

SHORT REVIEW

Interleukin-6 and severe COVID-19: a systematic review and meta-analysis

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Accepted for publication May 4, 2020

To cite this article: Mojtabavi H, Saghadzadeh A, Rezaei N. Interleukin-6 and severe COVID-19: a systematic review and meta-analysis. *Eur. Cytokine Netw.* 2020; 31(2): 44-49. doi: 10.1684/ecn.2020.0448

ABSTRACT. Background: Evidence links COVID-19 severity to hyper-inflammation. Treatment with tocilizumab, a monoclonal antibody directed against the interleukin-6 (IL-6) receptor, was shown to lead to clinical improvement in patients with severe COVID-19. We, therefore, performed the present systematic review and meta-analysis to investigate whether the circulating levels of IL-6 is a reliable indicator of disease severity among patients affected with COVID-19. **Methods:** A systematic search was conducted in PubMed, Scopus, Web of Science, and Google Scholar on April 19, 2020. **Results:** Eleven studies provided data of IL-6 levels in patients with severe to critical COVID-19 (severe) and patients with mild to moderate COVID-19 (non-severe). The included studies were of moderate to high quality. The mean patients' age was 60.9 years, ranging from 45.2 to 76.7 years in the severe group and 46.8 years, ranging from 37.9 to 61 years, in the nonsevere group. Fifty-two percent were male in the severe group, as compared to 46% in the non-severe group. An overall random effects meta-analysis showed significantly higher serum levels of IL-6 in the severe group than in the non-severe group with a mean difference of +23.1 pg/mL (95% CI: 12.42-33.79) and the overall effect of 4.24 (P -value < 0.001). Meta-regressions showed that neither age nor sex significantly influenced the mean difference of IL-6 between the groups. **Conclusions:** Meta-analysis and meta-regression reveal a reliable relationship between IL-6 and COVID-19 severity, independent of age and sex. Future research is, however, required to assess the effect of BMI on the pattern of IL-6 production in patients with COVID-19. Also, there might be confounding factors that influence the relationship between IL-6 and COVID-19 severity and remain as yet unknown.

Key words: age, COVID-19, severity, inflammation, interleukin-6, meta-analysis

INTRODUCTION

Originating in Wuhan, the novel coronavirus SARS-CoV-2, causing coronavirus disease (COVID-19), is the third member of the family of human coronaviruses that has brought us the global pandemic of the decade [1]. The virus is much similar in the clinical behavior and at the gene level to its prior two RNA viruses leading to outbreaks in 2003 and 2011 (SARS-COV and MERS-COV, respectively). The symptoms of COVID-19 mostly include fever, fatigue, and respiratory symptoms, including cough, sore throat, and shortness of breath [2]. The majority of the cases develop lymphopenia and pneumonia with the characterizing feature of pulmonary ground-glass opacity on chest computed tomography (CT) scan [3]. Also, high levels of pro-inflammatory cytokines are evident mostly in the severe to critical patients [4]. Multiorgan failure is the leading cause of death among patients with COVID-19 [2]. There is evidence that

higher levels of inflammatory markers correlate to this characteristic picture of the disease [5]. Ruan *et al.* suggest that virally driven hyper-inflammation could also be a possible predictor of fatality due to elevated levels of ferritin and IL-6 observed at 150 confirmed COVID-19 cases [6]. Hence, Mehta *et al.* impose the beneficial effect of earlier identifying and treating hyper-inflammation on reducing the overall mortality [7]. Based on previous outbreaks, corticosteroids are not among routine therapeutic guidelines and may diversely increase COVID-19-associated lung injury [8], while there are studies that show the beneficial effect of Tocilizumab, which is a monoclonal antibody (mAb) aiding the blockade of the IL-6 receptor, in patients with severe/critical COVID-19 [9, 10].

The current guidelines recommend the use of Tocilizumab for patients with severe COVID-19 who have warning signs of hyper-inflammation [11]. We, therefore, performed the present systematic review and

meta-analysis to investigate whether the peripheral level of interleukin-6 is a reliable indicator of disease severity among patients affected with COVID-19.

METHODS

We prepared the present systematic review and meta-analysis according to the Preferred reporting items for systematic reviews and meta-analyses (PRISMA) checklist and flow diagram [12].

Literature search and selection criteria

We identified relevant studies by searching keywords (IL-6 or interleukin-6) and (COVID or coronavirus) in PubMed, Scopus, and Web of Science on March 17, 2020. Owing to the special situation with a quick update in the papers, we also searched Google Scholar for the latest updates on April 19, 2020.

Observational studies that measured IL-6 among patients with severe and non-severe COVID-19 and provided sufficient data including the total number of subjects and mean and standard deviation (SD) of the IL-6 levels for each study group were eligible to be included.

Data extraction

We extracted the following data from each included publication; first-named author, location of study, number of subjects in each group, disease severity described as mild, moderate, or severe, demographic characteristics (e.g., age and gender) of both the groups, mean $\pm$ SD of the IL-6 levels, and the measurement scale (e.g., pg/mL, ng/mL, or ng/mg) of the IL-6.

Mentioned data were either extracted from the manuscript or converted from the provided tables and figures. We contacted the corresponding authors to share their results in case the first two approaches were unsuccessful. The excel spreadsheet containing details of extracted data is available on request.

Quality assessment

We applied the Newcastle-Ottawa scale (NOS) to assess the quality of included articles [13]. The NOS designed for observational studies rates them by three main aspects: sample selection, comparability of cases and controls, and exposure. NOS ranges from 0 to 9; studies that fall within 4 to 6 stars have a moderate risk of both bias and quality; while studies below four stars have the highest risk of bias and the lowest quality and those scoring 7-9 stars represent the highest quality with the lowest risk of bias.

Quantitative analysis

We used STATA MP 16 for all of the statistical analyses. We intended to use a standardized mean difference (SMD) as the effect size measure if the

included studies applied different measurement scales or assays [14]. Otherwise, we calculated the weighted mean difference (WMD) for the effect measurement as follows.

For each study included in the meta-analysis, we have:

$$D = X_1 - X_2$$

D indicates the raw difference in the means from two independent groups.

The variance of D is calculated as follows:

$$V_D = \frac{n_1 + n_2}{n_1 n_2} S_{Pooled}^2$$

where n_1 and n_2 are the sample size of the groups compared in the study and:

$$S_{Pooled}^2 = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

Then, the standardized mean difference d is estimated as follows:

$$d = \frac{D}{S_{Pooled}}$$

Heterogeneity was assessed by Q statistic tests and the I^2 index using Cochrane guidelines. An I^2 value of more than 40% indicates significant heterogeneity across studies. In contrast, the fixed effects model was considered the default model of analysis, it turned to the random effects model in the case of I^2 fluctuating more than 40%. We evaluated the risk of publication bias using the degree of funnel plot asymmetry and the test of Egger. The P -value of less than 0.05 was considered statistically significant.

RESULTS

The database search yielded a total of 255 records. After duplicate removal, 181 discrete search results remained for screening, which reduced to 26 after title/abstract screening. During the full-text review, fifteen articles did not meet the eligibility criteria and were excluded for the reasons, as outlined in *figure 1*. Finally, we included eleven studies in the present systematic review and meta-analysis.

Study characteristics

Eleven studies measured IL-6 in patients with severe to critical COVID-19 compared to patients with mild to moderate COVID-19 [15-25]. All the studies were conducted in China (*table 1*). Studies have reported the method of diseases severity classification as the international guidelines for community-acquired pneumonia [15], the seventh edition of the Chinese National Health Commission [20], the Guidelines for the Diagnosis and Treatment of Novel Coronavirus (2019-nCoV) Infection (Trial Version 5) issued by the National Health Commission of the People's Republic of China [21, 22, 24], the World Health Organization interim guidance [18, 19, 23], and the Guidelines of the Diagnosis and Treatment of New Coronavirus

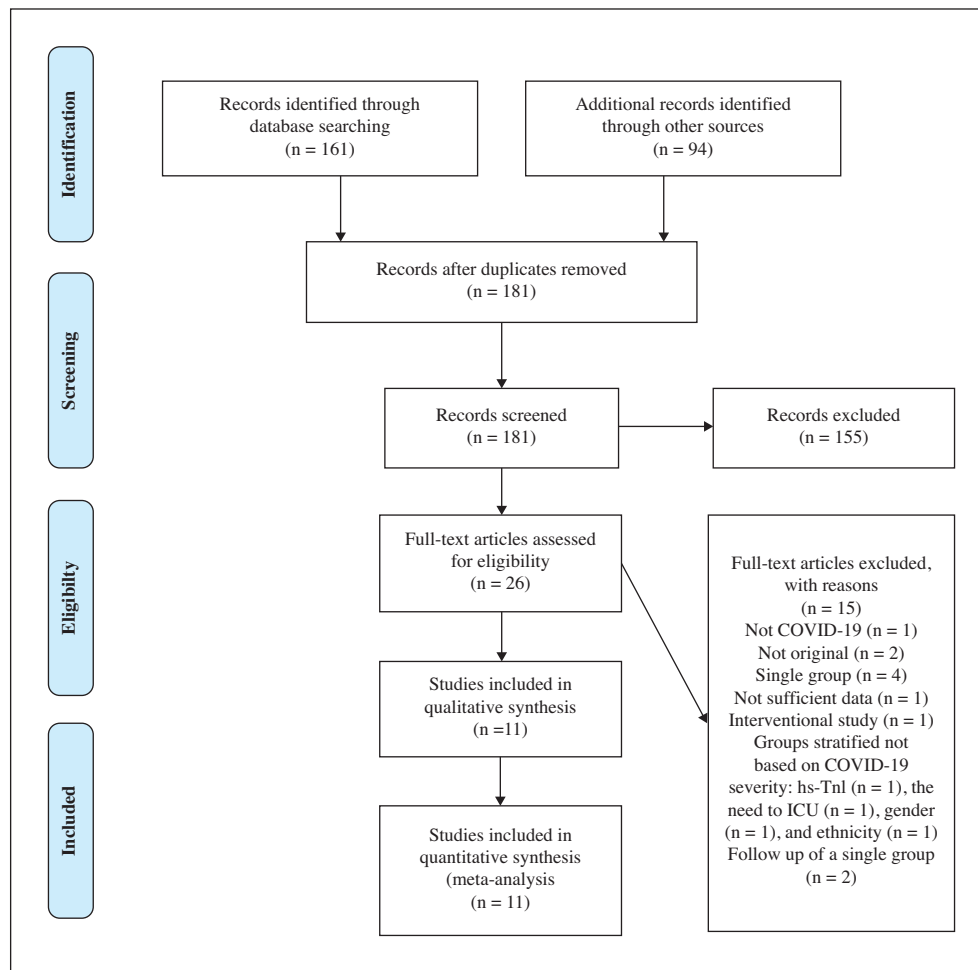


Figure 1
PRISMA flow diagram of study selection.

Pneumonia (version 6) published by the National Health Commission of China [17].

Quality assessment

As summarized in *table 2*, the includes studies were of moderate to high quality as rated from 6 to 8 (mean score: 7.6) on a nine-point scale.

Meta-analysis

A random effects meta-analysis of eleven studies involving 1357 patients with COVID-19 showed higher levels of IL-6 in patients with severe to critical COVID-19 ($n = 579$) compared to patients with mild to moderate COVID-19 ($n = 778$). The WMD was estimated as $+23.1$ pg/mL (95% CI: 12.42-33.79) with

Table 1
Characteristics of the included studies.

The first author, year	Location	IL-6 levels in severe			IL-6 levels in non-severe		
		No.	Male %	Mean age	No.	Male %	Mean age
Cai Q, 2020	Wuhan, China	58	56.9	62	240	46.3	42.3
Chen, L, 2020	Wuhan, China	14	NA	NA	15	NA	NA
Chen X, 2020	Wuhan, China	27	64.87	73.79	21	77.1	52.8
Gao Y, 2020	Fuyang, China	15	60	45.2	28	60.71	42.96
Jian-ya, 2020	Chongqing, China	7	57.1	52	44	63.7	42
Liu F, 2020	Wuhan, China	33	24.2	76.67	107	38.3	61
Liu T, 2020	Wuhan, China	69	47.83	56.3	19	9.09	38.3
Qin C, 2020	Wuhan, China	286	54.2	60.3	166	48.2	52.08
Xu Y, 2020	Wuhan, China	25	52	68	11	50	48.3
Yang Y, 2020	Shenzhen, China	34	64.7	59.8	44	47.3	50.3
Yuan J, 2020	Shenzhen, China	11	46	54.67	83	44.58	37.94

the overall effect of 4.24 (P -value < 0.001) (figure 2). There was significant heterogeneity across studies ($\text{Chi}^2 = 156.51$, P -value < 0.001 and $I^2 = 93.6\%$). Neither the funnel plot (figure 3) nor the Egger test (P -value = 0.282) revealed evidence of publication bias.

DISCUSSION

A systematic inflammatory response called cytokine release syndrome can occur due to immune-related disorders or during treatment with immune-related therapies. COVID-19 correlates with increased inflammation from the early stage of infection to the late stage of infection. In particular, increased release of cytokines, including IL2, IL7, G-CSF, IP-10, MCP-1, MIP-1 α , and TNF- α , is present in patients with severe

COVID-19 [26]. Moreover, extreme cases of COVID-19 surge IL-6 levels along with the progression to severe or critical condition [27]. Among hospitalized patients with COVID-19, patients with high IL-6 levels at admission are at increased risk of developing a severe form of the disease, requiring mechanical ventilation and ICU, and progressing to respiratory distress syndrome and multiorgan failure [28–30]. Such a pattern of cytokine activation induced by COVID-19 resembles a storm that crucially involves IL-6.

The exact mechanism through which COVID-19 causes inflammation remains to question. However, the novel coronavirus can bind to the ACE2 receptor expressed by alveolar epithelial cells and thereby induces the production of cytokines, in particular IL-6. IL-6 is a pleiotropic cytokine well documented in the

Table 2
Quality assessment of studies included in the quantitative synthesis (NOS).

The first author, year	Selection			Comparability			Exposure			Score
Cai Q, 2020	*	*	-	*	*	*	*	*	*	8
Chen, L, 2020	*	*	-	*	*	*	*	*	*	8
Chen X, 2020	*	*	-	*	*	*	*	*	*	8
Gao Y, 2020	*	*	-	*	-	*	*	*	*	7
Jian-ya, 2020	*	*	-	*	*	*	*	*	*	8
Liu F, 2020	-	*	-	*	-	*	*	*	*	6
Liu T, 2020	*	*	-	*	*	*	*	*	*	8
Qin C, 2020	*	*	-	*	*	*	*	*	*	8
Xu Y, 2020	*	*	-	*	*	*	*	*	*	8
Yang Y, 2020	*	*	-	*	*	*	*	*	*	8
Yuan J, 2020	*	*	-	*	-	*	*	*	*	7

The Newcastle-Ottawa Scale (NOS) was used for the assessment of the quality of the included studies. It is a nine-item scale that evaluates the quality of studies in three aspects: selection, comparability, and exposure. More precisely, it would assess whether the study can be awarded a score for four items in the selection part: 1. definition of cases, 2. representativeness of the cases, 3. selection of controls, and 4. definition of controls, two items in the comparability part: 1. age and 2. sex, and three items in the exposure part: 1. ascertainment of exposure, 2. same method of ascertainment for cases and controls, and 3. non-response rate.

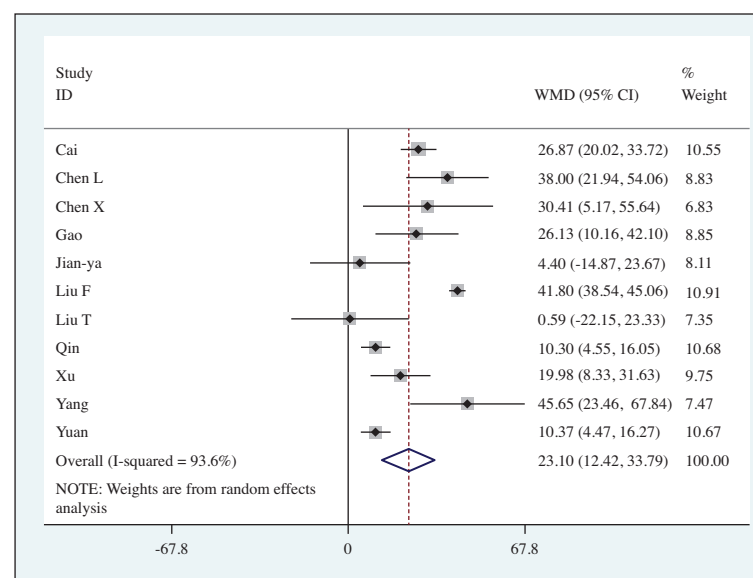


Figure 2

Meta-analysis of IL6 levels: patients with critical to severe COVID-19 vs. patients with mild to moderate COVID-19.

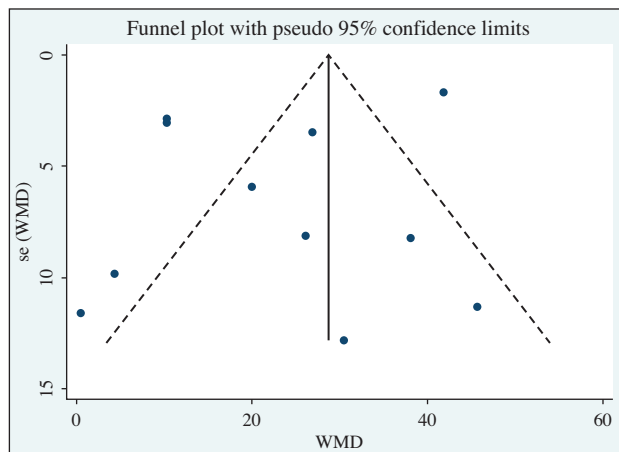


Figure 3
Funnel plot of studies included in the meta-analysis.

context of infectious diseases. Many immune cells and nonimmune cells that can produce IL-6 are a pivotal point for the broad role of this cytokine. Of note, toll-like receptors (TLRs) that help the innate immunity with the recognition of infectious pathogens can activate macrophages and monocytes to release IL-6. Besides, IL-6 takes part in different signal transduction pathways, including classical signal transduction, trans signal transduction, trans presentation, and the JAK-STAT, RAS-RAF, SRC-YAP-NOTCH, and AKT-PI3K pathways. Thereby, IL-6 can contribute to essential biological functions, including immune regulation [31].

COVID-19 is an example of a very fulminant immune dysregulation. The inflammation induced by COVID-19 can result in rapidly progressive respiratory failure. As evidenced by autopsy studies, lungs infected with COVID-19 represent as bronze, contain gray and white viscous fluids [32], and indicate the infiltration of alveolar macrophages as a measure of inflammation [1]. With the above evidence, IL-6 has the potential to be a therapeutic target for COVID-19-related hyperinflammation. Tocilizumab is a humanized monoclonal antibody directed toward the IL-6 receptor. It is applicable to inflammatory conditions, including rheumatoid arthritis systemic juvenile idiopathic arthritis, giant cell arthritis, and cytokine release syndrome. In a retrospective study, most patients with severe to critical COVID-19 ($n = 21$) who underwent treatment with tocilizumab showed improvement in the clinical, laboratory, and imaging parameters [9]. Another study of 15 patients confirmed the clinical benefits of tocilizumab in ill patients with COVID-19, along with a reduction in IL-6 levels following treatment in most patients [33]. Clinical trials are ongoing for the evaluation of the safety and efficacy of tocilizumab in patients with COVID-19 [34].

The present meta-analysis included eleven studies with moderate to the high quality of evidence and confirmed that patients with severe COVID-19 have higher concentrations of IL-6 than patients with non-severe COVID-19. The mean patients' age was 60.9 years ranging from 45.2 to 76.7 years in the severe group. It was 46.8 years ranging from 37.9 to 61 years in the non-severe group. 52% were male in the severe

group compared to 46% in the non-severe group. Meta-regressions showed that neither age nor sex could significantly influence the mean difference of IL-6 between groups. Few studies reported data of BMI, and therefore, it was not possible to enter that as a covariate in meta-regression.

CONCLUSIONS

Meta-analysis reveals a reliable relationship between IL-6 and COVID-19 severity. This relationship seems to exist, independent of age and sex. Future research is, however, required to assess the effect of BMI on the pattern of IL-6 production in patients with COVID-19. Although the research in this context has been devoted mainly to IL-6, there are other cytokines known to have a potential role in the cytokine release syndrome, for example, TNF- α . Therefore, it would be interesting to investigate other cytokines involved in the cytokine storm of COVID-19. In the end, there might be confounding factors that influence the relationship between IL-6 and COVID-19 severity and have hitherto remained unknown.

Acknowledgments and disclosures

Ethics approval and consent to participate: not applicable. *Consent for publication:* not applicable. *Availability of data and materials:* not applicable. *Conflicts of interests:* the authors declare that they have no competing interests. *Funding:* there is no funding for the present study. *Authors' contributions:* H.M. conceptualized the study, conducted database search, search results screening, detailed review, data extraction, quality assessment, and prepared the initial draft. A.S. prepared the final draft. N.R. supervised the project and critically appraised the manuscript. *Acknowledgments:* not applicable.

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