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REVIEW ARTICLE

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Deciphering the COVID-19 cytokine storm: Systematic review and meta-analysis

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Abstract

Introduction: The coronavirus pandemic has affected more than 20 million people so far. Elevated cytokines and suppressed immune responses have been hypothesized to set off a cytokine storm, contributing to ARDS, multiple-organ failure and, in the most severe cases, death. We aimed to quantify the differences in the circulating levels of major inflammatory and immunological markers between severe and non-severe COVID-19 patients.

Methods: Relevant studies were identified from PubMed, EMBASE, Web of Science, SCOPUS and preprint servers. Risk of bias was assessed for each study, using appropriate checklists. All studies were described qualitatively and a subset was included in the meta-analysis, using forest plots.

Results: Based on 23 studies, mean cytokine levels were significantly higher (IL-6: MD, 19.55 pg/mL; CI, 14.80, 24.30; IL-8: MD, 19.18 pg/mL; CI, 2.94, 35.43; IL-10: MD, 3.66 pg/mL; CI, 2.41, 4.92; IL-2R: MD, 521.36 U/mL; CI, 87.15, 955.57; and TNF-alpha: MD, 1.11 pg/mL; CI, 0.07, 2.15) and T-lymphocyte levels were significantly lower (CD4+ T cells: MD, −165.28 cells/μL; CI, −207.58, −122.97; CD8+ T cells: MD, −106.51 cells/μL; CI, −128.59, −84.43) among severe cases as compared to non-severe ones. There was heterogeneity across studies due to small sample sizes and non-uniformity in outcome assessment and varied definitions of disease severity. The overall quality of studies was sub-optimal.

Conclusion: Severe COVID-19 is characterized by significantly increased levels of pro-inflammatory cytokines and reduced T lymphocytes. Well-designed and adequately powered prospective studies are needed to amplify the current evidence and provide definitive answers to dilemmas regarding timing and type of anti-COVID-19 therapy particularly in severe patients.

KEYWORDS

CD4+ T cells, CD8+ T cells, COVID-19, cytokine storm, novel coronavirus, SARS-CoV-2

Prospero Registration number: CRD42020183246

1 | INTRODUCTION

In December of 2019, an outbreak of a novel strain of coronavirus named as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) originated in Wuhan, China, and rapidly spread across the globe. The disease caused by the virus is officially called coronavirus disease 19 (COVID-19). The outbreak was declared a Public Health Emergency of International Concern and a pandemic by the World Health Organization (WHO) in January 2020 and March 2020, respectively. As COVID-19 is raging around the globe having affected over 20 million people across more than 200 countries and causing more than 700 000 deaths,[1] deciphering the unique infection mode of this novel virus and its pathophysiology is a crucial step towards arresting the pandemic.

The current pandemic is distinctive in that the range of innate immune responses is unpredictable and varies; it is strong enough to kill in some, but others escape with a relatively mild infection. Infections with other subfamilies of the coronavirus are generally associated with upper respiratory tract infections (RTIs) although some patients may present with lower RTIs too. In contrast, the SARS-CoV-2 infection may remain asymptomatic in the early stages until the emergence of severe pneumonia, dyspnea, organ dysfunction and even death.[2] Pulmonary lesions in COVID-19 show pathological changes, degeneration, infiltration and hyperplasia consistent with inflammatory response throughout the course of the disease.[2] In certain cases, the disease triggers a hyper-inflammatory condition[3] that is potentially life-threatening and is often responsible for COVID-19 fatality.[4] The immune pathogenesis associated with an aberrant immune response results in lung damage, functional impairment, reduced pulmonary capacity and eventually death.[2] Such 'cytokine storm' syndromes are described by inappropriately elevated pro-inflammatory cytokines and chemokines produced by a dysregulated immune response with subsequent multi-organ failure. Interestingly, the COVID-19-associated cytokine storm is distinctive in being associated with early acute respiratory distress syndrome (ARDS) and coagulopathy, and biochemical parameters include elevated but lower serum ferritin and lower interleukin-6 levels as compared to those encountered in other cytokine storm syndromes.[5,6]

Given the novelty of the disease, definitive insights into the dynamics of the dysfunctional inflammatory reaction in the context of cellular immune responses in COVID-19 are much awaited and anticipated. Understanding the biological and clinical consequences of the role of pro-inflammatory cytokines in the pathogenesis of the disease is important for the scientific community as they are racing against time to develop therapeutics to treat patients. There has been some sporadic evidence to suggest that the level of immune response hyperactivity is significantly higher in patients with severe disease, as compared to patients with a mild infection.[7-9] However, it is crucial to generate systematic evidence from research that is adequately powered, to statistically compare the cytokine levels in patients at various stages of the illness. In this context, we sought to undertake a systematic review and meta-analysis of available evidence to understand the pattern of host immune response in patients diagnosed with COVID-19 and how the levels of inflammatory and immunological markers vary according to the severity or stage of the disease.

1.1 | Rationale for the current systematic review and meta-analysis

Systematic reviews and meta-analyses provide the highest level of evidence that is statistically meaningful and thus considered a gold standard. A few systematic reviews and meta-analyses have been conducted in the last few months, to understand the relationship between cytokines and the novel coronavirus disease 2019. However, they have been majorly centred on exploring the correlation between varying levels of interleukin-6 (IL-6) and the degree of severity of the illness.[10-12] A few others have provided a broader overview, albeit utilizing data from a very small number of studies for each outcome.[13,14] Moreover, individual studies with inadequate sample sizes have produced conflicting findings possibly due to a lack of statistical power. As we grapple with a pandemic and continue to get inundated with clinical data from numerous disease cohorts, it becomes imperative to collate existing data and provide the latest scientific evidence, which could be instrumental in informing healthcare professionals and devising appropriate treatment strategies.

Therefore, our review was aimed to summarize the relationship between circulating cytokine levels and COVID-19, and more specifically focusing on important inflammatory and immunological markers that are shown to be responsible for an exaggerated immune response that triggers respiratory distress, multi-organ failure and, in the worst cases, death. We undertook a systematic and comprehensive synthesis of the currently available literature, to obtain a detailed and holistic view of the dynamics between the host immune response and levels of disease severity among clinically confirmed patients of COVID-19.

2 | OBJECTIVE

To understand the pattern of host immune response and summarize evidence for the difference in the levels of immunological and inflammatory biomarkers associated with cytokine storm, between COVID-19 patient groups of varying disease severity.

3 | METHODS

The systematic review methodology has been described in detail, in the protocol registered with PROSPERO (registration number: CRD42020183246).[15] The reporting of this review is consistent with the Preferred Reporting of Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[16] as well as the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) recommendations.[17]

3.1 | Search strategy and inclusion of studies

We searched PubMed, EMBASE, Web of Science, SCOPUS, Cochrane Central, clinicaltrials.gov and the preprint server medRxiv, to obtain relevant articles on COVID-19 patient outcomes, published till April 2020. A sensitive search was designed using synonyms for the novel coronavirus 2019, along with keywords such as 'interleukins', 'cytokines', 'inflammation markers', 'IL-6', 'immunomodulatory', 'clinical features', 'patient outcomes', combined using boolean operators as appropriate. The strategies were prepared by one investigator (RM) and reviewed by the other two investigators (AK and TL) to ensure comprehensiveness. The complete search strategy prepared for PubMed is given in the File S1. It was modified for use according to the indexing style of the other databases. The reference list of the relevant papers was also hand-searched to obtain any additional articles.

3.2 | Study design

Articles eligible for inclusion in our review were observational studies (retrospective cohorts, prospective cohorts, case-control studies, and case series) or randomized controlled trials, characterizing and comparing severe and non-severe groups of COVID-19 patients.

3.3 | Study population and exposures

Studies measuring the immunological and inflammatory indicators of cytokine storm in adult patients with a confirmed diagnosis of COVID-19, and comparing them between severe and non-severe (mild-moderate) cases, were eligible to be included in our review. Disease assessment and clinical classification in each study were based on evaluation of symptoms by the study investigators as per the standard protocols for the diagnosis and clinical management of COVID-19.[18,19] Severe COVID-19 according to both the protocols was defined as having one of the following: a respiratory frequency ≥30/min; oxygen saturation ≤ 93%; and oxygenation index (PaO2/FiO2) ≤300 mm Hg. The disease severity groups created in every individual study were used as is, for our review. Additionally, for studies comparing outcomes of patients who had died and those who were alive, we considered dead as severe and alive as the non-severe category.

3.4 | Study outcomes

Outcomes included circulating levels of interleukins (IL-6, IL-8, IL-10 and others depending on availability of estimates), tumour necrosis factor-alpha and T-lymphocyte counts (CD4+ T cells and CD8+ T cells) that have been widely reported in relation to the cytokine storm in patients having severe COVID-19.

3.5 | Exclusion criteria

Studies published in non-English languages, those without a comparator group, clinical trials with a pre-post design, case reports and systematic and narrative reviews were not eligible for inclusion. Small case series and case reports involving less than 10 patients were excluded to minimize bias.

3.6 | Study selection

Eligible studies were imported into a reference manager (Zotero version 5.0) for sorting and removal of duplicates. At level 1, titles and abstracts of all the retrieved articles were individually screened by two reviewers (RM and AK; RM and TL). Full texts of studies eligible for inclusion were examined by two reviewers at level 2, and discrepancies were resolved through mutual consensus among the team members. In case of a suspected patient overlap across studies, the respective authors were contacted and requested for clarification. Two further reminders were sent in case no reply was received to the initial queries.

3.7 | Data extraction, assessment of quality and analysis

Data extraction was undertaken independently by RM and AK, and a data extraction form was prepared in Microsoft Excel based on the Cochrane Handbook of Recommendations. Information on author names, month of publication, country, study site, study design, enrolment duration, patient demographics (age and sex), comorbidities, sample size, levels of the requisite inflammatory and immunological markers and major study findings were recorded for each study. All the eligible studies were included in the narrative synthesis; and studies

reporting appropriate numeric estimates of the required markers were included in the quantitative synthesis.

Review Manager software (RevMan version 5.3) was used for a statistical pooling of estimates through a meta-analysis. Outcomes reported as medians and interquartile ranges (IQRs) were converted to means and standard deviations using standard methods,[20] for statistical uniformity across study data. The overall difference in the average value of each outcome between severe and non-severe groups was reported as mean difference (MD) along with 95% confidence intervals (CIs). Confidence intervals excluding the null value of 0 were considered to be significant. The pooled values were computed through random-effect models (REMs), using the inverse variance method by DerSimonian and Laird.[21] Forest plots were generated to compare the levels of and quantify the difference in each outcome, between the two patient groups. Articles that did not provide numeric estimates were excluded from this meta-analysis. The confidence intervals of each study of each outcome were visually observed for the presence of heterogeneity, indicated by an overlap. The magnitude of variation beyond chance was also objectively assessed using the Cochran's Q test,[22] to ascertain the level of overlap among the CIs of different studies. Variability in estimates was estimated by the Higgins I^2 statistic, used to determine the magnitude of heterogeneity across studies. A chi-square P-value smaller than .05 was considered statistically significant. The Cochrane Handbook cut-offs were used to ascertain the degree of heterogeneity. A sensitivity analysis was also undertaken to obtain pooled estimates where preprints were excluded, and only published studies were considered.

3.8 | Risk-of-bias assessment

The Newcastle-Ottawa Scale (NOS) for cohort studies, as recommended by Cochrane,[23] and the Joanna Briggs Institute (JBI) checklist[24] were used to assess the level of bias in the included observational studies. We used a modified version of the NOS for cohort studies and removed two questions on comparability of groups and selection of the non-exposed cohort based on relevance and to meet our study requirements. Two reviewers (RM and AK) independently graded each study, and disagreements were resolved by consensus.

Further, funnel plots were generated to visually assess the presence of a publication bias among studies. Bias was also detected statistically by Egger's test,[25] using Stata version 15.1.

4 | RESULTS

Our database search identified a total of 893 records (Figure 1). After removal of duplicates, 861 articles were screened on the basis of their titles and abstracts, following which full texts of 209 studies were assessed for eligibility in the review. A total of 40 studies were included in the narrative synthesis, and 25 of them contributed to the meta-analysis. A few studies with three different severity groups instead of two, as well as those that did not provide appropriate numeric estimates, were excluded from the quantitative synthesis. The following PRISMA diagram lists the common reasons for excluding studies and demonstrates the study selection process for this review.

4.1 | Narrative synthesis

The descriptive characteristics of the included studies are presented in Table 1. The sample size across all the studies ranged from 10 patients to 548 patients, and a total of 5209 males and females participated in the 40 studies included in our review. Of the total studies, 35 were case series[7,26-59] and five were retrospective cohorts,[60-64] based in hospitals in different parts of China. The participants were patients with a reverse transcription-polymerase chain reaction (RT-PCR)-confirmed diagnosis of the novel coronavirus 2019, enrolled in the hospital within the four-month duration of December 2019 to March 2020. Laboratory assessments of all confirmed COVID-19 cases were baseline measurements undertaken at the time of their hospitalization. The average age of the patients ranged from about 40-55 years in nearly three-quarters of the studies, to over 60 years in the remaining. Almost one-third of the patients in each study had a comorbid condition with heart disease, diabetes, hypertension, and kidney disease being the most prevalent ones. The most common symptoms across studies were fever, cough, fatigue, dyspnea, myalgia and headache.

A summary of major findings of each of the included studies is presented in Table 1. Older age, presence of comorbidities such as cardiovascular disease, hypertension and chronic obstructive pulmonary disease, and being male were found to be major risk factors for disease severity, higher complications and a greater likelihood of death. Additionally, patients in the severe groups were characterized by lymphocytopenia (low CD3+, CD4+ and CD8+ T-cell counts), leucocytosis, higher plasma levels of infection-related biomarkers such as erythrocyte sedimentation rate (ESR), C-reactive protein and procalcitonin, and enzymes such as lactate dehydrogenase (LDH) and alanine aminotransferase (ALT). The inflammatory cytokines, especially circulating interleukins 6, 8, 10, 2R and TNF-alpha levels were significantly elevated among severe/critical cases, as compared to the mild-to-moderate patients. Lymphocytopenia and a pro-inflammatory cytokine storm were therefore considered to be the most critical contributors to adverse clinical outcomes in patients of COVID-19.

The studies included in the meta-analysis reported various outcomes: IL-6 (n = 21),[7,27-33,35-37,39,40,44,46-49,54,60,62]

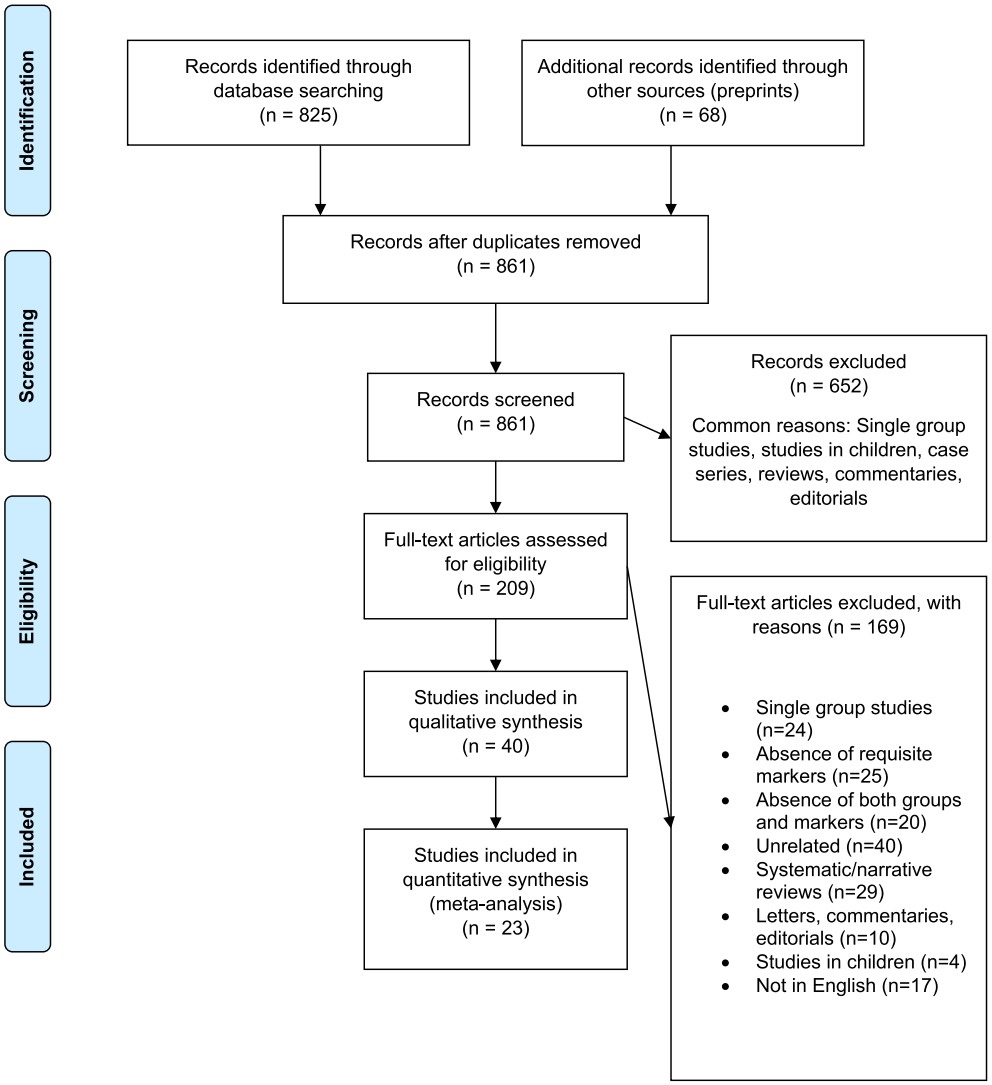

FIGURE 1 PRISMA flow diagram for study selection

IL-8 (n = 4),[7,30,37,54] IL-10 (n = 6),[7,30,33,36,37,46] IL-2R (n = 3),[7,30,37] TNF-alpha (n = 6),[7,30,33,36,37,46] CD4+ T cells (n = 7) [26,27,33,39,46,47,59] and CD8+ T cells (n = 6).[26,27,33,39,46,47]

4.2 | Risk of bias in included studies

The methodological quality of and the presence of bias among the studies was assessed using the JBI checklist for case series and the NOS for cohort studies in our review. Out of the 35 case series, 29 studies showed a low-moderate bias and six studies had a high risk of bias. Major factors contributing to the sub-optimal study quality were a lack of consecutive and complete inclusion of participants or unclear reporting of the same, inadequate description of the socio-demographic characteristics of patients and paucity of information about the study sites/hospitals. A high risk of bias emanated from the recruitment and selection process as well as ambiguity in reporting the same, across various studies. Since a majority of the studies included in this review were case series, the overall quality of evidence was not satisfactory. Out of the five retrospective cohorts, three studies had a moderate level of bias, one had a high risk of bias, and one study had a low risk of bias. The major risk of bias in these resulted from the non-representativeness of the study cohorts and gaps in reporting of study outcomes, in terms of timing, assessment and follow-up duration. The detailed assessment of bias is provided in File S2.

4.3 | Meta-analysis

We undertook a statistical pooling of estimates across 23 studies, to quantify the difference in the levels of various COVID-19-related inflammatory (IL-6, IL-8, IL-10, IL-2R and TNF-alpha) and immunological markers (CD4+ T and

TABLE 1 Descriptive characteristics of the included studies

Author	Month of publication	Country	Study setting	Study design	Enrolment duration	Study population	Sample size	Age
Yongli Zheng et al	April 2020	China	Chengdu Medical Centre	Retrospective case series	Jan 16-Feb 20	COVID-19-confirmed patients	99	Mean 49.4
Jia Ma et al	April 2020	China	Renmin Hospital of Wuhan University	Retrospective observational study	Jan 1-Mar 30	COVID-19-confirmed patients	37	Median 62
Ruirui Wang et al	March 2020	China	Anhui University	Retrospective descriptive study	Jan 20-Feb 9	COVID-19-confirmed patients	125	Mean 38.7
TieLong Chen et al		China	Zhongnan Hospital, Wuhan	Retrospective case series	Jan 1-Feb 10	COVID-19-confirmed patients	203	Median 54
Guang Chen et al	March 2020	China	Tongji Hospital, Wuhan	Retrospective observational study	till Jan 27	COVID-19-confirmed patients	21	Median 56
Tao Chen et al	March 2020	China	Tongji Hospital, Wuhan	Retrospective case series	till Feb 28	COVID-19-confirmed patients	274	Median 62
Pingzheng Mo et al		China	Zhongnan Hospital, Wuhan	Retrospective observational study	Jan 1-Feb 5	COVID-19-confirmed patients	155	Median 54
ChaominWu et al	March 2020	China	Jinyintan Hospital, Wuhan	Retrospective cohort	Dec 25-Jan 26	COVID-19-confirmed patients	201	Median 51
Yaqing Zhou et al		China	Huangshi Central Hospital	Retrospective case series	Jan 28-Mar 2	COVID-19-confirmed patients	21	Mean 66.1
Suxin Wan et al	March 2020	China	Chongqing Central Hospital	Prospective observational study	Jan 26-Feb 4	COVID-19-confirmed patients	123	Mean 43.1
Xiaohua Chen et al		China	General Hospital of Central Theater Command	Retrospective observational study	Feb 1-19	COVID-19-confirmed patients	48	Mean 64.6
Yong Gao et al	March 2020	China	Fuyang Second People's Hospital.	Retrospective observational study	Jan 23-Feb 2	COVID-19-confirmed patients	43	Mean 44

Gender	Comorbidities	No. of severe cases	No. of nonsevere cases	Major findings	Reference No.
51.5% M	41%	32	67	Elderly and those with CVD more prone to critical illness, decreased WBC count, CD4, CD8, high CRP and more myocardial damage.	[26]
54.05% M	32.40%	20	17	Increased neutrophils and IL-6 in severe patients.	[27]
57% M	27.20%	25	100	Old age, chronic disease and smoking could be risk factors; critical group had lower lymphocytes and higher CRP.	[28]
53.2% M	43.3%; 27% of all > 65 yrs and with more illness	19	36	Males, comorbidities, time from disease onset to hospitalization, abnormal kidney function and elevated procalcitonin levels were all significantly associated with death.	[29]
81% M	33.30%	11	10	Severe cases more frequently had dyspnea, lymphopenia and hypoalbuminaemia, with higher levels of alanine aminotransferase, lactate dehydrogenase and C-reactive protein; markedly higher IL-2R, IL-6, IL-10 and TNF-α; lower T lymphocytes, CD4 + T cells and CD8 + T; and lower IFN-y.	[7]
62% M	49%	113	161	Deceased were older males with comorbidities, ARDS, sepsis, cardiac injury, heart failure and kidney injury.	[30]
55.5 M	10% diabetes and CVD, and 23% hypertension	85	70	Refractory patients had an older age; male sex; more underlying comorbidities; lower fever incidence; higher incidence of breath shortness; high levels of neutrophil (AST), LDH and C-reactive protein; and higher incidence of bilateral pneumonia and pleural effusion.	[31]
63.7% M	4% diabetes, 10% CVD and 20% hypertension	84	117	Older age was associated with greater risk of ARDS and death. High fever was associated with better outcomes.	[60]
65.9% M	76.20%	13	8	The most common characteristics on chest CT were ground-glass opacity and bilateral patchy shadowing. The most common findings on laboratory measurements were lymphocytopenia, elevated levels of C-reactive protein and interleukin-6.	[32]
53.6% M	13%	21	102	Significant positive correlations between CD4 + T and CD8 + T, IL-6 and IL-10 in the mild group.	[33]
77.1% M	25% diabetes, 17% CVD and 50% hypertension	17 critical, 10 severe and 21 moderate		RNAaemia was diagnosed only in the critically ill group, reflected disease severity. Level of inflammatory cytokine IL-6 in critically ill patients increased almost 10 times than in other patients. Extremely high IL-6 level was closely correlated with the detection of RNAaemia.	[32]
60.46% M	16% diabetes, 70% CVD and 30% hypertension	15	28	IL-6 and D-D closely related to the occurrence of severe COVID-19 in the adults, and their combined detection had the highest specificity and sensitivity for early prediction of the severity.	[35]

(Continues)

Author	Month of publication	Country	Study setting	Study design	Enrolment duration	Study population	Sample size	Age
Zhongliang Wang et al		China	Union Hospital, Wuhan	Retrospective case series	Jan 16-29	COVID-19-confirmed patients	69	Median 42
Chuan Qin et al		China	Tongji Hospital, Wuhan	Retrospective observational study	Jan 10-Feb 12	COVID-19-confirmed patients	452	Median 58
Pan Luo et al	March 2020	China	Tongji Hospital, Wuhan	Retrospective observational study	Jan 27-Mar 5	COVID-19-confirmed patients	15	Mean 71.4
Lang Wang et al	March 2020	China	Renmin Hospital of Wuhan University	Retrospective observational study	Jan 1-Feb 6	COVID-19-confirmed patients	339	Median 71
Qiurong Ruan et al	March 2020	China	Jin Yin-tan Hospital and Tongji Hospital	Retrospective observational study	Not given	COVID-19-confirmed patients	150	Not given
Yun Feng et al		China	Jinyintan Hospital in Wuhan, Shanghai Public Health Clinical Center in Shanghai and Tongling People's Hospital in Anhui Province, China.	Retrospective observational study	Jan 1-Feb 15	COVID-19-confirmed patients	476	Median 53
Yulong Zhou et al	March 2020	China	Ninth Hospital of Nanchang	Retrospective observational study	Jan 28-Feb 6	COVID-19-confirmed patients	17	Mean 41.5
Jing Yuan et al	March 2020	China	Shenzhen Third People's Hospital	Retrospective observational study	Jan 5-Feb 13	COVID-19-confirmed patients	94	Median 40
Xiaochen Li et al	April 2020	China	Tongji Hospital, Wuhan	Ambispective cohort study	Jan 26-Feb 5	COVID-19-confirmed patients	548	Median 60

Gender	Comorbidities	No. of severe cases	No. of nonsevere cases	Major findings	Reference No.
46% M	10% diabetes, 12% CVD and 13% hypertension	14	55	Compared with SpO2 ≥ 90% group, patients of the SpO2 < 90% group had more comorbidities and dhigher plasma levels of IL-6, IL-10, lactate dehydrogenase and C-reactive protein.	36
52% M	44%	286	166	Severe cases had lower lymphocyte counts, higher leucocyte counts, lower percentages of monocytes, eosinophils and basophils. Most severe cases demonstrated elevated levels of infection-related biomarkers and inflammatory cytokines. T cells significantly decreased and were more hampered in severe cases.	37
80% M	66.70%	7 critical, 6 severe, 2 moderate		Tocilizumab could be effective for COVID-19 patients with a risk of cytokine storms. For critically ill patients with elevated IL-6, repeated dose of the TCZ recommended.	36
49% M	16% diabetes and CVD, and 41% hypertension	80 critical, 159 severe and 100 moderate		Dyspnea, cardiovascular disease chronic obstructive pulmonary disease and acute respiratory distress syndrome strong predictors of death. High level of lymphocytes predictive of better outcome. High proportion of severe to critical cases and high fatality rate observed in the elderly.	37
Not given	62% in severe and 41% in nonsevere group	68	82	Higher age in death group; significant differences in white blood cell counts, absolute values of lymphocytes, platelets, albumin, total bilirubin, blood urea nitrogen, blood creatinine, myoglobin, cardiac troponin, C-reactive protein (CRP) and interleukin-6 (IL-6) between the two groups.	40
57% M	43.10%	70 critical, 54 severe and 352 moderate		Compared with moderate group, higher comorbidities are in severe and critical groups. Patients over 75 years old had significantly lower survival rate. Multiple-organ dysfunction and impaired immune function are typical characteristics of severe and critical patients.	39
35.3% M	30%	5	12	Decreased total lymphocytes and CD4 in aggravation group, total lymphocyte count positively correlated with CD4 + T-cell count, and no significant differences were found between the 2 groups in WBC, CRP, albumin and LDH.	42
45% M	5.3% diabetes, 6.4% CVD and 9.6% hypertension	11 critical, 75 severe and 8 moderate		COVID-19 mRNA clearance ratio significantly correlated with the decline of serum creatine kinase (CK) and lactate dehydrogenase (LDH). Serum LDH or CK decline may predict a favourable response to treatment of COVID-19 infection.	41
51% M	15% diabetes, 6 CVD and 30% hypertension	269	279	Older age, underlying hypertension, high cytokine levels (IL-2R, IL-6, IL-10 and TNF-a) and high LDH level were significantly associated with severe COVID-19 on admission. Male sex, older age, leukocytosis, high LDH, cardiac injury, hyperglycaemia and high-dose corticosteroid use were associated with death in severe patients.	61

Author	Month of publication	Country	Study setting	Study design	Enrolment duration	Study population	Sample size	Age
Fei Zhou et al	March 2020	China	Jinyintan Hospital and Wuhan Pulmonary Hospital (Wuhan, China)	Retrospective cohort study	till Jan 31	COVID-19-confirmed patients	191	Median 56
Yang Xu et al		China	Zhongnan Hospital of Wuhan University, Chinese PLA General Hospital, Peking Union Medical College Hospital and affiliated hospitals of Shanghai University of Medicine & Health Sciences	Retrospective case series	Feb 7-28	COVID-19-confirmed patients	69	Median 57
Shuke Nie et al		China	Renmin Hospital, Wuhan	Retrospective observational study	Feb 9-28	COVID-19-confirmed patients	97	Median 39
Huizheng Zhang et al		China	Chongqing Medical Centre	Retrospective observational study	Feb 11-28	COVID-19-confirmed patients	43	Not given
Penghui Yang et al		China	PLA General Hospital	Retrospective case series	Dec 27-Feb 18	COVID-19-confirmed patients	55	Median 44
Yang Xu		China	Hospitals of Shanghai University	Retrospective observational study	Not given	COVID-19-confirmed patients	10	Not given
Lei Liu et al		China	Chongqing University Three Gorges Hospital	Retrospective case series	Jan 20-Feb 3	COVID-19-confirmed patients	51	Median 45
Qingxian Cai et al		China	Third People's Hospital of Shenzhen	Retrospective observational study	Jan 11-Feb 6	COVID-19-confirmed patients	298	Median 47
Chaomin Wu et al		China	Jinyintan Hospital, Wuhan	Retrospective cohort	Dec 25-Jan 27	COVID-19-confirmed patients	188	Mean 51.9
Sha Fu et al		China	Union Hospital, Tongji Medical College	Retrospective observational study	Feb 9-Mar 17	COVID-19-confirmed patients	50	Median 64

Gender	Comorbidities	No. of severe cases	No. of nonsevere cases	Major findings	Reference No.
62% M	48%	54	137	Age, lymphopenia, leucocytosis and elevated ALT, D-dimer, IL-6 and procalcitonin were associated with death.	62
50.7% M	Excluded	25	44	.Severe patients were older; LDH, D-dimer and IL-6 were higher in severe group	44
35% M	5% diabetes, 2% CVD and 15% hypertension	25	72	Hypoproteinaemia, hypoalbuminaemia, low high-density lipoproteinaemia and decreased ApoA1, CD3 + T% and CD8 + T% could predict severity of COVID-19.	45
51.2% M	14% diabetes and 9% hypertension	14	29	Severe patients were older; levels of IL-6, IL-10, ESR and D-dimer significantly were higher in severe patients, while the level of albumin was remarkably low.	46
60% M	11% diabetes and 20% hypertension	34	21	Those with pneumonia were older, with more comorbidities, higher IL-6 and lower CD8 cells.	47
Not given	Excluded	2	8	Lymphopenia, the depletion of T-lymphocyte subsets and higher IL-6 may be associated with disease severity linked to mortality.	48
62.7% M	8% diabetes and hypertension	7	44	Severe patients were older, had higher proportion of diabetics and more likely to have dyspnea.	49
50% M	6% diabetes, 4% CVD and 13% hypertension	58	240	Compared to the nonsevere cases, severe cases were associated with older age, underlying diseases, higher levels of CRP, IL-6 and ESR. Slower clearance of virus associated with higher risk of progression to severe clinical condition.	50
63.3% M	11% diabetes and 20% hypertension	62 high, 66 moderate and 60 low		Patients with high levels of high-sensitivity cardiac troponin I on admission had significantly higher mortality than patients with moderate or low levels of hs-TNI. hs-TNI level on admission was significantly negatively correlated with survival days.	61
54% M	24% diabetes, 22% CVD and 20% hypertension	29	21	Older age, hyperlipaemia, hypoproteinaemia and prolonged SARS-CoV-2 IgM-positive were all associated with poor recovery. The odds of impaired lung lesion resolutions were higher in patients with hypoproteinaemia, hyperlipaemia and elevated levels of IL-4 and ferritin.	51

Author	Month of publication	Country	Study setting	Study design	Enrolment duration	Study population	Sample size	Age
Yabo Ouyang et al	April 2020	China	Beijing Youan Hospital	Retrospective observational study	Jan 31-Feb 7	COVID-19-confirmed patients	11	Median 67
Jing Liu et al	April 2020	China	Wuhan Union Hospital, Tongji Medical College	Retrospective observational study	Jan 5-24	COVID-19-confirmed patients	40	Mean 48.7
Yang Liu et al		China	First Affiliated Hospital of Nanchang University	Retrospective observational study	Jan 22-Feb 15	COVID-19-confirmed patients	76	Median 45
Fang Liu et al	April 2020	China	General Hospital of Central Theater Command of People's Liberation Army	Retrospective cohort	Jan 18-Mar 12	COVID-19-confirmed patients	140	Median 65.5
Yanlei Li et al	April 2020	China	Tongji Hospital, Wuhan	Retrospective observational study	Jan 28- Feb 11	COVID-19-confirmed patients	54	Mean 65.8
Bo Xu et al	April 2020	China	Hubei Provincial Hospital of Traditional Chinese and Western Medicine	Retrospective observational study	Dec 26-Mar 19	COVID-19-confirmed patients	187	Median 62
Changcheng Zheng et al	March 2020	China	Cancer centre of Wuhan Union Hospital	Retrospective observational study	Admitted on Feb 15	COVID-19-confirmed patients	55	Median 60
Hong-Yi Zheng et al	March 2020	China	Yunnan Provincial Hospital of Infectious Diseases, Kunming, China	Retrospective observational study	Not given	COVID-19-confirmed patients	16	Not given
Meijuan Zheng et al	March 2020	China	The First Affiliated Hospital (Hefei) and Fuyang Hospital (Fuyang)	Prospective observational study	Not given	COVID-19-confirmed patients	68	Median 47.1

Gender	Comorbidities	No. of severe cases	No. of nonsevere cases	Major findings	Reference No.
50% M	50%	6	5	Older age, higher neutrophils, high CRP and decreased T cell found in severe cases; IL-10 level was significantly varied with disease progression and treatment.	52
37.5% M	35%	13	27	Severe cases showed significant and sustained decreases in lymphocyte counts, CD8 cells, increase in IL-6, IL-10, IL-2 and IFN-γ levels compared to mild cases. The degree of lymphopenia and a pro-inflammatory cytokine storm is higher in severe COVID-19 patients than in mild cases and is associated with the disease severity.	53
64.4% M	34.20%	30	46	The CD4 + T and CD8 + T-lymphocyte counts differed significantly between the two groups, as did differences in interleukin IL-2R, IL-6 and IL-8 levels. SARS-CoV-2 RNA load and lymphocyte count, CD4 + T-lymphocyte count and CD8 + T-lymphocyte count were linearly negatively correlated.	54
35% M	24% diabetes, 25% cardiopathy and 45% hypertension	33	107	The proportion of patients with increased IL-6 and CRP levels was significantly higher in the severe group compared to mild group. Cox proportional hazard model showed that IL-6 and CRP could be used as independent factors to predict the severity of COVID-19.	64
63% M	55.50%	31	23	Lymphocytes lower, IL-2R and IL-6 higher, and prolonged PT in more critical patients.	55
55% M	50.80%	28 died, 42 in-hospital and 117 discharged		All patients exhibited a significant drop of T-lymphocyte subset counts with remarkably increasing concentrations of CRP, IL-6 and IL-10 compared to normal values. The median lymphocyte, CD3 + T cell, CD4 + T cell, CD8 + T cell and B cell were significantly lower in patients who died. Lower counts (/uL) of T-lymphocyte subsets were associated with higher risks of in-hospital death.	54
43.6% M	Not given	21	34	Patients in the severe group had a lower lymphocyte count and CD3-T cells percentage than the nonsevere group. The severe group also had a higher interleukin-6 level than the nonsevere group.	57
Not given	37.50%	6	10	Among the differentially expressed functional molecules, the levels of interferon-γ and TNF-α in CD4 + T cells were lower in the severe group than in the mild group, whereas the levels of granzyme B and perforin in CD8 + T cells were higher in the severe group.	58
53% M	Not given	13	55	The number of T cells and CD8 + T cells was significantly lower in severe patients than that in the mild cases.	59

CD8+ T) in severely diseased patients, compared to the non-severe ones, through a meta-analysis. Severity was defined according to the WHO interim guidance or the Chinese protocol in a majority (78%) of the meta-analysed studies. One study defined an oxygen saturation of ≤90% as the severe group, while another used categories of dead and discharged. For three studies, categorization into severe and non-severe groups was not described. Forest plots were generated, and mean difference was calculated using the random-effects model.

Results from the meta-analysis showed significantly higher mean levels of IL-6 (21 studies (n = 2477); mean difference: 19.55 pg/mL; CI: 14.80, 24.30), IL-8 (4 studies (n = 823); mean difference: 19.18 pg/mL; CI: 2.94, 35.43), IL-10 (6 studies (n = 956); mean difference: 3.66 pg/mL CI: 2.41, 4.92), IL-2R (3 studies (n = 747); mean difference: 521.36 U/mL; CI: 87.15, 955.57) and TNF-Alpha (6 studies (n = 956); mean difference: 1.11 pg/mL; CI: 0.07, 2.15), and significantly lower mean levels of CD4+ T cells (7 studies (n = 754); mean difference: −165.28 cells/μL; CI: −207.58, −122.97) and CD8+ T cells (6 studies (n = 686); mean difference: −106.51 cells/μL; CI: −128.59, −84.43) in the severe/critical group of patients, in contrast to their levels in the mild-to-moderate groups.

However, there was substantial heterogeneity across estimates of all the inflammatory cytokine markers (I^2 values ranging from 92% to 97%; P-value < .05; Table 2).

A sensitivity analysis was undertaken to obtain pooled estimates for each outcome after excluding preprint articles. The results from this group of peer-reviewed studies were consistent with the overall analyses. The level of heterogeneity in these two groups was also comparable.

The forest plots for each of the individual outcomes included in the meta-analysis are depicted in Figures 2-8. Every plot depicts the mean values of the biomarker in the severe and moderate groups as well as the mean difference in each study, along with the sample sizes (study-wise and cumulative), the pooled mean difference and the corresponding confidence intervals.

4.4 | Assessment of publication bias

Funnel plots were created using RevMan, for a visual assessment of the presence of a possible publication bias among studies of each outcome (File S1). A few plots demonstrated some asymmetry which was further statistically assessed using Egger's test in Stata. There was a strong evidence to suggest the possibility of a publication bias among studies for the IL-6 outcome (P-value < .001), while the evidence for other outcomes was not found to be significant. It may be important to note that given the high heterogeneity among studies for a majority of the outcomes, as well as the fact that such bias detection tests are known to be underpowered, these observations need to be interpreted with caution.

5 | DISCUSSION

Based on the reviewed studies, we found that patients diagnosed with severe COVID-19 had significantly higher levels of circulating IL-6, IL-8, IL-10, IL-2R and TNF-alpha, as compared to the patients who had a mild-to-moderate form of the disease. Similarly, in the pooled analysis, CD4+ T cells as well as CD8+ T cells were significantly reduced in severely ill patients as compared to those with mild-to-moderate disease.

Our findings corroborate with the current understanding of the COVID-19 immunopathogenesis which is largely based on findings from studies on SARS coronavirus (SARS-CoV) that bears nearly 80% nucleic acid homology with SARS-CoV-2.[65,66] Following the virus entry into the lung epithelial cells, the innate immune response is triggered leading to the first wave of hypercytokinaemia. The delayed type I interferon (IFN) response in coronavirus infections is known to be associated with more severe forms of the disease resulting in rapid viral multiplication and paradoxical hyperinflammation induced by type I interferons.[67,68] Further activation of the type I IFN signalling pathways leads to a significant influx of neutrophils, inflammatory monocytes-macrophages, dendritic

TABLE 2 Pooled estimates from meta-analysis of studies for difference in each outcome

Outcome	Number of studies	Total sample size	Mean difference (severe-moderate)	Confidence interval	Heterogeneity
IL-6 (pg/ml)	21	2477	19.55	[14.80, 24.30]	95%
IL-8 (pg/ml)	4	823	19.18	[2.94, 35.43]	92%
IL-10 (pg/ml)	6	956	3.66	[2.41, 4.92]	92%
IL-2R (U/ml)	3	747	521.36	[87.15, 955.57]	97%
TNF-alpha (pg/ml)	6	956	1.11	[0.07, 2.15]	96%
CD4+ T (cells/microL)	7	754	-165.28	[−207.58, −122.97]	62%
CD8+ T (cells/microL)	6	686	-106.51	[−128.59, −84.43]	35%

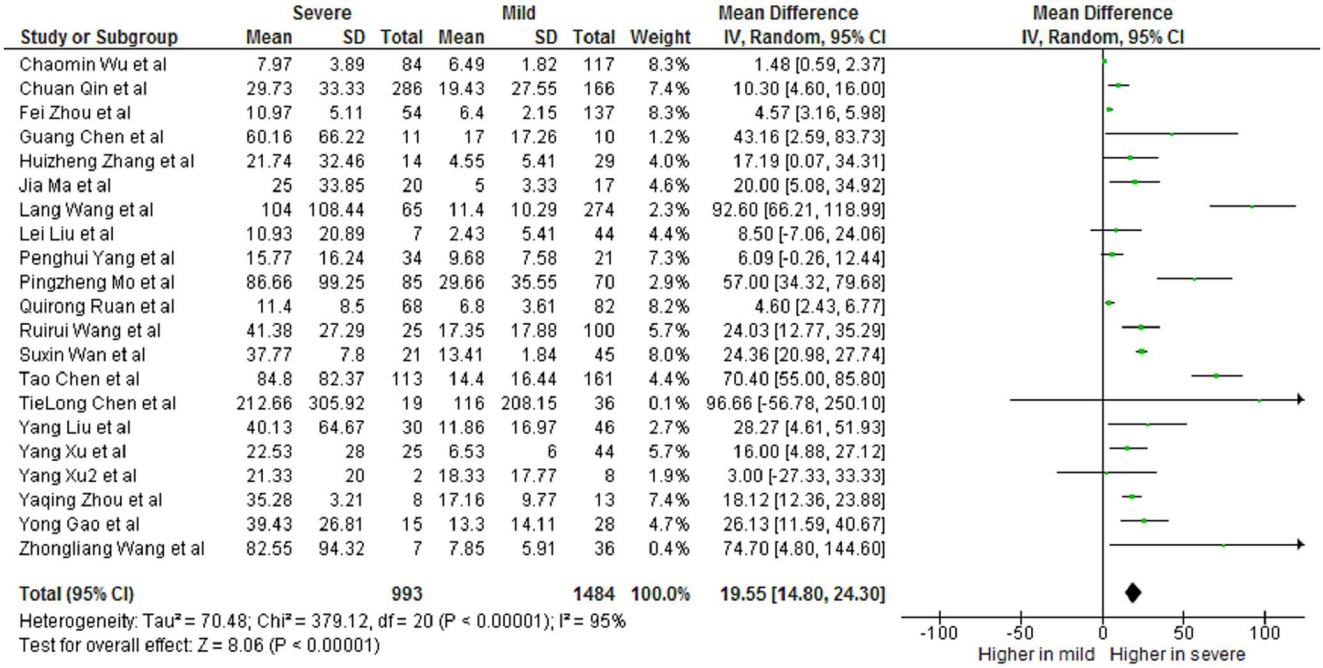

FIGURE 2 Forest plot for difference in IL-6 levels

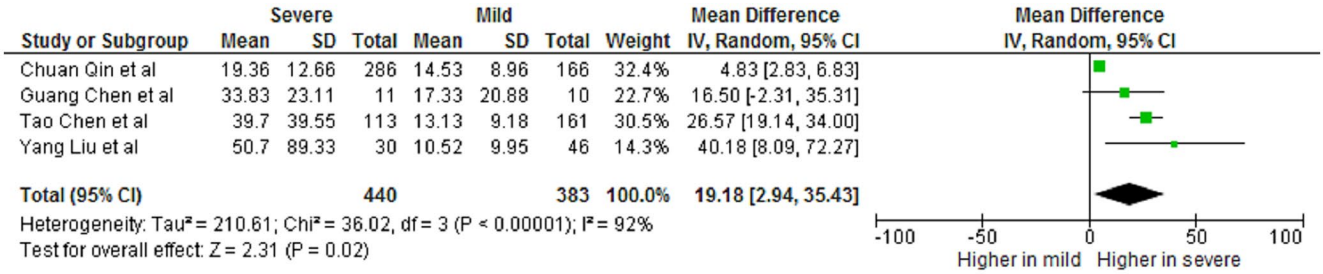

FIGURE 3 Forest plot for difference in IL-8 levels

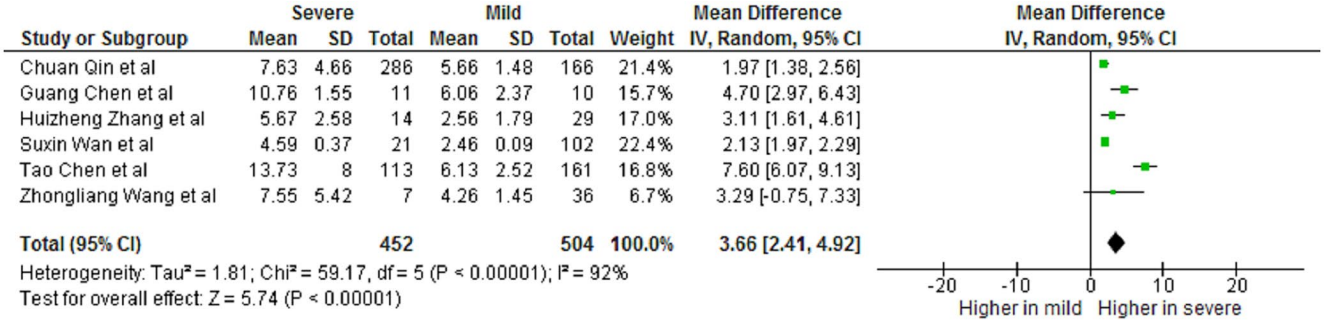

FIGURE 4 Forest plot for difference in IL-10 levels

cells and NK cells into the lungs. These infiltrating cells are the major source of inflammatory cytokines that set off the second wave—the dreaded cytokine storm.[69] A characteristic feature of severe COVID-19 is lymphopenia that has been ascribed to multiple plausible mechanisms including direct viral cytopathic effects, inhibitory effects of cytokines including TNF-alpha, IL-6 and IL-10, and immune cell redistribution into the lungs and lymphoid organs.[70-72] Diminished T-cell responses are known to further retard viral clearance, thus leading to a cytokine-driven vicious cycle. The hyperinflammatory state eventually leads to significant damage to the lung microvasculature and alveolar epithelium causing vascular leakage and alveolar oedema resulting in life-threatening acute respiratory distress syndrome (ARDS). These cytokines and chemokines have also been linked to extrapulmonary complications of COVID-19 including multiple-organ dysfunction syndrome.[73,74] In line with

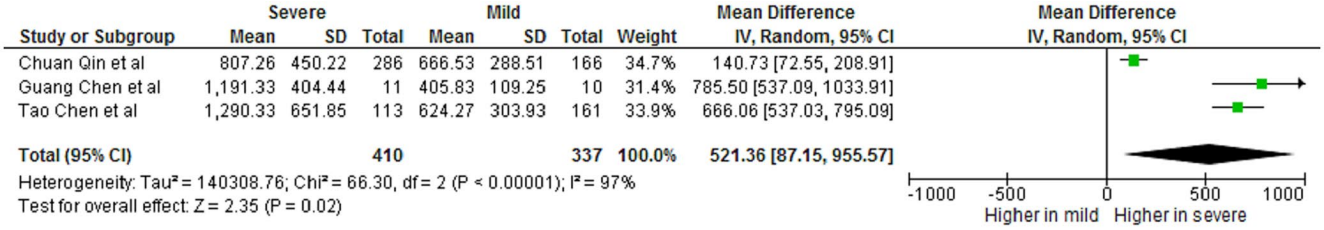

FIGURE 5 Forest plot for difference in IL-2R levels

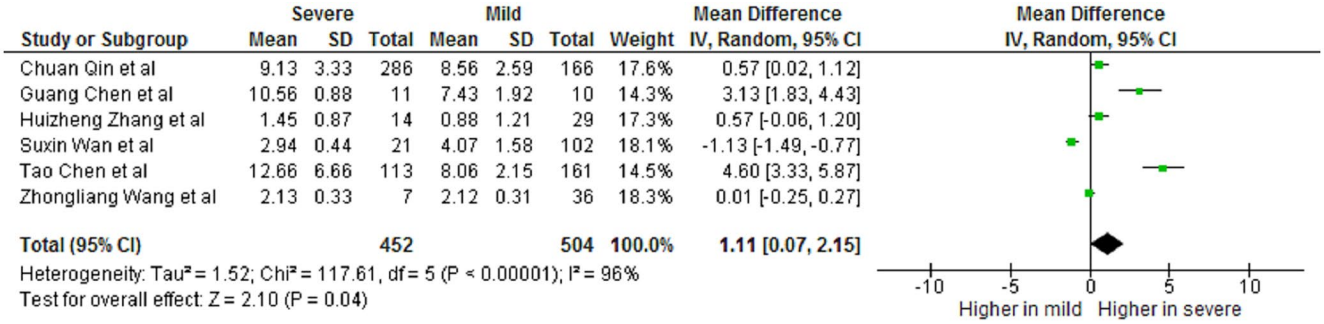

FIGURE 6 Forest plot for difference in TNF-alpha levels

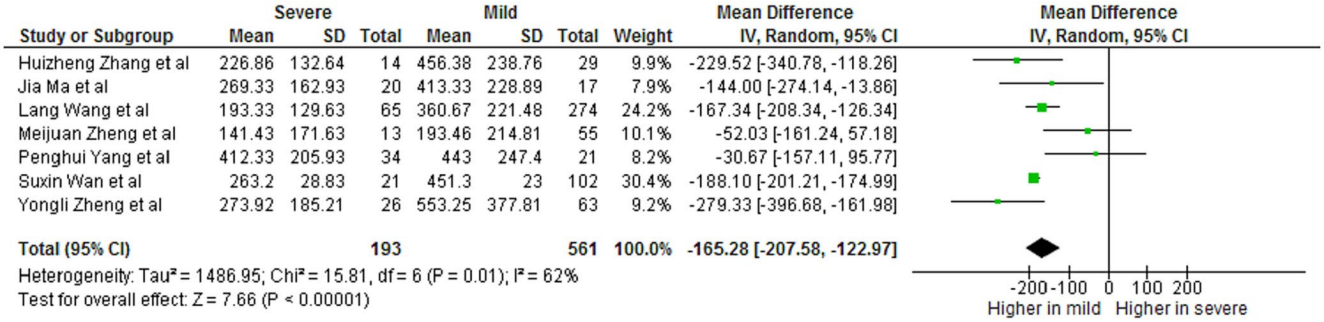

FIGURE 7 Forest plot for difference in CD4 + T-cell counts

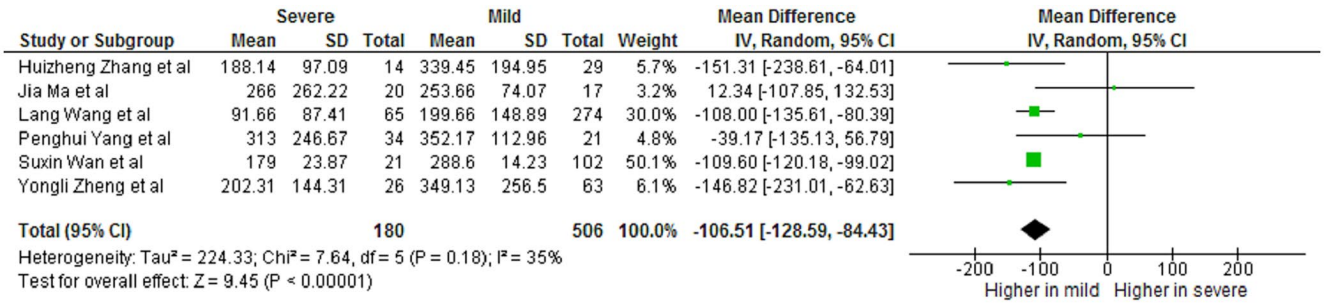

FIGURE 8 Forest plot for difference in CD8 + T-cell counts

these proposed mechanisms, we found significantly higher circulating levels of pro-inflammatory markers IL-6, IL-2R and TNF-alpha among patients diagnosed with severe COVID-19 as compared to the non-severe cases. IL-8, also known as CXCL8, is a key regulator of neutrophil and monocyte chemotaxis in the lungs and is considered to be a prognostic marker for the clinical course of ARDS.[75,76] In patients with acute lung injury, elevated plasma IL-8 levels have been associated with an increased risk of mortality, as well as reduced ventilator-free and organ failure–free days.[77] In contrast to SARS, we found elevated levels of IL-10, a T helper type 2 (Th2) cell–secreted cytokine and an inhibitor of inflammatory response, in severe COVID-19 patients as compared to non-severe cases. Higher levels of IL-10 have also been associated with increased

expression of T-cell exhaustion markers, PD-1 and Tim-3, in COVID-19 patients and their consequently impaired ability to clear viral infections especially in severely affected individuals.[78] The difference in levels of TNF-alpha though significant was not pronounced, which could be a consequence of lesser number of studies reporting the outcome as well as the rather small sample sizes of a majority of the included studies. We found a significant reduction in lymphocyte counts—both CD4+ and CD8+ in patients diagnosed with severe COVID-19. Similar findings have also been reported for SARS as well as MERS and have been linked to disease severity and adverse outcomes in these patients.[79-83] In follow-up studies in patients with SARS, it was shown that while CD8+ T lymphocytes return to normal levels in 2-3 months, memory CD4+ T cells can take up to a year for full recovery.[84]

Our findings can have important clinical implications for diagnostic as well as therapeutic purposes of COVID-19 management. Currently, there is no consensus on the utility of serial immune monitoring in patients with COVID-19. Based on our findings, cytokine—IL-6, IL-8, IL-10, IL-2R and TNF-alpha—levels can serve as potential prognostic biomarkers for risk stratification of COVID-19 patients. An important question that is yet to be answered is whether the therapeutic blockade of IL-6 is considered only in patients with elevated IL-6 levels since variability has been noted in individual studies comparing severe and non-severe cases. For this, the cut-off values for initiation of therapy will need to be defined based on well-designed and adequately powered prospective studies. Screening of COVID-19 patients for hyperinflammatory state using laboratory trends and severity grading systems such as HScore, devised originally for secondary haemophagocytic lymphohistiocytosis (sHLH), can be useful in identifying ideal candidates for immunosuppressive therapies.[85] Currently, there is significant interest in evaluating the efficacy and safety of tocilizumab, a humanized monoclonal antibody against IL-6, in COVID-19 patients as evidenced by more than 50 ongoing clinical studies across the globe.[86] The drug is currently approved for rheumatoid arthritis, giant cell arteritis and cytokine release syndrome as well as used off-label for COVID-19 as an investigational agent. However, the timing of the IL-6 blocking therapy in COVID-19 patients appears to be crucial since preclinical studies have demonstrated that IL-6 is required for viral clearance as well as control of lung inflammation.[87] On the contrary, high levels of IL-6 may also promote viral persistence through suppression of T-cell cytolytic activity.[88,89] This dilemma needs to be resolved through clinical trials aimed to determine whether early IL-6R blockade hampers viral clearance. Based on the available evidence, management of early stages of COVID-19 should focus on antiviral approaches and/or augmentation of IFN type I response. Remdesivir, an antiviral prodrug, has recently shown some benefit in patients with moderate COVID-19, indicating that targeting early stages of the illness and patients with moderate disease is perhaps a more rational approach.[90] Subsequently, in more advanced stages, use of immunomodulation strategies to control the infection-associated hyperinflammation could be critical in reducing COVID-19-linked mortality. The latter is also supported by the findings from SARS patients, where ARDS occurs despite declining viral loads, suggesting the role of hyperinflammation rather than pathogen virulence in determining adverse patient outcomes.[91] The recent findings from the RECOVERY (Randomised Evaluation of COVID-19 thERapY) trial, where low-dose dexamethasone reduced mortality by one-third in mechanically ventilated and one-fifth in oxygen-requiring patients, are in consonance with this approach. The trial enrolled more than 6000 patients, and evidently, no mortality benefit was observed in individuals not requiring respiratory support at randomization.[92]

However, our review findings need to be interpreted with caution, owing to the high heterogeneity among studies reporting inflammatory markers. Several factors could be responsible for this variation in effect estimates in some of the outcomes, like variability in sample sizes and non-uniform distribution of patients in the severe and non-severe groups. Heterogeneity might also be a consequence of weak study designs and other methodological shortcomings, studies employing varied cut-offs to categorize patients into disease severity groups and differences in the underlying comorbid conditions, as well as variability in the timing of measurement of the biomarkers. However, the fact that the statistical test for assessment of heterogeneity is largely underpowered in a meta-analysis [22] wherein studies are either very few in number or have small sample sizes should also be taken into consideration.

We undertook a sensitivity analysis with only studies that were peer-reviewed and published, excluding the preprints, and found that there was no major change in the direction and magnitude of the effects. This underscores the robustness and validity of our findings. Our results for IL-6 are consistent with the other recently published reviews comparing IL-6 levels between severe and moderate COVID-19 patients.[10-12] Nonetheless, there is a need for further high-quality prospective studies to contribute to the existing knowledge, enabling researchers to better understand the immunopathology of COVID-19.

5.1 | Strengths and limitations

To the best of our knowledge, ours is the first systematic review and meta-analysis to provide a detailed overview of the immunological and inflammatory response in severe and non-severe patients of COVID-19 and quantify the difference in various outcomes between the two groups studied. Secondly, a few previous reviews have primarily focused on the comparison of IL-6 levels between COVID-19 patients of varying disease severity, and our work provides updated evidence on the same, while exploring not just IL-6 but other

markers as well. Thirdly, considering that we have enormous literature being rapidly published, we covered 7 distinct databases and used a sensitive search strategy to obtain as many relevant studies as possible.

Our review has a few limitations. Some studies had not provided the necessary information on their methodological aspects which may have led to a possible underestimation of their quality. Also, authors were contacted regarding a possible overlap of participants in some studies conducted at a common hospital, but a majority (95%) of them did not respond to our queries. In addition, our analyses of immune responses in COVID-19 were limited by findings reported by individual studies and we could not evaluate the entire spectrum of immune-active molecules involved in the SARS-CoV-2-driven cytokine storm. Moreover, all the studies included in the meta-analysis are from China. Our literature search was conducted during the initial months of the pandemic, wherein most of the available data was from Chinese patients. This could limit the generalizability of our findings to some extent. Since levels of inflammatory markers can vary over the course of the infection, the timing of laboratory assessments can also impact the findings. However, the median time from symptom onset to hospitalization was reported by nearly one-third of the studies and it ranged from 7-11 days. Lastly, we could not include non-English articles due to unavailability of appropriate translators.

6 | CONCLUSIONS

Our findings showed that severe COVID-19 was characterized by elevated circulating levels of pro-inflammatory cytokines and lower levels of T lymphocytes, when compared to patients with mild-to-moderate disease. However, well-designed and adequately powered prospective studies are warranted to further strengthen the current evidence base. Prospective studies that follow a structured approach towards intensive immune monitoring along with daily clinical evaluations can further elucidate the mechanisms underlying COVID-19 immunopathology. Such data will be crucial in resolving the clinical dilemmas related to the timing and type of anti-COVID-19 therapy. Given the urgency of generating scientific data to help understand the disease and its short-term and long-term implications, it is understandable that efforts are underway to make research publicly available as soon as possible. But studies that are methodologically sound and involve thorough reporting of essential aspects are lacking. We believe the several ongoing studies evaluating the role of immunomodulation in severe COVID-19 patients can hopefully provide further clarity, and these therapies may prove instrumental in tackling hyperinflammation to improve patient management and clinical outcomes.

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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