Serum Krebs von den Lungen-6 for Predicting the Severity of COVID-19: A Systematic Review, Meta-Analysis, and Trial Sequence Analysis

Clinical Medicine Insights: Circulatory, Respiratory and Pulmonary Medicine
Volume 17: 1–13

DOI: 10.1177/11795484231152304
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Abhigan Babu Shrestha[1], Pashupati Pokharel[2], Harendra Singh[3], Sajina Shrestha[4] and Fioni[5]

[1]Department of Internal Medicine, M Abdur Rahim Medical College, Dinajpur, Bangladesh. [2]Maharajgunj Medical Campus, Institute of Medicine, Kathmandu, Nepal. [3]Department of Anesthesiology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal. [4]Department of Internal Medicine, KIST Medical College, Imadol, Nepal. [5]Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia.

ABSTRACT

OBJECTIVE: This systematic review and meta-analysis aimed to find the association between serum Krebs von den Lungen-6 (KL-6) and the severity of Coronavirus disease 2019 (COVID-19) infection.

DATA SOURCES: Databases of Embase, PubMed, Web of Science, Science Direct, and Google Scholar were searched for studies reporting KL-6 levels in COVID-19 patients, published between January 2020 and September 30 2022.

DATA SYNTHESIS: For comparison between the groups, standard mean difference (SMD) and 95% confidence intervals (CI) were computed as the effect sizes. Sensitivity, specificity, positive likelihood ratio (PLR), and negative likelihood ratio (NLR) were measured to assess the diagnostic power of KL-6. In addition, the summary receiver operating characteristics curve (sROC) was constructed to summarize the true positive (TP), and false positive (FP) rates. To validate the findings of meta-analysis, Trial Sequential Analysis (TSA) was conducted.

RESULTS: Altogether 497 severe COVID-19 patients and 934 non-severe (mild to moderate) COVID-19 patients were included. Pooling of 12 studies indicated that the serum KL-6 level had significant association with severity of COVID-19 infection: standard mean difference = 1.18 (95% CI: 0.93-1.43), p = 0.01; I^2: 58.56%]. Pooled diagnostic parameters calculated from eight studies were: sensitivity 0.53 (95% CI: 0.47-0.59); specificity 0.90 (95% CI: 0.88-0.93); positive likelihood ratio 4.80 (95% CI: 3.53-6.53); negative likelihood ratio 0.46 (95% CI: 0.32-0.68); and area under curve: 0.8841. Additionally, TSA verified the adequacy of sample size and robustness of the meta-analysis.

CONCLUSION: Serum KL-6 level has a moderate degree of correlation with the severity of COVID-19 infection but has low sensitivity. So, it is not recommended as a screening test for severe COVID-19 infection.

KEYWORDS: "Coronavirus disease", "COVID-19", "Krebs von den Lungen-6", "KL-6", "meta-analysis", "Severe COVID-19"

RECEIVED: May 2, 2022. **ACCEPTED:** January 4, 2023

TYPE: Meta-Analysis

CORRESPONDING AUTHOR: Abhigan Babu Shrestha, Department of Internal Medicine, M Abdur Rahim Medical College, Dinajpur, Bangladesh. Email: abigan17@gmail.com

Introduction

Coronavirus Disease 2019 (COVID-19) is a highly contagious infection due to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] The first case of COVID-19 was reported in Wuhan, Hubei Province, China, in late December 2019. As of 25 September 2022, more than 612 million confirmed cases of COVID-19, and more than 6.5 million deaths have been reported globally.[2] The median incubation period for SARS-CoV-2 is estimated to be 5.1 days, and the majority of patients will develop symptoms within 11.5 days of infection.[3] The clinical spectrum of COVID-19 varies from asymptomatic to acute respiratory failure requiring mechanical ventilation, septic shock, and multiple organ failure.[4]

Based on the severity, COVID-19 infection is categorized into asymptomatic, mild illness, moderate illness, severe illness, and critical illness.[5] Asymptomatic are those who have no symptoms of COVID-19 but are positive for SARS-CoV-2 using a nucleic acid amplification test [NAAT] or an antigen test. Mild illnesses are those with different signs and symptoms of COVID-19 (eg, fever, sore throat, malaise, headache, cough, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have dyspnea or chest imaging abnormalities. Moderate illness is those who have an oxygen saturation (SpO_2) ≥ 94% on room air at sea level and who show evidence of lower respiratory disease during imaging. Severe illness is those who have SpO_2 <94% on room air at sea level, a ratio of arterial partial pressure of oxygen to the fraction of inspired oxygen (PaO_2/FiO_2) <300 mm Hg, respiratory rate >30 breaths/min or pulmonary infiltrates >50%. Critical illness is those who have respiratory failure, multiple organ dysfunction, and septic shock.[5]

Since the global outbreak of COVID-19, the number of patients requiring intensive treatment has outnumbered the availability of intensive care unit (ICU) beds. So, the identification of reliable predictors of clinical deterioration is a major concern for clinicians to stratify the risk for each patient and focus the treatment efforts on those with a worse expected

clinical outcome.[6] Various parameters, such as C- reactive protein (CRP), procalcitonin, d-dimer, lactate dehydrogenase,[7] interleukin 6 (IL-6), lymphopenia, hypernatremia[8] and radiological characteristics have been used in clinical practice to assess the severity of COVID-19 infection. Among these Krebs von den Lungen-6 (KL-6) factor has also been established as a possible severity marker and prognostic tool by various articles.[9–21]

Krebs von den Lungen-6 (KL-6) is a high molecular weight transmembrane mucin protein that is secreted by proliferation or damage to type II alveolar epithelial cells.[22] KL-6 causes fibroblast accumulation in small airways lined by epithelial fluid resulting in intra-alveolar fibrosis.[23] With the worsening of COVID-19, a large amount of virus replication will destroy the alveolar epithelium, destroy the basement membrane, increase the permeability of pulmonary blood vessels, and lead to local pulmonary edema.[24] As type II pneumocytes also get injured in the process, this leads to increased KL-6 production. Concurrently, serum KL-6 also rises because of enhanced permeability of pulmonary vessels in response to inflammation caused by severe COVID-19 infection.

Serum KL-6 is a useful marker of interstitial lung diseases where alveolar epithelial proliferation and injury is a common phenomenon.[25,26] Previous meta-analyses have shown that KL-6 has moderate to limited diagnostic value in assessing the severity of COVID-19 infection.[27,28] However, another meta-analysis suggested KL-6 as a non-expensive, rapid screening tool for COVID-19 severity.[29] To overcome these hurdles due to an inadequate number of studies, and create more promising evidence, we aimed to conduct an updated meta-analysis between serum KL-6 and the severity of COVID-19 infection.

Methods

This meta-analysis and systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[30] Additionally, the study has been registered in PROSPERO with ID CRD42022371158 (link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022371158).
The PRISMA checklist detailing the process of meta-analysis is presented in Supplementary file (Appendix 1).

Search Strategy

We systematically searched the database of "Embase," "PubMed," "Google Scholar," "Web of Science," and "Science Direct" till September 2022 for all related articles on COVID-19 and KL-6. We used the keywords: "Coronavirus" OR "COVID-19" OR "SARS-COV-19" OR "SARS-COV-2" OR "2019-nCOV" AND "Krebs von den Lungen-6" OR "KL-6" OR "MUC1." The preliminary search strategy is shown in Supplementary file (Appendix 2).

Selection Criteria

Inclusion Criteria

(i) COVID-19 is diagnosed by standard microbiological tests such as polymerase chain reaction (PCR), and antigen test.
(ii) Studies that measured the serum/plasma level of KL-6 by standard methods such as chemiluminescence immunoassay, agglutination test, and ELISA.
(iii) Studies comparing the KL-6 levels in severe versus non-severe(mild/moderate) COVID-19 patients or healthy cases.

Exclusion Criteria

(i) Other diagnostic-related indices, but not KL-6.
(ii) Case reports, literature reviews, editorials, and conference papers.
(iii) Articles with irretrievable full text.

Data Extraction

Studies obtained from the electronic databases were exported to Mendeley version 1.19.8 reference software in compatible formats. Duplicate articles were screened first by automated and then manually. After that, duplicates were recorded and deleted. The titles and abstracts of the remaining studies were screened independently by four authors (ABS, PP, HS, and SS). Two authors (ABS and SS) retrieved the full text of potentially eligible studies and further screened for final inclusion. Disagreements between reviewers were resolved by the third author (HS).

Two reviewers (ABS and SS) independently scanned the results from the initial search and followed it up with a full-text review. The following data were extracted: author, year of publication, study design, country, KL-6 detection method, age, male, severe and mild-moderate/non-severe cases, KL-6 values for both, p-value, cut-off, sensitivity, specificity, AUC. True positive (TP), True Negative (TN), False Positive (FP), and False Negative (FN) were calculated from the data.

Quality Assessment

To evaluate the quality of the studies included in this review, the Newcastle-Ottawa Scale for non-randomized studies was used.[31] Using the tool as a checklist, the qualities of each of the original articles were evaluated independently by the authors (ABS and PP). The mean score of two authors was taken for the final decision, and articles (>4 of 10 across the three parts) were included in the analysis.

Statistical Analysis

Microsoft Excel version 2016 (Microsoft Corp., Redmond, WA, USA) was used for the basic calculation. The data

collected in a Microsoft Excel sheet was exported, and analysis was performed using the STATA software version 17 (StataCorp, College Station, TX, USA). For the studies, the standard mean difference (SMD) and 95% CI were computed as the effect size of comparison between patients with mild-moderate (non-severe) COVID-19 and severe COVID-19. The heterogeneity of the included studies was examined using the Cochrane Q test and I^2 statistic (I^2 of less than 25% defined as mild heterogeneity, I^2 of 25-50% as moderate heterogeneity, and I^2 of more than 50% as severe heterogeneity). With high heterogeneity (p-value for Q test $\leq$ 0.05, $I^2 \geq 50\%$), random effects models were performed as pooling methods. Whereas, with mild-moderate heterogeneity (p-value for Q test >.05, I^2 < 50%), the fixed-effects models were performed as pooling methods.

For sensitivity and specificity, data were extracted from eight studies with their baseline values.[9,12,15,17–19,21,32] The data for sensitivity, and specificity analysis is presented in Supplementary file (Appendix 4). For diagnostic studies, the Summary Receiver Operating Curve (sROC) was performed to summarize TP and FP rates. The diagnostic value of KL-6 associated with COVID-19 severity was assessed with the random effects model by MetaDisc V.1.4 (Metadisc, Madrid, Spain). A sensitivity analysis was performed by continuously excluding each study to determine the impact of a single study on the degree of heterogeneity. To assess publication bias, Egger's regression test was performed to see the impact of small studies. Using the trim and fill command of STATA 17, potential publication bias was presented in funnel plots of effect size and standard error. A p-value of <.10 was considered indicative of a statistically significant publication bias.

Due to the scarce data available, the results analyzed in a meta-analysis may show bias by the residence of systematic errors (bias) or random error (occur by chance). A false p-value for significance might be produced due to trials with low methodological quality, small sample size, and publication bias. So, to provide robust evidence for our meta-analysis, we used a novel statistical software, a Trial sequential analysis tool (Copenhagen Trial Unit, Center for Clinical Intervention Research, Denmark).[33] This tool helps to calculate the indispensable information size (sample number) by examining an overall type-I error of 5% and a type-II error of 20% with two-sided graph plots, in which the red straight line depicts the significance boundaries of the conducted meta-analysis, the blue line indicates the cumulative Z- score, and the inwards sloping red lines show adjusted p-value with trial sequential monitoring boundaries.

Results

Study Selection and Characteristics

The databases of "PubMed," "Embase," "Web of Science," "Science Direct," and "Google Scholar" were utilized for the search strategy. Altogether, we found 258 articles from five databases. After removing the duplicates, 138 articles were screened for titles and abstracts. Then 28 articles were screened with full-text reading. Finally, 15 articles were eligible based on the inclusion criteria. Additionally, one more article was included from a citation search of included articles. So, altogether 16 full-text articles were included in the systematic review. The PRISMA diagram tailoring the study selection process is shown in Figure 1.

Among the 16 articles: six were from China, four were from Italy, four were from Japan, one was from Belgium, and one was from Indonesia. Altogether, these studies included 497 severe COVID-19 patients and 934 non-severe (mild to moderate) COVID-19 patients. Moreover, 4 studies had a total of 225 healthy controls as well. Out of 16 studies, 7 studies used the agglutination method to measure KL-6 levels, 7 studies used the chemiluminescence immunoassay method, and 2 studies used the ELISA method. The detailed characteristics of the included studies are presented in Table 1. Also, the details of KL-6 assay are presented in Supplementary file (Appendix 3).

Out of the 16 full-text articles, in 3 studies (Peng et.al, Frix *et al*, and He *et al*),[15,16,34] serum KL-6 value was assessed only for severe COVID-19 cases. So, they were only included in the systematic review.

Study Quality

The risk of bias in all included studies was assessed using the Newcastle Ottawa scale. Studies with a mean score greater than or equal to 7 are considered "low risk," while studies with a mean score of 4-6 as "moderate/intermediate risk" and <4 are considered "high risk." Out of 16 studies, one study was of "high risk," two studies were "intermediate risk" and the others were of "low risk." The study of Suryananda *et al* was excluded due to the high risk of bias. The details of the quality assessment are illustrated in the Supplementary file (Appendix 5).

Meta-Analysis

Elevated KL-6 and Severity of COVID-19. The pooling of 12 studies for meta-analysis showed significant heterogeneity, hence the random effects model (DerSimonian and Laird method) was operated to calculate the pooled association between serum KL-6 and the severity of COVID-19 infection. Statistical analysis showed that serum KL- 6 levels were significantly elevated in severe COVID-19 cases [standard mean difference (SMD) = 1.18 (95% CI: 0.93-1.43), p = .01; I^2: 58.56%] compared to non-severe cases. This is shown in the forest plot in Figure 2. Additionally, the pooled parameters calculated from eight studies are as follows: sensitivity, 0.53 (95% CI: 0.47-0.59); specificity 0.90 (95% CI: 0.88-0.93); PLR 4.80 (95% CI: 3.53-6.53); NLR 0.46 (95% CI: 0.32-0.68).

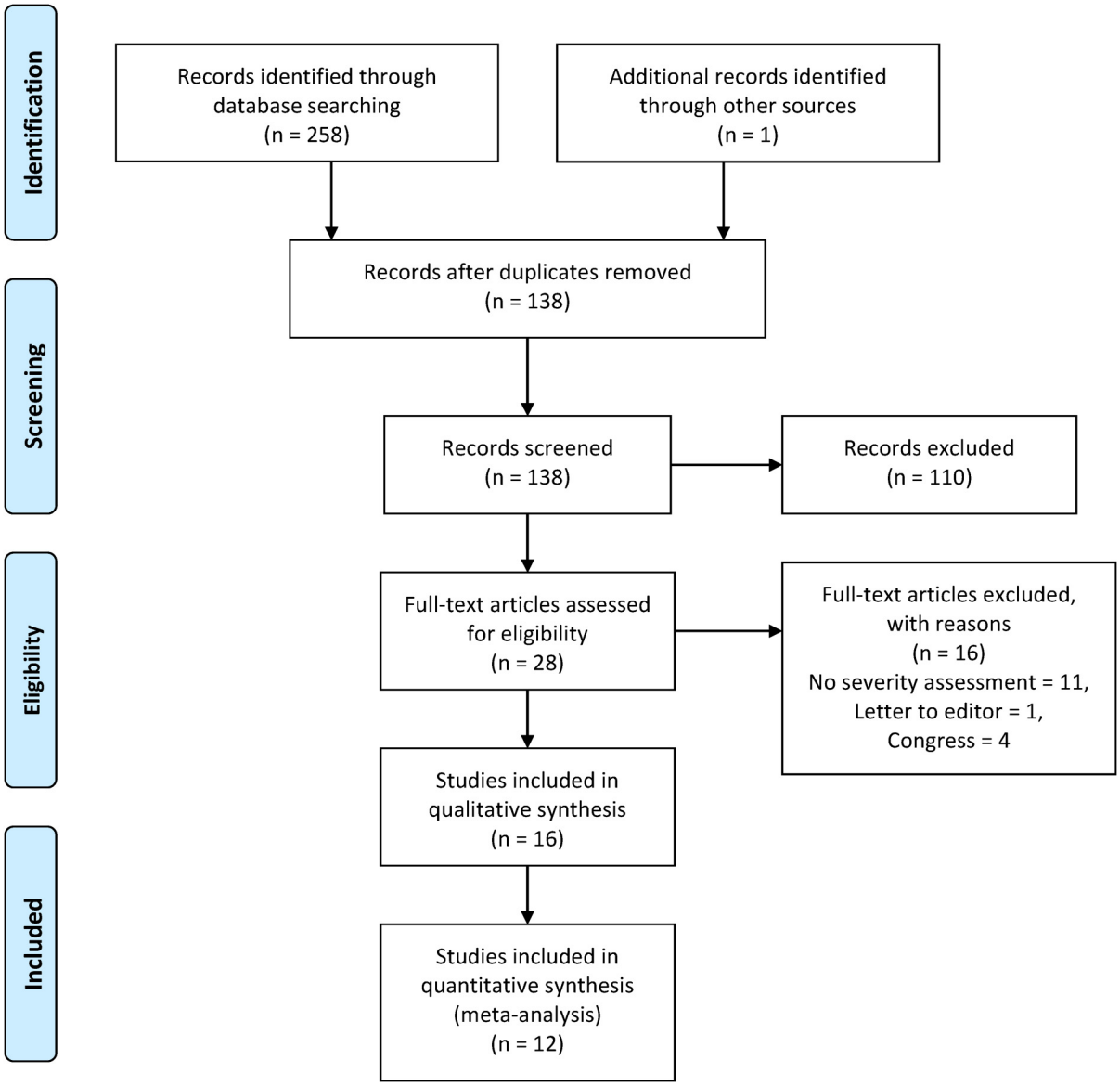

Figure 1. Prisma diagram for study selection process.

In the summary receiver operating characteristic curve (sROC), the area under the curve (AUC), was 0.8841. The sensitivity analysis, specificity analysis, PLR, NLR, and sROC curve are shown in Figures 3, 4, 5, 6, and 7, respectively.

Trial Sequential Analysis (TSA). The results of TSA were congruent with the conducted meta-analysis. TSA analysis showed that the red line sloping inwards (significance line of TSA) was crossed by the blue line (cumulative Z-score line), indicating a significant relationship between KL-6 and severe COVID-19 patients. Moreover, as the blue line crosses the information size (O'Brien-Fleming boundary) with study power adjusted to 80%, the required sample size or the number of studies is certainly adequate to establish a significant result. This is illustrated in Figure 8.

Sensitivity, Subgroup and Regression Analysis. A sensitivity analysis was performed to assess the influence of each study on the overall result of the meta-analysis. The results of the sensitivity analyses suggested that the overall point estimate was not affected by any single study, signifying the robustness of our meta-analysis. The detail of the sensitivity analysis is presented in the Supplementary file (Appendix 6).

To find the source of heterogeneity, subgroup analysis was done using the method utilized in the KL-6 assay. Compared with the chemiluminescence immunoassay method ($I^2 = 41.07\%$, $p = .15$), agglutination assay was responsible for heterogeneity ($I^2 = 66.11\%$, $p = .01$). Details of subgroup analysis are shown in the Supplementary file (Appendix 7).

Furthermore, to further explore the source of heterogeneity, regression analysis was done for the age of COVID-19 patients

Table 1. Characteristics of included studies.

			Study population							Serum KL-6 [median (IQR) U/ml]			
Author	Study design	Country	Sample size	Age in years (mean/ median)	Male	Severe cases	Non-severe cases	Healthy controls	KL-6 detection method	Non-severe cases	Severe cases	Healthy controls	p value
Yamaya *et al* 2021	Cross-sectional	Japan	356	69	218	60	296	–	Agglutination	267 (203–364)	549 (310–939)	–	<.001
Peng *et al* 2021	Cohort	China	Cases = 113, Healthy controls = 65	Cases = 50; Healthy controls = 56	Cases = 28, Healthy controls = 24	36	–	65	Agglutination	–	373.7 (269.9–428.1)	240.5 (217.5 –285.5)	<.001
Awano *et al* 2020	Cross-sectional	Japan	54	46	38	21	33	–	Agglutination	234 (194–282)	781 (429–1435)	–	<.001
Bergantini *et al* 2021	Cross –sectional	Italy	24	Severe COVID = 62.2; Non-severe COVID = 65.2	Severe = 8; Non-severe = 11	10	14	–	Agglutination	320 (226.3–927.8)	903 (333.8–1956)	–	.035
Scotto *et al* 2021	Cross-sectional	Italy	34	63	23	15	19	–	Chemiluminescence immunoassay	260 (125–421)	1188 (592–3608)	–	<.001
Wang *et al* 2020	Cross-sectional	China	64	43	35	12	52	–	Chemiluminescence immunoassay	317.07 (235.84 –440.51)	446.81 (350.86–728.58	–	.015
Xue *et al* 2020	Cross-sectional	China	63	Severe COVID = 55; Non-severe COVID = 57.2	Severe = 12; Non-severe = 2	15	6	43	Chemiluminescence immunoassay	241.2 + –207.9	676.6 + –506.70	–	.001
Deng *et al* 2021	Cross-sectional	China	166	Severe COVID = 48, Non-severe COVID = 55	Severe = 9; Non-severe = 65	17	149	–	Chemiluminescence immunoassay	452.1 (325.6–641.3)	898.0 (567.7–1278.9)	–	<.001
D'Alessandro *et al* 2020	Cross-sectional	Italy	32	63	16	12	10	–	Agglutination	293 (197–362)	1021 (473–1909)	–	.0118
Xue *et al* 2021	Cross-sectional	China	289	Severe COVID = 56; Non-severe COVID = 61	Severe = 31; Non-severe = 99	63	226	–	Agglutination	322.85 (234.27 –426.90)	688.75 (469.94 –991.41)	–	<.001

(continued)

Table 1. Continued.

			Study population							Serum KL-6 [median (IQR) U/ml]			
Author	Study design	Country	Sample size	Age in years (mean/median)	Male	Severe cases	Non-severe cases	Healthy controls	KL-6 detection method	Non-severe cases	Severe cases	Healthy controls	p value
Frix *et al* 2020	Cohort	Belgium	Cases = 83, Healthy controls = 70	Cases = 58; Healthy controls = 72	Cases = 35; Healthy controls = 52	83	–	70	Chemiluminescence immunoassay	–	405 (277–592)	254 (191–308)	<.001
D' Alessandro *et al* 2022	Cross-sectional	Italy	64	68	Severe = 20; Non-severe M = 28	29	35	–	Agglutination	239 (220–345)	827 (599–1103)	–	<.0001
He *et al* 2021	Case-control	China	Cases = 28, Healthy controls = 25	Cases = 64.93 ± 1.63; Healthy cases = 64.56 ± 1.55	Cases = 16; Healthy controls = 14	28	–	25	Chemiluminescence immunoassay	–	977.39 ± 136.93	251.96 ± 20.63	<.01
Suryananda *et al* 2021	Cross-sectional	Indonesia	75	48.04 ± 11.66	46	57	18	–	ELISA	44.85 (11.4–151.4)	45.70 (8.5–131.8)	–	–
Yamada *et al* 2022	Cross-sectional	Japan	135	50	69	27	42	–	ELISA	204.5 (168.8–264.3)	312.0 (210.0–410.0)	–	.001
Saito *et al* 2022	Cross-sectional	Japan	46	54.5	21	12	34	22	Chemiluminescence immunoassay	225 ± 84	396 ± 237	–	<.001

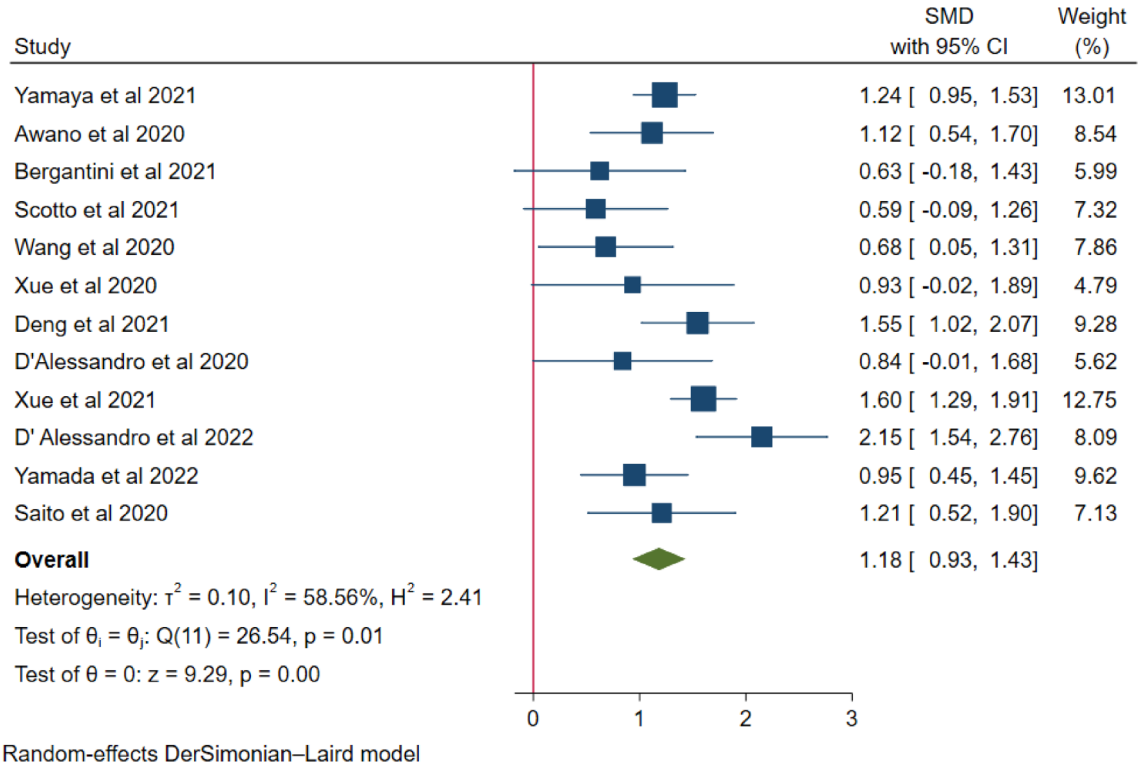

Figure 2. Forest plot showing the estimated pooled SMD of KL-6 in severe COVID-19 patients compared to non-severe patients.

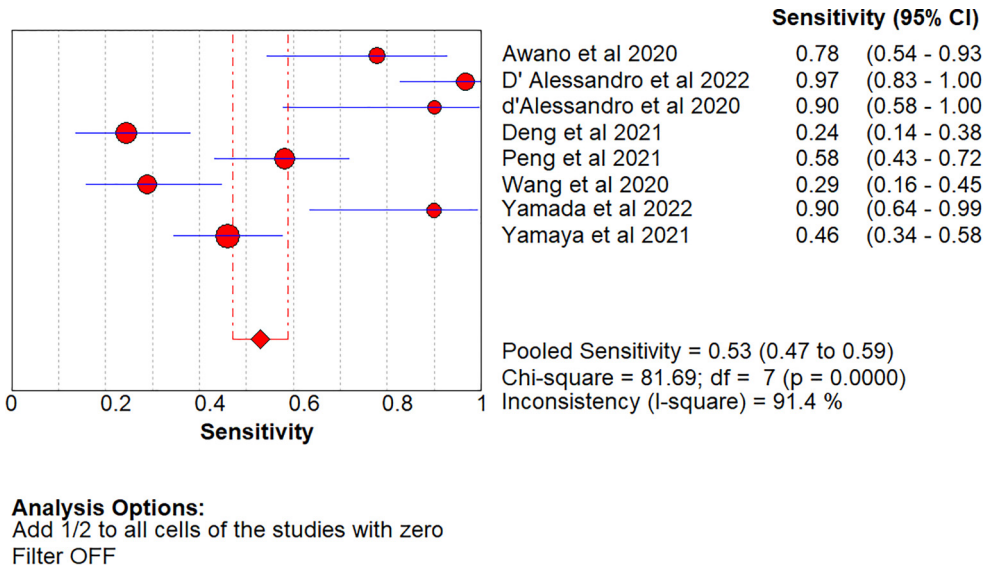

Figure 3. Sensitivity analysis.

and the male gender. However, both age (p = .475) and male gender (p = .504) were not the cause of heterogeneity. The details of meta-regression are depicted in the Supplementary file (Appendix 8).

Publication Bias. The funnel plot was qualitatively symmetrical for KL-6. Regression-based Egger's test did not show an indication of a small study effect (p = .11), confirming no evidence of publication bias in the meta-analysis. The detail of publication bias analysis is shown in the Supplementary file (Appendix 8).

Discussion

As the COVID-19 pandemic is affecting people more than ever and taking a large toll of human population, a large proportion of research is being dedicated globally toward this viral infection

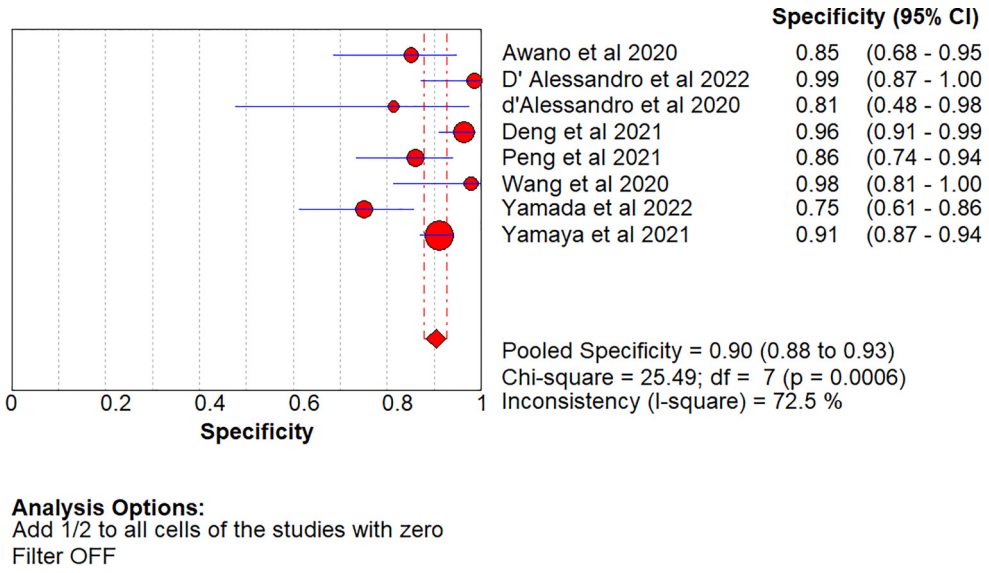

Figure 4. Specificity analysis.

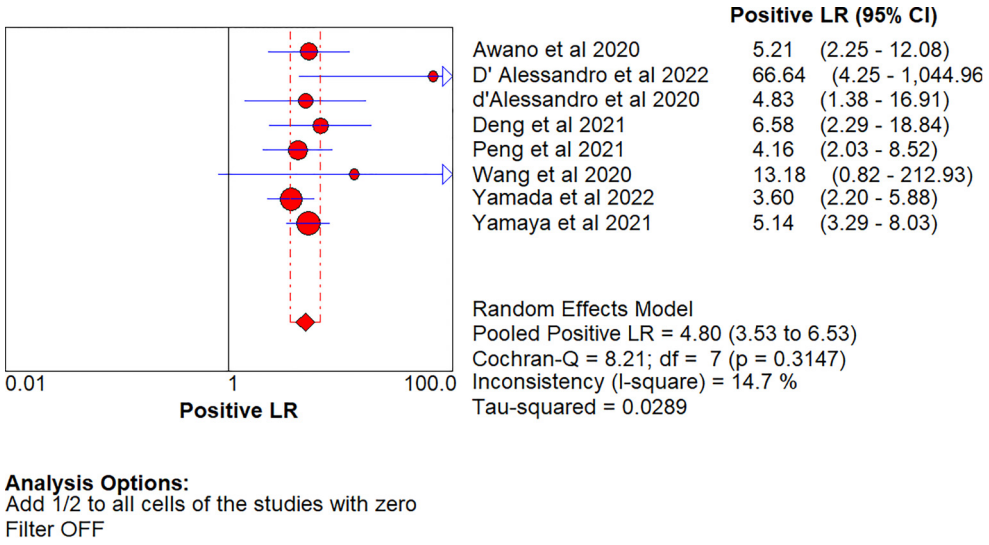

Figure 5. Positive likelihood ratio.

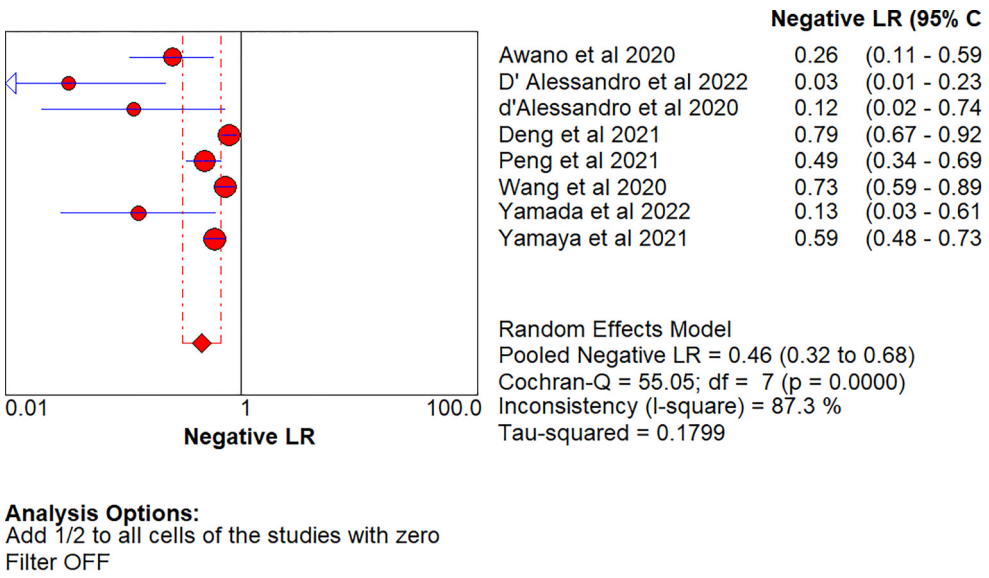

Figure 6. Negative likelihood ratio.

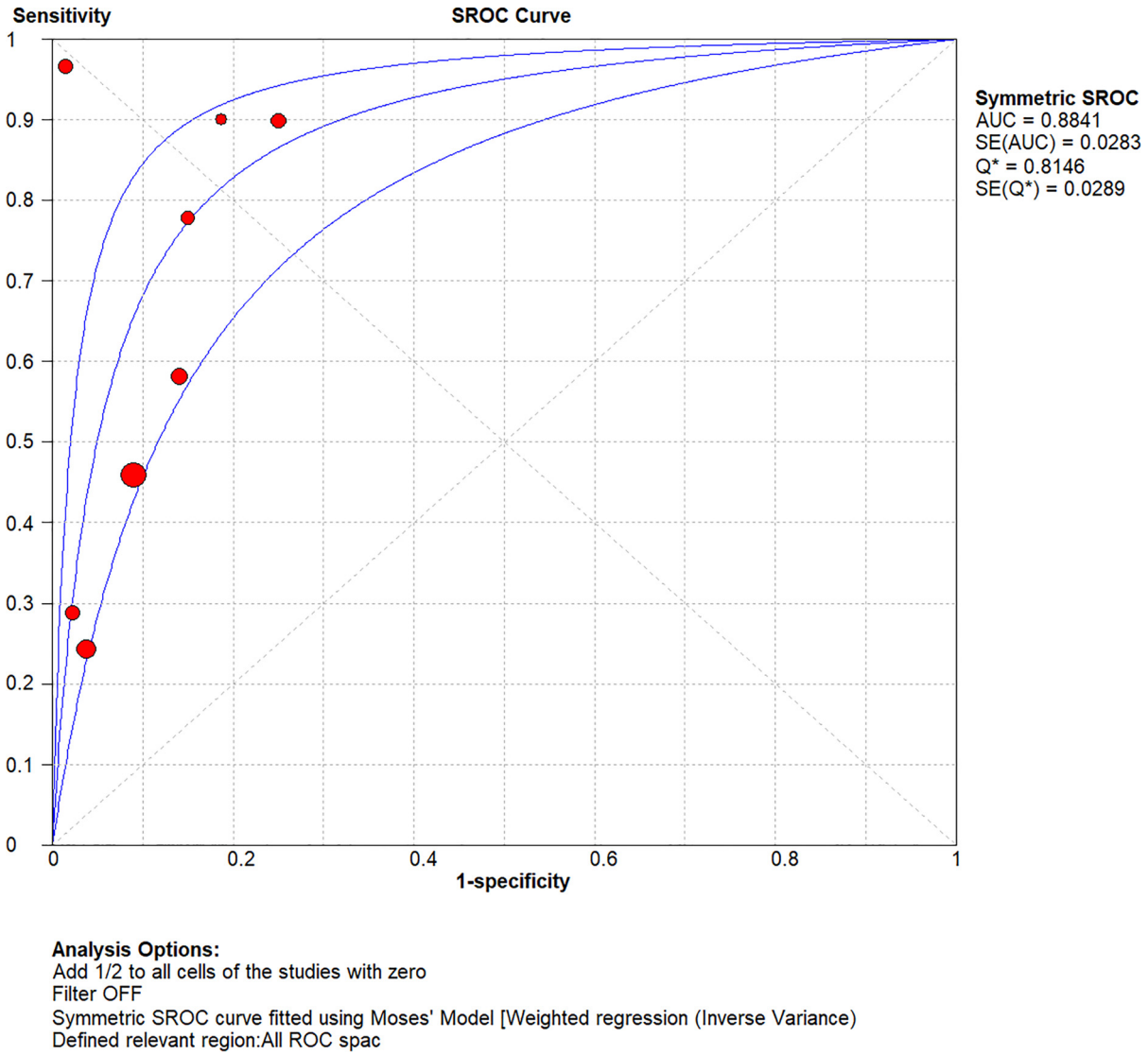

Figure 7. Summary receiver operating curve.

in the hope of making human life safer. To date, there is no effective treatment for COVID-19 and the vaccination rate in most countries throughout the world is not up to the mark. With the continuing spread of COVID-19 throughout the world, diagnostic tests that can predict the progression of patients to a severe state of disease with reasonable accuracy could greatly help in the management plan of these patients.

The mortality of COVID-19 infection is high, ranging from 3.1 to 61.5%, with higher mortality in severe COVID-19 patients.[35] So, to tackle high mortality, various studies have identified severity markers of COVID-19 infection. Serum CRP has been shown as a severity marker and a predictor of the progression of COVID-19 infection.[36–38] IL-6 is a widely accepted severity marker of COVID-19 infection.[36,39] Additionally, laboratory parameters such as ESR, LDH, and procalcitonin are also raised in severe COVID-19 infection.[39,40]

This meta-analysis was designed to accurately estimate the association between serum KL-6 and the severity of COVID-19 infection. In this meta-analysis, we demonstrated that the serum level of KL-6 is significantly elevated in the setting of severe COVID-19 patients [SMD = 1.18 (95% CI: 0.93-1.43)] compared to non-severe patients. Previous meta-analyses have also shown some association between serum KL-6 and the severity of COVID-19 patients, with the standard mean difference ranging from 1.16 to 1.34.[28,29] Such elevated levels have been associated with adverse clinical outcomes including ICU admission, acute respiratory distress syndrome (ARDS), post-COVID-19 fibrosis, and death.[6] If the KL-6 level is recorded in advance, the patient's management plan can be changed by predicting its severity. Compared with KL-6, the mean differences of other severity markers are; CRP = 1.48, IL-6 = 1.54, procalcitonin = 1.11.[41] Considering this, serum KL-6 is raised moderately in severe COVID-19 cases.

Our meta-analysis showed that the specificity and sensitivity of KL-6 for diagnosing severe COVID-19 infection are 0.90

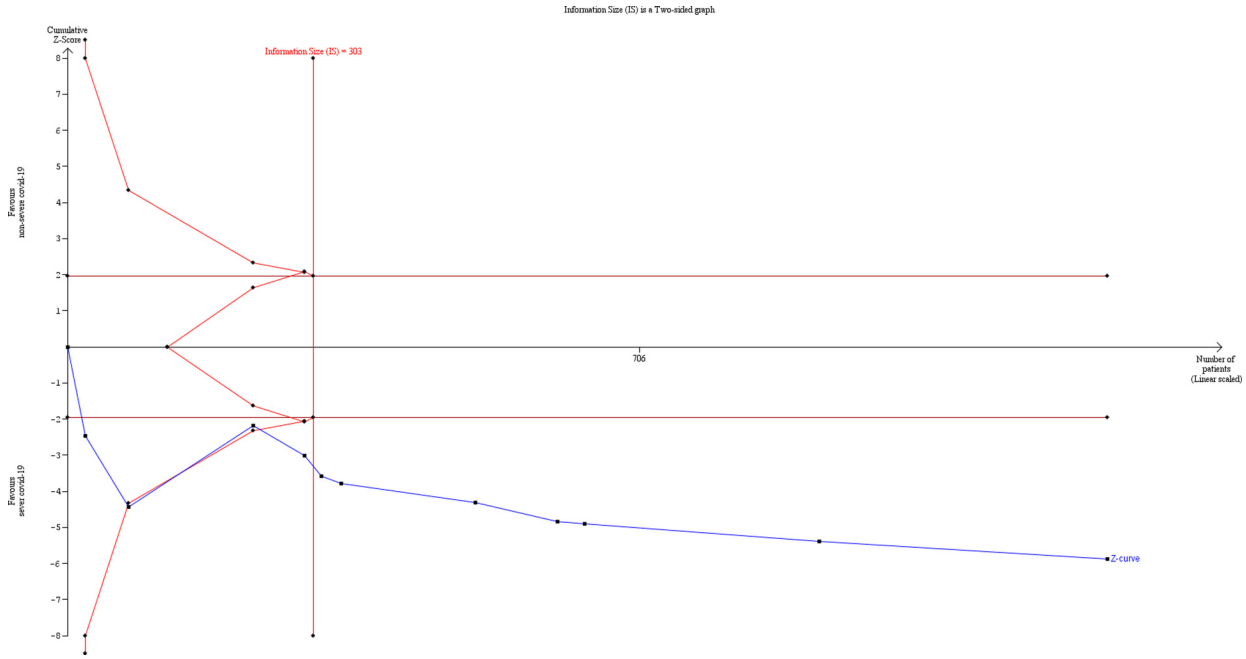

Figure 8. Trial sequential analysis of studies comparing KL-6 level of severe and non-severe COVID-19 patients. (Type-I error of 5%, Type-II error of 20% and power of study of 80%).

and 0.53, respectively. As the specificity is very high, so we can test serum KL-6 of COVID-19 patients at the initial stage to rule out severe infection. But the low sensitivity makes KL-6 assay a weaker screening test for severity assessment of COVID-19 infection. The sensitivity of procalcitonin is 0.54 making it a weaker screening test like KL-6.[42] However, the sensitivity of CRP, lymphopenia, and d-dimer are 0.82, 0.72, and 0.91, respectively, making them better screening tests for severe COVID-19 infection.[43]

The area under the curve (AUC) of KL-6 as a severity marker in the sROC curve is 0.88. Compared to this, AUC of other severity markers are neutrophil count = 0.79, lymphocyte count = 0.87, thrombocyte count = 0.76, D-dimer = 0.77, CRP = 0.88. So, we can draw an inference that the accuracy of KL-6 for diagnosing severe COVID-19 infection is 88%, which is comparable to CRP and lymphocyte count.[43] The meta-analysis of Liu *et al* showed that IL-6 is an adequate predictor of severe disease in patients infected with COVID-19.[44] The AUC was 0.875, which is similar to our study, suggesting the good diagnostic value of our study. Meta-analysis of Akbar *et al* showed that hyponatremia indicates a poor outcome in patients with COVID-19 infection. However, the study had low sensitivity and specificity compared to our meta-analysis.[45] Moreover, the meta-analysis of Silva *et al* showed that IFN-α cannot be used as a severity marker of COVID-19 infection.[46]

Various tools such as pneumonia severity index (PSI), CURB-65 (confusion, uremia, respiratory rate, BP, age ≥ 65 years), acute physiology and chronic health evaluation-2 (APACHE-II), Charlson comorbidity index (CCI), Simplified acute physiology score-3 (SAPS-3), and National Early Warning Score-2 (NEWS-2) have also been used to assess the severity of COVID-19 infection. ROC analysis showed the areas under the curve of the PSI, CURB-65, and APACHE-II scales were 0.83 (95% CI, 0.74-0.93), 0.80 (95% CI, 0.69-0.90), and 0.83 (95% CI, 0.75-0.92), respectively[47] demonstrating them as good tools to assess the severe COVID-19 pneumonia. The study by Christensen *et al* showed that increasing the CCI score was a good predictor of severe COVID infection.[48] The CCI demonstrated excellent discriminative ability, with an AUC of 0.854 ($p < .001$) and an optimal cut-off point of 3 (sensitivity 83.8%, specificity 69.6%, + LR 2.76).[49] Additionally, multiple studies have demonstrated SAPS-3 and NEWS-2 scoring systems as severity and mortality predictor tools of COVID-19 infection.[50–52] Despite serum KL-6 having a similar AUC to this severity assessment tool, the low sensitivity of KL-6 makes it a weaker test.

The CT severity score has an AUC of 0.824 ($p < .001$) and an optimal cut-off point of 53 (sensitivity 64.9%, specificity 84.4%, and PLR 4.17.[49] A study by Saeed *et al* suggested that the CT chest, despite having a pivotal role in severity assessment, is prone to interpreter bias.[53] However, compared with the detection of COVID-19 through chest CT and nucleic acid detection, the measurement of KL-6 levels is fast, inexpensive, noninvasive, no radiation hazard, and sensitive.[10] But, the higher sensitivity of CT chest still makes it a favorable test compared to KL-6.

The early severity assessment is extremely useful in triaging COVID-19 patients to provide optimal treatment, and to decide the need for antiviral drugs. In this case, assessment of

serum KL-6 can be an option for severity triaging. Elevated serum KL-6 is also an independent risk factor to prolong the duration of hospitalization in severe COVID-19 patients.[54] For early prevention of the development of pulmonary fibrosis, elevated concentrations of serum KL-6 in the early stage of COVID-19 should be paid close attention.[55]

To date, the relationship between serum KL-6 and COVID-19 vaccine efficacy has not been studied, but a case of COVID vaccine-induced interstitial lung disease has been reported.[56] Severe COVID-19 is an independent risk factor for the long covid syndrome,[57,58] and in severe COVID KL-6 values are raised. So, we can indirectly interpret that raised KL-6 is a predictor of a long covid syndrome. Moreover, severe COVID infection is a risk factor for thrombotic complications, which implies that higher KL-6 levels will have a similar risk.[59] However, currently there are no studies establishing the direct association of serum KL-6 levels with post-covid syndrome and thrombotic complications.

One study has shown that serum KL-6 levels are significantly higher in non-surviving COVID-19 patients than in surviving patients. The sensitivity and specificity of serum KL-6 values for predicting mortality in COVID-19 patients are 47.6% and 90.5%, respectively.[27] Moreover, at ICU admission, KL-6 serum level was significantly higher in most hypoxic COVID-19 patients, and was independently associated with ICU mortality.[60]

Our study has several strengths. First, compared with the past meta-analyses, our study has included a greater number of studies and more COVID-19 patients. This has given more power to our statistical analyses. Second, to show the robustness of our association, we conducted a trial sequential analysis which depicted that the result was not due to type-I and type-II error, as well as the sample size to conduct the meta-analysis was adequate. Third, the sensitivity analysis showed no single study effect. Fourth, subgroup and regression analysis were done to point out the source of heterogeneity. Fifth, there was no evidence of publication bias which further validated the findings of our meta-analysis.

However, our research has some limitations. Serum KL-6 and mortality association could not be assessed due to a scarce number of studies analyzing both parameters. Association with comorbidities could not be assessed as most of the studies had not reported comorbidities of COVID-19 patients. Heterogenous pooling might also have affected the overall results of the meta-analysis. So, the results of this meta-analysis should be interpreted with caution.

Conclusion

Our meta-analysis shows that serum KL-6 can be used as a severity and prognostic marker of COVID-19 infection. As the diagnostic power of KL-6 is limited, we suggest it as a supporting investigation, primarily to evaluate the extent of lung injury. Furthermore, large-scale studies aimed at assessing severity parameters in COVID-19 infection are warranted.

Abbreviations

KL-6	Krebs von den Lungen-6
COVID-19	Coronavirus Disease 2019
TSA	Trial Sequence Analysis
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
SMD	Standard Mean Difference
CI	Confidence Interval
PLR	Positive Likelihood Ratio
NLR	Negative Likelihood Ratio
DOR	Direct Odds Ratio
TP	True Positive
TN	True Negative
FP	False Positive
FN	False Negative
sROC	Summary Receiver Operating Characterisic Curve
AUC	Area Under Curve
ICU	Intensive Care Unit
CRP	C-Reactive Protein
IL-6	Interleukin-6
SARS CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2

Author contribution(s)

Abhigan Babu Shrestha: Conceptualization; Methodology; Validation; Data analysis; curation; Writing – original draft.
Pashupati Pokharel: Methodology; Validation; Writing – original draft; Writing – review & editing.
Harendra Singh: Data curation; Investigation; Methodology; Writing – original draft; Writing – review & editing.
Sajina Shrestha: Data curation; Investigation; Project administration; Writing – original draft; Writing – review & editing.
Fioni: Supervision; Visualization; Writing – review & editing.

Availability of Data and Materials

Data could be made available upon reasonable request to the corresponding author.

Supplemental Material

Supplemental material for this article is available online.

ORCID iDs

Abhigan Babu Shrestha https://orcid.org/0000-0002-0681-3825
Pashupati Pokharel https://orcid.org/0000-0002-9704-5883

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