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

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Review article

The role of cytokine profile and lymphocyte subsets in the severity of coronavirus disease 2019 (COVID-19): A systematic review and meta-analysis

Hamed Akbari[a,b,1], Reza Tabrizi[c,1], Kamran B. Lankarani[c], Hamid Aria[d,e], Sina Vakili[f], Fatemeh Asadian[g], Saam Noroozi[h], Pedram Keshavarz[i], Sanaz Faramarz[b,*,**]

[a] Department of Biochemistry, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[b] Student Research Committee, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
[c] Health Policy Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[e] Bioinformatics and Computational Biology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[f] Biochemistry Department, Medical School, Shiraz University of Medical Sciences, Shiraz, Iran
[g] Department of Medical Laboratory Sciences, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
[h] Department of Biochemistry, School of Medicine, Fasa University of Medical Sciences, Fasa, Iran
[i] Medical Imaging Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

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ABSTRACT

Aims: This study aimed to make a comparison between the clinical laboratory-related factors, complete blood count (CBC) indices, cytokines, and lymphocyte subsets in order to distinguish severe coronavirus disease 2019 (COVID-19) cases from the non-severe ones.

Materials and methods: Relevant studies were searched in PubMed, Embase, Scopus, and Web of Science databases until March 31, 2020. Cochrane's Q test and the I^2 statistic were used to determine heterogeneity. We used the random-effect models to pool the weighted mean differences (WMDs) and 95% confidence intervals (CIs).

Key findings: Out of a total of 8557 initial records, 44 articles (50 studies) with 7865 patients (ranging from 13 to 1582), were included. Our meta-analyses with random-effect models showed a significant decrease in lymphocytes, monocyte, CD4+ T cells, CD8+ T cells, CD3 cells, CD19 cells, and natural killer (NK) cells and an increase in the white blood cell (WBC), neutrophils, neutrophil to lymphocyte ratio (NLR), C-reactive protein (CRP)/hs-CRP, erythrocyte sedimentation rate (ESR), ferritin, procalcitonin (PCT), and serum amyloid A (SAA), interleukin-2 (IL-2), IL-2R, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (INF-γ) in the severe group compared to the non-severe group. However, no significant differences were found in IL-1β, IL-17, and CD4/CD8 T cell ratio between the two groups.

Significance: Decrease in total lymphocytes and lymphocyte subsets as well as the elevation of CRP, ESR, SAA, PCT, ferritin, and cytokines, but not IL-1β and IL-17, were closely associated with COVID-19 severity, implying reliable indicators of severe COVID-19.

1. Introduction

Breaking out for the first time in Wuhan, China, in December 2019, the new infectious primary atypical pneumonia pandemic has formally been named as Coronavirus Disease 2019 (COVID-19), and moreover, its causative virus as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) [1,2]. An overall number of 8,486,923 verified patients around the world were reported as of June 18, 2020, which involved 452,396 deaths [3].

Recent studies have shown, that apart from dyspnea, hypoxemia, and acute respiratory distress, lymphopenia, and cytokine release syndrome are among the characteristics of severe SARS-CoV-2 infection, suggesting role of cytokine storm in progression of disease. In cytokine storm syndrome, the amount of proinflammatory cytokine is

* Corresponding author at: Student Research Committee, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
** Corresponding author at: Department of Clinical Biochemistry, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
E-mail address: sanaz.faramarz68@gmail.com (S. Faramarz).
[1] Contributed equally to this article.

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dramatically elevated after microorganisms or medications stimulate the body, resulting in dysfunctions in the immune system [4]. Multiple organ dysfunction syndrome (MODS), acute respiratory distress syndrome (ARDS) and even death are the probable outcomes of this phenomenon [5,6].

Considering the fast dissemination of COVID-19 and the increased mortality in extreme cases, it is desperately required to better understand clinical features and to identify reliable laboratory inflammatory markers that can differentiate between severe-to-critical and mild-to-moderate infections. These data may also help to better understand pathogenesis of this emerging infection. Nonetheless, the precise role that cytokines, lymphocyte subsets, and infection-related factors play in the severity and progression of the disease is yet to be found. Therefore, the present study was conducted aiming to analyze the different characteristics of cytokine levels (Interleukin-1beta (IL-1β), IL-2, IL-2R, IL-4, IL-6, IL-8, IL-10, IL-17, tumor necrosis factor-alpha (TNF-α), and interferon-gamma (INF-γ)), lymphocyte subsets (CD3 cells, CD4+ T cells, CD8+ T cells, CD4/CD8 T cell ratio, CD19 cells, and natural killer (NK) cells), complete blood count (CBC) indices, and a number of infection-related factors (C-reactive protein (CRP)/hs-CRP, erythrocyte sedimentation rate (ESR), ferritin, procalcitonin (PCT), and serum amyloid A (SAA)) between mild/moderate and severe/critical patients, and further to screen out suitable indicators for the prediction of the disease severity in order to provide some insight into the subsequent clinical interventions.

2. Methods

2.1. Search strategy and selection criteria

This systematic reviews and meta-analyses was carried out in accordance with PRISMA [7]. The protocol for the review was registered with PROSPERO (Provisional registration number: CRD42020178847).

The relevant literature on the issue was identified through an online search in PubMed, Embase, Scopus, and Web of Science for studies published as of March 31, 2020. Furthermore, to improve search sensitivity, no filters or limits were used on time and language and all the included studies written in English or Chinese languages were adopted. It should be noted that the reviews in the Chinese language were translated by https://translate.google.com/ (the retrieval process is shown in Fig. 1). The medical subject headings (MESH) and the keywords searched included 'betacoronavirus' or 'betacoronavirus 1' or 'coronavirus Infection' or 'coronavirus' or 'SARS-2-CoV' or 'COVID-19' and 'inflammation' or 'cytokines' or 'C-reactive protein' or 'Interleukin-1beta' or 'interleukin-6' or 'Interleukins' or 'tumor necrosis factor-alpha' or 'antigens, CD' or 'lymphocyte subsets' or 'killer cells, natural' or 'procalcitonin' or 'blood sedimentation' or 'ferritins' or 'serum amyloid A protein'. The list of titles and abstracts and the full text of the selected manuscripts were independently examined by two reviewers (HA and SF). The disagreements as to what manuscripts to select during both title and abstract examination, and the subsequent full-text analysis, were addressed until a conclusion was reached. Besides the above-mentioned databases, the identification of any remaining relevant published studies was performed using citation tracking. Moreover, the studies which were not published were retrieved from the medRxiv website.

All the studies which have addressed the inflammatory-related laboratory factors in predicting severe COVID-19 infection were incorporated. All studies with various designs conducted since the outbreak (in December 2019) were considered as ineligible, however, repeat articles, case reports, case series, reviews, letters, editorials, short communication, animal trials, correspondence, guidance, radiology studies, meeting reports, and expert opinions were considered as illegible. The exclusion criteria included: (1) studies regarding particularly pediatric or pregnant cases due to the diverse presentation of COVID-19 in these groups, (2) inadequate information on inflammatory-related laboratory parameters in either severe or non-severe disease groups, (3) coronavirus strains other than COVID-19, (4) and studies with unusable data. Nonetheless, the diagnostic criteria for COVID-19 were explained on the basis of laboratory approved SARS-CoV-2 infection. If two or more studies were published by the same authors or institutions, only the study having the largest sample size was selected.

The data from the incorporated studies were extracted by two reviewers (SV and SF) independently. Also, a third reviewer (RT) was used to solve any arisen argument. The details of each study were collected which involve author, publication date, study location, study design, sample size, sample characteristics (age, gender, comorbidities), exposure characteristics (study definition of severity of COVID-19, the timing of classification of disease severity [on admission or otherwise], number of cases with non-severe COVID-19, number of cases with severe or critical COVID-19), the timing of blood sample collection (on admission or otherwise). Moreover, inflammatory-related laboratory factors, cytokines, lymphocyte subsets, and CBC indices were grouped by COVID-19 severity (mean [SD]) and finally, all the extracted data were transferred into Microsoft excel. Furthermore, through rechecking the primary studies, as well as discussions, any inconsistencies in the extracted data were resolved. It is worth mentioning that, using web plot digitizer online software, some graph data were converted to numerical data (https://apps.automeris.io/wpd/). In case the relevant data were missing, authors of selected studies were contacted via email. Also, it should be noted that due to inaccuracies in the research methodology for some of the studies, we reported the type of study in some articles, especially those submitted in the medRxiv, by inference.

The included studies differed in the way they defined patients' disease status, and classified the disease into 'mild, moderate, severe and critical', 'ordinary and severe/critical', 'common and severe', and 'non-severe and severe', categories. The first outcome measure adopted was severe (including both severe and critical cases) vs. non-severe disease. The definition provided for the severity of the disease was based on the New Coronavirus Pneumonia Prevention and Control Program (6th edition) published by the National Health Commission of China [8]. (1) mild: non-pneumonia patients as shown by imaging; (2) moderate: pneumonia-diagnosed patients as shown by their symptoms and the imaging examination; (3) severe: patients with any of the following factors: (i) respiratory rate equal to or higher than 30/min; (ii) resting pulse oxygen saturation (SpO_2) equal to or lower than 93%; (iii) oxygen partial pressure (PaO_2)/fraction of inspired oxygen (FiO2) equal to or lower than 300 mmHg (1 mmHg = 0.133 kPa); (iiii) imaging process showing a 50% progression in multiple pulmonary lobes of a lesion in 24–48 h; (4) critical: patients with any of the following factors: (i) the need for mechanical ventilation in case of respiratory failure (ii) shock; (iii) admission to the intensive care unit (ICU) due to simultaneous failure in another organ. It is noteworthy that mild or moderate patients were included in the non-severe group, while severe or critical patients were included in the severe one. The Newcastle-Ottawa Scale was used to evaluate quality, and moreover, assessment scores of 0–3, 4–6, and 7–9 represented poor, fair, and good studies, respectively. Additionally, discrepancies were resolved through consensus.

2.2. Statistical analysis

All statistical analyses were conducted using STATA version 12.0 (Stata Corp., College Station, TX). Laboratory factors were considered as the mean (SD) difference with 95% confidence intervals (CIs) between the severe group and the non-severe group. To pool the mean differences (SD), weighted mean difference (WMD) statistic with the random-effect model (DerSimonian–Laird method) were used. Cochrane's Q test or the I^2 statistic was used to assess heterogeneity among included studies. I^2 above 70% and Cochrane's Q test with $P < 0.05$ was considered as the existence of significant heterogeneity. Sensitivity analysis was used to evaluate the robustness of meta-

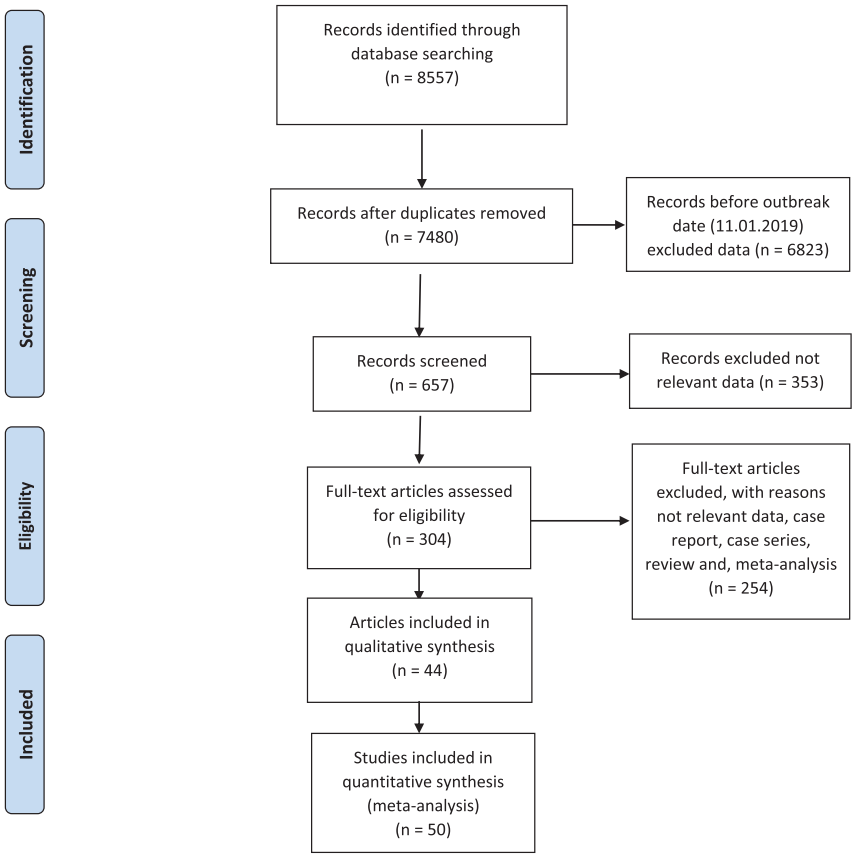

Fig. 1. The flowchart of study identification and study selection process.

analyses findings with applying the leave-one-out method after removing one by one included study on the pooled WMDs. Egger regression and Begg's rank correlation tests were applied to detect the potential evidence of publication bias between included studies.

3. Results

We yielded a total of 8557 records through initial online search in databases. Of these, 1077 were duplicate. After screening based on title and abstract, 304 articles were selected as the candidates for assess according to inclusion and exclusion criteria. Finally, 44 articles (50 studies) were identified to be eligible for current meta-analysis. Fig. 1 shows the flowchart of study identification and selection process.

All selected studies contained a total of 7865 (ranging from 13 to1582) patients including 2286 in the severe group and 5579 in the non-severe group. Forty-three of all included articles were conducted in China and one [9] of them was performed in USA.

Most of assessments on laboratory tests among included patients were conducted on admission period/before treatment. The characteristics of included studies are summarized in Table 1.

3.1. Pooled findings on blood cell counts

Using random-effects model, our meta-analyses indicated a significant decrease in the WMD of lymphocytes (n = 46, WMD = −0.41 × 109/L, 95% CI: −0.48, −0.34, I^2 = 85.9%, Q-value = 318.92, P < 0.001) and monocytes levels (n = 18, WMD = −0.04 × 109/L, 95% CI: −0.89, −0.01, I^2 = 55.9%, Q-value = 38.57, P = 0.002), and increased in the WMD of WBC (n = 42, WMD = 0.87 × 109/L, 95% CI: 0.50, 1.24, I^2 = 76.1%, Q-value = 171.58, P < 0.001) and neutrophils levels (n = 33, WMD = 1.27 × 109/L, 95% CI: 0.89, 1.65, I^2 = 71.9%, Q-value = 113.85, P < 0.001) in the severe group as compared to the non-severe group. The pooled finding of NLR showed a significant change in WMD (n = 5, WMD = 3.97, 95% CI: 1.97, 5.96, I^2 = 82.1%, Q-value = 22.37, P < 0.001) between two groups (Appendix 1a, Fig: A–E).

3.2. Pooled findings on infection-related biomarkers

Our results indicated a significant increase in the WMD of CRP (n = 37, WMD = 41.07 mg/L, 95% CI: 29.76, 52.38, I^2 = 98.5%, Q-value = 2430.53, P < 0.001), ESR (n = 13, WMD = 23.39 mm/h, 95% CI: 16.51, 30.27, I^2 = 77.1%, Q-value = 56.75, P < 0.001), PCT (n = 29, WMD = 0.07 ng/mL, 95% CI: 0.05, 0.09, I^2 = 96.5%, Q-value = 810.39, P < 0.001), SAA (n = 5, WMD = 90.45 mg/L, 95% CI: 28.69, 152.21, I^2 = 90.6%, Q-value = 42.37, P < 0.001), and ferritin levels (n = 8, WMD = 594.25 μg/L, 95% CI: 438.10, 750.39, I^2 = 76.9%, Q-value = 30.24, P < 0.001) in the severe group in comparison with the non-severe group (Appendix 1B, Fig: A–E).

3.3. Pooled findings on cytokines

The pooled finding on cytokines showed a significant increase in the WMD of IL-2: (n = 9, WMD = 0.28 U/mL, 95% CI: 0.19, 0.37, I^2 = 33.2%, Q-value = 11.97, P = 0.152), IL-2R: (n = 5, WMD = 339.75 pg/mL, 95% CI: 162.94, 516.55, I^2 = 95.5%, Q-value = 88.41, P < 0.001), IL-4 (n = 10, WMD = 0.15 pg/mL, 95% CI: 0.04, 0.26, I^2 = 67.9%, Q-value = 28.03, P = 0.001), IL-6 (n = 23, WMD = 17.79 pg/mL, 95% CI: 14.24, 21.33, I^2 = 98.6%, Q-value = 1583.20, P < 0.001), IL-8 (n = 4, WMD = 5.82 pg/mL, 95% CI: 1.20, 10.44, I^2 = 12.9%, Q-value = 3.44, P = 0.328), IL-10 (n = 16, WMD = 2.24 pg/mL, 95% CI: 1.94, 2.54, I^2 = 51.4%, Q-value = 30.87, P = 0.009), TNF-α (n = 17, WMD = 0.24 pg/mL, 95%

Table 1
Characteristics of included studies.

Authors (Ref)	Publication year	Country	Blood sampling time	Study type[a]	Severe patients	Non-severe patients	Severe, Non-Severe (Male/female)	Quality assessment (score)
Chen (Lei) (a) [12]	2020	China	On hospital admission	Cross-sectional	Critical	Ordinary	NR	3
Chen (Lei) (b)	2020	China	On hospital admission	Cross-sectional	Severe	Ordinary	NR	3
Wan (Suxin) #1 [13]	2020	China	On hospital admission	Retrospective single-center (case-control)	Severe	Mild	11/10, 55/47	4
Chen (Guang) [14]	2020	China	On hospital admission	Cross-sectional	Severe	Moderate	10/11, 7/10	3
Wang (Zhongliang) [15]	2020	China	On hospital admission	Retrospective (case-control)	SpO2 < 90%	SpO2 ≥ 90%	7/7, 25/30	6
Huang [16]	2020	China	After hospital admission (2–5 days)	Retrospective cross-sectional	ICU	Non-ICU	11/2, 19/9	5
Song [17]	2020	China	On hospital admission	Retrospective Cross-sectional	Severe/critical	Mild/moderate	46/27, 104/127	4
Qin [10]	2020	China	On hospital admission	Retrospective single-center (case-control)	Severe	Non-severe	155/131, 80/86	6
Yang [18]	2020	China	On hospital admission	Cross-sectional	Severe	Moderate	22/34, 9/19	5
Nie (a) [19]	2020	China	On hospital admission	Retrospective Cross-sectional	Severe	Mild 1 (14 days disease duration)	13/12, 21/51	4
Nie (b)	2020	China	On hospital admission	Retrospective Cross-sectional	Severe	Mild 2 (30 days disease duration)	NR	4
Zhang (Huizheng) [20]	2020	China	On hospital admission	Retrospective Cross-sectional	Severe	Mild	5/9, 17/12	4
Diao [21]	2020	China	On hospital admission	Retrospective Cross-sectional	ICU	Non-ICU	NR	5
Liu (Tao) [22]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Non-severe	33/36, 1/10	6
Liu (Jing) [23]	2020	China	On hospital admission (≤3 days)	Retrospective single-center (case-control)	Severe	Mild	7/6, 8/19	5
Lu [24]	2020	China	On hospital admission	Retrospective, single-center cohort	Severe/critical	Mild/moderate	NR	6
Cao (Min) [25]	2020	China	On hospital admission	Cross-sectional	ICU	Non-ICU	17/2, 84/95	7
Han [26]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Non-severe	17/7, 9/14	5
Ma [27]	2020	China	On hospital admission	Cohort	Severe	Non-severe	12/8, 36/28	6
Liu (Lei) [28]	2020	China	On hospital admission	Retrospective, single-center (case-control)	Severe	Non-severe	4/3, 28/16	5
Zhang (Jin-Jin) [29]	2020	China	On hospital admission	Cross-sectional	Severe	Non-severe	33/24, 38/44	6
Xu [30]	2020	China	On hospital admission	Retrospective, multi-center (case-control)	Severe/critical	Mild	13/12, 22/22	7
Gao [31]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Mild	9/6, 17/11	4
Liu (Zeming) [32]	2020	China	On hospital admission	Retrospective (case-control)	Severe/critical	Mild/moderate	NR	3
Shi [33]	2020	China	On hospital admission	None (case-control)	Severe	Mild	17/14, 18/7	5
Wang (Dawei) [15]	2020	China	On hospital admission	Retrospective (case-control)	ICU	Non-ICU	22/14, 53/49	6
Petrilli [9]	2020	USA	On hospital admission	Cross-sectional	Critical	No critical	560/208, 442/372	5
Li (Huan) (a) [34]	2020	China	On hospital admission	Retrospective (case-control)	Critically severe	Mild/moderate	44,110	6
Li (Huan) (b)	2020	China	On hospital admission	Retrospective (case-control)	Severe	Mild/moderate	37/19, 28/32	6
Wang (Fan) [35]	2020	China	On hospital admission	None (case-control)	Severe	Mild	NR	6
Zhang (Hongyan) [36]	2020	China	On hospital admission	Multicenter, retrospective, (case-control)	Severe	Mild	23/9, 18/17	4
Cao (Weiliang) [25]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Non-severe	12/9, 48/59	4
Qian [37]	2020	China	On hospital admission	Retrospective, multi-center (case-control)	Severe	Non-severe	NR	5
Liu (Min) [38]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Common	NR	5
wan (Suxin) #2 [39]	2020	China	On hospital admission	(Case-control)	Severe	Mild	21/19, 52/43	5
Li (Kunhua) [40]	2020	China	On hospital admission	Retrospective (case-control)	Severe/critical	Ordinary	15/10, 29/29	5
Guan [41]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Non-severe	73/100, 386/540	6
Zheng (F) [42]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Non-severe	14/16, 66/65	4
Zhu [43]	2020	China	On hospital admission	Single-center, retrospective, (case-control)	Severe	Non-severe	24/19, 43/28	8
Cai (Qingxian) [44]	2020	China	On hospital admission	Retrospective (case-control)	Severe	Non-severe	39/17, 106/134	8
Zhang (Fengqin) (a) [45]	2020	China	On hospital admission	Retrospective (case-control)	Critical	Common	2/7, 25/21	6
Zhang (Fengqin) (b)	2020	China	On hospital admission	Retrospective (case-control)	Severe	Common	18/8, 25/21	6

(*continued on next page*)

Table 1 (*continued*)

Authors (Ref)	Publication year	Country	Blood sampling time	Study type[a]	Severe patients	Non-severe patients	Severe, Non-Severe (Male/female)	Quality assessment (score)
Deng (a) [11]	2020	China	On hospital admission	Observational (Cross-sectional)	Critical	Ordinary	NR	4
Deng (b)	2020	China	On hospital admission	Observational (Cross-sectional)	Severe	Ordinary	NR	4
Zheng (Yishan) [46]	2020	China	On hospital admission	None (case-control)	Severe	Non-severe	5/3, 36/35	4
Liu (Jingyuan) [47]	2020	China	On hospital admission	Prospective single-center	Severe/critical	Common	10/7, 21/23	5
Liu (Songqiao) (a) [48]	2020	China	On hospital admission	Retrospective multicenter cohort study	severe/critical	Asymptomatic/Mild	35/18, 247/223	9
Liu (Songqiao) (b)	2020	China	On hospital admission	Retrospective multicenter cohort study	Severe/critical	Moderate	35/18, 44/53	9
Liu (Yanli) [49]	2020	China	On hospital admission	Retrospective (case-control)	ARDS	Non-ARDS	28/25, 31/25	8
Yudong [50]	2020	China	On hospital admission	Retrospective (case-control)	Critical	Ordinary	9/7, 44/52	5

[a] Study design whether stated clearly in methods or perceived from methods.

CI: 0.01, 0.47, I^2 = 83.4%, Q-value = 96.67, P < 0.001), and INF-γ levels (n = 11, WMD = 0.58 pg/mL, 95% CI: 0.30, 0.86, I^2 = 71.2%, Q-value = 34.70, P < 0.001) in the severe group in comparison with the non-severe group. No significant differences were observed in the levels of IL-17 (n = 3, WMD = 1.13 pg/mL, 95% CI: −1.39, 3.64, I^2 = 44.1%, Q-value = 3.58, P < 0.167) and IL-1β (n = 3, WMD = −0.09 pg/mL, 95% CI: −0.50, 0.33, I^2 = 0.0%, Q-value = 0.65, P = 0.723) between the two groups (Appendix 1c, Fig: A–J).

3.4. Pooled findings on lymphocyte subsets

Our meta-analyses indicated a significant decrease in the WMD of CD4+ T cells (n = 21, WMD = −218.34 (cell/μL), 95% CI: −253.09, −183.58, I^2 = 74.5%, Q-value = 78.38, P < 0.001), CD8+ T cells (n = 21, WMD = −148.23 (cell/μL), 95% CI: −176.92, −119.53, I^2 = 84.5%, Q-value = 129.06, P < 0.001), CD3 cells (n = 14, WMD = −441.71 (cell/μL), 95% CI: −551.96, −331.47, I^2 = 87.3%, Q-value = 102.29, P < 0.001), CD19 cells (n = 15, WMD = −33.27 (cell/μL), 95% CI: −44.79, −21.76, I^2 = 28.1%, Q-value = 19.46, P = 0.148), NK cells levels (n = 14, WMD = −43.75 (cell/μL), 95% CI: −57.22, −30.28, I^2 = 44.8%, Q-value = 23.54, P = 0.036) in the severe group as compared to the non-severe group. The pooled finding on CD4/CD8 T cell ratio showed not significant changes in WMD (n = 12, WMD = 0.08, 95% CI: −0.12, 0.28, I^2 = 74.9%, Q-value = 43.74, P < 0.001) between two groups (Appendix 1d, Fig: A–F).

3.5. Sensitivity analysis

We found no significant differences between the pre- and post-sensitivity pooled effect sizes by removing one by one study for WBC, neutrophil, lymphocyte, monocyte, NLR, CRP, ESR, PCT, SAA, ferritin, IL-1, IL-2, IL-2R, IL-4, IL-6, IL-10, INF-γ, IL-17, CD4+ T cells, CD8+ T cells, CD4/CD8 T cell ratio, CD3 cells, CD19 cells, and NK cells. But after excluding Qin et al., [10] the study on IL-8, (WMD = 10.56 pg/mL, 95%CI: −1.52, 22.64) and Deng et al. (b) [11], the study on TNF-α (WMD = 0.18 pg/mL, 95%CI: −0.03, 0.40), the sensitivity findings showed that there was a significant differences between pre- and post-sensitivity pooled WMD for these outcomes.

3.6. Publication bias

Potential publication bias across included studies was examined using the Egger's regression and Begg's rank correlation tests. These showed no significant evidence of publication bias for monocyte, NLR, IL-1, IL-2, IL-2R, IL-4, IL-8, IL-10, TNF-α, INF-γ, CD4+ T cells, CD4/CD8 T cell ratio, CD19 cells, and NK levels. Because there was evidence of publication bias on WBC [Egger (p < 0.01), Begg (P < 0.01)], neutrophil [Egger (p = 0.02), Begg (P < 0.01)], lymphocyte [Egger (p < 0.01), Begg (P = 0.38)], CRP [Egger (p < 0.01), Begg (P = 0.36)], ESR [Egger (p = 0.02), Begg (P = 0.87)], PCT [Egger (p < 0.01), Begg (P = 0.68)], SAA [Egger (p = 0.04), Begg (P = 0.14)], ferritin [Egger (p < 0.01), Begg (P = 0.21)], IL-6 [Egger (p < 0.01), Begg (P = 0.21)], IL-17 [Egger (p = 0.09), Begg (P = 0.11)], CD8+ T cells [Egger (p = 0.04), Begg (P = 0.09)], CD3 cells [Egger (p = 0.01), Begg (P = 0.29)], and LCR [Egger (p < 0.01), Begg (P < 0.01)], we used the Trimming estimator with linear method to include the findings of censored studies. There were no significant differences between before and after including censored studies for neutrophil, lymphocyte, CRP, ESR, PCT, SAA, ferritin, IL-6, IL-17, CD8+ T cells and CD3 cells, but there was significant difference for WBC before (WMD = 0.87 mg/L; 95% CI, 0.05, 1.24) and after (WMD = 0.23 mg/L; 95% CI, −0.16, 0.62).

4. Discussion

To the best of authors' knowledge, this is the first and the most comprehensive systematic review and meta-analysis that investigated the differences between severe and non-severe confirmed COVID-19 cases in terms of inflammatory-related laboratory tests along with cytokines, lymphocyte subsets and some CBC indices. According to the findings, the severity of COVID-19 has a significant, positive association with CRP/hs-CRP, ESR, PCT, SAA, and ferritin levels. Moreover, with respect to CBC indices, the findings revealed significantly higher levels in WBC and neutrophil, while lower lymphocyte and monocyte levels in severe than in non-severe confirmed COVID-19 patients. Furthermore, it was shown that, rather than IL-1β and IL-17, the circulating levels of all the investigated pro-inflammatory cytokines were significantly higher in severe vs. non-severe COVID-19 patients. Additionally, except for the CD4/CD8 T cell ratio, the levels of the investigated CD markers along with the total number of lymphocytes, were significantly lower in the severely infected cases than in non-severe ones. The number of NK cells and monocytes were also decreased in the severe group.

CRP/hs-CRP and ESR have been found to be increased in a vast number of inflammations/infections [46,51]. In this new pandemic pneumonia, the levels of CRP and ESR significantly increased in severe cases compared to non-severe COVID-19 patients [31,45], which greatly coincides with those found in the present systematic review and meta-analysis. In the present study, PCT concentrations were significantly higher in severe/critical patients than in non-severe cases. As it was previously shown, that is, the PCT does not increase with virus infections, it may indicate superimposed bacterial infection for the critically ill patients [47,52]. SAA, another important factor capable of improving inflammatory response through activation of chemokine and induction of chemotaxis even at a very low concentration [53], was found to have elevated circulating levels in severe patients and both were significantly related to COVID-19 severity. The critically ill patients were shown to have higher expressions of IL-1β, IL-6, TNF-α, and other cytokines, which boost SAA production by liver cells [16]. Likewise, induced by the activated macrophages, which produce TNF-α, ferritin was seen to undergo the same changes as SAA. An excessive amount of ferritin is also reflective of a surplus of TNF-α, which is a major apoptotic factor [16]. Consequently, these inflammatory-related factors might function as a biomarker to monitor the progression of respiratory diseases.

The higher level of IL-2 in COVID-19 patients is possibly indicative of T cell activation. An important pro-inflammatory cytokine, IL-6 can put an end to the activation of normal T cells, which may be a reason for the presence of lymphopenia. A study carried out by Gong et al. [54] showed that although levels of IL-2R and IL-10 were associated with the severity of the disease, they principally contributed to the inhibition of the inflammatory response. Consequently, this hypothesis developed further this idea that it may suggest the simultaneous inflammatory and anti-inflammatory reaction. The highly increased levels of IL-10 in severely infected patients might account for the negative feedback on the systemic and local inflammation. However, what role immunosuppression plays in the progression of the disease and whether IL-10 and IL-2R are possible therapeutic targets are yet to be answered by further research. Besides, COVID-19 infection was found to induce augmented secretion of T-helper-2 (Th2) cytokines (e.g., IL-4 and IL-10) that suppress inflammation; a finding which coincides with that of the present study but differs from SARS-CoV infection [55]. An important anti-viral cytokine generated by CD4+ T cells, CD8+ T cells, NK cells, and macrophages, IFN-γ has been reported to contribute to the cytokines storm in SARS patients [56,57]. The present study showed that levels of IFN-γ in severely infected cases were higher than those of the non-severe COVID-19 patients, suggesting that IFN-γ may efficiently indicate the status of the disease. Besides, IL-1β and IL-17 were found not to be significantly associated with the COVID-19 severity, which might be due to few numbers of included studies, hence the need for further studies to explain the role that these cytokines play in the progression of the disease. Albeit no significant association was found by some studies between the COVID-19 pneumonia severity and IL-6, IL-10, and TNF-α, this systematic review and meta-analysis indicated that IL-6, IL-10, and TNF-α could be used to assess the severity of COVID-19 and that they might be potential targets for immunotherapy of COVID-19.

The association found between lymphopenia and severity of the COVID-19 implies that, as does SARS-CoV, SARS-CoV-2 might act on lymphocytes, especially T types, hence possibly leading to the depletion of CD4+ T and CD8+ T cells [58]. The exhaustion of CD8+ T cells in severe patients may reduce their cellular immune response to SARS-CoV-2. The study conducted by Li et al. [59] showed that these multifunctional CD4+ T cells were much frequently seen in patients severely infected with SARS than in mild cases, suggesting the unique immune pathology of SARS-CoV-2 in comparison to other coronaviruses. Besides, given the significant NK cells decrease in the severe group compared to the non-severely infected cases, it can be said that the COVID-19 infection severity could be restricted by the activity of NK cells. Considering that immune adjuvant IL-2 can enhance the functioning of NK cells, there could be a new target for clinical treatment [55]. Moreover, the lower numbers of NK cells and monocytes in the severe group may also support the hypothesis that the greater role that innate immunity plays in the determination of the disease course and in the effectivity of acquired immunity in the control of this infection. This finding may have therapeutic implications as well as prophylactic importance. In this regard, any intervention promoting the innate immune system might have beneficial effects both as a preventive and therapeutic measure.

It was found that the ratio of CD4/CD8 T cell ratio in severe and non-severe COVID-19 infected groups did not change, which may indicate that CD4+ T and CD8+ T cells were equally reduced in both groups. Based on the current findings, lower levels of investigated CD markers represent inefficiency in the immune activation and a poor virus-specific T and B cell response accounts for the severe disease in SARS-CoV-2 infected patients. Collectively, these disorders in lymphocyte subsets might lead to the eventual reduction of the host antiviral immunity.

The present findings demonstrated a significant lymphopenia in severe/critical infected cases compared to mild/moderate COVID-19 ones across 46 studies. Lymphopenia assumes a high significance during infection with COVID-19, and it is under debate as to what its reasons are. It may be due to the direct contribution of the virus or redistribution of WBC via chemotaxis or apoptosis [16,41,60]. Compared to non-severe ones, severe cases are older and have comorbidity diseases [61–63], making them more susceptible to endothelial dysfunction and its associated lymphopenia. The fact that the present study showed elevated leukocyte levels in severe compared to non-severe patients across 42 studies is a novel and controversial finding. Moreover, neutrophils and monocytes decreased and increased in severe cases, respectively, which need further investigations in future studies. Regarded as a well-known marker for systemic inflammation and infection, NLR has been examined as a predictor of bacterial infection including pneumonia [52,64]. The elevated levels of NLR found in the present study suggest that the internal environment was seriously disturbed and that the severely infected cases were in a potentially critical condition. Liu et al. [47] revealed that the area under curve (AUC), c-index, sensitivity, and specificity for NLR were at a high level, suggesting that NLR is a reliable index of predicting the incidence of severe illness in an early time. These results point out that the easily accessible tests are potentially easy-to-use, low-cost for early screening and prognosis of the severe and/or critical COVID-19 infected cases.

Whereas COVID-2019 was initially recognized as a pulmonary disease followed by a storm of pro-inflammatory cytokines, resulting in ARDS, MODS, and death [65], recent evidence indicates that the disease is a systemic disorder affecting many organ systems including

kidneys, gastrointestinal tract, liver, nervous system, and skin among others [66–69]. The mechanism of these systemic effects is not clear yet and many might be related or mediated by the effects of the cytokines and dysregulated immune system [70].

There are several limitations for this review. As most of the evidence came from China, this lack of evidence from outside China might be a limitation in the way of generalizing our results, particularly with regard to the shortage of costly laboratory tests in the context of nations with low resources. The heterogeneity of the included studies was another limitation, and the need for further studies is greatly felt. One of the main causes might be the poor description of the analytical performance features of the methods applied among the included studies. Furthermore, aging as a condition related to the inflammation was the basis of the previous studies [62,71], showing that severe patients were older than non-severe ones. Since the proinflammatory response is believed to initiate SARS-CoV-2 infection, it is logically possible that the aged cases have an overwhelming inflammatory reaction. Additionally, as the age of some of the patients were not included in some included studies, the comparison of the age differences between the two groups was not possible, which would be another probable limitation. Accordingly, the findings showed that more comprehensive clinical studies are required, including cohort studies. Besides, the immune responses of an innate and/or adaptive nature are suppressed or dysregulated by long-term stress through changing the Type 1-Type 2 cytokine balance, as a result of which low-grade chronic inflammation is induced and numbers, trafficking, and function of immunoprotective cells are suppressed. Additionally, chronic stress can also contribute to the suppression of protective immune responses and/or exacerbation of pathological immune responses [72]. In order to clarify to see if the severity of the illness itself can have effects on any of the immunological parameters or other psychological parameters like stress, independent of the virus, requires a larger number of clinical studies which would shed light on the association of chronic stress with its possible immunosuppressive role in patients with COVID-19.

COVID-19 is considered as a global health threat, consequently it is essential that clinicians have access to reliable quick pathogen tests and feasible differential diagnoses based on the clinical descriptions in their first contact with suspected patients. Although it has not been witnessed that pro-inflammatory cytokines and chemokines are directly involved in lung pathology during COVID-19, the changes in laboratory parameters, including the reduced total lymphocytes, lymphocyte subsets, and the elevated NLR, IL-2, IL-4, IL-10, IL-6, and TNF-α, as well as the routine inflammatory-related parameters in infected patients were remarkably associated with the severity of the disease. Likewise, irrespective of the crucial role that hyper-inflammatory responses play in COVID-19 pathogenesis, there could be a protective role for the innate immune system.

Contributors

All authors contributed to the study conception. HA, SF, and SV did the literature search and data extraction. RT and HA did the data synthesis, created the tables and figures, and wrote the manuscript. All authors contributed to the interpretation of the data and revision of the manuscript.

Declaration of competing interest

None.

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Appendix A. Supplementary data

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