

Serum KL-6 concentrations as a novel biomarker of severe COVID-19

Miriana d'Alessandro BS¹  | Paolo Cameli MD¹  | Rosa Metella Refini MD¹  |
 Laura Bergantini BS¹  | Valerio Alonzi MD¹ | Nicola Lanzarone MD¹ |
 David Bennett MD¹ | Giuseppe Domenico Rana MD¹ |
 Francesca Montagnani MD, PhD^{2,3} | Sabino Scolletta MD⁴ | Federico Franchi MD⁴ |
 Bruno Frediani MD⁵ | Serafina Valente MD⁶ | Maria Antonietta Mazzei MD⁷ |
 Francesco Bonella MD⁸ | Elena Bargagli MD¹ 

¹Respiratory Diseases and Lung Transplantation, Department of Medical and Surgical Sciences & Neurosciences, Siena University Hospital, Siena, Italy

²Department of Specialized and Internal Medicine, Tropical and Infectious Diseases Unit, University Hospital of Siena, Siena, Italy

³Department of Medical Biotechnologies, University of Siena, Siena, Italy

⁴Department of Medicine, Surgery and Neuroscience, Anesthesia and Intensive Care Unit, University of Siena, Siena, Italy

⁵Research Center of Systemic Autoinflammatory Diseases and Behçet's Disease and Rheumatology-Ophthalmology Collaborative Uveitis Center, Department of Medical Sciences, Surgery and Neurosciences, University of Siena, Siena, Italy

⁶Department of Cardiovascular Diseases, University of Siena, Siena, Italy

⁷Diagnostic Imaging Section, Department of Medical and Surgical Sciences & Neurosciences, Siena University Hospital, Siena, Italy

⁸Center for Interstitial and Rare Lung Disease, Department of Pulmonology, Ruhrlandklinik University Hospital, Essen, Germany

Correspondence

Miriana d'Alessandro, BS, UOC Malattie Respiratorie, Dipartimento di Medicina Clinica e Scienze Immunologiche, Policlinico Le Scotte, Viale Bracci, 53100 Siena, Italy.
 Email: dalessandro.miriana@gmail.com

Abstract

Severe acute respiratory syndrome coronavirus 2-induced direct cytopathic effects against type I and II pneumocytes mediate lung damage. Krebs von den Lungen-6 (KL-6) is mainly produced by damaged or regenerating alveolar type II pneumocytes. This preliminary study analyzed serum concentrations of KL-6 in patients with coronavirus disease (COVID-19) to verify its potential as a prognostic biomarker of severity. Twenty-two patients (median age [interquartile range] 63 [59-68] years, 16 males) with COVID-19 were enrolled prospectively. Patients were divided into mild-moderate and severe groups, according to respiratory impairment and clinical management. KL-6 serum concentrations and lymphocyte subset were obtained. Peripheral natural killer (NK) cells/ μ L were significantly higher in nonsevere patients than in the severe group ($P = .0449$) and the best cut-off value was 119 cells/ μ L. KL-6 serum concentrations were significantly higher in severe patients than the non-severe group ($P = .0118$). Receiver operating characteristic analysis distinguished severe and nonsevere patients according to KL-6 serum levels and the best cut-off value was 406.5 U/mL. NK cell analysis and assay of KL-6 in serum can help identify severe COVID-19 patients. Increased KL-6 serum concentrations were observed in patients with severe pulmonary involvement, revealing a prognostic value and supporting the potential usefulness of KL-6 measurement to evaluate COVID-19 patients' prognosis.

KEYWORDS

biomarker, COVID-19, KL-6, prognosis

Abbreviations: ICU, intensive care unit; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6.

Francesco Bonella and Elena Bargagli are members of the European Reference Network for Rare Respiratory Diseases (ERN-LUNG). Miriana d'Alessandro and Paolo Cameli contributed equally to this study.

1 | INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan (China) in December 2019 and was declared pandemic by the World Health Organization (WHO) in March 2020. It has been hypothesized that CD4- and CD8-positive cells play a crucial role in defence against coronavirus, together with activated B cells producing specific antibodies and proinflammatory cytokines. Elevated serum concentrations of proinflammatory cytokines and oxidative stress mediators participate in lung injury, facilitating acute respiratory distress syndrome (ARDS) in severe patients. Host susceptibility and virus-induced direct cytopathic effects against type I and II pneumocytes mediate lung damage.

Krebs von den Lungen-6 (KL-6) is a high molecular weight glycoprotein, elevated in serum of patients with interstitial lung diseases (ILDs), such as idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. It is mainly produced by damaged or regenerating alveolar type II pneumocytes.¹ This mucin protein is recognized as a prognostic bioindicator of ILDs, predicting response to antifibrotic therapies.²⁻⁴ It has also been proposed as a prognostic marker of ARDS: high KL-6 concentrations have been demonstrated in ventilated patients and showed a correlation with risk of mortality.⁵ KL-6 serum levels in ARDS may reflect the alveolar epithelial cell damage induced by mechanical ventilatory support.⁵ High peripheral levels of this protein have also been reported in *Legionella*, *Pneumocystis jirovecii* infections,^{6,7} and measles-associated pneumonia,⁸ as well as in viral pneumonia.⁹

This preliminary study analyzed serum concentrations of KL-6 for the first time in a population of hospitalized coronavirus disease (COVID-19) patients to verify its potential as a prognostic biomarker of severity.

2 | MATERIALS AND METHODS

2.1 | Study population

Twenty-two patients (median age [interquartile range {IQR}] 63 [59-68] years, 16 males), hospitalized at Siena University Hospital with COVID-19, were enrolled prospectively. Patients were divided into mild-moderate and severe groups, according to respiratory impairment and clinical management. All patients in the severe group underwent intubation and mechanical ventilation in the COVID intensive care unit (ICU), while mild-moderate patients (not requiring intubation) were hospitalized for pharmacological treatment and oxygen supplementation or noninvasive ventilation. Patients in the severe group included nine patients with bilateral diffuse interstitial pneumonia and three patients with focal bilateral pneumonia. The 12 mild-moderate patients included three with bilateral diffuse pneumonia, one with monolateral pneumonia, and the other eight with focal bilateral pneumonia, all documented radiologically. Patients with pre-existing ILDs, chronic obstructive lung disease, or concomitant infections were excluded from the study.

Twenty-two healthy volunteers (median age [IQR] 54 [29-60] years, six males) were also enrolled. They had no history of concomitant pathologies and were not on any medication. They had normal lung function test parameters and normal chest X-ray.

All patients gave their written informed consent to the study. The study was approved by our local ethics committee (BIOBANCA-MIU-2010).

2.2 | KL-6 assay

Serum samples were obtained from all patients at hospital admission before any biological treatment or infusion of high-dose intravenous steroids or invasive ventilation. Serum concentrations of KL-6 (sKL-6) were measured by KL-6 reagent assay (Fujirebio Europe, UK), as previously reported.^{2,4,10} The principle of the assay is agglutination of sialylated carbohydrate antigen in samples with KL-6 monoclonal antibody by antigen-antibody reaction. The change in absorbance was measured to determine KL-6 concentrations, which were expressed in U/mL.

2.3 | Flow cytometry analysis

Blood samples were processed by flow cytometry using a panel of monoclonal antibodies (BD Multitest™ 6-color TBNK; BD-Biosciences, San Jose, CA), including fluorescein isothiocyanate-labelled CD3, phycoerythrin-labelled CD16 and CD56, PerCPCy5.5-labelled CD45, PE-Cy7-labelled CD4, APC-labelled CD19, and APCCy7-labelled CD8, according to the manufacturer's instructions. At least 30 000 events were collected for each sample. Data were analyzed using DIVA software (BD-Biosciences). Lymphocytes were phenotyped on the basis of forward (FSC) vs side (SSC) scatters and additional gating was applied using SSC vs CD45 to distinguish lymphocytes from cell debris. Specific panels were subsequently assessed to identify T lymphocytes, B lymphocytes, and natural killer (NK) cells. T lymphocyte subpopulations were gated to distinguish CD3⁺CD4⁺ (T-helper), CD3⁺CD8⁺ (T-cytotoxic), and CD3⁺CD16⁺/56⁺ (NK).

2.4 | Statistical analysis

The data did not show a normal distribution. One-way analysis of variance nonparametric test (Kruskal-Wallis test) and Dunn test were used for multiple comparisons. The Mann-Whitney test was used to compare pairs of variables. The χ^2 test was used for categorical variables as appropriate. Immunological data were also compared between the severe and nonsevere groups, assessing areas under the receiver operating characteristic (ROC) curves (AUC). $P < .05$ was considered statistically significant. Statistical analysis and graphic representation of the data were performed with GraphPad Prism 8.0 software.

3 | RESULTS

3.1 | Study population

Table 1 shows the main characteristics of our COVID-19 population together with lymphocyte subset results and KL-6 concentrations in serum, dividing patients into severe vs mild-moderate groups on the basis of whether mechanical ventilation requirements.

The total number of lymphocytes (CD45⁺) was found to be depleted in all patients (median [IQR] 797 [566-1150] cells/ μ L) and peripheral NK cells/ μ L were significantly higher in nonsevere patients than in the severe group (median [IQR] 141 [88-205] vs 74 [32-101]; $P = .0449$). ROC analysis distinguished these two groups with an AUC of 78.6% (95% confidence interval [CI]: 55-100; $P = .0425$; Figure 1A) and the best cut-off value was 119 cells/ μ L (71% sensitivity and 92% specificity).

KL-6 serum concentrations were significantly higher in severe patients than the nonsevere group (median [IQR] 1021 [473-1909] vs 293 [197-362], $P = .0118$; Figure 1B). ROC analysis distinguished severe and nonsevere patients according KL-6 serum levels with AUC 82.4% (95% CI: 62-100, $P = .0129$; Figure 1C) and the best cut-off value was 406.5 U/mL (83% sensitivity and 89% specificity).

When KL-6 concentrations were compared between COVID-19 patients and healthy controls, the latter showed serum concentrations of KL-6 similar to those of nonsevere patients (median [IQR] 239 [132-371] vs 293 [197-362], $P = .5277$; Figure 1B), while on the other hand, KL-6 concentrations were significantly higher in severe patients than in healthy controls (median [IQR] 1021 [473-1909] vs 239 [132-371], $P = .012$; Figure 1B).

4 | DISCUSSION

In this study, KL-6 was analyzed for the first time in a population of patients with COVID-19. Serum concentrations of KL-6 were only elevated in severe patients admitted to the ICU and requiring intubation and mechanical ventilation for diffuse interstitial pneumonia, and not in mild-moderate patients with less severe respiratory impairment. Patients with mild-moderate COVID interstitial pneumonia showed serum concentrations of KL-6 similar to those of healthy controls.

Although these results are preliminary (this was a monocentric study with a limited number of patients), they are of interest because KL-6 emerged as a potential prognostic biomarker readily detected in serum of COVID-19 patients and helpful for phenotyping patients according to disease severity.

This mucin protein has been widely studied in idiopathic pulmonary fibrosis and patients with ARDS but limited data have so far been available on its prognostic potential in infective pneumonia.^{1,5,9} No data have hitherto been available on its pattern in coronavirus-induced interstitial pneumonia. Our interest was aroused by the observation that this biomarker was correlated with prognosis in ILDs and ARDS, reflecting alveolar type I and type II pneumocyte damage. KL-6 concentrations at peripheral blood level can reflect severe interstitial lung damage, epithelial lung alterations and regenerative processes secondary to SARS-CoV-2 infection. In this context, a further aim will be to perform a serial evaluation of KL-6 in the follow-up of COVID-19 patients.

Another interesting finding, in line with previous studies on series of ILD and ARDS patients, was that KL-6 showed potential as a prognostic biomarker of COVID-19 pneumonia—patients with serum concentrations greater than 406 U/mL showed severe disease and a high risk of intubation. Likewise, Sato et al⁵ reported median serum concentrations of KL-6 of 537 U/mL in 28 patients with ARDS.

Parameters	Severe cases (n = 12)	Nonsevere cases (n = 10)	P-value
Age (median [IQR])	62 [60-68]	64 [51-64]	.5761
Sex, M/F	9/3	6/4	.6517
Lymphocyte subsets (median [IQR])			
CD45, cells/ μ L	792 [548-1156]	1039 [655-1612]	.2268
CD3%	73 [65-81]	72 [69-73]	.6504
CD3, cells/ μ L	495 [406-789]	794 [569-1014]	.1956
CD4%	45 [37-54]	40 [39-43]	.2894
CD4, cells/ μ L	356 [232-533]	458 [342-551]	.4695
CD8%	21 [15-33]	29 [25-33]	.4320
CD8, cells/ μ L	136 [89-307]	333 [217-432]	.1422
CD19%	12 [9-21]	15 [12-18]	.5358
CD19, cells/ μ L	109 [58-139]	135 [113-226]	.1883
NK cells %	10 [6-13]	14 [9-15]	.1148
NK, cells/ μ L	74 [32-101]	141 [88-205]	.0449
CD4/CD8	2.3 [1.1-3.4]	1.5 [1.2-1.8]	.3402
KL-6, U/mL	1021 [473-1909]	293 [197-362]	.0118

Abbreviation: IQR, interquartile range.

TABLE 1 Main characteristics of population including age (median [IQR]), sex (%), lymphocyte subsets, and KL-6 concentrations at the hospital admission

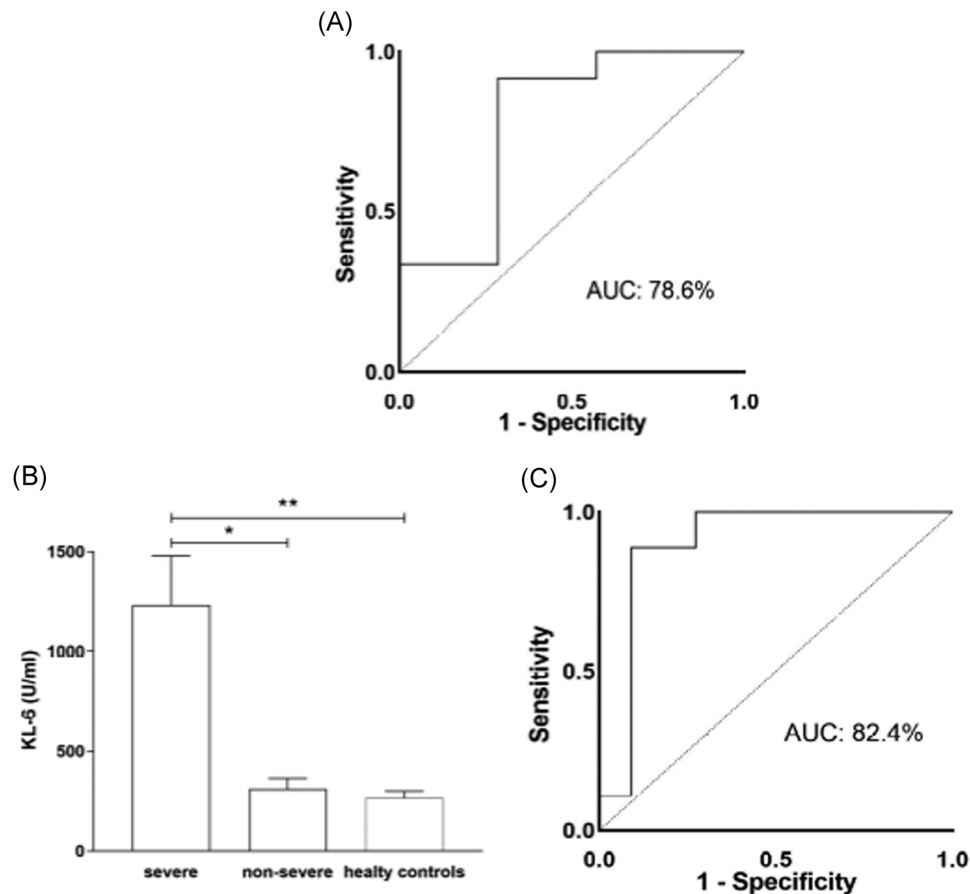


FIGURE 1 A, Receiver operating characteristic (ROC) analysis of natural killer cells/ μ L between severe and nonsevere coronavirus disease (COVID-19) patients. B, Krebs von den Lungen-6 (KL-6) concentrations in COVID-19 patients and healthy controls. ($P < .05$). C, ROC analysis of serum KL-6 concentrations between severe and nonsevere COVID-19 patients

These results are worthy of further validation in a larger cohort to define the cut-off value for identifying patients at high risk of severe respiratory failure. Moreover, these parameters may help clinicians establish correct timing of mechanical ventilation in this specific population. Lymphocyte depletion and decreased NK subsets in peripheral blood were accentuated in COVID-19 patients¹¹ with severe interstitial pneumonia and high serum concentrations of KL-6.

In conclusion, lymphocyte phenotyping including NK cell analysis and assay of KL-6 in serum can help identify severe COVID-19 patients. Serum concentrations of KL-6 were only elevated in patients with severe pulmonary involvement, revealing a prognostic value and supporting the potential usefulness of KL-6 measurement to evaluate COVID-19 patients' prognosis.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

MD conceived the study and supervised all aspects of study. MD, PC, RMR, LB, VA, NL, DB, GDR, FM, SS, FF, BF, SV, MAM, and EB collected data and built the database. MD, PC, LB, EB, and FB conducted the data analysis and interpretation of results. All authors drafted and revised the papers.

ORCID

Miriana d'Alessandro  <http://orcid.org/0000-0002-2368-5722>

Paolo Cameli  <https://orcid.org/0000-0001-8639-2882>

Rosa Metella Refini  <https://orcid.org/0000-0003-1753-5864>

Laura Bergantini  <https://orcid.org/0000-0002-1118-3223>

Elena Bargagli  <https://orcid.org/0000-0002-8351-3703>

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