Clinical characteristics, outcomes and immunity in patients with COVID-19

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Abstract

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Background The coronavirus disease 2019 (COVID-19) pandemic caused by the novel pathogen SARS-CoV-2 has spread rapidly around the world, causing massive hospitalisations and deaths, and placing an unprecedented burden on the world economy, globalisation and healthcare. Associations between characteristics of COVID-19 patients and clinical outcomes and immunity remained to be studied. This PhD work explored these associations to help understand COVID-19, inform prevention and control measures and reduce relevant burdens.

Methods This work used retrospectively collected data of all cases from 24 hospitals in Jiangsu province, China from January 10, 2020 to March 15, 2020; data from Huangshi city, Hunan province, China from January 21, 2020 to February 29, 2020; and data from a prospective longitudinal study conducted at Richmond Research Institute, St George's University of London, UK from March 19, 2020 to February 10, 2021. Adverse outcomes were severe/critical illness, disease deterioration (from asymptomatic/mild/moderate to severe/critically ill status) and respiratory failure during 14-day follow-up. Immunity status was assessed using anti-SARS-CoV-2 immunoglobulin G (IgG) levels measured repeatedly.

Results Of 625 patients in Jiangsu, 64 (10%) were severe/critically ill; 6% of patients had disease deterioration; 9% of patients had respiratory failure; and no patients died at the end of the study. Odds of being a severe/critically ill case were associated with age (year) (odds ratio [OR] 1.06, 95% CI 1.03–1.09), lymphocyte count (10^9/L) (OR 0.25, 95% CI 0.08–0.74), and pulmonary opacity in CT (per 5%) on admission (OR 1.31, 95% CI 1.15–1.51). Four variables were identified to be independently related to the occurrence of disease deterioration: age

(year) (OR 1.08, 95% CI 1.04–1.12), pulmonary opacity score (per 5%) (OR 1.32, 95% CI 1.12–1.57), lymphocyte count (10^9/L) (OR 0.28, 95% CI 0.09–0.91), and imported cases (exposed to the pandemic centre) (OR 2.45, 95% CI 1.03–5.80). Age (year) (OR 1.07, 95% CI 1.03–1.10), respiratory rate (breaths/minute) (OR 1.23, 95% CI 1.08–1.40), lymphocyte count (10^9/L) (OR 0.18, 95% CI 0.05–0.69), and pulmonary opacity score (per 5%) (OR 1.38, 95% CI 1.19–1.61) at admission were associated with respiratory failure. Predictors including age, lymphocyte count and pulmonary opacity score were selected to develop a nomogram to predict severe COVID-19. The nomogram exhibited good discrimination (area under the receiver operating characteristic curve [AUC] 0.93, 95% CI 0.90–0.96 in the derivation cohort; AUC 0.85, 95% CI 0.76–0.93 in the validation cohort) and satisfactory agreement. Anti-SARS-CoV-2 IgG levels declined non-linearly from month 2 to 11 and may be associated with gender (female vs. male; geometric mean ratio [GMR] 4.78, 95% CI 0.99–22.98), race (Caucasian vs. other races; GMR 0.19, 95% CI 0.03–1.02) and the loss of smell and taste (GMR 9.40, 95% CI 1.12–78.97).

Conclusion Age, lymphocyte count and lung opacity scores were associated with severe COVID-19, disease exacerbation and respiratory failure, and the nomogram composed of these three factors performs well in predicting the risk of severe COVID-19. This enables physicians to identify high-risk patients early and correctly, and take corresponding proactive interventions to reduce mortality and save lives. The potential association between anti-SARS-CoV-2 IgG levels and loss of smell and taste may need to be considered when developing targeted treatment and vaccine programs to reduce severe disease. Patients reporting loss of smell and taste may have higher IgG levels and require stricter monitoring when in isolation.

Publications resulting from this PhD work

Peer-reviewed research articles

Chapter 3.

Liu S, **Luo H**, Wang Y, Cuevas LE, Wang D, Ju S, Yang Y. Clinical characteristics and risk factors of patients with severe COVID-19 in Jiangsu province, China: a retrospective multicentre cohort study. *BMC Infect Dis*. 2020;20(1):584. doi:10.1186/s12879-020-05314-x. **(Co-first author)**

Chapter 4.

Luo H, Liu S, Wang Y, Mortimer K, Ju S, Yang Y, Wang D. Disease progression in patients with COVID-19: a retrospective cohort study in China. *Int J Tuberc Lung Dis*. 2020;24(10):1032-1037. doi:10.5588/ijtld.20.0386.

Chapter 5.

Wang Y, **Luo H**, Liu S, Hao T, Mortimer K, Yang Y, Wang D, Ju S. Respiratory failure among patients with COVID-19 in Jiangsu province, China: A multicentre retrospective cohort study. *Epidemiology and Infection*. 2021;149:e31. doi:10.1017/S0950268821000157. **(Co-first author)**

Chapter 6.

Luo H, Liu S, Wang Y, Phillips-Howard PA, Ju S, Yang Y, Wang D. Age differences in clinical features and outcomes in patients with COVID-19, Jiangsu, China: a retrospective, multicentre cohort study. *BMJ Open*. 2020;10(10):e039887. doi:10.1136/bmjopen-2020-039887.

Chapter 7.

Luo H, Wang Y, Liu S, Chen R, Chen T, Yang Y, Wang D, Ju S. Associations between CT pulmonary opacity score on admission and clinical characteristics and outcomes in patients with COVID-19. *Intern Emerg Med*. 2021;1-11. doi:10.1007/s11739-021-02795-9.

Chapter 8.

Liu S, **Luo H**, Lei Z, Xu H, Hao T, Chen C, Wang Y, Xie J, Liu L, Ju S, Qiu H, Wang D, Yang Y. A nomogram predicting severe COVID-19 based on a large study cohort from China. *The American Journal of Emergency Medicine.* 2021;50:218-223. doi:10.1016/j.ajem.2021.08.018. **(Co-first author)**

Chapter 9.

Luo H, Camilleri D, Garitaonandia I, Djumanov D, Chen T, Lorch U, Täubel J, Wang D. Kinetics of anti-SARS-CoV-2 IgG antibody levels and potential influential factors in subjects with COVID-19: A 11-month follow-up study. *Diagnostic Microbiology & Infectious Disease*. 2021. doi:10.1016/j.diagmicrobio.2021.115537.

Other publications during my PhD time

1. Liu H, Zhou Z, Fan X, **Luo H**, Wang D, Wang J, Shen C, Nawaz R. A mixed method study to examine the mental health problems of college students who had left-behind experiences. *Journal of Affective Disorders*. 2021;292:149-160. doi:10.1016/j.jad.2021.04.087.

2. Liu H, Fan X, **Luo H**, Zhou Z, Shen C, Hu N, Zhai X. Comparison of depressive symptoms and its influencing factors among the elderly in urban and rural areas: Evidence from the China Health and Retirement Longitudinal Study (CHARLS). *Int J Environ Res Public Health*. 2021;18(8):3886. doi:10.3390/ijerph18083886.

3. Wang YC, **Luo H**, Liu S, Huang S, Zhou Z, Yu Q, Zhang S, Zhao Z, Yu Y, Yang Y, Wang D, Ju S. Dynamic evolution of COVID-19 on chest computed tomography: experience from Jiangsu Province of China. *Eur Radiol*. 2020;30(11):6194-6203. doi:10.1007/s00330-020-06976-6.

4. **Luo H**, Qiu L, Wu Y, Zhang X. Growth in syphilis-exposed and -unexposed uninfected children from birth to 18 months of age in China: a longitudinal study. *Sci Rep*. 2019;9(1):4416. doi:10.1038/s41598-019-40134-3.

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List of abbreviations

ARDS	Acute respiratory distress syndrome
AUC	Area under the receiver operating characteristic curve
CCI	Charlson comorbidity index
CFR	Case fatality rate
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CRP	C-reactive protein
CT	Computer tomography
DAD	Diffuse alveolar damage
EEA	European Economic Area
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FiO_2	Fraction of inspired oxygen
GCPs	Good Clinical Practices
GLM	Generalised linear model
GLMMs	Generalised linear mixed models
GMR	Geometric mean ratio
HIV	Human immunodeficiency virus
HR	Heart rate
HR	Hazard ratio
ICC	Intraclass correlation coefficient
ICU	Intensive care unit
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMV	Invasive mechanical ventilation
IQR	Inter-quartile range
IVIG	Intravenous immunoglobulin
LFI	Lateral flow immunoassay
MERS-CoV	Middle East respiratory syndrome coronavirus
MMR	Measles, mumps, and rubella
NK	Natural killer
NRES	National Research Ethics Service
NSAIDs	Nonsteroidal anti-inflammatory drugs
OR	Odds ratio
PaO_2	Arterial blood oxygen partial pressure
PIs	Principal investigators
RBD	Receptor binding domain
RR	Respiratory rate
RT-PCR	Reverse transcriptase–polymerase chain reaction assay
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
SD	Standard deviations
SOPs	Standard Operating Procedures
SpO_2	Peripheral capillary oxygen saturation

S-RBD	Spike-receptor binding domain
WBC	White blood cell
WHO	World Health Organization

Chapter 1 Introduction

1.1 Introduction

Coronavirus disease 2019 (COVID-19) is caused by the etiological agent Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The disease was first reported in Wuhan, Hubei Province, China in December 2019. The World Health Organization (WHO) has conducted an assessment and declared that COVID-19 can be classified as a pandemic on the 11th March 2020 [1]. Epidemiological update from WHO revealed that the COVID-19 pandemic had rapidly spread with a large number of people died and many countries affected [2]. The pandemic has brought unprecedented burdens to the world economy (e.g. workforce, event cancellations, food and agriculture, and supply chain), globalization (e.g. mobility of individuals demonstrated by data on airline, seaport trade and travelling), and healthcare (demonstrated by healthcare systems indicators and responses of various countries) [3].

It is important to further understand the characteristics, outcomes, and immunity of COVID-19, which could support the early identification of patients at high risk of severe or critically ill COVID-19, provide information for prevention and control, and help reduce hospitalisation and mortality.

1.2 COVID-19

1.2.1 COVID-19

COVID-19 is a respiratory disease, caused by the novel etiological agent SARS-CoV-2. SARS-CoV-2 is an enveloped single-stranded positive-sense RNA virus belonging to β coronaviruses of the Coronaviridae family [4]. The disease was first reported from Wuhan, Hubei province,

China, in December 2019. Some coronaviruses such as the HCoV-OC43 and HCoV-229E viruses generally cause the common colds in humans, while some other coronaviruses such as the Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV can induce much severe respiratory disease [5]. The MERS-CoV, SARS-CoV and the novel highly pathogenic coronavirus SARS-CoV-2 are zoonotic coronaviruses considered to be transmitted from animals to humans [5].

The pathology and pathogenesis of COVID-19 have been discussed in the literature. When the host is exposed to the virus, the virus binds to the virus receptor expressing cells, leading to infection [6]. Angiotensin-convert enzyme 2 (ACE2) is one of the main receptors of SARS-CoV, and the other receptor is CD209 [6]. SARS infection can reduce the ACE2 expression, thereby destroying the function of the angiotensin renin system regulated by ACE2; on the other hand, SARS infection can also lead to increased inflammation [7,8]. Since the genetic sequences of SARS-CoV-2 and SARS-CoV are similar, SARS-CoV-2 is considered to use the same ACE2 receptor for infection [9-11]. These receptors bind to viral spike proteins, and then the virus enters the host cell [9]. After SARS-CoV enters the host cell, it produces a severe immune response, leading to a phenotype called cytokine storm syndrome characterised with uncontrolled increase of cytokines (e.g., IFN, IL-6) and chemokines [6,7]. The cytokine storm can cause the immune system to attack the infected organs and hence induce acute respiratory distress syndrome (ARDS) and severe failure of multiple organs such as lung [12,13], and the excessive levels of IL-6 can block lymphopoiesis and cause lymphocyte death [14,15].

Laboratory diagnostic methods of COVID-19, including nucleic acid, antibody and antigen tests, have been developed among suspected clinical cases conforming to the epidemiological history and clinical manifestations of COVID-19. The early and current clinical

diagnosis of SARS-CoV-2 is generally a nucleic acid test by using real-time reverse transcriptase-polymerase chain reaction (RT-PCR) to detect nucleic acid in nasopharyngeal and oropharyngeal specimens [16,17]. But for RT-PCR, sample contamination or technical failures can lead to negative results; and RNA extraction is time-consuming, requires trained laboratory technicians, and increases the risk of exposure to the virus [18-21]. So other rapid diagnostic tests, such as nucleic acid amplification testing and microarray hybridization testing, have been developed and are also in use [21,22]. In addition, for epidemiology and vaccine development, some serological and immunological assays are in use, such as enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFI), to detect the presence of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies based on blood sample analysis [21,23]. In addition to RT-PCR for detecting viral genetic material, antibody tests for detecting human antiviral antibodies, some developed antigen tests that recognise fragments of viral surface proteins provide a quick and simple method for early diagnosis of infection, but specificity and sensitivity are limited by factors such as antibody quality and the patient [24-26]. Furthermore, except for the above chemical diagnosis methods, CT can assist in screening for respiratory pneumonia caused by SARS-CoV-2 in suspected cases and judging the severity of the disease [26]. However, because the lung abnormalities displayed by CT may also appear in other diseases and are not specific to the COVID-19, CT testing is not suggested to be used as a diagnostic tool to confirm COVID-19 [26].

1.2.2 Emergence and spread

The WHO declared COVID-19 a pandemic [1], which has quickly spread from a region to all over the world with cumulative numbers of confirmed cases and deaths of around 228 million and

4.6 million respectively by the 19th September 2021 [2]. The person-to-person transmission is one of the potential transmission routes, including contact with patients' mucosal secretions from the nose, mouth and eyes; inhalation of droplets when patients cough or sneeze; and also maybe mother-to-fetus transmission [21,27-29]. Compared with SARS-CoV, SARS-CoV-2 has a higher reproductive (R) number, characterised with a much faster spread and higher infection rate [30,31]. The higher infection rate of SARS-CoV-2 than SARS-CoV may be due to the difference in the position of receptor binding domain (RBD) of viral spike proteins, resulting in the much higher binding tendency [7,32]. In addition, this rapid spread also results from the openness of 21st century geopolitics, ease of travel, and an initial underestimation of the seriousness of the virus [21].

Since droplets from a sneeze or cough of COVID-19 patients can spread as far as 2 meters, this underscores the 2-meter social distancing standard and the need to wear facial masks and avoid crowd [21,33,34]. SARS-CoV-2 can deposit on many surfaces and can survive for several days in some conditions, so people are also encouraged to wash their hands frequently to prevent infection [21,34,35].

1.2.3 Symptoms and mortality

Patients with COVID-19 may be asymptomatic or have various degrees of symptoms. The initial symptoms of COVID-19 are not specific, manifested as fever, and cough, and then can resolve spontaneously or quickly progress to shortness of breath, and pneumonia, leading to ARDS, renal failure, coagulation dysfunction, multiple organ failure, septic shock, metabolic acidosis, death or other poor outcomes [36-44]. The main clinical impact of COVID-19 infection is on the respiratory system, although other systems may also be affected [45,46]. COVID-19 can

lead to acute respiratory failure that requires mechanical ventilation and even death [37,47-49].

A study on the clinical course and mortality of adult hospitalised patients with COVID-19 in Wuhan, China found that the mortality rates of severe and critically ill patients were 22% and 78%, respectively [50]. Of the first 44,672 confirmed cases, 1023 patients died, with a crude case fatality rate (CFR) of 2.3%, and the mortality rate for critically ill patients was even higher, with a CFR of 49% [51]. Hubei has a higher proportion of severe COVID-19 cases than other provinces (17.7% and 7.0%, respectively) [51]. Compared with China, South Korea has a lower crude CFR for men (1.1%) and women (0.4%) [52]; Australia (1.4%) [53], and countries in the European Union (EU) and European Economic Area (EEA) also had a lower crude CFR (1.5%) [54]; while Italy's crude CFR was much higher (7.2%) [55].

The data from WHO show that the crude mortality rate (the number of reported deaths divided by the number of reported cases) may be between 3-4%, which seems to be higher than that of influenza, especially seasonal influenza with the mortality rate of below 0.1% [56]. However, WHO estimated that the infection mortality rate (the number of reported deaths divided by the number of infections) may be lower than the crude mortality ratio [56]. In addition, the mortality rate could be affected by factors such as regions, demographic and socioeconomic factors, levels of access and quality of healthcare, intervention strategies, and qualities of reported deaths and cases [56-58].

A study in Italy reported that the incidence of severe respiratory failure (defined as: SpO_2<93% with 100% FiO_2, respiratory rate [RR]>30 bpm, or respiratory distress) was around 29%-40% [59]. The 28-day mortality from time of intubation could be 26%-30% among patients with COVID-19 who had acute respiratory failure requiring invasive mechanical ventilation (IMV) [60].

1.3 Risk factors of adverse outcomes of COVID-19

1.3.1 Comorbidities and age

The evidence showed that people of all ages are susceptible to SARS-CoV-2, but the elderly had the higher positive rate of RT-PCR testing and a higher hospitalisation burden [61-63]. Like SARS, deaths and adverse clinical outcomes are more common in older adults with known comorbidities among patients with COVID-19 [64-66]. Compared with general patients, patients with severe/critical illness were older and had a higher incidence of comorbidities; medical conditions such as cancer, chronic kidney or liver or lung disease, dementia or other neurological conditions, type 1 or type 2 diabetes, obesity, coronary artery disease, high blood pressure (hypertension) or other heart conditions, human immunodeficiency virus (HIV) infection, depression, and so on can make people more likely to get severely ill due to COVID-19 [67-72]. Older age was found to be one of risk factors associated with ARDS development and progression from ARDS to death (hazard ratio [HR], 3.26; 95% confidence interval 2.08-5.11; and HR, 6.17; 95% CI, 3.26-11.67, respectively) [41]. Older patients have a higher risk of first-episode complications, more severe symptoms, multiple organ involvement, and death due to the possible factors such as the physiological changes of aging and a variety of age-related complications; so the threshold for suspicion and detection of SARS-CoV-2 in the elderly was suggested to be lowered, such as decreased lung function and shortness of breath [73-75].

The previous studies have shown a high frequency of hypertension and obesity among patients with COVID-19 and that patients with hypertension or obesity have a higher risk of morbidity and mortality from COVID-19 and the need for invasive mechanical ventilation,

possibly due to impaired pulmonary immune responses, systemic hyper-inflammatory responses, cytokine dysregulation, increased risk of thrombosis and increased viral load [39,76-81]. Similarly, COVID-19 patients with diabetes face an increased risk of severe course of disease and death (a pooled OR of 1.70, 95% CI 1.16-2.48; P = 0.006) due to several potential factors, notably an impaired immune response, an increased inflammatory response and a hypercoagulable state [72,73,82-87]; considering that diabetes is a complex disease associated with many metabolic disorders, the pathophysiology among COVID-19 patients with diabetes still needs further research [87].

Some studies also showed compared with age-matched noncancer patients confirmed with COVID-19, COVID-19 patients with cancer were at higher risks of severe events, including death, severe or critical illness, intensive care unit (ICU) admission, and the requirement of invasive mechanical ventilation, especially patients who received surgery or patients with lung cancer, hematologic cancer, or metastatic cancer [88,89]. The related finding, which is very puzzling, is that immunotherapy patients have the highest mortality rate and the highest severity of disease compared to cancer patients treated with other therapies, possibly because immunotherapy induces the cytokine storm which is reported to be the main reason for death of COVID-19 patients, leading to more severe disease [88,89].

A review study reported that HIV-related immunosuppression may increase the risk of COVID-19 severity rather than provide protection, but there is no excessive morbidity and mortality, especially for those on antiretroviral drugs to suppress viral load, but people living with HIV should still strictly follow the recommendations on how to protect themselves from SARS-CoV-2 infection [90].

1.3.2 Radiological characteristics

Although real-time reverse transcriptase-polymerase chain reaction assay (RT-PCR) is the standard diagnostic method for COVID-19, the chest computed tomography (CT) has important diagnostic values [91]. Some experts suggested that patients with typical CT findings, but negative RT-PCR results should be isolated because RT-PCR results can be false-negative [91]. Compared to radiologic images from X-ray examination, CT images have the advantage of high-resolution transversal imaging and accurate display of the extent and range of lung lesions. CT findings of COVID-19 are mainly characterised by patchy glass opacity in the peripheral area [92,93]. Correlation analysis detected a significant relationship between pulmonary inflammation index value and lymphocyte count, monocyte count, C-reactive protein, procalcitonin, days from illness onset and body temperature [94]. The median radiograph score was negatively correlated with oxygenation index, and patients with abnormal images were older, with higher rate of coexisting condition, fever, cough, expectoration, and headache, lower levels of lymphocytes, albumin, serum sodium and higher levels of total bilirubin, creatine kinase, lactate dehydrogenase and C-reactive protein and lower oxygenation index than non-pneumonia patients [95]. Compared with ordinary patients, severe/critical patients had higher incidence of lung consolidation, linear opacities, crazy-paving pattern, and bronchial wall thickening [67]. The higher radiograph score at admission was found to be a predictive factor for severe/critical subtype [67,95] and the automatically measured impaired lung volume was statistically significant correlated with the maximal respiratory severity score [96]. The severity score which quantifies the extent of COVID-19 lung involvement was associated with ICU admission [97]. The bilateral pneumonia on CT scan was correlated with a longer duration of hospitalisation [98].

However, it is important to note that because imaging features are not specific for COVID-19 and can be seen in other viral pneumonia, chest CT should not be used as a screening test in patients with suspected COVID-19 but to assess clinical deterioration in patients with confirmed COVID-19 [99-101].

1.3.3 Laboratory parameters

Abnormal changes have been found in some laboratory biomarkers of COVID-19 patients [102-104]. Previous studies show that the white blood cell (WBC) count was only slightly elevated in severe cases, which was higher than moderate cases, with the highest in critical cases; but the increase in WBC count in patients who died was more clinically significant, manifested as increased neutrophils, while decreased lymphocytes, monocytes and eosinophils [103,105]. Increased neutrophilia was associated with a greater risk of development of ARDS and death (HR, 1.14; 95% CI, 1.09-1.19; and HR, 1.08; 95% CI, 1.01-1.17) [41]. Lymphocyte count was significantly lower in severe COVID-19 patients compared with moderate cases [106]. Lymphopenia and elevated inflammation parameters were found to raise the risk of severe or critical COVID-19 pneumonia [67]. Leukocytes and neutrophils in ICU patients were higher than those in non-ICU patients, and lymphocytes gradually decreased after the disease onset in death cases [44,50]. Elevated neutrophils and neutrophil-to-lymphocyte ratio suggest severe or critical illness and poor prognosis [107]. The proportions of monocytes, eosinophils, and basophils dropped dramatically in severe cases [107,108].

Studies of platelets in COVID-19 patients have shown inconsistent results, which may be due to the complexity of the disease. Some studies have found a higher proportion of thrombocytopenia patients in severe cases than non-severe cases [50,109], and platelet count

was lower in non-survivors than in survivors [50,110-112]. However, other studies have found no significant difference in median platelet count between ICU patients and non-ICU patients [44,113], and no significant difference in platelet count between survivors and non-survivors of COVID-19 patients with ARDS [41,114]. In addition, a retrospective analysis of 30 patients with COVID-19 found that patients with higher platelet count and platelet-to-lymphocyte ratio had a longer average hospital stay [115]. Conflicting results on platelets have been interpreted by a study suggesting that decreased platelet counts may indicate thrombogenesis leading to platelet depletion, while increased platelet counts may indicate inflammation stimulating megakaryocyte production and increased platelet synthesis [116].

In severe and fatal COVID-19 patients, biomarkers of heart and muscle damage (including cardiac troponin levels), liver enzymes (alanine aminotransferase and aspartate aminotransferase), kidney biomarkers (blood urea nitrogen, creatinine), and coagulation indicators were also significantly elevated, suggesting the possibility of viral myocarditis, heart damage, and multiple organ failure such as kidney or liver [102,103]. The proportion of increased biochemical markers, including lactate dehydrogenase, aspartate aminotransferase, aspartate aminotransferase, creatine kinase and creatinine, gradually increased with the severity of the disease [109]. In addition, the levels of lactic acid, lactate dehydrogenase, aspartate aminotransferase, aspartate aminotransferase, creatine kinase, creatinine and cardiac troponin were significantly elevated in non-survivors compared with survivors [117]. On the other hand, the levels of lactate dehydrogenase, cardiac troponin, urea and creatinine were progressively increased after the disease onset among patients who died [44,50]. The proportion of elevated D-dimer indicating coagulation and fibrinolysis abnormalities increased gradually in patients with non-severe, severe and critical COVID-19, and was higher

in non-survivors than survivors [44,50]. The higher lactate dehydrogenase and D-dimer indicated organ and coagulation dysfunction and were associated with a greater risk of development of ARDS, critical illness and death [41,44,50,118]. Other coagulation and fibrinolytic biomarker abnormalities include the detection of significantly elevated fibrinogen and fibrin degradation products in non-survivors; higher fibrinogen level in patients with thrombotic complications than those without; and longer activated partial thromboplastin time and prothrombin time in non-survivors than in survivors at admission [118-120].

In terms of immune biomarkers, interleukin-6 and serum ferritin were found to increase significantly in patients with severe diseases compared with non-severe types and were recommended as indicators for prognosis of COVID-19 patients during hospitalisation [103]. The levels of interleukin-2, interleukin-7, interleukin-10, granulocyte-colony stimulating factor, tumor necrosis factor α and so on were increased in ICU patients compared with the non-ICU patients [48]. Levels of interleukin-6 and interleukin-2R were significantly higher in severe patients compared with moderate patients, and higher in non-survivors compared with survivors [50,106,108]. In addition to cytokines, levels of some inflammatory biomarkers such as serum ferritin, amyloid A protein, procalcitonin and C-reactive protein (CRP) were significantly higher in the severe and critical cases than in the moderate cases, indicating a higher risk of severe disease and poor prognosis [106,108,121]. Levels of serum amyloid A protein, procalcitonin, CRP and interleukin-6 increased according to the severity of the disease, and increased significantly in the non-survival group compared with the survival group [102,122]. Elevations of interleukin-6, serum ferritin and CRP can lead to cytokine storm and systemic inflammatory response syndrome, then severe illness such as acute lung injury, ARDS, other tissue damage and multiple organ failure [103]. In patients with severe COVID-19, the host's

immune system was over-activated and many cytokines such as interleukin-1ß, interleukin-7, interleukin-10, tumor necrosis factor α and interferon α were secreted to destroy the virus, but the cytokines concentration can get out of control and cause serious side effects leading to lung injury and even damage to other organs [102,123]. In addition, interleukin-10 has been observed to increase in severely ill patients, which is suspected to lead to a compensatory anti-inflammatory response contributing to secondary infection and sepsis [103].

1.4 Prediction models

1.4.1 Prediction models of severe COVID-19

While many countries have implemented public health responses to control the disease and slow its spread, the pandemic has resulted in a significant increase in the demand for hospital beds, shortages of medical equipment, infection of some health care workers, and a critical care crisis [124-126]. Effective predictive models combine several characteristics to estimate the risk of a person having severe disease after infection can help health care professionals and policy makers to identify patients at high risks of adverse outcomes to assist treatment and classify patients when allocating limited medical and human resources, and hence reduce the burden on the healthcare system while also providing the feasible and optimal health care to patients.

Some prediction models of severe COVID-19 are available, but those models are subject to various biases in terms of data quality, statistical analysis, and reporting [127-136]. The data quality issues were mainly on the presence of missing data and the handling strategies. The flaws in the statistical analysis were mainly on the lack of internal and external validation

of the predictive models, the categorisation of continuous predictors and hence loss of information, and weak assessment of model performance such as calibration and discrimination. The main reporting issue is that some studies did not mention the missing data. A retrospective analysis was performed on 123 young people diagnosed with COVID-19 at a tertiary hospital in Wuhan, China to establish a model for predicting the disease severity, but the model was not externally validated [134]. A model to predict progression to severe COVID-19 during hospitalisation using 366 confirmed COVID-19 patients collected in Sichuan province, China and a model using 139 patients with COVID-19 in Turkey were also not externally validated [135,136]. A retrospective cohort study of 80 COVID-19 patients admitted to a hospital in Beijing, China established a predictive scoring system and validated it in 22 subsequent COVID-19 patients [133]. A study developed a prediction model to identify severe COVID-19 patients with a regression coefficient of 48.8309 and an odds ratio with a confidence interval of >999.999 (>999.999, >999.999), which is nonsensical; and it is unclear how the missing data were handled and whether individuals with missing data or whether entire predictors were discarded [127,137]. A study retrospectively collected 220 clinical and laboratory records of COVID-19 patients in Shanghai, China to establish a predictive model for severe/critical symptoms [129]. Another retrospective multicentre study of 372 non-severe COVID-19 hospitalised patients constructed a nomogram for predicting the risk of severe COVID-19, but it is unclear how the perfect calibration was derived [128,138]. A study developed a model to identify COVID-19 patients with severe disease, but the study may not have enough events to evaluate the model performance and weak and used discredited approaches (not calibration plots) to assess the model calibration [130,139]. Xiao et al developed an artificial intelligence-assisted tool using CT imaging to predict disease severity and also established a HNC-LL (Hypertension, Neutrophil count, C-reactive protein, Lymphocyte count,

Lactate dehydrogenase) score using multivariate logistic regression analysis to predict disease severity with around 400 confirmed COVID-19 patients from two hospitals in China [131,132].

1.4.2 Nomogram

A nomogram is a two-dimensional graphical representation of a scoring prediction model, which aims to calculate the probability of having an outcome quickly and visually with acceptable accuracy. A nomogram is composed of multiple scale axes. The top axis is the point scale, followed by the scale axis of the selected predictor, the total point scale, and then the probability scale. Each value on the predictor axis corresponds vertically to a point on the point scale. After determining the points of all predictors from the point scale and adding them to get the total number of points, we can find the corresponding probability on the probability scale. The predictors can be continuous or categorical variables. The statistical methods can be multivariable logistic regression or Cox regression depending on the type of outcomes to be studied. Other than nomogram, there are also other risk estimation and decision aid tools, such as risk grouping, artificial neural network, probability table, and classification and regression tree analysis. Compared with other tools, the nomogram could provide consistent and reasonably accurate risk estimation, while also providing a user-friendly interface because it does not require computer software to calculate the results [140].

1.5 Clinical management

The treatment of COVID-19 is still under active research. In COVID-19 patients with ARDS, the treatment with methylprednisolone reduced the likelihood of death (HR, 0.38; 95%CI, 0.20-

0.72), suggesting the benefit for patients who have ARDS [41]. Prompt and adequate treatment of intravenous immunoglobulin (IVIG) was found to potentially be able to help reduce the mortality rate in COVID-19 patients with critical illness and diabetes [141]. The delayed treatment of severe COVID-19 may lead to longer hospital stays, increased mortality and a heavier financial burden [142,143]. Among COVID-19 patients, the incidences of bacterial co-infection and multidrug resistance were high, and consideration was suggested to be given to tests of other mixed infectious agents and timely treatment based on antimicrobial sensitivity results [144].

Several treatments have been proposed based on different aspects of the pathogenesis of COVID-19, including antiviral therapy (such as remdesivir), antimalarial drugs, anti-inflammatory drugs (such as barcitinib), angiotensin receptor blockers and statins, antithrombotic drugs (such as low molecular weight heparin), convalescent plasma treatment, stem cell therapies, immunoglobulins, etc. [101,145-151]. Related clinical trials have been completed or are underway with conflicting results, with some trials showing promise and others showing no benefit. Furthermore, patient heterogeneity, including the various comorbidities, disease severity, and multiple complications, remains a challenge for clinical trials [152]. Because of the lack of any approved effective drugs, health care providers have to use broad-spectrum antibiotics and antiviral drugs with a high and frequent dose for patients, leading to many serious adverse effects after patients recovered from COVID-19, such as mental illness, heart, liver and kidney complications [153].

Because the efficacy of existing antiviral drugs and the effectiveness of the standard of care have not been fully confirmed, especially for those patients with mild disease, prevention of transmission should be the priority [101]. Patients treated at home need close

monitoring and immediate expansion of care and treatment if their condition worsens [154]. To optimise clinical management, treatments are considered to vary depending on characteristics of the patients, such as for elderly patients, or patients with compromised immune systems, obesity, or hypertension [101,146]. Current treatment strategies for COVID-19 include considering corticosteroids only when severe disease occurs because data on the increased risk of viral replication and anti-inflammatory benefits of corticosteroids are inconclusive [155,156]; using inhalers rather than aerosols whenever possible to avoid increased airborne transmission of the virus [157]; choosing acetaminophen over nonsteroidal anti-inflammatory drugs (NSAIDs) whenever possible, as relief should vary from person to person and there is an increased risk of bleeding and kidney damage when using NSAIDs [101]; considering oxygen supplementation (nasal intubation and high-flow oxygen) for patients with moderate to severe disease, while non-invasive and invasive mechanical ventilation for those with acute respiratory failure [154]; and so on.

COVID-19 prevention largely relies on vaccine development. Hundreds of clinical trials have now been completed or are under way to test various novel and repurposed compounds against COVID-19, such as protein subunit vaccines, inactivated virus vaccines, adenovirus vaccines and gene vaccines [101,158-160]. Confirming vaccine safety and efficacy is important because ineffective vaccines may not protect people from infection and may also cause disease through increased antibody dependence or other mechanisms [161,162]. Despite of the lack of reliable clinical safety data, among the vaccines against COVID-19, BNT162 (from Pfizer/BioNTech), mRNA-1273 (from Moderna) and AZD-1222 (from AstraZeneca) quickly completed phase 3 clinical trials, with BNT162 and mRNA-1273 showing about 95% clinical efficacy in clinical trials and receiving emergency approval for use in the United States and

other countries [21,163]. Furthermore, future vaccine research needs to address another challenge, namely the high mutation rate of SARS-COV-2, with approximately 25 mutations per year [21,34,164]. In people with chronic comorbidities, annual influenza vaccination significantly prevented all-cause hospitalisation and in the elderly (65+), influenza vaccination prevented both all-cause hospitalisation and mortality [165]. There is growing evidence that SARS-CoV-2 may become seasonal and require annual vaccination [166]. People with pre-existing cardiovascular disease, congestive heart failure, hypertension, diabetes, chronic kidney disease and cancer are at higher risk of dying from COVID-19 and may therefore be prioritised for vaccine, especially when vaccine supplies are limited [71].

1.6 Immunity to SARS-CoV-2

1.6.1 Immune response

Viral infection can trigger humoral and cell-mediated immune responses. Humoral immunity is that virus and/or virus-infected cells can stimulate B lymphocytes to produce antibodies including immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA), all of which have been proven to exert antiviral activity [167]. The cell-mediated immunity refers to that leukocytes (cytotoxic T lymphocytes, natural killer (NK) cells, antiviral macrophages, and helper T cells) recognise and/or kill viruses and virus-infected cells, and produce different soluble factors (cytokines) when stimulated by viruses or virus-infected cells, which play important roles in regulating and developing antiviral immune function [167].

The assessment of the humoral and cell-mediated immune response to virus infection or vaccination is expected to help develop clinical applications and understand herd immunity. In particular, the kinetics of the humoral immune response against SARS-CoV-2 may

contribute to COVID-19 diagnosis, vaccine development, therapeutic immune plasma research, and epidemiological research including prevalence, exposure, and immunity. Decreased antibody levels may indicate a lack of protective immunity [168]. Most COVID-19 patients develop detectable IgM and IgG antibodies within a few weeks after infection, targeting SARS-CoV-2 nucleocapsid (N) or spike (S) protein [169,170].

The complex balance between host immunity and coronavirus load determines the degree of viral pathogenesis, and the potential correlation between the severity of refractory hypoxemia and reduced immune cell expression suggests that the immune compromised state may be one of the factors contributing to the severity of SARS-CoV-2 infection leading to adverse clinical outcomes [171].

Previous studies have shown that the IgG response to SARS-CoV-2 infection can last 3 to 8 months after the onset of symptoms [172-177]. However, the long-term kinetics of IgG antibodies remains to be studied, and most previous studies included limited sample sizes and narrow ranges of disease severity [178-181]. A longer follow-up study was conducted in the Umbria region of Italy, and 32 patients with COVID-19 who recovered from mild, moderate, and severe infection were followed for 14 months for anti-spike-receptor binding domain (S-RBD) IgG antibody titers [182].

1.6.2 Influential factors

Current reports have studied the associations between the IgG antibody response against SARS-CoV-2 and some potential influential factors. Older patients (over 35 years old) were found to have higher IgG antibody levels than younger patients (under 35 years old or equal to 35 years old) [183]. Body mass index and immunosuppression also appear to affect IgG

kinetics, with higher IgG levels in patients with higher body mass index and lower IgG levels in immunocompromised patients [184]. Subjects with one or more comorbidities, such as diabetes, developed better antibody titers than those without comorbidities [182,185-188]. A study suggested that comorbid cancer and treatment with systemic therapy may influence the immune response to SARS-CoV-2; for example, among patients, those who received chemotherapy had significantly lower levels of N-IgG than those who did not, and those who received immune checkpoint inhibitors had significantly higher levels of N-IgG and S-IgG than those who did not [189]. Prolonged and frequently more severe symptoms were found to be associated with elevated antibody levels [190], and similarly, a study found that non-severe clinical manifestation was the only factor associated with faster decay of IgG anti-spike antibodies after adjusting for sex and age >70 years [181]. Zejda et al found IgG positivity was statistically associated with age (negative correlation), contact history with COVID-19 patients, isolation history, fever, and loss of smell/taste [191]. To the best of my knowledge, as of February 2022, only one study preliminarily explored the relationship between IgG antibody levels and loss of smell and taste by comparing median anti-S-RBD IgG antibody titers at 14 months and found that subjects who experienced a loss of smell and taste during infection had higher antibody titers [182].

1.7 Research gap identified for the PhD work

Because COVID-19 is a new disease, healthcare workers and researchers are still learning. Although understanding of the disease is growing, some research gaps have been identified before the main chapters (Chapter 3 to 9) were completed, which urgently required the development of this PhD work. In the early stage of the pandemic, there were limited clinical

data on COVID-19 patients for research. As mentioned before, increased data and information were important to improve research reliability.

Before the publication of Chapter 3, the characteristics of patients with COVID-19 including age, comorbidities, C-reactive protein, albumin, cytokine, lactate dehydrogenase, D-dimer, albumin, platelet, lymphocyte, neutrophil, smoking, cough, expectoration, chest pain, dyspnea and CT manifestations had been reported to be associated with the severity of COVID-19 in some small sample size studies in China [67,192-197] and other countires.[40,76,198]. However, previous studies were mostly small and descriptive in nature. Chapter 3 used data from the whole Jiangsu province, China and made inferential statistical analyses by using multivariate regression model to control for possible confounding factors and identify independent risk factors of becoming a severe/critically ill case. Therefore, Chapter 3 could provide more accurate information on the risk factors to help reduce mortality and save lives. This had very real implications in the early stage of the pandemic.

Prior to the publication of Chapter 4, a study has reported risk factors for progression from ARDS to death in patients with COVID-19 [41]. However, the pattern of disease progression from "asymptomatic/mild/moderate" status to "severe/critical" status and its associated factors had not been adequately studied in patients with COVID-19. Assessing disease progression patterns and identifying factors associated with disease progression can help health care professionals prospectively identify patients at high risk for progression, help patients avoid a crisis phase that can lead to death, and help reduce the burden of healthcare, especially in intensive care. Chapter 4 can help fill in the gaps in this research topic.

COVID-19 can cause severe illness, one of which is of great concern is acute respiratory failure requiring mechanical ventilation, 26%-30% of respiratory failure patients necessitating

invasive mechanical ventilation may experience 28-day mortality [37,47-49,60]. Before Chapter 5 was published, research on risk factors for respiratory failure remained sparse. As far as I am aware, only two Italian studies had explored this topic at that time [59,199]. Nevertheless, the characteristics of patients could vary from country to country, as may the degree of respiratory failure and risk factors [200]. Therefore, information on respiratory failure in other regions outside of Italy was of great academic and clinical value. Considering this, Chapter 5 explored this topic based on a large multicentre retrospective cohort with rich information on demographic, epidemiological, clinical and laboratory characteristics, and CT imaging characteristics to prevent respiratory failure and even death and reduce the risk of infection of medical staff via medical procedures such as emergency intubation.

Before the publication of Chapter 6, it was known that age was associated with some patient characteristics. However, the quality of previous studies was insufficient, as most studies reporting these results were based on small or local studies [38,201-208]. Chapter 6 could provide robust and valuable relevant findings based on data of nearly all COVID-19 patients from multiple centres in Jiangsu Province, China, with very detailed information on patient characteristics at admission, disease severity, and clinical outcomes during hospitalisation. In terms of variables and outcomes studied, in addition to previously studied age differences in chronic medical history, lung opacities, and some abnormal laboratory parameters, Chapter 6, based on available data, could complement the previous studies by adding information on age differences in many other characteristics and clinical outcomes, such as exposure types, vital signs, and ICU monitoring.

Prior to the publication of Chapter 7, some studies provided some information on the association between lung opacity scores and demographic, epidemiological, clinical,

laboratory characteristics, and clinical management, but most of these studies had sample sizes between 50 and 200. Research on these associations in Chapter 7 based on a larger dataset (N = 496) can provide some novel and more reliable information. Whereas most previous studies have reported the effect of the lung opacity score on a single clinical outcome, Chapter 7 of this work assessed the effect of the lung opacity score on a number of important clinical outcomes (including disease severity, ICU admission, respiratory failure, and length of hospital stay), which can provide more comprehensive knowledge.

Prediction models of severe COVID-19 prior to the publication of Chapter 8 are subject to various biases in terms of data quality, statistical analysis, and reporting statistical issues [127]. The flaws included the presence of missing data and the processing strategies, lack of internal and external validation of the predictive models, weak assessment of model performance (e.g., calibration and discrimination), and reporting issues such as not mentioning the missing data. Chapter 8 aimed to address these flaws and develop a well-performed nomogram based on a relatively large dataset from 24 centres. No categorical data were missing, and missing continuous data were imputed with medians. The nomogram was internally validated on the derivation cohort using the bootstrap method and further externally validated on a separate independent validation cohort. Discrimination ability and calibration were used to assess the performance of the nomogram.

Although the human immune responses to previously emerged SARS-CoV and MERS-CoV have been carefully studied, the immune response to the novel SARS-CoV-2 has not been investigated in depth before Chapter 9 of this PhD work was done. Previous studies on the duration of immunity were limited by the follow-up time, generally 6-8 months, with a few reports of 1 year or 14 months, therefore, more studies with longer follow-up periods on

immunity in COVID-19 patients may be needed [173-177,182,183,209-211]. To the best of my knowledge, only one research studied the relationship between anti-SARS-CoV-2 IgG levels and loss of smell and taste, but that study only compared median anti-S-RBD IgG antibody titers at each month without using multiple regression to control for confounders and without explaining the underlying mechanism [182]. Knowledge of the immune response of SARS-CoV-2 and related influential factors is the basis for the development of vaccines and therapeutics to fight against the pandemic. Therefore, the aim of this PhD work was also to provide some data and evidence on this topic to improve understanding of SARS-CoV-2 serology, and identify determinants of long-term seroprotection.

This PhD work can provide important new insights into the factors that influence the severity of COVID-19, disease progression, the prediction of disease severity, profile and influential factors of post-onset immunity, and can provide a knowledge base for better management of COVID-19 and enable evidence-based policies during this pandemic. Patients around the world would benefit from this work because it can fill in the gaps in knowledge when the main chapters (Chapter 3 to 9) were completed. So this PhD work deserves better dissemination at the time to help health care providers, patients and policy makers.

1.8 Aims and objectives

This thesis aims to investigate the clinical characteristics, outcomes, and immunity of the population with COVID-19 and explore the accuracy of a prediction model of severe COVID-19 based on data from the UK and China.

More specifically, the objectives of the thesis are:

a. to identify independent risk factors of severe COVID-19, disease progression, and respiratory failure in COVID-19 patients based on all patients with COVID-19 in Jiangsu province, China from the 10 January 2020 up to the 15 March 2020;
b. to explore differences in clinical characteristics, disease severity, and clinical outcome burden in different age groups and different radiographic opacity groups of COVID-19 patients in Jiangsu province, China;
c. to construct a prediction model of severe COVID-19 based on all patients in Jiangsu province, China; and externally validate it by data in Hunan province, China from the 21 January 2020 up to the 29 February 2020;
d. to describe the IgG detectable/positive rate and the IgG level change profile over time after SARS-CoV-2 infection and identify the potential influential factors associated with IgG levels in the general population screened for SARS-CoV-2 infection.

1.9 Thesis outline

This chapter (Chapter 1) has outlined the general profile of COVID-19 pandemic, reviewed the known aspects of characteristics, outcomes, and immunity of COVID-19, identified some relevant research gaps before the main chapters (Chapter 3 to 9) were done, and stated the aims and specific objectives of this PhD work to fill these knowledge gaps. Chapter 2 will outline the materials and methods used in this PhD work for the accomplishment of these aims and specific objectives. As mentioned in the research gaps, before the publication of Chapter 3, the associations between characteristics of patients with COVID-19 and the severity of COVID-19 has been studied, while these studies were mostly small and descriptive

in nature. Therefore, Chapter 3, based on data from the whole Jiangsu province, will make inferential statistical analyses by means of multivariate regression model to control for possible confounding factors and identify independent risk factors of becoming a severe/critically ill case to inform prevention and control measures, potentially reduce mortality, and save lives. Similarly, further exploration of the risk factors for disease deterioration, that is, the dramatic progression of patients from asymptomatic or mild or moderate state to severe or critical state during the follow-up period, may help prevent patients with asymptomatic or mild or moderate disease from progressing into severe disease. Some patients can remain asymptomatic or mild or moderate state, but some patients may experience deterioration from asymptomatic or mild or moderate state to severe or critical state. However, relevant research was scarce, although I found a study that reported risk factors for progression from ARDS to death in patients with COVID-19 prior to the publication of Chapter 4. Therefore, Chapter 4 will describe the occurrence of disease progression in patients with COVID-19 and explore the factors associated with progression from “moderate or less” status to “severe or critical” illness. In addition, given that respiratory failure is one of the most severe illnesses that can occur after contracting COVID-19, further research into the risk factors of respiratory failure may provide some new insights. Before Chapter 5 was published, I found that only two Italian studies had explored this topic, so Chapter 5 will assess the incidence and potential risk factors for respiratory failure in patients with COVID-19 to prevent the occurrence of respiratory failure and deaths and reduce emergency intubation to protect medical staff from associated infections.

The findings from Chapter 3 to Chapter 5 showed that age and CT lung opacity score are important factors affecting the severity of COVID-19 patients, disease deterioration from

milder status to severe or critically ill status, as well as respiratory failure. As mentioned previously, before the publication of Chapter 6, previous studies had shown that age was associated with some patient characteristics. However, the quality of previous studies was low, as most studies reporting these results were based on small or local studies. Therefore, it was necessary to further understand how and why the characteristics and the incidence of adverse outcomes in patients with COVID-19 differ among various age groups, in order to provide a more effective reference for the management of COVID-19. Considering this, Chapter 6 will investigate differences in characteristics, disease severity, and clinical outcome burden in different age groups. Similarly, prior to the publication of Chapter 7, several studies provided some information on the association between lung opacity scores and characteristics and clinical outcomes, but most of these studies had relatively small sample sizes and only reported the effect of the lung opacity score on a single clinical outcome. Therefore, Chapter 7 will investigate differences in a number of characteristics and clinical outcome burden of different radiographic opacity groups of patients with COVID-19.

Chapter 3 to 5 looked at risk factors for severe or critical status, progression from milder status to severe or critical status, and respiratory failure in patients with COVID-19, and then Chapter 6 and 7 focused on two important factors, age and CT lung opacity score, so the next step was to develop a prediction tool to predict severe or critical disease to facilitate health care professionals stratifying patients and providing early and optimal therapies. Nevertheless, prediction models of severe COVID-19 prior to the publication of Chapter 8 are subject to various biases. Therefore, Chapter 8 will try to avoid these flaws, construct a nomogram to provide accurate personalised predictions of severe COVID-19 during the hospitalisation based on a large number of COVID-19 patients in Jiangsu province,

and externally validate the nomogram in a cohort from another province.

Chapter 3 to 8 pointed out that patients with underlying medical conditions and the elderly may have lower immunity after infection with SARS-COV-2 and were prone to severe illness and hence may be at higher risk of death from COVID-19, so it was necessary to learn more about the factors that influence immunity to inform the prevention and treatment of this new disease. However, I only found one study that examined the association between anti-SARS-CoV-2 IgG levels and loss of smell and taste, but that study only compared monthly median anti-S-RBD IgG antibody titers, did not use multiple regression controlling for confounding factors and had no explanation for the underlying mechanism. Chapter 9 will describe the IgG level change profile over time after SARS-CoV-2 infection and identify the potential influential factors associated with IgG levels including loss of smell and taste in the patients with SARS-CoV-2 infection. Finally, Chapter 10 will discuss possible explanations of the main findings of the PhD work, the novelty, the implications on COVID-19 prevention and control, and priorities for future related research.

1.10 Role in research

In this section, I list the roles of other authors and myself in each research.

Chapter 3, 4: **I** did the literature review, data analysis, data quality check, results presentation, results interpretation, drafting and review of the manuscripts. **Other authors** conceived and designed the studies; did the data collection, management and quality check; applied for ethical approval; reviewed and edited the manuscripts.

Chapter 5, 6, 7: **I** conceived and designed the studies; did the literature review, data analysis, data quality check, results presentation, results interpretation, drafting and review

of the manuscripts. **Other authors** did the data collection, management and quality check; applied for ethical approval; reviewed and edited the manuscripts.

Chapter 8: **I** did the literature review and data quality check; double checked results of data analysis and presentation; did the results interpretation, drafting and review of the manuscript. **Other authors** conceived and designed the study; did the data collection, management and quality check; applied for ethical approval; reviewed and edited the manuscript.

Chapter 9: **I** conceived and designed the study; did the literature review, data analysis, data quality check, results presentation, results interpretation, drafting and review of the manuscript. **Other authors** did the data collection, management and quality check; applied for ethical approval; and reviewed the manuscript.

Chapter 2 Materials and methods

2.1 Introduction

The work that is presented in the following chapters is based on two datasets from Jiangsu province and Hunan province, China, one dataset from the UK. This chapter describes the study design and subjects; the sites where to collect the data; the diagnostic criteria of COVID-19 and antibody measurements methods; the epidemiological, demographic, clinical, laboratory, and imaging data, including their identification and collection; the statistical methods and how they were used to investigate the associations between clinical outcomes and IgG levels and the other factors.

2.1 Study design and subjects

The datasets of the thesis are two retrospective cohort studies from Jiangsu province and Hunan province, China, and one prospective cohort study from the UK.

Jiangsu is a province in China with a population of 80 million, more than 600 km away from Hubei province without a common geographic boundary. The work hereby used retrospectively collected data of all cases from twenty-four hospitals in Jiangsu province from January 10, 2020, to March 15, 2020, including the demographic, epidemiological, clinical, laboratory, and imaging characteristics of the cases. Finally, 625 patients in Jiangsu province were included in the analyses.

This work also used retrospectively collected data from 105 patients from Huangshi, Hunan province, China, between January 21, 2020, and February 29, 2020.

For data from China, inclusion criterion was patients diagnosed with COVID-19.

Exclusion criterion was medical records unavailability.

In addition, this work used data from a prospective longitudinal study conducted at Richmond Pharmacology Ltd, London, UK and the Richmond Research Institute, St George's University of London. The participant inclusion criteria were (1) male or female aged 5 and older, (2) an understanding, ability, and willingness to fully comply with the project procedures and restrictions and (3) consent from a parent/legal guardian for participants aged 5 to 15 years. Finally 20 participants were included in this PhD work and were followed-up for a maximum of 11 months.

For Chapter 3-8, the data of all COVID-19 patients in Jiangsu province, China were collected by 24 hospitals designated for COVID-19 treatment in Jiangsu province, and centrally stored at the Data Centre of Jiangsu Provincial Health Commission from which the principal investigators (PIs) obtained the data for these studies. The medical team of Zhongda Hospital, Nanjing, China, where the PIs are based, travelled to Huangshi, Hunan province to support the treatment of COVID-19. The data from Huangshi were all collected and managed by Zhongda Hospital, and likewise stored at the Data Centre of Jiangsu Provincial Health Commission. The Ethics Committee of Zhongda Hospital Affiliated to Southeast University approved these studies (2020ZDSYLL013–P01 and 2020ZDSYLL019–P01).

For Chapter 9, the data were collected and stored at the Richmond Pharmacology Ltd, London, UK and the Richmond Research Institute, St George's University of London. The study was approved by the Committee of National Research Ethics Service (NRES) (West Midlands - Edgbaston) (IRAS ID: 281788).

All research units involved in the studies in this PhD work have extensive research experience in clinical trials, epidemiological studies and other types of medical research.

These studies in the PhD work followed all guidelines, Good Clinical Practices (GCPs) and Standard Operating Procedures (SOPs). For these studies, before conducting the analyses, I did data quality checks and found some logic errors. After discussions with colleagues who collected the data, they corrected those errors using original medical records, which I appreciate very much. Finally, apart from the missing data issue that cannot be resolved using original medical records, the quality of data used for analyses is good. For the issue of missing data, in the "2.5 Statistical analysis" section below, I show that the impact of the problem of missing covariates is mitigated by strategies of imputation and sensitivity analyses.

The data in the PhD work were permitted to be used for my PhD degree.

2.2 COVID-19 diagnosis and antibody measurements

For data from China, the diagnostic criteria of COVID-19 were in line with the “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)” released by China’s National Health Commission & National Administration of Traditional Chinese Medicine (Beijing, China). The diagnosis was based on epidemiological history, clinical manifestations, imaging manifestations of pneumonia in CT scans and laboratory confirmation (positive real-time reverse transcription-polymerase chain reaction assays [RT-PCR]) [212]. Exclusion criteria was medical records unavailability. For patients who presented to the hospital, those who had possible exposure to the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, the aetiological agent causing COVID-19) or had no identifiable exposure but clinical or imaging manifestations were tested for SARS-CoV-2.

For data from UK, RT-PCR testing of throat swab specimens for SARS-CoV-2-specific

RNA was performed repeatedly per participant to confirm the status of SARS-CoV-2 infection. The Abbott Laboratories (Illinois, USA) chemiluminescent microparticle immunoassay (CMIA) against the nucleocapsid protein (N) of SARS-CoV-2 was used to assess the anti-SARS-CoV-2 antibody IgG levels and IgG statuses (detectable/positive or undetectable/negative) of serum/plasma samples.

2.3 Outcomes

The outcomes in this thesis included severe/critically ill COVID-19, deterioration of COVID-19 (i.e., the dramatic progression from asymptomatic or mild or moderate status into severe or critically ill status during 14 days' follow-up), respiratory failure, and the IgG status (detectable/positive or undetectable/negative) and levels measured repeatedly during the follow up.

2.4 Other variables

2.4.1 Demographic features

Demographic features analysed in this thesis included age (year), gender, and race.

2.4.2 Epidemiologic features

Epidemiologic features included exposure types (imported case or local case) and types of disease onset (single onset or clustered onset).

2.4.3 Clinical features

Clinical features included initial symptoms (fever, cough, sputum, shortness of breath, anorexia, diarrhoea, and loss of smell and taste [loss of smell and taste, loss of smell only, loss of taste only, neither loss of smell nor taste]), medical history (hypertension, coronary heart disease, diabetes, stroke, smoking, and drinking), vital signs (temperature [°C], heart rate [HR, beats/minute], systolic blood pressure [SBP, mmHg], diastolic blood pressure [DBP, mmHg], respiratory rate [breaths/minute], and SpO_2 [%]), treatments (supportive treatments and medication) and so on.

2.4.4 Laboratory features

Laboratory features included blood test parameters (WBC count [10^9/L], neutrophil count [10^9/L], lymphocyte count [10^9/L], haemoglobin [g/L], and platelet count [10^9/L]), organ function parameters (albumin [g/L] and creatinine [umol/L]), inflammatory factors (C-reactive protein [mg/L]), coagulation function parameters (activated partial thromboplastin time [APTT, s], fibrinogen [g/L], and D-dimer [mg/L]) and so on.

2.4.5 Radiologic features

Radiologic features included lesion distribution (outer third of lung involved, middle third of lung involved, or inner third of lung involved), lesion density (below 20% consolidation, 20% to 80% consolidation, or above 80% consolidation), lesion border (well-defined border, moderately defined border, or ill-defined border), quadrant score, and pulmonary opacity score.

2.5 Statistical analysis

Continuous variables were described using means (standard deviations, SD) or medians (with inter-quartile range, IQR) and were compared using ANOVA or Kruskal-Wallis tests as appropriate. Categorical variables were summarised using frequencies and percentages and compared using χ^2 or Fisher exact tests. In addition, this research conducted inferential statistical analysis through multiple logistic regression models, control possible confounding factors, and identify independent risk factors that become severe/critical cases, experience disease deterioration, and have respiratory failure. Odds ratios (ORs) for having the severe/critical illness, disease progression, and respiratory failure for each variable were calculated along with 95% confidence intervals (CIs).

To assess the linear trend effect of age and pulmonary opacity score on baseline features and clinical variables, generalised linear models (GLMs) were employed with age and pulmonary opacity score as the only predictor, respectively. Normal distribution and identity link function were used for continuous variables whereas binomial distribution and logit link function were used for binary variables.

A nomogram for the prediction of severe COVID-19 was established and was internally validated on the derivation cohort from Jiangsu province using the bootstrap method and further externally validated on a separate independent validation cohort from Huangshi, Huanan province.

To explore potential factors associated with IgG levels in COVID-19, the generalised linear mixed models (GLMMs) with normal distribution and identity link function, predictive

variables as fixed effects, and subject as random effect, were employed. The natural logarithm of IgG level was the dependent variable. Geometric mean ratios (GMRs) and 95% (CIs) were estimated by taking an antilog transformation of estimates coming from the GLMMs. Half-life of IgG levels was also calculated from the model.

To deal with missing data and provide unbiased estimates of risk factors of severe/critical illness, disease progression and respiratory failure, missing covariates at admission were imputed with multiple imputation using a Markov Chain Monte Carlo simulation method with 10 iterations in logistic regression analysis. Sensitivity analyses were performed on the completed cases.

When constructing the nomogram for the prediction of severe COVID-19, no categorical data were missing, and missing continuous data were imputed with medians. When estimating of the potential influential factors of IgG antibody levels, missing data of baseline characteristics were imputed by median (continuous variables) and category which occupies the majority (categorical variables) in the generalised linear mixed model.

The STROBE guidelines for reporting cohort study and TRIPOD guidelines for transparent reporting of a multivariable prediction model for individual prognosis were followed.

The 2-tailed $P < 0.05$ was considered as statistically significant. Statistical analyses were performed using SAS 9.4 software (SAS Institute) and R software (version 3.6.0, http://www.R-project.org). The “rms” package was used to derive a user-friendly nomogram.

Chapter 3 Clinical characteristics and risk factors of patients with severe COVID-19 in Jiangsu province, China: a retrospective multicentre cohort study

Because understanding the factors associated with COVID-19 disease severity could support the early identification of patients with high risk for disease progression, inform prevention and control measures, as well as potentially reduce mortality, the first main chapter of this work (Chapter 3) aims to describe the characteristics of patients with COVID-19 and factors associated with severe or critically ill presentation.

3.1 Background

Coronavirus Disease 2019 (COVID-19), caused by the etiological agent Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), was first reported from Wuhan, Hubei province, China, in December 2019. The World Health Organization (WHO) declared a pandemic the 11th March 2020 [1]. The COVID-19 pandemic have spread quickly from a focal outbreak to over 7 410, 000 cases with more than 400, 000 deaths affecting more than 140 countries by the 12th June 2020 [2].

China had reported over 80, 000 confirmed cases by the 13th March 2020 [2]. Although the original epicentre was located in Wuhan, other provinces became affected in the following weeks. In a case series of the first 44,672 confirmed cases, 1023 patients had died, with a crude case fatality rate (CFR) of 2.3%, and mortality was higher among critically ill patients, who had a CFR of 49% [51]. In Hubei, the proportion of severe COVID-19 cases was higher than in other provinces (17.7% and 7.0%, respectively) [51]. Compared to China, the crude CFR in

South Korea is lower among both males (1.1%) and females (0.4%) [52], and the crude CFR in Australia (1.4%) [53] and in countries of the European Union (EU) and European Economic Area (EEA) was also lower (1.5%) [54], while the crude CFR in Italy was much higher (7.2%) [55].

COVID-19 initial symptoms are not specific, presenting with fever, and cough, which can then resolve spontaneously or progress to shortness of breath, dyspnoea, and pneumonia, leading to acute respiratory distress syndrome (ARDS), renal failure, coagulation dysfunction, multiple organ failure and death [36-40,44]. Demographic features (eg age and gender); epidemiological features (eg smoking and comorbidities); laboratory parameters (eg c-reactive protein, albumin, cytokine, lactate dehydrogenase, D-dimer, platelet, lymphocyte, and neutrophil); clinical manifestations (eg cough, expectoration, chest pain, and dyspnea); computer tomography (CT) image test results; and others, have been reported to be associated with the severity of COVID-19 in some small studies in China [67,192-197], and other countires [40,76,198].

Understanding the factors associated with COVID-19 disease severity could support the early identification of patients with high risk for disease progression and inform prevention and control activities and reduce mortality. Hubei was the COVID-19 epicentre of China at the time of data collection for this study (from the 10th January 2020 to the 15th March 2020), so patients in other parts of China, outside Hubei, may have different profiles of demographic, epidemiological, clinical characteristics, laboratory parameters, and image test results. Knowing those profiles may help predict the severity of COVID-19.

Jiangsu, a province in China over 600 km from Hubei without common geographical borders and 80 million population, reported over 600 patients infected with COVID-19. We report here an analysis of all cases in Jiangsu province from the 10th January 2020 to the 15th March

2020 to describe the demographic, epidemiological, clinical, laboratory, and imaging characteristics of cases and to identify risk factors for severe/critically ill COVID-19 presentation. Previous studies were mostly small and descriptive in nature. Our study will use data from the whole Jiangsu province and make inferential statistical analyses by means of multivariate regression model to control for possible confounding factors and identify independent risk factors of becoming a severe/critically ill case.

3.2 Methods

3.2.1 Study Design and Population

This is a multicentre retrospective cohort study. All patients were included if they (1) were clinically diagnosed and then confirmed to have COVID-19 in Jiangsu province from the 10th January 2020 up to the 15th March 2020, and (2) fulfilled the diagnostic criteria for the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" released by National Health Commission & National Administration of Traditional Chinese Medicine of China [212]. The diagnosis of COVID-19 was based on epidemiological history, clinical manifestations, and laboratory confirmation [58]. Patients without medical records were excluded.

The Ethics Committee of Zhongda Hospital, Affiliated to Southeast University, approved the study protocols (2020ZDSYLL013–P01 and 2020ZDSYLL019–P01). Patient informed consent was waived due to the retrospective study design.

3.2.2 Data Measures

The primary outcome was severe or critically ill within the follow up period. Patients were categorised by disease severity into (1) asymptomatic or mild, or moderate, and (2) severe or critically ill, according to "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" [212]. Asymptomatic infections were defined as the absence of clinical symptoms with a positive nucleic acid test (real-time reverse transcriptase–polymerase chain reaction assay, RT-PCR, for SARS-CoV-2). Mild COVID-19 disease was defined as the presence of mild clinical symptoms without respiratory distress and the absence of imaging manifestations of pneumonia. Moderate disease was defined as the presence of fever, with respiratory symptoms and an image of pneumonia in CT scans. Severe disease was defined as the presence of at least one of the three conditions: respiratory distress, a respiratory rate ≥ 30 beats / min; oxygen saturation in resting state ≤ 93% or an arterial blood oxygen partial pressure / oxygen concentration ≤ 300 mmHg (1 mmHg = 0.133kPa). Critically ill was defined as having respiratory failure requiring mechanical ventilation, shock or combined organ failure requiring intensive care unit (ICU) monitoring and treatment. A clustering onset was defined as the occurrence of two or more confirmed COVID-19 cases in the same cluster/group within 14 days, such as family, community, hospital, working place or public place, etc. A clustering onset could occur due to interpersonal transmission via close contact with or joint exposure to a confirmed COVID-19 case. Other cases not meeting the conditions of the clustering onset were classified as a single onset. All patients were followed up to the 15th March 2020.

Data were collected using case record forms and electronic medical record systems and included demographic, epidemiological, clinical, laboratory, and imaging information

provided by the Data Center of Jiangsu Provincial Health Commission without any patient's personal information. The day of admission to a COVID-19-designated hospital was considered the first day of hospitalisation. The severity of illness was assessed by two physicians. Severity was assessed at days 1, 2, 3, 4, 5, 6, 7 and 14 after admission and patients were followed for up to discharge. ICU admissions were recorded. Imaging grading was performed by two independent radiologists with more than 5 years' experience in pulmonary imaging. Chest CT axial sections were divided into quadrants (left and right, anterior and posterior) by drawing horizontal and vertical lines through the centre of the chest. Quadrant scores were estimated as the sum of quadrants with pulmonary opacities extending from the proximal to the distal end of the chest and ranged from 0 and 4. Pulmonary opacity was visually estimated and assigned a percentage of pulmonary opacity area in the area of bilateral lungs, rounded to the nearest 5%.

3.2.3 Statistical Analysis

Variables

Primary outcome variable was the occurrence of severe/critically ill case. Predictive variables included sex, age, exposure type, types of disease onset, initial symptoms, medical history, vital signs (including body temperature, heart rate (HR), respiratory rate, and peripheral capillary oxygen saturation (SpO_2)), CT image parameters (including quadrant score and pulmonary opacity), and laboratory parameters (including white blood cells count, neutrophil, lymphocyte, platelet, albumin, creatinine, C-reactive protein, activated partial thromboplastin time, fibrinogen, and D-dimer). Those variables were measured at hospital admission. The other variables including supportive treatments and medical drugs were

collected during the follow-up period.

Statistical Analysis

Continuous variables were described using means (standard deviations, SD) or medians (with inter-quartile range, IQR) by disease severity and were compared using ANOVA or Kruskal-Wallis tests as appropriate. Categorical variables were summarised using frequencies and percentages and compared using Fisher exact tests.

Logistic regression models were used to identify the risk factors for having a severe or critically ill status. Analysis was performed in 2 steps. Firstly, a univariate logistic regression model was fitted for each variable based on completed cases. As there are many potential predictors, we chose the variables for univariate regression analysis if a variable is significant at 5%. Respiratory rate and SpO_2 were not included in the regression analyses since they were part of criterion for classifying the disease severity. All variables selected for univariate regression analysis were also included in the second stage of the multivariate logistic regression. Missing covariates at admission were imputed with multiple imputation using a Markov Chain Monte Carlo simulation method with 10 iterations. In the logistic regression analysis, odds ratios (ORs) for having a disease progression for each variable were calculated along with 95% confidence intervals (CIs). A sensitivity analysis was performed on the completed cases. The 2-tailed $P < 0.05$ was considered as statistically significant for all analyses. The analyses were performed using SAS 9.4 (SAS Institute).

3.3 Results

From the 10th January 2020 to 15th March 2020, 721 suspected cases with possible COVID-19 were admitted in 24 hospitals in Jiangsu province, China, while 90 cases were excluded because of negative reverse transcriptase–polymerase chain reaction assay. 631 cases were diagnosed with COVID-19 totally. Of these, 625 (99.0%) had retrievable medical records and were included in the analysis (Figure 3.1).

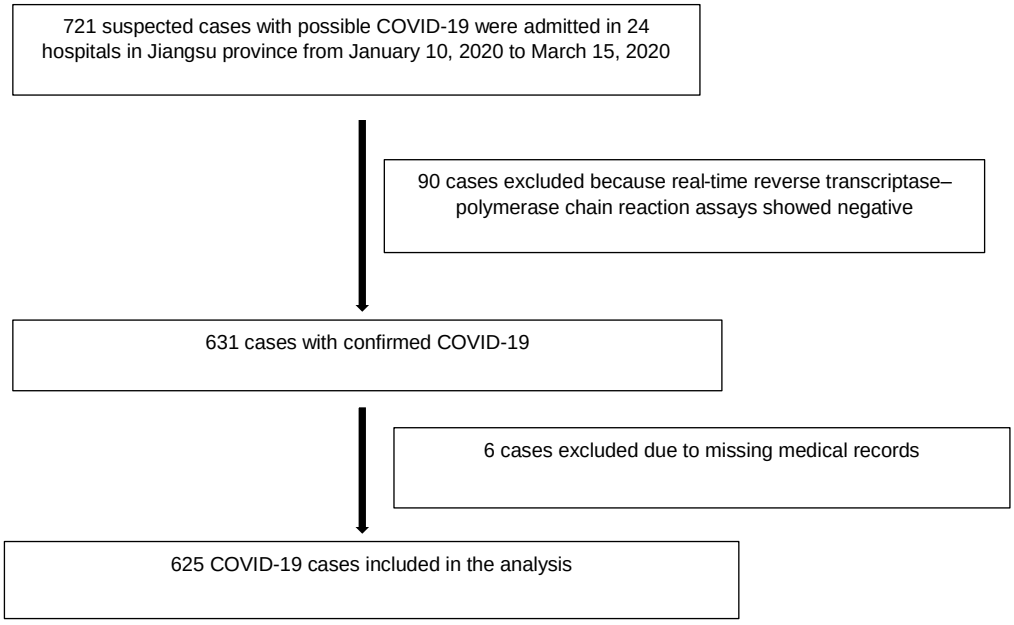

Figure 3.1 Study flow diagram

Table 3.1 describes the characteristics of patients at the time of admission by disease severity and Table S3.1 provides a more detailed description of the five severity categories. 561 (89.8%) patients were asymptomatic/mild/moderate and 64 (10.2%) patients were severe or critically ill. Patients with severe/critically ill COVID-19 were more likely to be older and to be single onset (i.e. not to a cluster of cases in family/community). Patients with severe/critically ill

presentation were more likely to have a medical history of hypertension and diabetes. Patients with severe/critically ill COVID-19 on admission had higher temperature, faster respiratory rates, lower SpO_2, and higher CT image quadrant scores and pulmonary opacity percentage (Table 3.1 and Table S3.1).

Table 3.1 Demographic and clinical characteristics of patients with COVID-19 at admission*

		Severe/Critically ill			
Category	Characteristics	Yes (N=64)	No (N=561)	All (N=625)	P-value
Demographic, , n/N(%), N,mean(SD)					
	Male	41/64(64.1%)	288/561(51.3%)	329/625(52.6%)	0.0534
	Female	23/64(35.9%)	273/561(48.7%)	296/625(47.4%)	
	Age (year)	64,59.53(13.43)	561,42.72(16.73)	625,44.44(17.19)	<.0001
	≤18 years	0/64(0.0%)	37/561(6.6%)	37/625(5.9%)	<.0001
	19-44 years	6/64(9.4%)	255/561(45.5%)	261/625(41.8%)	
	45-64 years	32/64(50.0%)	216/561(38.5%)	248/625(39.7%)	
	65+ years	26/64(40.6%)	53/561(9.4%)	79/625(12.6%)	
Exposure type, n/N(%)					
	Imported cases	25/64(39.1%)	194/561(34.6%)	219/625(35.0%)	0.4765
	Local cases	39/64(60.9%)	367/561(65.4%)	406/625(65.0%)	
Types of disease onset, n/N(%)					
	Single onset	40/64(62.5%)	270/561(48.1%)	310/625(49.6%)	0.0294
	Clustering onset	24/64(37.5%)	291/561(51.9%)	315/625(50.4%)	
Initial symptoms, n/N(%)					
	Fever	52/64(81.3%)	360/561(64.2%)	412/625(65.9%)	0.0063
	Cough	44/64(68.8%)	300/561(53.5%)	344/625(55.0%)	0.0200
	Sputum	25/64(39.1%)	141/561(25.1%)	166/625(26.6%)	0.0168
Medical history, n/N(%)					
	Hypertension	19/64(29.7%)	72/561(12.8%)	91/625(14.6%)	0.0003
	Diabetes	10/64(15.6%)	30/561(5.3%)	40/625(6.4%)	0.0015
	Stroke	2/64(3.1%)	8/560(1.4%)	10/624(1.6%)	0.2736
Vital signs, N,mean(SD)					
	Temperature (°C)	64,37.30(0.94)	561,37.02(0.70)	625,37.05(0.73)	0.0040
	HR (bpm)	64,89.98(15.06)	561,86.84(13.25)	625,87.17(13.46)	0.0772

		Severe/Critically ill			
Category	Characteristics	Yes (N=64)	No (N=561)	All (N=625)	P-value
	Respiratory rate (breath per min)	64,20.98(4.87)	561,18.87(2.04)	625,19.08(2.56)	<.0001
	SpO_2 (%)	64,95.53(4.70)	561,97.92(1.15)	625,97.68(1.99)	<.0001
CT image, N,median(IQR)					
	Quadrant score (1-4)	58,4.0(4.0-4.0)	438,2.0(1.0-4.0)	496,2.0(1.0-4.0)	<.0001
	Pulmonary opacity (%)	58,50.0(35.0-70.0)	438,20.0(5.0-30.0)	496,20.0(5.0-40.0)	<.0001
Laboratory test, N,median(IQR)					
	WBC Count (10^9/L)	52,4.3(3.4-5.8)	461,5.0(4.0-6.3)	513,4.9(3.9-6.2)	0.0440
	Neutrophil (10^9/L)	52,2.9(2.0-4.4)	455,3.0(2.2-4.0)	507,3.0(2.2-4.0)	0.7435
	Lymphocyte (10^9/L)	52,0.7(0.5-1.0)	453,1.4(1.0-1.8)	505,1.3(0.9-1.7)	<.0001
	Platelet (10^9/L)	48,154.0(118.0-185.0)	446,188.5(154.0-222.0)	494,183.5(151.0-219.0)	<.0001
	Albumin (g/L)	48,39.7(34.2-41.4)	432,42.0(38.0-45.7)	480,41.4(38.0-45.1)	<.0001
	Creatinine (umol/L)	49,64.0(51.0-83.0)	427,63.8(51.0-79.0)	476,63.9(51.0-79.0)	0.6950
	C-reactive protein (mg/L)	44,40.1(8.6-92.7)	430,10.0(2.6-19.3)	474,10.0(2.7-22.6)	<.0001
	Activated partial thromboplastin time (s)	54,32.6(28.5-36.5)	459,32.2(27.9-37.4)	513,32.2(28.0-37.2)	0.8158
	Fibrinogen (g/L)	53,4.3(3.2-5.9)	443,3.4(2.7-4.1)	496,3.5(2.7-4.2)	<.0001
	D-dimer (mg/L)	51,0.3(0.2-1.0)	424,0.2(0.1-0.4)	475,0.2(0.1-0.4)	0.0003

*Continuous variables: ANOVA or Kruskal-Wallis tests as appropriate; categorical variables: Fisher exact tests.

Table S3.1 Demographic and clinical characteristics of patients at admission by disease severity*

		Disease severity, total, mean(SD) or total, median(IQR) or n/total(%)						
Category	Characteristics	Asymptomatic (N=24)	Mild (N=35)	Moderate (N=502)	Severe (N=30)	Critically ill (N=34)	All (N=625)	P-value
Demographic	Male	11/24(45.8%)	13/35(37.1%)	264/502(52.6%)	16/30(53.3%)	25/34(73.5%)	329/625(52.6%)	0.0416
	Age (year)	24,36.75(24.91)	35,27.42(16.37)	502,44.07(15.70)	30,57.73(14.50)	34,61.12(12.40)	625,44.44(17.19)	<.0001
Exposure type	Imported cases	3/24(12.5%)	11/35(31.4%)	180/502(35.9%)	14/30(46.7%)	11/34(32.4%)	219/625(35.0%)	0.0936
	Local cases	21/24(87.5%)	24/35(68.6%)	322/502(64.1%)	16/30(53.3%)	23/34(67.6%)	406/625(65.0%)	
Types of disease onset	Single onset	7/24(29.2%)	4/35(11.4%)	259/502(51.6%)	19/30(63.3%)	21/34(61.8%)	310/625(49.6%)	<.0001
	Clustering onset	17/24(70.8%)	31/35(88.6%)	243/502(48.4%)	11/30(36.7%)	13/34(38.2%)	315/625(50.4%)	
Initial symptoms	Fever	0/24(0.0%)	11/35(31.4%)	349/502(69.5%)	27/30(90.0%)	25/34(73.5%)	412/625(65.9%)	<.0001
	Cough	0/24(0.0%)	18/35(51.4%)	282/502(56.2%)	19/30(63.3%)	25/34(73.5%)	344/625(55.0%)	<.0001
	Sputum	0/24(0.0%)	5/35(14.3%)	136/502(27.1%)	12/30(40.0%)	13/34(38.2%)	166/625(26.6%)	0.0005
Medical history	Hypertension	3/24(12.5%)	1/35(2.9%)	68/502(13.5%)	7/30(23.3%)	12/34(35.3%)	91/625(14.6%)	0.0018
	Diabetes	3/24(12.5%)	0/35(0.0%)	27/502(5.4%)	4/30(13.3%)	6/34(17.6%)	40/625(6.4%)	0.0056
Vital signs	Temperature (°C)	24,36.63(0.40)	35,36.79(0.62)	502,37.06(0.71)	30,37.09(0.80)	34,37.49(1.02)	625,37.05(0.73)	<.0001
	HR (bpm)	24,85.33(14.87)	35,89.54(15.80)	502,86.73(12.98)	30,86.50(14.01)	34,93.06(15.49)	625,87.17(13.46)	0.0699
	MAP (mmHg)	24,92.08(12.32)	35,92.31(12.40)	501,97.38(10.33)	30,93.78(11.48)	34,98.40(10.00)	624,96.77(10.67)	0.0035
	Respiratory rate (breath per min)	24,19.0(18.0-20.0)	35,18.0(18.0-20.0)	502,18.0(18.0-20.0)	30,19.0(18.0-22.0)	34,20.0(18.0-23.0)	625,19.0(18.0-20.0)	0.0081
	SpO_2 (%)	24,98.0(98.0-99.0)	35,98.0(98.0-98.0)	502,98.0(98.0-99.0)	30,98.0(95.0-98.0)	34,97.0(93.0-98.0)	625,98.0(97.0-99.0)	<.0001
CT image	Quadrant score (1-4)	14,0.0(0.0-1.0)	22,0.0(0.0-0.0)	402,2.0(1.0-4.0)	29,4.0(3.0-4.0)	29,4.0(4.0-4.0)	496,2.0(1.0-4.0)	<.0001
	Pulmonary opacity (%)	14,0.0(0.0-10.0)	22,0.0(0.0-0.0)	402,20.0(5.0-35.0)	29,50.0(30.0-70.0)	29,60.0(50.0-80.0)	496,20.0(5.0-40.0)	<.0001

*Continuous variables: ANOVA or Kruskal-Wallis tests as appropriate; categorical variables: Fisher exact tests.

Cases with severe/critically ill presentation were more likely to have increased C-reactive protein, fibrinogen, and D-dimer than asymptomatic/mild/moderate cases. Similarly, severe/critically ill cases had lower white blood cells, lymphocyte, and platelet counts and albumin (Table 3.1). As expected, severe cases were more likely to use supportive treatments and medical drugs, including antibiotics and antivirals, except interferon (Table 3.2).

Table 3.2 Clinical management and outcome

		n(%) or median(IQR)			
		Severe/Critically ill			
Category	Clinical management and outcome	Yes (N=64)	No (N=561)	All (N=625)	P-value
Supportive treatments	Inotropic and vasoconstrictive agents	5(7.8%)	0(0.0%)	5(0.8%)	<.0001
	Nasal cannula	53(82.8%)	168(29.9%)	221(35.4%)	<.0001
	Mask	12(18.8%)	2(0.4%)	14(2.2%)	<.0001
	High-flow nasal cannula oxygen therapy	24(37.5%)	1(0.2%)	25(4.0%)	<.0001
	Non-invasive ventilation	34(53.1%)	0(0.0%)	34(5.4%)	<.0001
	Intermittent mandatory ventilation	5(7.8%)	0(0.0%)	5(0.8%)	<.0001
	Prone position	17(26.6%)	1(0.2%)	18(2.9%)	<.0001
	Continuous renal replacement therapy	1(1.6%)	0(0.0%)	1(0.2%)	0.1024
	Extracorporeal membrane oxygenation	2(3.1%)	0(0.0%)	2(0.3%)	0.0103
	Lung transplantation	2(3.1%)	0(0.0%)	2(0.3%)	0.0103
Medical drugs	Traditional Chinese medicine	29(45.3%)	69(12.3%)	98(15.7%)	<.0001
	Immunoglobulin	50(78.1%)	106(18.9%)	156(25.0%)	<.0001
	Interferon	47(73.4%)	456(81.3%)	503(80.5%)	0.1363
	Antioxidants	35(54.7%)	117(20.9%)	152(24.3%)	<.0001
	Glucocorticoid	52(81.3%)	90(16.0%)	142(22.7%)	<.0001
	Thymosin	43(67.2%)	101(18.0%)	144(23.0%)	<.0001
	Neurotrophic drugs	21(32.8%)	81(14.4%)	102(16.3%)	0.0005
	Any antibiotics	59(92.2%)	277(49.4%)	336(53.8%)	<.0001
	Any antivirals	64(100%)	516(92.0%)	580(92.8%)	0.0098
Clinical outcome	Death	0(0.0%)	0(0.0%)	0(0.0%)	NC
	Hospital stay	21.5(15.0-29.0)	15.0(12.0-21.0)	16.0(12.0-22.0)	<.0001

None of the patients died and 625 (100%) of patients were discharged by the end of study (15th March 2020). The results from the univariate and multivariate logistic regression analyses are presented in Table 3.3. Factors independently associated with severe or critically ill infection included age (year) (OR 1.06, 95%CI 1.03-1.09), lymphocyte count (10^9/L) (OR 0.25, 95%CI 0.08-0.74), and pulmonary opacity in CT (per 5%) on admission (OR 1.31, 95%CI 1.15-1.51). Sensitivity analysis showed that they remained statistically significant in a logistic model with only above three variables based on the completed cases without missing data.

Table 3.3 Factors associated with severe/critically ill in patients with COVID-19: Results from logistic regression analysis

	Univariate analysis*		Multivariate analysis**	
Variables	Odds ratio (95%CI)	P-value	Odds ratio (95%CI)	P-value
Age (year)	1.07(1.05,1.09)	<.0001	1.06(1.03,1.09)	<.0001
Single onset	1.80(1.05,3.06)	0.0311	0.92(0.43,1.96)	0.8275
Fever	2.42(1.26,4.64)	0.0078	1.50(0.64,3.54)	0.3542
Cough	1.91(1.10,3.33)	0.0216	1.24(0.54,2.87)	0.6110
Sputum	1.91(1.12,3.27)	0.0183	1.12(0.48,2.60)	0.7994
Hypertension	2.87(1.59,5.18)	0.0005	1.06(0.47,2.40)	0.8874
Diabetes	3.28(1.52,7.07)	0.0025	1.64(0.52,5.22)	0.4004
Temperature (°C)	1.59(1.15,2.19)	0.0046	0.95(0.61,1.47)	0.8133
Lymphocyte (10^9/L)	0.03(0.01,0.08)	<.0001	0.25(0.08,0.74)	0.0161
Platelet (10^9/L)	0.99(0.98,0.99)	0.0003	1.00(0.99,1.00)	0.5147
Albumin (g/L)	0.91(0.87,0.96)	0.0002	0.99(0.92,1.07)	0.8344
C-reactive protein (mg/L)	1.02(1.01,1.02)	<.0001	1.00(0.99,1.01)	0.9789
Fibrinogen (g/L)	1.87(1.50,2.32)	<.0001	1.04(0.72,1.49)	0.8327
D-dimer (mg/L)	1.28(1.06,1.55)	0.0088	1.17(0.83,1.66)	0.3625
Quadrant score (1-4)	2.28(1.71,3.05)	<.0001	0.90(0.56,1.47)	0.6811
Pulmonary opacity (per 5%)	1.38(1.28,1.49)	<.0001	1.31(1.15,1.51)	0.0001

* Univariate analysis is based on the complete cases without missing value.

** Multivariate analysis is based on imputed values for missing data in Lymphocyte, Platelet, Albumin, C-reactive protein, Fibrinogen, D-dimer, Quadrant score and Pulmonary opacity using multiple imputation method.

3.4 Discussion

In this large multicentre cohort, 64 (10.2%) of 625 patients were severe or critically ill. This proportion of severe or critically ill cases is lower than the 17.7% reported among the 44 672 cases from Wuhan but similar to the 7.0% reported for areas outside Hubei province [51], which are lower than reported from several case series from Wuhan including 13 (32%) ICU admission among 41 cases with 6 (15%) deaths [48]; 11 (11%) deaths among 99 cases [37]; and 36 (26.1%) ICU admissions among 138 patients, with 6 deaths (4.3%) [44]. The lower proportion than that in Hubei is likely due to several factors, including more adequate medical resources, better disease recognition and testing capacity, earlier identification of asymptomatic and mild cases and a more informed supportive care in COVID-19-designated hospitals. The proportion of critically ill cases in early stage of COVID-19 outbreak in New York City, USA was much higher (22% [257]) [213] than in China, with 14.2% treated in the ICU [200] and 23.6% required mechanical ventilation reported in other studies [214]. Singapore also reported a higher proportion of severe cases in an early study (33.3% [6]) [215]. A more recent study from USA reported 5.8% (7,162) of cases suffered from severe COVID-19 [216], which is lower than in China. The proportions of severe COVID-19 in different countries vary a lot, which may result from quite different study designs, the COVID-19 outbreak stages when data were collected, population characteristics, health resources, government response measurements, et al.

Despite this being a hospital-based study, some patients had no symptoms. This is

likely due to testing of contacts after the identification of an index case and the policy of hospitalization of all infected individuals at the initial stages of the epidemic, independently of the presence of symptoms. This study found that fever, cough, and sputum were very common among patients with COVID-19 and more frequent in patients with severely or critically illness. Similar to COVID-19, fever and cough are the most common symptoms of the other two diseases caused by coronavirus, i.e. severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) [217]. Fever is a primary symptom for cytokine storms, with the production of high concentrations of cytokines stimulating abnormally excessive immune responses and inflammation [85,218-220]. Vital signs showed severe or critically ill patients had higher body temperature and respiratory rate, and lower SpO_2 on admission. SpO_2 <90% has been used as a marker for the use of glucocorticoids during the outbreak [221], and the oxygenation saturation index is associated with ARDS severity and increased mortality [222,223].

Gender have no effect on severity of COVID-19 patients. Although early reports from Wuhan indicated more men than women had severe COVID-19, recent studies reported similar proportions of men and women admitted to ICUs [37,38,48], suggesting gender differences disappeared with higher incidence. Earlier reports may have included more males due to a higher occupational infection risk for males in the markets and congregation places [44].

Our study found that age was independently associated with severe or critically ill presentation. Age is a well well-established factor for severe/critically ill COVID-19 for individuals > 60, and especially over 80 years old [224,225]. Similarly, previous reports

have indicated patients in ICUs are older than non-ICU patients [44], and that CFRs are higher among older individuals [37,38,48]. Older patients also have faster disease progression than younger patients [226], which is similar to the MERS and SARS presentations, in which, older age (> 60 or 45) is associated with disease severity (MERS) [227,228], and mortality [229,230]. Older age reflects a greater likelihood of underlying medical conditions such as hypertension and diabetes, which predisposes to immunological vulnerabilities. Also, age-related immunosenescence may also contribute to the severe disease [231].

In Wuhan, many asymptomatic patients had abnormal lung CT findings on admission, which then progressed to diffuse ground-glass opacities and consolidation [232]. In Jiangsu, several asymptomatic cases also had radiological changes presented as low quadrant scores and pulmonary opacity scores on admission and severe/critically-ill cases had higher CT quadrant and pulmonary opacity scores than moderate cases. Our study also identified pulmonary opacity as an independent predictor of severe/critical illness. This is consistent with the previous study reporting that the CT visual quantitative evaluation of acute lung inflammatory lesions involving each lobe in severe or critical cases was significantly higher than less severe cases [233].

We found severe or critically ill patients had more obvious damage of white blood cells and immune cells such as lymphocytes with lymphocytes identified as an independent predictor of more severe disease. COVID-19 may cause the reduced T lymphocytes, especially CD4 + T and CD8 + T cells, leading to reduced IFN-γ production, which may be related to the severity of disease [234]. In addition, severe or critically ill patients showed more serious organ dysfunction like reduced albumin on admission which may

be a sign of reduced liver production and increased gastrointestinal or renal loss, and increased fibrinogen on admission responding to systemic inflammation and tissue damage, and more fierce inflammatory response presented as much higher level of inflammatory markers, such as C-reactive protein [235-237].

This study has several strengths. Firstly, this is one of the largest studies describing the clinical characteristics of patients with COVID-19 and risk factors for severe/critically ill infection outside the Wuhan, epicentre of the epidemic in China at the time of data collection for this study (from the 10th January 2020 to the 15th March 2020). Secondly, the cohort includes almost all COVID-19 cases in the province, which may have reduced selection bias. Thirdly, Jiangsu province, which is far from Hubei, provides an opportunity to assess the demographic, epidemiological, clinical, laboratory, and imaging features of cases imported from other provinces and local cases. Fourthly, asymptomatic and mild cases were included, which provides a more comprehensive description of the characteristics of COVID-19 cases with a broad spectrum of disease severity.

There are also limitations that need mentioning. Firstly, laboratory and radiological data had a large amount of missing data preventing their integration in the analysis. Secondly, the predictive factors identified may be subject to uncontrolled confounders by unknown/unmeasured factors such as occupation and pregnancy. Medical staff and pregnant women may have different severity profiles. Thirdly, this is a retrospective observational study and data were susceptible to measurement and information bias.

3.5 Conclusion

The first main chapter of this work (Chapter 3) demonstrates that patients with COVID-19 in Jiangsu had a low rate of severe or critically ill presentation, with no deaths recorded. The COVID-19 severity is associated with epidemiological and clinical characteristics, laboratory test, and radiological findings. Age, lymphocyte count, and pulmonary opacity in CT on admission were independently associated with risk of severe or critically ill COVID-19 presentation. The information could understand patients with high risk of severe or critically ill COVID-19 and inform prevention and control activities and reduce mortality.

Similarly, it may be helpful to further explore risk factors of deterioration, i.e. the dramatic progression from asymptomatic or mild or moderate status into severe or critically ill status during follow-up. Some patients may stay in asymptomatic or mild or moderate status while other patients may experience disease deterioration, from asymptomatic or mild or moderate status into severe or critically ill status. Understanding what factors may affect the deterioration and taking appropriate preventive measures may reduce the likelihood of deterioration. This will be done in the next chapter (Chapter 4).

Chapter 4 Disease progression in patients with COVID-19: a retrospective cohort study in China

Chapter 3 provides insight into risk factors of being severe or critically ill status due to COVID-19. This helps in identifying patients with high risk of severe disease and taking early management measures. However, it is also worth investigating risk factors of another group, i.e. patients experiencing disease deterioration, from asymptomatic or mild or moderate status into severe or critically ill status. This may help prevent patients with asymptomatic or mild or moderate disease from progressing into severe disease and is the focus of this chapter (Chapter 4).

4.1 Introduction

The WHO declared COVID-19 a pandemic on 11 March 2020.[1] During their clinical course, some patients experienced deterioration in clinical symptoms, and some cases progressed rapidly to acute respiratory distress syndrome (ARDS), septic shock, metabolic acidosis, coagulopathy, multi-organ system failure, death or other poor outcomes [41-43]. A study on clinical course and mortality of adult inpatients with COVID-19 in Wuhan, China, found that the mortality of severe and critically ill patients was respectively 22% and 78% [42]. Another study has reported risk factors for progression from ARDS to death in patients with COVID-19 [41]. However, neither the pattern of disease progression from “moderate or less” status to “severe/critically-ill” status nor their associated factors has not been fully investigated in patients with COVID-19. Assessment of patterns of disease progression and identification of factors associated

with disease progression could help physicians to prospectively recognise patients at high risk of progression and help patients to avoid a crisis phase linked to oxygen desaturation profiles.

This multicentre retrospective cohort study set out to describe the occurrence of disease progression in patients with COVID-19 and explore the factors associated with progression from “moderate or less” status to “severe or critical” illness.

4.2 Methods

4.2.1 Study design and participants

This retrospective cohort study included all the patients who met the patient inclusion and exclusion criteria. Inclusion criteria were, as of 29 February 2020, all patients diagnosed with COVID-19 in Jiangsu according to the diagnostic criteria of “Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)” released by China’s National Health Commission & National Administration of Traditional Chinese Medicine (Beijing, China) [212]. The diagnosis of COVID-19 was based on epidemiological history, clinical manifestations, imaging manifestations of pneumonia in computer tomography (CT) scans and laboratory confirmation (positive real-time reverse transcription-polymerase chain reaction assays [RT-PCR]) [212]. Exclusion criteria was medical records unavailability. For patients who presented to the hospital, those who had possible exposure to the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, the aetiological agent causing COVID-19) or had no identifiable exposure but clinical or imaging manifestations were tested for SARS-CoV-2. The discharge standard was that body temperature was normal for more than 3 days, symptoms

were relieved for patients with any symptoms and RT-PCR (throat swab samples, at least 1 day for sampling interval) showed negative for two consecutive times.

4.2.2 Data collection and definition of variables

The epidemiological, clinical, laboratory and radiological parameters were collected on admission. Data on disease severity were available at Days 1, 2, 3, 4, 5, 6, 7 and 14 after admission, except for those who were discharged, and data on mortality and hospitalisation status were available until 29 February 2020. The primary outcome was disease deterioration, i.e., dramatic progression from “asymptomatic” or “mild or moderate” status on admission, to “severe or critically” ill status during 2 weeks’ follow-up. Dramatic progression in our study does not include fragile progression such as progression from “asymptomatic” to “mild” status or from “mild” status to “moderate” status or “severe” status to “critically ill” status. Two attending physicians were invited to determine disease severity. Asymptomatic infection was defined as the absence of clinical symptoms but a positive nucleic acid test result. Mild disease was defined as having mild clinical symptoms and the absence of imaging manifestations of pneumonia in CT scans. Moderate disease was defined as the presence of fever, respiratory tract symptoms or other symptoms and imaging manifestations. Severe disease was defined as the presence of at least one of the following: respiratory distress, respiratory rate ≥30 breaths/min, oxygen saturation in resting state (SpO_2) ≤93%, or arterial blood oxygen partial pressure (PaO_2)/fraction of inspired oxygen (FiO_2) ≤300 mmHg (1 mmHg = 0.133 kPa). Critically ill was defined as having respiratory failure requiring mechanical ventilation, shock or combined organ

failure requiring intensive care unit (ICU) monitoring and treatment.

All patients in Jiangsu underwent high-resolution CT thorax examination to determine lung lesions. CT images were assessed visually by two radiologists with more than 5 years of experience in chest imaging. The radiologists were blinded to the patient characteristics. Quadrant scores were the sum of the number of quadrants containing pulmonary opacities extending from the proximal to the distal end of the chest and had a score between 0 and 4. For pulmonary opacity, bilateral lungs were scored manually and assigned an estimated proportion of pulmonary opacity relative to the whole lung, rounded to the nearest 5%.

4.2.3 Statistical analysis

A summary table was generated to present dynamic patterns of disease progression in severity at each follow-up day by three categorised disease severity groups (1 = asymptomatic/mild, 2 = moderate and 3 = severe/critically ill) on admission. We also generated a table to present the disease progression to worst severity during 14-day hospitalisation among COVID-19 patients. Continuous variables were reported as means ± standard deviation (SD) or median (interquartile range [IQR]) by group (patients with and without disease deterioration) and compared using Student's *t*-test or Mann-Whitney *U*-test depending on their distributions. Categorical variables were summarised using frequency and percentage and compared using χ^2 or Fisher's Exact test.

Logistic regression models were used to identify the risk factors for experiencing disease deterioration. Variables that were significant at the significance

level of 5% in the univariate logistic regression analysis were included in the multivariate logistic regression. Missing covariates at admission were imputed in multivariate regression model analysis with multiple imputation using a Markov Chain Monte Carlo simulation method with 10 iterations. In the logistic regression analysis, odds ratios (ORs) for having a disease progression for each variable were calculated along with their 95% confidence intervals (CIs). Two-tailed $P < 0.05$ was considered statistically significant for all analyses. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA).

4.2.3 Ethics approval

The study was approved by the Ethics Committee of Zhongda Hospital Affiliated to Southeast University, Zhongda, China (2020ZDSYLL013-P01 and 2020ZDSYLL019-P01). Requirement for patient informed consent was waived due to the retrospective study design.

4.3 Results

From 10 January to 29 February 2020, 721 presumptive cases of COVID-19 were admitted in 24 hospitals in Jiangsu Province, China; 90 were excluded because of negative RT-PCR result and 631 cases were diagnosed with COVID-19 in total. This study included 625 cases who had complete medical records (Figure 4.1). The median age was 46 years (IQR 32–57; range 0.75–96); 329 (52.6%) were male. No deaths were reported during the study.

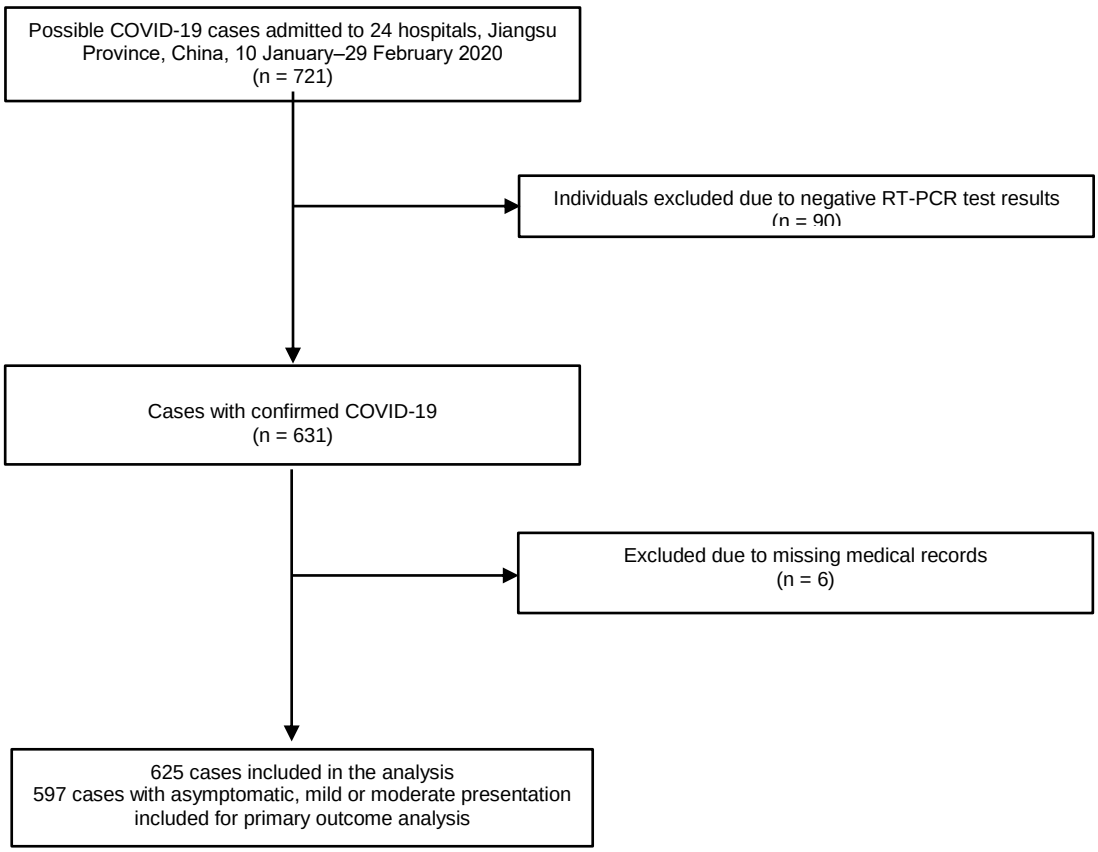

Figure 4.1 Study flow diagram. RT-PCR = real-time reverse transcription-polymerase chain reaction.

Supplementary Table S4.1 shows the dynamic patterns of disease progression by follow-up day among patients with COVID-19. On admission (Day 1), overall 109 (17.4%) patients were asymptomatic or had mild status, 488 (78.1%) had moderate status and 28 (4.5%) were severely or critically ill. Overall, changes in disease severity from admission showed an increased proportion of moderate cases deteriorating into severe or critically ill cases, with 1.6% (*n* = 8) at Day 2 progressively increasing up to 5.2% (*n* = 25) at Day 7.

Table S4.1 Disease progression by day among patients with COVID-19

		Disease severity at Day 1			
Day	Statistics	Asymptomatic/Mild	Moderate	Severe/Critically ill	All
Day 2	n	109	488	28	625
	Asymptomatic or mild	101(92.7%)	0(0.0%)	0(0.0%)	101(16.2%)
	Moderate	7(6.4%)	480(98.4%)	0(0.0%)	487(77.9%)
	Severe or critically ill	1(0.9%)	8(1.6%)	28(100%)	37(5.9%)
Day 3	n	109	488	28	625
	Asymptomatic or mild	90(82.6%)	6(1.2%)	0(0.0%)	96(15.4%)
	Moderate	17(15.6%)	469(96.1%)	0(0.0%)	486(77.8%)
	Severe or critically ill	2(1.8%)	13(2.7%)	28(100%)	43(6.9%)
Day 4	n	109	488	28	625
	Asymptomatic or mild	83(76.1%)	3(0.6%)	0(0.0%)	86(13.8%)
	Moderate	24(22.0%)	465(95.3%)	0(0.0%)	489(78.2%)
	Severe or critically ill	2(1.8%)	20(4.1%)	28(100%)	50(8.0%)
Day 5	n	109	488	28	625
	Asymptomatic or mild	77(70.6%)	8(1.6%)	0(0.0%)	85(13.6%)
	Moderate	31(28.4%)	458(93.9%)	1(3.6%)	490(78.4%)
	Severe or critically ill	1(0.9%)	22(4.5%)	27(96.4%)	50(8.0%)
Day 6	n	105	481	28	614
	Asymptomatic or mild	57(54.3%)	15(3.1%)	0(0.0%)	72(11.7%)
	Moderate	48(45.7%)	443(92.1%)	4(14.3%)	495(80.6%)
	Severe or critically ill	0(0.0%)	23(4.8%)	24(85.7%)	47(7.7%)
Day 7	n	105	481	28	614
	Asymptomatic or mild	76(72.4%)	20(4.2%)	0(0.0%)	96(15.6%)
	Moderate	29(27.6%)	436(90.6%)	6(21.4%)	471(76.7%)
	Severe or critically ill	0(0.0%)	25(5.2%)	22(78.6%)	47(7.7%)
Day 14	n	65	328	24	417
	Asymptomatic or mild	35(53.8%)	50(15.2%)	1(4.2%)	86(20.6%)
	Moderate	30(46.2%)	260(79.3%)	10(41.7%)	300(71.9%)
	Severe or critically ill	0(0.0%)	18(5.5%)	13(54.2%)	31(7.4%)

Table 4.1 presents disease progression in severity from admission to the worst severity during the 14-day hospital stay among COVID-19 patients. Of the 625 patients, 83.7% (*n* = 523) had a stable condition or became better during 14 days' hospitalisation, whereas 16.3% (*n* = 102) patients progressed by at least one degree in disease severity. Some patients had disease deterioration, i.e., dramatic progression

from asymptomatic or mild or moderate status on admission, to severe or critically ill status, during 2 weeks of hospital stay. Of 597 patients, 36 (6%) had dramatic progression from Day 2 to 14 after admission.

Table 1 Disease progression to worst severity during 2-week follow-up from admission among patients with COVID-19

	Worst severity during 2-week follow-up					
	Asymptomatic	Mild	Moderate	Severe	Critically ill	
Severity at admission	*n/N* (%)	*n/N* (%)	*n/N* (%)	*n/N* (%)	*n/N* (%)	Total
Asymptomatic	24/55 (44)	7/55 (13)	23/55 (42)	1/55 (2)	0/55 (0)	55
Mild	0/54 (0)	28/54 (52)	25/54 (46)	1/54 (2)	0/54 (0)	54
Moderate	0/488 (0)	0/488 (0)	454/488 (93)	19/488 (4)	15/488 (3)	488
Severe	0/20 (0)	0/20 (0)	0/20 (0)	9/20 (45)	11/20 (55)	20
Critically ill	0/8 (0)	0/8 (0)	0/8 (0)	0/8 (0)	8/8 (100)	8
Total	24/625 (4)	35/625 (6)	502/625 (80)	30/625 (5)	34/625 (5)	625/625 (100)

Compared to patients without dramatic progression ($n = 561$) during the 14-day hospitalisation, patients with dramatic progression ($n = 36$) were significantly older (mean age: 60.97 years, SD 12.67 vs. 42.72 years, SD 16.73; $P < 0.0001$); more likely to be imported cases who had a contact history with the pandemic centre in Wuhan (having been to or contacted with a visitor from Wuhan) (52.8% vs. 34.6%; $P = 0.0272$); more likely to have prior histories of hypertension (27.8% vs. 13.5%; $P = 0.0184$) and diabetes (16.7% vs. 5.3%; $P = 0.0057$); more likely to have lower mean SpO_2 (97.17%, SD 1.81% vs. 97.92%, SD 1.15%; $P = 0.0003$); and more likely to have higher median CT quadrant score (4.0, IQR 0.0–4.0 vs. 2.0, IQR 0.0–4.0; $P < 0.0001$); and more likely to have higher median proportion of pulmonary opacity volume (50.0%, IQR 0.0–80.0 vs. 20.0%, IQR 0.0–80.0; $P < 0.0001$) (Table 4.2). Patients with disease deterioration had also significantly lower median lymphocyte count (0.8 10^9/L, IQR 0.2–1.5 vs. 1.4 10^9/L,

IQR 0.3–3.6; $P < 0.0001$) and median platelet count (155.5 10^9/L, IQR 92.0–236.0 vs. 188.5 10^9/L, IQR 51.0–530.0; $P = 0.0004$). In addition, patients with disease deterioration had significantly higher median levels of C-reactive protein (26.2 mg/L, IQR 0.5–250.4 vs. 10.0 mg/L, IQR 0.5–208.2; $P = 0.0020$) and median fibrinogen levels (4.2 g/L, IQR 1.5–7.0 vs. 3.4 g/L, IQR 0.9–8.2; $P = 0.0175$).

Table 4.2 Demographic and clinical characteristics of patients with COVID-19 at admission

			Disease progression*		
		All (*N* = 597)	Yes (*N* = 36)	No (*N* = 561)	
Category	Characteristics	*n* (%)	*n* (%)	*n* (%)	*P* value†
Demographic	Male	309 (51.8)	21 (58.3)	288 (51.3)	0.4924
	Age, years, mean ± SD	43.82 ± 17.07	60.97 ± 12.67	42.72 ± 16.73	<0.0001
Exposure type	Imported cases	213 (35.7)	19 (52.8)	194 (34.6)	0.0272
	Local cases	384 (64.3)	17 (47.2)	367 (65.4)	
Types of disease onset	Single onset	293 (49.1)	23 (63.9)	270 (48.1)	0.0667
	Clustering onset	304 (50.9)	13 (36.1)	291 (51.9)	
Initial symptoms	Fever	388 (65.0)	28 (77.8)	360 (64.2)	0.0971
	Cough	322 (53.9)	22 (61.1)	300 (53.5)	0.3730
	Sputum	153 (25.6)	12 (33.3)	141 (25.1)	0.2747
Medical history	Hypertension	86 (14.4)	10 (27.8)	76 (13.5)	0.0184
	Diabetes	36 (6.0)	6 (16.7)	30 (5.3)	0.0057
Vital signs, mean ± SD	Temperature	37.04 ± 0.72	37.26 ± 0.89	37.02 ± 0.70	0.0507
	HR, bpm	86.88 ± 13.39	87.39 ± 15.73	86.84 ± 13.25	0.8135
	Respiratory rate, breaths per min	18.87 ± 2.05	19.00 ± 2.32	18.87 ± 2.04	0.7051
	SpO_2, %	97.88 ± 1.21	97.17 ± 1.81	97.92 ± 1.15	0.0003
CT image, *n*; median [IQR]	Quadrant score (1–4)	471; 2.0 [0.0–4.0]	33; 4.0 [0.0–4.0]	438; 2.0 [0.0–4.0]	<0.0001
	Pulmonary opacity, %	471; 20.0 [0.0–80.0]	33; 50.0 (0.0–80.0]	438; 20.0 (0.0–80.0]	<0.0001
Laboratory test, *n*; median [IQR]	Lymphocyte, 10^9/L	481; 1.3 [0.2–3.6]	28; 0.8 [0.2–1.5]	453; 1.4 [0.3–3.6]	<0.0001
	Platelet, 10^9/L	472; 184.5 [51.0–530.0]	26; 155.5 [92.0–236.0]	446; 188.5 [51.0–530.0]	0.0004
	C-reactive protein, mg/L	455; 10.0 [0.5–250.4]	25; 26.2 [0.5–250.4]	430; 10.0 [0.5–208.2]	0.0020
	Fibrinogen, g/L	473; 3.4 [0.9–8.2]	30,; 4.2 [1.5–7.0]	443; 3.4 [0.9–8.2]	0.0175

*The primary outcome was disease deterioration, i.e., dramatic progression from asymptomatic or mild or moderate status on admission to severe or critically ill status at 2 weeks' follow-up.

†From testing whether these characteristics are different between patients with and without disease deterioration.

SD = standard deviation; HR = heart rate; SpO_2 = oxygen saturation; IQR = interquartile range.

Eleven variables were selected for univariate and multivariate logistic regression analyses (Table 4.3). For multivariable logistic regression model, four variables measured at admission were identified to be independently related to the occurrence of disease progression: age (in years) (OR 1.08, 95% CI 1.04–1.12; $P < 0.0001$); pulmonary opacity score (per 5%) (OR 1.32, 95% CI 1.12–1.57; $P = 0.0015$); lymphocyte count (10^9/L) (OR 0.28, 95% CI 0.09–0.91; $P = 0.0357$); and imported cases (exposed to the pandemic centre in Wuhan) (OR 2.45, 95% CI 1.03–5.80; $P = 0.0421$).

Table 4.3 Factors associated with disease progression in patients with COVID-19: results from logistic regression analysis (*n* = 597)

	Univariate analysis*			Multivariate analysis†		
Variables	OR (95%CI)	*P* value	χ^2	OR (95%CI)	*P* value	$\chi^{2‡}$
Age, years	1.08 (1.05–1.11)	<0.0001	33.0	1.08 (1.04–1.12)	<0.0001	17.1
Pulmonary opacity, per 5%	1.36 (1.24–1.49)	<0.0001	41.7	1.32 (1.12–1.57)	0.0015	10.4
Lymphocyte, 10^9/L	0.06 (0.02–0.18)	<0.0001	23.6	0.28 (0.09–0.91)	0.0357	4.5
Imported cases	2.11 (1.07–4.16)	0.0302	4.7	2.45 (1.03–5.80)	0.0421	4.1
SpO_2, per 5%	0.14 (0.04–0.41)	0.0004	12.4	0.31 (0.07–1.33)	0.1147	2.5
Platelet, 10^9/L	0.99 (0.98–0.99)	0.0012	10.6	1.00 (0.99–1.00)	0.3187	1.0
Diabetes	3.54 (1.37–9.16)	0.0091	6.8	1.84 (0.49–6.93)	0.3685	0.8
Quadrant score (1–4)	2.45 (1.64–3.67)	<0.0001	19.0	0.83 (0.47–1.47)	0.5275	0.4
Fibrinogen, g/L	1.54 (1.16–2.04)	0.0029	8.9	0.91 (0.59–1.41)	0.6871	0.2
C-reactive protein, mg/L	1.01 (1.01–1.02)	0.0002	13.7	1.00 (0.99–1.01)	0.7880	0.1
Hypertension	2.99 (1.41–6.33)	0.0043	8.2	0.89 (0.33–2.40)	0.8138	0.1

* Based on the complete cases without missing values.

† Based on imputed values for missing data in quadrant score, pulmonary opacity score, lymphocyte, platelet, C-reactive protein and fibrinogen using multiple imputation method.

‡ Factors are ranked according to χ^2 values to indicate their relative importance.

OR = odds ratio; CI = confidence interval; SpO_2 = oxygen saturation.

Supplementary Table S4.2 shows that oxygen was delivered to patients with disease deterioration via nasal cannulae (*n* = 31, 86.1%), simple face masks (*n* = 7, 19.4%), high-flow nasal cannulae (*n* = 11, 30.6%) or in prone position (*n* = 6, 16.7%).

Ventilatory support was used in approximately 50% of patients with clinical progression.

Table S4.2 Clinical management and outcome of patients with COVID-19 during hospital stay

		Disease progression, n(%)			
Category	Clinical management/outcome	All (N=597)	Yes (N=36)	No (N=561)	P-value
Supportive treatments	Inotropic and vasoconstrictive agents	4(0.7%)	4(11.1%)	0(0.0%)	<.0001
	Nasal cannula	199(33.3%)	31(86.1%)	168(29.9%)	<.0001
	Mask	9(1.5%)	7(19.4%)	2(0.4%)	<.0001
	High-flow nasal cannula oxygen therapy	12(2.0%)	11(30.6%)	1(0.2%)	<.0001
	Non-invasive ventilation	16(2.7%)	16(44.4%)	0(0.0%)	<.0001
	Invasive mechanical ventilation	3(0.5%)	3(8.3%)	0(0.0%)	0.0002
	Prone position	7(1.2%)	6(16.7%)	1(0.2%)	<.0001
Medical drugs	Chinese medicine	86(14.4%)	17(47.2%)	69(12.3%)	<.0001
	Immunoglobulin	133(22.3%)	27(75.0%)	106(18.9%)	<.0001
	Interferon	481(80.6%)	25(69.4%)	456(81.3%)	0.0857
	Antioxidants	132(22.1%)	15(41.7%)	117(20.9%)	0.0063
	Glucocorticoid	120(20.1%)	30(83.3%)	90(16.0%)	<.0001
	Thymosin	123(20.6%)	22(61.1%)	101(18.0%)	<.0001
	Neurotrophic drugs	94(15.7%)	13(36.1%)	81(14.4%)	0.0017
	Any antibiotics	310(51.9%)	33(91.7%)	277(49.4%)	<.0001
	Any antivirals	552(92.5%)	36(100%)	516(92.0%)	0.0995
Clinical outcome	Death	0(0.0%)	0(0.0%)	0(0.0%)	NC
	ICU	20(3.4%)	19(52.8%)	1(0.2%)	<.0001
	Shock	0(0.0%)	0(0.0%)	0(0.0%)	NC
	Respiratory failure	32(5.4%)	31(86.1%)	1(0.2%)	<.0001
	Renal failure	1(0.2%)	1(2.8%)	0(0.0%)	0.0603

4.4 Discussion

This is one of the largest studies to describe disease progression in patients hospitalised with COVID-19, with 625 cases included. On admission to hospital, 17.4%

patients had asymptomatic or mild disease, 78.1% had moderate disease and 4.5% were severely or critically ill. During the study period (up to 29 February 2020) there were no deaths; 81.6% had been discharged, and fewer than 1% required ongoing ICU care. Jiangsu Province reported no deaths mainly due to the early recognition of high-risk and critically ill patients, early intervention, hierarchical management strategies, and reasonable allocation of materials and human resources [58].

We found that over four fifths of patients with COVID-19 had a stable or improving clinical course, with a minority deteriorating during a 14-day follow-up period. This is consistent with a previous study, which found that 2 weeks after admission, 14.1% (11) of patients had worsened status and 85.9% (*n* = 67) of patients had improved or stable status [238]. Several studies showed clinical deterioration may occur within 2 weeks after onset of illness [42,48,239,240]. In comparison, other zoonotic coronavirus diseases, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), progress rapidly to respiratory failure and organ injury [241]. Within 7 days of admission, CT scans showed clinical signs that 31% (*n* = 4) of patients progressed, while within 14 days, 85.7% (*n* = 54) of patients progressed [232,242,243].

Studies on SARS and MERS have suggested a tri-phasic pattern of disease progression, combined with time course of viral load. For SARS, Week 1 is associated with increasing viral load, which may be related to mild symptoms; Week 2 shows a fall in viral load, but a more severe clinical response and immunopathological damage (as a result of an overexuberant host response, rather than uncontrolled viral replication); and Phase 3 with either resolution of symptoms or further deterioration

[244]. MERS showed a similar pattern [245]. For SARS-CoV-2, viral loads were reported to peak at around 5–6 days after symptom onset and a patient presented an extremely high viral load [246]; viral loads in asymptomatic patients has been found to be similar to that in symptomatic patients [247]. Except for severe cases, most of the patients with COVID-19 were able to clear the virus and their disease progression fits the biphasic model (i.e., first phase characterised by fever and other systemic symptoms, followed by symptoms relief in Week 2) [248].

Our study found that only 6% (*n* = 36) patients experienced disease deterioration, i.e., progression from "moderate or less" status on admission, to "severe or critically" ill status within 2 weeks of admission. This study showed that features, including symptoms and abnormal radiological and laboratory presentation on admission, may be early signs of deterioration of respiratory, immune and the coagulation systems. In particular, age, pulmonary opacity score on CT, lymphocyte count and exposure to the pandemic centre in Wuhan were independent predictors for disease progression. This is in line with a study that identified several risk factors for disease progression of COVID-19, including age, respiratory failure and C-reactive protein levels [238]. The severity of opacity evaluated from an initial CT scan of patients with COVID-19 was closely related to the progression of opacity presented in the subsequent CT, which are of value for monitoring disease progression [249]. Old age and coagulation dysfunction were associated with progression from ARDS to death in patients with COVID-19 [41]. Old age and severe lymphopenia seem to be statistically significant in predicting clinical deterioration in patients with SARS [244,250]. Patients who have been to Wuhan may have been exposed to a large amount of virus, so the disease may be more likely to deteriorate.

The progress and outcome of SARS may be associated with specific temporal patterns of development in combination with several non-specific signs and symptom complexes [251]. Further study suggests that clinical progression at Week 2 may not be associated with uncontrolled viral replication, but with immunopathological damage [244]. This evidence indirectly supports our study results: symptoms and abnormal laboratory and radiological manifestations on admission provided early indicators for short-term immunopathological damage and progression of COVID-19.

This cohort consisted of almost all COVID-19 patients in this province (population: over 80 million) and its results should be generalisable to other similar places outside Hubei Province. Our study also had some limitations. First, severity data were only available for the first 14 days of hospital stay, and we were unable to assess disease progression and its risk factors beyond this period. Second, as the data were collected retrospectively, we could not assess the impact of some important predictive variables such as clinical management before disease progression (e.g., oxygen support and drug treatments), viral load at admission (e.g., the quantity of viral RNA in blood), some other laboratory parameters (e.g., lactate dehydrogenase) and host genetic factors due to the lack of available data. As a result, observed risk factors may still be subject to unobserved confounders.

4.5 Conclusion

In this multicentre cohort of 625 patients with COVID-19, we found that 16.3% of patients experienced a deterioration in their clinical condition and 6% of patients with "moderate or less" status deteriorated to being "severe or critically ill", but ultimately

survived. Age, pulmonary opacity score, lymphocyte count on admission and exposure to the pandemic centre in Wuhan were identified as independent risk factors for disease deterioration. Careful attention to these risk factors may help guide clinical care.

Chapter 3 and Chapter 4 identify risk factors of being severe or critically ill status due to COVID-19, and risk factors of experiencing disease deterioration from asymptomatic or mild or moderate status into severe or critically ill status. These risk factors include some epidemiological and clinical characteristics, laboratory parameters, and radiological parameters. This helps health care providers and policy makers to pay more attention on patients with these factors to prevent patients from having severe disease or progressing from asymptomatic or mild or moderate disease into severe disease, and hence reduce the risk of death. Respiratory failure is one of the most severe illnesses that can occur after contracting COVID-19. Further research into risk factors of respiratory failure may provide some new insights, and the next chapter (Chapter 5) will do this.

Chapter 5 Respiratory failure among patients with COVID-19 in Jiangsu province, China: a multicentre retrospective cohort study

Chapter 3 and Chapter 4 identify risk factors of two dangerous situations: being severe or critically ill status, and experiencing disease deterioration from asymptomatic or mild or moderate status into severe or critically ill status. Several factors such as age, pulmonary opacity score, lymphocyte count, and exposure to the pandemic centre were identified, which helps identifying patients with high risk of severe disease or disease deterioration, and hence taking early management measures. Based on these contents, this chapter (Chapter 5) aims to extend these studies by exploring the risk factors of respiratory failure, one of the most serious diseases caused by COVID-19, which deserves a better understanding.

5.1 Introduction

The major clinical effects of COVID-19 infection are on the respiratory system although other systems can be affected [45,46]. COVID-19 may result in acute respiratory failure requiring mechanical ventilation and even leading to death [37,47-49]. A study in Italy reported rates of respiratory failure as high as 29%-40% [59]. The 28-day mortality could occur in 26%-30% of patients with COVID-19 who had respiratory failure necessitating invasive mechanical ventilation (IMV) [60]. Since the COVID-19 pandemic is still evolving, the true mortality has not been defined, but the crude mortality ratio (the number of reported deaths divided by the number of reported cases) has been estimated to be 3%-4%, which appears to be higher than that for influenza [56]. However, the infection

mortality rate (the number of reported deaths divided by the number of infections) is lower than the crude mortality ratio; and the mortality rate varies among different regions, demographic and socioeconomic characteristics, levels of healthcare access and quality, intervention methods, and qualities of reported deaths and cases [56-58]. Identifying risk factors of respiratory failure in patients with COVID-19 could help clinicians recognise patients at high risk of respiratory failure and hence take active treatment for them to prevent further worse outcomes and reduce emergency intubation or cardiopulmonary resuscitation to protect medical staff from related infections.

For patients with COVID-19, risk factors of severe COVID-19, admission to intensive care unit (ICU) and death have been reported in many studies [252-255], but studies on risk factors of respiratory failure remain scarce. To the best of our knowledge, only two studies are available in the literature identifying the predictors of respiratory failure and both studies were conducted in Italy [59,199]. However, demographic characteristics may vary across different countries, and the levels and risk factors of respiratory failure may also differ [200]. Therefore, information on the respiratory failure in other settings than Italy will be of great academic and clinical value.

Based on a large multicentre retrospective cohort with rich information on demographic, epidemiologic, clinical and laboratory features as well as CT imaging features, this study aims to assess the level and identify potential risk factors of respiratory failure in patients with COVID-19.

5.2 Methods

5.2.1 Study Design and Participants

The study design and participants have been described in our previous study [256]. Here we briefly describe it. This is a retrospective multicentre cohort study. Patient inclusion criterion was as of 15th March 2020, all patients diagnosed with COVID-19 at 24 hospitals designated to treat COVID-19 in Jiangsu province, China according to the diagnostic criteria of the "Diagnosis and treatment protocol for novel coronavirus pneumonia (trial version 7)" released by National Health Commission and National Administration of Traditional Chinese Medicine of China [212]. The diagnosis of COVID-19 was based on epidemiological history, clinical and CT manifestations, and laboratory confirmation (real–time reverse transcriptase–polymerase chain reaction assays [RT-PCR] to detect etiological agent severe acute respiratory syndrome coronavirus-2, SARS-CoV-2, which caused COVID-19) [58,212]. The exclusion criterion was patients with no available medical records. The criteria for discharge were: the patient's body temperature remained normal for more than 3 days, the symptoms were relieved (if there were symptoms), and the results of two consecutive RT-PCR assays were negative (throat swab samples, at least 1 day apart).

5.2.2 Data collection and definition of variables

The primary outcome was the occurrence of acute respiratory failure during 14-day follow-up (days 1, 2, 3, 4, 5, 6, 7 and 14 from admission). The respiratory failure was defined as oxygen saturation (SpO_2) <93% and/or partial pressure of oxygen in arterial blood (PaO_2) <60 mmHg on room air and/or requirement of high-flow nasal cannula

oxygen therapy (HFNC), non-invasive or invasive mechanical ventilation. Type 1 (hypoxemic) respiratory failure refers to the hypoxemia (PaO_2 <60 mmHg [8 kPa]) with the normal (normocapnia) or low (hypocapnia) partial pressure of carbon dioxide ($PaCO_2$) in arterial blood. Type 2 (hypercapnic) respiratory failure refers to the hypoxemia with the hypercapnia ($PaCO_2$ >45 mm Hg [6 kPa]).

Demographic features analysed in this study included sex and age (year). Epidemiologic features included exposure types (imported case or local case) and types of disease onset (single onset or clustered onset). Clinical features included initial symptoms (fever, cough, sputum, shortness of breath, anorexia, and diarrhoea), medical history (hypertension, coronary heart disease, diabetes, stroke, smoking, and drinking), and vital signs (temperature [°C], heart rate [HR, beats/minute], systolic blood pressure [SBP, mmHg], diastolic blood pressure [DBP, mmHg], respiratory rate [breaths/minute], and SpO_2 [%]). Laboratory features included blood test parameters (white blood cell count [WBC, 10^9/L], neutrophil count [10^9/L], lymphocyte count [10^9/L], haemoglobin [g/L], and platelet count [10^9/L]), organ function parameters (albumin [g/L] and creatinine [umol/L]), inflammatory factors (C-reactive protein [mg/L]), and coagulation function parameters (activated partial thromboplastin time [APTT, s], fibrinogen [g/L], and D-dimer [mg/L]). Radiologic features included lesion distribution (outer third of lung involved, middle third of lung involved, or inner third of lung involved), lesion density (below 20% consolidation, 20% to 80% consolidation, or above 80% consolidation), lesion border (well-defined border, moderately defined border, or ill-defined border), quadrant score, and pulmonary opacity score. Data of features listed above were collected at admission. Data of treatments (supportive treatments and medication) were obtained within the whole study period.

Imported cases were defined as those who had been to the pandemic centre of China (Wuhan city), or had contact with people or patients with COVID-19 who had been to Wuhan; and other cases were classified as “local cases”. A clustered onset is defined as the occurrence of two or more confirmed COVID-19 cases in the same cluster/group within 14 days, such as family, community, hospital, workplace or public place. A clustered onset may occur from interpersonal transmission via close contact with or joint contact with a confirmed COVID-19 case. Other cases not meeting the criteria for a clustered onset were classified as “single onset”.

Radiologic features were evaluated visually, by two radiologists with more than 5 years’ working experience. The radiologists were blinded to patients’ other characteristics and would reach agreements on different assessments of radiologic features. Chest CT axial sections were divided into four quadrants (left, right, anterior and posterior) by drawing horizontal and vertical lines through the centre of the chest. The quadrant score was defined as the number of quadrants with pulmonary opacities extending from the proximal end to the distal end of the chest, ranging from 0 to 4; and pulmonary opacity score was defined as the percentage of pulmonary opacity area in the area of bilateral lungs, rounded to the nearest 5%.

5.2.3 Statistical analysis

Normally distributed continuous variables and skewed distributed continuous variables of patients were reported as mean (standard deviation, SD) and median (interquartile range, IQR) by group (patients with and without respiratory failure) and compared using Student's t-test or Mann-Whitney U test, respectively. Categorical

variables were summarised using frequency and percentage and compared by χ^2 or Fisher exact test.

Logistic regression models were used to identify risk factors of having respiratory failure. Univariate logistic regression models were fitted first to evaluate associations between each variable measured at admission and respiratory failure on the complete cases (without missing value). Variables that were statistically significant in the univariate analysis were then included in the multivariate logistic regression model. In the multivariate analysis, missing covariates were imputed with multiple imputation using a Markov Chain Monte Carlo simulation method with 10 iterations. A sensitivity analysis was performed on complete cases. Odds ratio (OR) of having respiratory failure for each variable was calculated along with 95% confidence interval (CI). The 2-tailed $P < 0.05$ was considered as statistically significant. The analyses were performed using SAS 9.4 (SAS Institute).

5.3 Results

Of the 721 suspected cases with possible COVID-19 in Jiangsu province from 10th January to 15th March 2020, 90 cases were excluded since RT-PCR tests showed negative results and 6 cases were excluded due to no available medical records. Finally 625 cases (52.6% male; median age 46 years old [IQR 32-57]) in 24 hospitals were included for analysis, mainly from the Second Hospital of Nanjing, Suzhou Infectious Disease Hospital and Huai'an No.4 People's Hospital (113 [18.1%], 86 [13.8%] and 79 [12.6%], respectively). The remaining cases were disproportionately from the other hospitals. The hospital to which a patient was admitted was mainly determined by

geographic location. Of the 625 patients, 56 (9%) had respiratory failure, mainly type I (hypoxemic). At the study end point (15th March 2020), no patients died and all patients were discharged from hospitals.

At admission to hospital, compared with patients without respiratory failure, those with respiratory failure were significantly older (mean age 59.70 vs. 42.93 years, $P < 0.0001$); were more likely to be single onset (62.5% vs. 48.3%, $P = 0.0498$); were more likely to have symptoms including fever (82.1% vs. 64.3%, $P = 0.0074$), cough (71.4% vs. 53.4%, $P = 0.0110$), sputum (42.9% vs 25.0%, $P = 0.0064$), and shortness of breath (12.5% vs. 2.3%, $P = 0.0010$); were more likely to have prior histories of hypertension (32.1% vs. 13.9%, $P = 0.0014$), coronary heart disease (7.1% vs. 1.6%, $P = 0.0224$), and diabetes (17.9% vs. 5.3%, $P = 0.0015$); had higher mean temperature (37.25 vs. 37.03 °C, $P = 0.0347$), greater mean heart rate (90.71 vs. 86.82 beats/minute, $P = 0.0387$), greater mean respiratory rate (21.13 vs. 18.88 breaths/minute, $P < 0.0001$), and lower mean SpO_2 (95.27% vs. 97.92%, $P < 0.0001$) (Table 5.1).

Table 5.1 Epidemiological and clinical characteristics of patients at admission

			Respiratory failure		
Category	Characteristics	All (N=625)	Yes (N=56)	No (N=569)	P-value
Demographic	Male, n(%)	329(52.6%)	36(64.3%)	293(51.5%)	0.0700
	Age (year), mean(SD)	44.44(17.21)	59.70(13.55)	42.93(16.80)	<.0001
Exposure type, n(%)	Imported case	219(35.0%)	23(41.1%)	196(34.4%)	0.3784
	Local case	406(65.0%)	33(58.9%)	373(65.6%)	
Types of disease onset, n(%)	Single onset	310(49.6%)	35(62.5%)	275(48.3%)	0.0498
	Clustered onset	315(50.4%)	21(37.5%)	294(51.7%)	
Initial symptoms, n(%)	Fever	412(65.9%)	46(82.1%)	366(64.3%)	0.0074
	Cough	344(55.0%)	40(71.4%)	304(53.4%)	0.0110

			Respiratory failure		
Category	Characteristics	All (N=625)	Yes (N=56)	No (N=569)	P-value
	Sputum	166(26.6%)	24(42.9%)	142(25.0%)	0.0064
	Shortness of breath	20(3.2%)	7(12.5%)	13(2.3%)	0.0010
	Anorexia	13(2.1%)	1(1.8%)	12(2.1%)	1.0000
	Diarrhoea	53(8.5%)	6(10.9%)	47(8.3%)	0.4516
Medical history, n(%)	Hypertension	97(15.5%)	18(32.1%)	79(13.9%)	0.0014
	Coronary heart disease	13(2.1%)	4(7.1%)	9(1.6%)	0.0224
	Diabetes	40(6.4%)	10(17.9%)	30(5.3%)	0.0015
	Stroke	9(1.4%)	2(3.6%)	7(1.2%)	0.1900
	Smoke	25(4.0%)	0(0.0%)	25(4.4%)	0.1547
	Drinking	30(4.8%)	1(1.8%)	29(5.1%)	0.5066
Vital signs, mean(SD)	Temperature (°C)	37.05(0.73)	37.25(0.90)	37.03(0.71)	0.0347
	HR (beats/minute)	87.17(13.46)	90.71(15.24)	86.82(13.24)	0.0387
	SBP (mmHg)	128.83(15.66)	131.16(18.89)	128.60(15.31)	0.2430
	DBP (mmHg)	81.47(10.51)	78.95(9.67)	81.72(10.57)	0.0596
	Respiratory rate (breaths/minute)	19.08(2.56)	21.13(5.03)	18.88(2.07)	<.0001
	SpO_2 (%)	97.68(1.99)	95.27(4.95)	97.92(1.16)	<.0001

SD, standard deviation; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood; SpO2, peripheral capillary oxygen saturation.

Patients with respiratory failure had significantly lower median lymphocyte count ($0.7*10^9$/L vs. $1.3*10^9$/L, $P < 0.0001$), median platelet count ($155.5*10^9$/L vs. $186.5*10^9$/L, $P = 0.0008$), and median albumin levels (40.0 vs. 41.9 g/L, $P = 0.0005$). In addition, patients with respiratory failure had significantly higher median levels of C-reactive protein (40.6 vs. 10.0 mg/L, $P < 0.0001$), median fibrinogen levels (4.3 vs. 3.4 g/L, $P < 0.0001$), and median D-dimer levels (0.3 vs. 0.2 mg/L, $P = 0.0004$) (Table 5.2).

Table 5.2 Laboratory parameters at hospital admission

		N,median(IQR)			
			Respiratory failure		
Category	Parameters	All	Yes	No	P-value
Blood test	WBC count (10^9/L)	513,4.9(3.9-6.2)	45,4.5(3.4-5.8)	468,4.9(3.9-6.3)	0.1742
	Neutrophil (10^9/L)	507,3.0(2.2-4.0)	45,3.1(2.1-4.6)	462,3.0(2.2-4.0)	0.3912
	Lymphocyte (10^9/L)	505,1.3(0.9-1.7)	45,0.7(0.5-0.9)	460,1.3(1.0-1.8)	<.0001
	Haemoglobin (g/L)	510,137.0(124.0-150.0)	43,129.0(121.0-142.0)	467,137.0(124.0-150.0)	0.0772
	Platelet (10^9/L)	494,183.5(151.0-219.0)	40,155.5(121.5-188.5)	454,186.5(153.0-220.0)	0.0008
Organ function	Albumin (g/L)	480,41.4(38.0-45.1)	41,40.0(34.0-41.4)	439,41.9(38.0-45.6)	0.0005
	Creatinine (umol/L)	476,63.9(51.0-79.0)	42,62.4(49.1-85.0)	434,64.0(51.0-78.8)	0.9785
Inflammatory factors	C-reactive protein (mg/L)	474,10.0(2.7-22.6)	39,40.6(7.6-106.6)	435,10.0(2.6-19.7)	<.0001
Coagulation function	APTT (s)	513,32.2(28.0-37.2)	47,32.0(28.0-36.6)	466,32.2(28.0-37.3)	0.7284
	Fibrinogen (g/L)	496,3.5(2.7-4.2)	46,4.3(3.0-6.1)	450,3.4(2.7-4.2)	<.0001
	D-dimer (mg/L)	475,0.2(0.1-0.4)	44,0.3(0.2-1.1)	431,0.2(0.1-0.4)	0.0004

IQR, inter-quartile range; WBC, white blood cell; APTT, activated partial thromboplastin time.

For visually evaluated CT features at hospital admission, patients with respiratory failure had significantly greater median of CT quadrant score (4.00 vs. 2.00, P < 0.0001) and median of pulmonary opacity score (52.50% vs. 20.00%, *P* < 0.0001) (Table 5.3).

Table 5.3 Visually evaluated CT features at hospital admission

			Respiratory failure, n(%) or median(IQR)		
Category	Measurements	All	Yes	No	P-value
Lesion distribution*	Outer third of lung involved	400(82.6%)	39(84.8%)	361(82.4%)	0.6873
	Middle third of lung involved	278(57.4%)	27(58.7%)	251(57.3%)	0.8561
	Inner third of lung involved	123(25.4%)	17(37.0%)	106(24.2%)	0.0587
Lesion density*	Below 20% consolidation	205(42.4%)	21(45.7%)	184(42.0%)	0.6343
	20% to 80% consolidation	89(18.4%)	10(21.7%)	79(18.0%)	0.5375
	Above 80% consolidation	116(24.0%)	9(19.6%)	107(24.4%)	0.4622
Lesion border*	Well-defined border	90(18.6%)	9(19.6%)	81(18.5%)	0.8589

			Respiratory failure, n(%) or median(IQR)		
Category	Measurements	All	Yes	No	P-value
	Moderately defined border	82(16.9%)	8(17.4%)	74(16.9%)	0.9320
	Ill-defined border	235(48.6%)	23(50.0%)	212(48.4%)	0.8365
Other findings**	Quadrant score (0-4)	2.00(1.00-4.00)	4.00(4.00-4.00)	2.00(1.00-4.00)	<.0001
	Pulmonary opacity (%)	20.00(5.00-40.00)	52.50(35.00-75.00)	20.00(5.00-35.00)	<.0001

* The total number of cases is 484 (43 respiratory failures and 441 no respiratory failures)

** The total number of cases is 496 (47 respiratory failures and 449 no respiratory failures).

CT, computer tomography; IQR, inter-quartile range.

Oxygen was delivered to patients with respiratory failure via nasal cannula (80.4%), simple face masks (23.2%), HFNC (39.3%), non-invasive mechanical ventilation (NIV) (53.6%), IMV (8.9%), and in prone position (30.4%) (Table 5.4). Patients with respiratory failure were more likely to receive supportive treatments and medications (all $P < 0.05$), except for the interferon.

Table 5.4 Clinical management during hospitalisation

			Respiratory failure		
Category	Clinical management	All (N=625)	Yes (N=56)	No (N=569)	P-value
Supportive treatments, n(%)	Inotropic and vasoconstrictive agents	5(0.8%)	5(8.9%)	0(0.0%)	<.0001
	Nasal cannula	221(35.4%)	45(80.4%)	176(30.9%)	<.0001
	Mask	14(2.2%)	13(23.2%)	1(0.2%)	<.0001
	High-flow nasal cannula oxygen therapy	25(4.0%)	22(39.3%)	3(0.5%)	<.0001
	Non-invasive ventilation	34(5.4%)	30(53.6%)	4(0.7%)	<.0001
	Invasive mechanical ventilation	5(0.8%)	5(8.9%)	0(0.0%)	<.0001
	Prone position	18(2.9%)	17(30.4%)	1(0.2%)	<.0001
Medical drugs, n(%)	Traditional Chinese medicine	98(15.7%)	29(51.8%)	69(12.1%)	<.0001
	Immunoglobulin	156(25.0%)	41(73.2%)	115(20.2%)	<.0001
	Interferon	503(80.5%)	40(71.4%)	463(81.4%)	0.0787

			Respiratory failure		
Category	Clinical management	All (N=625)	Yes (N=56)	No (N=569)	P-value
	Antioxidants	152(24.3%)	30(53.6%)	122(21.4%)	<.0001
	Glucocorticoid	142(22.7%)	48(85.7%)	94(16.5%)	<.0001
	Thymosin	144(23.0%)	35(62.5%)	109(19.2%)	<.0001
	Neurotrophic drugs	102(16.3%)	19(33.9%)	83(14.6%)	0.0009
	Any antibiotics	336(53.8%)	53(94.6%)	283(49.7%)	<.0001
	Any antivirals	580(92.8%)	56(100%)	524(92.1%)	0.0258

Twenty variables at admission were found to be related to the occurrence of respiratory failure in univariate logistic regression analysis (Table 5.5). When they were included in the multivariate logistic regression model simultaneously, four variables were independently related to the occurrence of respiratory failure: age (in years) (OR, 1.07; 95% CI: 1.03–1.10; $P = 0.0002$), respiratory rate (breaths/minute) (OR, 1.23; 95% CI: 1.08–1.40; $P = 0.0020$), lymphocyte count (10^9/L) (OR, 0.18; 95% CI: 0.05–0.69; $P = 0.0157$), and pulmonary opacity score (per 5%) (OR, 1.38; 95% CI: 1.19–1.61; $P < 0.0001$).

Table 5.5 Factors associated with respiratory failure in patients with COVID-19: Results from logistic regression analysis

	Univariate analysis*		Multivariate analysis**	
Variables	OR (95%CI)	P-value	OR (95%CI)	P-value
Age (year)	1.07(1.05,1.09)	<.0001	1.07(1.03,1.10)	0.0002
Clustered onset	0.56(0.32,0.99)	0.0453	1.11(0.45,2.71)	0.8202
Fever	2.55(1.26,5.16)	0.0092	1.69(0.60,4.71)	0.3176
Cough	2.18(1.19,3.98)	0.0113	1.20(0.46,3.16)	0.7081
Sputum	2.26(1.29,3.96)	0.0046	1.45(0.55,3.86)	0.4520
Shortness of breath	6.11(2.33,16.02)	0.0002	0.82(0.15,4.59)	0.8242
Hypertension	2.94(1.60,5.40)	0.0005	0.80(0.30,2.13)	0.6589
Diabetes	3.91(1.80,8.49)	0.0006	2.13(0.59,7.68)	0.2469

	Univariate analysis*		Multivariate analysis**	
Variables	OR (95%CI)	P-value	OR (95%CI)	P-value
Coronary heart disease	4.79(1.43,16.08)	0.0113	4.60(0.78,27.20)	0.0925
Temperature (°C)	1.44(1.02,2.03)	0.0362	0.67(0.39,1.17)	0.1607
HR (beats/minute)	1.02(1.00,1.04)	0.0394	1.00(0.96,1.03)	0.7733
Respiratory rate (breaths/minute)	1.25(1.15,1.37)	<.0001	1.23(1.08,1.40)	0.0020
Lymphocyte (10^9/L)	0.02(0.01,0.07)	<.0001	0.18(0.05,0.69)	0.0157
Platelet (10^9/L)	0.99(0.98,1.00)	0.0026	1.00(0.99,1.01)	0.9590
Albumin (g/L)	0.92(0.87,0.96)	0.0007	1.02(0.93,1.11)	0.7318
C-reactive protein (mg/L)	1.02(1.01,1.03)	<.0001	1.00(0.99,1.01)	0.6707
Fibrinogen (g/L)	1.88(1.50,2.37)	<.0001	0.95(0.64,1.41)	0.8005
D-dimer (mg/L)	1.33(1.10,1.60)	0.0035	1.15(0.78,1.71)	0.4824
Quadrant score (0-4)	2.37(1.71,3.28)	<.0001	0.80(0.46,1.40)	0.4331
Pulmonary opacity (per 5%)	1.40(1.29,1.52)	<.0001	1.38(1.19,1.61)	<.0001

* Univariate analysis is based on the complete cases without missing value.

** Multivariate analysis is based on imputed values for missing data in lymphocyte, platelet, albumin, C-reactive protein, fibrinogen, D-dimer, quadrant score and pulmonary opacity using multiple imputation method.

OR, odds ratio; CI, confidence interval; HR, heart rate.

The sensitivity logistic regression model with only above four significant variables was estimated on complete cases (without missing data), and these variables remained statistically significant.

5.4 Discussion

This study assessed the level and risk factors of respiratory failure among patients with COVID-19. During the 14-day follow-up, 9% (56 out of 625) of patients with COVID-19 suffered from respiratory failure (mainly type I, hypoxemic) in Jiangsu province, China. At the end of the study (15th March 2020), no patients died and all patients were discharged from hospitals. Among many factors explored in this study, four of them

(older age, increased respiratory rate, decreased lymphocyte count and greater pulmonary opacity score) were identified as independent risk factors of respiratory failure after controlling for other confounders. Therefore, patients with such factors need to be carefully and thoroughly managed by physicians.

Jiangsu province is non-neighbouring with and geographically distant around 600 km from Hubei province, where Wuhan city (the epicentre of COVID-19 pandemic in China) located in. The rate of respiratory failure in Jiangsu province is similar to the figure in the national report from China [109,257] but this rate is much lower than reported earlier in Wuhan city (26%–32%) [48,238]. This may be due to the early responses and measures adopted by the Jiangsu provincial health authorities to deal with the disease during the pandemic, including more adequate medical resources, deeper understanding and better management of respiratory failure for patients with COVID-19 [58]. The rate of respiratory failure in this study is also lower than reported in other countries, including Italy (29%–40%) [59] and the United States (~14%) [200], which may result from variable population demographics [200], and the early identification and early treatment of patients at high risk of respiratory failure in Jiangsu province [58].

Our study found that age was associated with respiratory failure. This is consistent with previous studies reporting that middle-aged and elderly patients with COVID-19 were susceptible to respiratory failure [47,59], and is also similar to the results for patients with severe acute respiratory syndrome (SARS) [258-260]. A previous study showed that the older population have a higher incidence of comorbidities and hence possible poorer immune response to COVID-19 and poorer clinical outcomes [261]. This study also found that patients with respiratory failure tended to have more

comorbidities including hypertension, coronary heart disease and diabetes.

Increased respiratory rate as a risk factor of respiratory failure identified in this study is simple and convenient to apply for clinicians in their practice. This result is consistent with previous studies [59,262].

In our study, reduced lymphocyte count at admission was strongly associated with respiratory failure. Previous studies also show that the lymphocyte count of COVID-19 patients admitted to the ICU continued to decline [38,44], and the reduced lymphocyte count was a risk factor for respiratory failure [59]. Reduced lymphocytes may be part of the pathogenesis of COVID-19 [27,263]. Therefore, more attention needs to be paid to patients whose lymphocyte count decline more severely. Laboratory abnormalities including lymphopenia have been previously reported in severe cases of other respiratory viral diseases, including SARS, Middle East respiratory syndrome (MERS), and influenza [228,258,264-266].

All radiologic images collected in our study were CT which have the advantage of high-resolution transversal imaging and accurate display of the extent and range of lung lesions. The potential measurement bias and misclassification bias resulted from visual assessment of CT images were controlled by double assessments of CT images by two independent radiologists with more than 5 years' experience in pulmonary imaging, and by discussion and reaching agreements on different assessment results. This study showed that the pulmonary opacity score, one of the radiologic features, was strongly and independently associated with respiratory failure in patients with COVID-19, indicating that the more severe abnormality of lung function is an important factor to identify patients at high risks of respiratory failure. Former studies

demonstrate that CT lung lesions can predict death and ICU admission in patients with COVID-19 [253,267]. On the other hand, this study shows that between patients with and without respiratory failure, there were no significant differences in the distribution of lung lesions (both were more likely to involve the outer third of lungs), lesion density (both were more likely to have less than 20% of consolidation of lungs), and lesion boundary definition (both were more likely to have the ill-defined border of lungs).

In Jiangsu province with adequate medical material and human resources, all COVID-19 patients with respiratory failure received ICU monitoring. In comparison, due to limited resources at disease outbreak in Spain, some comorbid patients with respiratory failure requiring mechanical ventilation and ICU treatment did not receive these treatments which were reserved for non-comorbid patients, hence a large number of patients with respiratory failure finally died [268]. For similar settings with limited resources, the experience in Jiangsu province may be beneficial to COVID-19 patients with respiratory failure: in Jiangsu, a large proportion of patients with respiratory failure conducted prone position (30%) and received HFNC (40%) or NIV (~50%), which help reduce the further use of IMV (~10%) and mortality (no death occurred and all patients were discharged at the end of the study). This confirms the findings from several previous small studies [269,270].

This study addressed several limitations of previous studies by (1) adding radiologic features and several epidemiologic, clinical, and laboratory features into analysis to reduce residual confounding; (2) using multiple imputation method to provide unbiased estimates of levels and risk factors; and (3) conducting a sensitivity analysis to confirm that results from multivariate logistic regression analysis, from which our

main conclusion was drawn, were insensitivity to missing data, and hence were robust and credible. This study included 625 patients from 24 hospitals, i.e. nearly all patients in Jiangsu province, China (a province with a population of 80 million; only 6 cases were excluded due to missing medical records), to make the findings from this study be subject to less selection bias and be more generalisable to populations in similar settings.

Our study has several limitations. Firstly, this is a retrospective observational study and its results may be subject to measurement bias and information bias, and some unobserved confounders (e.g. obesity, gene cluster) [199,271]. Secondly, some data on laboratory test were missing and hence fewer laboratory parameters were included in the analysis. Thirdly, we were unable to analyse the impact of medical management on respiratory failure including supportive treatments and medication, because of the chronological order and treatment information collected before, when, or after respiratory failure occurred.

5.5 Conclusions

This chapter (Chapter 5) provides some new knowledge. This large cohort study based on a representative sample of 625 patients with COVID-19 shows that the rate of respiratory failure in Jiangsu province, China (9%), was similar to the national level in China, but much lower than in Wuhan city (the epicentre of COVID-19 pandemic in China) and some other countries. The study has also identified four independent risk factors of respiratory failure in patients with COVID-19 including older age, increased respiratory rate, decreased lymphocyte count and greater pulmonary opacity score at

admission. For successful control of mortality related to COVID-19, patients with COVID-19 having these risk factors need to be intensively managed during hospitalisation.

Chapter 3 to Chapter 5 identify risk factors of three life-threatening situations: being severe or critically ill status, experiencing disease deterioration from asymptomatic or mild or moderate status into severe or critically ill status, and developing respiratory failure, to help provide evidence for health care professionals and policy makers to make better decision on the clinical management and allocation of medical and social resources. In particular, Chapter 3 to Chapter 5 suggest that age is one of the independent risk factors of severe or critically ill status, progression from milder disease to more severe status, and respiratory failure in patients with COVID-19. So in Chapter 6, I complete more comprehensive analysis on differences in clinical characteristics, disease severity, and clinical outcome burden in various age groups of patients with COVID-19.

Chapter 6 Age differences in clinical features and outcomes in patients with COVID-19, Jiangsu, China: a retrospective, multi-centre cohort study

From Chapter 3 to Chapter 5, we know that age is a very important factor on whether suffering severe disease, disease deterioration from milder disease to severe or critically ill status, and respiratory failure in patients with COVID-19. It is therefore important to further understand how and why characteristics and incidence of adverse outcomes among patients with COVID-19 differ among various age groups. To provide a more effective reference for managing COVID-19, this will be investigated in Chapter 6.

6.1 Introduction

COVID-19 infection causes a wide spectrum of disease which may lead to respiratory failure and organ failure, leading to death. Most of the available studies prior to the COVID-19 pandemic found that people of all ages are susceptible to SARS-CoV-2 infection but noted higher positivity rates in real-time reverse transcriptase–polymerase chain reaction (RT-PCR) assays and hospitalisation burden in older people [61-63]. Similar to SARS, deaths and adverse clinical outcomes have been found to be more common in the elderly with known comorbidities for patients with COVID-19 [64-66]. Evidence suggests that asymptomatic carriers were more common among middle-aged people in close contact with infected family members [272]. One study found that elderly patients with COVID-19 had some different clinical features compared with younger patients [273]. Because age is a host factor that leads to a higher risk of severe

COVID-19 and worse prognosis, it is important to better understand age-related susceptibility and pathology. However, published data on age differences in clinical features and clinical outcomes associated with COVID-19 remain scarce. This study aimed to investigate differences in clinical characteristics, disease severity, and clinical outcome burden in different age groups of patients with COVID-19.

6.2 Methods

6.2.1 Study design and participants

This retrospective cohort study included all patients who met the study's inclusion and exclusion criteria. The inclusion criterion was from March 15, 2020, all patients diagnosed with COVID-19 in Jiangsu Province according to the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" released by the National Health Commission & National Administration of Traditional Chinese Medicine of China [212]. The only exclusion criterion was any patient with no available medical records. A standard criterion was set for discharge: a patient's body temperature must be normal for more than 3 days, symptoms were resolved (if there were symptoms), and RT-PCR assays (throat swab samples, with at least 1 day sampling interval) showed two consecutive negative results.

6.2.2 Data collection and definition of variables

Epidemiological, clinical, laboratory, and radiologic parameters on admission; disease severity (asymptomatic, mild, moderate, severe, and critically ill); and clinical

outcomes data were extracted from medical records. A clustered onset was defined as the occurrence of two or more confirmed COVID-19 cases in the same cluster/group within 14 days, such as family, community, hospital, working place or public place, etc. A clustered onset could occur from interpersonal transmission via close contact with, or joint exposure to, a confirmed COVID-19 case. Other cases not meeting the conditions of a clustered onset were classified as 'single onset'. Disease severity was assessed at Days 1, 2, 3, 4, 5, 6, 7 and 14 after admission, except for those who were discharged, and the highest degree of disease severity during the 14-day follow-up was selected to be analysed. Data on mortality and hospitalisation status and other clinical outcomes were available until March 15, 2020. Asymptomatic infection was defined as the absence of clinical symptoms but with a positive nucleic acid test result. Mild disease was defined as having mild clinical symptoms and the absence of imaging manifestations of pneumonia in computer tomography (CT) scans. Moderate disease was defined as the presence of fever, respiratory tract symptoms, or other symptoms and imaging manifestations of pneumonia. Severe disease was defined as the presence of at least one of the following conditions: respiratory distress, respiratory rate ≥ 30 breaths/min; oxygen saturation in resting state (SpO_2) ≤93%; or arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) ≤300 mmHg (1 mmHg = 0.133kPa). Critically ill was defined as having respiratory failure requiring mechanical ventilation, shock or combined organ failure requiring intensive care unit (ICU) monitoring and treatment. We categorised the population into four age groups: children (18 years or younger), young adults (19-44 years), middle-aged adults (45-64 years), and elderly adults (65 years or older).

All of the patients in Jiangsu had a high-resolution CT thorax examination to identify

lung lesions. CT images were assessed visually by two radiologists who had more than 5 years of working experience in chest imaging. The radiologists were blinded to the patients' characteristic. Quadrant scores were the sum of the number of quadrants containing pulmonary opacities extending from the proximal to the distal end of the chest, with a score ranging from 0 to 4. For pulmonary opacity, bilateral lungs were scored manually and assigned an estimated percentage of pulmonary opacity relative to the whole lung, rounded to the nearest 5%.

6.2.3 Statistical analysis

Normally distributed variables were summarised using mean (standard deviation [SD]) by group and compared using ANOVA tests, while skewed distributed variables were summarised using median (interquartile range [IQR]) by group and compared using Kruskal–Wallis tests. Categorical variables were summarised using frequency and percentage and compared using Chi-squared/Fisher exact test. To assess the linear trend effect of age on demographic and clinical variables, generalised linear models were employed with age (year) as the only predictor. Normal distribution and identity link function were used for continuous variables whereas binomial distribution and logit link function were used for binary variables. Analyses were performed using SAS 9.4 (SAS Institute), and a two-sided $P < 0.05$ was considered statistically significant.

6.2.4 Patient and public involvement

Patients and members of the public were not involved in the design, conduct,

reporting, or dissemination plans of this research.

6.3 Results

Of the 721 suspected cases with possible COVID-19 during the study period, 631 patients were found to be RT-PCR positive for COVID-19. Only 625 patients were included in the study with complete data (Figure 6.1). The median age was 46 years (IQR, 32-57; range, 0.75-96 years), and 329 (52.6%) were men (Table 6.1). Thirty-seven (5.9%) were children, 261 (40%) young adults of 19-44 years, 248 (39.7%) middle-aged of 45-64 years, and 79 (12.6%) elderly of 65 years or older.

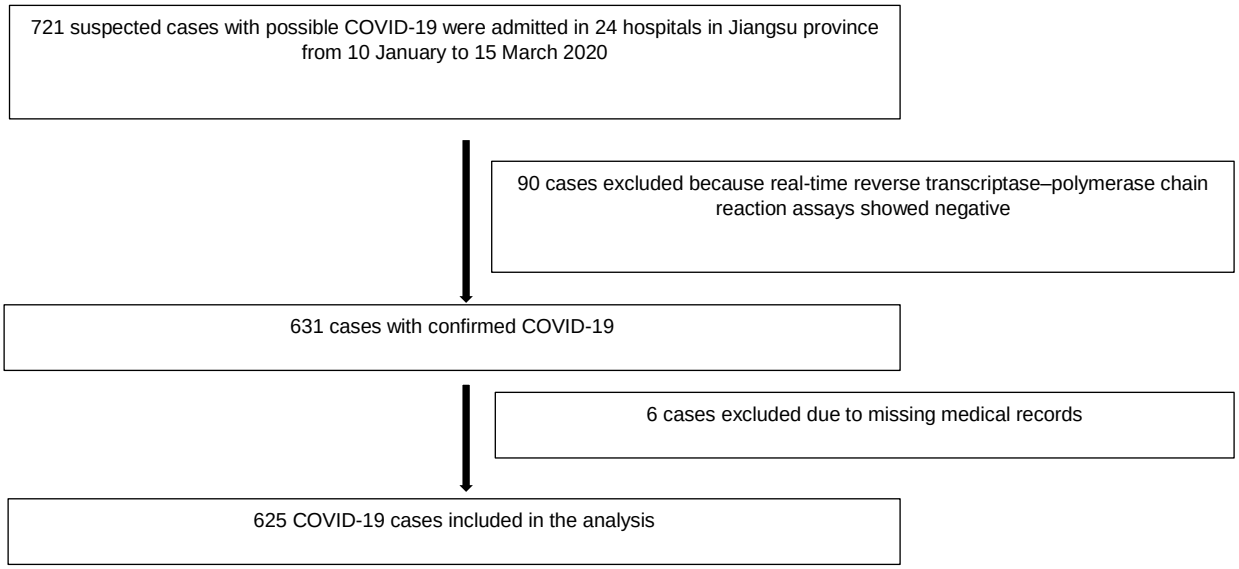

Figure 6.1: Study flow diagram

Table 6.1 Demographic and clinical characteristics of patients with COVID-19 at admission by age group

			Age group					
Category	Characteristics*	All (N=625)	Children (≤18 years) (N=37)	Young adulthood (19-44 years) (N=261)	Middle adulthood (45-64 years) (N=248)	Elderly (≥65 years) (N=79)	P-value**	P-value***
Demographic, n(%)	Male	329(52.6%)	23(62.2%)	137(52.5%)	132(53.2%)	37(46.8%)	0.4844	0.7235
Exposure type, n(%)	Imported cases	219(35.0%)	10(27.0%)	98(37.5%)	88(35.5%)	23(29.1%)	0.3908	0.5508
	Local cases	406(65.0%)	27(73.0%)	163(62.5%)	160(64.5%)	56(70.9%)		
Types of disease onset, n(%)	Single onset	310(49.6%)	8(21.6%)	148(56.7%)	117(47.2%)	37(46.8%)	0.0005	0.5335
	Clustered onset	315(50.4%)	29(78.4%)	113(43.3%)	131(52.8%)	42(53.2%)		
Initial symptoms, n(%)	Fever	412(65.9%)	13(35.1%)	177(67.8%)	170(68.5%)	52(65.8%)	0.0008	0.0341
	Cough	344(55.0%)	11(29.7%)	151(57.9%)	139(56.0%)	43(54.4%)	0.0146	0.3678
	Sputum	166(26.6%)	3(8.1%)	70(26.8%)	68(27.4%)	25(31.6%)	0.0548	0.2217
	Shortness of breath	20(3.2%)	0(0.0%)	5(1.9%)	8(3.2%)	7(8.9%)	0.0282	0.0016
Medical history, n(%)	Hypertension	91(14.6%)	0(0.0%)	11(4.2%)	49(19.8%)	31(39.2%)	<.0001	<.0001
	Coronary heart disease	13(2.1%)	0(0.0%)	1(0.4%)	5(2.0%)	7(8.9%)	0.0006	0.0003
	Chronic obstructive pulmonary disease	9(1.4%)	0(0.0%)	0(0.0%)	3(1.2%)	6(7.6%)	0.0003	<.0001
	Diabetes	40(6.4%)	1(2.7%)	4(1.5%)	21(8.5%)	14(17.7%)	<.0001	<.0001
	Current smoker	38(6.1%)	0(0.0%)	8(3.1%)	25(10.1%)	5(6.3%)	0.0035	0.0034
	Drinking alcohol	33(5.3%)	1(2.7%)	6(2.3%)	20(8.1%)	6(7.6%)	0.0137	0.0032
Vital signs, mean(SD)	Temperature (°C)	37.05(0.73)	36.86(0.44)	37.03(0.69)	37.09(0.81)	37.07(0.73)	0.3558	0.2324
	HR (bpm)	87.17(13.46)	91.43(17.84)	86.90(13.01)	86.92(12.77)	86.84(14.62)	0.2672	0.2721
	SBP (mmHg)	128.83(15.66)	120.11(16.17)	123.14(12.37)	132.85(13.94)	139.06(20.38)	<.0001	<.0001

			Age group					
Category	Characteristics*	All (N=625)	Children (≤18 years) (N=37)	Young adulthood (19-44 years) (N=261)	Middle adulthood (45-64 years) (N=248)	Elderly (≥65 years) (N=79)	P-value**	P-value***
	DBP (mmHg)	81.47(10.51)	72.41(11.24)	80.87(9.98)	83.60(10.30)	80.99(10.16)	<.0001	<.0001
	Respiratory rate (breath per min)	19.08(2.56)	19.86(3.15)	18.74(1.99)	19.38(3.00)	18.92(2.21)	0.0086	0.8381
	SpO_2 (%)	97.68(1.99)	98.05(1.05)	98.03(1.24)	97.58(1.92)	96.65(3.57)	<.0001	<.0001
CT image, N,median(IQR)	Quadrant score (1-4)	496,2.0(1.0-4.0)	27,0.0(0.0-1.0)	205,2.0(1.0-3.0)	196,3.0(2.0-4.0)	68,3.0(2.0-4.0)	<.0001	<.0001
	Pulmonary opacity (%)	496,20.0(5.0-40.0)	27,0.0(0.0-5.0)	205,15.0(5.0-30.0)	196,30.0(12.5-45.0)	68,27.5(10.0-50.0)	<.0001	<.0001

*Missing data only occurred to two CT parameters.

** P value from testing differences in proportions or means or distributions among different age groups

*** P value from trend tests of linear age effect in generalised linear model in which age (year) was included as the only predictor.

There was no significant difference in the proportion of men and women in each age group (Table 6.1). The disease onset was clustered among a significantly higher proportion of young patients aged 18 years and below compared with older patients (aged 18 years and younger, young adult, middle-aged, and elderly patients: 78.4%, 43.3%, 52.8%, 53.2%, respectively, P = 0.0005). The comorbidities incidence of hypertension, coronary heart disease, chronic obstructive pulmonary disease, and diabetes increased with age (trend test, $P < 0.0001$, $P = 0.0003$, $P < 0.0001$, and $P < 0.0001$ respectively). Fever, cough, and shortness of breath occurred significantly more commonly among young adult, middle-aged, and elderly patients compared to children (67.8%, 68.5%, 65.8% vs 35.1%, $P = 0.0008$; 57.9%, 56%, 54.4%, vs 29.7%, $P = 0.0146$; 1.9%, 3.2%, 8.9%, vs 0%, $P = 0.0282$, respectively). The frequencies of these symptoms were similar among different age groups of adults except for shortness of breath which was dramatically more common (8.9%) in elderly adult patients aged 65 years or older. Compared to children and young adult patients, the incidence of smoking and drinking alcohol was significantly (twice the rate) higher among middle-aged and elderly patients. Three vital sign parameters on admission: systolic blood pressure (SBP) (mmHg), diastolic blood pressure (DBP) (mmHg) and SpO_2 (%), changed linearly with age (trend tests, all $P < 0.0001$). The quadrant score and pulmonary opacity score increased with age (trend tests, both $P < 0.0001$).

Significant differences were observed in some laboratory test results (Table 6.2). Older patients tended to have lower PaO_2 (mmHg) and partial pressure of carbon dioxide ($PaCO_2$) (mmHg) in blood gas analysis. In blood tests they had lower white blood cell (WBC) count (10^9/L), lymphocyte count (10^9/L), haemoglobin level (g/L), and platelets count (10^9/L). In organ function tests these patients had higher levels of alanine

aminotransferase (U/L) and creatinine (umol/L), and lower albumin level (g/L). In inflammatory factor tests they had higher level of C-reactive protein (mg/L); and in coagulation function tests lower activated partial thromboplastin time (s), and higher levels of fibrinogen (g/L) and D-dimer (mg/L).

Table 6.2 Laboratory parameters of patients with COVID-19 at admission by age group

		Age group, N, median(IQR)						
Category	Parameters	All (N=625)	Children (≤18 years) (N=37)	Young adulthood (19-44 years) (N=261)	Middle adulthood (45-64 years) (N=248)	Elderly (≥65 years) (N=79)	P-value*	P-value**
Blood gas analysis	pH	252,7.4(7.4-7.4)	9,7.4(7.4-7.4)	92,7.4(7.4-7.4)	117,7.4(7.4-7.5)	34,7.4(7.4-7.5)	0.0213	0.0007
	PaO_2 (mmHg)	252,94.0(78.0-110.0)	9,94.0(86.7-113.0)	92,102.0(87.0-124.5)	117,89.0(76.2-106.0)	34,81.7(74.0-101.0)	0.0002	0.0009
	$PaCO_2$ (mmHg)	252,39.0(36.0-42.0)	9,45.0(42.0-49.0)	92,39.5(37.2-42.0)	117,39.0(35.6-42.0)	34,37.2(34.6-42.0)	0.0016	0.0087
	Lactate (mmol/L)	260,2.1(1.3-2.8)	17,2.3(1.5-3.1)	98,2.1(1.3-2.8)	106,1.9(1.3-2.8)	39,2.3(1.3-3.1)	0.5056	0.5554
Blood test	WBC (10^9/L)	513,4.9(3.9-6.2)	33,6.0(4.8-7.2)	213,4.9(3.9-6.1)	205,4.8(3.8-6.1)	62,4.6(3.9-6.4)	0.0119	0.1275
	Neutrophil (10^9/L)	507,3.0(2.2-4.0)	33,3.2(2.0-4.6)	210,2.8(2.1-3.9)	203,3.1(2.2-4.1)	61,3.4(2.4-4.3)	0.4466	0.2909
	Lymphocyte (10^9/L)	505,1.3(0.9-1.7)	33,1.8(1.5-2.5)	209,1.4(1.0-1.9)	202,1.1(0.9-1.6)	61,1.0(0.7-1.3)	<.0001	<.0001
	Haemoglobin (g/L)	510,137.0(124.0-150.0)	33,138.0(126.0-148.0)	212,139.5(123.0-154.0)	204,136.5(125.0-148.0)	61,129.0(121.0-140.0)	0.0250	0.0050
	Platelet (10^9/L)	494,183.5(151.0-219.0)	33,236.0(192.0-298.0)	203,197.0(161.0-228.0)	198,171.5(143.0-206.0)	60,152.0(125.0-186.5)	<.0001	<.0001
Organ function	Alanine aminotransferase (U/L)	425,25.0(16.5-38.0)	33,19.0(16.0-30.0)	169,25.0(16.0-39.3)	170,26.8(19.0-42.0)	53,22.0(15.0-33.0)	0.0499	0.3126
	Albumin (g/L)	480,41.4(38.0-45.1)	33,45.0(40.1-47.9)	194,42.1(38.0-46.6)	193,41.5(38.0-44.4)	60,38.9(35.4-43.1)	0.0001	<.0001
	Total bilirubin (umol/L)	465,9.8(6.0-14.7)	33,7.7(5.0-10.8)	186,9.8(6.1-14.8)	188,10.0(6.3-14.9)	58,10.4(6.6-15.3)	0.1141	0.3401
	Creatinine (umol/L)	476,63.9(51.0-79.0)	32,54.5(36.0-61.0)	193,64.0(51.3-78.0)	191,64.0(51.0-80.0)	60,67.0(56.0-85.1)	0.0003	0.0004
Inflammatory factors	C-reactive protein (mg/L)	474,10.0(2.7-22.6)	30,5.1(1.2-10.0)	193,9.6(2.0-16.2)	189,10.0(4.4-26.3)	62,16.8(5.0-51.1)	<.0001	<.0001
	Procalcitonin (ng/mL)	408,0.0(0.0-0.2)	28,0.0(0.0-0.2)	170,0.0(0.0-0.1)	157,0.1(0.0-0.2)	53,0.0(0.0-0.2)	0.8557	0.0980
Coagulation function test	Activated partial thromboplastin time (s)	513,32.2(28.0-37.2)	31,37.2(30.1-41.8)	217,32.2(27.9-36.5)	203,31.8(28.0-36.6)	62,33.0(27.9-38.1)	0.0448	0.2740
	Fibrinogen (g/L)	496,3.5(2.7-4.2)	30,2.6(2.2-2.9)	209,3.3(2.6-4.1)	198,3.8(3.0-4.4)	59,3.7(2.6-4.8)	<.0001	<.0001
	D-dimer (mg/L)	475,0.2(0.1-0.4)	30,0.2(0.1-0.3)	191,0.2(0.1-0.3)	195,0.2(0.1-0.4)	59,0.4(0.2-0.7)	0.0016	0.0049

* P value from Kruskal-Wallis tests of differences in distributions among different age groups ** P value from trend tests of linear age effect in generalised linear model in which age (year) was included as only predictor.

The proportion of patients who received supportive treatment and antiviral and antibiotic therapy increased significantly with patients' age, except for the very rare procedure of continuous renal replacement therapy and extracorporeal membrane oxygenation treatment, and the very common use of interferon among different age groups (Supplementary Table S6.1).

Table S6.1 Clinical management of patients with COVID-19 during hospital stay

		Age group, n(%)						
Category	Clinical management	All (N=625)	Children (≤18 years) (N=37)	Young adulthood (19-44 years) (N=261)	Middle adulthood (45-64 years) (N=248)	Elderly (≥65 years) (N=79)	P-value*	P-value**
Supportive treatments	Inotropic and vasoconstrictive agents	5(0.8%)	0(0.0%)	0(0.0%)	1(0.4%)	4(5.1%)	0.0018	0.0063
	Nasal cannula	221(35.4%)	5(13.5%)	76(29.1%)	103(41.5%)	37(46.8%)	<.0001	<.0001
	Mask	14(2.2%)	0(0.0%)	2(0.8%)	6(2.4%)	6(7.6%)	0.0090	0.0041
	High-flow nasal cannula oxygen therapy	25(4.0%)	0(0.0%)	4(1.5%)	11(4.4%)	10(12.7%)	0.0005	0.0001
	Non-invasive ventilation	34(5.4%)	0(0.0%)	2(0.8%)	16(6.5%)	16(20.3%)	<.0001	<.0001
	Invasive mechanical ventilation	5(0.8%)	0(0.0%)	0(0.0%)	1(0.4%)	4(5.1%)	0.0018	0.0038
	Prone position	18(2.9%)	0(0.0%)	2(0.8%)	8(3.2%)	8(10.1%)	0.0008	0.0010
	Continuous renal replacement therapy	1(0.2%)	0(0.0%)	0(0.0%)	0(0.0%)	1(1.3%)	0.1856	0.0999
	Extracorporeal membrane oxygenation	2(0.3%)	0(0.0%)	0(0.0%)	1(0.4%)	1(1.3%)	0.2313	0.0951
Medical drugs	Traditional Chinese medicine	98(15.7%)	0(0.0%)	26(10.0%)	49(19.8%)	23(29.1%)	<.0001	<.0001
	Immunoglobulin	156(25.0%)	2(5.4%)	40(15.3%)	79(31.9%)	35(44.3%)	<.0001	<.0001
	Interferon	503(80.5%)	28(75.7%)	209(80.1%)	205(82.7%)	61(77.2%)	0.5612	0.9408
	Antioxidants	152(24.3%)	4(10.8%)	49(18.8%)	71(28.6%)	28(35.4%)	0.0012	<.0001
	Glucocorticoid	142(22.7%)	1(2.7%)	40(15.3%)	70(28.2%)	31(39.2%)	<.0001	<.0001
	Thymosin	144(23.0%)	1(2.7%)	37(14.2%)	74(29.8%)	32(40.5%)	<.0001	<.0001
	Neurotrophic drugs	102(16.3%)	1(2.7%)	32(12.3%)	48(19.4%)	21(26.6%)	0.0008	<.0001
	Any antibiotics	336(53.8%)	5(13.5%)	136(52.1%)	142(57.3%)	53(67.1%)	<.0001	<.0001
	Any antivirals	580(92.8%)	25(67.6%)	245(93.9%)	234(94.4%)	76(96.2%)	<.0001	<.0001

* P value from testing differences in proportions among different age groups

** P value from trend test of linear age effect in generalised linear model in which age (year) was included as only predictor.

The proportion of patients with severe or critical illness was 33% among elderly patients, compared with 13% among middle-aged patients, 2.3% among young adult patients, and 0% among children (*P* < 0.0001) (Table 6.3).

Table 6.3 Highest degree of disease severity of patients with COVID-19 during hospital stay by age group

		Age group, n(%)				
Worst disease severity	All (N=625)	Children (≤18 years) (N=37)	Young adulthood (19-44 years) (N=261)	Middle adulthood (45-64 years) (N=248)	Elderly (≥65 years) (N=79)	P-value
Asymptomatic	24(3.8%)	8(21.6%)	5(1.9%)	7(2.8%)	4(5.1%)	<.0001
Mild	34(5.4%)	11(29.7%)	18(6.9%)	4(1.6%)	1(1.3%)	
Moderate	503(80.5%)	18(48.6%)	232(88.9%)	205(82.7%)	48(60.8%)	
Severe	30(4.8%)	0(0.0%)	4(1.5%)	16(6.5%)	10(12.7%)	
Critically ill	34(5.4%)	0(0.0%)	2(0.8%)	16(6.5%)	16(20.3%)	

By the end of the study, none of the patients had died and all 625 patients had been discharged. The ICU rate (trend test, *P* < 0.0001), respiratory failure rate (trend test, *P* < 0.0001), and length of hospital stays (trend test, *P* < 0.0001) increased with age (Table 6.4). The proportion of patients requiring ICU care (*P* < 0.0001) and developing respiratory failure (P < 0.0001) among elderly patients was 35.4% and 31.6% respectively, compared to 14.5% and 12.5% among middle-aged patients, 2.3% and 1.9% among young adult patients, and none among children, respectively. Elderly patients also had longer hospital stays (median [IQR], 21.0 [14.0-26.0] days) than all other age groups (15.0 [11.0-21.0] for children, 14.0 [11.0-19.0] for young adults, and 17.0 [13.0-22.0] for middle-aged adults, *P* < 0.0001).

Table 6.4 Clinical outcome of patients with COVID-19 by age group

	Age group, n(%) or median(IQR)						
Clinical outcome	All (N=625)	Children (≤18 years) (N=37)	Young adulthood (19-44 years) (N=261)	Middle adulthood (45-64 years) (N=248)	Elderly (≥65 years) (N=79)	P-value*	P-value**
ICU	70(11.2%)	0(0.0%)	6(2.3%)	36(14.5%)	28(35.4%)	<.0001	<.0001
Shock	2(0.3%)	0(0.0%)	0(0.0%)	1(0.4%)	1(1.3%)	0.3581	0.0672
Respiratory failure	61(9.8%)	0(0.0%)	5(1.9%)	31(12.5%)	25(31.6%)	<.0001	<.0001
Renal failure	2(0.3%)	0(0.0%)	0(0.0%)	0(0.0%)	2(2.5%)	0.0031	0.0266
Hospital stay (day)	16.0(12.0-22.0)	15.0(11.0-21.0)	14.0(11.0-19.0)	17.0(13.0-22.0)	21.0(14.0-26.0)	<.0001	<.0001

* P value from testing differences in proportions or distributions among different age groups

** P value from trend test of linear age effect in generalised linear model in which age (year) was included as only predictor.

6.4 Discussion

To our knowledge, this is the largest cohort of 625 patients to date, to assess age differences in clinical features and clinical outcomes associated with COVID-19. We observed that the majority (~80%) of COVID-19 cases were among young adult patients of 19-44 years and middle-aged patients of 45-64 years, with the remainder of patients comprising children of 18 years or under (5.9%), and elderly patients of 65 years or over (12.6%). This is consistent with a previous study from Korea which reported 6.3% of cases with COVID-19 were children under 19 years old, a study which had tested the broadest and hence the most representative population during this study period [202].

Our study showed that compared to adults, children were more likely to get infected through cluster gatherings. A previous study reported that COVID-19 in children was mainly caused by family transmission [203].

Our study in Jiangsu, found that all patients were discharged with no deaths. Elderly patients were more than twice as likely to have severe or critical illness compared with middle-aged

patients, while a smaller proportion of young adult patients had severe symptoms, and child patients exhibited none. The ICU use and respiratory failure rate, and length of hospital stay increased with age. Many case studies have shown that older patients are refractory (not yielding to treatment or not significantly improved after treatment) and likely to be at higher risk of more severe disease including acute respiratory distress syndrome (ARDS), respiratory failure, and death [38,41,42,47,274-277], while younger patients are more likely to have mild or moderate type of COVID-19 [203,205,278]. This is similar to SARS characteristics that, compared to adults and adolescents, disease appears to be less severe in younger children [66].

We found that initial symptoms including fever, cough, and shortness of breath occurred more frequently among adults compared to child patients and especially shortness of breath was dramatically more common in elderly adult patients aged 65 years or older. This is consistent with the previous study showing that the older group (≥60y) had a higher rate of shortness of breath than the younger group (<60y) [279]. However, another study showed that only sore throat showed a significant difference between age groups but fever, cough, sputum and diarrhoea did not show differences, probably due to the small sample size [205]. Another study which evaluated age difference only found 4 patients in total with symptoms of chest tightness or difficulty of breathing; however, the sample size (56 patients) was too small to draw any conclusions [273]. Such symptoms may be the early signs of more severe illness and poorer outcomes in older patients. The frequency of these symptoms among adult patients in different age groups were similar except for shortness of breath, which was dramatically more common in elderly patients than younger adult groups in our study. This is different from the characteristics of influenza, where the initial clinical manifestations of frail elderly patients are usually subtle compared to young patients [280,281]. In our study, the difference in

vital sign of SBP and DBP on admission for different age groups were statistically significant (increasing with age) but may not be clinically significant.

Our study showed that the age differences in clinical outcomes may also have partially resulted from the increased incidence of comorbidities with age including hypertension, coronary heart disease, chronic obstructive pulmonary disease, and diabetes. This may have increased susceptibility to the virus infection; such comorbidities are identified as risk factors of more severe disease including respiratory failure and death in patients with COVID-19 [38,47,276,277,282]. Other explanations about why older people suffer poorer outcomes may be due to the higher prevalence (twice the rate) of smoking and alcohol drinking in older patients with COVID-19 in Jiangsu. A history of smoking has been identified as a factor contributing to the progression of COVID-19 pneumonia [238].

Our study demonstrated that the quadrant score and pulmonary opacity score increased with age, suggesting more severe abnormal imaging manifestation on admission among these older patients. This is consistent with the finding that the proportion of multiple lobe involvement in older patients was higher than in younger cases [273]. Previous reports have also found some imaging differences by age groups; for example, primarily elderly patients were reported to have atypical imaging findings of consolidative opacities superimposed on ground-glass opacification [283], while paediatric patients showed more modest pulmonary involvement and less commonly reported consolidation complicated peripheral halo signs, compared with adults [206-208].

More abnormal manifestation in laboratory parameters in older patients may also be an early sign of, and a contribution to, severe illness and poor outcomes. This is consistent with previous studies showing the level of lymphocytes and albumin in the older cases was

significantly lower and the level of C-reactive protein was higher than that in the younger patients [205,273]. Studies showed that levels of albumin and C-reactive protein were associated with the progression of COVID-19 pneumonia [238], greater D-dimer on admission increased risk of in-hospital death [42], and organ and coagulation dysfunction (e.g., higher D-dimer) contributed to the development of ARDS and progression from ARDS to death [41].

Our study indicated that lymphopenia (normal range: [0.3-3.0]*10^9/L) was shown in all age groups, but white blood cell and lymphocytes counts were lower in the older patients, indicating that live SARS-COV-2 virus stimulated poorer responses in the older patients. The mechanism in old patients with a severe COVID-19 illness may be that older patients have a diminished immune response to the novel SARS-COV-2 virus which is a defence mechanism against respiratory viruses and contributes to virus clearance. The thymic involution in older patients causes age-related reduction of T cell repertoire diversity and defects in CD4+ and CD8+ T cell function and hence significantly reduces immune function (known as immunosenescence) [231,284]. Immunosenescence makes many viral infections worse in older patients [285]. But further research is necessary to investigate whether age differences in disease severity and outcomes of COVID-19 result from aging of the immune system and reduced responsiveness. This is because some respiratory viruses could escape antiviral mechanisms and immune responses [286]. For example, a study of H1N1pdm (respiratory viruses) on ferrets found no significant difference in viral clearance between young and adult subjects [287]. However, studies found that the pulmonary pathology improved earlier in young ferrets, regulatory interleukin-10 (which is mainly produced by monocytes and lymphocytes) and interferon responses were more robust in young ferrets [287-289]. Also H1N1pdm infection triggered formation of lung structures that resembled inducible bronchus-associated

lymphoid tissues (iBALTs) in young ferrets which contributes to pulmonary immune responses and were not seen in the adult ferrets with severe disease [287-289]. Some other studies demonstrated aged ferrets infected with influenza viruses had reduced antibody production and delayed peripheral blood T-cell responses compared to adult comterparts [231,284,290]. Another study reported that, except for immunosenescence in older people, age-related increases in levels of phospholipase could also result in a delay of immune response and poor outcomes after SARS-CoV infection [285]. Overall, research focused on innate immune-related mechanisms and viral clearance in patients with COVID-19 of different age groups may help determine the underlying mechanisms of disease severity.

Some other reasonable mechanisms of mild presentation in children include qualitatively different response to SARS-CoV-2, or different expression of angiotensin-converting enzyme (ACE) 2 receptors required for SARS-CoV-2 infection, or different virus-to-virus interaction and competition from other viruses limiting SARS-CoV-2 growth [202].

A larger proportion of older patients in this study received supportive treatment and antiviral and antibiotic therapies which was due in part to the increasing proportion of severe or critical illness in older adults. Previous studies similarly showed that treatment was statistically different by age group [205,273].

We believe that the findings from this study are generalisable to populations in similar settings (e.g. outside the initial pandemic centre) for two reasons: (1) we included nearly all patients in Jiangsu Province, China; (2) the study population consisted of cases confirmed by laboratory tests including those who were screened from suspected cases who had been to the pandemic centre (Wuhan), or had contact with people who had been to Wuhan or who had a confirmed diagnoses of COVID-19.

Our study has several limitations. First, the relatively short follow-up time and a very small proportion of patients who remained in hospital after the 14-day follow-up period may have resulted in incomplete estimates for disease severity. This would limit our interpretation of age differences in the burden associated with COVID-19. However, this impact is minor and may not strongly affect the study results because we included analyses of clinical outcomes at the end of the study and actually all patients in this study were observed to discharge. Second, we were unable to perform multiple regression analysis to control for possible bias in the observed age impact on clinical features and outcomes. As a result, the observed age differences may still be subject to possible confounding factors. Third, this is an observational and exploratory analysis in which many statistical tests have been performed. As a result, there may be some false positive results.

Strengths and limitations of this study

This cohort consists of almost all COVID-19 patients in Jiangsu Province, with a population over 80 million. Findings are therefore representative of the hospitalised patient population in the whole province. They are inclusive across the range of disease severity, so are subject to less selection bias.

The study includes imported and local cases with different types of exposure.

This is an observational exploratory study and our results may thus be subject to possible confounding factors and false positive error.

6.5 Conclusions

This chapter shows that older patients outside the pandemic centre of Wuhan, China, tended to have relatively more severe clinical infections and poorer clinical outcomes associated with COVID-19 compared to younger patients. Elderly patients aged 65 and older were at a much higher risk of developing severe or critical illness than other age groups. The ICU and respiratory failure rate, and length of hospital stay increased with age. Older patients had worse clinical outcomes, in part due to comorbidities in older people, and higher rates of smoking and drinking habits, and immune, organ, and coagulation dysfunction on admission. In studying the pathogenesis and developing management strategies of COVID-19, age is confirmed as a critical factor in severity of infection.

Cumulatively, Chapter 3 to 5 suggest that both age and CT pulmonary opacity score are important factors of developing severe or critical illness and having adverse outcomes with Chapter 6 providing useful information on age factor. Similar to the previous chapter, it is meaningful to focus on CT pulmonary opacity scores in relation to characteristics and outcomes, which will be completed in Chapter 7.

Chapter 7 Associations between CT pulmonary opacity score on admission and clinical characteristics and outcomes in patients with COVID-19

Chapter 3 to Chapter 5 suggest that beside of age factor, greater pulmonary opacity score in CT is also one of the independent risk factors of severe or critically ill status, progression from milder disease to more severe status, and respiratory failure in patients with COVID-19. Therefore, similar to Chapter 6, Chapter 7 here will present the information of differences in clinical characteristics, disease severity, and clinical outcome burden in different pulmonary opacity score groups of patients with COVID-19, which is helpful for us to understand the novel disease of COVID-19.

7.1 Introduction

Computed tomography (CT) is important in the diagnosis and monitoring of emerging infectious diseases caused by viral infections and manifested mainly in the respiratory tract (such as H1N1 and severe acute respiratory syndrome [SARS]) [291,292]. COVID-19 is a clinical infectious disease resulting in bilateral pneumonia and rapid deterioration of lung function [293]. Although real-time reverse transcriptase-polymerase chain reaction assay (RT-PCR) is the standard diagnostic method for COVID-19, chest CT plays an important supplementary role in the diagnosis [91] with a high sensitivity but a lower specificity [294]. CT findings of COVID-19 are mainly patchy glass opacity in the peripheral area [92,93]. Previous studies have shown that the abnormal imaging of COVID-19 patients was correlated with main clinical symptoms [295], demographic, epidemiologic, clinical, laboratory characteristics, treatments, severe/critical

pneumonia [95], maximal respiratory severity score [96], intensive care unit (ICU) admission [97], and length of hospitalization [98].

This large multi-centre cohort study aims to systematically investigate associations between CT pulmonary opacity score on admission and clinical features and outcomes in COVID-19 patients.

7.2 Material and methods

7.2.1 Study population

Inclusion criteria were as of March 15, 2020, all patients (N=631) diagnosed with COVID-19 in all 24 hospitals designated for COVID-19 treatment in Jiangsu province, China according to the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" released by the National Health Commission & National Administration of Traditional Chinese Medicine of China [212]. Exclusion criteria: Patient with no available medical records (N=6) or CT pulmonary opacity score (N=129, did not take CT but X-ray examination). The study ultimately included 496 cases.

7.2.2 Data collection and definition of variables

Data were extracted from medical records provided by the Data Centre of Jiangsu Provincial Health Commission of China. Variables analyzed were demographic features (age, sex), epidemiological features (type of disease onset [single onset or clustering onset]), clinical features on admission (initial symptoms, medical history, vital signs), radiologic features on admission (CT pulmonary opacity score), laboratory features on admission (hematology,

organ function, inflammatory factors, coagulation function indicators), clinical management during hospitalization (supportive treatments and medical drugs), and clinical outcomes (disease severity, ICU admission, respiratory failure, length of hospital stay). A clustering onset was defined as the occurrence of two or more confirmed COVID-19 cases in the same cluster/group within 14 days, such as family and hospital. Other cases were classified as single onset. Two radiologists (YW and SJ) with more than 5 years of working experience in chest imaging performed visual evaluation of CT images and the agreement was reached through consultation if discrepancies of pulmonary opacity score occurred. Pulmonary opacity score was defined as the percentage of pulmonary opacity (ground-glass opacities [GGO] or consolidation) area relative to the entire lung on CT images (range: 0-100%), rounded to the nearest 5%. GGO was defined as an area of hazy increased attenuation, in which vessels and bronchial markings may still be observable. Consolidation was more opaque than GGO, in which such markings were obscured. On CT image, GGO and consolidation looked more grey or hazy compared to the normal dark appearance of the lung. Figure 1 showed an example of CT image for a patient with pulmonary opacity score being 70%.

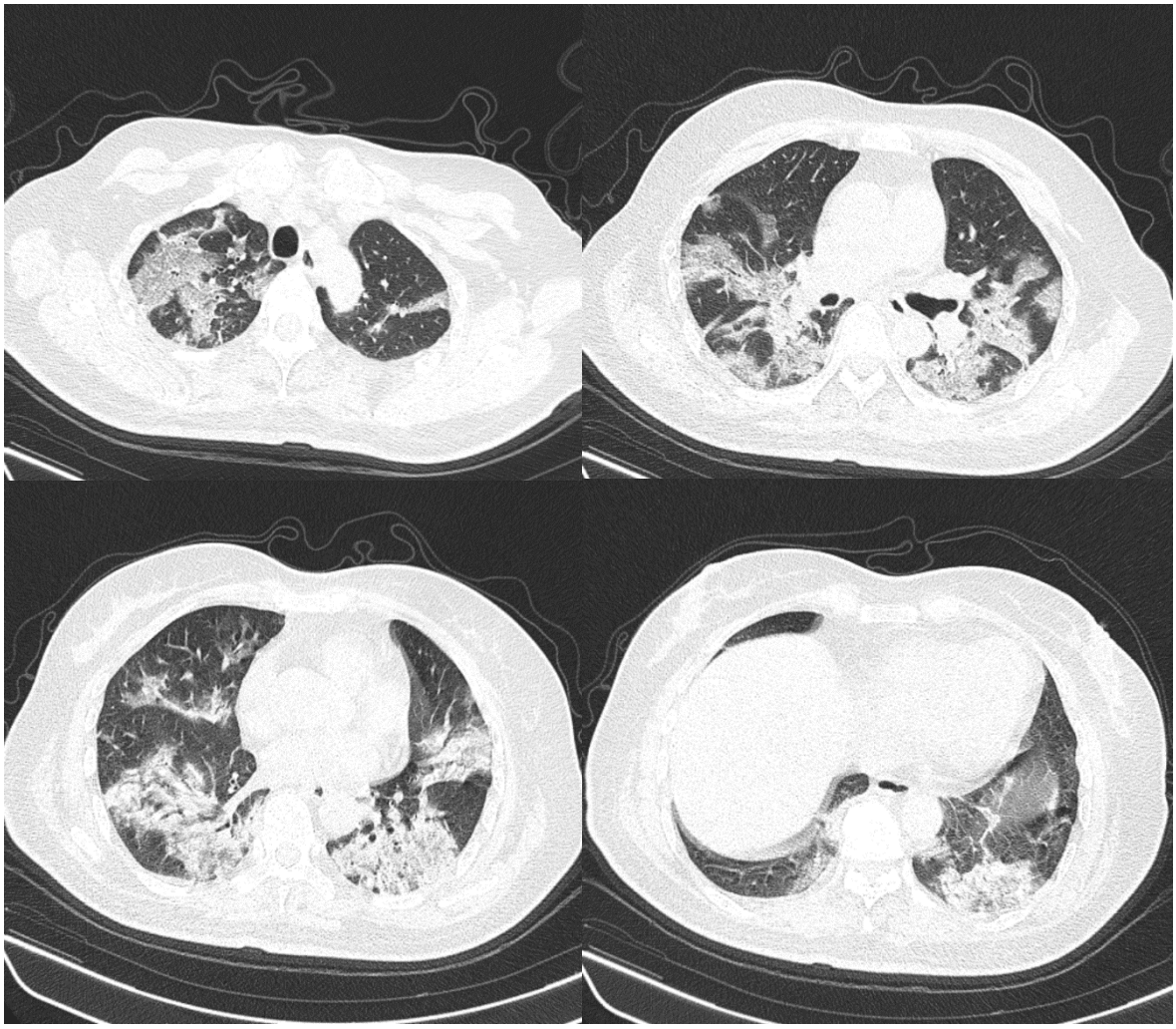

Figure 1: CT images of a patient with pulmonary opacity score of 70%. Details: Female, 61 years old. Her CT was performed 1 day after admission. She was diagnosed with respiratory failure on the next day.

Disease severity were measured at days 1, 2, 3, 4, 5, 6, 7 and 14 after admission, mortality and hospitalization status were available until March 15, 2020. Asymptomatic infection was defined as the absence of clinical symptoms but a positive RT-PCR result. Mild disease was defined as having mild clinical symptoms without respiratory distress and the absence of imaging manifestations of pneumonia. Moderate disease was the presence of fever, respiratory tract symptoms and imaging manifestations of pneumonia. Severe disease

was the presence of at least one of the follows: respiratory distress, respiratory rate ≥ 30 breaths/minute; peripheral capillary oxygen saturation (SpO_2) ≤ 93%; or arterial blood oxygen partial pressure (PaO_2) / fraction of inspired oxygen (FiO_2) ≤ 300 mmHg (1 mmHg = 0.133kPa). Critically ill was having respiratory failure requiring mechanical ventilation, shock or combined organ failure requiring ICU monitoring and treatment.

7.2.3 Statistical analysis

Patients were divided into four groups based on the quartile of baseline CT pulmonary opacity score: <=5%, 6-20%, 21-40% and 41%+. Continuous variables of baseline features and clinical management during hospitalization were reported as mean (standard deviation [SD]) or median (interquartile range [IQR]) by group and compared using ANOVA test or Kruskal-Wallis test depending on their distributions. Binary variables were summarized using frequency and percentage and compared using χ^2/Fisher exact test. To assess the linear trend effect of pulmonary opacity score on baseline features and clinical management, generalized linear model (GLM) was employed with pulmonary opacity score as the only predictor (continuous variable). For GLM analysis of continuous variables, normal distribution and identity link function were used, whereas for GLM analysis of binary variables, binomial distribution and logit link function were used. The analysis of inter-observer agreement for pulmonary opacity scores was conducted using an intraclass correlation coefficient (ICC) calculated from a generalized linear mixed model.

To explore associations between pulmonary opacity score with characteristics on admission, univariate and multivariate linear regression analysis was conducted, with

pulmonary opacity score as dependent variable (continuous variable). To assess effects of pulmonary opacity score on clinical outcome, GLMs were performed, in which pulmonary opacity score was treated as a continuous and categorical variable, controlling for baseline characteristics.

Two-tailed $P < 0.05$ was considered statistically significant. The analyses were performed using SAS 9.4 (SAS Institute).

7.3 Results

Of 496 patients (270 [54.4 %] men, mean age 45.10 [SD 17.13] years), 244 (49.2%) were single onset, 339 (68.3%) had fever, 279 (56.3%) had cough, 130 (26.2%) had sputum, 71 (14.3%) had hypertension, 31 (6.3%) had diabetes, 30 (6.0%) smoked, mean temperature was 37.09 °C (SD 0.76), mean heart rate was 87.70 beats/minute (SD 13.42), mean respiratory rate was 19.10 breaths/minute (SD 2.49) and mean peripheral capillary oxygen saturation (SpO_2) was 97.60% (SD 2.16) (Table 7.1). The ICC value for pulmonary opacity score from the two radiologists was 0.93. With the rise of pulmonary opacity score, age and body temperature statistically significantly increased (trend test) while SpO_2 decreased, and the proportion of men and patients who were single onset and who had initial symptoms, hypertension comorbidity and smoking habit increased.

Table 7.1 Demographic and clinical characteristics of patients with COVID-19 at admission

		Pulmonary opacity group, mean (SD) or n (%)						
Category	Characteristics	All (N=496)	<=5% (N=142)	6-20% (N=123)	21-40% (N=135)	41%+ (N=96)	P-value*	P-value†
Demographic	Male	270(54.4%)	68(47.9%)	63(51.2%)	78(57.8%)	61(63.5%)	0.0791	0.0094
	Age (year)	45.10(17.13)	37.38(19.44)	44.52(16.95)	48.57(13.64)	52.36(13.27)	<.0001	<.0001
Types of disease onset	Single onset	244(49.2%)	37(26.1%)	62(50.4%)	77(57.0%)	68(70.8%)	<.0001	<.0001
	Clustering onset	252(50.8%)	105(73.9%)	61(49.6%)	58(43.0%)	28(29.2%)	<.0001	<.0001
Initial symptoms	Fever	339(68.3%)	66(46.5%)	86(69.9%)	107(79.3%)	80(83.3%)	<.0001	<.0001
	Cough	279(56.3%)	66(46.5%)	65(52.8%)	80(59.3%)	68(70.8%)	0.0019	<.0001
	Sputum	130(26.2%)	31(21.8%)	22(17.9%)	38(28.1%)	39(40.6%)	0.0009	<.0001
Medical history	Hypertension	71(14.3%)	11(7.7%)	11(8.9%)	24(17.8%)	25(26.0%)	0.0002	<.0001
	Diabetes	31(6.3%)	6(4.2%)	4(3.3%)	8(5.9%)	13(13.5%)	0.0088	0.0009
	Current smoker	30(6.0%)	5(3.5%)	6(4.9%)	8(5.9%)	11(11.5%)	0.1011	0.0237
Vital signs	Temperature (°C)	37.09(0.76)	36.84(0.56)	37.07(0.73)	37.21(0.78)	37.33(0.91)	<.0001	<.0001
	Heart rate (beat/minute)	87.70(13.42)	86.64(12.29)	85.25(13.66)	88.09(13.69)	91.88(13.51)	0.0023	0.0012
	Respiratory rate (breath/minute)	19.10(2.49)	18.87(1.67)	18.68(1.81)	19.20(2.70)	19.85(3.57)	0.0031	<.0001
	Peripheral capillary oxygen saturation (SpO_2, %)	97.60(2.16)	97.97(1.18)	97.88(1.11)	97.76(1.88)	96.49(3.74)	<.0001	<.0001

* P value from testing differences in proportions or means or distributions among different pulmonary opacity groups.

† P value from trend test of linear pulmonary opacity effect in generalized linear model in which pulmonary opacity (%) was included as the only predictor.

SD, standard deviation.

As pulmonary opacity score raised, lymphocyte count, platelet count and albumin level statistically significantly declined, while the level of CRP and fibrinogen elevated (trend test, Table 7.2). The proportion of patients receiving medical treatment and oxygen support increased with the increase in pulmonary opacity score, except for the rarely used continuous renal replacement therapy and extracorporeal membrane oxygenation, and the commonly used interferon and antivirals (trend test, Table 7.3).

Table 7.2 Laboratory parameters of patients with COVID-19 at admission

			Pulmonary opacity group, N, median (IQR)					
Category	Parameters	All (N=496)	<=5% (N=142)	6-20% (N=123)	21-40% (N=135)	41%+ (N=96)	P-value*	P-value†
Blood test	White blood cell count (10^9/L)	413,4.8(3.9-6.0)	124,5.2(4.3-6.6)	93,4.6(3.5-5.4)	113,4.4(3.6-5.9)	83,4.9(3.9-6.2)	0.0009	0.9967
	Neutrophil (10^9/L)	407,2.9(2.1-3.9)	117,3.0(2.3-4.1)	94,2.7(2.0-3.5)	113,2.9(2.1-3.6)	83,3.3(2.5-4.6)	0.0071	0.0028
	Lymphocyte (10^9/L)	405,1.3(0.9-1.7)	117,1.6(1.2-2.1)	93,1.3(1.0-1.6)	112,1.3(0.9-1.6)	83,1.0(0.7-1.3)	<.0001	<.0001
	Hemoglobin (g/L)	410,135.5(123.0-150.0)	124,135.5(124.0-149.0)	93,135.0(121.0-154.0)	111,134.0(118.0-146.0)	82,138.0(124.0-154.0)	0.4568	0.9862
	Platelet (10^9/L)	395,182.0(149.0-218.0)	123,199.0(160.0-235.0)	91,184.0(154.0-211.0)	107,175.0(144.0-208.0)	74,160.0(123.0-211.0)	0.0026	0.0002
Organ function	Alanine aminotransferase (U/L)	341,24.9(16.0-37.5)	103,22.0(15.0-33.0)	82,24.0(14.5-39.3)	95,27.0(19.0-38.0)	61,26.4(19.0-44.0)	0.0280	0.0003
	Albumin (g/L)	396,41.0(37.5-44.3)	119,42.0(38.0-46.7)	91,41.6(38.8-45.5)	107,41.0(37.5-43.7)	79,39.8(34.0-42.0)	0.0003	<.0001
	Total bilirubin (umol/L)	381,9.9(6.0-14.3)	114,9.6(5.0-15.6)	85,9.0(6.6-14.1)	106,10.1(6.4-14.9)	76,10.1(5.3-12.9)	0.7289	0.8663
	Creatinine (umol/L)	392,64.0(51.0-78.4)	118,61.0(48.0-78.0)	91,65.0(51.0-76.0)	103,60.7(47.0-76.0)	80,70.5(57.5-86.0)	0.0125	0.0426
Inflammatory factors	C-reactive protein (mg/L)	377,10.0(4.0-25.2)	112,5.1(1.0-10.0)	89,10.0(4.5-16.2)	96,13.4(5.4-26.9)	80,33.6(10.2-67.0)	<.0001	<.0001
	Procalcitonin (ng/mL)	355,0.0(0.0-0.2)	100,0.1(0.0-0.2)	85,0.0(0.0-0.1)	102,0.0(0.0-0.1)	68,0.1(0.0-0.4)	0.0007	0.1307
Coagulation function test	Activated partial thromboplastin time (s)	407,33.0(28.0-38.0)	121,32.8(27.9-38.0)	93,32.9(28.2-37.3)	111,32.4(28.0-38.0)	82,34.6(28.8-38.3)	0.8155	0.9293
	Fibrinogen (g/L)	393,3.6(2.8-4.4)	115,3.0(2.5-3.7)	90,3.1(2.6-3.8)	109,4.1(3.4-4.4)	79,4.5(3.8-5.5)	<.0001	<.0001
	D-dimer (mg/L)	393,0.3(0.2-0.4)	113,0.3(0.2-0.4)	91,0.2(0.2-0.4)	108,0.3(0.2-0.4)	81,0.3(0.2-0.5)	0.5765	0.4164

* P value from testing differences in distributions among different pulmonary opacity groups.

† P value from trend test of linear pulmonary opacity effect in generalized linear model in which pulmonary opacity (%) was included as the only predictor.

IQR, interquartile range.

Table 7.3 Clinical management of patients with COVID-19 during hospital stay

		Pulmonary opacity group, n (%)						
Category	Clinical management	All (N=496)	<=5% (N=142)	6-20% (N=123)	21-40% (N=135)	41%+ (N=96)	P-value*	P-value†
Supportive treatments	Inotropic and vasoconstrictive agents	5(1.0%)	0(0.0%)	0(0.0%)	1(0.7%)	4(4.2%)	0.0079	0.0014
	Nasal cannula	178(35.9%)	34(23.9%)	33(26.8%)	58(43.0%)	53(55.2%)	<.0001	<.0001
	Mask	13(2.6%)	0(0.0%)	1(0.8%)	2(1.5%)	10(10.4%)	<.0001	<.0001
	High-flow nasal cannula oxygen therapy	20(4.0%)	2(1.4%)	4(3.3%)	2(1.5%)	12(12.5%)	0.0002	<.0001
	Non-invasive ventilation	29(5.8%)	2(1.4%)	3(2.4%)	4(3.0%)	20(20.8%)	<.0001	<.0001
	Invasive mechanical ventilation	5(1.0%)	0(0.0%)	0(0.0%)	1(0.7%)	4(4.2%)	0.0079	0.0007
	Prone position	15(3.0%)	1(0.7%)	2(1.6%)	2(1.5%)	10(10.4%)	0.0003	<.0001
	Continuous renal replacement therapy	1(0.2%)	0(0.0%)	0(0.0%)	0(0.0%)	1(1.0%)	0.1935	0.1339
	Extracorporeal membrane oxygenation	2(0.4%)	0(0.0%)	0(0.0%)	1(0.7%)	1(1.0%)	0.4553	0.0507
Medical drugs	Traditional Chinese medicine	79(15.9%)	8(5.6%)	14(11.4%)	27(20.0%)	30(31.3%)	<.0001	<.0001
	Immunoglobulin	134(27.0%)	15(10.6%)	33(26.8%)	33(24.4%)	53(55.2%)	<.0001	<.0001
	Interferon	387(78.0%)	117(82.4%)	94(76.4%)	99(73.3%)	77(80.2%)	0.2906	0.4539
	Antioxidants	134(27.0%)	22(15.5%)	27(22.0%)	38(28.1%)	47(49.0%)	<.0001	<.0001
	Glucocorticoid	123(24.8%)	12(8.5%)	19(15.4%)	41(30.4%)	51(53.1%)	<.0001	<.0001
	Thymosin	130(26.2%)	19(13.4%)	27(22.0%)	39(28.9%)	45(46.9%)	<.0001	<.0001
	Neurotrophic drugs	95(19.2%)	15(10.6%)	17(13.8%)	35(25.9%)	28(29.2%)	0.0002	<.0001
	Any antibiotics	283(57.1%)	53(37.3%)	65(52.8%)	89(65.9%)	76(79.2%)	<.0001	<.0001
	Any antivirals	471(95.0%)	135(95.1%)	116(94.3%)	128(94.8%)	92(95.8%)	0.9762	0.6806

* P value from testing differences in proportions among different pulmonary opacity groups.

† P value from trend test of linear pulmonary opacity effect in generalized linear model in which pulmonary opacity (%) was included as the only predictor.

Pulmonary opacity score on admission was independently associated with age, single onset type, presentation of symptoms including fever and cough, vital signs including temperature and SpO_2 (Table 7.4).

Table 7.4 Factors associated with pulmonary opacity score in patients with COVID-19: Results from linear regression analysis*

	Univariate analysis		Multivariate analysis	
Variables	Estimate (95%CI)	P-value	Estimate (95%CI)	P-value
Male	5.16(1.30,9.02)	0.0088	1.20(-2.22,4.63)	0.4917
Age (year)	0.41(0.30,0.52)	<.0001	0.29(0.19,0.39)	<.0001
Single onset	13.22(9.53,16.91)	<.0001	10.29(6.86,13.72)	<.0001
Fever	12.27(8.26,16.29)	<.0001	4.67(0.89,8.46)	0.0158
Cough	8.29(4.46,12.13)	<.0001	4.13(0.32,7.94)	0.0340
Sputum	8.88(4.54,13.21)	<.0001	2.32(-1.96,6.59)	0.2895
Hypertension	11.62(6.19,17.06)	<.0001	2.45(-2.65,7.55)	0.3471
Diabetes	14.02(6.12,21.92)	0.0005	3.42(-3.73,10.57)	0.3492
Smoker	9.48(1.40,17.56)	0.0215	4.14(-2.91,11.19)	0.2506
Temperature (°C)	6.58(4.09,9.06)	<.0001	3.42(1.06,5.79)	0.0047
Heart rate (beat/minute)	0.23(0.09,0.38)	0.0014	0.03(-0.10,0.17)	0.6166
Respiratory rate (breath/minute)	1.54(0.77,2.30)	<.0001	0.41(-0.29,1.11)	0.2504
Peripheral capillary oxygen saturation (SpO_2, per 5%)	-15.23(-19.51,-10.94)	<.0001	-10.44(-14.57,-6.32)	<.0001

* The estimates were coefficients.

CI, confidence interval.

Pulmonary opacity score on admission was independently associated with severe or critical illness, ICU admission and respiratory failure (Table 7.5). Especially, compared to patients with pulmonary opacity score <=5%, those who with score >=41% had a statistically significant increased odds of severe or critical illness (OR, 15.58, 95% CI: 3.82-63.53, p = 0.0001), ICU admission (OR, 6.26, 95% CI: 2.15-18.23, p = 0.0008) and respiratory failure (OR, 19.49, 95% CI: 4.55-83.40, p < 0.0001). Pulmonary opacity score was also associated with the length of hospital stay and in the third quartile versus the first quartile, the duration of hospitalization raised significantly (coefficient, 2.59, 95% CI: 0.46-4.72, *p* = 0.0170).

Table 7.5 Effects of pulmonary opacity score on clinical outcomes in patients with COVID-19: Results from generalized linear model analysis

			Unadjusted analysis		Adjusted analysis*	
Clinical outcome†	Pulmonary opacity score	n/N(%) or N,Mean(SD)	Estimate (95%CI)	P-value	Estimate (95%CI)	P-value
Severe or critically ill (Yes or no)	Score as continuous variable (per 5%)	58/496(11.7%)	1.38(1.28,1.49)	<.0001	1.35(1.22,1.49)	<.0001
	Score as categorical variable					
	<=5%	3/142(2.1%)	1.00		1.00	
	6-20%	6/123(4.9%)	2.38(0.58,9.71)	0.2282	1.79(0.37,8.53)	0.4665
	21-40%	11/135(8.1%)	4.11(1.12,15.07)	0.0330	2.19(0.50,9.61)	0.2998
	41%+	38/96(39.6%)	30.36(9.01,102.28)	<.0001	15.58(3.82,63.53)	0.0001
ICU admission (Yes or no)	Score as continuous variable (per 5%)	64/496(12.9%)	1.31(1.22,1.39)	<.0001	1.27(1.16,1.38)	<.0001
	Score as categorical variable					
	<=5%	7/142(4.9%)	1.00		1.00	
	6-20%	7/123(5.7%)	1.16(0.40,3.42)	0.7824	0.77(0.22,2.62)	0.6713
	21-40%	12/135(8.9%)	1.88(0.72,4.93)	0.1986	0.91(0.29,2.86)	0.8700
	41%+	38/96(39.6%)	12.64(5.33,29.95)	<.0001	6.26(2.15,18.23)	0.0008
Respiratory failure (Yes or no)	Score as continuous variable (per 5%)	55/496(11.1%)	1.39(1.28,1.50)	<.0001	1.39(1.25,1.55)	<.0001
	Score as categorical variable					
	<=5%	3/142(2.1%)	1.00		1.00	
	6-20%	5/123(4.1%)	1.96(0.46,8.39)	0.3626	1.66(0.32,8.54)	0.5419
	21-40%	10/135(7.4%)	3.71(1.00,13.77)	0.0504	2.37(0.52,10.90)	0.2658
	41%+	37/96(38.5%)	29.06(8.62,97.96)	<.0001	19.49(4.55,83.40)	<.0001
Hospital stay (Continuous variable, day)	Score as continuous variable (per 5%)	496,17.62(7.53)	0.32(0.17,0.47)	<.0001	0.24(0.07,0.41)	0.0047
	Score as categorical variable					
	<=5%	142,15.94(6.36)	0.00		0.00	
	6-20%	123,17.36(7.45)	1.42(-0.37,3.21)	0.1192	1.13(-0.65,2.91)	0.2148
	21-40%	135,18.20(7.84)	2.26(0.52,4.01)	0.0110	1.37(-0.45,3.18)	0.1397
	41%+	96,19.65(8.27)	3.71(1.79,5.63)	0.0002	2.59(0.46,4.72)	0.0170

* All variables in Table 1 were included in the adjusted analysis.

† For severe or critically ill, intensive care unit admission and respiratory failure, the estimates were odds ratios; while for hospital stay, the estimates were coefficients.

SD, standard deviation; ICU, intensive care unit.

7.4 Discussion

To our knowledge, this is by far one of the largest studies that systematically assessed associations between CT pulmonary opacity score and clinical features and outcomes in COVID-19 patients. The study demonstrated that demographic, epidemiological, clinical, laboratory features on admission, and clinical management during hospitalization showed a linear trend by the quartile CT pulmonary opacity score. CT score correlated with poor clinical outcomes including severe and critical illness, ICU admission, respiratory failure and prolonged hospital stay. Our study introduced a simple quantitative parameter of pulmonary opacity score from CT to describe lung involvement which is reproducible for radiologists.

To facilitate better communication between radiologists and physicians, different classification systems of COVID-19 incorporating imaging findings (e.g. ground-glass opacities (GGO), consolidation, crazy-paving pattern and fibrosis) have been developed [296]. CT imaging findings play a role in the classification and staging of COVID-19, early detection in relation to a serological test, disease severity and guide to therapy, surveillance with the response to therapy, prediction of secondary bacterial infection, differentiation from simulating lesions, and screening with prevention and control [296]. Follow-up CT scans were used for longitudinal evaluation by allowing the detection of disease progression, complications, and suspected acute respiratory deteriorations [297]. Advanced imaging modalities, such as quantitative CT techniques may also be valuable in delineation of the pulmonary distribution (GGO and consolidations) to assess the disease severity and detect disease progression on follow-up [296,297]. To calculate the opacity extent, we employed a more precise CT scoring system that used the percentage of pulmonary opacity area relative to the entire lung zone on CT image in the unit of 5%. Other studies applied crude CT scoring

systems. For example, Wang et al. used 0:0%; 1:1-49%; 2:50-75%; 3:>75%; range 0-3; global score 0-15 [298]; Hu et al. used 0:0%; 1:1-25%; 2:26-50%; 3:51-75%; 4:76-100%; range 0-4; global score 0-20) [233,299-302]; Francone et al. used 0:0%; 1:< 5%; 2:5-25%; 3:26-50%; 4:51-75%; 5:> 75%; range 0-5; global score 0-25) [303,304]; Zhao et al. used 0:0%; 1:< 25%; 2:25-49%; 3:50-74%; 4:>= 75%; range 0-4; global score 0-24) [100,305,306]; Aalinezhad et al. used another system (0:0%; 1:< 50%; 2:> 50%; range 0-2; global score 0-40) [307,308]. Similarly, Guillo et al. used a scoring system of 0-10%, 11-25%, 26-50%, 51-75% and 76-100% ground glass opacities and consolidation [309]. The ICC value for pulmonary opacity score from the two radiologists (YW and SJ) in our study was closed to 1, indicating high inter-observer consistency and reliability. Due to the good agreement of pulmonary opacity score assessed by the two radiologists, the average of pulmonary opacity scores was used when discrepancies occurred.

The study found that age, single onset, initial symptoms, body temperature and SpO_2 on admission in patients with COVID-19 were independent predictors of CT pulmonary opacity score, indicating more severe lung function injury. This is consistent with the previous studies reporting that clinical characteristics including patient age and coexisting condition, immune status, body temperature and exposure history may be related to CT imaging in patients with viral pneumonia including SARS and COVID-19 [95,292,310,311]. In addition, Aalinezhad et al.'s study also showed a significant inverse relationship between CT severity score and SpO_2 [308].

Our study showed that in patients with COVID-19, as CT pulmonary opacity score increased, platelet count and albumin level decreased, while the level of CRP and fibrinogen elevated, and >=41% of lung involvement was associated with more severe lymphopenia,

suggesting signs of viral infection and inflammation, abnormal coagulation function and liver function. Previous studies reported that CT severity showed a positive association with CRP level and negative correlation with lymphocyte count [312,313]. Francone et al. found the semi-quantitative CT score was significantly correlated with CRP (correlation coefficient r = 0.62) and D-dimer (r = 0.66) levels [303] while our data provided some evidence to support the association with CRP but not with D-dimer. A study demonstrated that age and monocyte-lymphocyte ratio may predict imaging progression on chest CT in COVID-19 patients [314]. Other studies indicated moderate positive correlations between CT severity score and transferrin, lactate dehydrogenase, troponin, and inflammation-related factors of leucocytes, neutrophils, and IL-2R (r range: 0.45-0.60) [299,315], although we were unable to verify those correlations due to lack of data.

The study presented that the proportion of patients receiving medical treatment and oxygen support increased with the rise of pulmonary opacity score. Khosravi et al. also found patients with baseline CT severity score > 8 had 3-fold higher risk of intubation [305]. Our data showed that pulmonary opacity score, especially when >=41%, may be an accurate indicator of severe or critical illness, acute respiratory failure and intensive care requirements. Zhao et al. evaluated lung involvement using another CT scoring system and also found mean score was higher in the emergency group (mild and common types) than in the nonemergency group (severe and fatal types) [100]. More studies showed the CT score calculated by their scoring systems was significantly higher in critical and severe than in mild and common category [233,300,303]. Other studies found that the overall lung involvement score had predictive value for clinical severity and ICU admission, and higher chest CT score was significantly associated with an increase in requirement of oxygen and even mechanical

ventilation [298,305-307,316,317]. CT involvement score can help early diagnosis, severity assessment and treatment of COVID-19 [95,318,319] and may indicate the progression and recovery of the disease [92,320]. These findings are similar to that of SARS that the extent of lung opacification was an objective prognostic indicator of death, disease severity and requirements of aggressive therapy for assisted ventilation or oxygen supplementation [321-323], and pulmonary opacity resolved over time in convalescent patients [324,325]. We found patients with more pulmonary opacity stayed longer in hospital. This may be due to more severe illness and more medical treatment and oxygen support associated with higher pulmonary opacity score. Previous evidence suggested that patients having bilateral pneumonia were hospitalized longer than those with normal CT scan results [98] and patients with higher CT scores may have more prolonged disease course and hospital stay [302,326,327]. Our study did not assess the association between pulmonary opacity score and mortality because of no recorded death in the study sample as a result of early recognition and intervention [58].

The study had several merits compared with previous studies. First, all eligible COVID-19 patients with and without symptoms, including asymptomatic, mild, moderate, severe and critically ill, in Jiangsu province, were included in the current study whereas most of the previous studies focused on the patients with moderate, severe or critically ill symptoms. Therefore, results from this study are more generalizable to the COVID-19 patients with a wide spectrum of infections, particularly in the post-COVID-19 era when severe cases are supposed to decrease. Second, the study provided some novel and robust information on associations between pulmonary opacity score and demographic, epidemiological, clinical, laboratory features and clinical management based on a larger

dataset (N=496) than most previous studies on the similar topic (mostly N=50-200). Third, the present study assessed impacts of pulmonary opacity score at admission on a number of important clinical outcomes (including disease severity, ICU admission, respiratory failure, and hospital stay), instead of a single clinical outcome alone in most previous reports, thus providing a more comprehensive perspective of the relationships between pulmonary opacity score and various clinical outcomes in patients with COVID-19. Fourth, to assess effects of pulmonary opacity score on clinical outcomes, the pulmonary opacity score was measured in the unit of 5% and treated as both continuous and categorical variables in the generalised linear models, and the results were robust regardless of CT score function forms. This is in contrast to most previous studies in which CT score was treated as either a continuous variable or binary variable, which may have generated biased statistical results due to possibly wrong specification of CT score.

This study had some limitations. First, the study excluded 129 patients who did not take CT but X-ray examination and hence had no CT pulmonary opacity score, so selection bias may occur. Second, although this study analyzed effects of pulmonary opacity scores at admission on disease severity, ICU admission and respiratory failure during the whole study period, we only recorded CT at the time of admission; hence it was impossible to analyze the change in pulmonary opacity scores throughout the study period. Third, due to lack of data, we did not analyze associations between CT pulmonary opacity score and some other characteristics, e.g. transferrin, leucocytes and platelet-lymphocyte ratios, which had been reported to be associated with pulmonary opacity score in the previous studies [299,315,328]. For the same reason, we did not analyze association between CT findings and pulmonary embolism although a previous study has showed a high pulmonary embolism prevalence at

CT pulmonary angiography in patients testing positive for COVID-19 [329]. Lastly, this was an observational study and the observed results may still be subject to possible unobserved confounding factors.

7.5 Conclusion

This chapter (Chapter 7) indicates that the degree of CT pulmonary opacity was closely related to age, single onset, fever, cough, SpO_2, lymphocyte count, platelet count, albumin level, CRP level and fibrinogen level. Patients with high pulmonary opacity score, in particular, >=41%, had a high risk of severe or critical illness, ICU admission, respiratory failure and long hospital stay. More attention may need to be paid to patients with high pulmonary opacity score to reduce the adverse impact of COVID-19.

Chapter 3 to Chapter 5 suggest several risk factors of severe or critically ill status, progression from milder disease to more severe status, and respiratory failure in patients with COVID-19, including age, exposure to the pandemic centre, respiratory rate, lymphocyte count, pulmonary opacity score in CT, and so on. Chapter 6 and Chapter 7 provide further information of the impact of two important factors, i.e. age and pulmonary opacity score in CT, on the novel disease of COVID-19. Therefore, based on these understandings, we could further build a prediction model to provide accurate, personalised predictions of the risk of being severe or critically ill status, which can help early identify those at high risks of severe disease and death. This will be carried out in Chapter 8.

Chapter 8 A nomogram predicting severe COVID-19 based on a large study cohort from China

Chapter 3 to Chapter 7 investigate several risk factors of severe or critically ill status, progression from milder disease to more severe status, and respiratory failure in patients with COVID-19, and comprehensively look into age factor and pulmonary opacity score in CT. Basing on this knowledge, Chapter 8 here builds a nomogram which can be a reliable prediction tool for assessing the probability of being severe or critically ill and may facilitate clinicians stratifying patients and providing early and optimal therapies.

8.1 Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory disease caused by the novel coronavirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Patients with COVID-19 may be asymptomatic, have mild to moderate symptoms (such as cough, fever, dyspnea, and pneumonia), be in severe or critically ill condition, or even die. The COVID-19 pandemic has placed an unprecedented burden on the world economy and health care [3]. Delayed treatment for severe COVID-19 in particular can lead to a prolonged hospitalization duration, increased mortality and a heavier financial burden [142,143]. Risk factors for severe COVID-19 are currently considered to be age, comorbidities, dyspnea, chest pain, cough, expectoration, lower lymphocyte and higher leukocyte counts, blood urea nitrogen/creatinine ratios and serum ferritin, pulmonary opacity, and so on [67,136,141,144,330,331]. As there are many related risk factors, the use of accurate prediction tools and early intervention are important in

addressing severe COVID-19.

However, the prediction models of severe COVID-19 available to date are subject to various biases related to data quality (the presence and handling of missing data), flaws in the statistical analysis (lack of internal and external validation and categorization of continuous predictors and hence loss of information and weak assessment of model calibration and discrimination) and poor reporting (no mention of missing data) [127-136].

A nomogram is a two-dimensional graphical representation of a scoring model consisting of multiple scale axes designed to quickly, visually calculate the probability of having an outcome with acceptable accuracy. The axis on the top is the point scale, which is followed by scale axes for the selected predictors, the total point scale, and then the probability scale. Each value on a predictor axis corresponds vertically to a point on the point scale. After determining the points for all the predictors from the point scale and adding them to obtain the total number of points, we can find a corresponding probability on the probability scale. Compared with other risk estimates and decision-making aid tools (risk groupings, artificial neural networks, probability tables, classification and regression tree analyses), nomograms provide a user-friendly interface (no computer software is required for interpretation and prediction) with consistent, highly accurate risk estimates [140].

Therefore, this study aimed to construct a nomogram based on a large number of COVID-19 patients to provide accurate, personalized predictions of severe COVID-19.

8.2 Methods

8.2.1 Study design and subjects

This study was based on a large, multicenter retrospective derivation cohort and a validation cohort. Patients were included if they fulfilled the diagnostic criteria of the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" released by the National Health Commission & National Administration of Traditional Chinese Medicine of China [212]. Patients without medical records or computed tomography (CT) information were excluded. The derivation cohort consisted of 496 patients from Jiangsu Province, China, between January 10, 2020 and March 15, 2020, and the validation cohort contained 105 patients from Huangshi, Hunan Province, China, between January 21, 2020, and February 29, 2020.

8.2.2 Variables measured

The primary outcome was severe or critical illness within the follow-up period. According to disease severity, patients were categorized into two groups: (1) the asymptomatic/mild/moderate group and (2) the severely or critically ill group [212]. Asymptomatic disease was defined as the absence of clinical symptoms and a positive nucleic acid test (real-time reverse transcriptase–polymerase chain reaction assay (RT-PCR) for SARS-CoV-2). Mild disease was defined as the presence of mild clinical symptoms without respiratory distress and the absence of imaging manifestations of pneumonia. Moderate disease was the presence of fever with respiratory symptoms and imaging manifestations of pneumonia. Severe disease was the presence of at least one of the three following conditions: respiratory distress, a respiratory rate ≥30 breaths/min; oxygen saturation (SpO_2) ≤93%; or

arterial blood oxygen partial pressure (PaO_2)/fraction of inspired oxygen (FiO_2) ≤300 mmHg (1 mmHg = 0.133 kPa). Critical illness was having respiratory failure requiring mechanical ventilation, shock or combined organ failure requiring intensive care unit (ICU) monitoring and treatment.

Disease severity was assessed at days 1, 2, 3, 4, 5, 6, 7 and 14 after admission (except for those who were discharged before day 14), the highest degree of severity was selected for analysis, and patients were followed up to discharge. Data were collected using medical records. Predictive baseline variables included sex, age, time from illness onset to admission (days), fever, cough, dyspnea, Charlson comorbidity index (CCI, a weighted index considering comorbidities associated with mortality [332]), white blood cell count, lymphocyte count, platelet count, creatinine level, C-reactive protein (CRP) level, procalcitonin level, D-dimer level, radiologic quadrant score and pulmonary opacity score.

Imaging grading was performed by two independent radiologists with more than 5 years of experience in pulmonary imaging. Axial chest CT sections were divided into quadrants (left, right, anterior, and posterior) by drawing horizontal and vertical lines through the center of the chest. Quadrant scores were estimated as the sum of quadrants with pulmonary opacities extending from the proximal to the distal end of the chest and ranged from 0 to 4. The pulmonary opacity score was visually assessed as the percentage of bilateral lung area with pulmonary opacity rounded to the nearest 5%.

8.2.3 Statistical analysis

Baseline characteristics of the derivation and validation cohorts are summarized as the median (interquartile range [IQR]) or frequency (percentage) and were compared using the

Mann–Whitney U test for continuous variables and the χ^2 test or Fisher exact test for categorical variables.

Univariate binary logistic regression analysis was performed to select potential predictors of severe COVID-19. In addition to considering the clinical significance, collinearity analysis was conducted to exclude variables with slight collinearity (Spearman's rank correlation coefficient >0.2) before univariate analysis. To determine the optimal combination of baseline characteristics for predicting severe COVID-19, a multivariate logistic regression model with variables selected via a backward stepwise process based on the smallest Akaike information criterion was established. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to evaluate associations between predictors and severe COVID-19. No categorical data were missing, and missing continuous data were imputed with medians. A nomogram for the prediction of severe COVID-19 was established with the determined predictors.

The nomogram was internally validated on the derivation cohort using the bootstrap method and further externally validated on a separate independent validation cohort from Huangshi. Discrimination ability and calibration were used to assess the performance of the nomogram. The area under the receiver operating characteristic curve (AUC) and its 95% CI were used to evaluate model discrimination (0.5–1.0, the higher the better). Calibration plots were presented using the bootstrap resampling method (1000 resamples). Calibration plots are a good way to visually compare the accordance (agreement) between the predicted and actual absolute risk. The ideal calibration curve is located on the 45-degree diagonal, which reflects perfect consistency. Hosmer-Lemeshow tests were also conducted ($P \geq 0.05$ indicates that the model fits the data well [well-calibrated]).

The 2-tailed $P < 0.05$ was set as the significance level. Statistical analyses were performed using R software (version 3.6.0, http://www.R-project.org). The "rms" package was used to derive a user-friendly nomogram.

8.3 Results

A total of 601 patients were included in the study, with 496 patients in the derivation cohort and 105 patients in the external validation cohort. During the 14-day hospitalization period, in the derivation and validation cohorts, 58 (11.7%) and 27 (25.7%) patients had severe COVID-19, and 438 (88.3%) and 78 (74.3%) patients had nonsevere COVID-19, respectively ($P < 0.001$). The patients' median age was 49 years (IQR, 35–60), and 53.9% were male (Table 8.1). Most of the baseline characteristics were significantly different between the two cohorts, including age, time from illness onset to admission, dyspnea, CCI, lymphocyte count, platelet count, CRP level, procalcitonin level, D-dimer level, radiologic quadrant score and pulmonary opacity score (all $P < 0.05$).

Table 8.1 Patient baseline characteristics for the derivation and validation cohorts*

Characteristics	Overall cohort (N=601)	Derivation cohort (N=496)	Validation cohort (N=105)	*P* value
Sex				0.650
Female	277 (46.1%)	226 (45.6%)	51 (48.6%)	
Male	324 (53.9%)	270 (54.4%)	54 (51.4%)	
Age (years)	49.0 (35.0–60.0)	47.0 (32.5–57.0)	60.0 (50.0–69.0)	<0.001
Time from onset to admission (days)	5.0 (2.5–8.0)	5.00 (2.0–8.0)	6.0 (5.0–9.0)	<0.001
Fever				0.124
No	199 (33.1%)	157 (31.7%)	42 (40.0%)	
Yes	402 (66.9%)	339 (68.3%)	63 (60.0%)	
Cough				0.094

No	253 (42.1%)	217 (43.8%)	36 (34.3%)	
Yes	348 (57.9%)	279 (56.2%)	69 (65.7%)	
Dyspnea				<0.001
No	566 (94.2%)	493 (99.4%)	73 (69.5%)	
Yes	35 (5.8%)	3 (0.6%)	32 (30.5%)	
CCI				<0.001
0	511 (85.0%)	440 (88.7%)	71 (67.6%)	
≥1	90 (15.0%)	56 (11.3%)	34 (32.4%)	
WBC (10^9/L)	4.84 (3.88–6.01)	4.84 (3.88–5.96)	4.95 (3.92–6.23)	0.473
Lymphocyte count (10^9/L)	1.20 (0.79–1.63)	1.28 (0.90–1.72)	0.95 (0.62–1.33)	<0.001
Platelet count (10^9/L)	165 (130–208)	182 (149–218)	127 (120–136)	<0.001
Creatinine (μmol/L)	63.0 (51.0–78.0)	64.0 (51.0–78.2)	60.5 (50.4–74.1)	0.325
C-reactive protein (mg/L)	11.1 (4.67–34.4)	10.0 (4.04–25.2)	30.8 (13.5–68.7)	<0.001
Procalcitonin (ng/mL)	0.06 (0.02–0.20)	0.04 (0.02–0.20)	0.10 (0.07–0.15)	<0.001
D-dimer (mg/L)	0.25 (0.13–0.41)	0.26 (0.16–0.42)	0.11 (0.04–0.35)	<0.001
Quadrant score (0–4)	3.00 (1.00–4.00)	2.00 (1.00–4.00)	4.00 (4.00–4.00)	<0.001
Pulmonary opacity score (%)	20.0 (5.00–40.0)	20.0 (5.00–40.0)	25.0 (10.0–45.0)	0.030

* Descriptive statistics: frequency (percentage), median (interquartile range).

Abbreviations: CCI, Charlson comorbidity index; WBC, white blood cell.

Considering the clinical significance and collinearity shown in supplementary Figure S8.1, the radiologic quadrant score was excluded in the univariate logistic regression analysis due to the high correlation with pulmonary opacity score. The univariate analysis revealed that age, time from illness onset to admission, fever, cough, dyspnea, CCI, lymphocyte count, platelet count, CRP, D-dimer and pulmonary opacity score were statistically significant risk factors for severe COVID-19 (Table 8.2, all $P < 0.05$).

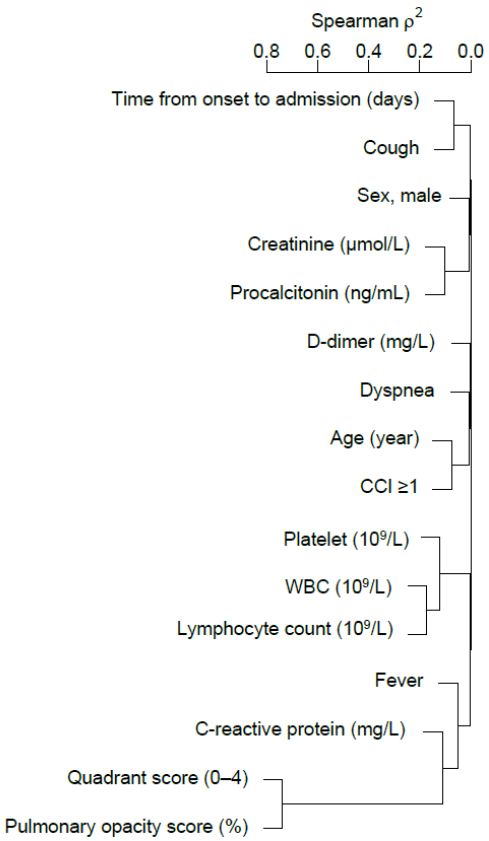

Figure S8.1. Results of the collinearity analysis. Abbreviations: CCI, Charlson comorbidity index; WBC, white blood cell.

Table 8.2 Factors associated with severe COVID-19 in the derivation cohort (univariate logistic regression)

Characteristics	OR (95% CI)	*P* value
Sex, Male vs. Female	1.42 (0.81–2.54)	0.218
Age (years)	1.07 (1.05–1.09)	<0.001
Time from onset to admission (days)	1.07 (1.01–1.13)	0.031
Fever, Yes vs. No	2.11 (1.10–4.42)	0.024
Cough, Yes vs. No	2.01 (1.13–3.75)	0.018
Dyspnea, Yes vs. No	14.52 (1.16–460.18)	0.039
CCI, ≥1 vs. 0	2.95 (1.53–5.51)	0.002
WBC (10^9/L)	0.96 (0.82–1.12)	0.595
Lymphocyte count (10^9/L)	0.04 (0.02–0.10)	<0.001
Platelet count (10^9/L)	0.99 (0.99–1.00)	0.001
Creatinine (umol/L)	1.01 (1.00–1.02)	0.165
C-reactive protein (mg/L)	1.02 (1.01–1.03)	<0.001
Procalcitonin (ng/mL)	1.03 (0.98–1.07)	0.244
D-dimer (mg/L)	1.33 (1.11–1.59)	0.002
Pulmonary opacity score (%)	1.07 (1.05–1.08)	<0.001

Abbreviations: OR, odds ratio; CCI, Charlson comorbidity index; WBC, white blood cell.

Subsequent multivariate analyses identified significant independent predictors of severe COVID-19, including age, lymphocyte count, and pulmonary opacity score (Table 8.3). Since the variance inflation factor value of predictors in the final model was less than 10, the multicollinearity was considered acceptable.

Table 8.3 The final multivariate logistic regression model on which the nomogram was based*

Variable	Coefficient	OR	95% CI	*P* value
Age (years)	0.059	1.061	1.028–1.095	<0.001
Lymphocyte count (10^9/L)	-2.567	0.077	0.023–0.257	<0.001
Pulmonary opacity score (%)	0.053	1.055	1.035–1.075	<0.001

*AUC: 0.93 (95% CI, 0.90–0.96) in the derivation cohort; 0.85 (95% CI, 0.76–0.93) in the validation cohort.

Abbreviations: OR, odds ratio; CI, confidence interval; AUC, area under the receiver operating characteristic curve.

A nomogram was established based on the above three variables, which could predict the probability of severe COVID-19 in an individual patient (Figure 8.1). Lymphocyte count

had the largest regression coefficient absolute value and was used as a reference, and its range (3.5–0) corresponded vertically to the point range (0–100) of the point scale. According to the absolute value of the regression coefficient, each value of the remaining predictors (age and pulmonary opacity score) also corresponds to a point on the point scale vertically. The probability of severe COVID-19 in an individual patient can be determined on the probability scale, which corresponds vertically to the total point scale.

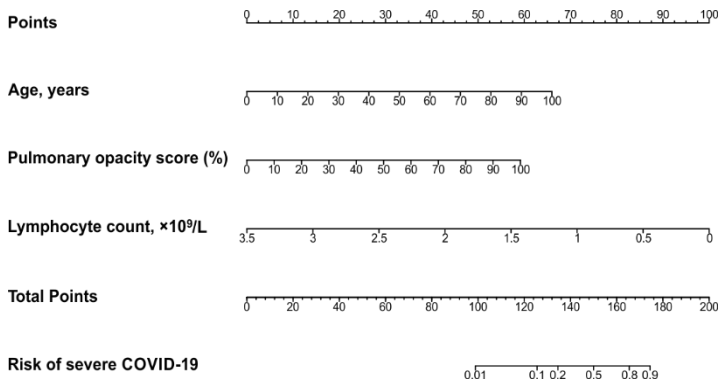

Figure 8.1 Predictive nomogram for the probability of severe COVID-19.

The proposed nomogram showed good discrimination for predicting severe COVID-19 (Figure 8.2; AUC 0.93, 95% CI 0.90–0.96 in the derivation cohort; AUC 0.85, 95% CI 0.76–0.93 in the validation cohort). Furthermore, calibration plots and Hosmer-Lemeshow tests (P = 0.66 in the derivation cohort; P = 0.59 in the validation cohort) revealed that the nomogram was well calibrated and that the actual risks of severe COVID-19 were in good agreement with the predicted risks of severe COVID-19 in both the derivation and validation cohorts (Figure 8.3).

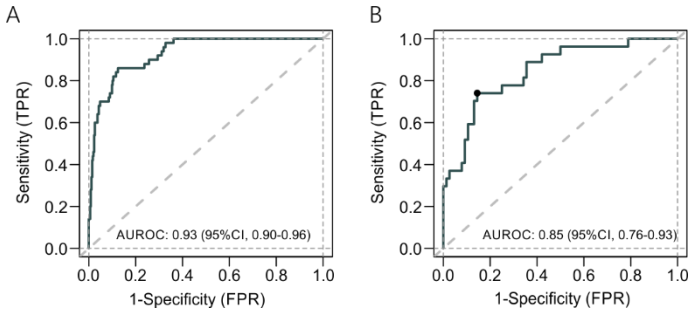

Figure 8.2 Receiver operating characteristic curves of the nomogram in the derivation and external validation cohorts.

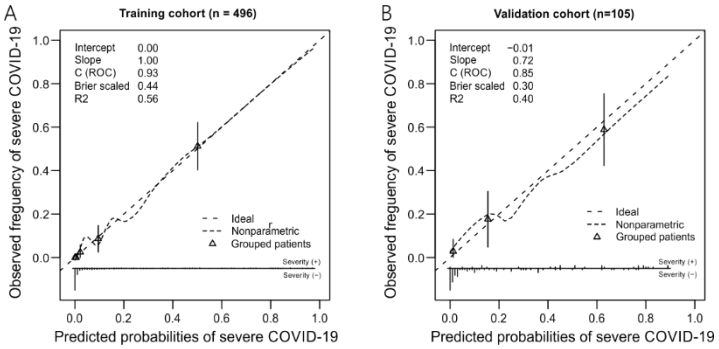

Figure 8.3 Calibration plots of the nomogram in the derivation and external validation cohorts. The 45-degree straight line represents ideal agreement between the actual and predicted probability. The vertical bars represent the 95% confidence interval of the actual probability.

An online tool for automatically calculating prediction probabilities (http://www.China-critcare.com/covid/calculate_en.html) (Figure 8.4) was created to make the prediction model easier to use.

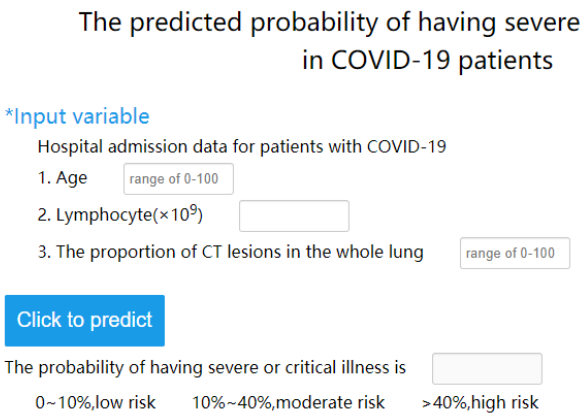

Figure 8.4 Screenshot of the nomogram website.

8.4 Discussion

By employing a large, multicenter retrospective cohort, we constructed a practical nomogram comprised of a few readily available baseline demographic, clinical and CT features (age, lymphocyte count and pulmonary opacity score) to predict severe COVID-19. The model quantifies the individual probability of having severe COVID-19 with good discrimination and agreement, which enables physicians to identify patients with high risk early and correctly and take proactive measures accordingly.

The incidences of severe COVID-19 among our derivation and validation cohorts were significantly different (11.7% vs. 25.7%). Most of the baseline characteristics were also significantly different between the two cohorts, which may be due to the difference in the incidence of severe COVID-19. A meta-analysis showed that compared with patients with nonsevere COVID-19, patients with severe COVID-19 had elevated levels of procalcitonin, CRP, and D-dimer but lower albumin levels [333]. External validation partially identified the general applicability of our nomogram.

In the current prediction model, age was one of the predictors of severe COVID-19. Two

previously developed nomograms also incorporated older age in early risk estimations for severe COVID-19 [130,138]. The relationship between age and severe disease may be related to ACE2. A study showed that ACE2 has an important salutary function: ACE2 limits several detrimental effects, including vasoconstriction and enhanced inflammation and thrombosis, but it is markedly downregulated by the entry of SARS-CoV-2 into cells, which may be especially detrimental in elderly individuals with age-related baseline ACE2 deficiency [334]. In addition, compared with younger COVID-19 patient groups, the elderly (≥65 years) patient population had the highest risk of severe or critical illness, intensive care use, and respiratory failure and the longest hospital stay, which may be partly due to their higher incidence of comorbidities (such as dementia and Parkinson's disease) and age-related degeneration of the immune system (known as immunosenescence) and hence impaired immunity to SARS-CoV-2 [261,335,336].

This study showed that a prolonged time from illness onset to admission may increase the risk of severe COVID-19, which is likely attributed to the delay of treatment. This is consistent with previous research [117,337]. Having symptoms (fever, cough and dyspnea) and a greater CCI (a weighted index considering comorbidities) on admission may also increase the risk of severe COVID-19. Previous studies have largely reported the association between comorbidities and COVID-19 severity [67,141,331].

Laboratory parameters, including lymphocyte count, platelet count, CRP level and D-dimer level, were found to be associated with severe COVID-19 in the univariate logistic regression analysis of this study, which is in accordance with previous research [67,136,330,331]. Among these laboratory parameters, only lymphocyte count was identified as an independent predictor of severe COVID-19. Two previously developed clinical risk scoring

systems also included lymphocyte count in the prediction of COVID-19 severity [130,338]. A previous study proposed four potential mechanisms for reduced lymphocyte levels in COVID-19: lymphocytes are a direct target of viruses because they express the coronavirus receptor ACE2, lymphatic organs are destroyed by SARS-CoV-2, lymphocyte deficiency is induced by pro-inflammatory cytokines, and lymphocyte inhibition results from metabolic disorders [339]. A study showed that the antiviral protein interferon-inducible transmembrane protein 3 (IFITM3) is low in immune cells (including lymphocytes), indicating that SARS-CoV-2 may attack lymphocytes and induce cytokine release syndrome [340].

In terms of radiologic features, the pulmonary opacity score was identified as a predictor of severe COVID-19 in this study. A deep learning-based model also demonstrated that CT imaging can accurately predict the severity of COVID-19 [131]. The mechanism of COVID-19-induced organ damage may be related to ACE2. ACE2 is widely expressed in the lungs (particularly in type 2 pneumocytes and macrophages) [334]. SARS-CoV-2 enters its host cell through the receptor ACE2 and causes diseases [341]. In the lungs, after viral invasion via ACE2, the dysregulation resulting from ACE2 deficiency promotes inflammation and thrombosis triggered by local angiotensin II hyperactivity, leading to cell death and lung damage [334]. In patients infected with SARS-CoV-2, angiotensin II levels were positively linearly correlated with viral load and lung injury [342]. A mouse model demonstrated that severe acute respiratory syndrome coronavirus (SARS-CoV) replicated more efficiently and that pulmonary lesions were more severe in the lungs of transgenic mice with the human gene for ACE2 than in those of wild-type mice [343]. Another mouse model showed that pathologic alterations in the lungs were reduced in ACE2 knockout mice with SARS-CoV compared to wild-type mice with SARS-CoV [344]. Several possible treatment options related to ACE2 have been proposed [345-347]. On

the other hand, the expression of the antiviral protein IFITM3 in the lung is much lower than that in other tissues, which may be associated with severe lung symptoms in COVID-19 [340].

The current nomogram was built based on a relatively large, representative dataset from 24 centers, was externally and independently validated and had good prediction accuracy. Although most of the baseline characteristics were significantly different between the derivation and validation cohorts, the nomogram had decent generalizability for the data obtained outside of Jiangsu Province where the nomogram was established. All patients in this study were discharged from the hospital at the end of the study, so the severity data did not change and were correct. In addition, collinearity analysis was conducted to select variables to avoid having too many candidate variables for the multivariate logistic regression analysis.

This study has several limitations. First, the model needs to be verified by larger studies and international studies. Second, the derivation cohort, on which the nomogram was based, was composed only of those from Jiangsu Province who had CT information available. Nevertheless, this nomogram performed well in predicting severe COVID-19 in both the derivation and validation cohorts. Third, due to the nature of retrospective research, other potential factors (such as lactate dehydrogenase and erythrocyte sedimentation rate) were unavailable for analysis. Fourth, the pulmonary opacity score was visually estimated and hence was a subjective measurement. However, the scores were estimated by two radiologists with rich experience in pulmonary imaging, and agreement was reached through consultation if discrepancies in pulmonary opacity score occurred, which may have reduced the measurement bias. Finally, the study used CCI ≥1 as a measurement of comorbidities, rather than information on specific comorbidities, and thus some information may be lost.

8.5 Conclusion

We established a nomogram with age, lymphocyte count, and pulmonary opacity score for predicting severe COVID-19 during a 14-day hospitalization. When externally verified, the nomogram performed well in discrimination ability and calibration, but it still needs to be verified by larger studies and international studies. The nomogram enables clinicians to accurately estimate the probability of developing severe COVID-19 and conduct beneficial preventive management for individual patients.

We know from Chapter 3 to Chapter 8 that patients with underlying diseases and older adults may have lower immunity and be prone to severe illness after infection with SARS-CoV-2, leading to a higher risk of death from COVID-19. These patients may require active clinical care and treatment to reduce deaths and save lives. This also shows that it is necessary for us to learn more about the immunity caused by infection with this novel disease and explore what factors may affect the level of immunity. These will be completed in Chapter 9.

Chapter 9 Kinetics of anti-SARS-CoV-2 IgG antibody levels and potential influential factors in subjects with COVID-19: A 11-month follow-up study

From Chapter 3 to Chapter 8, it is understood that more attention from health care providers and policy makers may need to be paid to those who are at high risks of having severe illness induced by COVID-19, such as those with comorbidities and weak immunity, to ease pressure on the health care systems while avoiding delays in treating those patients and reducing mortalities. This also evokes the need of Chapter 9 which is to understand how immunity changes after contracting this new disease and what factors may affect the immunity in patients with COVID-19. A good understanding of immunity may help build herd immunity, fight COVID-19, and limit the further impact of the pandemic on society and patients.

9.1 Introduction

As the infectious disease COVID-19 continues to spread, it is vitally important to understand well the pattern of immune response and its influential factors. Anti-SARS-CoV-2 humoral response kinetics can aid in COVID-19 diagnosis, vaccine development, therapeutic immune plasma studies, and epidemiologic studies including prevalence, exposure, and immunity. Decrease in antibody levels is likely to indicate a lack of protective immunity [168]. Most COVID-19 patients develop detectable immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies targeting the nucleocapsid (N) or the spike (S) protein of SARS-CoV-2 within several weeks post infection [169,170].

Previous studies have shown that IgG responses against SARS-CoV-2 infection can

persist for 3 to 8 months post-symptom onset [172,173]. But longer-term kinetics of IgG antibodies remains to be investigated. In addition, previous studies mostly included limited sample sizes and narrow spectrums of disease severity [178-181]. More data from asymptomatic and mild COVID-19 cases is necessary to better understand anti-SARS-CoV-2 IgG antibody detectable/positive rate and IgG level kinetics in the general population screened for SARS-CoV-2 infection. Previous reports have examined the associations between IgG antibody response against SARS-CoV-2 and potential influential factors including disease severity [178,179], comorbidities [348], and immunocompromised status [181], but the evidence on predictive factors of IgG levels was still limited.

Hence, we aimed to provide more information on the IgG detectable/positive rate and the IgG level changes over time after SARS-CoV-2 infection for up to 11 months and identify the potential influential factors associated with IgG levels in the general population screened for SARS-CoV-2 infection.

9.2 Material and methods

9.2.1 Study design and participants

The study was a prospective longitudinal study conducted at Richmond Pharmacology Ltd, London, UK and the Richmond Research Institute, St George's University of London. The participant inclusion criteria were (1) male or female aged 5 and older, (2) an understanding, ability, and willingness to fully comply with the project procedures and restrictions and (3) consent from a parent/legal guardian for participants aged 5 to 15 years. Informed written consent was obtained from each participant/guardian. The study complied with the principles of the World Medical Assembly (Helsinki 1964) and subsequent amendments.

Questionnaires were used to collect participant baseline characteristics. Polymerase Chain Reaction (PCR) testing of throat swab specimens for SARS-CoV-2-specific RNA were performed repeatedly per participant to confirm the status of SARS-CoV-2 infection. The Abbott Laboratories (Illinois, USA) chemiluminescent microparticle immunoassay (CMIA) against the nucleocapsid protein (N) of SARS-CoV-2 was used to assess the anti-SARS-CoV-2 antibody IgG levels and IgG statuses (detectable/positive or undetectable/negative) of serum/plasma samples. The cut-off value of Abbott CMIA for SARS-CoV-2 positive has been set at 1.4 signal/cut-off (S/CO) units [349], which was calculated to maximise positive predictive values and minimise false positives, according to the manufacturer. Public Health England assessed that the assay had a specificity of 100% but sensitivity of 93% [350].

9.2.2 Variables

The primary outcome was the IgG level measured repeatedly during the follow up. The secondary outcome was the IgG status (detectable/positive or undetectable/negative). Predictive variables measured at screening included time, age, gender, race, fever, and loss of smell and taste (loss of smell and taste, loss of smell only, loss of taste only, neither loss of smell nor taste). Race was classified as Caucasian, Black African, and other races (Hispanic, Indian, Pakistani, other Asian than Chinese and Japanese).

9.2.3 Statistical analysis

Characteristics of subjects with at least one positive PCR result were summarised as n, median (interquartile range [IQR]) and minimum-maximum or frequency (percentage). IgG levels and

the statuses of whether IgG was detectable or positive were recorded by day, but to make the trend information more concise, we summarised them by month. The IgG statuses (detectable/positive or undetectable/negative) were described as frequency and percentage, and IgG levels were as n, median (IQR), and minimum-maximum.

To explore potential factors associated with IgG levels in COVID-19, the generalized linear mixed models (GLMMs) with normal distribution and identity link function, predictive variables as fixed effects, and subject as random effect were employed. The natural logarithm of IgG level was the dependent variable. Time (month), age (year), gender, race, fever, and loss of smell and taste were predictive variables. All predictive variables were included in univariate GLMMs separately and in multivariate GLMM simultaneously. Geometric mean ratios (GMRs) and 95% confidence intervals (CIs) were estimated by taking an antilog transformation of estimates coming from the GLMM. The half-life was calculated from the GLMM using the formula $-\ln(2)/\beta_1$ where β_1 was the coefficient of day. The half-life was defined as the time elapsed (days) for the IgG level to reduce to half of its initial level. The graph comprised of the daily change of IgG levels since positive PCR and the fit curve for the predicted day effect from the GLMM was presented. Missing data of baseline characteristics were imputed by median (continuous variables) and category which occupies the majority (categorical variables) in the GLMM.

Statistical analyses were performed using SAS 9.4 software (SAS Institute).

9.2.3 Ethical approval

The study was approved by the Committee of National Research Ethics Service (NRES) (West Midlands - Edgbaston) (IRAS ID: 281788).

9.3 Results

9.3.1 Participants included in the analysis

From 19 March 2020 up to 10 February 2021, 2216 participants were screened for PCR for 18884 times; 510 participants were tested for IgG for 899 times (Figure 9.1). Twenty five participants had at least one positive PCR testing results and IgG data afterwards, 1 participant was excluded from the analyses due to incomplete data, 4 participants were excluded due to reinfection during the study period (who may have different patterns of IgG kinetics), and finally 20 participants were included. The analyses were based on 77 serum/plasma samples with a mean of 4 serum/plasma samples per participant (range 1-18).

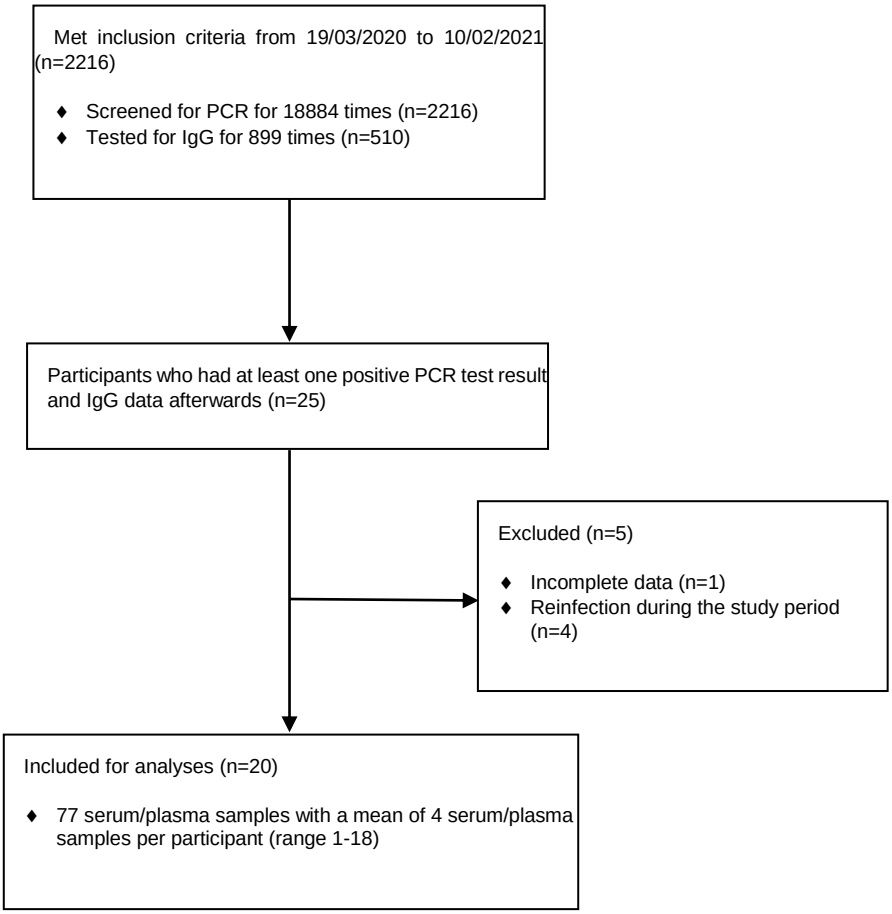

Figure 9.1 Consort diagram

9.3.2 Characteristics of participants

Median age in the study sample was 34.5 years (IQR 28.5-52.0), and most of the subjects were male (65.0%) (Table 9.1). Approximately half of the subjects were Caucasian (52.6%), 15.8% were Black African, and 31.6% were other races (including Hispanic, Indian, Pakistani, other Asian than Chinese and Japanese). Around half of the subjects (47.4%) had fever; the majority of subjects (68.4%) had lost their smell and taste, and one third of subjects had neither lost smell nor taste (31.6%). The median follow-up time post initial positive PCR testing was 2 months (IQR 1-2).

Table 9.1 Demographic characteristics of subjects with at least one positive PCR result

Characteristics	Statistics	All
Age (year)	n	20
	Median (IQR)	34.5 (28.5-52.0)
	Min-Max	24.0-66.0
Gender (n/N [%])	Female	7/20 (35.0%)
	Male	13/20 (65.0%)
Race (n/N [%])	Caucasian	10/19 (52.6%)
	Black African	3/19 (15.8%)
	Other races*	6/19 (31.6%)
Fever (n/N [%])	Yes	9/19 (47.4%)
	No	10/19 (52.6%)
Loss smell taste (n/N [%])**	Loss of smell and taste	13/19 (68.4%)
	Neither loss of smell nor taste	6/19 (31.6%)
Time (month)	n	20
	Median (IQR)	2.0(1.0-2.0)
	Min-Max	1.0-11.0

* Including Hispanic, Indian, Pakistani and other Asian than Chinese and Japanese.

** No participant in the study only lost smell or only lost taste.

Abbreviation: IQR, interquartile range.

9.3.3 Percentage of participants with detectable or positive IgG

The percentage of the subjects who had detectable or positive IgG decreased over time. At month 1 post initial positive PCR testing, 75.0% (9 subjects) of the subjects had detectable or positive IgG, while 25.0% (3) had not (Table 9.2). At month 2, 70.0% (14) of the subjects still had detectable or positive IgG. At month 3, the percent dropped to only 42.9% (3); from month 4 to 7, only 10% to 20% (1); from month 8 to 11, our data did not show any subjects who had detectable or positive IgG.

Table 9.2 Percent of participants with detectable or positive IgG since positive PCR by month

Month	Detectable or positive, n/N(%)*
1	9/12 (75.0%)
2	14/20 (70.0%)
3	3/7 (42.9%)
4	1/6 (16.7%)
1-4	27/45 (60%)
5	1/5 (20.0%)
6	1/7 (14.3%)
7	1/5 (20.0%)
8	0/5 (0%)
5-8	3/22 (13.6%)
9	0/5 (0%)
10	0/4 (0%)
11	0/1 (0%)
9-11	0/10 (0%)

* n, numbers of participants with detectable or positive IgG since positive PCR; N, numbers of participants tested IgG status since positive PCR; %, percent of participants with detectable or positive IgG since positive PCR.

9.3.4 IgG kinetics and potential influential factors

IgG levels showed a decreasing pattern over time within 11 months with an individual

heterogeneity in quantity and speed (Figure 9.2). The median IgG level at month 1 was 4.05 S/CO (IQR 1.71-6.54), then decreased to 2.31 (IQR 0.83-5.27) at month 2, 1.23 (IQR 0.51-4.57) at month 3, and then below 1 from month 4 to month 11 (Table 9.3).

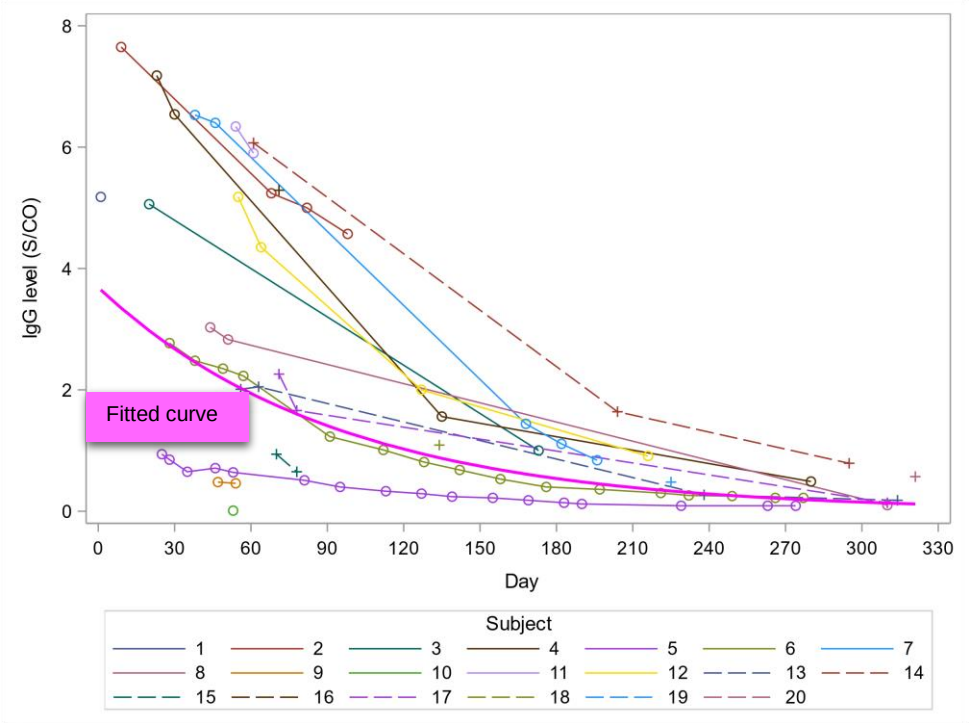

Figure 9.2 Daily change of IgG levels since positive PCR per subject and fitted curve of IgG levels from the generalized linear mixed model (thick magenta curve)

Table 9.3 IgG levels (S/CO) since positive PCR by month

Month	Statistics	All
1	n	12
	Median (IQR)	4.05 (1.71-6.54)
	Min-Max	0.65-7.65
2	n	20
	Median (IQR)	2.31 (0.83-5.27)
	Min-Max	0.01-6.40
3	n	7
	Median (IQR)	1.23 (0.51-4.57)
	Min-Max	0.40-5.00
4	n	6
	Median (IQR)	0.91 (0.33-1.09)
	Min-Max	0.29-2.00
1-4	n	45
	Median (IQR)	2.23(0.81-5.18)
	Min-Max	0.01-7.65
5	n	5
	Median (IQR)	0.53 (0.24-0.68)
	Min-Max	0.22-1.56
6	n	7
	Median (IQR)	0.40 (0.14-1.11)
	Min-Max	0.12-1.44
7	n	5
	Median (IQR)	0.84 (0.36-0.91)
	Min-Max	0.30-1.64
8	n	5
	Median (IQR)	0.26 (0.25-0.27)
	Min-Max	0.09-0.48
5-8	n	22
	Median (IQR)	0.38(0.24-0.91)
	Min-Max	0.09-1.64
9	n	5
	Median (IQR)	0.22 (0.09-0.22)
	Min-Max	0.09-0.49
10	n	4
	Median (IQR)	0.16 (0.12-0.49)
	Min-Max	0.10-0.79
11	n	1
	Median (IQR)	0.57 (0.57-0.57)
	Min-Max	0.57-0.57
9-11	n	10
	Median (IQR)	0.20(0.10-0.49)
	Min-Max	0.09-0.79

Abbreviation: IQR, interquartile range.

IgG level declined non-linearly with the follow-up time (per month; GMR 0.73; 95% CI, 0.72-0.74; Table 9.4). There was some evidence on the association between IgG level and loss of smell and taste (GMR 9.40; 95% CI, 1.12-78.97) but weak evidence on the associations between IgG level and gender and race: female vs. male (GMR 4.78; 95% CI, 0.99-22.98), Caucasian vs. other races (including Hispanic, Indian, Pakistani, other Asian than Chinese and Japanese; GMR 0.19; 95% CI, 0.03-1.02). There was insufficient evidence on the associations between IgG level and age or fever. In addition, the calculated IgG half-life was 65 days (95% CI, 62-68). The fit curve of IgG levels from the generalized linear mixed model fitted the data well, showing a non-linear decreasing trend (Figure 9.2).

Table 9.4 Estimates of geometric mean ratios and 95% CI of IgG from the univariate linear mixed models and multivariate linear mixed model

Characteristics*	Crude GMR (95% CI)	P-value	Adjusted GMR (95% CI)	P-value
Time (month)	0.73(0.72,0.74)	<.0001	0.73(0.72,0.74)	<.0001
Age (per 5 years)	1.01(0.81,1.25)	0.95	0.83(0.60,1.15)	0.25
Female vs. Male	1.15(0.34,3.89)	0.82	4.78(0.99,22.98)	0.05
Caucasian vs. Other races†	0.33(0.09,1.12)	0.07	0.19(0.03,1.02)	0.05
Black African vs. Other races†	0.41(0.08,2.19)	0.29	0.12(0.01,1.22)	0.07
Fever vs. No fever	0.88(0.28,2.81)	0.83	0.57(0.12,2.61)	0.46
Loss of smell and taste vs. Neither loss of smell nor taste††	3.38(0.95,12.00)	0.06	9.40(1.12,78.97)	0.04

* Missing data of categorical variables of baseline characteristics were imputed by the category which occupies the majority, and continuous variables had no missing data: race: 1 missing data was replaced by Caucasian; fever: 1 missing data was replaced by no; loss of smell and taste: 1 missing data was replaced by loss of smell and taste.

† Including Hispanic, Indian, Pakistani and other Asian than Chinese and Japanese.

†† No participant in the study only lost smell or only lost taste.

Abbreviation: GMR, geometric mean ratio.

9.4 Discussion

We longitudinally characterized the detectable/positive rate of IgG antibody and the dynamic

changes of IgG level over time after the onset (positive PCR for SARS-CoV-2), allowing a better understanding of the immune response in the general population with SARS-CoV-2 infection. Our study showed that IgG antibodies could be detected in up to 70% of infections in the first two months after a positive PCR, and the detectable/positive rate of IgG antibody responses in subjects gradually decreased within 3-7 months. IgG antibody levels continued to wane from the second month to the eleventh month with an individual heterogeneity in quantity and speed. Gender, race and loss of smell and taste may be associated with IgG levels.

The IgG detectable/positive rate in the PCR positive population can help estimate the proportion of individuals that has antibodies against SARS-CoV-2. Here we report that among 20 subjects with noncritical disease, a high proportion of individuals had detectable or positive IgG in the first two months while a growing proportion of individuals lost their detectable or positive IgG from month 3. Previous studies have shown high rates of seroconversion of IgG to detectable or positive levels between 4 and 14 days after symptoms onset in SARS-CoV-2-infected patients [169,178,351-353]. A study described that substantial amounts of IgG antibody in hospitalized and non-hospitalized patients with COVID-19 were detectable up to 60 days after symptom onset [178]. Similar results were reported in another serological study showing that except for the patients who failed to produce detectable levels of IgG with commercial assays, irrespective of the severity of symptoms, other patients still had detectable IgG levels >75 days post symptom onset [354]. A longer-term study of anti-SARS-CoV-2 IgG levels reported that IgG can be detected in most recovered patients at 3-4 months after infection [172]. Another study detected a high percentage of subjects with seropositive IgG at 6 to 8 months post-symptom onset [173]. By contrast, for the SARS-CoV-1 infection that occurred in 2003, previous studies have shown that a high proportion (>70%) of patients' IgG

levels were detectable after 1, 2, and 3 years [355,356]. However, to understand the IgG detectable/positive rate and kinetics, the performance of the serological tests used (e.g. sensitivity to detect IgG) needs to be taken into consideration [357]. In addition, the specific positive proportion values in our study need to be interpreted with caution and may be underestimated, because validation of the assay we used may have been performed in COVID-19 patients with severe symptoms and the fixed cut-off for a positive diagnosis may be set too high for the general population, which is also a problem previously encountered in the SARS-CoV-2 antibody tests [358].

On the other hand, our study found 4 reinfections among 25 PCR-positive participants within the 11 months study period. This may suggest immunity can rapidly decline over time and improving immune persistence through vaccines is necessary. The declined immunity may be due to the wane antibody response which represents part of the immune system, or the falling T cell response which is the other part [172,173]. In addition, some SARS-CoV-2 variants, such as B.1.617, may evade antibodies induced by prior infections and lead to reinfections [359].

The daily change plot of IgG levels showed extensive individual heterogeneity in quantity and changing speed over time in COVID-19 positive subjects, so we used a generalised linear mixed model in which random effects were fitted to handle with between-subject and within-subject variabilities. We demonstrated a decreasing tendency of IgG antibody levels from the second month to the eleventh month. Previous reports presented that antibody response peaked between the 2-5 weeks after infection and declined afterwards [186,360,361]. A study observed no drastic decline in IgG levels 3-4 months after infection [172]. Nevertheless, our results are in line with previous studies indicating the decline for IgG was statistically significant at month 2-3 [360], most patients showed a variable degree

of reduction in antibody levels within 6 months post-illness onset [362], and a progressive decline of IgG values was observed at about 6 months later [170]. In addition, the calculated IgG half-life in our data was 65 days post positive PCR (95% CI, 62-68), which was similar to a previous study of 68 days, suggesting that IgG may wane from 2 month post-infection [173].

Our study provided some evidence on the association between higher IgG levels and loss of smell and taste in subjects with SARS-CoV-2 infection but insufficient evidence on the association between IgG levels and fever. To the best of our knowledge, the studies on the association between immune responses and loss of smell and taste are currently rare, highlighting the novelty and impact of the present study. A study showed that among patients with COVID-19, those reporting loss of smell and taste developed higher antibody titers [182]; another study demonstrated that among patients with upper respiratory tract infection, COVID-19 IgG antibody titers were higher in patients with olfactory disorders than those without [363]; but both studies did not further discuss the potential mechanisms. De Melo et. al. investigated the interaction between SARS-CoV-2 and the olfactory system and its pathophysiological mechanisms based on patients and animal models with SARS-CoV-2 related anosmia/ageusia [364]. They observed the expression of cleaved caspase-3 in the olfactory mucosa, indicating cell damage and death caused by SARS-CoV-2 infection. They found the cleaved caspase-3 in both infected and uninfected cells, suggesting that cell damage and death are not only caused by cytopathic effects of SARS-CoV-2, but also possibly by the inflammation and immune responses to infection, and observed some up-regulated genes which were mainly involved in inflammatory and immune responses and functions associated with chemokine signalling. In addition, they did not observe cell death or immune cells in the olfactory mucosa in a COVID-19 patient without loss of smell, suggesting the

importance of assessing the associations between inflammation, immune responses, and cell and tissue damage and smell loss using larger cohorts to validate their observations. However, since different variants of the SARS-CoV-2 may have different symptoms, loss of smell and taste may not always be a dominant feature and associated with IgG levels. A previous study showed that in several asymptomatic cases, the antibody levels were lower, and the IgG seroconversion was delayed compared to the symptomatic cases [362]. Among studies exploring the relationship between disease severity and humoral immunity against SARS-CoV-2, some studies reported IgG seroconversion time, positive rates, and levels were associated with more severe forms of the disease [178,179,186,365-367] but others did not [180,181,188,368]. Some publications proposed that higher IgG levels in patients with more severe disease may be due to the high amounts of SARS-CoV-2 RNA [369], and a strong and uncontrolled humoral response may be a feature of over-activation of the immune system in patients with severe disease and may contribute to the disease pathogenesis of a severe systemic inflammatory response (called "cytokine storm") and organ damage [170,370]. On the other hand, another study stated that the IgG levels in critically ill patients were lower than moderate and severe patients, which may be the result of longer virus exposure or a severely impaired immune response in these patients [371].

We found weak evidence on the association between IgG levels and gender. Caution needs to be taken when interpreting the result and further studies are warranted to verify the association. Legros et al.'s longitudinal study of 140 COVID-19 patients revealed that the IgG response can be used as a marker for neutralizing antibody activity and found that gender was not associated with neutralizing antibody activity [170]. In agreement with Legros et al., other studies did not show gender differences in the antibody response [351,372,373]. By contrast,

a study observed gender differences on anti-nucleocapsid IgG antibody response at weeks 6-7 during a 10-week follow-up, but did not test the gender differences on the overall trend of IgG [360].

In addition, our study looked at whether there was a difference in the generation of antibodies against SARS-CoV-2 infection in individuals from different ethnicities. We provided weak evidence on the difference on IgG levels between Caucasian and other races (including Hispanic, Indian, Pakistani, other Asian than Chinese and Japanese) but insufficient evidence on the difference between Black African and other races. However, currently the studies exploring this question are rare.

Our study provided insufficient evidence on differences in immune response in relation to age. However, a study covering COVID-19 patients from 16 to over 65 years old found that antibody levels were age-related, showing that higher antibody levels correlated with older patients [374]. Another study detected a moderate association between age and neutralizing activity [375]. However, Legros et al.'s study found no association when examining whether age was related to neutralizing antibody activity in the same disease severity group of COVID-19 patients, indicating that disease severity may be the main factor explaining the neutralizing activity [170]. Other studies did not find a clear correlation between IgG levels and age [351,372,376].

This study has several limitations. First, although the study provided insight into the IgG response and potential influential factors in PCR-confirmed COVID-19 subjects, the sample size of this study is still modest and the study findings need to be corroborated by larger studies. But the generalised mixed model we employed allowed us to efficiently use the information by combining measurements from different subjects. Second, while our study

described the longer-term kinetics of IgG up to 11 months, we only characterized the decreasing phase and did not have enough data to model the early growth phase and peaking point which was supposed to happen around the first month. Third, due to lack of data, we did not analyse the impact of other potential factors on antibody kinetics, e.g. Asian race including Chinese and Japanese, disease severity, comorbidities [348], laboratory features such as C-reactive protein [377], and virus neutralization titre [180]. For the same reasons, we were unable to investigate the kinetics of IgG responses to the spike protein of coronavirus.

9.5 Conclusion

This chapter (Chapter 9) demonstrated that in people confirmed with SARS-CoV-2 infection, a high proportion of individuals had detectable or positive IgG antibody levels in the first two months while a growing proportion of individuals lost their detectable or positive IgG after that, highlighting the importance of developing long-term immunity, which may be achieved from effective vaccine strategies. IgG levels declined non-linearly from month 2 to 11 with individual heterogeneity in quantity and changing speed and tended to be associated with gender, race, and the loss of smell and taste. These factors may need to be taken into consideration when making efforts to build herd immunity and fight against COVID-19. To reduce the further impact of the pandemic on society and patients with different characteristics, the long-term monitoring on the efficacy of COVID-19 vaccines in these different groups may be required.

The main chapters of this work including Chapter 3 to Chapter 9 provide new insights into the novel disease COVID-19. Chapter 3 to Chapter 5 carefully investigate risk factors associated with three life-threatening situations: being severe or critically ill status,

experiencing disease deterioration from asymptomatic or mild or moderate status into severe or critically ill status, and developing respiratory failure. This can help provide evidence for health care professionals and policy makers to make better decision on the clinical management and allocation of medical and social resources and prevent potential mortalities. Based on these much-needed information, Chapter 6 to Chapter 7 provide more comprehensive information on two factors: age factor and pulmonary opacity score in CT. With this knowledge, the following chapter (Chapter 8) builds a nomogram which can be a reliable prediction tool for assessing the probability of being severe or critically ill and may facilitate clinicians stratifying patients and providing early and optimal therapies. In particular, Chapter 3 to Chapter 8 provide evidence that older patients with underlying diseases may have lower immunity and be prone to severe illness after infection with SARS-CoV-2, which can cause death. This indicates that these patients may require active clinical care and treatment to reduce deaths and save lives, and also leads to Chapter 9 that investigates the immunity caused by infection of COVID-19 and explores what factors may affect the level of immunity.

Chapter 10 Discussion

10.1 Introduction

The research presented in the preceding chapters used datasets from Jiangsu province and Hunan province, China, and also from the UK; explored potential factors associated with severe/critical illness, disease deterioration, respiratory failure, and IgG antibody levels change; and systematically assessed associations between age and CT pulmonary opacity score and baseline features and clinical variables in COVID-19 patients. This chapter discusses the main findings of these investigations, and the potential explanations for the associations observed.

10.2 Summary of findings

This PhD work aimed to investigate the characteristics of COVID-19 and their associations with clinical outcomes and immunity. Despite the limitations which were detailed in previous chapters, the investigations provided evidence of the associations between characteristics of COVID-19 and clinical outcomes and immunity. Specifically the study has:

a. demonstrated that patients with COVID-19 in Jiangsu had a low rate of severe or critically ill presentation, with no deaths recorded;
b. identified that age, CT pulmonary opacity and lymphocytes were independently associated with severe or critically ill presentation;
c. found that 16.3% of patients experienced a deterioration in their clinical condition and 6% of patients with “moderate or less” status deteriorated to being “severe or critically ill”;

d. indicated that age, pulmonary opacity score, lymphocyte count on admission and exposure to the pandemic centre in Wuhan were independent risk factors for disease deterioration;
e. showed that the rate of respiratory failure in Jiangsu province, China (9%), was similar to the national level in China, but much lower than in Wuhan city (the epicentre of COVID-19 pandemic in China) and some other countries;
f. identified four independent risk factors of respiratory failure in patients with COVID-19 including older age, increased respiratory rate, decreased lymphocyte count and greater pulmonary opacity score at admission;
g. confirmed that older patients had worse clinical outcomes, in part due to comorbidities in older people, and higher rates of smoking and drinking habits, and immune, organ, and coagulation dysfunction on admission;
h. demonstrated that the degree of CT pulmonary opacity was closely related to age, single onset, fever, cough, SpO_2, lymphocyte count, platelet count, albumin level, CRP level and fibrinogen level;
i. demonstrated that a high proportion of the general population confirmed with SARS-CoV-2 infection had detectable or positive IgG antibody levels in the first two months while a growing proportion of individuals lost their detectable or positive IgG after that, and IgG levels tended to be associated with gender, race, and the loss of smell and taste.

As a result, the study has contributed to further understanding of the potential risk factors underlying adverse outcomes and immunity of COVID-19, and may help the

prevention and control of COVID-19, and help reduce mortality.

10.3 Mortality

The zero mortality amongst patients in the Jiangsu dataset may be unexpected. The main reasons have been detailed in the literature (mainly due to the early recognition of high-risk and critically ill patients, early intervention, hierarchical management strategies, and reasonable allocation of materials and human resources) [58]. China attaches great importance to curbing the spread of the virus through public health measures including detection, tracing, isolation, and encouraging social distancing and wearing masks to reduce the mortality rate, although early recognition and early intervention of high-risk and critically ill patients are important to reduce mortality in the early stage of pandemic and in settings adopting a policy of coexisting with the virus. On the other hand, according to the bulletin from the National Health Commission and the Provincial Health Commission in China (http://2019ncov.chinacdc.cn/2019-nCoV/), up to the 23rd December 2020, no or few deaths occur in most provinces of China, e.g. 0 death in Shanxi, Xizang (Tibet) and Ningxia province, and 1 death in Zhejiang, Fujian and Jiangxi province, respectively. Only four provinces and regions have more than 10 deaths, including Hubei (4512 deaths), Henan (22 deaths), Heilongjiang province (13 deaths) and Hong Kong Special Administrative Region (133 deaths). In addition, the quality of data from Jiangsu province has been recognised by discussion and confirmation with the department collecting the data. The department has confirmed that all patients in Jiangsu conformed to the discharge standard without COVID-19 and were alive when they were discharged (the discharge standard was that body temperature was normal for more than 3 days, symptoms were relieved for patients with any symptoms and RT-PCR

for SARS-CoV-2 [throat swab samples, at least 1 day for sampling interval] showed negative for two consecutive times).

This research could not address the risk factor and prediction issues of mortality, because there was no mortality dataset available for this work. However, this thesis addressed the issues of prediction of severe or critical illness of COVID-19. Patients with severe or critical illness are at high risk of death, especially in settings with limited medical resources, and need early identification and early intervention. On the other hand, strong immunity is the basis to combat COVID-19. Therefore, the further information and understanding on risk factors of severe or critical illness and immunity, as well as the prediction model on severe or critical illness provided in this PhD work could help address the issues of mortality and help health care professionals and policy makers to make evidence-based decisions.

10.4 Predictors of adverse outcomes of COVID-19

For successful control of mortality related to COVID-19, patients with COVID-19 having risk factors of adverse outcomes of COVID-19 need to be carefully managed during hospitalisation. The practical nomogram constructed in this work was comprised of a few readily available baseline demographic, clinical and CT features (age, lymphocyte count and pulmonary opacity score) and may predict severe COVID-19 with good discrimination and agreement, which enables physicians to identify patients with high risk early and correctly and take proactive measures accordingly.

10.4.1 Comorbidities and age

In studying the pathogenesis and developing management strategies of COVID-19, age is confirmed as a critical factor in the severity of infection. This work found that initial symptoms including fever, cough, and shortness of breath occurred more frequently among adults compared to child patients. This work also showed that the ICU and respiratory failure rate, and length of hospital stay increased with age. In addition, this study found that age was independently associated with severe or critically ill presentation, disease progression, and also respiratory failure. Age was the most consistent factor among factors affecting the risk of severe COVID-19 in different studies, including several studies that used multivariate regression models to conclude that age was an independent risk [378-381]. One of the clearest analyses of age data using different age groups was a univariate regression model analysis in which individuals aged 65 years and older had a 3.26-fold higher risk of developing ARDS than those under 65 [382]. In the prediction model of this work, age was one of the predictors of severe COVID-19. These results are consistent with some previous studies [41,59,138,225,238]. COVID-19 mortality rates vary widely by age group, with those aged 65 to 74 having a 95-fold higher mortality rate compared to those aged 18-29, 230-fold higher among those aged 75-84, and 600-fold higher among those aged 85 and above [383].

This study demonstrated that the quadrant score and pulmonary opacity score increased with age, suggesting more severe abnormal imaging manifestation on admission among these older patients, which is consistent with the previous finding [273]. More abnormal manifestation in laboratory parameters in older patients may also be an early sign of, and a contribution to, immune, organ, and coagulation dysfunction, and hence severe illness and poor outcomes, which is in line with other studies [205,273].

Poorer clinical outcomes among the elderly may also be partly due to their higher incidence of comorbidities (such as hypertension, coronary heart disease and diabetes) and hence immunological vulnerabilities, also the age-related degeneration of the immune system (known as immunosenescence) and hence impaired immunity to SARS-CoV-2 which is a defence mechanism against respiratory viruses and contributes to virus clearance [261,335,336]. Several studies have linked the risk of severe illness in people with COVID-19 to any comorbidities, such as hypertension [41,44,378,379,384-386], diabetes [41,85,378-380,384,385,387-389], and some other comorbidities [44,50,384,386-388,390-392]. The role of comorbidities in COVID-19 may be correlated to the underlying pathology of the SARS-CoV-2 [382]. SARS-CoV-2 can infect all the major cell types in the respiratory tract mainly via host cell receptor ACE2, and the corresponding inflammatory response of the release of cytokines such as the interleukins 1β and 6 can cause cell death and systemic effects leading to disseminated intravascular coagulation, pulmonary oedema, ARDS, shock and other severe diseases [382,393-395]. ACE2 is involved in the renin-angiotensin system that controls blood pressure, and viral infection can interfere with this function and aggravate the cells damage by changing vasoconstriction and sodium homeostasis and causing pro-inflammatory state and an increase in vascular permeability [382]. Renal disease, cardiovascular dysfunction and other conditions, as the incentives of hypertension, also affect renin-angiotensin system and may aggravate the pathology of SARS-CoV-2 [382]. Any comorbidity that produces a pro-inflammatory state (e.g. type II diabetes or pre-existing infection) or that involves autoimmunity (e.g. type I diabetes) may also lead to increased pathology [382]. Comorbidities such as HIV infection and cancer requiring inflammatory cells or the use of immunosuppressant drugs (e.g. chemotherapy or steroids) may also worsen the disease severity [382]. Additionally, SARS-CoV-2 infection may exacerbate the level of cells damage if the target tissue is already compromised by the

underlying disease [382].

The thymic involution in older patients causes age-related reduction of T cell repertoire diversity and defects in CD4+ and CD8+ T cell function and hence significantly reduces immune function (immunosenescence) [231,284,382]. Another study reported that, except for immunosenescence in older people, age-related increases in levels of phospholipase could also result in a delay of immune response and poor outcomes after SARS-CoV infection [285]. Research focused on innate immune-related mechanisms and viral clearance in patients with COVID-19 of different age groups may help determine the underlying mechanisms of disease severity.

Other explanations about why older people suffer poorer outcomes may be due to the higher prevalence (twice the rate) of smoking and alcohol drinking in older patients with COVID-19 in Jiangsu. A history of smoking has been identified as a factor contributing to the progression of COVID-19 pneumonia [238]. Another small study also shows that 100% of current smokers suffered from severe diseases [384]. But it is worth noting that, some other studies do not support smoking as a risk factor for disease severity and found the distribution in historical/current and non-current/non-smokers similar across disease severity using descriptive or statistical tests [379,387,390].

In addition, the relationship between age and severe disease may be related to angiotensin converting enzyme-2 (ACE2). A study showed that ACE2 had an important salutary function: ACE2 limits several detrimental effects, including vasoconstriction and enhanced inflammation and thrombosis, but it is markedly downregulated by the entry of SARS-CoV-2 into cells, which may be especially detrimental in elderly individuals with age-related baseline ACE2 deficiency [334]. Some other reasonable mechanisms of mild presentation

in children include qualitatively different responses to SARS-CoV-2, or different virus-to-virus interaction and competition from other viruses limiting SARS-CoV-2 growth [202].

On the other hand, most of the deaths were due to ARDS, histologically presenting as diffuse alveolar damage (DAD); however, in older adults with COVID-19, increased circulating inflammatory cytokines and age-related epigenetic abnormalities prevent the general response to DAD (downregulation of ACE2 expression) and even lead to upregulation of ACE2, ultimately leading to poorer clinical outcomes [383,396]. Furthermore, 1,25(OH)2D3, as the active form of vitamin D, can induce ACE2/Ang-(1-7)/MasR axis activity and inhibit renin and ACE/Ang II/AT1R axis, thus potentially exerting protective effect on ARDS; nevertheless, vitamin D synthesis is significantly reduced in people aged 60 and above, possibly attenuating the protective effects of ARDS [383,397].

Another study proposed that children usually had had vaccines against polio and measles, mumps, and rubella (MMR) more recently and hence may have higher titers of nonspecific anti-viral antibodies to protect children from developing severe illness from COVID-19 [398,399]. Similarly, among children and young adults, the infection rates of seasonal human coronavirus are generally higher so these populations may have more cross-reactive antibodies [400]. Therefore, pneumococcal vaccination is recommended by CDC for the elderly and children under the age of two [401]. In addition, a strong response to type I interferon is a key factor in people with mild or no response to SARS-COV-2, so the older individuals with reduced type I interferon may hence show more severe illness [398,402].

The PhD work supports previous research that older people with pre-existing conditions are at higher risk of severe illness, so policy makers may need to be careful to prioritise these individuals for immunization when allocating vaccines, especially when

vaccines volumes are limited [71].

10.4.2 Radiological characteristics

This study demonstrated that the degree of CT pulmonary opacity indicating the severity of lung dysfunction was closely related to age, single onset, fever, cough, SpO_2, which is consistent with the previous studies [95,292,308,310,311,403,404]. This study also showed that in patients with COVID-19, as CT pulmonary opacity score increased, platelet count and albumin level decreased, while the level of CRP and fibrinogen elevated, and >=41% of lung involvement was associated with more severe lymphopenia, suggesting signs of viral infection and inflammation, abnormal coagulation function and liver function [303,312-314].

The data here showed that pulmonary opacity score, especially when >=41%, may be an accurate indicator of severe or critical illness, acute respiratory failure, intensive care requirements and disease progression, which is in accordance with previous studies [233,298,303,305,307,317]. CT involvement score can help early diagnosis, severity assessment and treatment of COVID-19 [95,318,319] and may indicate the progression and recovery of the disease [92,320]. This work found patients with more pulmonary opacity stayed longer in hospital, which may be due to more severe illness and more medical treatment and oxygen support associated with a higher pulmonary opacity score [98,302,326,327,405].

The role of CT pulmonary opacity in COVID-19 severity may also be correlated to the pathology of the SARS-CoV-2. The internalization of the virus into lung cells and hence the cytokine storm can cause acute pulmonary interstitial injury which may lead to GGO and parenchymal changes, manifested as increased CT pulmonary opacity. ACE2 is widely

expressed in the lungs (particularly in type 2 pneumocytes and macrophages) [334]. SARS-CoV-2 enters its host cell through the receptor ACE2 and causes diseases [341]. In the lungs, after viral invasion via ACE2, the dysregulation resulting from ACE2 deficiency promotes inflammation and thrombosis triggered by local angiotensin II hyperactivity, leading to cell death and lung damage [334]. The sub-segmental pulmonary vasodilatation surrounding parenchymal abnormalities has also been demonstrated to be possibly attributed to the release of pro-inflammatory factors [92,294,406]. In patients infected with SARS-CoV-2, angiotensin II levels were positively linearly correlated with viral load and lung injury [342]. Several possible treatment options related to ACE2 have been proposed [345-347]. On the other hand, the expression of the antiviral protein interferon-inducible transmembrane protein 3 (IFITM3) in the lung is much lower than that in other tissues, which may be associated with severe lung symptoms in COVID-19 [340].

However, other viral pneumonia may also have imaging findings, so chest CT should not be used as a screening test to confirm COVID-19 but to assess disease severity or progression, and many patients actually didn't take CT test [99-101,407]. Some patients in this PhD work also didn't take CT tests. On the other hand, clinical manifestations, epidemiological characteristics (such as a history of exposure), laboratory characteristics, and imaging characteristics can raise suspicions of COVID-19 infection, but the presence of SARS-CoV-2 RNA in clinical specimens by microbiological tests, such as RT-PCR, should be employed to confirm diagnosis [101].

10.4.3 Laboratory parameters

This PhD work found severely or critically ill patients had more obvious damage of white blood

cells and immune cells with lymphopenia identified as an independent predictor of severe COVID-19, disease progression and respiratory failure. Some studies have also shown that in severe cases, the number of peripheral blood lymphocytes was significantly reduced [106,339,408]. Patients with acute respiratory distress syndrome and patients requiring intensive care had lower numbers of lymphocytes than patients with a better prognosis [48]. Approximately 85% of severely or critically ill patients with COVID-19 had lymphopenia [38,48]. Significant reductions in lymphocytes have also been shown to be positively associated with in-hospital mortality and disease severity [50,409]. The level of lymphocytes in individuals who died of COVID-19 was significantly lower than that in survivors [112]. Lymphopenia was found to raise the risk of severe or critical COVID-19 pneumonia [44,59,67,130,338]. The flow cytometry analyses showed that SARS-CoV-2 can significantly reduce the number of lymphocyte subsets such as B cells, CD4+ T cells, CD8+ T cells, and NK cells [48,108,410,411]. The reduction of CD8+ and CD4+ T cells was more significant in elderly patients over 60 years old and patients requiring intensive care units [412]. CD8+ T cells and CD4+/CD8+ ratio were significantly associated with the inflammatory status of COVID-19 and were independent predictors of poor prognosis [413]. Regulatory T cell levels were low in COVID-19 patients, mostly in those who had severe diseases [414]. Notably, Zheng et al showed that NK cells were significantly reduced in COVID-19 patients, but then were remade after recovery [415].

Severe complications of COVID-19 are generally attributed to virus-induced overactivation of the immune system and overproduction of pro-inflammatory cytokines known as cytokine storm, which may be used by the host immune system to compensate for lymphocyte deficiency and dysfunction [416]. Lymphocytes are the main immune cells against SARS-CoV-2, and abnormalities in these cells may contribute to the exacerbation of COVID-

19. Therefore, lymphocytes play an important role in the pathogenesis of COVID-19. The potential mechanisms for reduced lymphocyte levels in COVID-19 may be: lymphocytes are a direct target of viruses because they express the coronavirus receptor ACE2, lymphatic organs are destroyed by SARS-CoV-2, lymphocyte deficiency is induced by pro-inflammatory cytokines, and lymphocyte inhibition results from metabolic disorders [339]. Cytokine storms can wreak havoc on the body's immune system, leading to ARDS, organ failure, other severe diseases, and even death [417]. In addition to antiviral activity, synergistic effects of most inflammatory cytokines have been demonstrated to be antiproliferative and proapoptotic, and induce the expression of cytokines and cytokine receptors, thereby inhibiting lymphocytes proliferation [418-421]. SARS-CoV-2 may directly attack lymphocytes or destroy lymphoid organs, resulting in lymphopenia [417]. Also, the lymphopenia in severe COVID-19 patients may be due to some metabolic molecules, such as the observed increased blood lactic acid levels [339]. In addition, the antiviral protein IFITM3 is low in immune cells (including lymphocytes), indicating that SARS-CoV-2 may attack lymphocytes and induce cytokine release syndrome [340]. Another proposed mechanism is that genes such as annexin V and other depletion-related genes are upregulated and may be associated with lymphocyte apoptosis [38,413,422].

Combining the roles of cytokine storm and inflammation-mediated lymphopenia in the pathogenesis of COVID-19, especially in severe cases, new therapeutic strategies aiming at controlling these events are under active research, such as blockade of pro-inflammatory cytokines, modulation of lymphocyte exhaustion, immunomodulators aiming at eliminating the number of pathogens, and NK cell-based therapy [416,423-427]. However, the efficacy and safety of these new therapeutic strategies should still be evaluated through more precise

investigations [416].

10.5 Clinical management

The clinical management including oxygen supportive treatments and medical drugs treatments might impact the disease. But this work could not analyse it further since no appropriate data were available. The available data of clinical management were collected at the end of follow-up, so the clinical management could happen after the disease progression has already occurred. Since it was unable to determine the chronological order, i.e. whether the clinical management happened first or the disease progression happened first, this work could not analyse whether the clinical management impacts the disease. This is a limitation of the study.

This study showed that a prolonged time from illness onset to admission may increase the risk of severe COVID-19, which is likely attributed to the delay of treatment. This is consistent with previous research [117,337]. For settings similar to Spain with limited resources to receive mechanical ventilation and ICU treatment at disease outbreak stage [268], the experience in Jiangsu province may be beneficial to COVID-19 patients with respiratory failure: in Jiangsu, a large proportion of patients with respiratory failure conducted prone position and received HFNC or NIV, which help reduce the further use of IMV and mortality (no death occurred and all patients were discharged at the end of the study). This confirms the findings from several previous small studies [269,270].

All the centres in Jiangsu province, China shared the same quite successful experience of management of COVID-19, including the diagnosis and treatment, and complied with the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia" released by National

Health Commission & National Administration of Traditional Chinese Medicine of China. Although those 24 hospitals in Jiangsu province are different in terms of medical resources and other aspects, they were well selected high-quality hospitals from the whole Jiangsu province, and were required to follow the same guidelines of management of patients with COVID-19. So the centre differences are expected to be small and the centre factor was therefore not included in the analysis.

Antiviral drugs, protease inhibitors, and drugs targeted to the viral genome or human ACE2 receptors were considered to help alleviate SARS-CoV-2 infection [428]. However, it has been suggested that the most efficient way to control the pandemic is building antibody-mediated immune responses by vaccination [429]. Currently, drugs used to treat critically ill patients are mostly repurposed drugs, and these drugs are considered life- and time-saving and cost-effective during the pandemic, but the long term efficacy and safety (such as dose optimization and dose-related toxicity) of the drugs for this new disease still warrant further confirmation [4]. Some novel treatments, such as convalescent plasma therapy, are also under active research [101,430]. In addition, when developing effective and safe treatments, different characteristics of the patients and the relevant immune responses need to be taken into consideration [146].

Because the patients in China included in this study fell ill at the very early stage of the pandemic, there was basically no treatment experience and not much research evidence for the disease at that time. Therefore, Chinese medical experts made efforts to treat the disease based on their previous experience and patients' clinical manifestations, considered to give more active oxygen supportive treatments and medical drugs (including antibiotics and antivirals) for patients with severe illness, and in particular, considered to increase medical

treatment and oxygen support with the increase in patients' age and pulmonary CT opacity score. However, from the point of view of zero mortality, the treatment strategy at that time was relatively scientific. In addition, based on the latest research evidence and information on COVID-19 treatment, the treatment management strategies in China reported in this PhD work have reference significance for the world to combat the pandemic.

10.6 Immunity to SARS-CoV-2

This work discussed the immunity to SARS-CoV-2 from an epidemiological perspective, as well as clinical and immunological aspects, and made efforts to explain potential mechanisms to the association between anti-SARS-CoV-2 IgG levels and loss of smell and taste.

10.6.1 Immune response

The humoral immune response against enveloped viruses and hampering the replication of the virus involves the production of a broad spectrum of immunoglobulins, mainly IgM, IgG, and IgA, which are primarily specific to the enveloped glycoproteins and nucleoproteins [416]. Immunoglobulins are highly diverse autologous molecules capable of enhancing immune function and facilitate the development and function of lymphocytes [431]. Lymphocytes deficiencies can lead to suppression of immunity and severe diseases due to bacterial, fungal, or viral infections. IgG is the main effector molecule of the humoral immune response [431]. The IgG detectable/positive rate in the PCR positive population can help estimate the proportion of individuals that has antibodies against SARS-CoV-2. Unexposed individuals are expected to test negative for anti-SARS-CoV-2 IgM or IgG prior to SARS-CoV-2 infection. Following

exposure to infection, SARS-CoV-2 elicits the humoral immune response, starting with the production of IgM indicating an acute or ongoing infection status, manifested as a positive result for IgM or IgM/IgG serology test [432]. Multiple studies show that IgM levels continue to rise until they peak two to five weeks post symptom onset, after which IgM levels decrease and was no more detectable over time [432-437]. On the other hand, IgG levels generally increase and peak between three and seven weeks post symptom onset [211,432-434,436,438-440]. This PhD work showed that among 20 subjects with a noncritical disease, a high proportion of individuals had detectable or positive IgG in the first two months while a growing proportion of individuals lost their detectable or positive IgG from month 3.

In addition, this study demonstrated a decreasing tendency of IgG antibody levels from the second month to the eleventh month, and estimated an IgG half-life of 65 days post positive PCR. A study reported a decrease in antibody levels after 8 weeks of symptom onset [441]. Another study with a longer follow-up period demonstrated variable persistence of virus-specific IgG 12 weeks after symptom onset [442]. Some studies followed up for 6-8 months and showed the persistence of seropositivity [173-177]. Other studies reported 1-year duration of IgG antibody in convalescent COVID-19 patients [183,209]. Varona et al estimated the mean time to loss of IgG antibodies to be 375 (95% CI: 342–408) days from the start of the study [210]. A longitudinal analysis in 32 patients with COVID-19 who recovered from mild and moderately-severe infection in the Umbria region of Italy found that anti-S-RBD IgG persisted for up to 14 months in 31 patients [182]. The date of the last detection of antibodies in studies was limited by the length of study follow-up, not to confirm the disappearance of detectable antibody [211]. By contrast, for the SARS-CoV-1 infection that occurred in 2003, previous studies have shown that a high proportion (>70%) of patients' IgG levels were detectable after 1, 2, and 3 years

[355,356]. Hence, longer follow-up studies on immunity of a larger number of COVID-19 patients may be necessary.

This PhD work included 4 reinfections among 25 PCR-positive participants within the 11 months study period, which may suggest that immunity can rapidly decline over time and improving immune persistence through vaccines is necessary. The declined immunity may be due to the wane antibody response which represents part of the immune system, or the falling T cell response which is the other part [172,173]. One study shows a case of reinfection was clearly related to the lack of seroconversion after initial infection [443]. In addition, some SARS-CoV-2 variants, such as B.1.617, may evade antibodies induced by prior infections and lead to reinfections [359].

Existing antiviral therapy of immunoglobulin therapy, cannot significantly increase the chances of survival of severely ill COVID-19 patients [416]. However, a successful strategy of convalescent plasma from virus-infected patients without serious adverse events has been explored [444,445]. Convalescent plasma therapy uses IgG antibodies collected from recovered COVID-19 patients from surrounding areas (where these donor subjects have naturally encountered the virus) to increase virus neutralization and passive immunity [446]. A study on convalescent plasma therapy, using 5 critically ill patients with COVID-19 received plasma with high virus-specific IgG and IgM antibodies from recovered patients, preliminarily showed that convalescent plasma may help clear the SARS-CoV-2 and improve symptoms [149].

10.6.2 Influential factors

This study provided some evidence on the association between higher IgG levels and loss of smell and taste in subjects with COVID-19, which is consistent with the research of 32 patients

in Italy [182]. The study in Italy found that patients who reported a loss of smell and taste during the clinical course of the disease developed significantly higher anti-S-RBD IgG antibody titers, but that study only compared median anti-S-RBD IgG antibody titers at each month while did not control for confounding factors using multiple regressions and did not explain the underlying mechanism [182]. As of February 2022, apart from this study, I have not found any other studies on the relationship between IgG levels and loss of smell and taste in COVID-19 patients. Another study found that the IgG antibody positive rate was 22.2% in the control group (patients with mild upper respiratory tract infection symptoms but no reported smell or taste disturbances), and the positive rate in case group (patients with olfactory disorders and mild upper respiratory tract infection symptoms) was twice that of the control group, suggesting that it is important to isolate patients during a pandemic if they develop symptoms of smell and taste disturbances, with or without other symptoms [363]. A cohort study reported that loss of smell was more common in participants with SARS-CoV-2 antibodies (93% vs. 78%) compared with those without antibodies, and loss of taste was equally common (90% vs. 89%); furthermore, participants with acute anosmia were up to 3-fold more seropositive for SARS-CoV-2 compared with those with taste loss [447].

Loss of smell is one of the symptoms of patients infected with SARS-CoV-2, but the mechanism behind this symptom has not been clearly elucidated until the study from Zazhytska et al was published online in the journal Cell in February 2022 [448]. In the new study, researchers from institutions including Columbia University and the Icahn School of Medicine at Mount Sinai have discovered a mechanism that may explain why patients with COVID-19 lose their sense of smell. Specifically, they found that infection with the SARS-CoV-2 indirectly downregulates the role of olfactory receptors, proteins on the surface of nerve cells (i.e.

neurons) in the nose that detect odor-related molecules. Experiments showed that the presence of the SARS-CoV-2 near neurons in olfactory tissue brought an influx of immune cells (microglia and T cells) that sense and fight infection. These immune cells release proteins called cytokines that alter the genetic activity of olfactory neurons even though SARS-CoV-2 cannot infect them, the authors said. According to their theory, immune cell activity otherwise dissipates rapidly, and in the brain, the persistence of immune signalling reduces the activity of genes that express olfactory receptors. This mechanism may also indirectly explain the relationship between anosmia and elevated IgG levels in COVID-19 patients. The study by Zazhytska et al partially validates the pathophysiological mechanism between SARS-CoV-2 and the olfactory system proposed by De Melo et al, that cell damage and death in the olfactory neuroepithelium may be caused not only by the cytopathic effects of SARS-CoV-2, but also by the inflammation and an overactive immune response to infection [364]. De Melo et al observed up-regulated genes that are primarily involved in inflammatory and immune responses and functions related to chemokine signalling. The mechanisms of taste disorder in COVID-19 are not fully understood, but multiple theories have been proposed. For example, the peripheral neurotropism of the olfactory nerve leads to viral invasion, taste bud cells are rich in ACE2 receptors that support viral infection and subsequent inflammation, and angiotensin II imbalance can affect taste sensation [449-454].

Since different variants of the SARS-CoV-2 may have different symptoms, loss of smell and taste may not always be a dominant feature and associated with IgG levels. So far, research on the relationship between loss of smell and taste and IgG levels is still limited. Previous studies reported asymptomatic individuals had lower neutralizing antibody titers than symptomatic individuals [174], and symptomatic infection was identified as an

independent factor associated with longer durability of antibodies in COVID-19 patients [210]. Therefore, loss of smell and taste may be associated with IgG levels as a symptom of SARS-CoV-2 infection indicating a more active immune response. This finding may have implications for the development of targeted and effective treatment and vaccine programs to handle the pandemic outbreak. On the other hand, because COVID-19 patients reporting smell or taste disturbances had higher levels of IgG antibodies, health policy makers may need to consider the necessity of isolation and stricter monitoring of these patients.

10.7 Efforts to address possible sources of bias

The multicentre study design involved nearly all patients from Jiangsu province, China, yielding a fully representative sample of this province free from selection bias. As stated in the preceding sections, all patients in Jiangsu province were included if they (1) were clinically diagnosed and then confirmed to have COVID-19 up to the 15th March 2020, and (2) fulfilled the diagnostic criteria for the "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" released by National Health Commission & National Administration of Traditional Chinese Medicine of China [212], and only patients without medical records were excluded.

The measurement bias and misclassification bias were also controlled as stated in the preceding section that disease severity was categorised according to the same standard "Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7)" [212]. In addition, the severity of illness was assessed by two physicians and imaging grading was performed by two independent radiologists with more than 5 years' experience in pulmonary imaging.

Biases estimates could have occurred if an analysis was performed on the completed cases. Considering this, the multiply imputation method in logistic regression analysis was used to provide unbiased estimates of risk factors of being a severe/critically ill COVID-19. Sensitivity analysis was also performed to confirm that the results from multivariate logistic regression analysis, from which the main conclusion was drawn, were insensitivity to missing data and were not due to random error. On the other hand, in addition to descriptive and univariate analyses, this PhD work also employed multiple regression analyses to control for confounding factors. Confounding can lead to biased estimates of exposure effects and is, therefore, a major problem in causal studies; unlike selection or information bias, confounding is a bias that can be adjusted using statistical methods after data collection, generally stratification and multivariate approaches [455,456].

10.8 Novelty and impact of the study

When and after completing the main chapters of this PhD work, other relevant studies have been carried out. I have added the findings of those studies in Chapter 1 and 10 to give us a more complete picture of COVID-19. In section 10.9, I list the novelty and impact of the PhD work.

The current pandemic, caused by SARS-CoV-2, is one of the deadliest outbreaks of the 21st century, sweeping the world and bringing a great disaster to human life and health [4]. The mortality mostly occurred in patients with severe disease from COVID-19. To control the pandemic, building immunity is crucial [429]. This PhD work provides much-needed information and new insights into severe disease and immunity related to COVID-19 for further basic and clinical research into the disease and immunity and may contribute to better clinical

management of patients with COVID-19. In particular, this PhD work identifies the role of comorbidities, age, CT, lymphocytes, and symptoms of loss of smell and taste on severe disease and immunity of COVID-19, and discusses the potential related underlying mechanism and pathology, as well as the relevant treatment and management strategies. I have received the necessary multidisciplinary training in medicine, statistics and clinical epidemiology, and have experience in multiple studies, which provides the foundation for the effective and timely implementation of this work. Although this work informs the public health response and helps save lives, its impact may be amplified if the relevant publications have been published in open access repositories and been better promoted online (such as Twitter).

The human coronavirus was first found in the 1960s [457]. There have been multiple outbreaks of coronavirus pneumonia in recent years, including the SARS outbreak in 2002 and 2003, the MERS outbreak in 2012, and the SARS-CoV-2 pandemic that began in 2019 and continues to now 2022 and is still ongoing, all of which have spread to many countries around the world [458,459]. Besides several occurred SARS-CoV-2 variants which are of concern, we may have to face other outbreaks of new coronavirus pneumonia in the future. The outbreaks of coronavirus pneumonia have resulted in severe infectious diseases and caused high morbidities and even mortalities. We need to learn from known epidemics to prepare for the early identification of new ones and the prevention of severe illness and death. Thus this PhD work is of importance because it provides a reference for research methods of possible new infectious diseases in the future, as well as helps to fill the gap of unknown knowledge based on individual patients data from one of the most severe epidemics in history for the academic research community, healthcare professionals, and policy makers, which may improve patient's health and save lives.

For the research methods of future new infectious diseases, as presented in this PhD work, investigating risk factors of severe illness and then constructing its prediction nomogram can provide evidence and guidance for clinical management including risk classification and corresponding treatment. Previous studies mainly used basic comparative statistical methods but were unable to assess the linear trend with age or pulmonary opacity score. In comparison, this study used generalised linear models to examine whether there were any linear trends in clinical characteristics and outcomes with age and pulmonary opacity score. On the other hand, the use of accurate prediction tools and early intervention is important for addressing severe COVID-19. However, the prediction models for severe COVID-19 available before this PhD work were subject to various biases. This study developed and well-delineated a practicable and relatively well-validated nomogram to help healthcare professionals to predict the risk of severe COVID-19, which is interesting and innovative. This work identified a number of variables for the risk stratification model that may serve as clinical predictors of severe and critically ill patients with COVID-19. The website to predict the risk of severe COVID-19 created from this work may need more publicity to expand its influence and usage. On the other hand, given the very small number of people who had IgG testing between 8 and 11 months, using a coarser categorisation of periods (e.g., periods of 3 or 4 months) would provide more information. In the future, if the study includes a small number of people due to constraints, such as when it is urgent in the early stage of an epidemic, perhaps this method can better display information, and if the researchers also want to show monthly results, they can add a column with the coarser categorisation.

Compared with studies of similar research topics prior to the publication of Chapter 3 to Chapter 7, this PhD work has made a more in-depth analysis of COVID-19 data including

cases who were asymptomatic, mild, moderate, severe, and critically ill, and hence provided more comprehensive, generalisable and robust information on risk factors of severe/critical illness, disease deterioration and respiratory failure, and age and imaging differences in a number of baseline features and adverse outcomes in patients with COVID-19, whereas most of the previous studies focused on the patients with moderate, severe or critical symptoms, although some other studies with large sample sizes were conducted when and after publishing Chapter 3 to Chapter 7.

Before the publication of Chapter 3, several characteristics of patients with COVID-19 had been reported to be associated with the severity of COVID-19 in some small sample size studies in China [67,192-197] and other countires.[40,76,198]. Chapter 3 differs from previous studies, because previous studies were mostly small and descriptive in nature, while Chapter 3 used data from the whole Jiangsu province of China and made inferential statistical analyses by means of multivariate regression model to control for possible confounding factors and identify independent risk factors of becoming a severe or critically ill case. Chapter 3 presents that about one-tenths of patients with COVID-19 in Jiangsu had a severe or critically ill presentation. Chapter 3 indicated that the proportion of severe COVID-19 cases varies widely across countries [200,213-216], likely due to widely varying study designs, the stage of the COVID-19 outbreak at the time the study was conducted, demographic characteristics, health resources, and government responses. Patients who were severe or critically ill required careful management to prevent potential death. Chapter 3 identified risk factors of severe or critical COVID-19 including older age, low lymphocyte count and high pulmonary opacity score on admission, and highlighted the importance of early identification and intervention of patients with these risk factors to reduce the preventable and unacceptable toll of deaths

and save lives. Careful attention to these risk factors may help guide clinical care, especially intensive care, in the early stages of an epidemic.

Prior to the publication of Chapter 4, a study has reported risk factors for progression from ARDS to death in patients with COVID-19 [41]. However, the pattern of disease progression in patients with COVID-19 from "asymptomatic/mild/moderate" to "severe/critical" state and its associated factors remained to be studied. Chapter 4 supplements the information with a multicentre study. The strength of Chapter 4 was its relatively large number and inclusion of all the COVID-19 patients in the province of China. Chapter 4 described clinical deterioration in 16.3% of patients, with 6% of patients with "moderate or mild" worsening as "severe or critical". Preventing exacerbations in these patients could help improve clinical outcomes and reduce the risk of death. Age, lung opacity score, lymphocyte count on admission, and exposure at the pandemic centre were identified as independent risk factors for disease progression. This suggests to healthcare professionals that good management of patients with these characteristics may help prevent disease progression from asymptomatic/mild/moderate status to severe/critical status.

Acute respiratory failure, one of the severe diseases caused by COVID-19, can lead to a mortality rate of up to 26%-30% [37,47-49,60], while before Chapter 5 was published, research on risk factors for respiratory failure remained sparse and I only found two relevant studies both of which were conducted in Italy [59,199]. Considering the discrepancies of characteristics of patients among different countries and regions [200], there was academic and clinical value in adding respiratory failure information from other regions outside of Italy. Therefore, Chapter 5 added data from a relatively large multicentre retrospective cohort and found that approximately 9% of patients had respiratory failure. Managing these patients is a key point

in critical care management. Older age, increased respiratory rate, decreased lymphocyte count and greater pulmonary opacity score at admission were independent risk factors of respiratory failure in patients with COVID-19. This indicates that respiratory failure patients with these risk factors require intensive management during hospitalisation to prevent further worse outcomes and even deaths and reduce emergency intubation or cardiopulmonary resuscitation to protect medical staff from related infections.

Before the publication of Chapter 6, it is already known that older age is associated with more chronic histories, pulmonary opacity, and some abnormal laboratory parameters. However, Chapter 6 has some important innovations and strengths. 1. Study design and quality of research. I have searched for up-to-date publications and have found that although some of the results of Chapter 6 were known, most of the studies reporting these were based on small or local studies [38,201-208]. Chapter 6 was based on a large database comprising nearly all patients with COVID-19 from multiple centres in Jiangsu province, China, with very detailed information on patient characteristics at admission, disease severity and clinical outcomes during hospitalisation, and a systematic investigation of age differences in clinical characteristics and outcomes, which provides robust and comprehensive results of value to the readers. 2. Study variables and outcomes. In addition to studying age differences in chronic histories, pulmonary opacity, and some abnormal laboratory parameters, Chapter 6 also studied age differences in other features and clinical outcomes associated with COVID-19: (1) Exposure types (imported cases and local cases), types of disease onset (single onset and clustered onset); initial symptoms (fever, cough, sputum and shortness of breath); current smoker; drinking alcohol; vital signs (temperature, heart rate, systolic blood pressure, diastolic blood pressure, respiratory rate and SpO_2); and detailed treatment information with

18 variables; (2) The highest disease severity during the 14-day follow-up; (3) Clinical outcomes at the end of the study (intensive care unit monitoring, shock, respiratory failure, renal failure and hospital stay duration). 3. Quantification of age differences. Chapter 6 aimed to quantify age effects on various parameters and outcomes in terms of direction and magnitude. Although previous studies have shown age differences in chronic histories, pulmonary opacity, and some abnormal laboratory parameters [38,201-208]. I found only two small studies that quantified age differences: one is a multicentre study on 221 patients [205] and the other is a single hospital-based study on 56 patients [201]. Small sample sizes in these two studies suggest that their results on age differences may be subject to larger random errors than mine. In addition, previous studies mainly used basic comparative statistical methods but were unable to assess the linear trend with age. In comparison, Chapter 6 used generalised linear models to examine whether there were any linear trends in clinical characteristics and outcomes with age.

Chapter 7 provides some novel and robust information on the association between lung opacity scores and demographic, epidemiological, clinical, laboratory characteristics, and clinical management, based on a larger dataset (N = 496), rather than most studies on similar topics prior to the publication of Chapter 7 (mostly N=50-200). Chapter 7 assessed the impact of the lung opacity score on a number of important clinical outcomes, including disease severity, ICU admission, respiratory failure, and length of hospital stay, rather than the single clinical outcome in most previous reports before the publication of Chapter 7, thereby providing a more comprehensive understanding of the relationship between lung opacity scores and various clinical outcomes in COVID-19 patients. To assess the impact of lung opacity scores on clinical outcomes, lung opacity scores were measured in 5% units and

treated as continuous and categorical variables in generalised linear models, and the results were robust regardless of the functional form of the CT score. This is in stark contrast to most previous studies before the publication of Chapter 7 in which CT scores were treated as either a continuous or binary variable, which may have produced biased statistical results due to potential misspecification of CT scores.

In addition, Chapter 8 is an interesting and innovative study with potentially very useful implications. The use of accurate prediction tools and early intervention is important for addressing COVID-19. However, the prediction models for severe COVID-19 prior to the publication of Chapter 8 have some flaws including the presence of missing data and the processing strategies, lack of internal and external validation of the predictive models, weak assessment of model performance (e.g., calibration and discrimination), and reporting issues such as not mentioning the missing data [127]. To fill in these gaps, Chapter 8 developed a practicable nomogram to provide accurate, personalised predictions of the risk of severe COVID-19. In terms of missing data, no categorical data were missing, and missing continuous data were imputed with medians. In addition, the nomogram was based on a relatively large, multicentre retrospective derivation cohort and a validation cohort. The discrimination and calibration of the nomogram were evaluated with the area under the receiver operating characteristic curve and calibration plots and Hosmer-Lemeshow tests, respectively. Three predictors, namely, age, lymphocyte count, and pulmonary opacity score, were selected to develop the nomogram. These predictors of severe COVID-19 were identified as risk factors of the severe or critical illness, disease deterioration and respiratory failure in Chapter 3 to Chapter 7, and hence support the findings of the first five chapters. The nomogram exhibited good discrimination and satisfactory agreement in both the derivation and the validation

cohorts, so it should perform well in predicting the risk of severe COVID-19. Therefore, this predictive nomogram may help health care professionals and policy makers to identify patients at high risks of adverse outcomes to assist treatment and classify patients when allocating limited medical and human resources. In this case, this nomogram could help ease pressures such as hospital bed demand, shortages of medical equipment, infections among some healthcare workers, and the critical care crisis triggered by the pandemic [124-126].

Although there is considerable research on the mechanisms of immune response of other coronaviruses, more investigations on the novel SARS-CoV-2 are required to develop effective treatment and vaccine strategies. Before the completion of this PhD work, the follow-up time for studies on the duration of immunisation was limited, generally 6-8 months, and a few were around 1 year [173-177,182,183,209-211], so data with longer follow-up time for immunization in patients with COVID-19 should be valuable. The decreasing trend in participants' IgG antibody levels from month 2 to month 11 and the occurrence of reinfection presented in this PhD work prompts policymakers to consider vaccination policies to proactively improve immunity persistence. Furthermore, the association between anti-SARS-CoV-2 IgG levels and loss of smell and taste, which was statistically significant in multivariable analysis, may be considered a novel and impacting finding; only the small numbers of subjects and samples examined do not necessarily limit the novelty and impact. In fact, until I updated the literature again in February 2022, I still found only one study on the relationship between anti-SARS-CoV-2 IgG levels and loss of smell and taste, but that study only compared monthly median anti-S-RBD IgG antibody titers without using multiple regression to control for confounders and without explaining the underlying mechanism [182]. This PhD work may be crucial as it can aid researchers in better understanding the kinetics and influential factors of

anti-SARS-CoV-2 IgG antibody levels in subjects with COVID-19 for up to 11 months and hence immune responses associated with SARS-CoV-2 to develop targeted and effective treatment and vaccine programs to handle the pandemic outbreak. Because COVID-19 patients reporting smell or taste disturbances had higher levels of IgG antibodies, health policy makers may need to consider the necessity of isolation and stricter monitoring of these patients. On the other hand, the issue of the biological mechanisms underlying the association of the immune response to SARS-CoV-2 infection with loss of smell and taste is understudied, and this PhD work calls out the importance of the comprehensive immunological evaluation and discussed potential mechanisms to explain the association between anti-SARS-CoV-2 IgG levels and loss of smell and taste using the latest literature including the one published in the journal Cell in February 2022 [448]. Moreover, I discussed the contribution of rapidly declining immunity to reinfection, and also the resistance of some SARS-CoV-2 variants to durable immunity acquired from initial infection, which may have implications for vaccine development strategies. Therefore, this PhD work not only made discussion from epidemiological perspective, but also gave comments digging into clinical and immunological aspects.

This PhD work confirmed that older people and those with one or more comorbidities are at higher risk of developing a severe or critical illness, disease deterioration and respiratory failure, and tried to explain the underlying mechanisms. This provides evidence for clinicians and policy makers to support the use of additional protection measures for older people with comorbidities. This PhD work may also help the investigation of effective and safe treatments by providing information on different characteristics of the patients and the relevant immune responses. Treatments are considered to vary depending on the

characteristics of the patients, such as elderly patients and patients with obesity, hypertension or compromised immune systems [146]. In addition, the treatment management strategies in China presented in this PhD work have implications for the world's response to COVID-19, especially for other outbreaks that may follow. In the absence of treatment experience and research evidence, Chinese medical experts, based on their previous experience and clinical manifestations of patients, have made efforts to treat them and considered giving more oxygen support therapy and drugs (including antibiotics and antiviral drugs) to severe or critically ill patients. In particular, Chinese medical experts have considered individualised treatment based on the severity of the disease, age and lung CT opacity scores, and have delivered a zero mortality rate in Jiangsu province to the world.

Based on the knowledge provided by this PhD work, I have some considerations. In the early stage of a new outbreak or in settings with limited medical and social resources and adopting a policy of coexisting with the virus instead of a zero-COVID policy that aim to eradicate the COVID-19, health care providers and policy makers may need to consider that priority should be given and more attention should be paid to high-risk groups who may suffer from severe illness, such as those in long-term care facilities with weak immunity, rather than general asymptomatic infection, to ease pressure on the health care systems while avoiding delays in treating patients with high-risk factors such as diabetes. In settings with medical and social resources, although until now, the full implementation of COVID-19 vaccination strategy is still in dispute [460,461], considering the waning antibody levels over time, it may be safer for policy makers to promote effective COVID-19 vaccination policies to build longer-term herd immunity and prevent more infections and pandemics.

10.9 Future studies

In the current analysis, clinical outcomes include binary outcomes and continuous outcome but do not include survival data. Therefore, it was unable to provide a Kaplan–Meier curve for any clinical outcome. In addition, survival analysis on the clinical outcomes was unable to perform for the following reasons. (1) The data on the timing of the occurrence of clinical outcomes are missing for some patients, yielding a smaller number of events in survival analysis than that in the current generalised linear model analysis. If survival analysis were performed, biased statistical estimates of the effect of CT score on clinical outcomes would be generated. (2) Since the follow-up time is short in the current retrospective cohort study, it can be reasonably assumed that the timing of a clinical outcome is less important and could be ignored as currently done in the generalised linear model analysis. However, ROC analysis, Kaplan–Meier curve and other survival analysis methods may be worth doing in further studies that include more complete survival data, especially the follow-up time for the occurrence of clinical outcomes.

This work has also evoked future studies on immunity to COVID-19, which may have an impact on policy and prevention. The future studies may be a prospective cohort study to recruit subjects who have received vaccine once or twice, to include a larger sample size of subjects (e.g., 2000 subjects including staff, healthcare workers, et. al.), to collect more baseline information, such as vitamin D status, occupation, medical history (previous virus infection [e.g., 2003 SARS], bacterial co-infection, HIV, diabetes), treatment history, mental health, knowledge, attitude, behaviour. The studies may further assess the outcomes including death, reinfection, cost-effective outcomes, and continuous outcomes such as levels of IgG antibody and T cell. The studies may employ the machine learning method to do

exploratory studies.

In addition, clinical trials on the effective clinical management of COVID-19, including interventions of specific medicine and vaccinations, are very interesting and meaningful, which may extend the depth of this work to a large extent.

10.10 Conclusion

This study investigated the characteristics of COVID-19 and their associations with clinical outcomes and immunity, as well as the potential mechanisms and practical significance. The work identified that age, CT pulmonary opacity, lymphocyte count and pulmonary opacity score at admission may predict severe or critically ill presentation, disease deterioration, and respiratory failure in patients with COVID-19. This study also confirmed that older patients had worse clinical outcomes, in part due to comorbidities in older people, and higher rates of smoking and drinking habits, and immune, organ, and coagulation dysfunction on admission; and demonstrated that the degree of CT pulmonary opacity was closely related to age, symptoms, vital signs and laboratory parameters. In addition, this research demonstrated that a high proportion of patients confirmed with SARS-CoV-2 infection had detectable or positive IgG antibody levels in the first two months while a growing proportion of individuals lost their detectable or positive IgG after that, and IgG levels tended to be associated with gender, race, and the loss of smell and taste. It is necessary to carefully consider those potential risk factors underlying adverse outcomes and immunity of COVID-19 and take specific management to help the prevention and control of COVID-19.

Reference

1. World Health Organization. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. *Accessed 13 June 2020.* https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020.
2. World Health Organization. Coronavirus disease (COVID-19) situation report – 144. *Accessed 09 October 2020.* https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
3. Shrestha N, Shad MY, Ulvi O, et al. The impact of COVID-19 on globalization. *One Health.* 2020:100180.
4. Singh S, Pandey R, Tomar S, Varshney R, Sharma D, Gangenahalli G. A brief molecular insight of COVID-19: epidemiology, clinical manifestation, molecular mechanism, cellular tropism and immuno-pathogenesis. *Mol Cell Biochem.* 2021;476(11):3987-4002.
5. Westheim AJF, Bitorina AV, Theys J, Shiri-Sverdlov R. COVID-19 infection, progression, and vaccination: Focus on obesity and related metabolic disturbances. *Obes Rev.* 2021;22(10):e13313.
6. Zarandi PK, Zinatizadeh MR, Zinatizadeh M, Yousefi MH, Rezaei N. SARS-CoV-2: From the pathogenesis to potential anti-viral treatments. *Biomed Pharmacother.* 2021;137:111352.
7. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LFP. The trinity of COVID-19: Immunity, inflammation and intervention. *Nat Rev Immunol.* 2020;20(6):363-374.
8. Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. *Am J Pathol.* 2007;170(4):1136-1147.
9. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): Current status and future perspectives. *Int J Antimicrob Agents.* 2020;55(5):105951.
10. Hui DS, E IA, Madani TA, et al. The continuing 2019-nCoV epidemic threat of novel coronaviruses to global health - The latest 2019 novel coronavirus outbreak in Wuhan, China. *Int J Infect Dis.* 2020;91:264-266.
11. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature.* 2020;579(7798):270-273.
12. Saghazadeh A, Rezaei N. Immune-epidemiological parameters of the novel coronavirus - a perspective. *Expert Rev Clin Immunol.* 2020;16(5):465-470.
13. Yazdanpanah F, Hamblin MR, Rezaei N. The immune system and COVID-19: Friend or foe? *Life Sci.* 2020;256:117900.
14. Maeda K, Baba Y, Nagai Y, et al. IL-6 blocks a discrete early step in lymphopoiesis. *Blood.* 2005;106(3):879-885.
15. Notz Q, Schmalzing M, Wedekink F, et al. Pro- and anti-inflammatory responses in severe covid-19-induced acute respiratory distress syndrome-an observational pilot study. *Front Immunol.* 2020;11:581338.
16. Ozma MA, Maroufi P, Khodadadi E, et al. Clinical manifestation, diagnosis, prevention and control of SARS-CoV-2 (COVID-19) during the outbreak period. *Infez Med.* 2020;28(2):153-165.
17. Udugama B, Kadhiresan P, Kozlowski HN, et al. Diagnosing COVID-19: The disease and tools for detection. *ACS Nano.* 2020;14(4):3822-3835.
18. Li Y, Yao L, Li J, et al. Stability issues of RT-PCR testing of SARS-CoV-2 for hospitalized patients clinically diagnosed with COVID-19. *J Med Virol.* 2020;92(7):903-908.
19. Xu Q, Liu H, Yuan P, et al. Development of a simplified RT-PCR without RNA isolation for rapid detection of RNA viruses in a single small brown planthopper (Laodelphax striatellus Fallén). *Virol J.* 2017;14(1):90.
20. Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): A Review. *JAMA.* 2020;324(8):782-793.

21. Chilamakuri R, Agarwal S. COVID-19: Characteristics and therapeutics. *Cells.* 2021;10(2).
22. Carter LJ, Garner LV, Smoot JW, et al. Assay techniques and test development for COVID-19 diagnosis. *ACS Cent Sci.* 2020;6(5):591-605.
23. Li Z, Yi Y, Luo X, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *J Med Virol.* 2020;92(9):1518-1524.
24. Diao B, Wen K, Zhang J, et al. Accuracy of a nucleocapsid protein antigen rapid test in the diagnosis of SARS-CoV-2 infection. *Clin Microbiol Infect.* 2021;27(2):289.e281-289.e284.
25. Porte L, Legarraga P, Vollrath V, et al. Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples. *Int J Infect Dis.* 2020;99:328-333.
26. Benzigar MR, Bhattacharjee R, Baharfar M, Liu G. Current methods for diagnosis of human coronaviruses: pros and cons. *Anal Bioanal Chem.* 2021;413(9):2311-2330.
27. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet.* 2020;395(10223):514-523.
28. Li H, Liu Z, Ge J. Scientific research progress of COVID-19/SARS-CoV-2 in the first five months. *J Cell Mol Med.* 2020;24(12):6558-6570.
29. Rimmer A. Covid-19: Doctors in final trimester of pregnancy should avoid direct patient contact. *BMJ.* 2020;368:m1173.
30. Billah MA, Miah MM, Khan MN. Reproductive number of coronavirus: A systematic review and meta-analysis based on global level evidence. *PLoS One.* 2020;15(11):e0242128.
31. Petrosillo N, Viceconte G, Ergonul O, Ippolito G, Petersen E. COVID-19, SARS and MERS: Are they closely related? *Clin Microbiol Infect.* 2020;26(6):729-734.
32. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science.* 2020;367(6483):1260-1263.
33. Setti L, Passarini F, De Gennaro G, et al. Airborne transmission route of COVID-19: Why 2 meters/6 feet of inter-personal distance could not be enough. *Int J Environ Res Public Health.* 2020;17(8).
34. Bhat EA, Khan J, Sajjad N, et al. SARS-CoV-2: Insight in genome structure, pathogenesis and viral receptor binding analysis - An updated review. *Int Immunopharmacol.* 2021;95:107493.
35. Acter T, Uddin N, Das J, Akhter A, Choudhury TR, Kim S. Evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as coronavirus disease 2019 (COVID-19) pandemic: A global health emergency. *Sci Total Environ.* 2020;730:138996.
36. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of 2019 novel coronavirus infection in China. *medRxiv.* 2020.
37. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. *Lancet.* 2020;395(10223):507-513.
38. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study [published correction appears in Lancet Respir Med. 2020 Apr;8(4):e26]. *Lancet Respir Med*. 2020;8(5):475-481.
39. Goyal P, Choi JJ, Pinheiro LC, et al. Clinical characteristics of COVID-19 in New York City. *N Engl J Med.* 2020;382(24):2372-2374.
40. Mitra AR, Fergusson NA, Lloyd-Smith E, et al. Baseline characteristics and outcomes of patients with COVID-19 admitted to intensive care units in Vancouver, Canada: A case series. *CMAJ*. 2020;192(26):E694-E701.
41. Wu C, Chen X, Cai Y, et al. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China [published correction appears in JAMA Intern Med. 2020 Jul 1;180(7):1031]. *JAMA Intern Med*. 2020;180(7):934-943.

42. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study [published correction appears in Lancet. 2020 Mar 28;395(10229):1038] [published correction appears in Lancet. 2020 Mar 28;395(10229):1038]. *Lancet*. 2020;395(10229):1054-1062.
43. Zuo MZ, Huang YG, Ma WH, et al. Expert recommendations for tracheal intubation in critically ill patients with noval coronavirus disease 2019. *Chin Med Sci J*. 2020;35(2):105-109.
44. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China [published correction appears in JAMA. 2021 Mar 16;325(11):1113]. *JAMA*. 2020;323(11):1061-1069.
45. Chang L, Yan Y, Wang L. Coronavirus disease 2019: Coronaviruses and blood safety. *Transfus Med Rev*. 2020;34(2):75-80.
46. Li YC, Bai WZ, Hashikawa T. The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J Med Virol*. 2020;92(6):552-555.
47. Liu K, Fang YY, Deng Y, et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl)*. 2020;133(9):1025-1031.
48. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet.* 2020;395(10223):497-506.
49. MacLaren G, Fisher D, Brodie D. Preparing for the most critically ill patients with covid-19: the potential role of extracorporeal membrane oxygenation. *JAMA*. 2020;323(13):1245-1246.
50. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study. *Lancet.* 2020;395(10229):1054-1062.
51. Chinese Center for Disease Control and Prevention. The epidemiological characteristics of an outbreak of 2019 novel coronavirus diseases (COVID-19) in China. *Zhonghua Liu Xing Bing Xue Za Zhi.* 2020;41(2): 145-151.
52. Shim E, Tariq A, Choi W, Lee Y, Chowell G. Transmission potential and severity of COVID-19 in South Korea. *Int J Infect Dis.* 2020;93:339-344.
53. COVID-19 National Incident Room Surveillance Team. COVID-19, Australia: Epidemiology report 17 (Fortnightly reporting period ending 24 May 2020). *Commun Dis Intell (2018).* 2020;44.
54. European Centre for Disease Prevention and Control. Coronavirus disease 2019 (COVID-19) in the EU/EEA and the UK – eighth update. *Accessed 13 June 2020.* https://www.ecdc.europa.eu/sites/default/files/documents/covid-19-rapid-risk-assessment-coronavirus-disease-2019-eighth-update-8-april-2020.pdf.
55. Livingston E, Bucher K. Coronavirus disease 2019 (COVID-19) in Italy. *JAMA*. 2020;323(14):1335.
56. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report – 46. *Accessed 13 June 2020.* https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
57. Valls J, Clèries R, Gálvez J, et al. RiskDiff: a web tool for the analysis of the difference due to risk and demographic factors for incidence or mortality data. *BMC Public Health.* 2009;9:473.
58. Sun Q, Qiu H, Huang M, Yang Y. Lower mortality of COVID-19 by early recognition and intervention: experience from Jiangsu Province. *Ann Intensive Care.* 2020;10(1):33.
59. Bartoletti M, Giannella M, Scudeller L, et al. Development and validation of a prediction model for severe respiratory failure in hospitalized patients with SARS-CoV-2 infection: A multicentre cohort study (PREDI-CO study). *Clin Microbiol Infect*. 2020;26(11):1545-1553.
60. Ferguson J, Volk S, Vondracek T, Flanigan J, Chernaik A. Empiric therapeutic anticoagulation and mortality in critically ill patients with respiratory failure from SARS-CoV-2: A retrospective cohort study. *J Clin Pharmacol*. 2020;60(11):1411-1415.

61. Wang S, Guo L, Chen L, et al. A case report of neonatal 2019 coronavirus disease in China. *Clin Infect Dis*. 2020;71(15):853-857.
62. Sun D, Li H, Lu XX, et al. Clinical features of severe pediatric patients with coronavirus disease 2019 in Wuhan: A single center's observational study. *World J Pediatr*. 2020;16(3):251-259.
63. Liu R, Han H, Liu F, et al. Positive rate of RT-PCR detection of SARS-CoV-2 infection in 4880 cases from one hospital in Wuhan, China, from Jan to Feb 2020. *Clin Chim Acta.* 2020;505:172-175.
64. Porcheddu R, Serra C, Kelvin D, Kelvin N, Rubino S. Similarity in case fatality rates (CFR) of COVID-19/SARS-CoV-2 in Italy and China. *J Infect Dev Ctries.* 2020;14(2):125-128.
65. Wong RS, Wu A, To KF, et al. Haematological manifestations in patients with severe acute respiratory syndrome: Retrospective analysis. *BMJ.* 2003;326(7403):1358-1362.
66. Hon KL, Leung CW, Cheng WT, et al. Clinical presentations and outcome of severe acute respiratory syndrome in children. *Lancet.* 2003;361(9370):1701-1703.
67. Li K, Wu J, Wu F, et al. The clinical and chest CT features associated with severe and critical COVID-19 pneumonia. *Invest Radiol.* 2020;55(6):327-331.
68. Center for Disease Control. COVID-19: People with certain medical condition. *Accessed 01 February 2022.* https://wwwcdcgov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditionshtml?CDC_AA_refVal=https%3A%2F%2Fwwwcdcgov%2Fcoronavirus%2F2019-ncov%2Fneed-extra-precautions%2Fgroups-at-higher-riskhtml.
69. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. *Nature.* 2020;584(7821):430-436.
70. Deng G, Yin M, Chen X, Zeng F. Clinical determinants for fatality of 44,672 patients with COVID-19. *Crit Care.* 2020;24(1):179.
71. Ssentongo P, Ssentongo AE, Heilbrunn ES, Ba DM, Chinchilli VM. Association of cardiovascular disease and 10 other pre-existing comorbidities with COVID-19 mortality: A systematic review and meta-analysis. *PLoS One.* 2020;15(8):e0238215.
72. Zheng YY, Ma YT, Zhang JY, Xie X. COVID-19 and the cardiovascular system. *Nat Rev Cardiol.* 2020;17(5):259-260.
73. Wu ZH, Tang Y, Cheng Q. Diabetes increases the mortality of patients with COVID-19: A meta-analysis. *Acta Diabetol.* 2021;58(2):139-144.
74. Feng Y, Ling Y, Bai T, et al. COVID-19 with different severities: A multicenter study of clinical features. *Am J Respir Crit Care Med.* 2020;201(11):1380-1388.
75. Chen T, Dai Z, Mo P, et al. Clinical characteristics and outcomes of older patients with coronavirus disease 2019 (COVID-19) in Wuhan, China: A single-centered, retrospective study. *J Gerontol A Biol Sci Med Sci.* 2020;75(9):1788-1795.
76. Simonnet A, Chetboun M, Poissy J, et al. High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation [published correction appears in Obesity (Silver Spring). 2020 Oct;28(10):1994]. *Obesity (Silver Spring)*. 2020;28(7):1195-1199.
77. Stefan N, Birkenfeld AL, Schulze MB, Ludwig DS. Obesity and impaired metabolic health in patients with COVID-19. *Nat Rev Endocrinol.* 2020;16(7):341-342.
78. Sattar N, McInnes IB, McMurray JJV. Obesity is a risk factor for severe COVID-19 infection: Multiple potential mechanisms. *Circulation.* 2020;142(1):4-6.
79. Perez A, Naljayan M, Shuja I, Florea A, Reisin E. Hypertension, obesity, and COVID-19: A collision of pandemics. *Curr Hypertens Rep.* 2021;23(6):36.
80. Kulkarni S, Jenner BL, Wilkinson I. COVID-19 and hypertension. *J Renin Angiotensin Aldosterone Syst.* 2020;21(2):1470320320927851.
81. Fang L, Karakiulakis G, Roth M. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? *Lancet Respir Med.* 2020;8(4):e21.

82. Gupta R, Hussain A, Misra A. Diabetes and COVID-19: Evidence, current status and unanswered research questions. *Eur J Clin Nutr.* 2020;74(6):864-870.
83. Moradi-Marjaneh R, Asgharzadeh F, Khordad E, Marjaneh MM. Diabetes and COVID-19; a review of possible mechanisms. *Curr Pharm Des.* 2021;27(21):2522-2527.
84. Mahrooz A, Muscogiuri G, Buzzetti R, Maddaloni E. The complex combination of COVID-19 and diabetes: pleiotropic changes in glucose metabolism. *Endocrine.* 2021;72(2):317-325.
85. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: Summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA.* 2020;323(13):1239-1242.
86. Ghosh A, Gupta R, Misra A. Telemedicine for diabetes care in India during COVID19 pandemic and national lockdown period: Guidelines for physicians. *Diabetes Metab Syndr.* 2020;14(4):273-276.
87. Knapp S. Diabetes and infection: is there a link?--A mini-review. *Gerontology.* 2013;59(2):99-104.
88. Miyashita H, Mikami T, Chopra N, et al. Do patients with cancer have a poorer prognosis of COVID-19? An experience in New York City. *Ann Oncol.* 2020;31(8):1088-1089.
89. Dai M, Liu D, Liu M, et al. Patients with cancer appear more vulnerable to SARS-CoV-2: A multicenter study during the COVID-19 outbreak. *Cancer Discov.* 2020;10(6):783-791.
90. Kanwugu ON, Adadi P. HIV/SARS-CoV-2 coinfection: A global perspective. *J Med Virol.* 2021;93(2):726-732.
91. Long C, Xu H, Shen Q, et al. Diagnosis of the coronavirus disease (COVID-19): rRT-PCR or CT? *Eur J Radiol.* 2020;126:108961.
92. Ye Z, Zhang Y, Wang Y, Huang Z, Song B. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): A pictorial review. *Eur Radiol*. 2020;30(8):4381-4389.
93. Dai H, Zhang X, Xia J, et al. High-resolution Chest CT Features and Clinical Characteristics of Patients Infected with COVID-19 in Jiangsu, China. *Int J Infect Dis*. 2020;95:106-112.
94. Wu J, Wu X, Zeng W, et al. Chest CT findings in patients with coronavirus disease 2019 and its relationship with clinical features. *Invest Radiol*. 2020;55(5):257-261.
95. Zhang X, Cai H, Hu J, et al. Epidemiological, clinical characteristics of cases of SARS-CoV-2 infection with abnormal imaging findings. *Int J Infect Dis*. 2020;94:81-87.
96. Noll E, Soler L, Ohana M, et al. A novel, automated, quantification of abnormal lung parenchyma in patients with COVID-19 infection: Initial description of feasibility and association with clinical outcome. *Anaesth Crit Care Pain Med.* 2020:100780.
97. Cozzi D, Albanesi M, Cavigli E, et al. Chest X-ray in new coronavirus disease 2019 (COVID-19) infection: findings and correlation with clinical outcome. *Radiol Med.* 2020;125(8):730-737.
98. Wu S, Xue L, Legido-Quigley H, et al. Understanding factors influencing the length of hospital stay among non-severe COVID-19 patients: A retrospective cohort study in a Fangcang shelter hospital. *PLoS One.* 2020;15(10):e0240959.
99. Shi H, Han X, Jiang N, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. *Lancet Infect Dis.* 2020;20(4):425-434.
100. Zhao W, Zhong Z, Xie X, Yu Q, Liu J. Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) pneumonia: A multicenter study. *AJR Am J Roentgenol.* 2020;214(5):1072-1077.
101. Salian VS, Wright JA, Vedell PT, et al. COVID-19 transmission, current treatment, and future therapeutic strategies. *Mol Pharm.* 2021;18(3):754-771.
102. Chen Z, Xu W, Ma W, et al. Clinical laboratory evaluation of COVID-19. *Clin Chim Acta.* 2021;519:172-182.
103. Henry BM, de Oliveira MHS, 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;58(7):1021-1028.

104. Zheng Y, Xu H, Yang M, et al. Epidemiological characteristics and clinical features of 32 critical and 67 noncritical cases of COVID-19 in Chengdu. *J Clin Virol.* 2020;127:104366.
105. Liu K, Fang YY, Deng Y, et al. Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. *Chin Med J (Engl).* 2020;133(9):1025-1031.
106. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest.* 2020;130(5):2620-2629.
107. Zhang B, Zhou X, Zhu C, et al. Immune phenotyping based on the neutrophil-to-lymphocyte ratio and igg level predicts disease severity and outcome for patients with COVID-19. *Front Mol Biosci.* 2020;7:157.
108. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with coronavirus 2019 (COVID-19) in Wuhan, China. *Clin Infect Dis.* 2020;71(15):762-768.
109. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* 2020;382(18):1708-1720.
110. Liu Y, Sun W, Guo Y, et al. Association between platelet parameters and mortality in coronavirus disease 2019: Retrospective cohort study. *Platelets.* 2020;31(4):490-496.
111. Yang X, Yang Q, Wang Y, et al. Thrombocytopenia and its association with mortality in patients with COVID-19. *J Thromb Haemost.* 2020;18(6):1469-1472.
112. 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;46(5):846-848.
113. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID-19 infection. *Am J Hematol.* 2020;95(6):E131-e134.
114. Wu C, Chen X, Cai Y, 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;180(7):934-943.
115. Qu R, Ling Y, Zhang YH, et al. Platelet-to-lymphocyte ratio is associated with prognosis in patients with corona virus disease-19. *J Med Virol.* 2020.
116. Amgalan A, Othman M. Hemostatic laboratory derangements in COVID-19 with a focus on platelet count. *Platelets.* 2020;31(6):740-745.
117. Chen T, Wu D, Chen H, et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *BMJ.* 2020;368:m1091.
118. Al-Samkari H, Karp Leaf RS, Dzik WH, et al. COVID-19 and coagulation: bleeding and thrombotic manifestations of SARS-CoV-2 infection. *Blood.* 2020;136(4):489-500.
119. Levi M. COVID-19 coagulopathy vs disseminated intravascular coagulation. *Blood Adv.* 2020;4(12):2850.
120. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost.* 2020;18(4):844-847.
121. Li H, Xiang X, Ren H, et al. Serum Amyloid A is a biomarker of severe coronavirus disease and poor prognosis. *J Infect.* 2020;80(6):646-655.
122. Pieri M, Ciotti M, Nuccetelli M, et al. Serum Amyloid A Protein as a useful biomarker to predict COVID-19 patients severity and prognosis. *Int Immunopharmacol.* 2021;95:107512.
123. Cabler SS, French AR, Orvedahl A. A cytokine circus with a viral ringleader: SARS-CoV-2-associated cytokine storm syndromes. *Trends Mol Med.* 2020;26(12):1078-1085.
124. Xie J, Tong Z, Guan X, Du B, Qiu H, Slutsky AS. Critical care crisis and some recommendations during the COVID-19 epidemic in China. *Intensive Care Med.* 2020;46(5):837-840.
125. Grasselli G, Pesenti A, Cecconi M. Critical care utilization for the COVID-19 outbreak in lombardy, Italy: Early experience and forecast during an emergency response. *JAMA.* 2020;323(16):1545-1546.
126. Arabi YM, Murthy S, Webb S. COVID-19: A novel coronavirus and a novel challenge for critical care. *Intensive Care Med.* 2020;46(5):833-836.

127. Collins GS, Wilkinson J. Statistical issues in the development of COVID-19 prediction models. *J Med Virol*. 2021;93(2):624-625.
128. Wynants L, Van Calster B, Collins GS, et al. Prediction models for diagnosis and prognosis of covid-19 infection: systematic review and critical appraisal. *BMJ.* 2020;369:m1328.
129. Sun L, Song F, Shi N, et al. Combination of four clinical indicators predicts the severe/critical symptom of patients infected COVID-19. *J Clin Virol.* 2020;128:104431.
130. Wu G, Yang P, Xie Y, et al. Development of a clinical decision support system for severity risk prediction and triage of COVID-19 patients at hospital admission: An international multicentre study. *Eur Respir J.* 2020;56(2).
131. Xiao LS, Li P, Sun F, et al. Development and validation of a deep learning-based model using computed tomography imaging for predicting disease severity of coronavirus disease 2019. *Front Bioeng Biotechnol.* 2020;8:898.
132. Xiao LS, Zhang WF, Gong MC, et al. Development and validation of the HNC-LL score for predicting the severity of coronavirus disease 2019. *EBioMedicine.* 2020;57:102880.
133. Zhang C, Qin L, Li K, et al. A novel scoring system for prediction of disease severity in COVID-19. *Front Cell Infect Microbiol.* 2020;10:318.
134. Zhou C, Huang Z, Tan W, et al. Predictive factors of severe coronavirus disease 2019 in previously healthy young adults: A single-center, retrospective study. *Respir Res.* 2020;21(1):157.
135. Zhou Y, He Y, Yang H, 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.
136. Ok F, Erdogan O, Durmus E, Carkci S, Canik A. Predictive values of blood urea nitrogen/creatinine ratio and other routine blood parameters on disease severity and survival of COVID-19 patients. *J Med Virol.* 2020.
137. Yang P, Wang P, Song Y, Zhang A, Yuan G, Cui Y. A retrospective study on the epidemiological characteristics and establishment of an early warning system of severe COVID-19 patients. *J Med Virol.* 2020;92(10):2173-2180.
138. Gong J, Ou J, Qiu X, et al. A tool for early prediction of severe coronavirus disease 2019 (COVID-19): A multicenter study using the risk nomogram in Wuhan and Guangdong, China. *Clin Infect Dis.* 2020;71(15):833-840.
139. Collins GS, van Smeden M, Riley RD. COVID-19 prediction models should adhere to methodological and reporting standards. *Eur Respir J.* 2020;56(3).
140. Shariat SF, Capitanio U, Jeldres C, Karakiewicz PI. Can nomograms be superior to other prediction tools? *BJU Int.* 2009;103(4):492-495; discussion 495-497.
141. Huang J, Zhu L, Bai X, et al. Multidimensional analysis of risk factors for the severity and mortality of patients with COVID-19 and diabetes. *Infect Dis Ther.* 2020:1-22.
142. Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: A narrative review on potential mechanisms. *J Mol Histol.* 2020:1-16.
143. Rees EM, Nightingale ES, Jafari Y, et al. COVID-19 length of hospital stay: A systematic review and data synthesis. *BMC Med.* 2020;18(1):270.
144. Ramadan HK, Mahmoud MA, Aburahma MZ, et al. Predictors of severity and co-infection resistance profile in COVID-19 patients: First report from upper Egypt. *Infect Drug Resist.* 2020;13:3409-3422.
145. Sanders JM, Monogue ML, Jodlowski TZ, Cutrell JB. Pharmacologic treatments for coronavirus Disease 2019 (COVID-19): A Review. *JAMA.* 2020;323(18):1824-1836.
146. Voutouri C, Nikmaneshi MR, Hardin CC, et al. In silico dynamics of COVID-19 phenotypes for optimizing clinical management. *Proc Natl Acad Sci U S A.* 2021;118(3).
147. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: A randomised, double-blind, placebo-controlled, multicentre trial. *Lancet.* 2020;395(10236):1569-1578.
148. Beigel JH, Tomashek KM, Dodd LE. Remdesivir for the treatment of COVID-19 - preliminary report. Reply. *N Engl J Med.* 2020;383(10):994.

149. Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. *JAMA.* 2020;323(16):1582-1589.
150. Marston HD, Paules CI, Fauci AS. Monoclonal antibodies for emerging infectious diseases - borrowing from history. *N Engl J Med.* 2018;378(16):1469-1472.
151. Atluri S, Manchikanti L, Hirsch JA. Expanded Umbilical Cord Mesenchymal Stem Cells (UC-MSCs) as a therapeutic strategy in managing critically ill covid-19 patients: The case for compassionate use. *Pain Physician.* 2020;23(2):E71-e83.
152. Iwashyna TJ, Burke JF, Sussman JB, Prescott HC, Hayward RA, Angus DC. Implications of heterogeneity of treatment effect for reporting and analysis of randomized trials in critical care. *Am J Respir Crit Care Med.* 2015;192(9):1045-1051.
153. Siddique R, Bai Q, Shereen MA, et al. Evidence and speculations: vaccines and therapeutic options for COVID-19 pandemic. *Hum Vaccin Immunother.* 2021;17(4):1113-1121.
154. Centers for Disease Control and Prevention. Interim guidelines for collecting, handling, and testing clinical specimens for COVID-19. *Accessed 13 September 2020.* https://wwwcdcgov/coronavirus/2019-ncov/lab/guidelines-clinicalspecimenshtml.
155. Attaway A, Hatipoğlu U. Management of patients with COPD during the COVID-19 pandemic [published online ahead of print, 2020 Jul 17]. *Cleve Clin J Med*. 2020;10.3949/ccjm.87a.ccc007.
156. Singh AK, Majumdar S, Singh R, Misra A. Role of corticosteroid in the management of COVID-19: A systemic review and a clinician's perspective. *Diabetes Metab Syndr.* 2020;14(5):971-978.
157. Wilson NM, Norton A, Young FP, Collins DW. Airborne transmission of severe acute respiratory syndrome coronavirus-2 to healthcare workers: A narrative review. *Anaesthesia.* 2020;75(8):1086-1095.
158. Das G, Das T, Chowdhury N, Chatterjee D, Bagchi A, Ghosh Z. Repurposed drugs and nutraceuticals targeting envelope protein: A possible therapeutic strategy against COVID-19. *Genomics.* 2021;113(1 Pt 2):1129-1140.
159. De P, Chakraborty I, Karna B, Mazumder N. Brief review on repurposed drugs and vaccines for possible treatment of COVID-19. *Eur J Pharmacol.* 2021;898:173977.
160. Fathi A, Mellinghoff SC. Vaccines against COVID-19. *Internist (Berl).* 2021;62(11):1191-1201.
161. Graham BS. Rapid COVID-19 vaccine development. *Science.* 2020;368(6494):945-946.
162. Kalita P, Padhi AK, Zhang KYJ, Tripathi T. Design of a peptide-based subunit vaccine against novel coronavirus SARS-CoV-2. *Microb Pathog.* 2020;145:104236.
163. Thanh Le T, Andreadakis Z, Kumar A, et al. The COVID-19 vaccine development landscape. *Nat Rev Drug Discov.* 2020;19(5):305-306.
164. Toyoshima Y, Nemoto K, Matsumoto S, Nakamura Y, Kiyotani K. SARS-CoV-2 genomic variations associated with mortality rate of COVID-19. *J Hum Genet.* 2020;65(12):1075-1082.
165. Remschmidt C, Wichmann O, Harder T. Vaccines for the prevention of seasonal influenza in patients with diabetes: Systematic review and meta-analysis. *BMC Med.* 2015;13:53.
166. Moriyama M, Hugentobler WJ, Iwasaki A. Seasonality of respiratory viral infections. *Annu Rev Virol.* 2020;7(1):83-101.
167. Klimpel GR. Immune Defenses. In: Baron S, ed. *Medical Microbiology*. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston; 1996.
168. Bauer G, Struck F, Schreiner P, Staschik E, Soutschek E, Motz M. The challenge of avidity determination in SARS-CoV-2 serology. *J Med Virol*. 2021;93(5):3092-3104.
169. Lynch KL, Whitman JD, Lacanienta NP, et al. Magnitude and kinetics of anti-severe acute respiratory syndrome coronavirus 2 antibody responses and their relationship to disease severity. *Clin Infect Dis.* 2021;72(2):301-308.
170. Legros V, Denolly S, Vogrig M, et al. A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. *Cell Mol Immunol.* 2021;18(2):318-327.

171. Muralidar S, Ambi SV, Sekaran S, Krishnan UM. The emergence of COVID-19 as a global pandemic: Understanding the epidemiology, immune response and potential therapeutic targets of SARS-CoV-2. *Biochimie.* 2020;179:85-100.
172. Jiang XL, Wang GL, Zhao XN, et al. Lasting antibody and T cell responses to SARS-CoV-2 in COVID-19 patients three months after infection. *Nat Commun.* 2021;12(1):897.
173. Dan JM, Mateus J, Kato Y, et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science.* 2021;371(6529).
174. He Z, Ren L, Yang J, et al. Seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies in Wuhan, China: A longitudinal, population-level, cross-sectional study. *Lancet.* 2021;397(10279):1075-1084.
175. den Hartog G, Vos ERA, van den Hoogen LL, et al. Persistence of antibodies to severe acute respiratory syndrome coronavirus 2 in relation to symptoms in a nationwide prospective study. *Clin Infect Dis.* 2021;73(12):2155-2162.
176. Pradenas E, Trinité B, Urrea V, et al. Stable neutralizing antibody levels 6 months after mild and severe COVID-19 episodes. *Med (N Y).* 2021;2(3):313-320.e314.
177. Sherina N, Piralla A, Du L, et al. Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6-8 months after the infection. *Med (N Y).* 2021;2(3):281-295.e284.
178. Vogelzang EH, Loeff FC, Derksen NIL, et al. Development of a SARS-CoV-2 total antibody assay and the dynamics of antibody response over time in hospitalized and nonhospitalized patients with COVID-19. *J Immunol.* 2020;205(12):3491-3499.
179. Semmler G, Traugott MT, Graninger M, et al. Assessment of S1-, S2-, and NCP-specific IgM, IgA, and IgG antibody kinetics in acute SARS-CoV-2 infection by a microarray and twelve other immunoassays. *J Clin Microbiol*. 2021;59(5):e02890-20.
180. To KK, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: An observational cohort study. *Lancet Infect Dis.* 2020;20(5):565-574.
181. Sakhi H, Dahmane D, Attias P, et al. Kinetics of anti-SARS-CoV-2 IgG antibodies in hemodialysis patients six months after infection. *J Am Soc Nephrol*. 2021;32(5):1033-1036.
182. Dehgani-Mobaraki P, Zaidi AK, Yadav N, Floridi A, Floridi E. Longitudinal observation of antibody responses for 14 months after SARS-CoV-2 infection. *Clin Immunol.* 2021;230:108814.
183. Zeng F, Wu M, Wang J, Li J, Hu G, Wang L. Over 1-year duration and age difference of SARS-CoV-2 antibodies in convalescent COVID-19 patients. *J Med Virol.* 2021;93(12):6506-6511.
184. De Greef J, Scohy A, Zech F, et al. Determinants of IgG antibodies kinetics after severe and critical COVID-19. *J Med Virol.* 2021;93(9):5416-5424.
185. Huang M, Lu QB, Zhao H, et al. Temporal antibody responses to SARS-CoV-2 in patients of coronavirus disease 2019. *Cell Discov.* 2020;6:64.
186. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis.* 2020;71(16):2027-2034.
187. Terpos E, Politou M, Sergentanis TN, et al. Anti-SARS-CoV-2 antibody responses in convalescent plasma donors are increased in hospitalized patients; subanalyses of a phase 2 clinical study. *Microorganisms*. 2020;8(12):1885.
188. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral immune response to SARS-CoV-2 in Iceland. *N Engl J Med.* 2020;383(18):1724-1734.
189. Yazaki S, Yoshida T, Kojima Y, et al. Difference in SARS-CoV-2 antibody status between patients with cancer and health care workers during the COVID-19 pandemic in Japan. *JAMA Oncol.* 2021;7(8):1141-1148.
190. Horton DB, Barrett ES, Roy J, et al. Determinants and dynamics of SARS-CoV-2 infection in a diverse population: 6-month evaluation of a prospective cohort study. *J Infect Dis.* 2021;224(8):1345-1356.

191. Zejda JE, Brożek GM, Kowalska M, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in a random sample of inhabitants of the Katowice region, Poland. *Int J Environ Res Public Health*. 2021;18(6):3188.
192. Chen W, Zheng KI, Liu S, Yan Z, Xu C, Qiao Z. Plasma CRP level is positively associated with the severity of COVID-19. *Ann Clin Microbiol Antimicrob.* 2020;19(1):18.
193. Gao F, Zheng KI, Wang XB, et al. Obesity is a risk factor for greater COVID-19 severity. *Diabetes Care*. 2020;43(7):e72-e74.
194. Li J, Li M, Zheng S, et al. Plasma albumin levels predict risk for nonsurvivors in critically ill patients with COVID-19. *Biomark Med*. 2020;14(10):827-837.
195. Li X, Xu S, Yu M, et al. Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. *J Allergy Clin Immunol*. 2020;146(1):110-118.
196. Li Y, Zhao K, Wei H, et al. Dynamic relationship between D-dimer and COVID-19 severity. *Br J Haematol*. 2020;190(1):e24-e27.
197. Lippi G, Plebani M, Henry BM. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. *Clin Chim Acta.* 2020;506:145-148.
198. Bhatraju PK, Ghassemieh BJ, Nichols M, et al. Covid-19 in critically ill patients in the seattle region - case series. *N Engl J Med.* 2020;382(21):2012-2022.
199. Rottoli M, Bernante P, Belvedere A, et al. How important is obesity as a risk factor for respiratory failure, intensive care admission and death in hospitalised COVID-19 patients? Results from a single Italian centre. *Eur J Endocrinol.* 2020;183(4):389-397.
200. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City Area. *JAMA.* 2020;323(20):2052-2059.
201. Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *J Infect.* 2020;80(6):e14-e18.
202. Brodin P. Why is COVID-19 so mild in children? *Acta Paediatr*. 2020;109(6):1082-1083.
203. Su L, Ma X, Yu H, et al. The different clinical characteristics of corona virus disease cases between children and their families in China - the character of children with COVID-19. *Emerg Microbes Infect.* 2020;9(1):707-713.
204. Mo P, Xing Y, Xiao Y, et al. Clinical characteristics of refractory coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis.* 2021;73(11):e4208-e4213.
205. Liu Y, Mao B, Liang S, et al. Association between age and clinical characteristics and outcomes of COVID-19. *Eur Respir J*. 2020;55(5):2001112.
206. Xia W, Shao J, Guo Y, Peng X, Li Z, Hu D. Clinical and CT features in pediatric patients with COVID-19 infection: Different points from adults. *Pediatr Pulmonol*. 2020;55(5):1169-1174.
207. Liu H, Liu F, Li J, Zhang T, Wang D, Lan W. Clinical and CT imaging features of the COVID-19 pneumonia: Focus on pregnant women and children. *J Infect*. 2020;80(5):e7-e13.
208. Li W, Cui H, Li K, Fang Y, Li S. Chest computed tomography in children with COVID-19 respiratory infection. *Pediatr Radiol*. 2020;50(6):796-799.
209. Masiá M, Fernández-González M, Telenti G, et al. Durable antibody response one year after hospitalization for COVID-19: A longitudinal cohort study. *J Autoimmun.* 2021;123:102703.
210. Varona JF, Madurga R, Peñalver F, et al. Kinetics of anti-SARS-CoV-2 antibodies over time; results of 10 month follow up in over 300 seropositive Health Care Workers. *Eur J Intern Med.* 2021;89:97-103.
211. Post N, Eddy D, Huntley C, et al. Antibody response to SARS-CoV-2 infection in humans: A systematic review. *PLoS One.* 2020;15(12):e0244126.
212. National Health Commission & National Administration of Traditional Chinese Medicine. Diagnosis and treatment protocol for novel coronavirus pneumonia (Trial Version 7). *Chin Med J (Engl)*. 2020;133(9):1087-1095.

213. Cummings MJ, Baldwin MR, Abrams D, et al. Epidemiology, clinical course, and outcomes of critically ill adults with COVID-19 in New York City: A prospective cohort study. *Lancet.* 2020;395(10239):1763-1770.
214. Petrilli CM, Jones SA, Yang J, et al. Factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: Prospective cohort study. *BMJ.* 2020;369:m1966.
215. Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA.* 2020;323(15):1488-1494.
216. Preliminary estimates of the prevalence of selected underlying health conditions among patients with coronavirus disease 2019 - United States, February 12-March 28, 2020. *MMWR Morb Mortal Wkly Rep.* 2020;69(13):382-386.
217. Xu XW, Wu XX, Jiang XG, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: Retrospective case series. *BMJ.* 2020;368:m606.
218. Zhang W, Zhao Y, Zhang F, et al. The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The Perspectives of clinical immunologists from China. *Clin Immunol.* 2020;214:108393.
219. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood.* 2014;124(2):188-195.
220. Cron R, Behrens EM. *Cytokine storm syndrome.* 1 ed: Springer International Publishing; 2019.
221. Zhang J, Zhou L, Yang Y, Peng W, Wang W, Chen X. Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics. *Lancet Respir Med*. 2020;8(3):e11-e12.
222. DesPrez K, McNeil JB, Wang C, Bastarache JA, Shaver CM, Ware LB. Oxygenation saturation index predicts clinical outcomes in ARDS. *Chest.* 2017;152(6):1151-1158.
223. Chen WL, Lin WT, Kung SC, Lai CC, Chao CM. The value of oxygenation saturation index in predicting the outcomes of patients with acute respiratory distress syndrome. *J Clin Med*. 2018;7(8):205.
224. Gralinski LE, Menachery VD. Return of the coronavirus: 2019-nCoV. *Viruses*. 2020;12(2):135.
225. Wu J, Li W, Shi X, et al. Early antiviral treatment contributes to alleviate the severity and improve the prognosis of patients with novel coronavirus disease (COVID-19). *J Intern Med*. 2020;288(1):128-138.
226. Wang W, Tang J, Wei F. Updated understanding of the outbreak of 2019 novel coronavirus (2019-nCoV) in Wuhan, China. *J Med Virol.* 2020;92(4):441-447.
227. Feikin DR, Alraddadi B, Qutub M, et al. Association of higher MERS-CoV virus load with severe disease and death, Saudi Arabia, 2014. *Emerg Infect Dis.* 2015;21(11):2029-2035.
228. Ko JH, Park GE, Lee JY, et al. Predictive factors for pneumonia development and progression to respiratory failure in MERS-CoV infected patients. *J Infect.* 2016;73(5):468-475.
229. Majumder MS, Kluberg SA, Mekaru SR, Brownstein JS. Mortality risk factors for middle east respiratory syndrome outbreak, South Korea, 2015. *Emerg Infect Dis.* 2015;21(11):2088-2090.
230. Chan JWM, Ng CK, Chan YH, et al. Short term outcome and risk factors for adverse clinical outcomes in adults with severe acute respiratory syndrome (SARS). *Thorax.* 2003;58(8):686-689.
231. Maue AC, Yager EJ, Swain SL, Woodland DL, Blackman MA, Haynes L. T-cell immunosenescence: Lessons learned from mouse models of aging. *Trends Immunol.* 2009;30(7):301-305.
232. Shi H, Han X, Jiang N, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: A descriptive study. *Lancet Infect Dis*. 2020;20(4):425-434.
233. Li K, Fang Y, Li W, et al. CT image visual quantitative evaluation and clinical classification of coronavirus disease (COVID-19). *Eur Radiol*. 2020;30(8):4407-4416.

234. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest*. 2020;130(5):2620-2629.
235. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. *Front Immunol.* 2018;9:754.
236. Singh T, Newman AB. Inflammatory markers in population studies of aging. *Ageing Res Rev.* 2011;10(3):319-329.
237. Jousilahti P, Salomaa V, Rasi V, Vahtera E, Palosuo T. The association of c-reactive protein, serum amyloid a and fibrinogen with prevalent coronary heart disease--baseline findings of the PAIS project. *Atherosclerosis.* 2001;156(2):451-456.
238. Liu W, Tao ZW, Wang L, et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. *Chin Med J (Engl)*. 2020;133(9):1032-1038.
239. Holshue ML, DeBolt C, Lindquist S, et al. First case of 2019 novel coronavirus in the United States. *N Engl J Med.* 2020;382(10):929-936.
240. Young BE, Ong SWX, Kalimuddin S, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore [published correction appears in JAMA. 2020 Apr 21;323(15):1510]. *JAMA*. 2020;323(15):1488-1494.
241. Mackay IM, Arden KE. MERS coronavirus: diagnostics, epidemiology and transmission. *Virol J.* 2015;12:222.
242. Pan Y, Guan H, Zhou S, et al. Initial CT findings and temporal changes in patients with the novel coronavirus pneumonia (2019-nCoV): A study of 63 patients in Wuhan, China. *Eur Radiol*. 2020;30(6):3306-3309.
243. Song F, Shi N, Shan F, et al. Emerging 2019 novel coronavirus (2019-nCoV) pneumonia [published correction appears in Radiology. 2020 Dec;297(3):E346]. *Radiology*. 2020;295(1):210-217.
244. Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: A prospective study. *Lancet.* 2003;361(9371):1767-1772.
245. Al-Tawfiq JA, Hinedi K. The calm before the storm: clinical observations of Middle East respiratory syndrome (MERS) patients. *J Chemother.* 2018;30(3):179-182.
246. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis*. 2020;20(4):411-412.
247. 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):1177-1179.
248. Chen J, Qi T, Liu L, et al. Clinical progression of patients with COVID-19 in Shanghai, China. *J Infect*. 2020;80(5):e1-e6.
249. Xiong Y, Sun D, Liu Y, et al. Clinical and high-resolution CT features of the COVID-19 infection: comparison of the initial and follow-up changes. *Invest Radiol*. 2020;55(6):332-339.
250. Yan J, Feng X, Tian JH, et al. Predictors associated with clinical deterioration in SARS patients. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi.* 2003;17(3):222-224.
251. Li S, Wang R, Zhang Y, et al. Symptom combinations associated with outcome and therapeutic effects in a cohort of cases with SARS. *Am J Chin Med.* 2006;34(6):937-947.
252. Zhao Z, Chen A, Hou W, et al. Prediction model and risk scores of ICU admission and mortality in COVID-19. *PLoS One.* 2020;15(7):e0236618.
253. Ruch Y, Kaeuffer C, Ohana M, et al. CT lung lesions as predictors of early death or ICU admission in COVID-19 patients. *Clin Microbiol Infect*. 2020;26(10):1417.e5-1417.e8.
254. Figliozzi S, Masci PG, Ahmadi N, et al. Predictors of adverse prognosis in COVID-19: A systematic review and meta-analysis. *Eur J Clin Invest*. 2020;50(10):e13362.
255. Noor FM, Islam MM. Prevalence and associated risk factors of mortality among COVID-19 patients: A meta-analysis. *J Community Health*. 2020;45(6):1270-1282.

256. Luo H, Liu S, Wang Y, et al. Disease progression in patients with COVID-19: A retrospective cohort study in China. *Int J Tuberc Lung Dis*. 2020;24(10):1032-1037.
257. Guan WJ, Ni ZY, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382(18):1708-1720.
258. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med.* 2003;348(20):1986-1994.
259. Joynt GM, Yap HY. SARS in the intensive care unit. *Curr Infect Dis Rep*. 2004;6(3):228-233.
260. Tsui PT, Kwok ML, Yuen H, Lai ST. Severe acute respiratory syndrome: Clinical outcome and prognostic correlates. *Emerg Infect Dis*. 2003;9(9):1064-1069.
261. Luo H, Liu S, Wang Y, et al. Age differences in clinical features and outcomes in patients with COVID-19, Jiangsu, China: A retrospective, multicentre cohort study. *BMJ Open*. 2020;10(10):e039887.
262. Murphy DL, Johnson NJ, Hall MK, Kim ML, Shapiro NI, Henning DJ. Predicting prolonged intensive care unit stay among patients with sepsis-induced hypotension. *Am J Crit Care.* 2019;28(6):e1-e7.
263. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. *J Autoimmun*. 2020;109:102433.
264. Choi KW, Chau TN, Tsang O, et al. Outcomes and prognostic factors in 267 patients with severe acute respiratory syndrome in Hong Kong. *Ann Intern Med.* 2003;139(9):715-723.
265. Lupovitch A. White cell differential count and influenza A. *Am J Med.* 2005;118(11):1306-1307; author reply 1307-1309.
266. Chu H, Zhou J, Wong BH, et al. Middle east respiratory syndrome coronavirus efficiently infects human primary t lymphocytes and activates the extrinsic and intrinsic apoptosis pathways. *J Infect Dis*. 2016;213(6):904-914.
267. Davarpanah AH, Asgari R, Moharamzad Y, et al. Risk factors for poor outcome in patients with severe viral pneumonia on chest ct during the COVID-19 outbreak: A perspective from Iran. *SN Compr Clin Med*. 2020;2(9):1366-1376.
268. Yarza R, Bover M, Paredes D, et al. SARS-CoV-2 infection in cancer patients undergoing active treatment: Analysis of clinical features and predictive factors for severe respiratory failure and death. *Eur J Cancer.* 2020;135:242-250.
269. Patel M, Gangemi A, Marron R, et al. Retrospective analysis of high flow nasal therapy in COVID-19-related moderate-to-severe hypoxaemic respiratory failure. *BMJ Open Respir Res*. 2020;7(1):e000650.
270. Sivaloganathan AA, Nasim-Mohi M, Brown MM, et al. Noninvasive ventilation for COVID-19-associated acute hypoxaemic respiratory failure: experience from a single centre. *Br J Anaesth*. 2020;125(4):e368-e371.
271. Severe COVID-19 GWAS Group, Ellinghaus D, Degenhardt F, et al. Genomewide association study of severe COVID-19 with respiratory failure. *N Engl J Med*. 2020;383(16):1522-1534.
272. Wang Y, Liu Y, Liu L, Wang X, Luo N, Li L. Clinical Outcomes in 55 Patients with severe acute respiratory syndrome coronavirus 2 who were asymptomatic at hospital admission in Shenzhen, China. *J Infect Dis*. 2020;221(11):1770-1774.
273. Liu K, Chen Y, Lin R, Han K. Clinical features of COVID-19 in elderly patients: A comparison with young and middle-aged patients. *J Infect*. 2020;80(6):e14-e18.
274. Mo P, Xing Y, Xiao Y, et al. Clinical characteristics of refractory coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis*. 2021;73(11):e4208-e4213.
275. Shim E, Tariq A, Choi W, Lee Y, Chowell G. Transmission potential and severity of COVID-19 in South Korea. *Int J Infect Dis*. 2020;93:339-344.
276. Lai CC, Liu YH, Wang CY, et al. Asymptomatic carrier state, acute respiratory disease, and pneumonia due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): Facts and myths. *J Microbiol Immunol Infect*. 2020;53(3):404-412.

277. Guo YR, Cao QD, Hong ZS, 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.
278. Qiu H, Wu J, Hong L, Luo Y, Song Q, Chen D. Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: An observational cohort study. *Lancet Infect Dis*. 2020;20(6):689-696.
279. Lian J, Jin X, Hao S, et al. Analysis of epidemiological and clinical features in older patients with coronavirus disease 2019 (COVID-19) outside Wuhan. *Clin Infect Dis*. 2020;71(15):740-747.
280. Pop-Vicas A, Gravenstein S. Influenza in the elderly: A mini-review. *Gerontology.* 2011;57(5):397-404.
281. Wong PL, Sii HL, P'ng CK, et al. The effects of age on clinical characteristics, hospitalization and mortality of patients with influenza-related illness at a tertiary care centre in Malaysia. *Influenza Other Respir Viruses*. 2020;14(3):286-293.
282. Leung C. Clinical features of deaths in the novel coronavirus epidemic in China. *Rev Med Virol*. 2020;30(3):e2103.
283. Salehi S, Abedi A, Balakrishnan S, Gholamrezanezhad A. Coronavirus disease 2019 (COVID-19): A systematic review of imaging findings in 919 patients. *AJR Am J Roentgenol.* 2020:1-7.
284. Yager EJ, Ahmed M, Lanzer K, Randall TD, Woodland DL, Blackman MA. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J Exp Med.* 2008;205(3):711-723.
285. Vijay R, Hua X, Meyerholz DK, et al. Critical role of phospholipase A2 group IID in age-related susceptibility to severe acute respiratory syndrome-CoV infection. *J Exp Med.* 2015;212(11):1851-1868.
286. Vareille M, Kieninger E, Edwards MR, Regamey N. The airway epithelium: Soldier in the fight against respiratory viruses. *Clin Microbiol Rev.* 2011;24(1):210-229.
287. Huang SS, Banner D, Degousee N, et al. Differential pathological and immune responses in newly weaned ferrets are associated with a mild clinical outcome of pandemic 2009 H1N1 infection. *J Virol.* 2012;86(24):13187-13201.
288. Hwang JY, Randall TD, Silva-Sanchez A. Inducible bronchus-associated lymphoid tissue: Taming inflammation in the lung. *Front Immunol.* 2016;7:258.
289. Randall TD. Bronchus-associated lymphoid tissue (BALT) structure and function. *Adv Immunol.* 2010;107:187-241.
290. Paquette SG, Huang SSH, Banner D, et al. Impaired heterologous immunity in aged ferrets during sequential influenza A H1N1 infection. *Virology.* 2014;464-465:177-183.
291. Jardon M, Mohammad SF, Jude CM, Pahwa A. Imaging of emerging infectious diseases. *Curr Radiol Rep.* 2019;7(9):25.
292. Koo HJ, Lim S, Choe J, Choi SH, Sung H, Do KH. Radiographic and CT features of viral pneumonia. *Radiographics.* 2018;38(3):719-739.
293. Cao Y, Liu X, Xiong L, Cai K. Imaging and clinical features of patients with 2019 novel coronavirus SARS-CoV-2: A systematic review and meta-analysis. *J Med Virol*. 2020;92(9):1449-1459.
294. Caruso D, Zerunian M, Polici M, et al. Chest CT features of COVID-19 in Rome, Italy. *Radiology*. 2020;296(2):E79-E85.
295. Wu J, Wu X, Zeng W, et al. Chest CT findings in patients with coronavirus disease 2019 and its relationship with clinical features. *Invest Radiol.* 2020;55(5):257-261.
296. Razek A, Fouda N, Fahmy D, et al. Computed tomography of the chest in patients with COVID-19: what do radiologists want to know? *Pol J Radiol.* 2021;86:e122-e135.
297. Razek A, El Badrawy MK, Alnaghy E. Interstitial lung fibrosis imaging reporting and data system: What radiologist wants to know? *J Comput Assist Tomogr.* 2020;44(5):656-666.

298. Wang X, Hu X, Tan W, et al. Multicenter study of temporal changes and prognostic value of a ct visual severity score in hospitalized patients with coronavirus disease (COVID-19). *AJR Am J Roentgenol*. 2021;217(1):83-92.
299. Hu Y, Zhan C, Chen C, Ai T, Xia L. Chest CT findings related to mortality of patients with COVID-19: A retrospective case-series study. *PLoS One.* 2020;15(8):e0237302.
300. Liu N, He G, Yang X, et al. Dynamic changes of Chest CT follow-up in coronavirus disease-19 (COVID-19) pneumonia: Relationship to clinical typing. *BMC Med Imaging.* 2020;20(1):92.
301. Tabatabaei SMH, Rahimi H, Moghaddas F, Rajebi H. Predictive value of CT in the short-term mortality of coronavirus disease 2019 (COVID-19) pneumonia in nonelderly patients: A case-control study. *Eur J Radiol.* 2020;132:109298.
302. Zhan J, Li H, Yu H, et al. 2019 novel coronavirus (COVID-19) pneumonia: CT manifestations and pattern of evolution in 110 patients in Jiangxi, China. *Eur Radiol.* 2021;31(2):1059-1068.
303. Francone M, Iafrate F, Masci GM, et al. Chest CT score in COVID-19 patients: Correlation with disease severity and short-term prognosis. *Eur Radiol.* 2020;30(12):6808-6817.
304. Pan F, Ye T, Sun P, et al. Time course of lung changes at chest CT during recovery from coronavirus disease 2019 (COVID-19). *Radiology.* 2020;295(3):715-721.
305. Khosravi B, Aghaghazvini L, Sorouri M, et al. Predictive value of initial CT scan for various adverse outcomes in patients with COVID-19 pneumonia. *Heart Lung.* 2021;50(1):13-20.
306. Li S, Liu S, Wang B, et al. Predictive value of chest CT scoring in COVID-19 patients in Wuhan, China: A retrospective cohort study. *Respir Med.* 2021;176:106271.
307. Yang R, Li X, Liu H, et al. Chest CT severity score: An imaging tool for assessing severe COVID-19. *Radiol Cardiothorac Imaging.* 2020;2(2):e200047.
308. Aalinezhad M, Alikhani F, Akbari P, Rezaei MH, Soleimani S, Hakamifard A. Relationship between CT severity score and capillary blood oxygen saturation in patients with COVID-19 infection. *Indian J Crit Care Med.* 2021;25(3):279-283.
309. Guillo E, Bedmar Gomez I, Dangeard S, et al. COVID-19 pneumonia: Diagnostic and prognostic role of CT based on a retrospective analysis of 214 consecutive patients from Paris, France. *Eur J Radiol.* 2020;131:109209.
310. Paul NS, Chung T, Konen E, et al. Prognostic significance of the radiographic pattern of disease in patients with severe acute respiratory syndrome. *AJR Am J Roentgenol.* 2004;182(2):493-498.
311. Chen Z, Fan H, Cai J, et al. High-resolution computed tomography manifestations of COVID-19 infections in patients of different ages. *Eur J Radiol.* 2020;126:108972.
312. Wang K, Kang S, Tian R, Zhang X, Zhang X, Wang Y. Imaging manifestations and diagnostic value of chest CT of coronavirus disease 2019 (COVID-19) in the Xiaogan area. *Clin Radiol*. 2020;75(5):341-347.
313. Tan C, Huang Y, Shi F, et al. C-reactive protein correlates with computed tomographic findings and predicts severe COVID-19 early. *J Med Virol.* 2020;92(7):856-862.
314. Yang Z, Shi J, He Z, et al. Predictors for imaging progression on chest CT from coronavirus disease 2019 (COVID-19) patients. *Aging (Albany NY)*. 2020;12(7):6037-6048.
315. Azab SM, Zytoon AA, Kasemy ZAA, et al. Learning from pathophysiological aspects of COVID-19 clinical, laboratory, and high-resolution CT features: A retrospective analysis of 128 cases by disease severity. *Emerg Radiol.* 2021:1-15.
316. Kodama T, Obinata H, Mori H, et al. Prediction of an increase in oxygen requirement of SARS-CoV-2 pneumonia using three different scoring systems. *J Infect Chemother.* 2021;27(2):336-341.
317. Liu S, Nie C, Xu Q, et al. Prognostic value of initial chest CT findings for clinical outcomes in patients with COVID-19. *Int J Med Sci.* 2021;18(1):270-275.
318. Xu YH, Dong JH, An WM, et al. Clinical and computed tomographic imaging features of novel coronavirus pneumonia caused by SARS-CoV-2. *J Infect.* 2020;80(4):394-400.

319. Liu KC, Xu P, Lv WF, et al. CT manifestations of coronavirus disease-2019: A retrospective analysis of 73 cases by disease severity. *Eur J Radiol.* 2020;126:108941.
320. Wang Y, Dong C, Hu Y, et al. Temporal Changes of CT findings in 90 patients with COVID-19 pneumonia: A longitudinal study. *Radiology*. 2020;296(2):E55-E64.
321. Antonio GE, Ooi CG, Wong KT, et al. Radiographic-clinical correlation in severe acute respiratory syndrome: Study of 1373 patients in Hong Kong. *Radiology.* 2005;237(3):1081-1090.
322. Chau TN, Lee PO, Choi KW, et al. Value of initial chest radiographs for predicting clinical outcomes in patients with severe acute respiratory syndrome. *Am J Med.* 2004;117(4):249-254.
323. Ooi CG, Khong PL, Ho JC, et al. Severe acute respiratory syndrome: Radiographic evaluation and clinical outcome measures. *Radiology.* 2003;229(2):500-506.
324. Chang YC, Yu CJ, Chang SC, et al. Pulmonary sequelae in convalescent patients after severe acute respiratory syndrome: Evaluation with thin-section CT. *Radiology.* 2005;236(3):1067-1075.
325. Hsu HH, Tzao C, Wu CP, et al. Correlation of high-resolution CT, symptoms, and pulmonary function in patients during recovery from severe acute respiratory syndrome. *Chest.* 2004;126(1):149-158.
326. Cai Y, Liu J, Yang H, et al. Correlation between early features and prognosis of symptomatic COVID-19 discharged patients in Hunan, China. *Sci Rep.* 2021;11(1):4304.
327. Kaya F, Konya PŞ, Demirel E, Demirtürk N, Orhan S, Ufuk F. Visual and quantitative assessment of COVID-19 pneumonia on chest CT: The relationship with disease severity and clinical findings. *Curr Med Imaging*. 2021;17(9):1142-1150.
328. Çakmak V, Yılmaz A, Sarı T, et al. Evaluation of the chest computed tomography and hemogram data in patients with COVID-19: The importance of thymus. *Turk J Med Sci*. 2021;51(3):991-1000.
329. Kaminetzky M, Moore W, Fansiwala K, et al. Pulmonary embolism at CT pulmonary angiography in patients with COVID-19. *Radiol Cardiothorac Imaging.* 2020;2(4):e200308.
330. Huang G, Kovalic AJ, Graber CJ. Prognostic value of leukocytosis and lymphopenia for coronavirus disease severity. *Emerg Infect Dis.* 2020;26(8):1839-1841.
331. Liu S, Luo H, Wang Y, et al. Clinical characteristics and risk factors of patients with severe COVID-19 in Jiangsu province, China: A retrospective multicentre cohort study. *BMC Infect Dis.* 2020;20(1):584.
332. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *J Chronic Dis.* 1987;40(5):373-383.
333. Hariyanto TI, Japar KV, Kwenandar F, et al. Inflammatory and hematologic markers as predictors of severe outcomes in COVID-19 infection: A systematic review and meta-analysis. *Am J Emerg Med.* 2021;41:110-119.
334. Verdecchia P, Cavallini C, Spanevello A, Angeli F. The pivotal link between ACE2 deficiency and SARS-CoV-2 infection. *Eur J Intern Med.* 2020;76:14-20.
335. Hariyanto TI, Putri C, Situmeang RFV, Kurniawan A. Dementia is a predictor for mortality outcome from coronavirus disease 2019 (COVID-19) infection. *Eur Arch Psychiatry Clin Neurosci.* 2021;271(2):393-395.
336. Putri C, Hariyanto TI, Hananto JE, Christian K, Situmeang RFV, Kurniawan A. Parkinson's disease may worsen outcomes from coronavirus disease 2019 (COVID-19) pneumonia in hospitalized patients: A systematic review, meta-analysis, and meta-regression. *Parkinsonism Relat Disord.* 2021;87:155-161.
337. Linton NM, Kobayashi T, Yang Y, et al. Incubation period and other epidemiological characteristics of 2019 novel coronavirus infections with right truncation: A statistical analysis of publicly available case data. *J Clin Med*. 2020;9(2):538.

338. Dong Y, Zhou H, Li M, et al. A novel simple scoring model for predicting severity of patients with SARS-CoV-2 infection. *Transbound Emerg Dis*. 2020;67(6):2823-2829.
339. Tan L, Wang Q, Zhang D, et al. Lymphopenia predicts disease severity of COVID-19: A descriptive and predictive study. *Signal Transduct Target Ther.* 2020;5(1):33.
340. Dai YJ, Zhang WN, Wang WD, He SY, Liang CC, Wang DW. Comprehensive analysis of two potential novel SARS-CoV-2 entries, TMPRSS2 and IFITM3, in healthy individuals and cancer patients. *Int J Biol Sci.* 2020;16(15):3028-3036.
341. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell.* 2020;181(2):271-280.e278.
342. Liu Y, Yang Y, Zhang C, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci.* 2020;63(3):364-374.
343. Yang XH, Deng W, Tong Z, et al. Mice transgenic for human angiotensin-converting enzyme 2 provide a model for SARS coronavirus infection. *Comp Med.* 2007;57(5):450-459.
344. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med.* 2005;11(8):875-879.
345. Offringa A, Montijn R, Singh S, Paul M, Pinto YM, Pinto-Sietsma SJ. The mechanistic overview of SARS-CoV-2 using angiotensin-converting enzyme 2 to enter the cell for replication: Possible treatment options related to the renin-angiotensin system. *Eur Heart J Cardiovasc Pharmacother.* 2020;6(5):317-325.
346. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Med.* 2020;46(4):586-590.
347. Pang X, Cui Y, Zhu Y. Recombinant human ACE2: Potential therapeutics of SARS-CoV-2 infection and its complication. *Acta Pharmacol Sin.* 2020;41(9):1255-1257.
348. Lee YL, Liao CH, Liu PY, et al. Dynamics of anti-SARS-Cov-2 IgM and IgG antibodies among COVID-19 patients. *J Infect.* 2020;81(2):e55-e58.
349. Bryan A, Pepper G, Wener MH, et al. Performance characteristics of the abbott architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. *J Clin Microbiol*. 2020;58(8):e00941-20.
350. Evaluation of the Abbott SARS-CoV-2 IgG for the detection of anti-SARS-CoV-2 antibodies. Public Health England. *Accessed 01* June 2021. https://www.gov.uk/government/publications/covid-19-laboratory-evaluations-of-serologicalassays.
351. Cameron A, Porterfield CA, Byron LD, et al. A multiplex microsphere IgG assay for SARS-CoV-2 using ACE2-mediated inhibition as a surrogate for neutralization. *J Clin Microbiol.* 2021;59(2).
352. Bavaro DF, Laghetti P, Milano E, et al. Anti-spike S1 receptor-binding domain antibodies against SARS-CoV-2 persist several months after infection regardless of disease severity. *J Med Virol*. 2021;93(5):3158-3164.
353. Suthar MS, Zimmerman MG, Kauffman RC, et al. Rapid generation of neutralizing antibody responses in COVID-19 patients. *Cell Rep Med.* 2020;1(3):100040.
354. Marklund E, Leach S, Axelsson H, et al. Serum-IgG responses to SARS-CoV-2 after mild and severe COVID-19 infection and analysis of IgG non-responders. *PLoS One.* 2020;15(10):e0241104.
355. Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of antibodies to SARS-associated coronavirus after recovery. *N Engl J Med.* 2007;357(11):1162-1163.
356. Wu LP, Wang NC, Chang YH, et al. Duration of antibody responses after severe acute respiratory syndrome. *Emerg Infect Dis.* 2007;13(10):1562-1564.
357. Tuaillon E, Bolloré K, Pisoni A, et al. Detection of SARS-CoV-2 antibodies using commercial assays and seroconversion patterns in hospitalized patients. *J Infect.* 2020;81(2):e39-e45.

358. Deeks JJ, Dinnes J, Takwoingi Y, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Database Syst Rev.* 2020;6(6):Cd013652.
359. Hoffmann M, Hofmann-Winkler H, Krüger N, et al. SARS-CoV-2 variant B.1.617 is resistant to bamlanivimab and evades antibodies induced by infection and vaccination. *Cell Rep.* 2021;36(3):109415.
360. Korte W, Buljan M, Rösslein M, et al. SARS-CoV-2 IgG and IgA antibody response is gender dependent; and IgG antibodies rapidly decline early on. *J Infect.* 2021;82(1):e11-e14.
361. Long QX, Liu BZ, Deng HJ, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med.* 2020;26(6):845-848.
362. Zhang X, Lu S, Li H, et al. Viral and antibody kinetics of COVID-19 patients with different disease severities in acute and convalescent phases: A 6-month follow-up study. *Virol Sin.* 2020;35(6):820-829.
363. Taziki Balajelini MH, Vakili MA, Saeidi M, Tabarraei A, Hosseini SM. Using anti-SARS-CoV-2 IgG and IgM antibodies to detect outpatient cases with olfactory and taste disorders suspected as mild form of COVID-19: A retrospective survey. *SN Compr Clin Med.* 2020:1-7.
364. de Melo GD, Lazarini F, Levallois S, et al. COVID-19-related anosmia is associated with viral persistence and inflammation in human olfactory epithelium and brain infection in hamsters. *Sci Transl Med.* 2021;13(596).
365. den Hartog G, Schepp RM, Kuijer M, et al. SARS-CoV-2-specific antibody detection for seroepidemiology: A multiplex analysis approach accounting for accurate seroprevalence. *J Infect Dis.* 2020;222(9):1452-1461.
366. Okba NMA, Müller MA, Li W, et al. Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. *Emerg Infect Dis.* 2020;26(7):1478-1488.
367. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med.* 2020;26(8):1200-1204.
368. Hu WT, Howell JC, Ozturk T, et al. Antibody profiles according to mild or severe SARS-CoV-2 infection, Atlanta, Georgia, USA, 2020. *Emerg Infect Dis.* 2020;26(12):2974-2978.
369. Kwon JS, Kim JY, Kim MC, et al. Factors of severity in patients with COVID-19: cytokine/chemokine concentrations, viral load, and antibody responses. *Am J Trop Med Hyg.* 2020;103(6):2412-2418.
370. Qin X, Shen J, Dai E, et al. The seroprevalence and kinetics of IgM and IgG in the progression of COVID-19. *BMC Immunol.* 2021;22(1):14.
371. Zhang B, Yue D, Wang Y, Wang F, Wu S, Hou H. The dynamics of immune response in COVID-19 patients with different illness severity. *J Med Virol.* 2021;93(2):1070-1077.
372. Graham NR, Whitaker AN, Strother CA, et al. Kinetics and isotype assessment of antibodies targeting the spike protein receptor-binding domain of severe acute respiratory syndrome-coronavirus-2 in COVID-19 patients as a function of age, biological sex and disease severity. *Clin Transl Immunology.* 2020;9(10):e1189.
373. Zeng F, Dai C, Cai P, et al. A comparison study of SARS-CoV-2 IgG antibody between male and female COVID-19 patients: A possible reason underlying different outcome between sex. *J Med Virol.* 2020;92(10):2050-2054.
374. Ojeda DS, Gonzalez Lopez Ledesma MM, Pallarés HM, et al. Emergency response for evaluating SARS-CoV-2 immune status, seroprevalence and convalescent plasma in Argentina. *PLoS Pathog.* 2021;17(1):e1009161.
375. Wu F, Liu M, Wang A, et al. Evaluating the association of clinical characteristics with neutralizing antibody levels in patients who have recovered from mild covid-19 in Shanghai, China. *JAMA Intern Med.* 2020;180(10):1356-1362.
376. Wang X, Guo X, Xin Q, et al. Neutralizing antibody responses to severe acute respiratory syndrome coronavirus 2 in coronavirus disease 2019 inpatients and convalescent patients. *Clin Infect Dis.* 2020;71(10):2688-2694.

377. Sun B, Feng Y, Mo X, et al. Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. *Emerg Microbes Infect.* 2020;9(1):940-948.
378. Liang W, Guan W, Chen R, et al. Cancer patients in SARS-CoV-2 infection: A nationwide analysis in China. *Lancet Oncol.* 2020;21(3):335-337.
379. Shi Y, Yu X, Zhao H, Wang H, Zhao R, Sheng J. Host susceptibility to severe COVID-19 and establishment of a host risk score: Findings of 487 cases outside Wuhan. *Crit Care.* 2020;24(1):108.
380. Cai Q, Huang D, Ou P, et al. COVID-19 in a designated infectious diseases hospital outside Hubei Province, China. *Allergy.* 2020;75(7):1742-1752.
381. Chen J, Qi T, Liu L, et al. Clinical progression of patients with COVID-19 in Shanghai, China. *J Infect.* 2020;80(5):e1-e6.
382. Flook M, Jackson C, Vasileiou E, et al. Informing the public health response to COVID-19: A systematic review of risk factors for disease, severity, and mortality. *BMC Infect Dis.* 2021;21(1):342.
383. Chen F, Zhang Y, Li X, Li W, Liu X, Xue X. The impact of ACE2 polymorphisms on COVID-19 disease: Susceptibility, severity, and therapy. *Front Cell Infect Microbiol.* 2021;11:753721.
384. Zhang R, Ouyang H, Fu L, et al. CT features of SARS-CoV-2 pneumonia according to clinical presentation: A retrospective analysis of 120 consecutive patients from Wuhan city. *Eur Radiol.* 2020;30(8):4417-4426.
385. Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis*. 2020;71(15):769-777.
386. Pan W, Zhang J, Wang M, et al. Clinical features of COVID-19 in patients with essential hypertension and the impacts of renin-angiotensin-aldosterone system inhibitors on the prognosis of COVID-19 patients. *Hypertension.* 2020;76(3):732-741.
387. Wan S, Xiang Y, Fang W, et al. Clinical features and treatment of COVID-19 patients in northeast Chongqing. *J Med Virol.* 2020;92(7):797-806.
388. Zhu L, She ZG, Cheng X, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. *Cell Metab.* 2020;31(6):1068-1077.e1063.
389. Mehta HB, Li S, Goodwin JS. Risk factors associated With SARS-CoV-2 Infections, hospitalization, and mortality among US nursing home residents. *JAMA Netw Open.* 2021;4(3):e216315.
390. Zhang JJ, Dong X, Cao YY, et al. Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*. 2020;75(7):1730-1741.
391. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in the New York City area. *JAMA.* 2020;323(20):2052-2059.
392. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. *BMJ.* 2020;368:m1295.
393. Gengler I, Wang JC, Speth MM, Sedaghat AR. Sinonasal pathophysiology of SARS-CoV-2 and COVID-19: A systematic review of the current evidence. *Laryngoscope Investig Otolaryngol.* 2020;5(3):354-359.
394. Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-mediated inflammatory responses: From mechanisms to potential therapeutic tools. *Virol Sin.* 2020;35(3):266-271.
395. Chu H, Chan JF, Wang Y, et al. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: An ex vivo study with implications for the pathogenesis of COVID-19. *Clin Infect Dis.* 2020;71(6):1400-1409.
396. Baker SA, Kwok S, Berry GJ, Montine TJ. Angiotensin-converting enzyme 2 (ACE2) expression increases with age in patients requiring mechanical ventilation. *PLoS One.* 2021;16(2):e0247060.
397. Heaney RP. Barriers to optimizing vitamin D3 intake for the elderly. *J Nutr.* 2006;136(4):1123-1125.

398. Zaman MS, Sizemore RC. Diverse manifestations of COVID-19: Some suggested mechanisms. *Int J Environ Res Public Health.* 2021;18(18).
399. Gold JE, Baumgartl WH, Okyay RA, et al. Analysis of Measles-Mumps-Rubella (MMR) titers of recovered COVID-19 patients. *mBio.* 2020;11(6).
400. Ng KW, Faulkner N, Cornish GH, et al. Preexisting and de novo humoral immunity to SARS-CoV-2 in humans. *Science.* 2020;370(6522):1339-1343.
401. CDC (Center for Disease Control). Pneumococcal vaccination: What everyone should know. *Accessed 06 February 2022.* https://wwwcdcgov/vaccines/vpd/pneumo/public/indexhtml.
402. Meffre E, Iwasaki A. Interferon deficiency can lead to severe COVID. *Nature.* 2020;587(7834):374-376.
403. Zhou Z, Guo D, Li C, et al. Coronavirus disease 2019: Initial chest CT findings. *Eur Radiol*. 2020;30(8):4398-4406.
404. Song F, Shi N, Shan F, et al. Emerging 2019 novel coronavirus (2019-nCoV) pneumonia. *Radiology.* 2020;295(1):210-217.
405. Lieveld AWE, Azijli K, Teunissen BP, et al. Chest CT in COVID-19 at the ED: Validation of the COVID-19 Reporting and Data System (CO-RADS) and CT severity score: A prospective, multicenter, observational study. *Chest.* 2021;159(3):1126-1135.
406. Bai HX, Hsieh B, Xiong Z, et al. Performance of radiologists in differentiating COVID-19 from non-COVID-19 viral pneumonia at chest CT. *Radiology.* 2020;296(2):E46-e54.
407. Verma HK. Radiological and clinical spectrum of COVID-19: A major concern for public health. *World J Radiol.* 2021;13(3):53-63.
408. Jiang Y, Wei X, Guan J, et al. COVID-19 pneumonia: CD8(+) T and NK cells are decreased in number but compensatory increased in cytotoxic potential. *Clin Immunol.* 2020;218:108516.
409. Xu B, Fan CY, Wang AL, et al. Suppressed T cell-mediated immunity in patients with COVID-19: A clinical retrospective study in Wuhan, China. *J Infect.* 2020;81(1):e51-e60.
410. Tan M, Liu Y, Zhou R, et al. Immunopathological characteristics of coronavirus disease 2019 cases in Guangzhou, China. *Immunology.* 2020;160(3):261-268.
411. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Respir Med.* 2020;8(4):420-422.
412. Diao B, Wang C, Tan Y, et al. Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). *Front Immunol.* 2020;11:827.
413. Wang F, Nie J, Wang H, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. *J Infect Dis.* 2020;221(11):1762-1769.
414. Misra DP, Agarwal V, Gasparyan AY, Zimba O. Rheumatologists' perspective on coronavirus disease 19 (COVID-19) and potential therapeutic targets. *Clin Rheumatol.* 2020;39(7):2055-2062.
415. Zheng M, Gao Y, Wang G, et al. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. *Cell Mol Immunol.* 2020;17(5):533-535.
416. Delshad M, Tavakolinia N, Pourbagheri-Sigaroodi A, Safaroghli-Azar A, Bagheri N, Bashash D. The contributory role of lymphocyte subsets, pathophysiology of lymphopenia and its implication as prognostic and therapeutic opportunity in COVID-19. *Int Immunopharmacol.* 2021;95:107586.
417. Fathi N, Rezaei N. Lymphopenia in COVID-19: Therapeutic opportunities. *Cell Biol Int.* 2020;44(9):1792-1797.
418. Chiba Y, Mizoguchi I, Hasegawa H, et al. Regulation of myelopoiesis by proinflammatory cytokines in infectious diseases. *Cell Mol Life Sci.* 2018;75(8):1363-1376.
419. Jeffery LE, Burke F, Mura M, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol.* 2009;183(9):5458-5467.

420. Wandrer F, Falk CS, John K, et al. Interferon-mediated cytokine induction determines sustained virus control in chronic hepatitis C virus infection. *J Infect Dis.* 2016;213(5):746-754.
421. Dierckx T, Khouri R, Menezes SM, et al. IFN-β induces greater antiproliferative and proapoptotic effects and increased p53 signaling compared with IFN-α in PBMCs of Adult T-cell Leukemia/Lymphoma patients. *Blood Cancer J.* 2017;7(1):e519.
422. Hadjadj J, Yatim N, Barnabei L, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. *Science.* 2020;369(6504):718-724.
423. Hotchkiss RS, Colston E, Yende S, et al. Immune checkpoint inhibition in sepsis: A phase 1b randomized study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of nivolumab. *Intensive Care Med.* 2019;45(10):1360-1371.
424. Chiappelli F, Khakshooy A, Greenberg G. CoViD-19 Immunopathology and immunotherapy. *Bioinformation.* 2020;16(3):219-222.
425. Kuri-Cervantes L, Pampena MB, Meng W, et al. Comprehensive mapping of immune perturbations associated with severe COVID-19. *Sci Immunol.* 2020;5(49).
426. Alijotas-Reig J, Esteve-Valverde E, Belizna C, et al. Immunomodulatory therapy for the management of severe COVID-19; beyond the anti-viral therapy: A comprehensive review. *Autoimmun Rev.* 2020;19(7):102569.
427. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome [published correction appears in Lancet Respir Med. 2020 Feb 25]. *Lancet Respir Med*. 2020;8(4):420-422.
428. Suganya S, Divya S, Parani M. Severe acute respiratory syndrome-coronavirus-2: Current advances in therapeutic targets and drug development. *Rev Med Virol.* 2021;31(3):e2174.
429. Li G, De Clercq E. Therapeutic options for the 2019 novel coronavirus (2019-nCoV). *Nat Rev Drug Discov.* 2020;19(3):149-150.
430. Chen L, Xiong J, Bao L, Shi Y. Convalescent plasma as a potential therapy for COVID-19. *Lancet Infect Dis.* 2020;20(4):398-400.
431. Nabih HK. Importance of immunoglobulin therapy for COVID-19 patients with lymphocytopenia. *Bull Natl Res Cent.* 2021;45(1):46.
432. Xiao AT, Gao C, Zhang S. Profile of specific antibodies to SARS-CoV-2: The first report. *J Infect.* 2020;81(1):147-178.
433. Fathi M, Vakili K, Sayehmiri F, et al. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 virus: A systematic review and meta-analysis study. *Iran J Immunol.* 2021;18(1):34-46.
434. Arkhipova-Jenkins I, Helfand M, Armstrong C, et al. Antibody response after SARS-CoV-2 infection and implications for immunity: A rapid living review. *Ann Intern Med.* 2021;174(6):811-821.
435. Hou H, Wang T, Zhang B, et al. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019. *Clin Transl Immunology.* 2020;9(5):e01136.
436. Du Z, Zhu F, Guo F, Yang B, Wang T. Detection of antibodies against SARS-CoV-2 in patients with COVID-19. *J Med Virol.* 2020;92(10):1735-1738.
437. Qu J, Wu C, Li X, et al. Profile of immunoglobulin G and IgM antibodies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *Clin Infect Dis.* 2020;71(16):2255-2258.
438. Zhang Y, Xu J, Jia R, et al. Protective humoral immunity in SARS-CoV-2 infected pediatric patients. *Cell Mol Immunol.* 2020;17(7):768-770.
439. Jin Y, Wang M, Zuo Z, et al. Diagnostic value and dynamic variance of serum antibody in coronavirus disease 2019. *Int J Infect Dis.* 2020;94:49-52.
440. Ma H, Zeng W, He H, et al. Serum IgA, IgM, and IgG responses in COVID-19. *Cell Mol Immunol.* 2020;17(7):773-775.
441. Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: Current state of the science. *Immunity.* 2020;52(6):910-941.

442. Li K, Huang B, Wu M, et al. Dynamic changes in anti-SARS-CoV-2 antibodies during SARS-CoV-2 infection and recovery from COVID-19. *Nat Commun.* 2020;11(1):6044.
443. To KK, Hung IF, Ip JD, et al. Coronavirus disease 2019 (COVID-19) Re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing. *Clin Infect Dis.* 2021;73(9):e2946-e2951.
444. Altuntas F, Ata N, Yigenoglu TN, et al. Convalescent plasma therapy in patients with COVID-19. *Transfus Apher Sci.* 2021;60(1):102955.
445. Xi Y. Convalescent plasma therapy for COVID-19: A tried-and-true old strategy? *Signal Transduct Target Ther.* 2020;5(1):203.
446. Jawhara S. Could intravenous immunoglobulin collected from recovered coronavirus patients protect against COVID-19 and strengthen the immune system of new patients? *Int J Mol Sci.* 2020;21(7).
447. Makaronidis J, Mok J, Balogun N, et al. Seroprevalence of SARS-CoV-2 antibodies in people with an acute loss in their sense of smell and/or taste in a community-based population in London, UK: An observational cohort study. *PLoS Med.* 2020;17(10):e1003358.
448. Zazhytska M, Kodra A, Hoagland DA, et al. Non-cell autonomous disruption of nuclear architecture as a potential cause of COVID-19 induced anosmia. *Cell. In Press, Corrected Proof, Published online: February 1, 2022*.
449. Mahmoud MM, Abuohashish HM, Khairy DA, Bugshan AS, Khan AM, Moothedath MM. Pathogenesis of dysgeusia in COVID-19 patients: A scoping review. *Eur Rev Med Pharmacol Sci.* 2021;25(2):1114-1134.
450. Butowt R, von Bartheld CS. Anosmia in COVID-19: Underlying mechanisms and assessment of an olfactory route to brain infection. *Neuroscientist.* 2021;27(6):582-603.
451. Mastrangelo A, Bonato M, Cinque P. Smell and taste disorders in COVID-19: From pathogenesis to clinical features and outcomes. *Neurosci Lett.* 2021;748:135694.
452. Eshraghi AA, Mirsaeidi M, Davies C, Telischi FF, Chaudhari N, Mittal R. Potential mechanisms for COVID-19 induced anosmia and dysgeusia. *Front Physiol.* 2020;11:1039.
453. Aziz M, Perisetti A, Lee-Smith WM, Gajendran M, Bansal P, Goyal H. Taste changes (dysgeusia) in COVID-19: A systematic review and meta-analysis. *Gastroenterology.* 2020;159(3):1132-1133.
454. Keyhan SO, Fallahi HR, Cheshmi B. Dysosmia and dysgeusia due to the 2019 Novel Coronavirus; a hypothesis that needs further investigation. *Maxillofac Plast Reconstr Surg.* 2020;42(1):9.
455. Pourhoseingholi MA, Baghestani AR, Vahedi M. How to control confounding effects by statistical analysis. *Gastroenterology and hepatology from bed to bench.* 2012;5(2):79-83.
456. McNamee R. Regression modelling and other methods to control confounding. *Occupational and Environmental Medicine.* 2005;62(7):500.
457. Woo PC, Lau SK, Lam CS, et al. Discovery of seven novel Mammalian and avian coronaviruses in the genus deltacoronavirus supports bat coronaviruses as the gene source of alphacoronavirus and betacoronavirus and avian coronaviruses as the gene source of gammacoronavirus and deltacoronavirus. *J Virol.* 2012;86(7):3995-4008.
458. World Health Organization. Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. *Accessed 03 February 2022.* http://wwwwhoint/csr/sars/country/table2004_04_21/en/indexhtml.
459. Al-Rabiaah A, Temsah MH, Al-Eyadhy AA, et al. Middle East Respiratory Syndrome-Corona Virus (MERS-CoV) associated stress among medical students at a university teaching hospital in Saudi Arabia. *J Infect Public Health.* 2020;13(5):687-691.
460. Lucia VC, Kelekar A, Afonso NM. COVID-19 vaccine hesitancy among medical students. *J Public Health (Oxf).* 2021;43(3):445-449.
461. Dror AA, Eisenbach N, Taiber S, et al. Vaccine hesitancy: The next challenge in the fight against COVID-19. *Eur J Epidemiol.* 2020;35(8):775-779.

Awards and grants

Feb 2019-Jan 2022	36-month scholarship from China Scholarship Council
Feb 2019-Jan 2021	2-year funding to cover PhD tuition from Richmond Pharmacology Ltd

Experience with design and analysis of clinical trials

- Involved as a statistician in 10 clinical trials including trials on glucose and moxifloxacin, copper balance, Malaria drug, psychological well-being, coronary intervention, and so on.
- Experienced in biometric support, including data management, statistical analysis and reporting activities for clinical studies.

1. **(2021, Liverpool School of Tropical Medicine) Trial name:** A randomised placebo-controlled double-blind phase II trial to determine the effects of metformin versus placebo on glycaemia in HIV-infected persons with pre-diabetes in Tanzania.

   Statistical analysis: Estimates of treatment differences in means of primary and secondary outcomes from linear mixed models (LMMs); Estimates of odds ratios of primary outcome and secondary outcomes from LMMs; Covariate adjusted analysis; Sensitivity analysis of the primary outcome with imputation using the multiple imputation strategy and the last observation carried forward method; Subgroup analysis; Safety analysis.

2. **(2021, West China Hospital of Sichuan University) Trial name:** A prospective randomized multi-centre clinical trial comparing different fibrinolysis-transfer percutaneous coronary intervention strategies in acute ST-segment elevation myocardial infarction.

   Development of statistical analysis plan (SAP). Statistics in the SAP include estimates of relative risk of primary binary outcome from a log binomial model (a generalized linear model [GLM]); Covariate adjusted analysis; Subgroup analysis; GLM analysis of secondary binary and continuous outcomes; Kaplan Meier curves, log rank test, and estimates of hazard ratios using Cox regression model for time-to-event outcome; Estimates of odds ratios and mean differences using generalized linear mixed models (GLMMs) to analyse the outcomes with repeated measurements.

3. **(2021, University of Liverpool) Trial name:** Evaluating a multi-component group intervention for improving psychological well-being of trainee civil servants in Pakistan: A randomised controlled study.

Statistical analysis: Estimates of treatment differences in means of primary and secondary outcomes from GLMMs; Estimates of odds ratios of secondary outcomes from GLMMs; Covariate adjusted analysis; Imputation analysis using the last observation carried forward method; Subgroup analysis.

4. **(2020, University of Liverpool) Trial name:** Scaling-up training of nurses in an evidence-based psychosocial intervention for perinatal depression in China: a randomized trial of electronic vs face-to-face training methods.

   Statistical analysis: Same as the second trial.

5. **(2020, Richmond Pharmacology Ltd, St George's University of London) Trial name:** A phase 1, open-label study to assess copper balance in healthy participants following administration of ALXN 1840.

   Statistical analysis: Stepwise testing of linear trend by mixed effect linear models to determine the attainment of and time to steady state of the trough concentration of plasma total copper and plasma ultrafiltrate copper.

6. **(2020, Richmond Pharmacology Ltd, St George's University of London) Trial name:** A phase 1, randomised, open-label, 3-way, 3-period, crossover relative bioavailability study to assess the single-dose pharmacokinetics of FOR-6219 in capsule and tablet formulations in postmenopausal women.

   Statistical analysis: Pharmacokinetics (PK) analyses including the bioavailability assessments and metabolic ratios of enzyme inhibitor FOR-6219; adverse events tables and listings by system organ class and preferred term, maximum severity, relationship, maximum Common Terminology Criteria of Adverse Events (CTCAE) Grade.

7. **(2019, Richmond Pharmacology Ltd, St George's University of London) Trial name:** A single centre, placebo controlled, phase I study to evaluate the effect of glucose and moxifloxacin on cardiac repolarisation in male and female patients with type I diabetes.

   Development of SAP and implementation of statistical analysis: Concentration-response models including mixed effects linear and nonlinear model, mixed effects Emax model and sigmoid Emax model, based on both single difference (baseline-corrected) and double difference (time-matched placebo-corrected change from

average baseline) to evaluate effects on electrocardiogram (ECG) intervals (QTc, J-Tpeak and Tpeak-Tend).

8. **(2019, Richmond Pharmacology Ltd, St George's University of London) Trial name:** A Phase I study to investigate the safety, tolerability and pharmacokinetic profile and food effect of P218 in healthy adult volunteers.

   Statistical analysis: Same as the sixth trial.

9. **(2019, Richmond Pharmacology Ltd, St George's University of London) Trial name:** A randomised, double blind, placebo controlled Phase I study to investigate the effects of systemically absorbed Cortexolone 17α-propionate (and its metabolites) on QT interval following repeat topical administration in healthy volunteers.

   Statistical analysis: Same as the sixth trial.

10. **(2019, Liverpool School of Tropical Medicine) Trial name:** Malaria chemoprevention with monthly treatment with dihydroartemisinin-piperaquine for the post-discharge management of severe anaemia in children aged less than 5 years in Uganda and Kenya: a 3-year, multi-centre, parallel-group, two-arm randomised placebo controlled superiority trial.

    Statistical analysis: Did validation analysis of conditional risk set modelling model, also known as Prentice Williams Peterson – Total Time (PWP-TT) model to analyse recurrent time-to-event data (i.e. the composite primary outcome of all-cause mortality or all-cause hospital readmissions determined by the time from randomization until week-26 inclusive).

Manuscripts under review or preparation

1. **Luo H,** et al. Racial and diurnal variability of biochemical parameters used for diagnosis or prediction of diabetes and cardiovascular disease: an analysis of pooled trial data from healthy volunteers. Under preparation.

2. **Luo H,** et al. Statistical analysis plan for the OPTIMAL-REPERFUSION trial: a prospective randomized multi-center clinical trial comparing different

fibrinolysis-transfer percutaneous coronary intervention strategies in acute ST-segment elevation myocardial infarction. Under preparation.

3. Wei S, **Luo H,** et al. Predicting the risk of drug non-adherence in epilepsy populations of Tropical China: development and evaluation of a new predictive nomogram. Under review by the *Journel of Advanced Nursing.* **(Co-first author)**
4. Nitta Y, **Luo H,** et al. Association between baseline anaemia severity and mortality among patients with HIV-associated cryptococcal meningitis in sub-Saharan Africa. Under preparation. **(Co-first author)**
5. Fan X, Zhao Y, **Luo H**, Zhou Z, James B, Wang D, Hellman B. Smartphone-based study on non-motor symptoms in patients with Parkinson's disease and their association with health-related quality of life. Under review by the journal of *PLOS ONE*.
6. Nisar A, Yin J, Nan Y, **Luo H**, Han D, Li J, Yang L, Wang D, Rahman A, Li X. Scaling-up training of nurses in an evidence-based psychosocial intervention for perinatal depression in China: a randomized trial of electronic vs face-to-face training methods. Under preparation.

Conferences

2019 The 5th International Clinical Trials Methodological Conference, Brighton, UK. Poster.