Risk Factors and Laboratory Test Results Associated with Severe Illness and Mortality in COVID-19 Patients: A systematic review

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ABSTRAK

Latar belakang: *tinjauan sistematik ini dilakukan untuk mereviu studi-studi relevan tentang faktor risiko dan hasil pemeriksaan laboratorium yang berhubungan dengan penyakit berat dan kematian pada pasien COVID-19.* ***Metode:*** *kami menggunakan studi systematic review / meta-analisis, studi kohort dan kasus-kontrol yang mencakup kasus supek dan/atau terkonfirmasi COVID-19 yang ditemukan dari penelusuran sistematis di PubMed, Scopus, ProQuest, Wiley Online Library, ScienceDirect dan MedRxiv, serta beberapa studi tambahan yang dicari secara manual. Kami memasukan faktor risiko serta hasil pemeriksaan laboratorium. Risiko bias dinilai menggunakan tool ROBIS-I dan Newcastle-Ottawa Scale. Tipe studi, risiko bias, dan presisi hasil menentukan sufisiensi bukti.* ***Hasil:*** *dari 26 studi, bukti sufisien menunjukkan hubungan antara usia >60 tahun, hipertensi, penyakit jantung koroner, diabetes melitus, level LDH serum 250-500 U/L, LDH > 500 U/L, dan limfopenia (jumlah limfosit darah absolut ≤1.0 109/L) dan penyakit COVID-19 berat. Jumlah sel CD3+CD8+ darah absolut ≤ 75 sel/µl, D-dimer >1 mg/L, AKI stadium 2 dan 3, proteinuria ≥1, hematuria ≥1+, dan level kreatinin serum puncak > 13,26 µmol/L berhubungan dengan kematian.* ***Kesimpulan:*** *usia >60 tahun, hipertensi dan penyakit jantung koroner adalah faktor risiko penyakit COVID-19 berat. Hasil pemeriksaan laboratorium yang berhubungan dengan penyakit berat adalah level LDH serum 250-500 U/L, LDH > 500 U/L dan limfopenia, sedangkan yang berhubungan dengan kematian adalah jumlah sel CD3+CD8+ darah absolut ≤ 75 sel/µl, D-dimer > 1 mg/L, AKI stadium 2 dan 3, proteinuria ≥1, hematuria ≥1+, dan level kreatinin serum puncak > 13,26 µmol/L.*

Kata kunci: *COVID-19, penyakit berat, kematian, faktor risiko, pemeriksaan laboratorium*

ABSTRACT

Background: *we aimed to systematically review all relevant studies related to the risk factors and laboratory test results associated with severe illness and mortality in COVID-19 patients.* ***Methods:*** *we utilised PubMed, Scopus, ProQuest, Wiley Online Library, ScienceDirect and MedRxiv to search for studies, with additional hand-searched journals. We included systematic reviews/meta-analyses, cohort and case control studies of suspected and/or confirmed COVID-19 cases with severe illness and/or mortality as outcomes. We included laboratory test*

results and risk factors. We assessed risk of bias using ROBIS-I and Newcastle-Ottawa Scale assessment tool. Type of study, risk of bias, and precision of results determined evidence sufficiency. ***Results:*** *of 26 records included, sufficient evidence suggested the association between age >60 years, hypertension, coronary heart disease, DM, serum LDH 250-500 U/L, LDH >500 U/L, and lymphopenia (lymphocyte count ≤1.0 x 109 /L) and severe illness of COVID-19. CD3+CD8+ cell count ≤ 75 cell/µl, D-dimer > 1 mg/L, AKI stage 2 and 3, proteinuria ≥1+, hematuria ≥1+, and peak serum creatinine > 13.26 µmol/L are associated with mortality.* ***Conclusion:*** *age >60 years, hypertension, DM, and coronary heart disease are the risk factors for severe illness of COVID-19. Laboratory test results associated with severe illness are serum LDH 250-500 U/L, LDH >500 U/L, and lymphopenia, whereas test results associated with mortality are CD3+CD8+ cell count ≤ 75 cell/µl, AKI stage 2 and 3, proteinuria ≥1+, hematuria ≥1+, D-dimer > 1 mg/L, peak serum creatinine > 13.26 µmol/L.*

Keywords: *COVID-19, severe illness, mortality, risk factor, laboratory test.*

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is a problem in more than 210 countries. As of 12 Aug 2020, the global case fatality rate as high as 3.6%.[1] The case fatality rate was much higher in early pandemic era in various developing and developed countries. In severe cases, patients with SARS-CoV-2 infection may require respiratory support or cardio-respiratory support in the form of mechanical ventilation or extra-corporeal membrane oxygenation (ECMO), respectively. Moreover, previous studies reported that respiratory failure was found in nearly half of the fatalities of COVID-19 patients.[2,3]

The great interest in COVID-19 has led researchers to conduct studies about the risk factors for adverse outcomes in COVID-19. Although numerous studies of varying study design and quality reported potential risk factors and laboratory test results associated with severe illness and mortality, the results remain inconclusive.[4-7] Several studies were not able to provide causal mechanisms to link risk factors and outcomes.[4,6] Furthermore, several systematic reviews/ meta-analyses (SR/MA) in this field provided substandard quality of analyses. High case fatality rate, inconclusive study results, and substandard quality of analysis led us us to conduct a systematic review in this field and to assess the quality of evidence in a careful manner subsequently.

In this systematic review, we examined primary observational studies as well as existing SR/MA. Due to the ever-growing body of evidence, we also took into account common laboratory results that indicated clinical conditions associated with unfavourable outcomes of COVID-19. We adopted systematic approach to confirm and summarise the evidence regarding risk factors and laboratory test results associated with severe illness and mortality in COVID-19 patients. Finally, we elucidated the proposed mechanisms of the risk factors affecting the course of the disease and their influence on disease outcomes.

METHODS

Search strategy and selection criteria

We registered our review protocol on PROSPERO with registration number CRD42020185424.[8] A literature search was conducted systematically using electronic databases, namely PubMed, Scopus, ProQuest, Wiley Online Library, ScienceDirect, and MedRxiv. Articles hand-searched from the authors' personal files were also included. Only full-text articles published in English between 1 January and 13 April 2020 were taken into account. The search strategies are shown in **Table 1**.

Selection of Studies

We selected studies based on predefined eligibility criteria. We included all systematic reviews, meta-analyses, and primary observational studies (cohort and case–control studies) of patients of any age with suspected and/or confirmed COVID-19. The selected risk factors included, but were not limited to: (1)

Table 1. Keywords for search strategies

Database	Search query	Hits
Pubmed	Search ((((((((((SARS-CoV-2[Title/Abstract)) OR SARS-CoV-2[MeSH Terms]) OR 2019-nCOV[Title/Abstract]) OR 2019-nCOV[MeSH Terms]) OR COVID-19[Title/Abstract]) OR COVID-19[MeSH Terms]) OR Wuhan coronavirus[Title/Abstract]) OR Wuhan coronavirus[MeSH Terms])))) AND ((((((((((((((((((((mortality[Title/Abstract]) OR mortality[MeSH Terms]) OR death[Title/Abstract]) OR death[MeSH Terms]) OR ventilators[Title/Abstract]) OR ventilators[MeSH Terms]) OR ARDS[Title/Abstract]) OR acute respiratory distress syndrome[Title/Abstract]) OR ARDS[MeSH Terms]) OR acute respiratory distress syndrome[MeSH Terms]) OR ECMO[Title/Abstract]) OR ECMO[MeSH Terms]) OR Extracorporeal Membrane Oxygenation[Title/Abstract]) OR Extracorporeal Membrane Oxygenation[MeSH Terms]) OR severe illness[Title/Abstract]) OR severe illness[MeSH Terms]))))))	373
Scopus	TITLE-ABS-KEY ((sars-cov-2 OR 2019-ncov OR wuhancoronavirus OR covid-19) AND (mortality OR death OR ventilators OR ards OR acuterespiratorydistresssyndrome OR ecmo OR extracorporealmembraneoxygenation OR severeillness))	208
ProQuest	ab(SARS-CoV-2 OR 2019-nCOV OR Wuhancoronavirus OR COVID-19) AND ab(mortality OR death OR ventilators OR ARDS OR acute respiratory distress syndrome OR ECMO OR Extracorporeal Membrane Oxygenation OR severe illness)	153
MedRxiv	"(SARS-CoV-2 OR 2019-nCOV OR Wuhancoronavirus OR COVID-19) AND (mortality OR death OR severe illness OR ARDS OR ECMO)"	810
Wiley Online Library	((sars-cov-2 OR 2019-ncov OR wuhancoronavirus OR covid-19) AND (mortality OR death OR ventilators OR ards OR acuterespiratorydistresssyndrome OR ecmo OR extracorporealmembraneoxygenation OR severeillness))	91
ScienceDirect	((SARS-CoV-2 OR 2019-nCOV OR Wuhancoronavirus OR COVID-19) AND (mortality OR death OR ventilators OR ARDS OR acute respiratory distress syndrome OR ECMO OR Extracorporeal Membrane Oxygenation OR severe illness))Filter:2020	158

clinical characteristics (e.g., age, sex, history of smoking, body mass index [BMI]); (2) clinical symptoms (e.g., dyspnoea, fever, cough); (3) duration of symptoms; (4) time from first medical visit to admission; (5) comorbidities (e.g., cardio-vascular disease and chronic obstructive pulmonary disease [COPD]), current or history of treatment (e.g., history of chest operation, ongoing chemotherapy); (6) healthcare resource constraint; (7) blood type; (8) coinfections (e.g., other viral or bacterial infections); and (9) low presenting oxygen saturation. We also considered laboratory test results such as serum lactate, platelet count, neutrophil-to-lymphocyte-ratio (NLR), acute cardiac injury markers, C-reactive protein (CRP), coagulation markers, and serum cytokines.

The main outcomes were severe illness and mortality of COVID-19. We defined COVID-19 severe illness as SARS-CoV-2 infection resulting in severe COVID-19 disease, acute respiratory distress syndrome (ARDS) based on the Berlin definition,[9] intensive care unit (ICU) admission, mechanical ventilation requirement, and/or ECMO requirement. Severe COVID-19 disease in the teenage, adult, and older adult population was defined as a suspected or confirmed case of COVID-19 with at least one of the following symptoms: respiratory rate ≥30 bpm; pulse oximeter <93% saturation in room air; and PaO_2/FiO_2 ratio ≤300 mm Hg. Severe COVID-19 disease in children was defined as cough or shortness of breath, with at least one of the following symptoms: central cyanosis or pulse oximeter <90% saturation in room air, severe respiratory distress, abnormal chest retractions; sign(s) of severe pneumonia, such as poor feeding, inability to tolerate oral intake, lethargy, change in mental status, and seizure.

First, we excluded duplications from the articles collected from the initial electronic databases. Second, we selected articles based on the predetermined eligibility criteria. Selection was done through initial title and abstract screening, followed by full-text screening. The selection process involved a minimum of two independent reviewers. Conflicting decisions were resolved by discussion between two

reviewers, or consultation with a third reviewer, if required.

Quality Assessment and Data Extraction

Risk of bias of each study was assessed for each outcome, namely severe illness and death. Risk of bias assessment was performed on all articles chosen through a careful selection process. Two independent reviewers conducted the assessment using certain assessment tools. Systematic review was assessed using ROBIS-I,[10] whereas the assessment of the observational studies relied on the Newcastle-Ottawa Scale (NOS) assessment tool.[11] There are four domains in ROBIS-I (i.e., study eligibility criteria, identification and selection of studies, data collection and study appraisal, and synthesis and findings) consisting of 21 items in total. Risk of bias was determined by the final three domains in ROBIS-I. The interpretation of ROBIS-I was categorised as high, low, or unclear risk of bias.

The NOS assessment has three domains consisting of selection, comparability, and exposure. There are four items, one item, and two items in the selection, comparability, and exposure domain, respectively. In the selection and exposure domains, there are several possible answers to each question and the highest answer is marked with a "star", presented as number in this systematic review. In the comparability domain, there are two possible stars given if there is adjustment of other controlled factors. In this review, we classified the results of NOS assessment as low risk and high risk of bias. Only studies with full stars in all domains were considered as having a low risk of bias.

The data extraction process involved at least two independent reviewers. Disagreement required discussion and subsequent involvement of the third reviewer as needed. In the case of incomplete data of the study, the systematic review team contacted the author. The measures of effect of interest were limited to relative risk (RR), odds ratio (OR), or hazard ratio (HR).

Data Synthesis

We planned to pool primary studies providing usable data in any single meta-analysis as clinically homogeneous with Review Manager 5 using a fixed-effect model. If a single true effect was not obtained due to the variety of population and exposures or substantial heterogeneity, we planned to use a random-effect or narrative review method. Subgroup analysis was planned to be performed on the data of special populations of COVID-19 patients, e.g., patients with underlying malignancy. Sufficiency of evidence was determined by the type of

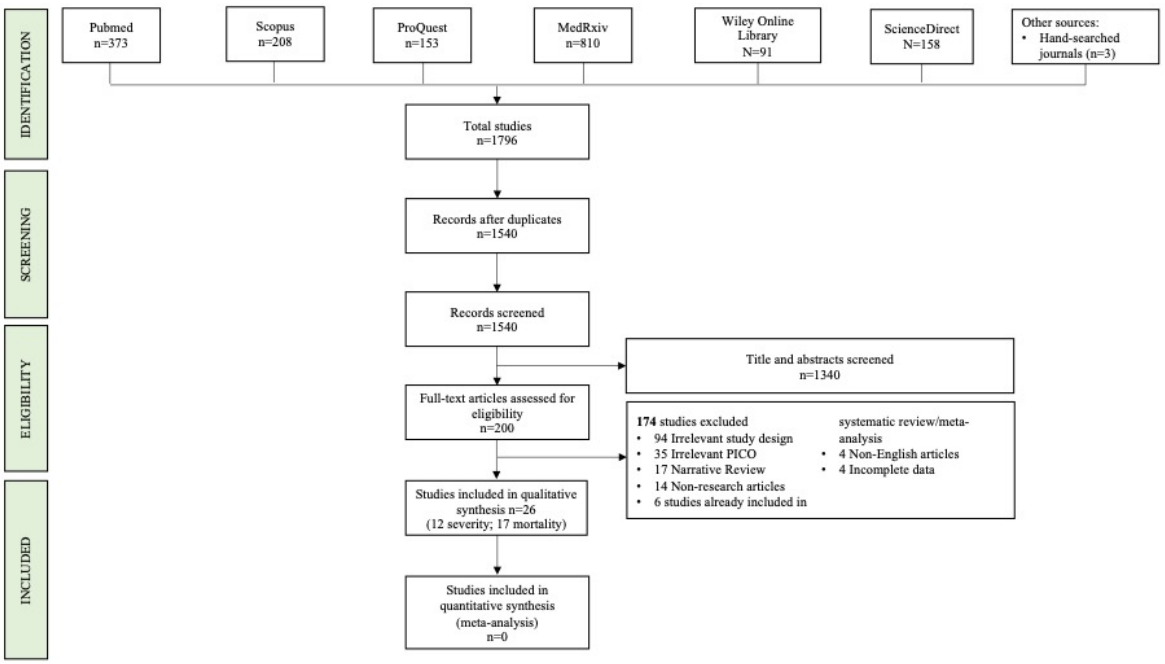

Figure 1. PRISMA diagram of this systematic review.

study, risk of bias, and precision of results. We considered the evidence was sufficient if supported by the best study design with low risk of bias and precise results.

RESULTS

We retrieved 1,796 records, consisting of 1,793 records from four electronic databases and three from other hand-searched journals (**Figure 1**).

We used a PRISMA flow diagram to illustrate our literature searching strategy.[12] After removing duplicates, each of 1,540 title and abstract records was assessed by at least two of nine reviewers (SS, SRFS, KH, EDS, RR, YP, WW, MKA, JM) independently. There were 200 records identified by the full text for further assessment of eligibility. We excluded 170 studies that did not meet our eligibility criteria as well as excluding primary studies identified in SR/MA. In addition, four other studies with incomplete outcome data were excluded. We have contacted the authors but received no response. Finally, there were 26 records included in this study.

Most of the studies were conducted in China, followed by France, Germany, Singapore, and the USA. There were ten journal pre-proofs (e.g. in medRxiv) as noted in **Table 2**. We did not find any articles that included a paediatric population.

Table 2. List of included studies and databases

Author (Publication Year)	Title	Journal
Zuin (2020)	Arterial hypertension and risk of death in patients with COVID-19 infection: systematic review and meta-analysis	Journal of Infection
Tang (2020)	Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease 2019 patients with coagulopathy	J Thromb Haemost
Zhou (2020)	A New Predictor of Disease Severity in Patients with COVID-19 in Wuhan, China	Pre-proof (Medrxiv)
Zhou (2020)	Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study	The Lancet
Fu (2020)	Influence factors of death risk among COVID-19 patients in Wuhan, China: a hospital-based case-cohort study	Pre-proof (Medrxiv)
Shi (2020)	Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China	JAMA
Lippi (2020)	Hypertension and its severity or mortality in Coronavirus Disease 2019 (COVID-19): a pooled analysis	Polish Arch Intern Med
Cheng (2020)	Kidney disease is associated with in-hospital death of patients with COVID-19	Kidney International
Simonnet (2020)	High prevalence of obesity in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) requiring invasive mechanical ventilation	Obesity (Silver Spring)
Ma (2020)	COVID-19 Myocarditis and Severity Factors: An Adult Cohort Study	Pre-proof (Medrxiv)
Zhang (2020)	Myocardial injury is associated with in-hospital mortality of confirmed or suspected COVID-19 in Wuhan, China: A single center retrospective cohort study	Pre-proof (Medrxiv)
Barrasa (2020)	SARS-Cov-2 in Spanish Intensive Care: Early Experience with 15-day Survival In Vitoria	Anaesth Crit Care Pain Med
Lippi (2020)	Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis	Clin Chim Acta
Chen (2020)	Effects of hypertension, diabetes and coronary heart disease on COVID-19 diseases severity: a systematic review and meta-analysis	Pre-proof (Medrxiv)
Matsushita (2020)	The relationship of COVID-19 severity with cardiovascular disease and its traditional risk factors: A systematic review and meta-analysis	Pre-proof (Medrxiv)
Roncon (2020)	Diabetic patients with COVID-19 infection are at higher risk of ICU admission and poor short-term outcome	Journal of Clinical Virology
Xie (2020)	Development and external validation of a prognostic multivariable model on admission for hospitalized patients with COVID-19	Pre-proof (Medrxiv)

Table 2. List of included studies and databases

Author (Publication Year)	Title	Journal
Jain (2020)	Systematic review and meta-analysis of predictive symptoms and comorbidities for severe COVID-19 infection	Pre-proof (Medrxiv)
Parohan (2020)	Risk factors for mortality of adult inpatients with Coronavirus disease 2019 (COVID-19): a systematic review and meta-analysis of retrospective studies	Pre-proof (Medrxiv)
Alqahtani (2020)	Prevalence, Severity and Mortality associated with COPD and Smoking in patients with COVID-19: A Rapid Systematic Review and Meta-Analysis	Plos One
Du (2020)	Predictors of Mortality for Patients with COVID-19 Pneumonia Caused by SARS-CoV-2: A Prospective Cohort Study	European Respiratory Journal
Ji (2020)	Prediction for Progression Risk in Patients with COVID-19 Pneumonia: the CALL Score	Clinical Infectious Diseases
Wang (2020)	Coronavirus Disease 2019 in elderly patients: characteristics and prognostic factors based on 4-week follow-up.	Journal of Infection
Zhang (2020)	Comorbid Diabetes Mellitus was Associated with Poorer Prognosis in Patients with COVID-19: A Retrospective Cohort Study	Pre-proof (Medrxiv)
Liu (2020)	Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19	Journal of Infection
Lippi (2020)	Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis	Clin Chim Acta

Table 3. Characteristics of included study of severe illness and mortality.

First author	Publication year	Country	Study design	Number of participants	Follow-up Period	Risk Factors
Severe Illness						
Alqahtani[14]	2020	China and USA	SR/MA	2473	N/A	COPD, Smokers
Chen[15]	2020	China	SR/MA	1936	N/A	Hypertension, Diabetes mellitus, Coronary heart disease
Lippi (a)[16]	2020	China	SR/MA	2552	N/A	Hypertension
Lippi (b)[17]	2020	China and Singapore	SR/MA	1289	N/A	Thrombocytopenia
Lippi (c)[13]	2020	China	SR/MA	N/R	N/A	PCT
Matsushita[4]	2020	China	SR/MA	51845	N/A	Male, Smokers, Hypertension, Diabetes mellitus
Roncon[18]	2020	China	SR/MA	1382	N/A	Diabetes Mellitus
Jain[5]	2020	China	SR/MA	1813	N/A	Male, Dyspnoea, Cough, Fever, Fatigue, Myalgia, Expectoration, Headache, COPD, Diabetes mellitus, CVD, Hypertension
Ma[19]	2020	China	Case-control	84	41 days	Age, Diabetes mellitus, Ct value of SARS-CoV-2, PIIa
Zhou (b)[29]	2020	China	Case-control	377	N/R	Agea, NLRa, CRP, D-dimera
Simonnet[21]	2020	France	Prospective Cohort	124	2-40 days	BMI (obese vs non obese), BMI (severe obese vs non severe obese), Age, Male, Diabetes mellitus, Hypertension, Dyslipidaemia
Ji[22]	2020	China	Retrospective Cohort	208	18-58 days	Age (>60 years old), Lymphocyte (≤1.0x10^9/L), D-dimer (>0.55 mg/L), LDH (250-500 U/L), LDH (>500 U/L)

Table 3. Characteristics of included study of severe illness and mortality.

First author	Publication year	Country	Study design	Number of participants	Follow-up Period	Risk Factors
Mortality						
Alqahtani[14]	2020	China and USA	SR/MA	2473	N/A	COPD
Parohan[23]	2020	China	SR/MA	22350	N/A	Age (≥ 65 years old), Male, Hypertension, Diabetes mellitus, COPD, CVD
Lippi (a)[16]	2020	China	SR/MA	2552	N/A	Hypertension
Roncon[18]	2020	China	SR/MA	1382	N/A	Diabetes mellitus
Zuin[24]	2020	China	SR/MA	302	N/A	Arterial hypertension
Tang[25]	2020	China	Case-control	449	29-72 days	Agea, Sex ratioa, PTa, D-dimer[a], Platelet Count[a], treating with heparin
Wang[6]	2020	China	Case-control	339	28 days	COPD, Cardiovascular disease, Cerebrovascular disease, Acute cardiac injury, Arrhythmia, AKI, ARDS, Cardiac insufficiency, Bacterial infection
Barrasa[26]	2020	Spain	Prospective Cohort	48	15 days	PCT >0.5 vs ≤0.5 µmol/L and PCT >1.0 vs ≤1.0 µmol/L
Cheng[27]	2020	China	Prospective Cohort	701	18-32 days	Proteinuria, Haematuria, Elevated BUN[a], Elevated Serum Cr[a], AKI Stage 1-3
Fu[28]	2020	China	Prospective Cohort	200	N/R	Age (> 70 years old), Male, Smokers, Hypertension, Diabetes mellitus, Cardiac disease, Chronic pulmonary disease, Oxygenation index on admission, Myoglobin, Alanine aminotransferase[a], Total bilirubin[a], Creatininea, Urea nitrogena, Uric acid[a], Creatine kinase[a], LDH[a], Aspartate aminotransferase[a], Aspartate/alanine ratio[a]
Shi[7]	2020	China	Prospective Cohort	416	5-26 days	Age[a], CAD, CVD, Diabetes mellitus, COPD, Chronic renal failure, Cancer, ARDS, Cardiac injury, Creatinine ≥ 13.26 µmol/L, nt-pro-BNP ≥ 106,42 pmol/L
Du[29]	2020	China	Retrospective Cohort	179	Minimum 46 days	Age (≥ 65 years old), CVD, CD3CD8+≤ 75 cell/µL, TnI ≥ 0.05 ng/mL
Liu[30]	2020	China	Retrospective Cohort	245	1-59 days	NLR Tertile 2 (2.21-4.82), NLR Tertile 3 (4.85-88.09)
Zhang (a)[31]	2020	China	Retrospective Cohort	258	29-43 days	Diabetes mellitus
Zhang (b)[32]	2020	China	Retrospective Cohort	48	N/R	Age[a], SpO2%[a], Serum Cr[a], D-dimer per 1 mg/L increase, hs-TnI ≥ 0.026 mcg/L
Zhou (a)[33]	2020	China	Retrospective Cohort	191	33 days	Age per 1 year increase, CAD, SOFA[a], Lymphocyte (per 1x109/L increase), D-dimer (> 0.5 or >1) vs ≤0.5 mg/L
Xie[34]	2020	China	Retrospective Cohort	299	60 days	Age[a], LDH[a], Log Lymphocyte count[a], SpO2%[a]

SR/MA: Systematic Review/Meta-analysis, PCT: procalcitonin, CVD: cardiovascular disease, LDH: lactate dehydrogenase, N/R: not reported, N/A: not applicable, PT: prothrombin time, AKI: acute kidney injury, ARDS: acute respiratory distress syndrome, BUN: blood urea nitrogen, Cr: creatinine, TnI: troponin I, hs-cTnI: high sensitivity troponin I, CAD: coronary artery disease, SOFA: Sequential Organ Failure Assessment.
[a] The article did not mention the cut-off point.

There were 12 and 17 included studies describing the factors related to severe illness and mortality of COVID-19 infection, respectively (**Table 3**). Among the studies reporting severe illness, there were eight SR/MA, two case–control, and two cohort studies. Most studies were conducted in China. The number of subjects included was between 84 and 51,845, with one study not mentioning the number of participants.[13]

There were five SR/MA, two case–control, four prospective cohort, and six retrospective cohort studies reporting risk factors related to the mortality of COVID-19 patients. The number of subjects ranged from 48 to 22,350.

There were four out of 12 cohort studies with low risk of bias[22,27,29,33] (**Table 4**). All of the case–control studies had high risk of bias (**Table 5**). Only one in ten SR/MA had low risk of bias (**Table 6**),[15] whereas the remainder had high risk of bias due to lack of information regarding study eligibility and identification and selection of patients.

Risk Factors for Severe Illness and Mortality of COVID-19

We identified several risk factors related to severe illness and mortality of COVID-19

Table 4. The assessment of risk of bias of the cohort study using Newcastle Ottawa scale[a]

Study	Selection				Comparability (C)	Outcome			Risk of Bias
	REC	SNEC	AE	DO		AO	FU	AFU	
Severe Illness									
Ji, 2020[22]	1	1	1	1	2	1	1	1	Low
Simonnet, 2020[21]	0	1	1	1	1	0	0	1	High
Mortality									
Du, 2020[29]	1	1	1	1	2	1	1	1	Low
Zhang, 2020b[32]	1	1	1	1	2	1	0	1	High
Liu, 2020[30]	1	1	1	1	2	1	0	1	High
Barrasa, 2020[26]	0	1	1	1	0	1	1	1	High
Xie, 2020[34]	1	1	1	1	1	1	1	0	High
Zhang, 2020a[31]	0	1	1	1	1	1	1	1	High
Fu, 2020[28]	1	1	1	1	1	1	0	1	High
Cheng, 2020[27]	1	1	1	1	2	1	1	1	Low
Shi, 2020[7]	0	1	1	1	1	1	0	1	High
Zhou, 2020[33]	1	1	1	1	2	1	1	1	Low

[a]1 representing 1 star
REC: Representativeness of the exposed cohort, SNEC: Selection of the non-exposed cohort, AE: Ascertainment of exposure, DO: Demonstration that outcome of interest was not present at start of study, C: Comparability of cohorts on the basis of the design or analysis, AO: Assessment of outcome, FU: Was follow-up long enough for outcomes to occur, AFU: Adequacy of follow up of cohorts.

Table 5. The assessment of risk of bias of the case-control study using Newcastle Ottawa scale[a]

Study	Selection				Comparability (C)	Outcome			Risk of Bias
	ACD	RC	SC	DC		AE	SMA	NRR	
Severe Illness									
Ma, 2020[19]	1	1	0	1	1	1	1	0	High
Zhou, 2020b[20]	1	0	0	1	0	1	1	0	High
Mortality									
Tang, 2020[25]	1	1	0	1	0	1	1	0	High
Wang, 2020[6]	1	1	0	1	2	1	1	0	High

[a]1 representing 1 star
ACD: Adequate case definition, RC: Representativeness of the cases, SC: Selection of Controls, DC: Definition of Controls, C: Comparability of cases and controls on the basis of the design or analysis, AE: Ascertainment of exposure, SMA: Same method of ascertainment for cases and controls, NRR: Non-response rate.

Table 6. The assessment of risk of bias of the systematic review/meta-analysis using ROBIS-I

Outcome	Phase 2				Phase 3
	Study eligibility criteria	Identification and selection of studies	Data collection and study appraisal	Synthesis and findings	Risk of bias in the review
Severe Illness					
Alqahtani, 2020[14]	H	H	L	L	H
Chen, 2020[15]	L	H	L	L	L
Jain 2020[5]	L	H	H	H	H
Lippi, 2020a[16]	L	L	H	H	H
Lippi, 2020b[17]	L	H	H	H	H
Lippi, 2020c[13]	H	H	H	H	H
Matsushita, 2020[4]	H	H	L	L	H
Roncon, 2020[18]	H	H	L	L	H
Mortality					
Alqahtani, 2020[14]	H	H	L	L	H
Lippi, 2020a[16]	L	L	H	L	H
Parohan, 2020[23]	L	H	L	L	H
Roncon, 2020[18]	H	H	L	L	H
Zuin, 2020[24]	H	H	L	L	H

H: High risk of bias; L: Low risk of bias

that consisted of signs and symptoms, clinical characteristics, and comorbidities (**Table 7**).

The included studies that related to clinical characteristics reported four risk factors, namely older age, male sex, high BMI, and history of smoking. Age >60 years was associated with severe illness with OR 3.00 (95% confidence interval [CI) 1.40–6.00).[22] One study with high risk of bias reported older age linked to mortality with HR,OR 2.39 (1.75–3.28).[2]

Other reported risk factors were male sex and high BMI. Two of three studies reported an association between male sex and severe illness,[4,21] but there was no significant association reported by two included studies for mortality.[23,28] One observational study reported that a high BMI ($\geq$35 kg/m^2) is associated with severe illness.[21] We found varying results pertaining to smoking history.[4,14,28]

Reported comorbidities related to severe illness and mortality of COVID-19 were hypertension, diabetes mellitus (DM), cardio-vascular disease, and COPD. Coronary heart disease was also reported to have an association with severe illness with OR 2.85 (95% CI 1.68–4.84).[15] In addition, other comorbidities related to mortality were cerebrovascular disease, chronic renal failure, and cancer. Four out of five studies reported that hypertension was associated with severe illness,[4,5,15,16] whereas three out of four studies reported an association with mortality.[16,18,23] Four out of five studies reported that DM was associated with severe illness,[4,5,15,18] and three out of five studies reported an association with mortality.[18,23,31] Most of the studies had high risk of bias except one study by Chen and colleagues.[15] Several studies supporting the role of other comorbidities, such as COPD, cardio-vascular disease, and cerebrovascular disease, had high risk of bias and variable conclusions.[6,7,14,23,28,29,33]

Systematic review/meta-analyses with high risk of bias, reported several signs and symptoms as risk factors for severe infection, such as dyspnoea, cough, fever, expectoration, headache, fatigue, and myalgia.[5] Among the suggested risk factors, dyspnoea and cough had OR of 3.70 (95% CI 1.83–7.46) and 1.63 (95% CI 1.03–2.60), respectively.[5]

We identified several studies related to cardio-vascular disease as a risk factor for mortality. However, we did not conduct a meta-analysis for this variable due to the potential heterogeneity among the studies, such as study design and follow-up period.

Table 7. Risk factors for severe illness and mortality

Type of Variable	Type of Study	Author	Total (N)	Severe Illness (N)	Type of Estimate Effect	Effect Estimate (95% CI)	Risk of Bias
Severe Illness							
Clinical Characteristics							
Age > 60 years old	Observational	Ji[22]	N/R	N/R	HR	3.0 (1.40 to 6.00)	Low
Age per 10 year increase	Observational	Ma[19]	84	N/R	OR	2.35 (1.21 to 4.58)	High
Male	SR/MA	Matsushita[4]	43396	N/R	OR/HR	1.70 (1.52 to 1.89)	High
	SR/MA	Jain[5]	908	104	OR	1.15 (0.89 to 1.48)	High
	Observational	Simonnet[21]	124	64	OR	2.83 (1.02 to 7.85)	High
BMI > 30 kg/m^2	Observational	Simonnet[21]	89	48	OR	3.45 (0.83 to 14.31)	High
BMI ≥ 35 kg/m^2	Observational	Simonnet[21]	65	30	OR	7.36 (1.63 to 33.14)	High
Current smokers[a]	SR/MA	Alqahtani[14]	916	31	RR	1.45 (1.03 to 2.04)	High
Smokers[a]	SR/MA	Matsushita[4]	1342	N/R	OR/HR	2.01 (0.83 to 4.86)	High
Comorbidities							
Hypertension	SR/MA	Lippi (a)[16]	2552	243	OR	2.49 (1.98 to 3.12)	High
	SR/MA	Matsushita[4]	24351	N/R	OR/HR	2.74 (2.12 to 3.54)	High
	SR/MA	Chen[15]	1936	117	OR	2.3 (1.76 to 3.00)	Low
	SR/MA	Jain[5]	212	16	OR	1.97 (1.40 to 2.77)	High
	Observational	Simonnet[21]	124	48	OR	2.29 (0.89 to 5.84)	High
Diabetes mellitus	SR/MA	Matsushita[4]	24403	N/R	OR/HR	2.81 (2.01 to 3.93)	High
	SR/MA	Chen[15]	1936	67	OR	2.67 (1.91 to 3.74)	Low
	SR/MA	Roncon[18]	1380	41	OR	2.79 (1.85 to 4.22)	High
	SR/MA	Jain[5]	105	7	OR	3.12 (1.00 to 9.75)	High
	Observational	Simonnet[21]	124	23	OR	1.6 (0.44 to 5.83)	High
Cardiovascular disease	SR/MA	Matsushita[4]	22612	N/R	OR/HR	3.58 (2.06 to 6.21)	High
	SR/MA	Jain[5]	53	2	OR	2.7 (1.52 to 4.80)	High
Coronary heart disease	SR/MA	Chen[15]	335	28	OR	2.85 (1.68 to 4.84)	Low
COPD	SR/MA	Jain[5]	19	1	OR	6.42 (2.44 to 16.9)	High
	SR/MA	Alqahtani[14]	35	22	RR	1.88 (1.40 to 2.40)	High
Dyslipidaemia	Observational	Simonnet[21]	124	24	OR	0.68 (0.24 to 1.97)	High
Sign and Symptoms							
Dyspnoea	SR/MA	Jain[5]	262	37	OR	3.70 (1.83 to 7.46)	High
Cough	SR/MA	Jain[5]	1040	157	OR	1.63 (1.03 to 2.60)	High
Fever	SR/MA	Jain[5]	913	129	OR	1.17 (0.88 to 1.56)	High
Expectoration	SR/MA	Jain[5]	392	24	OR	1.75 (0.63 to 4.83)	High
Headache	SR/MA	Jain[5]	181	4	OR	1.16 (0.78 to 1.74)	High
Fatigue	SR/MA	Jain[5]	586	57	OR	1.44 (0.76 to 2.72)	High
Myalgia	SR/MA	Jain[5]	187	7	OR	1.32 (0.89 to 1.96)	High
Mortality							
Clinical Characteristics							
Age (≥ 65 vs <65 years)	SR/MA	Parohan[23]	22350	N/R	HR,OR	2.39 (1.75 to 3.28)	High
Age (≥ 65 vs <65 years)	Observational	Du[29]	179	17	OR	3.765 (1.15 to 17.39)	Low

Table 7. Risk factors for severe illness and mortality

Type of Variable	Type of Study	Author	Total (N)	Severe Illness (N)	Type of Estimate Effect	Effect Estimate (95% CI)	Risk of Bias
Age (50-59 vs <49 years)	Observational	Fu[28]	102	8	RR	3.698 (0.83 to 16.57)	High
Age (60-69 vs <49 years)	Observational	Fu[28]	108	7	RR	2.907 (0.63 to 13.36)	High
Age (>70 vs <49 years)	Observational	Fu[28]	88	17	RR	10.679 (2.62 to 43.46)	High
Age per 1 Unit increase	Observational	Zhou[33]	191	N/R	OR	1.10 (1.03 to 1.17)	Low
Male	SR/MA	Parohan[23]	22086	N/R	HR,OR	1.25 (0.75 to 2.09)	High
	Observational	Fu[28]	200	16	RR	0.907 (0.49 to 1.68)	High
Smokers[a]	Observational	Fu[28]	170	16	RR	0.809 (0.44 to 1.48)	High
Comorbidities							
Hypertension	SR/MA	Zuin[24]	302	47	OR	3.36 (1.96 to 7.74)	High
	SR/MA	Parohan[23]	21640	652	HR,OR	3.29 (1.54 to 7.05)	High
	SR/MA	Lippi (a)[16]	341	55	OR	2.42 (1.51 to 3.90)	High
	Observational	Fu[28]	200	22	RR	1.797 (0.94 to 3.43)	High
Diabetes mellitus	SR/MA	Roncon[18]	354	26	OR	3.21 (1.82 to 5,64)	High
	SR/MA	Parohan[23]	21376	634	HR,OR	3.11 (1.10 to 8.80)	High
	Observational	Zhang (b)[31]	258	7	HR	2.84 (1.01 to 8.01)	High
	Observational	Fu[28]	200	26	RR	1.495 (0.72 to 3.11)	High
	Observational	Shi[7]	416	60	HR	0.75 (0.38 to 1.50)	High
COPD	SR/MA	Alqahtani[14]	167	10	RR	1.1 (0.60 to 1.80)	High
	SR/MA	Parohan[23]	21175	590	OR	7.69 (5.65 to 10.47)	High
	Observational	Wang[6]	339	11	HR	2.24 (1.12 to 4.50)	High
	Observational	Shi[7]	416	12	HR	0.39 (0.04 to 3.68)	High
Cardiovascular disease	Observational	Wang[6]	339	21	HR,OR	1.858 (1.06 to 3.26)	High
	Observational	Fu[28b]	200	2	RR	0.719 (0.19 to 2.73)	High
	Observational	Shi[7]	416	44	HR	1.40 (0.65 to 3.03)	High
	Observational	Zhou[33]	191	13	OR	2.14 (0.26 to 17.79)	Low
Cerebrovascular disease	SR/MA	Parohan[23]	21175	590	OR	7.39 (2.88 to 18.96)	High
	Observational	Wang[6]	339	10	HR,OR	1.379 (0.65 to 2.93)	High
Cardiovascular or cerebrovascular diseases	Observational	Du[29]	179	12	OR	2.464 (0.76 to 8.04)	Low
Chronic pulmonary disease	Observational	Fu[28]	200	4	RR	3.2 (1.486 to 6.89)	High
Chronic renal failure	Observational	Shi[7]	416	17	HR	0.66 (0.29 to 1.46)	High
Cancer	Observational	Shi[7]	416	9	HR	0.82 (0.18 to 3.65)	High

[a] There is uncertainty of the length of exposure
[b] The author described as cardiac disease

Laboratory Test Results Associated with Severe Illness and Mortality of COVID-19

The laboratory test results associated with severe illness and mortality are shown in **Table 8**. The role of thrombocytopenia as a test result associated with severe illness was reported by one SR/MA with OR 5.13 (95% CI 1.81–14.58).[17] There was also a reported association between

Table 8. Laboratory and other test results for severe illness and mortality of COVID-19.

Type of Variable	Type of Study	Author	Total (N)	Severe Illness (N)	Type of Estimate Effect	Effect Estimate (95% CI)	Risk of Bias
Severe Illness							
Thrombocytopeniaa	SR/MA	Lippi (b)[17]	1289	113	OR	5.13 (1.81 to 14.58)	High
LDH 250-500 U/L	Observational	Ji[22]	202	24	HR	2.5 (1.20 to 5.20)	Low
LDH >500 U/L	Observational	Ji[22]	131	5	HR	9.8 (2.80 to 33.80)	Low
PCT ≥ 0.50 µmol/L	SR/MA	Lippi (c)[13]	N/A	N/R	OR	4.76 (2.74 to 8.29)	High
Lymphocyte (≤1.0x10^9/L)	Observational	Ji[22]	208	N/R	HR	3.7 (1.80 to 7.80)	Low
D-dimer >0.55 mg/L	Observational	Ji[22]	208	16	HR	1.0 (0.50 to 2.10)	Low
CT-Value of SARS-CoV-2 ≤36.67 vs moreb	Observational	Ma[19]	84	N/R	OR	0.158 (0.03 to 0.99)	High
Mortality							
Lymphocyte count per 1 Unit increase (x109/L)	Observational	Zhou[33]	191	N/R	OR	0.19 (0.02 to 1.62)	Low
CD3+CD8+ ≤ 75 cell/mcL	Observational	Du[29]	179	17	OR	5 (1.32 to 18.96)	Low
NLR tertile 2 (2.21-4.82)	Observational	Liu[30]	163	5	OR	1.71 (0.14 to 21.38)	High
NLR tertile 3 (4.85-88.09)	Observational	Liu[30]	164	26	OR	16.61 (1.58 to 74.66)	High
Acute cardiac injuryc	Observational	Wang[6]	339	39	HR	1.547 (0.75 to 3.193)	High
	Observational	Shi[7]	416	42	HR	3.41 (1.62 to 7.16)	High
Cardiac Troponin I ≥ 0.05 ng/mL	Observational	Du[29]	179	13	OR	7.2 (1.52 to 34.14)	Low
Myoglobin Positive	Observational	Fu[28]	200	13	OR	0.643 (0.23 to 1.82)	High
hs-cTropI ≥ 0.026 mcg/L	Observational	Zhang (a)[32]	48	10	HR	10.902 (1.28 to 92.93)	High
Arrhythmia	Observational	Wang[6]	339	13	HR	0.754 (0.37 to 1.53)	High
Cardiac Insufficiencyd	Observational	Wang[6]	339	25	HR	1.105 (0.59 to 2.06)	High
nt-proBNP ≥ 106,42 pmol/L	Observational	Shi[7]	416	N/A	HR	1.52 (0.74 to 3.10)	High
AKI	Observational	Wang[6]	339	17	HR	1.159 (0.55 to 2.41)	High
AKI stage 1e	Observational	Cheng[27]	701	13	HR	1.90 (0.76 to 4.75)	Low
AKI stage 2e	Observational	Cheng[27]	701	9	HR	3.53 (1.50 to 8.27)	Low
AKI stage 3e	Observational	Cheng[27]	701	14	HR	4.72 (2.55 to 8.75)	Low
Proteinuria 1+ vs Negative	Observational	Cheng[27]	701	N/R	HR	2.47 (1.15 to 5.33)	Low
Proteinuria 2+/3+ vs Negative	Observational	Cheng[27]	701	N/R	HR	6.80 (2.97 to 15.56)	Low
Hematuria 1+ vs Negative	Observational	Cheng[27]	701	N/R	HR	3.05 (1.43 to 6.49)	Low
Hematuria 2+/3+ vs Negative	Observational	Cheng[27]	701	N/R	HR	8.89 (4.41 to 17.94)	Low
Peak Serum Cr >13.26 µmol/L	Observational	Cheng[27]	701	N/R	HR	3.09 (1.95 to 4.87)	Low
Creatinine ≥13.26 µmol/L	Observational	Shi[7]	416	N/R	HR	1.22 (0.60 to 2.50)	High
ARDS	Observational	Wang[6]	339	56	HR	29.332 (12.36 to 69.58)	High

Table 8. Laboratory and other test results for severe illness and mortality of COVID-19.

Type of Variable	Type of Study	Author	Total (N)	Severe Illness (N)	Type of Estimate Effect	Effect Estimate (95% CI)	Risk of Bias
Elevated D-dimer per 1 mg/L	Observational	Zhang (a)[32]	48	N/R	HR	1.103 (1.03 to 1.18)	High
D-dimer > 0.5 vs ≤0.5 mg/L	Observational	Zhou[33]	100	6	OR	2.14 (0.21 to 21.39)	Low
D-dimer > 1 vs ≤0.5 mg/L	Observational	Zhou[33]	127	44	OR	18.42 (2.64 to 128.55)	Low
Bacterial Infectionf	Observational	Wang[6]	339	49	HR	1.517 (0.77 to 3.24)	High
PCT >0.5 vs ≤0.5 µmol/L	Observational	Barrasa[26]	46	4	HR	2.7 (0.50 to 13.50)	High
PCT >1 vs ≤1 µmol/L	Observational	Barrasa[26]	46	2	HR	2.4 (0.30 to 20.90)	High

nt-proBNP: N-terminal (NT)-pro hormone BNP
[a] There are 3 included studies with endpoint of mortality
[b] Cycle threshold (Ct) value data of real-time PCR (RT-PCR)
[c] Acute cardiac injury was defined as cardiac injury was defined if the serum level of cardiac troponin I (cTnI) was above the 99th percentile upper reference limit (Wang), regardless of new abnormalities in electrocardiography and echocardiography (Shi)
[d] Cardiac insufficiency was defined when the serum level of NT-pro BNP exceeded the normal range and the presence of associated symptoms, such as dyspnea, orthopnea and edema of lower extremity
[e] The stage of AKI was determined using the peak serum creatinine level after AKI detection, with increases of 1.5 to 1.9, 2.0 to 2.9, and 3 times baseline being defined as AKI stage 1, 2, and 3, respectively (definition by KDIGO)
fBacterial infection was defined as an increased in PCT (the normal range is <0.1 µmol/L.

higher serum lactate dehydrogenase (LDH) (≥250 U/L) and low lymphocyte count (≤1.0 x 10^9/L) with severe illness, with HR 9.80 (95% CI 2.80–33.80) and 3.70 (95% CI 1.80–7.80), respectively.[22] One SR/MA with high risk of bias reported that increased PCT (≥0.50 µmol/L) was associated with severe illness.

Several studies reported an association between higher serum D-dimer levels and both outcomes with variable cut-off points.[22,33] An observational study related to mortality reported serum D-dimer >1 mg/L with OR 18.42 (95% CI 2.64–128.55).33 Low CD3+ CD8+ cell count, ≤75 cell/µL, was reported to be associated with mortality with OR 5.00 (95% CI 1.32–18.96; low risk of bias).29 Another finding associated with mortality was tertile 3 NLR (4.85–88.09) with OR 16.61 (95% CI 1.58–174.66).[30]

Acute cardiac injury was defined by various indicators, including hs-TropI and myoglobin. There were varying results among studies related to acute cardiac injury.[6,7,28,29,32] Only one in five studies reporting the acute cardiac injury marker high-sensitive Troponin I (hs-TnI) showed an association with mortality, with OR 7.20 (95% CI 1.52–34.14).[29] Studies of cardiac insufficiency (defined as serum levels of nt-proBNP exceeding the normal range with symptoms of acute heart failure) as well as studies on its marker alone (nt-proBNP), showed no association between acute heart failure and risk of mortality.[6,7] On the other hand, an observational study with low risk of bias showed an association between COVID-19 patients with kidney-related conditions on admission, including acute kidney injury (stage 2–3), proteinuria, haematuria , peak serum creatinine >13.26 µmol/L, and risk of mortality.[27] We also found a study reporting an association between ARDS and mortality of COVID-19 patients with OR 29.332 (95% CI 12.36–69.58).[6]

DISCUSSION

Age >60 years, hypertension, coronary heart disease, and DM are the risk factors for severe illness of COVID-19.15,22 Laboratory test results associated with severe illness include serum LDH 250–500 U/L, serum LDH >500 U/L, and lymphopenia (lymphocyte count ≤1.0 x 10^9/L).[22] On the other hand, laboratory test results associated with mortality include a CD3+ CD8+ cell count ≤75 cell/µL,[29] D-dimer >1 vs ≤0.5 mg/L,(33) AKI stage 2, AKI stage 3, proteinuria

≥1+, haematuria ≥1+, and peak serum creatinine >13.26 μmol/L.27 The role of other reported risk factors for both severe illness and mortality is mostly supported by insufficient evidence.

Age >60 years is a risk factor for severe illness of COVID-19, supported by an observational study with low risk of bias.[22] The pattern of increasing severe illness of SARS-CoV-2 infection with age is consistent with the epidemiology of MERS-CoV and SARS-CoV-1.[35] In general, there is a progressive decline in immunological competence as one ages.[36] Older people are more likely to develop a dysfunctional immune response resulting in pathological conditions as well as the failure to eradicate pathogens. The ageing lung microenvironment leads to an alteration of dendritic cell maturation and migration of cells to the lymphoid organs. Dysfunction of dendritic cells in turn causes a defective activation of T-cells.[35] Patients with immune dysfunction may generally have a heightened risk of immunologic failure in the initial phase of clinical SARS-CoV-2 infection, followed by a hyperinflammation phase instead of recovery from the disease.[37] From an endocrinological perspective, older people have decreased levels of oestrogens and androgens, which provides an alternative explanation for the link between older age and greater severe illness and mortality in COVID-19. Testosterone is important for the downregulation of inflammation. It has been hypothesised to play a role in the cascade of events resulting in the progression of COVID-19 infection due to cytokine storm. In addition, normal serum testosterone levels have a protective role for several respiratory outcomes. In contrast, low testosterone levels result in reduced respiratory muscle activity and overall diminished exercise capacity and strength.[38]

Based on sufficient evidence, hypertension is a risk factor for severe illness of COVID-19 patients.[15] The link between hypertension and disease outcome is possibly explained by T cell dysfunction observed in patients with hypertension in general. As a result, dysfunctional CD8+ T cells cannot fight against the viral infection and also contribute to overproduction of cytokines.[39] The mechanism of SARS-CoV-1 infection causing reduced ACE2 function and subsequent renin angiotensin system (RAS) dysfunction may also apply to SARS-CoV-2 infection. RAS dysfunction will in turn influence electrolyte and fluid balance as well as blood pressure.[35]

We also suggest a clear link between the presence of coronary heart disease in COVID-19 patients and a threefold increase in risk for severe illness.[4] Interestingly, the presence of cardio-vascular disease affects mortality rate in COVID-19 to a greater extent than a history of COPD, which has not been the case in SARS-CoV-1 infection.44 An acute systemic inflammatory response in COVID-19 patients at the coronary artery level can trigger plaque rupture causing myocardial infarction. Furthermore, several metalloproteinases related to cytokine recruitment and inflammation may mediate the function and effects of ACE2 in atherosclerotic diseases.[48]

Sufficient evidence also supported the role of DM as a risk factor for severe illness.4 In general, diabetic patients are prone to infection due to the impairment of neutrophil chemotaxis and phagocytosis. On the other hand, several specific factors in DM identified in animal and human studies may explain the higher risk for severe illness and mortality in COVID-19, including increased furin, upregulation of ACE2, T cell function impairment, and elevated IL-6 level.40 In addition, IL-6 levels increase over time in severely ill COVID-19 patients requiring intensive care unit (ICU) admission, and IL-6 levels are more elevated in non-survivors compared with survivors.[35] As pancreatic islets express ACE2 receptors, cohort studies of COVID-19 have yet to confirm de novo development of hyperglycaemia/diabetes as seen in patients infected by SARS-CoV-1.[40]

Serum LDH was found to be significantly high in refractory COVID-19 patients.[41] The risk of severe illness was nearly three and ten times higher among COVID-19 patients with serum LDH 250–500 U/L and >500 U/L, respectively.[22] Similarly, a high initial LDH level independently correlates with an adverse clinical outcome of patients with SARS-CoV-1 infection.[42] LDH is essential for pyruvate conversion into lactate in

glucose metabolism. The secretion of LDH is induced by cell membrane necrosis, indicating lung damage or viral infection.[42]

COVID-19 patients with lymphopenia are likely to have an increased risk of severe illness.[22] CD3, as a marker of mature T lymphocytes, helps in the activation of CD4+ T cells and CD8+ T cells.[43] Both CD4+ and CD8+ T cells are critical in controlling influenza virus, SARS-CoV-1, and MERS-CoV infection. Since SARS-CoV-2 is highly homologous to SARS-CoV-1 and MERS-CoV, these types of T cells are hypothesised to play a role in infection control as well.[44] The decline in CD8+ T cell count often precedes radiographic changes in SARS-CoV-1 infection. T cell counts in severe COVID-19 patients are hypothesised to fall progressively through the viraemia phase, acute (pneumonia) phase, and finally in the severe phase of the disease.[37] Thus, it may be important to check for lymphocyte levels early, specifically CD3+ CD8+ T cells and trend the cell count in the COVID-19 disease course to stratify the risk of severe illness and fatality.

Elevated serum D-dimer levels, >1 vs ≤0.5 g/L, may indicate higher risk of death in infected patients.[33] An elevated level of D-dimer signifies a hypercoagulable state in patients with COVID-19.[36] An exceptionally high percentage of aberrant coagulation cases was noticed in severe and critical COVID-19 patients. Such abnormal coagulation was also reported in severe influenza, but it was a rare finding for other coronavirus infections. In the hypothetical pathogenesis of COVID-19, D-dimer levels keep increasing steadily in severely ill patients starting from the initial viraemia phase. The increasing trend may be explained by conjecture. First, direct viral attack in the lung is an important activator of coagulation. Second, dysfunction of endothelial cells in viral infection may result in excess thrombin generation.[37] Third, COVID-19-related hypoxia also stimulates an increase in blood viscosity and hypoxia-inducible transcription factor-dependent signalling pathways.[32] Fourth, certain cytokines, including IL-6, could suppress the fibrinolytic system and activate the coagulation system. In conjunction with the activation of the coagulation system via exposure to tissue factors and other pathways following the viral attack in the lung, these processes may act in a feed-forward manner towards an uncontrolled end-point.[37]

Renal involvement during the course of COVID-19 disease is common[45] and certain degrees of AKI are associated with mortality.[6,27] Studies of AKI in COVID-19 patients in general suggest imprecision of CI.[6] However, if COVID-19 patients are classified as proposed by Cheng and colleagues, stage 2 and stage 3 AKI patients have nearly fourfold and fivefold increased risk of death.[27] Both sepsis- and non-sepsis-related mechanisms may explain AKI in SARS-CoV-2 infection.[46] More studies are needed to provide information related to these mechanisms. The mechanisms leading to acute tubular necrosis (ATN) were hypothesised to be direct viral invasion, cytokine release syndrome, rhabdomyolysis, renal hypoperfusion, cardio-renal syndrome due to viral myocarditis, and hypoxia of renal medulla secondary to alveolar damage.[47] It is plausible that hypercoagulation as a characteristic complication of severe COVID-19 could promote the evolution of ATN becoming irreversible cortical necrosis.[48] The pathological features of renal injury in the setting of COVID-19 include a type of nephrotic syndrome, namely collapsing focal segmental glomerulosclerosis (FSGS) and ATN. Collapsing FSGS is a known complication in patients with another viral infection causing cytopathic effect, namely human immunodeficiency virus (HIV). ACE2 as a SARS-CoV-2 viral entry receptor was also found in podocytes45 and the apical membrane of proximal tubular cells in the kidney.[46] Although PCR result for SARS-CoV-2 in kidney biopsy samples was negative, viral particles were found in the podocytes and proximal tubular cells. This finding suggested the probable involvement of a direct cytopathic effect of SARS-CoV-2 in the development of the kidney injury.[45]

A significant number of patients had proteinuria and a smaller proportion of patients developed haematuria.[46] A proteinuria dipstick test result of 1+ signifies a threefold risk of death, and more massive proteinuria results (2+/3+) indicate a seven times heightened

mortality risk in COVID-19 patients. Similarly, a haematuria test result of 1+ and 2+/3+ indicate a three and nine times higher risk of death, respectively.[27] Both proteinuria and haematuria probably develop from infection-mediated glomerulonephritis.[47]

Collectively, the findings suggest that the worse the AKI, proteinuria, and haematuria each COVID-19 patient has, the higher the risk for mortality may become. Interestingly, in our review, underlying chronic renal failure in COVID-19 patients was not found to be a significant risk factor of COVID-19-related severe illness and death.[7] A single measurement of high serum creatinine level is less valuable in determining the increase in mortality risk.[27] Thus, it may be important to trend the serum creatinine to obtain the peak level and the acute progression of kidney failure. The higher risk of mortality in those with severe AKI, even with renal replacement therapy (RRT), may result from lung–kidney crosstalk in COVID-19 infections.[49] Uncontrolled inflammation in COVID-19 generally could cause multi-organ damage and subsequently bring about organ failure.[35]

Among studies with insufficient evidence, there were two reported conditions, namely ARDS and acute cardiac injury, that we found to have potential association with mortality. ARDS itself may result from a massive pro-inflammatory response,[50] and microthrombotic disease.[51] Direct cardiac injury is theoretically possible since the heart also expresses ACE2. Circulatory failure and myocardial injury observed in several patients may also result from a cytokine storm involving tumour necrosis factor (TNF).[35] Moreover, the dysfunction in cardiac endothelial cells and pericytes due to either direct viral infection or global inflammation in COVID-19 disease course are also hypothesised to cause coronary microcirculatory disruption.[52] Due to insufficient evidence, more high-quality studies are required.

Similarly, the role of a PCT level ≥0.50 g/L as a laboratory test result associated with severe illness was supported by insufficient evidence.[13] In general, viral infection per se attenuates the upregulation of PCT by interferon-alpha release in response to the viral illness. Procalcitonin is actually more specific for bacterial infection and may help to distinguish viral and bacterial infections.[53] Thus, a substantial increase in PCT in COVID-19 patients indicates bacterial coinfection in those developing severe infection.[13] Such a phenomenon is also seen in bacterial coinfections in paediatric patients with viral lower respiratory tract infections, whose infectious causes include coronavirus.[54] More high-quality studies are required to support the significance of an elevated PCT level.

After considering relevant evidence and conjectures, we propose a concise hypothesis of the COVID-19 disease course leading to severe illness and death (**Figure 2**). Concurrent bacterial infection may bring about severe illness in

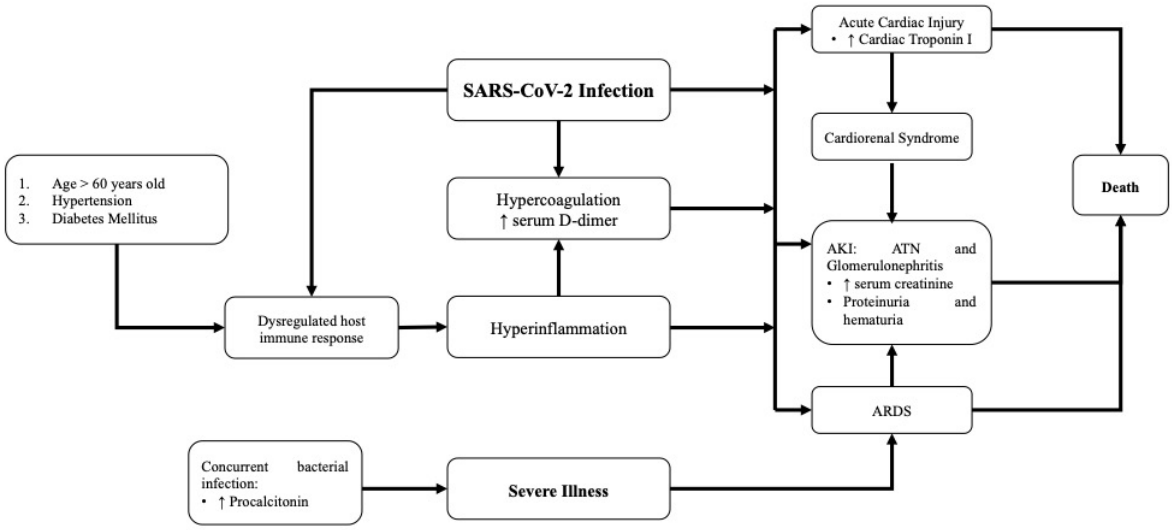

Figure 2. Hypothesis model of COVID-19 disease course leading to severe illness and death.

COVID-19 patients. People aged ≥60 years, with hypertension or with DM have an underlying dysregulation of the immune system. Patients with immune dysfunction may generally have a heightened risk for failure in the initial phase of the clinical course of SARS-CoV-2 infection, followed by a hyperinflammation phase. Both direct viral attack in the lung and inflammation will in turn cause hypercoagulation as shown by elevated serum D-dimer levels. Inflammation and hypercoagulation, together with the hypothesised direct viral infection in lung, heart, and kidney cells, may then lead to ARDS and acute cardiac injury, as well as AKI related to ATN and glomerulonephritis, respectively. Hypercoagulation, hyperinflammation, and/or organ failure(s) play a crucial role in causing death of COVID-19 patients.

Limitations

To date, we believe that our study is the only review collecting and summarising both SR/MA and observational studies and using a systematic approach. However, we also acknowledge the limitations of this review. First, since the evidence pertaining to COVID-19 is growing rapidly, the data collected in this review were restricted to early April 2020. This limitation may impact the collection of data from certain regions in the world in the early period of the pandemic. Second, we may not have retrieved all the existing studies due to our search restriction on studies published in English. To overcome these limitations, we plan to regularly update our review by utilising more robust and comprehensive methods in retrieving all relevant existing studies. Lastly, we also included journal pre-proofs from medRxiv that have not been peer reviewed. However, we carefully appraised all included studies with appropriate quality assessment tools.

In this review, there was only one meta-analysis with low risk of bias. The insufficient evidence is mainly caused by high risk of bias of the available meta-analyses and the lack of meta-analysis of studies related to certain risk factors. Wide CI was in part due to the small sample sizes of the studies. We also found insufficient information in several cohort studies in terms of duration of observational period and time of sample collection.

CONCLUSION

Age >60 years, hypertension, coronary heart disease, and DM are the risk factors for severe illness of COVID-19. Laboratory test results associated with severe illness are serum LDH 250–500 U/L, serum LDH >500 U/L, and lymphopenia (lymphocyte count ≤1.0 x 10^9/L). Test results associated with mortality are CD3+ CD8+ cell count ≤75 cells/μL, D-dimer >1 vs ≤0.5 mg/L, AKI stage 2, AKI stage 3, proteinuria ≥1+, haematuria ≥1+, and peak serum creatinine >13.26 μmol/L. It is crucial to regularly update the review by utilising more robust and comprehensive methods in retrieving all relevant existing studies. Future studies need to specify the duration of observational period and time of sample collection with a larger sample size.

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CONFLICT OF INTEREST

All authors declare no competing interests, other than the research grant.

AUTHOR CONTRIBUTIONS

SS, EDS, RWR, SRFS, MKA and JM contributed equally in drafting the protocol, selecting studies for inclusion, extracting data, assessing risk of bias, carrying out and interpreting the analysis. Both SS and KH contributed in carrying out and interpreting the analysis and providing clinical expertise in the study. Both YP and WW contributed equally in developing the search strategy and running the search and selecting studies for inclusion. YP also contributed in data extraction and assessed the risk of bias. All authors have read and approved the manuscript.

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