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ORIGINAL RESEARCH

Clinical Utility of Circulating Pneumoproteins as Diagnostic and Prognostic Biomarkers in COVID-19: A Systematic Review and Meta-analysis

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ABSTRACT

Introduction: This study explored circulating pneumoproteins in the diagnosis, severity, and prognosis of COVID-19 by meta-analysis.
Methods: We searched five databases and other sources until December 16, 2021. Standardized mean difference (SMD) and 95% confidence interval (CI) were the overall outcomes. RevMan 5.3, Stata 16, and Meta-DiSc 1.4 were utilized for pooled analysis.

Results: A total of 2432 subjects from 26 studies were included. Patients with COVID-19 had higher circulating KL-6, SP-D, and SP-A levels (SMD 1.34, 95% CI [0.60, 2.08]; SMD 1.74, 95% CI [0.64, 2.84]; SMD 3.42, 95% CI [1.31, 5.53], respectively) than healthy individuals. Circulating SP-D levels were not significantly different in survivors and non-survivors (SMD − 0.19, 95% CI [− 0.78, 0.40]). Circulating KL-6, SP-D, and RAGE levels in patients with mild to moderate COVID-19 were significantly lower (SMD − 0.93, 95% CI [− 1.22, − 0.65]; SMD − 1.32, 95% CI [− 2.34, − 0.29]; SMD − 1.17, 95% CI [− 2.06, − 0.28], respectively) than in patients with severe COVID-19. Subgroup analysis suggested that country and total number may be related to the heterogeneity when analyzing SP-D in patients with mild to moderate vs. severe COVID-19. The

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meta-analysis of diagnostic accuracy including KL-6 for severity, KL-6 for mortality, and SP-D for severity demonstrated that they all had limited diagnostic value.
Conclusion: Therefore, circulating pneumoproteins (KL-6, SP-D, and RAGEs) reflect the diagnosis, severity, and prognosis of COVID-19, and follow-up studies are still needed.

Keywords: COVID-19; Coronavirus disease; Pneumoproteins; Krebs von den Lungen-6; Surface protein D; Meta-analysis

Key Summary Points

The roles of circulating pneumoproteins KL-6/SP-D/SP-A/RAGEs/CC-16 in the diagnosis, severity, and prognosis of COVID-19 are controversial
Patients with COVID-19 had higher circulating KL-6, SP-D, and SP-A levels than healthy individuals
Circulating KL-6, SP-D, and RAGE levels in patients with mild to moderate COVID-19 were significantly lower than in patients with severe COVID-19
The meta-analysis results of KL-6 for severity, KL-6 for mortality, and SP-D for severity demonstrated that they had limited diagnostic and prognostic value
Circulating pneumoproteins KL-6/SP-D/RAGEs may reflect the diagnosis, severity, and prognosis of COVID-19

INTRODUCTION

The first ongoing coronavirus disease 2019 (COVID-19) infection was detected in Wuhan, China and subsequently spread globally in a short time [1]. The outbreak was declared a pandemic by the World Health Organization (WHO) in March 2020, and various responses to prevent infection were quickly put in place. As of April 5, 2022, more than 493 million cases and 6.17 million patient deaths have been recorded worldwide (https://coronavirus.jhu.edu/). People infected with COVID-19 can be asymptomatic [2], but can also have fever, cough, and even severe respiratory failure (SRF) [3, 4], along with sore throat, fatigue, joint pain, and loss of smell and taste [5]. Multiple research studies [6, 7] have indicated that serum markers such as ferritin, C-reactive protein (CRP), and lactate dehydrogenase (LDH) are elevated in patients with severe disease compared to those with mild disease, helping to predict disease progression. Nevertheless, the accurate prognostic biomarkers for assessing the severity of COVID-19 have not yet been established. Therefore, it is critical to find appropriate biomarkers to assess the severity and prognosis of patients with COVID-19.

Pneumoproteins, namely lung-specific proteins, are proteins that originate from the lung and are released into the circulation following damage to lung tissues [8, 9]. Changes in their expression levels are considered to be markers of lung injury and inflammation and are closely related to pulmonary homeostasis [10, 11]. The most common measured pneumoproteins are Krebs von den Lungen-6 (KL-6), surface protein D (SP-D), surface protein A (SP-A), advanced glycation end-products (RAGEs), and Clara cell secretory protein-16 (CC-16). Recently, an increasing number of studies have explored the relationship between pneumoproteins and common respiratory diseases [12, 13]. Furthermore, Pramana et al. [14] conducted a meta-analysis of serum KL-6 to depict more severe COVID-19 with high sensitivity and specificity, and Naderi et al. [15] performed a meta-analysis to reveal the potential significance of KL-6 as a predictive biomarker in severe COVID-19. Therefore, we hypothesized that there is a relationship between circulating pneumoproteins and disease progression of COVID-19.

This study conducted a systematic review and meta-analysis of circulating pneumoproteins (including KL-6, SP-A, SP-D, RAGEs, and CC-16) to evaluate their value in the diagnosis of COVID-19 and to clarify their correlations with the severity and prognosis of COVID-19.

METHODS

Following the PRISMA guidelines (2020) [16], the whole retrieval process was performed independently by two members. The protocol of this meta-analysis was registered in PROSPERO (No. CRD42021283569) (Supplementary Material 1). This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Search Strategy

We searched PubMed, EMBASE, Cochrane Library, clinical.gov, GreyLit.org, and other sources. The search scope was from database construction to December 16, 2021. We used free words and MeSH words with no language limitation as shown in Supplementary Material 2, and the search strategy was as follows: ("COVID-19" OR "COVID19" OR "SARS-CoV-2" OR "Sars-CoV-2 infection" OR "2019 nCoV" OR "2019-nCoV infection" OR "coronavirus" OR "coronavirus disease 2019" OR "SARS-CoV-2" OR "Novel coronavirus" OR "nCoV" OR "nCoV pneumonia" OR "Emerging Coronavirus" OR "new coronavirus" OR "corona-virus") AND ("pneumoproteins" OR "KL-6" OR "Krebs von den Lungen-6" OR "SP-A" OR "surfactant protein A" OR "Pulmonary Surfactant Associated Protein A" OR "SP-D" OR "surfactant protein D" OR "Pulmonary Surfactant Associated Protein D" OR "CC-16" OR "club cell secretory protein 16" OR "RAGEs" OR "receptor for advanced glycation end products"). Language limitation was not set during the retrieval. The references of included studies were evaluated for any omission. For articles with missing data, we emailed the corresponding authors.

Study Selection

The study selection was checked by two researchers (YNK and YQZ) independently. A third researcher (SL) decided on the disputed part according to the established protocol. In case of incomplete data or unclear expression, we contacted the original authors via email.

Association Between Pneumoproteins (Including KL-6, SP-A, SP-D, RAGEs, or CC-16) and COVID-19

The inclusion criteria were as follows: (1) studies concerned with patients diagnosed with COVID-19; (2) outcomes that included at least one of the biomarkers (KL-6, SP-A, SP-D, RAGEs, or CC-16); and (3) cohort or case–control studies. In cohort studies, the high-exposure group was severe COVID-19 or non-survivors; the low-exposure group was the mild to moderate COVID-19 group or survivors. In case–control studies, patients with COVID-19 were the case group, and healthy people were the control group. The exclusion criteria were as follows: (1) patients with suspected COVID-19; (2) healthy group with other lung diseases, such as pneumonia, interstitial lung disease (ILD), and acute respiratory distress syndrome (ARDS), in case–control studies; (3) unclear or repeated classification of different groups in cohort studies; (4) pneumoprotein (KL-6, SP-D, SP-A, RAGEs, or CC-16) levels not in the plasma or serum; (5) articles with missing important data and no reply from the corresponding author; and (6) case report, review literature, or duplicate articles.

Diagnostic and Prognostic Value of Pneumoproteins (Including KL-6, SP-A, SP-D, RAGEs, or CC-16) in COVID-19

The inclusion criteria were as follows: (1) patients with COVID-19 diagnosed by PCR detection of nucleic acid and/or radiological evaluation; (2) patients with COVID-19 diagnosed with pneumoproteins (KL-6, SP-D, SP-A, RAGEs, or CC-16); and (3) sufficient outcomes to analyze the sensitivity, specificity, positive likelihood ratio (PLR), and negative likelihood ratio (NLR). The exclusion criteria were as follows: (1) other diagnostic related indices, but not pneumoproteins (KL-6, SP-D, SP-A, RAGEs, or CC-16); (2) pneumoprotein (KL-6, SP-D, SP-A, RAGEs, or CC-16) levels not in the plasma or serum; (3) incomplete diagnostic test data in four grids and no reply from the corresponding author; (4) case report, review literature, or duplicate articles.

Data Extraction and Quality Assessment

Two researchers (YNK and YQZ) completed the data extraction independently, and the disputed parts were decided by a third researcher (SL). The extracted data included the first author's last name, publication date, country of origin, Newcastle–Ottawa scale (NOS) score [17], number of cases and controls, basic information of cases and controls (such as age and sex), diagnostic methods, pneumoprotein level measurement method, levels of pneumoproteins (KL-6, SP-D, SP-A, RAGEs, CC-16), and sensitivity, specificity and area under ROC curve (AUC) of the receiver operating characteristic (ROC) curve in some studies. The NOS score [18] was used for quality evaluation, which includes assessment of selection, comparability, and exposure. We further evaluated the quality of our study using the grading of recommendation, assessment, development, and evaluation (GRADE) approach (https://gdt.gradepro.org).

Statistical Analysis

RevMan 5.3, Stata 16, and Meta-DiSc 1.4 were utilized to analyze and integrate the data. All studies used standardized mean difference (SMD) as the outcome. Data following a normal distribution are presented in the form of mean ± SD, while data with a non-normal distribution were converted to mean ± SD through an online tool (https://www.math.hkbu.edu.hk/~tongt/papers/median2mean.html) [19, 20]. The I^2 test and Cochran's Q test were used to evaluate the heterogeneity among studies. When the heterogeneity was low ($I^2 < 50\%$), the fixed-effects model was the first choice. If high heterogeneity was found ($I^2 > 50\%$), the random-effects model was applied [21–23]. Furthermore, we explored the sources of high heterogeneity using subgroup analysis (study number ≥ 5). Diagnostic meta-analysis included the results of pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and summary ROC (sROC) curve drawing. Different diagnostic thresholds in each single diagnostic test can cause a threshold effect, which was an important source of heterogeneity in diagnostic meta-analysis. Spearman correlation coefficient between sensitivity and specificity was calculated to explore this problem: if there was a strong negative correlation between them, it indicated the existence of a threshold effect. The accuracy was defined according to AUC: ≤ 0.5, poor accuracy; > 0.5 and ≤ 0.7, moderate accuracy; > 0.7 and ≤ 0.9, good accuracy; 1, perfect accuracy [24]. When the number of studies was too limited to calculate the pooled AUC, DOR can be referred to. A higher DOR also indicated a better diagnostic value. For the sensitivity analysis, omission of each study was performed. In addition, Egger's test [25] was used to evaluate publication bias.

RESULTS

Study Selection

The study was completed according to the PRISMA guidelines. After screening 417 articles from five databases and other sources, we further assessed 26 studies [26–51] on the basis of the eligibility criteria. The included studies represented broad geographic representations with mixed populations. Most studies were performed exclusively in hospital settings, mainly in Europe (UK, Italy, Germany, Belgium, Portugal, Russia, the Netherlands, and Denmark), North America (USA and Mexico), and Asia (China, Japan, and Turkey). A total of 2432 subjects were included, including 310 healthy people. In terms of survival, 569 survivors and 81 non-survivors with COVID-19 were included in this part. According to the WHO COVID-19 guidelines (respiratory symptoms and respiratory status), 1250 patients with mild to moderate COVID-19 and 389 patients with severe COVID-19 were included when analyzing the severity of disease. The study screening flowchart is shown in Fig. 1, and the characteristics of each study are presented in Table 1.

Quality Assessment

By using the NOS score, we found that the average score was 5.73 (Supplementary Material 2), indicating that most articles adopted a reasonable methodology. Additionally, the results from the GRADE system are shown in Supplementary Material 3. The certainty of evidence was very low in our study. As all included studies were observational studies, the certainty of evidence can only start from low. Moreover, the NOS score, high heterogeneity among studies, and some uncontrollable confounding factors further affect the certainty of evidence, making it from low to very low.

Meta-analysis of Circulating KL-6 Levels

Seven sets of data from four studies were analyzed for the association of KL-6 levels in patients with COVID-19 and healthy controls, as shown in Fig. 2a. Patients with COVID-19 had significantly higher circulating KL-6 levels than healthy individuals (SMD 1.34 [0.60, 2.08]). Only one study provided data related to KL-6 levels in surviving and non-surviving patients with COVID-19. Circulating KL-6 levels were significantly lower in surviving patients than in non-surviving patients (SMD − 0.93 [− 1.65, − 0.22]). A pooled analysis of nine sets of data from eight studies analyzed KL-6 levels in patients with mild to moderate COVID-19 and patients with severe COVID-19. As shown in Fig. 2b, circulating KL-6 levels in patients with mild to moderate COVID-19 were significantly lower (SMD − 1.32 [− 1.50, − 1.14]) than those in patients with severe COVID-19.

Meta-analysis of Circulating SP-D Levels

As shown in Fig. 2c, the pooled analysis of SP-D levels showed that circulating SP-D levels in patients with COVID-19 were significantly higher (SMD 1.74 [0.64, 2.84]) than in healthy individuals. Figure 2d shows that there was no significant difference in circulating SP-D levels between surviving patients and non-surviving patients (SMD − 0.19 [− 0.78, 0.40]). Figure 2e shows that patients with mild to moderate COVID-19 exhibited significantly lower SP-D levels than those with severe COVID-19, with SMD − 1.32 [− 2.34, − 0.29].

Subgroup analysis was performed because of the high heterogeneity (Table 2). Patients with mild to moderate COVID-19 had no significant difference in circulating SP-D levels for the Chinese subgroup (SMD − 0.72 [− 1.73, 0.28]), but significantly lower circulating SP-D levels in mild to moderate COVID-19 than those with severe COVID-19 in other countries (SMD − 2.19 [− 3.85, − 0.80]). Patients with mild to moderate COVID-19 with total number ≤ 40 had significantly lower circulating SP-D levels than patients with severe COVID-19 (SMD − 2.47 [− 3.36, − 1.57]), whereas there was no significant difference with total number > 40 (SMD − 0.58 [− 1.39, 0.23]).

Meta-analysis of Circulating SP-A Levels

A pooled analysis for SP-A levels in COVID-19 was performed in Fig. 2f. Circulating SP-A levels were significantly higher in patients with COVID-19 than in healthy individuals (SMD 3.42 [1.31, 5.53]). For SP-A levels and survival, only one study was included; thus, the reproducibility of the results was very low. Surviving patients may have significantly lower circulating SP-A levels than non-surviving patients, with SMD − 0.94 [− 1.54, − 0.34]. Two studies provided data related to circulating SP-A and severity (Fig. 2g). Circulating SP-A levels in patients with mild to moderate COVID-19 were not significantly different from those with severe COVID-19 (SMD − 3.93 [− 8.89, 1.03]).

Meta-analysis of Circulating RAGE Levels

A pooled analysis of RAGE levels and COVID-19 was presented in Fig. 2h. No significant difference exists in circulating RAGE levels between patients with COVID-19 and healthy individuals (SMD 0.94 [− 0.52, 2.39]). Circulating RAGE levels in patients with mild to moderate COVID-19 were significantly lower (SMD − 1.17 [− 2.06, − 0.28]) than in those with severe COVID-19 (Fig. 2I).

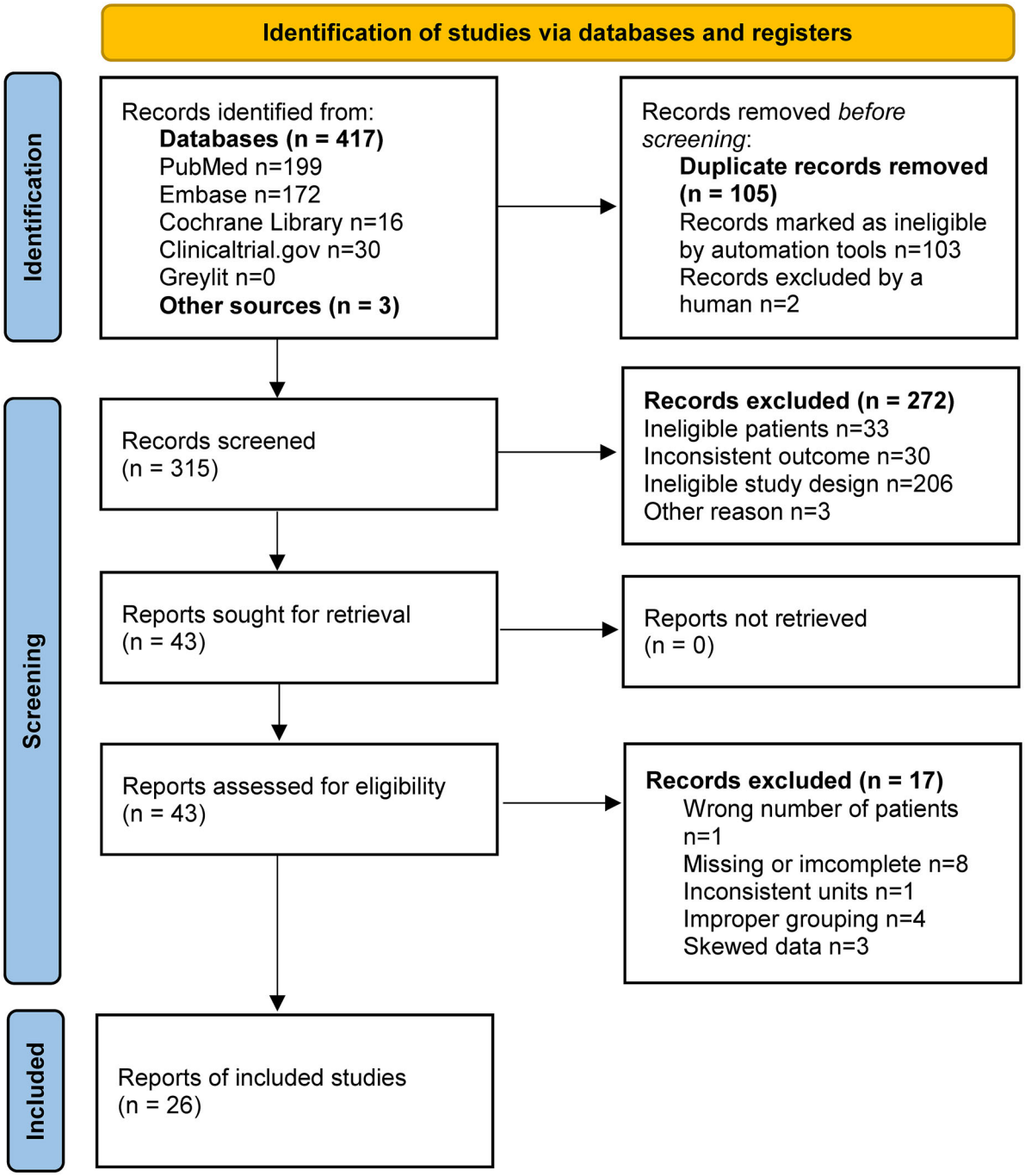

Fig. 1 Flowchart of study inclusion and exclusion

Meta-analysis of Circulating CC-16 Levels

Only one study was included for CC-16 levels. The level of circulating CC-16 in the surviving group was not significantly lower than that in the non-surviving group (SMD − 0.33 [− 0.83, 0.16]).

Table 1 Baseline characteristics of studies included in the meta-analysis

No.	Author	Year	Country	Protein	No. of patients		Gender		Age		Diagnostic criteria	Detection method	Data
					COVID-19 patients	Healthy people	COVID-19 patients	Healthy people	COVID-19 patients	Healthy people			
1	Alay [26]	2021	Turkey	SP-D	64[a]	50	29/35	28/22	55.17 ± 16.1	51.3 ± 14.5	RT-PCR	SP-D ELISA kit (Elabscience, HUMAN SP-D: catlog no. E-EL-H1269)	③
2	Awano [27]	2020	Japan	KL-6	33[b]		23/10		40 (33–50)		RT-PCR	Nanopia KL-6 reagent kit (Sekisui Medical Co., Ltd, Tokyo, Japan)	③④
					21[c]		15/6		64 (56–78)				
3	Bergantini [28]	2021	Italy	KL-6	14[b]		11/3		62.6 ± 15.6		Clinical and radiological evaluation	KL-6 mAb reagent (Fujirebio Europe, UK)	③④
					10[c]		8/2		65.2 ± 8				
4	D'Alessandro [29]	2020	Italy	KL-6	22[a]	22	15/7	6/16	63 [59–68]	54 [29–60]	Hospitalized patients with COVID-19	KL-6 reagent (Fujirebio Europe, UK)	②③④
					10[b]		6/4		64 [51–64]				
					12[c]		9/3		62 [60–68]				
5	D'Alessandro [30]	2021	Italy	KL-6	40[b]		21/19		64 (58–72)		PCR-positive	Chemiluminescence assay	③
					14[c]		12/2		65 (59–71)				
6	Deng [31]	2021	China	KL-6	149[b]		65/84		48 (34.5–62)		Diagnosis and treatment scheme	Diagnostic kit (catalog no. 200309; Kangrun Biotech)	③
					17[c]		9/8		55 (53–68)				
7	Frix [32]	2020	Belgium	KL-6	83[a]	70	52/31	35/35	72 (58–82)	58 (52–64)	PCR-positive	Fujirebio Lumipulse 1200 instrument (Tokyo, Japan)	②
8	Gomes [33]	2021	Portugal	RAGEs	20[a]	9	16/4	6/3	56 [40–65]	58 [48–61]	PCR-positive	Sandwich ELISA kits (RayBiotech, GA)	②
9	He [34]	2021	China	SP-A KL-6	28[a]	25	16/12	14/11	64.93 ± 1.63	64.56 ± 1.55	–	KL-6 chemiluminescent enzyme immunoassay method (LUMIPULSE G2100, FUJIREBIO INC, Japan) SP-A ELISA kit (OKAN05694, Aviva Systems Biology, San Diego, USA)	②

Table 1 continued

No.	Author	Year	Country	Protein	No. of patients		Gender		Age		Diagnostic criteria	Detection method	Data
					COVID-19 patients	Healthy people	COVID-19 patients	Healthy people	COVID-19 patients	Healthy people			
10	Herr [35]	2021	Germany	SP-D	35[a]	28	26/9	28/0	63.86 ± 3.18	62.42 ± 2.14	Hospitalized patients with COVID-19	–	①②
11	Kerget B [36]	2020	Turkey	SP-D	88[a]	20	41/47	8/12	49.1 ± 21.1	35.2 ± 6.9	PCR-positive	ELISA kit (Elabscience human ELISA kit, UK)	①②
12	Kerget F [37]	2021	Turkey	SP-A	88[a]	20	41/47	8/12	49.1 ± 21.1	35.2 ± 6.9	RT-PCR	–	②
13	Khadzhieva [38]	2021	Russia	SP-A	90[d]		49/41		45.5 (40–55.25)		Interim guidelines	Elisa kit (Elisa Alisei analyzer, Radim, Italy)	①
				SP-D									
				CC16	19[e]		11/8		61 (57–72)				
14	Lim [39]	2021	Germany	RAGEs	75[b]	15	32/43	6/9	57 (53–60)	36 (34–52)	PCR-positive	ELISA kit (Minneapolis, MN, USA)	③
					57[b]		31/26		57 (55–64)				
					32[c]		26/6		69 (64–77)				
15	Manoppo [40]	2021	Indonesia	SP-D	28[a]						–	–	①
16	Peng [41]	2021	China	KL-6	49[b]	65	25/24	28/37	45 (16–72)	50 (22–69)	PCR-positive	Latex agglutination assay (Nanopia KL-6, Sekisui Medical Co., Ltd., Tokyo, Japan)	②③
					28[b]		12/16		51 (27–75)				
					26[c]		24/12		56 (28–86)				
17	Saito [42]	2020	Japan	SP-A	34[b]	22	14/20		49.6 ± 15.7		RT-PCR	KL-6 electrochemiluminescence immunoassay (Sekisui Medical Co., Ltd., Tokyo, Japan)	②③④
				SP-D	12[c]		7/5		65.1 ± 10.7				
				KL-6								SP-A ELISA kit (Brno, Czech Republic)	
												SP-D ELISA kit (Yamasa Co., Japan)	
18	Scotto [43]	2021	Italy	KL-6	34[a]		23/11		63 [54–71]		PCR-positive	KL-6 antibody kit (LUMIPULSE G1200, Fujirebio)	①

Table 1 continued

No.	Author	Year	Country	Protein	No. of patients		Gender		Age		Diagnostic criteria	Detection method	Data
					COVID-19 patients	Healthy people	COVID-19 patients	Healthy people	COVID-19 patients	Healthy people			
19	Shao [44]	2020	China	RAGEs SP-D	115[a]	30	60/55	15/15	49 [37–62]	51 [44–55]	PCR-positive	SP-D AT2 cell marker RAGE AT1 cell marker	②③
20	Tong [45]	2021	China	SP-D	30[b] 9[c]		16/14 4/5		49 (25–55) 54 (47–75)		PCR-positive	ELISA (Boster Biological Technology Co. Ltd., Wuhan, China)	③
21	Wang [46]	2021	China	KL-6 SP-D	52[b] 12[c]		26/26 9/3		42 [31, 51.75] 53.5 [49, 66.75]		RT-PCR	Chemiluminescent methods (Kangrun Biotech)	③④
22	Xue [47]	2020	China	KL-6	6[b] 15[c]	43	2/4 12/3	30/13	55 ± 18.84 57.2 ± 14.25	51.70 ± 3.27	PCR-positive	Chemiluminescence immunity (KAESER 1000)	③
23	Xue [48]	2021	China	KL-6	226[b] 63[c]		99/127 31/32		56 [41, 66] 61 [54.5, 69]		PCR-positive	KL-6 kit (Shanghai Medconn Diagnostics Technology Co., Ltd. China)	③④
24	Yalcin [49]	2021	Turkey	RAGEs	35[#] 23[#]	19	22/13 14/9	10/9	42.7 ± 21.6 62.2 ± 11.9	42.42 ± 10.88	PCR-positive	ELISA Kit (No. E0031Hu, Bioassay Technology Laboratory, Shanghai, China)	②③
25	Yamaya [50]	2021	Japan	KL-6	296[b]/350[d] 60[c]/6[e]						PCR-positive	Nanopia KL-6 Reagent kit (Sekisui Medical Co., Ltd., Tokyo, Japan)	①③④

Table 1 continued

No.	Author	Year	Country	Protein	No. of patients		Gender		Age		Diagnostic criteria	Detection method	Data
					COVID-19 patients	Healthy people	COVID-19 patients	Healthy people	COVID-19 patients	Healthy people			
26	Yosuke [51]	2021	Japan	SP-D	50[b]		30/20		40 (29.7–58)		PCR-positive	ELISA kit (R&D Systems)	③
				SP-A	22[c]		18/4		61 (56.7–69)				

Included data: ① Circulating pneumoproteins levels between patients with COVID-19 and healthy group. ② Circulating pneumoproteins levels between surviving patients and non-surviving patients with COVID-19. ③ Circulating pneumoproteins levels between patients with mild to moderate COVID-19 and patients with severe COVID-19. ④ Meta-analysis for diagnostic value of circulating pneumoproteins levels

[a]Total patients with COVID-19

[b]Patients with mild to moderate COVID-19

[c]Patients with severe COVID-19

[d]Surviving patients with COVID-19

[e]Non-surviving patients with COVID-19

[#]Patients with COVID-19 divided by other methods

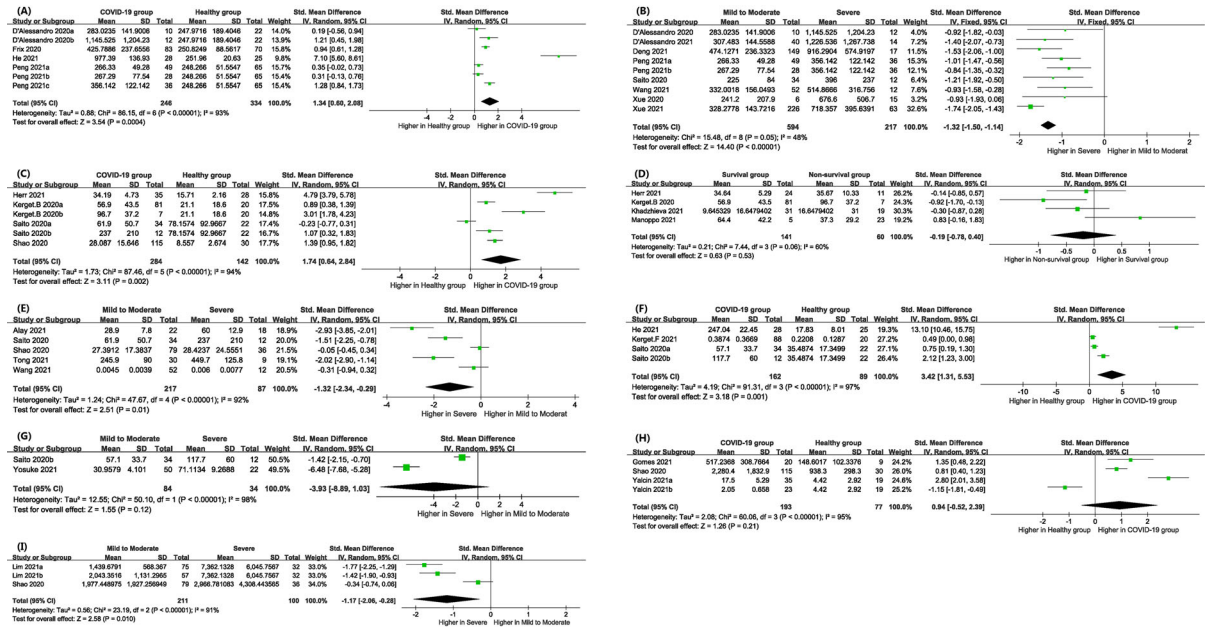

Fig. 2 Forest plot of circulating **a** KL-6, **c** SP-D, **f** SP-A, and **h** RAGE levels between patients with COVID-19 and healthy group. Forest plot of circulating **b** KL-6, **e** SP-D, **g** SP-A, and **i** RAGE levels between patients with mild to moderate COVID-19 and patients with severe COVID-19. **d** Forest plot of circulating SP-D levels between surviving patients and non-surviving patients with COVID-19

Table 2 Subgroup analysis of circulating SP-D levels in patients with mild to moderate COVID-19 and patients with severe COVID-19

Subgroup	Data sets	Number of participants	Model	SMD (95% CI)	*P*	I^2 (%)
Country						
China	3	218	–	− 0.72 [− 1.73, 0.28]	0.0003	87
Other countries	2	86	–	− 2.19 [− 3.58, − 0.80]	0.02	82
Total	5	304	Random	− 1.32 [− 2.34, − 0.29]	< 0.001	92
Total number						
≤ 40	2	79	–	− 2.47 [− 3.36, − 1.57]	0.16	49
> 40	3	225	–	− 0.58 [− 1.39, 0.23]	0.003	83
Total	5	304	Random	− 1.32 [− 2.34, − 0.29]	< 0.001	92

SMD standard mean difference, *CI* confidence intervals

Meta-analysis Results for Diagnostic and Prognostic Value

Diagnostic Value of Circulating KL-6 for Severity

Seven studies were pooled for the meta-analysis of the diagnostic accuracy. The overall sensitivity was 0.67 [0.60, 0.73], and the overall specificity was 0.82 [0.79, 0.85] (Table 3). Meanwhile, the pooled PLR and NLR were estimated to be 3.27 [1.90, 5.62] and 0.42 [0.32, 0.55]. The pooled DOR was 11.20 [7.57, 16.57] and the pooled AUC was 0.844 [0.787, 0.902]. Significant heterogeneity was observed in many outcomes of the diagnostic analysis (sensitivity: $I^2 = 66.9\%$, $P = 0.0059$; specificity: $I^2 = 88.8\%$, $P < 0.001$; PLR: $I^2 = 87.0\%$, $P < 0.001$). The sROC curve showed a shoulder–arm-shaped distribution. The corresponding Spearman correlation coefficient was 0.821 ($P = 0.023$; $\alpha = 0.5$), suggesting that a threshold effect contributed to the heterogeneity. All the results indicated that circulating KL-6 may be an indicator of good accuracy for the severity of COVID-19, and the threshold effect may be the main cause of high heterogeneity.

Prognostic Value of Circulating KL-6 for Mortality

Only two studies were included in Table 3. The pooled sensitivity, specificity, PLR, and NLR were 0.476 [0.257, 0.702], 0.905 [0.871, 0.933], 5.792 [1.106, 30.336], and 0.422 [0.059, 3.047], respectively. Additionally, the pooled DOR was estimated to be 13.235 [1.221, 143.49]. Because of the limited number of included studies, we did not further explore the heterogeneity and threshold effects. This indicates that circulating KL-6 may be an indicator for predicting the mortality of COVID-19.

Diagnostic Value of Circulating SP-D for Severity

Table 3 shows the results for the diagnostic value of circulating SP-D to identify disease severity. The pooled sensitivity and specificity were 0.810 [0.581, 0.946] and 0.859 [0.750, 0.934], respectively. The pooled PLR, NLR, and DOR were estimated to be 5.773 [3.044, 10.948], 0.244 [0.102, 0.584], and 24.821 [6.582, 93.600]. The pooled DOR is high, indicating that circulating SP-D may be an indicator for the severity of COVID-19.

Sensitivity Analysis

Sensitivity analysis was performed for outcomes that included more than two studies. After each study was excluded sequentially, outcomes were found to be unchanged, meaning the results were robust (Supplementary Material 4).

Publication Bias

According to the Egger's test, the *P* values were all greater than 0.05, meaning low possibilities

Table 3 Meta-analysis results for diagnostic and prognostic value

Objective	Se [95% CI]	Sp [95% CI]	PLR [95% CI]	NLR [95% CI]	DOR [95% CI]	AUC [95% CI]
KL-6 for severity	0.67 [0.60, 0.73]	0.82 [0.79, 0.85]	3.27 [1.90, 5.62]	0.42 [0.32, 0.55]	11.20 [7.57, 16.57]	0.844 [0.787, 0.902]
KL-6 for mortality	0.476 [0.257, 0.702]	0.905 [0.871, 0.933]	5.792 [1.106, 30.336]	0.422 [0.059, 3.047]	13.235 [1.221, 143.49]	–
SP-D for severity	0.810 [0.581, 0.946]	0.859 [0.750, 0.934]	5.773 [3.044, 10.948]	0.244 [0.102, 0.584]	24.821 [6.582, 93.600]	–

Se sensitivity, *Sp* specificity, *PLR* pooled positive likelihood ratio, *NLR* negative likelihood ratio, *DOR* diagnostic odds ratio, *AUC* area under ROC curve

of obvious publication bias, except for the results of circulating SP-D levels in patients with mild to moderate vs. severe COVID-19 and circulating SP-A levels in patients with COVID-19 vs. healthy people (Supplementary Material 4). We further carried out metatrim for the two comparisons with *P* value less than 0.05, and the results indicated a non-negligible existence of publication bias.

DISCUSSION

The pathogen responsible for the worldwide pandemic of COVID-19 is the 2019 novel coronavirus (SARS-CoV-2) [52]. It has been confirmed that SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the surface of the host cell membrane through Spike protein [53–55], invades the host cells, and eventually leads to the occurrence of the disease [56]. ACE2 is widely expressed on human tissues, especially in the alveolar epithelial cells [57]. The lungs are undoubtedly the primary target of SARS-CoV-2. When the patient's inflammatory response continues to progress, cellular responses induce apoptosis of normal lung tissue and further damage to the alveolar structure. Studies have observed that some patients with COVID-19 have pulmonary interstitial fibrosis and type II alveolar epithelial cell hyperplasia [58]. In addition, both immunohistochemical and electron microscopy findings suggest damage of type II alveolar epithelial cells with mild to moderate hyperplasia [59].

KL-6 is a high molecular weight glycoprotein secreted by type II alveolar and bronchiolar epithelial cells [60, 61], which promotes chemotactic activity and anti-apoptotic effects in human lung fibroblasts. SP-A and SP-D are collagen glycoproteins mainly secreted by type II alveolar cells and have an innate immune defense function [62]. They can participate in the host's innate defense against microorganisms and regulate the adaptive immune response. RAGEs are 35-kDa proteins that are recognized as members of the immunoglobulin superfamily and a hallmark of alveolar epithelial injury [63]. Meanwhile, the RAGE pathway is involved in the pathogenesis of some lung diseases [64]. CC-16 is a 16-kDa pneumoprotein produced predominantly by club cells [65] found in respiratory bronchioles and from the non-ciliated columnar cells of the large and small airways [66].

It has been speculated that SARS-CoV-2 can induce cytopathic effects on type II lung cells, leading to lung injury. When pulmonary epithelial lesions are present, alveolar capillary leakage leads to elevated or decreased levels of circulating pneumoproteins. Therefore, the expression levels of circulating pneumoproteins can reflect the condition and prognosis of COVID-19 to some degree (Fig. 3). Exploring the link between COVID-19 and pneumoproteins could be of great clinical value.

Of the 417 articles we retrieved from five databases and other sources, we included only 26, which originated from multiple continents (Europe, North America, and Asia). We explored the relationship between circulating pneumoproteins (including KL-6, SP-A, SP-D, RAGEs, CC-16) and COVID-19 and their diagnostic and prognostic value in terms of disease severity and mortality. The pooled results showed that the levels of circulating KL-6, SP-D, and SP-A in patients with COVID-19 were significantly higher than those in healthy individuals, but no significant difference existed in RAGE levels. The non-surviving group had non-significantly different circulating SP-D levels compared with the surviving group. Finally, circulating KL-6, SP-D, and RAGE levels were significantly lower in patients with mild to moderate COVID-19 than in those with severe COVID-19, while there was no significant difference in circulating SP-A levels between the two groups. For the groups with non-significant results (RAGE levels between patients with COVID-19 and healthy individuals, SP-D levels between surviving and non-surviving patients, SP-A levels between patients with mild to moderate and severe COVID-19), we found that the number of studies included was relatively small. The results of the diagnostic meta-analysis demonstrated that circulating KL-6 levels had good accuracy in diagnosing disease severity, and there was a threshold effect leading to heterogeneity. Pramana's study [14] was limited by the number of

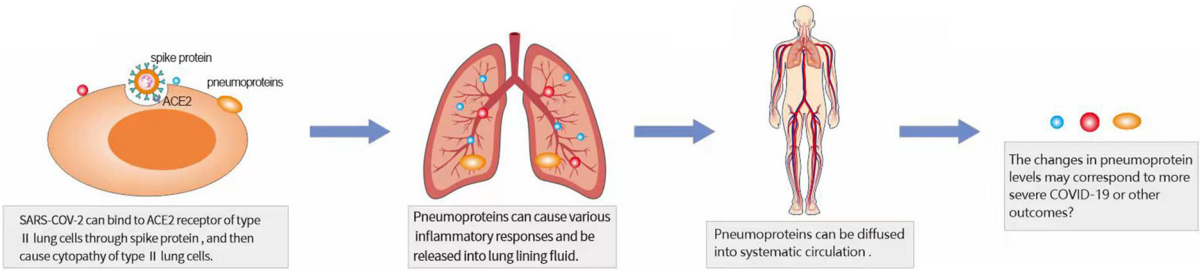

Fig. 3 Possible mechanism of circulating pneumoproteins in COVID-19

studies and they could not draw a definitive conclusion on the diagnostic value of KL-6 for disease severity. We included more studies and concluded that KL-6 has a good diagnostic value for disease severity according to the pooled AUC. However, considering the threshold effect and the inability to conduct subgroup analysis, the diagnostic value of KL-6 for disease severity is still limited. In addition, like Wang et al. [67], we also referred to the pooled DOR value for the part with insufficient numbers of studies. The DORs of KL-6 for mortality and SP-D for severity are relatively high and the heterogeneity is also high, so they may only have limited diagnostic value in this way.

Most of the studies included have high heterogeneity. Subgroup analysis was conducted to explore the reason for heterogeneity. However, as a result of the limited number of studies, it was uncertain whether country and total number may be correlated with the high heterogeneity. In addition, the sensitivity analysis results suggest that all results are robust. Publication bias was measured using Egger's test. Most studies have no publication bias, except for two comparisons. Because of the limited number of included studies, we cannot conduct meta-regression to explore the sources of heterogeneity and report a more detailed discussion. Additionally, this study's certainty of evidence was evaluated to be very low according to the GRADE system. All of these suggest issues that follow-up studies should be carried out under the condition of multiregional cooperation and large population base.

Limitations

The present study has some limitations. First, the included studies were limited. Many studies may have some relevance, but they were excluded because the data were incomplete. Only one or two studies were included in some parts, and the pooled results may lack strong reproducibility of the results. Similarly, there are few studies to analyze diagnostic accuracy. Perhaps follow-up studies could focus on the accuracy of pneumoproteins in the diagnosis of COVID-19. Second, our research may have some regional bias, as studies were mainly from Europe, North America, and Asia. COVID-19 has become a global disease in the last 2 years; thus, it is better to summarize the situations of all regions in the world to obtain more accurate conclusions. Third, there may be many confounding factors among the included studies. As a result of differences in race, testing technology, and experimental operation in various countries, the values included in our study also varied greatly, even if we had converted the same indicator into the same unit. Therefore, we finally selected SMD to analyze the results. Meanwhile, we tried to include studies in which the circulating pneumoproteins levels were detected at admission or within 1–3 days after admission. However, there may still be differences at different times of measurement in each study, which cannot be ignored. Fourth, COVID-19 may often appear at the same time with other diseases or symptoms. However, in order to avoid the influence of too many confounding factors, we selected patients only with COVID-19 and without other serious diseases, which is prevents us from drawing conclusions

related to comorbidity status. Finally, there is obviously high heterogeneity among many parts, and we only analyzed one part because of the limited number of studies. Each study had different concerns, and there was little common basis for subgroup classification. For the part related to severity, the classification standards of different countries and regions may also be different. We referred to WHO COVID-19 guidelines, and mainly considered respiratory symptoms (including fever, cough, throat pain, polypnea, dyspnea, etc.) and respiratory status (including character and rate of resuscitation, oxygen saturation, monetary imaging, etc.). However, there may be slight differences in the specific clinical classification of each study, and with the growing understanding and prevention of coronavirus, the classification guidelines on the severity are constantly updated, which may be an important source of high heterogeneity in our study.

CONCLUSIONS

We explored the relationship between circulating pneumoproteins and COVID-19 in terms of disease, mortality, and disease severity. The results showed that circulating KL-6, SP-D, and SP-A levels in patients with COVID-19 were significantly higher than those in healthy people. The non-surviving group had non-significantly different SP-D levels compared with the surviving group. The levels of circulating KL-6, SP-D, and RAGEs in patients with mild to moderate COVID-19 were significantly lower than those in patients with severe COVID-19, and the results of subgroup analysis suggest that country and total number may be responsible for the high heterogeneity. Furthermore, the pooled meta-analysis of circulating KL-6 in disease severity and in mortality, and SP-D in disease severity demonstrated that they all had limited diagnostic value. As the number of included studies is limited and regional, multi-regional, multi-aspect, and multicenter studies are still necessary to explore the influence of various pneumoproteins on the pathogenesis and prognosis of COVID-19 and their diagnostic value.

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Authors' Contributions. SL and JH: Study concept, drafting the article, final quality assessment, making critical revisions. YNK and YQZ: quality assessment, data collection, analysis, revision of the article. SHC, LW and RLC: data collection, analysis, making and modifying charts. All authors read and approved the final manuscript.

Compliance with Ethics Guidelines. This article is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

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