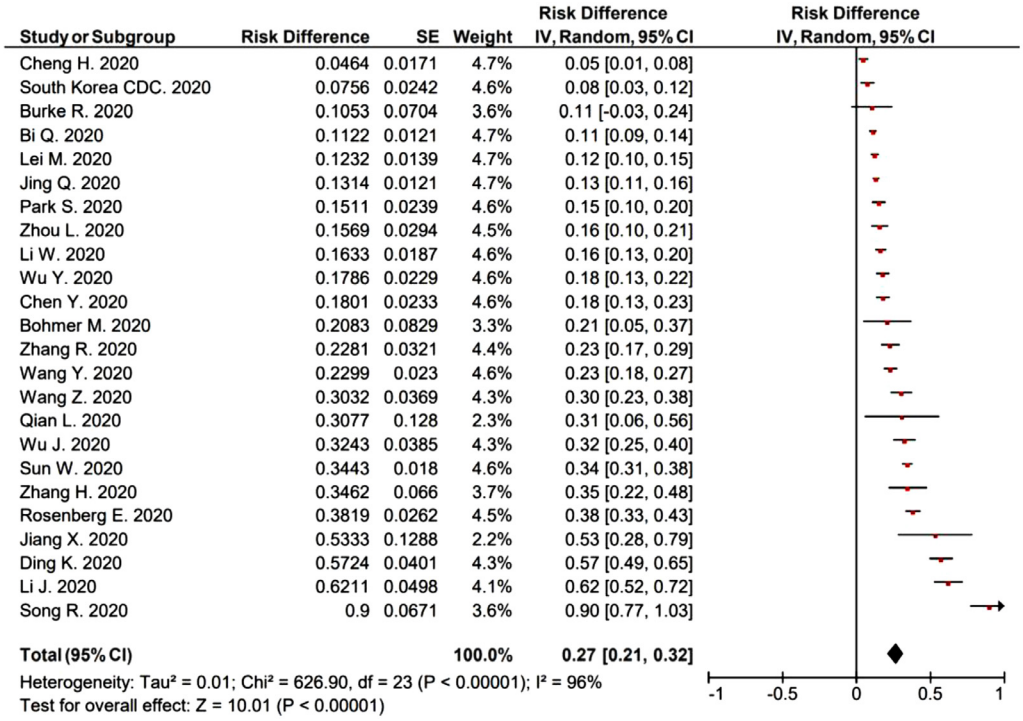

Fig. 1. Secondary attack rates (SIRs) among household contacts.

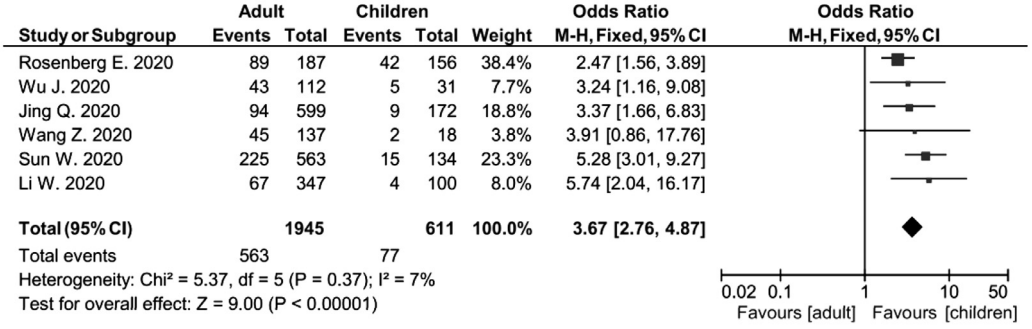

Fig. 2. Transmission risk of COVID-19 to adults and children in household.

the households was not available in most studies, the quantitative analysis of the SARs and age of the households were not done.

For SARS-CoV, the SAR was estimated to be 6.2–10.2% in Beijing, Hong Kong, Toronto and Singapore.[6–8] Information about the household transmission of MERS-CoV is relatively rare. In a study in Saudi Arabia, the household SAR of MERS-CoV was estimated to be 4% (95% CI: 2–7).[9] We conclude that SARS-CoV-2 is more transmissible than SARS-CoV and MERS-CoV in households. In addition, pre-symptomatic and asymptomatic cases were estimated to contribute 53% of COVID-19 transmission.[10] All these challenge the value of home isolation for COVID-19 patients, as it may put household members at high risk of infection, propagating the disease. When the hospital isolation of all cases becomes unfeasible, other sheltering facilities, such as the Fangcang Shelter Hospital used in Wuhan, China, might be a better option.[2]

Author's contributions

HL and XX contributed equally. HL conceived the study, HL and XX designed the study. YS and XW supervised the study. HL, XX and SX searched the references, collected and cleaned the data. HL wrote the drafts of the manuscript. YS, XW, XX and SX commented on and revised drafts of the manuscript. All authors read and approved the final report.

Declaration of Competing Interest

All authors declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.08.033.

References

1. Wang Z., Ma W., Zheng X., Wu G., Zhang R. Household transmission of SARS–CoV-2. *J Infect* 2020;**81**(1):179–82.
2. Chen S., Zhang Z., Yang J., et al. Fangcang shelter hospitals: a novel concept for responding to public health emergencies. *Lancet* 2020;**395**:1305–14.
3. Lau L.L.H., Nishiura H., Kelly H., et al. Household transmission of 2009 pandemic influenza A(H1N1): a systematic review and meta-analysis. *Epidemiol* 2012;**23**(4):531–42.
4. Zhang J., Litvinova M., Liang Y., et al. Changes in contact patterns shape the dynamics of the COVID-19 outbreak in China. *Science* 2020 DOI: 11.1126/science.abb8001.
5. Li J., Gong X., Wang Z., et al. Clinical features of familial clustering in patients infected with 2019 novel coronavirus in Wuhan, China. *Virus Res* 2020;**286**:198043.
6. China C.D.C.. Efficiency of quarantine during an epidemic of severe acute respiratory syndrome—Beijing, China, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**:1037.
7. Wilson-Clark S.D., Deeks S.L., Gournis E., et al. Household transmission of SARS, 2003. *CMAJ* 2006;**175**(10):1219–23.
8. Goh D.L.M., Lee B.W., Chia K.S., et al. Secondary Household transmission of SARS, Singapore. *Emerg Infect Dis* 2004;**10**(2):232–4.
9. Drosten C., Meyer B., Müller M.A., et al. Transmission of MERS-coronavirus in household contacts. *N Engl J Med* 2014;**371**:828–35.
10. Ferretti L., Wymant C., Kendall M., et al. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. *Science* 2020;**368**(619):eabb6936.

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Detection of PB2 627 K mutation in two highly pathogenic isolates of the H7N9 subtype Influenza a virus from chickens in Northern China

Dear Editor,

H7N9 avian influenza virus (AIV) first emerged in February 2013 in China. Early H7N9 isolates are all low pathogenic (LP) until September 2017 when a few highly pathogenic (HP) H7N9 mutants were identified.[1] Phylogenetic analyses suggest that the HP viruses are a tri-reassortant of H7, N9, and H9N2 subtypes of AIV. This new reassortant carries several amino acid substitutions in its hemagglutinin (HA) gene to allow the virus to bind to its mammalian receptor with a high affinity.[2,3]

Since the first case of human infection with the H7N9 influenza virus was reported in China in March 2013,[4] there have been five H7N9 epidemic waves that sickened 1568 individuals and caused 616 fatalities according to the WHO report (http://www.who.int/influenza/human_animal_interface). Previous studies have proved that the PB2 E627K substitution played an important role in the emergence of the rapid cross-species transmission of the H7N9 AIV from avian sources to human hosts, and functions of this substitution in mammalian adaptation have also been well documented, including enhancing polymerase activity, virus replication, pathogenicity, and transmission of AIV in humans and other mammals.[5,6] As summarized in the Table 1, >70% of H7N9 human isolates (896/1273) acquired the PB2 627 K mutation, and approximately 27% (343/1273) remained 627E. In contrast, >99% of avian isolates (786/791) retained 627E, while only three LP isolates gained 627 K mutation. Since H9N2 provides all the internal genes to generate the prototype of H7N9 viruses, we also noticed that H9N2 isolates obtaining the PB2 627 K mutation had been reported recently.[7]

In late 2018, we conducted routine surveillance of AIV infections in poultry and isolated two strains of H7N9 subtype AIV from breeder farms in northern China. We found that these two chicken-origin H7N9 isolates, A/chicken/Northern China/SX4748/2018 (SX4748) and A/chicken/Northern China/SD3866/2018 (SD3866), were HP based on the sequence of the HA gene. Deduced amino acid sequences of the HA gene revealed that both strains had a four-amino-acid (KRTA) insertion at position 339–342 and an I335V mutation in the cleavage site to make the motif PEVPKRKRTAR↓GLF. These amino acid changes concur with the alterations that took place in human isolates such as A/Guangdong/Th008/2017(Th008) and A/Taiwan/1/2017(TW1).

Table 1
Amino acids at position 627 of PB2 protein of the H7N9 viruses isolated from 2013 to 2019.

Isolated year	Human isolates				Avian isolates		
	E	K	V	Q	E	K	V
2013	49/378	328/378	1/378	0/378	191/191	0/191	0/191
2014	72/264	190/264	2/264	0/264	313/314	1/314	0/314
2015	51/128	74/128	2/128	1/128	100/102	2/102	0/102
2016	46/123	74/123	3/123	0/123	64/64	0/64	0/64
2017	124/374	225/374	25/374	0/374	115/117	0/117	2/117
2018	1/1	0/1	0/1	0/1	3/3	0/3	0/3
2019	0/5	5/5	0/5	0/5	0/0	0/0	0/0
Total	343/1273	896/1273	33/1273	1/1273	786/791	3/791	2/791

Sequences of H7N9 viruses were obtained from the public database "Global Initiative on Sharing Avian Influenza Data" and GenBank from 2013 to 2019. The number on the left of the slash shows the number of viruses bearing the indicated amino acid, and the number on the right of the slash shows the total number of viruses analyzed.

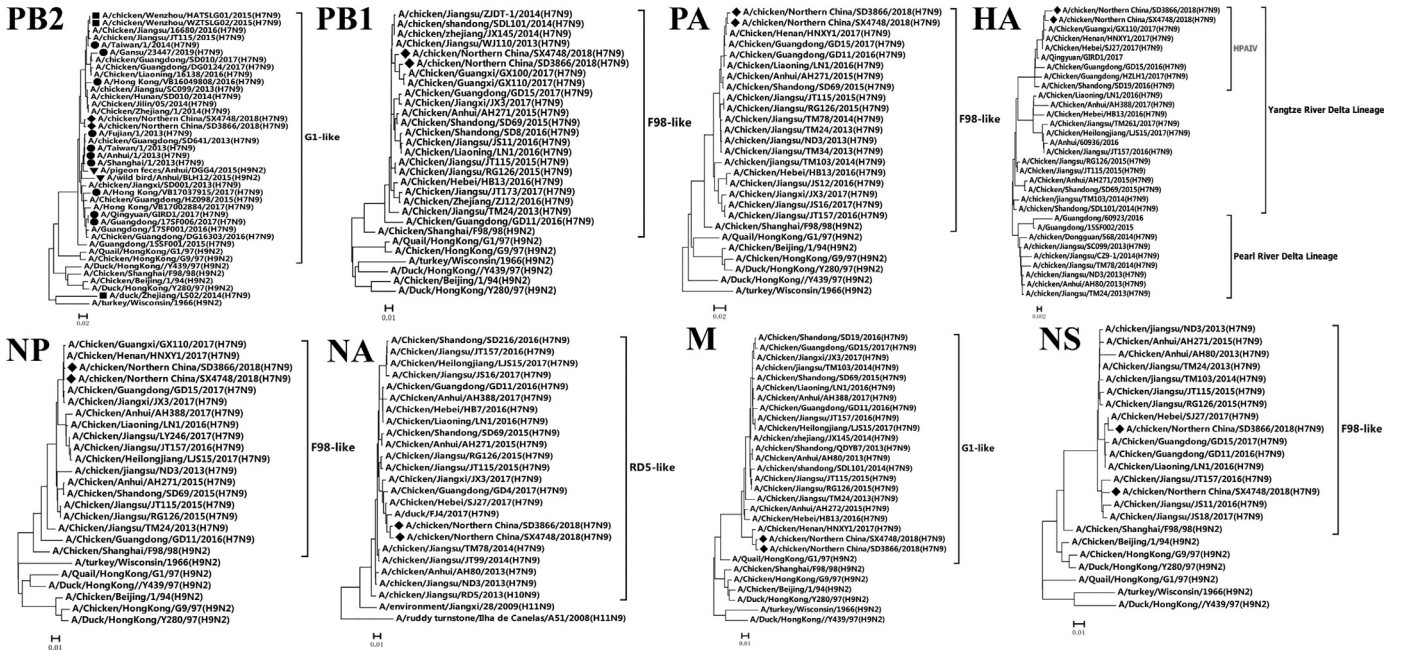

Fig. 1. Phylogenetic trees of eight genes between the two viruses (SX4748 and SD3866) and some representative isolates obtained from GISAID and GenBank. The isolates in this study are marked with black diamonds; LP H7N9 isolates harboring 627 K mutation in the PB2 protein are marked with black squares; human isolates harboring 627 K mutation in the PB2 protein are marked with black spots; H9N2 isolates harboring 627 K mutation in the PB2 protein are marked with black triangle. The tree was inferred by MEGA 6.0 using the Maximum Likelihood method based on the Tamura-Nei model with 1000 bootstrap replicates.

The HA gene-based phylogenetic analysis revealed that these two strains clustered to the HP H7N9 clade belonging to Yangtze River Delta lineage, whereas the NA gene of these two strains was in the RD5-like (H10N9) branch. Their internal genes were closely related to the dominant S genotype (G57-like) of the H9N2 subtype[8,9] (Fig. 1). Remarkably, the sequences of the internal genes revealed that both strains gained the E627K mutation in their PB2 gene. Since this substitution reportedly attributes to increased virulence and adaptation in mammalian hosts, we evaluated the biological characteristics and pathogenicity of these two strains in BALB/c mice. Unexpectedly, based on the 50% mouse lethal dose (MLD_{50}) (Supplementary Table) and percent survival curves, we found that these two H7N9 viruses were low virulent. Only one of five mice infected with the SX4748 strain died at the dose of 10^6EID_{50}, while none of the mice infected with the SD3866 strain died. Furthermore, body weight measurement revealed that mice inoculated with the SD3866 strain only experienced a transient infection at the dose of 10^5EID_{50} and 10^6EID_{50} at the end of the experiment (Supplementary Figure). These results suggest that the PB2 627 K mutation is not the only alteration in the virus genome that dictates the virulence and adaptation of influenza viruses in mammalian hosts. Several amino acid alterations in other proteins of AIV have been identified to play an important role in the pathogenicity of the H7N9 virus in mice.[10]

In summary, we report here for the first time the isolation of two highly pathogenic H7N9 strains that harbor the E627K mutation in the PB2 gene from chickens in northern China. These H7N9 variants are likely derived from the reassortment with H9N2 viruses that have already harbored the PB2 E627K substitution or evolved from spontaneous mutations. We also provide evidence that the PB2 627 K in these two variants did not render them a highly pathogenic phenotype in mice, suggesting that PB2 627 K mutation alone is not sufficient for its high virulence in mammals.

Declaration of Competing Interest

No competing financial interest from all authors.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2020.08.024.

References

1. Shi J., Deng G., Ma S., Zeng X., Yin X., Li M., et al. Rapid evolution of H7N9 highly pathogenic viruses that emerged in China in 2017. *Cell Host Microbe* 2018;**24**(4):558–68. doi:10.1016/j.chom.2018.08.006.
2. Zhang Q., Shi J., Deng G., Guo J., Zeng X., He X., et al. H7N9 influenza viruses are transmissible in ferrets by respiratory droplet. *Science* 2013;**341**(6144):410–14. doi:10.1126/science.1240532.
3. Shi J., Deng G., Liu P., Zhou J., Guan L., Li W., et al. 2013. Isolation and characterization of H7N9 viruses from live poultry markets-Implication of the source of current H7N9 infection in humans. *Chin Sci Bull.* 2013;**58**:1857–63. doi:10.1007/s11434-013-5873-4.
4. Gao R., Cao B., Hu Y., Feng Z., Wang D., Hu W., et al. Human infection with a novel avian-origin influenza A (H7N9) virus. *N Engl J Med* 2013;**368**:1888–97. doi:10.1056/NEJMoa1304459.
5. Subbarao E.K., London W., Murphy B.R.. A single amino acid in the PB2 gene of influenza A virus is a determinant of host range. *J Virol* 1993;**67**(4):1761–4.
6. Mok C.K., Lee H.H., Lestra M., Nicholls J.M., Chan M.C., Sia S.F., et al. Amino acid substitutions in polymerase basic protein 2 gene contribute to the pathogenicity of the novel A/H7N9 influenza virus in mammalian hosts. *J Virol* 2014;**88**(6):3568–76. doi:10.1128/JVI.02740-13.
7. Ge Y., Yao Q., Chai H., Hua Y., Deng G., Chen H.. A 627 K variant in the PB2 protein of H9 subtype influenza virus in wild birds. *Influenza Other Respir Viruses* 2018;**12**(6):728–41. doi:10.1111/irv.12592.
8. Gu M., Xu L., Wang X., Liu X.. Current situation of H9N2 subtype avian influenza in China. *Vet Res* 2017;**48**(1):49. doi:10.1186/s13567-017-0453-2.
9. Pu J., Wang S., Yin Y., Zhang G., Carter R.A., Wang J., et al. Evolution of the H9N2 influenza genotype that facilitated the genesis of the novel H7N9 virus. *Proc Natl Acad Sci U S A* 2015;**112**(2):548–53. doi:10.1073/pnas.1422456112.

10. Ma S., Zhang B., Shi J., Yin X., Wang G., Cui P., et al. Amino acid mutations A286V and T437M in the Nucleoprotein Attenuate H7N9 viruses in Mice. *J Virol* 2020;**94**(2):e01530 −19. doi:10.1128/JVI.01530-19.

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