Discriminant models for the prediction of postponed viral shedding time and disease progression in COVID-19

Wen-Yang Li¹, Daqing Wang², Yuhao Guo³, Hong Huang¹, Hongwen Zhao¹, Jian Kang¹ and Wei Wang¹*

Abstract
Background: COVID-19 infection can cause life-threatening respiratory disease. This study aimed to fully characterize the clinical features associated with postponed viral shedding time and disease progression, then develop and validate two prognostic discriminant models.

Methods: This study included 125 hospitalized patients with COVID-19, for whom 44 parameters were recorded, including age, gender, underlying comorbidities, epidemiological features, laboratory indexes, imaging characteristics and therapeutic regimen, et al. Fisher’s exact test and Mann–Whitney test were used for feature selection. All models were developed with fourfold cross-validation, and the final performances of each model were compared by the Area Under Receiving Operating Curve (AUROC). After optimizing the parameters via L2 regularization, prognostic discriminant models were built to predict postponed viral shedding time and disease progression of COVID-19 infection. The test set was then used to detect the predictive values via assessing models’ sensitivity and specificity.

Results: Sixty-nine patients had a postponed viral shedding time (> 14 days), and 28 of 125 patients progressed into severe cases. Six and eleven demographic, clinical features and therapeutic regimen were significantly associated with postponed viral shedding time and disease progressing, respectively (p < 0.05). The optimal discriminant models are: 

\[ y_1 (\text{postponed viral shedding time}) = -0.244 + 0.2829x_1 (\text{the interval from the onset of symptoms to antiviral treatment}) + 0.2306x_4 (\text{age}) + 0.234x_{28} (\text{Urea}) - 0.2847x_{34} (\text{Dual-antiviral therapy}) + 0.3084x_{38} (\text{Treatment with antibiotics}) + 0.3025x_{21} (\text{Treatment with Methylprednisolone}) \]

\[ y_2 (\text{disease progression}) = -0.348 - 0.099x_2 (\text{interval from Jan 1st, 2020 to individualized onset of symptoms}) + 0.0945x_4 (\text{age}) + 0.1176x_5 (\text{imaging characteristics}) + 0.0398x_8 (\text{short-term exposure to Wuhan}) - 0.1646x_{19} (\text{lymphocyte counts}) + 0.0914x_{32} (\text{Neutrophil counts}) + 0.1254x_{31} (\text{Neutrophil/lymphocyte ratio}) + 0.1397x_{33} (\text{C-Reactive Protein}) + 0.0814x_{35} (\text{Procalcitonin}) + 0.1294x_{24} (\text{Lactic dehydrogenase}) + 0.1099x_{29} (\text{Creatine kinase}) \]

The output ≥ 0 predicted postponed viral shedding time or disease progressing to severe/critical state. These two models yielded the maximum AUROC and fared best in terms of prognostic performance (sensitivity of 78.6%, 75%, and specificity of 66.7%, 88.9% for prediction of postponed viral shedding time and disease severity, respectively).

Conclusion: The two discriminant models could effectively predict the postponed viral shedding time and disease severity and could be used as early-warning tools for COVID-19.

Keywords: Prognostic discriminant model, Postponed viral shedding time, Disease progression, COVID-19
Background
Summary: this study fully characterizes the clinical features associated with postponed viral shedding time and disease progression, then develop and validate two prognostic models with satisfactory discriminant performance.

The prevalence of coronavirus disease 2019 (COVID-19) has put a huge burden to medical resources [1]. Although patients with COVID-19 infection mostly manifested as non-severe cases, it can also cause life-threatening conditions before or during hospitalization, such as severe pneumonia, adult respiratory distress syndrome or multiple organ failure, which are all related to worse outcomes [2]. Compare to the other epidemiological disease, such as the previous outbreaks of SARS-CoV and MERS-CoV, COVID-19 progresses and spreads more rapidly, with peculiar epidemiological traits. High viral loads of SARS-CoV-2 were observed in the upper respiratory specimens of patients with little or no symptoms, this indicated that inapparent-transmission plays a major but underestimated role in sustaining the outbreak of COVID-19 [3].

Since the first case emerged in Liaoning province in Jan 22th, 2020, there is an urgent need to construct a simple, efficient and accurate “early-warning prediction model” for disease progression at early stage once the patients were admitted to the hospital. This will facilitate the medical staff to make critical time-sensitive decisions regarding patients and treatments. Traditional evaluation scoring tools, such as CURB-65, qSOFA, and NEWS, could be adopted to assess disease severity, but not for the early assessment of COVID-19 severity [4]. Thus, to promptly predict and identify patients with postponed viral shedding time and disease severity is required but challenging. To date, dozens of prediction models of COVID-19 have been established to respond quickly to this healthcare crisis [5, 6]. Unfortunately, the quality of some of the identified models is uniformly poor and none can be recommended for clinical use, as demonstrated by one systemic review [7]. This study aimed to fully characterize the demographic, epidemiological, clinical features and therapeutic regimens and to detect their association with postponed viral shedding time and disease progression among patients with COVID-19 in Liaoning province, China. Furthermore, another purpose of this study was to specifically design and validate two prognostic discriminant models incorporating the associated features. These new mathematical models can serve as early-warning prediction tools to estimate the postponed viral shedding time and to identify the risk of progressing to severe stage in advance among patients with COVID-19 infection.

Methods
Study design
This retrospective multi-center cohort study included consecutive patients, they were laboratory-confirmed with COVID-19 infection and enrolled from Jan 22th to Mar 22th 2020 in eight designated hospitals throughout Liaoning province. All the enrolled patients were diagnosed with COVID-19 according to the WHO interim guidance [8]. Laboratory confirmation of COVID-19 was achieved by the nucleic acid test using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay at Liaoning municipal Center for Disease Prevention and Control (CDC). Samples were collected using a nose swab and/or throat swab from each suspected patient. This study was approved by the human research ethics committee of the first hospital of CMU (committee’s reference number: AF-OG-20-1.1-02), Shenjing hospital of CMU (committee’s reference number: 2020041002), Liaoning province peoples’ hospital, Shenyang Sixth People’s Hospital, Jinzhou Infectious Disease Hospital, Tieling infectious Disease Hospital, Fuxin Infectious Disease Hospital, Central Hospital of Huludao City. The other committee’s reference number were not available. All the written informed consent was waived. Permissions were obtained to access the data, as this was included as a part of the formal ethics approval. The data used in this study was anonymized before its use.

Data collection
The date of disease onset (defined as the day when any symptom was noticed by the patients) and hospital admission date, the first day for nucleic acid detected to be positive or negative were all recorded. All the patients were hospitalized, and the clinical outcomes were monitored for at least 8 weeks. All the clinical data on epidemiology (recent exposure history), symptoms, signs, underlying comorbidities, laboratory results (on admission), imaging findings (on admission) and clinical progression were recorded and retrospectively double-extracted from electronic medical records, with two independent reviewers extracted the data and evaluated the eligibility of the original data. Long-term exposure to Wuhan was defined by Wuhan residence or study, work for at least one-month in Wuhan. Short-term exposure to Wuhan was defined by meeting, transfer or travel history to Wuhan temporarily. Considering that one of the isolation release and discharge criteria for hospitalized patients is a sputum/oral swab testing negative twice with 24 h interval [5], virus detection was repeated twice every 24 h. It was deemed as viral clearance when virus was detected to be negative for two consecutive times.

Regarding antiviral treatment, 14 patients with confirmed COVID-19 were treated with lopinavir–ritonavir
(400 mg/100 mg), 11 with arbidol (200 mg t.i.d.). Dual-antiviral therapy of nebulized Interferon-α (IFN-α) (5 mU b.i.d.) with lopinavir–ritonavir or arbidol were used in 42 and 8 patients. Triple-antiviral therapy of IFN-α, lopinavir–ritonavir and arbidol were used in 20 patients. Other therapies such as antibiotics and corticosteroids were used in 38 and 22 patients.

Definition of disease progression and postponed virus shedding time
Disease progression were recorded for at least 8 weeks after admission. Severity of COVID-19 was defined according to the American Thoracic Society (ATS) guidelines for community-acquired pneumonia (CAP) [9]. Severe/critical cases of COVID-19 should meet one major criterion (septic shock with need for vasopressors or respiratory failure requiring mechanical ventilation) or at least three minor criteria: (a) respiratory distress with respiratory frequency ≥ 30/min; (b) oxygenation index (partial pressure of oxygen/inspired oxygen fraction, PaO₂/FiO₂) ≤ 250 mmHg; (c) multilobe infiltrates, confusion/disorientation; (d) uremia (blood urea nitrogen ≥ 20 mg/dL); (e) leucopenia (white blood cell count < 400 cells/μL); (f) thrombocytopenia (platelet count < 100,000/μL); (g) hypothermia (body temperature < 36 °C); (h) hypotension requiring aggressive fluid resuscitation. Non-severe patients were defined as a confirmed case with fever, respiratory symptoms, with or without radiographic evidence of pneumonia.

The median viral shedding time was 14 days (IQR, 11–19), those ≥ 14 days were deemed as postponed virus shedding.

Variables
Discriminant variables for disease severity and postponed virus shedding time were determined according to the assessment of the existing medical records (Additional file 1: Table S1) listed as follows: the interval from onset of symptoms to antiviral treatment (x₁), interval from Jan 1st, 2020 (the day for the first case emerged in Liaoning) to individualized onset of symptoms (x₂), gender (x₃), age (x₄), imaging characteristics (x₅), long-term exposure to Wuhan (x₆), local transmitted history (x₇), short-term exposure to Wuhan (x₈), respiratory symptoms (x₉), digestive symptoms (x₁₀), general malaise (x₁₁), fever (x₁₂), comorbidities with chronic respiratory disease (x₁₃), hypertension (x₁₄), diabetes mellitus (x₁₅), surgery history (x₁₆), other comorbidities (x₁₇), White blood cell (WBC) (x₁₈), lymphocyte counts (x₁₉), neutrophil counts (x₂₀), neutrophil/lymphocyte ratio (N/L) (x₂₁), C-Reactive Protein (CRP) (x₂₂), Procalcitonin (PCT) (x₂₃), Lactic dehydrogenase (LDH) (x₂₄), Aspartate amino transferase (AST) (x₂₅), Alanine aminotransferase (ALT) (x₂₆), Creatinine (Cr) (x₂₇), Urea nitrogen (Urea) (x₂₈), Creatine kinase (CK) (x₂₉), oxygenation index (x₃₀), disease severity (x₃₁), treatment with Lopinavir/ritonavir alone (x₃₂), treatment with Arbidol alone (x₃₃), treatment of nebulized IFN-α with Lopinavir–ritonavir (x₃₄), combined treatment of nebulized IFN-α, with Arbidol (x₃₅), combined treatment of nebulized IFN-α, lopinavir–ritonavir and Arbidol (x₃₆), treatment with Oseltamivir phosphate alone (x₃₇), Treatment with antibiotics (x₃₈), treatment with ribavirin (x₃₉), treatment with Chinese traditional medicine (Xuebijing) (x₄₀), treatment with Methylprednisolone (x₄₁), treatment with γ-globulin (x₄₂), antiviral treatment course (x₄₃). The outcomes were postponed viral shedding time (y₁) and disease progression (y₂).

Discriminate factors were also quantitatively assigned, some variables such as the imaging characteristics were assigned from 0, and the order was based on their influence on the progression of disease.

Establish the optimal discriminant models for disease progression and postponed viral shedding time
Setting disease progression and postponed viral shedding time as the goal for discriminant models, logistic regression, linear discriminant analysis, K-nearest neighbor, support vector machine (SVM) and decision tree were constructed through Python 3.6 software (Numpy and Sklearn package). The dataset was split 4:1 by stratified random sampling and four-fifths was used as a training group to establish models. After comparing effectiveness among the models by analyzing total accuracy in both training and testing data set, the most optimal discriminant models incorporating multiple related factors were established to reflect the probability of disease progressing to severe stage or postponed viral shedding time. The models were constructed using the output as an outcome, while the output ≥ 0 indicated disease progressing to severe/critical stage or postponed viral shedding time.

Verification of the discriminant models
The precision of the prediction models was further evaluated and validated. Due to the resampling methods, bootstrapping or cross-validation were more powerful than splitting the sample for internal validation. We applied cross-validation on the basis of random stratification. By comparing the area under the receiver-operating characteristic curve (AUROC) value, sensitivity, specificity, accuracy and recall rate, et al., the optimal primary screening model was chosen. Multicollinearity was calculated to assess the feasibility of the optimal model. Receiver operating characteristic (ROC) curves and confusion matrix were constructed to describe the screening
effectiveness of the optimal model. By analyzing total accuracy, the optimal discriminant model was chosen.

Statistical analysis
Categorical variables were summarized as frequencies and percentages. Continuous variables were described using median and interquartile ranges (IQR) values. Data were then compared between groups divided by disease severity (non-severe vs. severe group) or viral shedding time (with cutoff value 14 days) using F test for continuous variable, or by Mann–Whitney test or $\chi^2$ test for categorical data. Features significantly different ($p < 0.05$) in both algorithms were selected into the model. In order to eliminate the overfitting effect and regularize the models, z-score standardization $[x^* = (x - \text{mean})/\text{standard deviation}]$ was conducted on all continuous variables in the data set. Thus, each corresponding feature was converted into a normal distribution with mean value of 0 and variance of 1, to eliminate the dimensional influence.

Results
Demographic and clinical characteristics of patients with COVID-19 infection
A total of 125 hospitalized patients diagnosed as COVID-19 infection were included in this study. Disease progression was recorded during the 8-weeks follow-up after admission. Ten patients were categorized into mild cases, 97 developed into moderate cases and 28 patients developed into severe cases (including 3 critically ill) during hospitalization. Symptoms were shown in Table 1. Among 125 patients enrolled, 75 (62%) were imported cases (with an exposure history to Wuhan), which further divided into 37 (29.6%) short-term exposure (meeting, transfer or travel et al.) and 38 (30.4%) long-term exposure (residence or study, work in Wuhan) history to Wuhan. The rest 50 (40%) cases had no history of Wuhan exposure, thus were categorized into local transmitted cases who had contact with symptomatic cases. The median age for all patients were 44 years (IQR, 34–57), for non-severe patients was 41 years old (IQR, 34–55), and for severe patients was 50 years old (IQR, 38–63). About half (55.2%) of patients were male. Among all patients, up to 61 patients had at least one underlying comorbidity, the most common of which were chronic diseases, such as hypertension, et al. The median interval from the onset of symptoms to hospital admission was 4 days (IQR, 2–7). The median viral shedding time was 14 days (IQR, 11–19).

Clinical and laboratory features associated with disease progression or postponed viral shedding time
A total of 44 laboratory and clinical records on admission and during hospitalization were obtained and analyzed, including but not limited to the demographics, symptoms, signs, images, blood routine, immunocytochemistry, enzymatic and liver/renal function. These data were acquired within 24 h on admission. For the postponed viral shedding time discriminant model, six features (including one clinical feature, one demographic feature, one laboratory index and three therapeutic regimens) were selected to be the significant indicators of the postponed viral shedding time. They were listed as follows: the interval from the onset of symptoms to antiviral treatment (days) ($x_2$), age ($x_4$), CK ($x_{29}$), Combined treatment of nebulized IFN-$\alpha$ with lopinavir/ritonavir ($x_{34}$), Treatment with antibiotics ($x_{38}$), Treatment with Methylprednisolone ($x_{41}$) (Table 2).

For the disease progression discriminant model, eleven features (including one demographic, two epidemiological features and one imaging, seven laboratory indexes) were significantly associated with the progression of severe disease. These features include the interval from Jan 1st, 2020 (the day for the first case emerged in Liaoning) to individualized onset of symptoms ($x_2$), age ($x_4$), imaging characteristics ($x_5$), epidemiological history of short-term exposure to Wuhan ($x_9$), and immune features [Lymphocyte counts ($x_{19}$), leukocyte counts ($x_{20}$), N/L ratio ($x_{21}$), CRP ($x_{22}$), PCT ($x_{23}$)], Lactic dehydrogenase ($x_{28}$), creatine kinase ($x_{29}$).

No multi-collinearity was found in the screened variables, since VIF (Variance Inflation Factor) values of the screened dependent variables were all less than 10. Thus, the model passed the multi-collinearity test (Additional file 1: Tables S2, S3).

Establishment and verification of the optimal discriminant models for disease progression and postponed viral shedding time
All samples were stratified and randomly divided into training and testing datasets, and all models were developed with fourfold cross-validation [10]. During construction of the discriminant model of postponed, the training datasets contained 55 postponed virus clearance cases and 45 non-postponed cases, while the testing dataset was consisted of 14 postponed virus clearance cases and 12 postponed cases. During construction of the discriminant model of disease progression, the training datasets contained 31 severe/critical cases and 69 non-severe cases, while the testing dataset was consisted of 8 severe/critical cases and 18 non-severe cases. Then, the discriminant models were constructed in training set via the selected indicators using several methods, including logistic regression, linear discriminant analysis, decision tree, K-nearest neighbor, and support vector machine method. The outcome was predicted in testing data set.
| Demographic and baseline characteristics of patients with different severities and virus shedding of COVID-19 infection | Total (N=125) | Non-severe (N=97) | Severe/critical (N=28) | p | Virus shedding < 14 days (N=56) | Virus shedding ≥ 14 days (N=69) |
|---|---|---|---|---|---|---|
| Age | 44 (34–57) | 41 (34–55) | 50 (38–63) | 0.001 | 41 (29–54) | 45 (37–59) |
| Gender | | | | | | |
| Male | 69 (55.4%) | 51 (52.6%) | 18 (64.3%) | 0.49 | 34 (59.6%) | 35 (50.7%) |
| Female | 56 (44.6%) | 46 (47.4%) | 10 (35.7%) | 22 (40.4%) | 34 (49.3%) | 0.50 |
| Epidemic features | | | | | | |
| Long-term exposure to Wuhan | 38 (30.4%) | 33 (34.0%) | 5 (17.9%) | 0.16 | 16 (29.8%) | 22 (31.9%) |
| Short-term exposure to Wuhan | 37 (29.6%) | 22 (22.7%) | 15 (53.6%) | 0.04 | 19 (33.3%) | 18 (26.1%) |
| Local transmitted cases | 50 (40%) | 42 (43.3%) | 8 (28.6%) | 0.65 | 21 (36.8%) | 29 (42.0%) |
| Comorbidity | | | | | | |
| Hypertension | 22 (17.8%) | 15 (15.5%) | 7 (25%) | 0.14 | 8 (15.8%) | 7 (10.1%) |
| Diabetes mellitus | 9 (7.2%) | 6 (6.2%) | 3 (10.7%) | 0.88 | 2 (3.5%) | 7 (10.1%) |
| Hypertension | 12 (9.6%) | 7 (7.2%) | 5 (17.9%) | 0.15 | 5 (8.8%) | 7 (10.1%) |
| Other (ex. kidney stone) | 13 (10.4%) | 8 (8.2%) | 5 (17.9%) | 0.24 | 5 (8.8%) | 7 (11.6%) |
| Interval from symptom onset to diagnosis (days) | 4 (2–7) | 4 (2–7) | 3 (1.7–7.9) | 0.01 | 3 (1.7–7.9) | 3 (2–6) |
| COVID-19 viral RNA shedding time (days) | 14 (11–19.3) | 14 (11–18) | 16 (13–22) | 0.15 | 10 (8–12) | 19 (15–25) |
| Interval between outbreak in Liaoning and diagnosis (days) | 11 (7–16) | 13 (7.5–17.5) | 10 (5.5–14.0) | 0.01 | 12 (7–18) | 10 (7–16) |
| Symptoms | | | | | | |
| Fever | 103 (82.4%) | 75 (77.3%) | 28 (100%) | 0.006 | 44 (77.2%) | 59 (85.5%) |
| Cough | 78 (62.4%) | 54 (55.7%) | 24 (85.7%) | 0.004 | 38 (66.7%) | 40 (58.0%) |
| Dyspnea/chest tightness | 31 (%) | 5 (5.2%) | 26 (92.9%) | <0.001 | 19 (33.3%) | 22 (31.9%) |
| Diarrhea/nausea | 15 (12%) | 12 (12.3%) | 3 (10.7%) | 0.36 | 7 (12.3%) | 8 (11.6%) |
| Fatigue | 35 (28%) | 22 (22.7%) | 13 (46.4%) | 0.014 | 18 (31.6%) | 17 (24.6%) |
| Signs | | | | | | |
| Breath rate > 24/min | 10 (8%) | 0 | 10 (35.7%) | 0.00 | 4 (7.0%) | 6 (8.7%) |
| Laboratory characteristics | | | | | | |
| White blood cell, ×10⁹/L | 5.16 (3.91–6.30) | 5.1 (3.0–6.1) | 5.5 (4.6–6.9) | 0.20 | 5.0 (3.6–6.4) | 5.2 (4.4–6.2) |
| Neutrophil count, ×10⁹/L | 3.20 (2.25–4.00) | 3.1 (2.1–3.7) | 3.8 (2.7) | 0.002 | 3.1 (2.0–3.8) | 3.3 (2.6–4.0) |
| Lymphocyte count, ×10⁹/L | 1.20 (0.80–1.70) | 1.42 (1.0–1.8) | 0.8 (0.5–1.1) | <0.001 | 1.2 (0.8–6.4) | 1.2 (0.7–16) |
| Neutrophil–lymphocyte ratio | 2.36 (1.70–4.17) | 2.1 (1.6–2.9) | 4.9 (2.1–7.8) | <0.001 | 2.1 (1.7–4.0) | 2.5 (1.8–4.2) |
| C-Reactive Protein, mg/dL | 5.40 (1.30–24.0) | 3.6 (1.0–9.7) | 25 (5.4–66) | <0.001 | 4.6 (1.0–21.9) | 5.4 (2.0–25.0) |
| Procalcitonin, ng/mL | 0.05 (0.00–0.08) | 0.04 (0.00–0.05) | 0.06 (0.03–0.11) | <0.001 | 0.05 (0.00–0.05) | 0.05 (0.00–0.09) |
| Lactic dehydrogenase, U/L | 320.50 (200–456) | 267 (177.5–412.0) | 400 (321–590) | <0.001 | 312 (200–428) | 348 (200–460) |
| Creatine kinase, U/L | 63.5 (40.0–107.8) | 56.0 (38.0–89.5) | 109.5 (76–125.9) | <0.001 | 52 (39.5–120) | 93.5 (54–213) |
| ALT, U/L | 29.0 (18.0–45.8) | 27.0 (17.0–40.5) | 42.0 (24.2–59.3) | 0.02 | 32 (21–45) | 27.2 (17.0–46.0) |
| AST, U/L | 25.9 (20.0–35.0) | 24.0 (20.0–30.5) | 29.1 (24.4–55.5) | 0.03 | 26 (20–35) | 24 (20–39) |
| Urea nitrogen, mmol/L | 3.7 (3.4–4.7) | 3.6 (3.0–4.7) | 4.2 (3.8–5.3) | 0.85 | 3.5 (3.0–4.4) | 4 (3–5) |
| Creatinine, mmol/L | 57.0 (48.0–66.8) | 56.0 (45.5–66.0) | 59.5 (49.7–80.5) | 0.60 | 60 (48–67) | 55 (47–66) |
| Oxygenation index, mmHg | 371.4 (324.1–377.9) | 371.4 (371.0–381.0) | 274.6 (225.0–362) | <0.001 | 371.4 (342.9–371.4) | 371.4 (323.8–381.0) |
| Imaging characteristics | | | | | | |
| Normal | 17 (13.6%) | 17 (17.5%) | 0 | 0.01 | 7 (12.3%) | 4 (5.8%) |
| Unilateral GGO | 24 (19.2%) | 23 (23.7%) | 1 (3.6%) | <0.001 | 13 (22.8%) | 12 (2.9%) |
| Bilateral GGO | 87 (69.6%) | 62 (63.9%) | 25 (89.3%) | <0.001 | 36 (63.2%) | 50 (72.5%) |
| Diffuse lesions in both lungs | 3 (2.4%) | 0 | 3 (10.7%) | 0.02 | 1 (1.8%) | 3 (4.3%) |

Data are median (IQR) or No (%). GGO, ground glass opacities
By analyzing total accuracy in both training and testing data set, the optimal discriminant models for the prediction of disease progression and postponed viral shedding time of COVID-19 infection were established via the logistic regression with the selected eleven and six features used as independent variables. In order to eliminate the influence of model overfitting on the prediction results and to improve the generalization ability of the model, L2 regularization was used to constrain the objective function in the logistic regression optimization process. Regarding the term of L2 regularization, $\beta$ represents the coefficient in front of a variable. $C$ represents the regularization parameter in cost function, and it controls the strength of L2 regularization. $X_i$ represents the value of the attribute variable and $c$ represents the constant term.

"Viral shedding postponed or not" was used as a dependent variable ($y_1$) for logistic regression, which yielded the equation:

$$\ln\left(\frac{p}{1-p}\right) = -0.348 - 0.099x_2 + 0.0945x_4 + 0.1176x_5 + 0.0398x_8 - 0.1646x_{19} + 0.0914x_{20} + 0.1254x_{21} + 0.1397x_{22} + 0.0814x_{23} + 0.1294x_{24} + 0.1099x_{29}.$$

Within the formula, $p$ represents the probability of the patient being judged positive (severe disease). Notably, all data needs to be equally standardized before being plugged into the equation, and the corresponding mean and standard deviation were determined and shown in Additional file 1: Tables S4, S5.

**Performance of the discriminant models**

When applying the discriminant model of postponed viral shedding time onto the validation of the training set (Tables 3, 4; Additional file 1: Table S6), sensitivity was 0.727, specificity was 0.733, positive predictive value was 0.769, and negative predictive value was 0.688. In the test set, sensitivity was 0.786, specificity was 0.667, with positive predictive value 0.733 and negative predictive value 0.727. Recall rate was 78.6%. Accuracy was 0.732. The AUROC of the combinations of 7 clinical features was 0.73.
Table 3  The summary of primary discriminant screening models for virus shedding of Covid-19 infection

| Discriminant models for postponed viral shedding time | Training set with cross-validation | Test set with cross-validation |
|-------------------------------------------------------|-----------------------------------|--------------------------------|
|                                                       | Logistic regression | Linear discriminant analysis | K-nearest neighbor | Support vector machine | Decision tree | Logistic regression | Linear discriminant analysis | K-nearest neighbor | Support vector machine | Decision tree |
| AUC                                                   | 73.0                 | 59.1                          | 357                | 45.0                   | 40.9           | 73.0                 | 57.9                          | 40.6                      | 446                     | 39.0         |
| Sensitivity, %                                         | 72.7                 | 62.2                          | 459                | 53.9                   | 39.5           | 78.6                 | 59.3                          | 50.4                      | 309                     | 42.0         |
| Specificity, %                                         | 73.3                 | 36.4                          | 399                | 40.7                   | 35.5           | 66.7                 | 39.4                          | 49.9                      | 49.7                    | 25.0         |
| Positive predictive value, %                           | 76.9                 | 60.1                          | 489                | 50.3                   | 25.2           | 73.3                 | 50.4                          | 45.2                      | 41.5                    | 32.9         |
| Negative predictive value, %                           | 68.8                 | 43.4                          | 304                | 39.9                   | 30.4           | 72.7                 | 40.0                          | 49.8                      | 55.4                    | 29.3         |
| Accuracy                                               | –                    | –                             | –                  | –                      | –              | 73.2                 | 50.3                          | 60.3                      | 304                     | 20.3         |
| Recall rate                                            | –                    | –                             | –                  | –                      | –              | 78.6                 | 51.3                          | 45.0                      | 349                     | 29.9         |

AUC area under the curve
Table 4  The summary of primary discriminant screening models for different severities of Covid-19 infection

| Discriminant models for disease progression | Training set with cross-validation | Test set with cross-validation |
|-------------------------------------------|-----------------------------------|-------------------------------|
|                                           | Logistic regression | Linear discriminant analysis | K-nearest neighbor | Support vector machine | Decision tree | Logistic regression | Linear discriminant analysis | K-nearest neighbor | Support vector machine | Decision tree |
| AUC                                       | 82.9                 | 60.9                         | 476                 | 26.3                   | 43.4          | 81.9              | 62.3                         | 46.0                  | 390                  | 36.9          |
| Sensitivity, %                            | 77.4                 | 52.0                         | 403                 | 36.5                   | 49.1          | 75                | 50.4                         | 49.8                  | 401                  | 41.9          |
| Specificity, %                            | 88.4                 | 69.9                         | 344                 | 24.4                   | 30.4          | 88.9              | 60.2                         | 35.1                  | 352                  | 29.8          |
| Positive predictive value, %              | 75                   | 46.7                         | 456                 | 45.3                   | 51.2          | 75                | 59.1                         | 55.5                  | 330                  | 30.5          |
| Negative predictive value, %              | 89.7                 | 55.9                         | 403                 | 30.4                   | 37.4          | 88.9              | 58.3                         | 40.1                  | 503                  | 40.3          |
| Accuracy                                  | –                    | –                            | –                   | –                      | –             | 84.6              | 55.4                         | 35.4                  | 41.3                | 34.5          |
| Recall rate                               | –                    | –                            | –                   | –                      | –             | 75                | 60.0                         | 40.1                  | 299                  | 35.3          |
in the training dataset (Fig. 1a) and 0.73 in the testing dataset (Fig. 1b).

According to the confusion matrix of discriminant model for disease progression (Tables 3, 4; Additional file 1: Table S7), during the validation of the training set, sensitivity was 0.774, specificity was 0.884, positive predictive value was 0.75, and negative predictive value was 0.897. In the test set, sensitivity was 0.75, specificity was 0.889, with positive predictive value 0.75 and negative predictive value 0.889. The recall rate was 75%, and accuracy was 0.846. The AUROC was also constructed to evaluate the effectiveness of the discriminant models. The AUROC of the combinations of 11 demographic, clinical and imaging/laboratory features was 0.829 (Fig. 2a) in the training dataset and 0.819 (Fig. 2b) in the testing dataset.

Discussion

This retrospective study tentatively developed two discriminant models consisting of several clinical and epidemiological features that could be quickly obtained on admission. To date, a certain number of models predict the risk of severe COVID-19 has been developed [5, 11]. However, there is geographic discrepancy of severity and mortality rate in patients with COVID-19 infection, and most of the previous prediction models were established to predict survival risk or progression to severe or critical state in the south part of China [5, 11]. Few of them were designed to predict the postponed viral shedding time. Besides, there is no consensus about the therapeutic regimen of COVID-19 [12], the models established previously mainly consisted of variables that extracted from the clinical and laboratory parameters, with few of them incorporated epidemiological features or therapeutic regimen [11]. To the best of our knowledge, for the first time, the impact of epidemiological features and therapeutic regimen on the disease progression and postponed viral shedding time were described and integrated into the predicted polynomial equations among patients confirmed as COVID-19 infection.

Overall, these two discriminant models in the present study was demonstrated to have satisfactory sensitivity (> 72.00%) and specificity (> 73.00%), and they can be used as early warning tools to robustly and effectively predict the postponed viral shedding time and the severe/critical progression in patients with COVID-19 infection upon admission. A medical staff can easily predict in advance using these two discriminant models and conduct a timely and optimal medical intervention at an early stage.

SARS-CoV-2 in the respiratory tract, especially sputum, has been observed to be associated with a prolonged viral shedding and high viral load, when compared with the stool specimens [13]. In this study, the median viral shedding time was 14 days (IQR, 11–19). In order to effectively control the transmission resources on the imported cities or regions, it is essential to identify factors that associated with the COVID-19 PCR negative conversion time and to establish a prediction model that could individually estimate the risk of postponed viral shedding time among patients with COVID-19 upon admission to hospitals or shelters. In this study, we initially selected 43 variables probably associated with disease progression and postponed viral shedding time respectively according to the published literatures [4–7], then detected the risk factors by F-test and χ² test analysis. As a result, 6 variables were identified as discriminatory factors and were devised to discriminant models for prediction of postponed viral shedding time of COVID-19 infection. Both older age and delayed antiviral treatment could give rise to the postponed viral shedding time. Consistently, these results were in accordance with another previous study indicated that the time from symptom onset to viral clearance slightly increased with age [14]. Besides, the association between delayed initiation of antiviral treatment and the prolonged virus shedding for influenza A (H7N9) and SARS-CoV-2 was also observed in previous studies, indicating that timely initiation of antiviral treatments necessary for viral clearance [15–17]. In addition, dual-antiviral therapy of nebulized IFN-α with lopinavir/ritonavir (x₃₃) was negatively associated with the viral shedding time, whereas treatment with antibiotics (x₃₇) and methylprednisolone (x₉₀) were related to postponed viral shedding time. This was consistent with previous studies which demonstrated that SARS-CoV-2 was more susceptible to IFNs when compared to SARS-CoV, as the inhalation of Interferon-α (IFN-α) 2b could reduce the infection rate significantly and it can be used for prophylaxis of SARS-CoV-2 infection [18–20]. In addition, although Lopinavir/ritonavir (Kalera) presented controversial therapeutic effects as compared to the standard care in vivo [22–25], it was found to have anti-SARS-CoV efficacy in vitro [21], thus Lopinavir/ritonavir has been recommended by the National Health Commission of China for the treatment of COVID-19 in the early period of 2020 [26]. To the best of our knowledge, for the first time this study observed that combined treatment of IFN-α inhalation and lopinavir/ritonavir was related to the shortened viral shedding time of COVID-19. Nevertheless, there is no additional benefit on virus clearance from an extra use of Arbidol, when combined with IFN-α and lopinavir/ritonavir. Anyway, statistical analysis can only stress association but cannot explain causality. A recent study also observed that early initiation of dual-antiviral treatment with lopinavir/ritonavir + IFN-α combination therapy could help shorten the duration of SARS-CoV-2 shedding when
compared with triple antiviral treatment (opinavir/ritonavir + IFN-α + arbidol) [17]. This conclusion may provide a rationale for clinicians to optimize and to early initiate the antiviral treatments. In a previous study, the administration of corticosteroids has been observed to bring benefits for patients infected by COVID-19, since will prevent the use of mechanical ventilation and reduce the mortality of them [27]. Conversely, in this study, treatment with antibiotics ($x_{37}$) and methylprednisolone ($x_{40}$) could give rise to the postponed viral shedding time.

Fig. 1 AUROC curve of the postponed viral shedding discriminant model for training set (a) and testing set (b). AUROC area under receiving operating curve

Fig. 2 AUROC curve of the disease progression discriminant model for training set (a) and testing set (b). AUROC area under receiving operating curve
Some previous studies have also observed that high-dose corticosteroids was associated with increased mortality and longer viral shedding in patients with influenza A (H7N9) viral pneumonia and MERS [28–30]. Systemic corticosteroids could increase the risk of opportunistic infections (such as bacterial or fungal) that occur secondary to immunosuppression, and eventually hinders the virus clearance ability [31]. Besides, potential bacterial infections secondary to influenza viral infection that has been commonly seen in this study (32.8%) could also prolong the viral shedding time, as indicated by the evidence that the use of antibiotics was associated with postponed viral shedding time.

Noteworthy, this study for the first time observed that intervals from the first case emerged in Liaoning province (Jan 22st, 2020) to the individualized onset of symptoms (x2) could serve as an important prognostic feature in our early-warning model of disease progression. Indeed, nearly half of the confirmed cases at the early stage (in January) of COVID-19 outbreak were severe cases, whereas in the latter period (after February), the percentage of non-severe cases became dominant (76.9%) in Liaoning province. One explanation is that pathogens tend to reduce their virulence overtime in order to maximize their between-host transmission, which could result in a gradually lowered severity of COVID-19 infection on the imported regions [32, 33]. Besides, the human intervention efforts in China, such as to promptly admit suspected patients to the designated shelter hospitals, have effectively contributed to the decreased number of severe cases of COVID-19. Interestingly, short-term exposure to the epidemic area (Wuhan) during traveling or transfer could also result in a higher likelihood of progressing into severe stage of disease. Those transiently migrant individuals might have been primed by one or more prior coronavirus exposures during traveling or transfer around the epidemic area, thus have experienced the effects of antibody dependent enhancement (ADE) antigenic epitope heterogeneity due to antigenic epitope heterogeneity [34]. ADE hinders the ability to manage inflammation and result in disease progression. Another explanation would be attributed to the SARS-CoV-2 strains of L type, which are evolutionarily more aggressive and contagious. This virus strain of L type with altered virulence could probability be the underlying causal pathogen for patients who acquired infections via short-term exposure to the epidemic area [35].

In our discriminant models, both immune features (lymphocytes, neutrophils, N/L ratio, CRP) and enzymatic index (LDH, CK) obtained on admission were observed as the most significant prognostic factors for disease severity. These are consistent with the well-established within-host model in previous literature, which describes the interactions between SARS-CoV-2, host pulmonary epithelial cells and cytotoxic T lymphocyte cells [36]. Our results illustrate an earlier exhibition of abnormal laboratory features prior to the disease progression [37, 38]. Consistent with previous studies, our study revealed that advanced age in a strong risk factor for more severe COVID-19 infection [39–41]. This suggests that more intensive surveillance is necessary in elderly patients.

Anyway, some limitations should be noted in this study. First, the study design was retrospective, and the sample size may be insufficient for characterization of an entire population. However, by including all patients from eight designated hospitals throughout Liaoning province, we considered patients recruited in this study are representative of cases diagnosed with COVID-19 in Liaoning, China. Secondly, not enough severe/critical cases were recruited for the present study. This was possibly because the fatality rate of patients infected by COVID-19 in Liaoning province was lower (1.6%) than the whole national average level (3.2%) [42], and not resembling the previous studies from Wuhan [2, 43]. Thirdly, we only included the initial antiviral treatments as factors for prolonged shedding duration, so as to minimize the bias of different efficacy caused by different treatment courses as much as possible. This discrepancy may have had an unknown influence on the efficacy of the models.

Conclusion
The discriminant models reported here is the first attempt of its kind to develop an early warning tool for both postponed viral shedding time and disease progression in the northeast area of China. We believe that these models can help to judge the disease progression early enough in a great number of patients with COVID-19 infection, and this early judgment can facilitate a timely medical intervention, which will ultimately reduce the mortality of COVID-19.

Abbreviation
COVID-19: Coronavirus disease 2019.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12879-022-07338-x.

Additional file 1: The variable assignment and multi-collinearity analysis of independent variables in two models.
Acknowledgements
We thank all the efforts from staff of Liaoning provincial health commission and Liaoning COVID-19 treatment team in the management of the confirmed patients. We also appreciate the editor and the reviewers for their constructive suggestions.

Author contributions
WYL: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing—original draft. YG: Formal analysis, Methodology, HH: Formal analysis, Investigation, Supervision. HZ: Formal analysis, Supervision. JK: Validation, Visualization. DW: Writing original draft, review and editing. WW: Conceptualization, Funding acquisition, Investigation, Validation, Visualization, Writing original draft, review and editing. All authors have read and approved the manuscript and ensure that this is the case.

Funding
This study was supported by the emergency project of Liaoning provincial science and technology department, No. 2020H2/10300003. Liaoning provincial science and technology department had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

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

Declarations
Ethics approval and consent to participate
This study was approved by the human research ethics committee of the first hospital of CMU (committee's reference number: AF-OG-20-1.1-02), Shenzhen hospital of CMU (committee's reference number: 2020041002), Liaoning province's hospital, Shenyang Sixth People's Hospital, Jinhzhou Infectious Disease Hospital, Tieling Infectious Disease Hospital, Fuxin Infectious Disease Hospital, Central Hospital of Huludao City. The other committee's reference number were not available. All the written informed consent was waived. Permissions were obtained to access the data, as this was included as a part of the formal ethics approval. The data used in this study was anonymized before its use.

Consent for publication
Not applicable.

Competing interests
All authors declare no competing interests.

Author details
1 Respiratory and Critical Care Department, The First Hospital of China Medical University, Shenyang, China. 2 Department of Respiratory Disease, Liaoning Province People's Hospital, Shenyang, China. 3 Department of Mathematics and Statistics, Xian Jiaotong University, Xian 710049, China.

Received: 13 July 2020 Accepted: 2 April 2022
Published online: 11 April 2022

References
1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N Engl J Med. 2020;382(13):1199–207.
2. 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(10223):507–13.
3. Wang Y, Wang Y, Chen Y, Quin Q. Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicates special control measures. J Med Virol. 2020;92(6):568–76.
4. Ito A, Ishida T, Tokumaru H, Washio Y, Yamazaki A, Ito Y, et al. Prognostic factors in hospitalized community-acquired pneumonia: a retrospective study of a prospective observational cohort. BMC Pulm Med. 2017;17(1):78.
5. Zhou Y, He Y, Yang H, Yu H, Wang T, Chen Z, et al. Development and validation a nomogram for predicting the risk of severe COVID-19: a multi-center study in Sichuan. China. PLoS ONE. 2020;15(5):e0233328.
6. Sun L, Song F, Shi N, Liu F, Li L, Li P, et al. Combination of four clinical indicators predicts the severe/critical symptom of patients infected COVID-19. J Clin Virol. 2020;128:104431.
7. Wynants L, Van Calster B, Collins GS, Riley RD, Heinze G, Schuit E, et al. Prediction models for diagnosis and prognosis of covid-19 infection: systematic review and critical appraisal. BMJ. 2020;369:m2204.
8. World Health Organization. 2020. Clinical management of COVID-19: interim guidance, 27 May 2020. World Health Organization. https://apps.who.intiris/handle/10665/332196.
9. Metlay JP, Waterer GW, Long AC, Anzueto A, Brozek J, Crotthers K, et al. Diagnosis and treatment of adults with community-acquired pneumonia An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases Society of America. Am J Resp Crit Care. 2019;200(7):E45–67.
10. Wolff RR, Moons KG, Riley RD, Whiting PF, Woodhead MC, Gary SR, for the PROBAST Group, et al. PROBAST: a tool to assess the risk of bias and applicability of prediction model studies. Ann Intern Med. 2019;170:51–8.
11. Liang W, Liang H, Ou L, Chen B, Chen A, Li C, China Medical Treatment Expert Group for COVID-19, et al. Development and validation of a clinical risk score to predict the occurrence of critical illness in hospitalized patients with COVID-19. JAMA Intern Med. 2020;180:e202033.
12. Siemieniuk RA, Bartoszko JJ, Ge L, Zeraatkar D, Izcovich A, Kum E, et al. Drug treatments for covid-19 living systematic review and network meta-analysis. BMJ. 2020;30(370):m2980.
13. Wolff R, Corman VM, Guggemos W, Seilmaer M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-19. Nature. 2020.
14. Mancuso P, Venturrelli F, Vicentini M, Perulli C, Larosa E, Bisaccia E, et al. Temporal profile and determinants of viral shedding and of viral clearance confirmation on nasopharyngeal swabs from SARS-CoV-2-positive subjects: a population-based prospective cohort study in Reggio Emilia, Italy. BMJ Open. 2020;10(8):e040386.
15. Wang Y, Guo Q, Yan Z, Zhou D, Cao B. Factors associated with prolonged viral shedding in patients with avian influenza A(H7N9) virus infection. J Infect Dis. 2018;217(11):1708–17.
16. Zheng S, Wang Y, Yu F, Wang Y, Yu F, Cui D, et al. Benefit of early initiation of neuraminidase inhibitor treatment to hospitalized patients with avian influenza A(H7N9) virus. Clin Infect Dis. 2018;66(7):1054–60.
17. Zuo Y, Liu Y, Zhong Q, Zhang K, Xu Y, Wang Z. Lopinavir/ritonavir and interferon combination therapy may help shorten the duration of viral shedding in patients with COVID-19: a retrospective study in two designated hospitals in Anhui. China J Med Virol. 2020. https://doi.org/10.1002/jmv.26127.
18. Sheahan TP, Sims AC, Leist SR, Schaffer A, Won J, Brown AL, et al. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020;11:222.
19. Shen K, Yang Y. Diagnosis and treatment of 2019 novel coronavirus infection in children: a pressing issue. World J Pediatr. 2020;16:219.
20. Lokugamage KG, Schindewolf C, Menachery VD. SARS-CoV-2 sensitive to type I interferon pretreatment. bioRxiv. 2020.
21. Chu CM, Cheng VCC, Hung IFN, Wong MLM, Chan KH, Chan KS, et al. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorac. 2004;59:252–6.
22. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, et al. A trial of lopinavir-ritonavir in adults hospitalized with severe Covid-19. N Engl J Med. 2020.
23. Group RC. Lopinavir–ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2020.
24. Li Y, Xie Z, Lin W, Cai W, Wen G, Cui D, et al. An exploratory randomized, controlled study on the efficacy and safety of lopinavir/ritonavir or arbidol treating adult patients hospitalized with mild/moderate COVID-19 (ELACOI). medRxiv. 2020;13:e0195068.
25. Lim J, Jeon S, Shin HY, Kim MJ, Seong YM, Lee WJ, et al. Case of the index patient who caused tertiary transmission of COVID-19 infection in Korea: the application of lopinavir/ritonavir for the treatment of COVID-19.
infected pneumonia monitored by quantitative RT-PCR. J Korean Med Sci. 2020;35(6):e79.

26. National Health Commission of China. Guideline for COVID-19 pneumonia of China. 2020.

27. Edalatifard M, Akhtari M, Salehi M, Naderi Z, Jamshidi A, Mostafaei S, et al. Intravenous methylprednisolone pulse as a treatment for hospitalised severe COVID-19 patients: results from a randomised controlled clinical trial. Eur Respir J. 2020;56(6):2002808.

28. 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.

29. Hu Y, Lu S, Song Z, Wang W, Hao P, Li J, et al. Association between adverse clinical outcome in human disease caused by novel influenza A H7N9 virus and sustained viral shedding and emergence of antiviral resistance. Lancet. 2013;381:2273–9.

30. Arabi YM, Mandourah Y, Al-Hameed F, Sindi AA, Almekhlafi GA, Hussein MA, et al. Corticosteroid therapy for critically ill patients with middle east respiratory. Am J Respir Crit Care Med. 2018;197(6):757–67.

31. Rice TW, Rubinson L, Uyeki TM, Vaughn FL, John BB, Miller RR 3rd, et al. NHLBI ARDS Network: critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. Crit Care Med. 2012;40:1487–98.

32. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, et al. First case of 2019 novel coronavirus in the United States. N Engl J Med. 2020;382(10):929–36.

33. Lei H, Li Y, Xiao S, Lin CH, Norris SL, Wei D, et al. Routes of transmission of influenza A H1N1, SARS-CoV, and norovirus in air cabin: comparative analyses. Indoor Air. 2018;28:394–403.

34. Jason A. Tetro, is COVID-19 receiving ADE from other coronaviruses? Microbes Infect. 2020;22(2):72–3.

35. Guo YR, Cao QD, Hong ZS, Tan YY, Chen S-D, Jin H-J, et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak—an update on the status. Mil Med Res. 2020;7(1):11.

36. Hattaf K, Yousfi N. Dynamics of SARS-CoV-2 infection model with two modes of transmission and immune response. Math Biosci Eng. 2020;17(5):5326–40.

37. Sun L, Liu G, Song F, Shi N, Shi Y. Combination of four clinical indicators predicts the severe/critical symptom of patients infected COVID-19. J Clin Virol. 2020;128:104431.

38. Zhang H, Zhou P, Wei Y, Yue H, Wang Y, Hu M, et al. Histopathologic changes and SARS-CoV-2 immunostaining in the lung of a patient with COVID-19. Ann Intern Med. 2020;172(9):629–32.

39. Chen R, Liang W, Jiang M, Guan W, Zhan C, Wang T. Medical Treatment Expert Group for COVID-19, et al. Risk factors of fatal outcome in hospitalized subjects with coronavirus disease 2019 from a nationwide analysis in China. Chest. 2020;158(1):97–105. https://doi.org/10.1016/j.chest.2020.04.010.

40. 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;323(1):1061–9.

41. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Med. 2020;368(2):m641–3.

42. National Health Commission of the People’s Republic of China home page (http://www.nhc.gov.cn).

43. Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497–506.