Evaluation of the Mucin Glycoprotein (KL-6) as a Prognostic Factor in COVID-19 Patients

Giuseppe Perruolo[1] *, Oriana Catapano[2], Lorenzo Ferraro[2], Francesca Velino[3], Annamaria Sessa[3], Natalia Gargiulo[4], Aldo Contina[2], Claudio Saglioccolo[2], Michele Francesco Di Tolla[1], Carlo Sepe[2], Annita Furgi[2], Claudia Passaretti[2], Francesca Perna[5], Maurizio D'Amora[2]

1. *Università degli Studi di Napoli Federico II, Dipartimento di Scienze Mediche Traslazionali.*
2. *ASL Napoli 1 Centro.*
3. *University Hospital "San Giovanni di Dio e Ruggi d'Aragona".*
4. *ASL Napoli 3 Sud.*
5. *Istituto Nazionale Tumori IRCSS Fondazione Pascale.*

Corresponding author: giuseppe.perruolo@unina.it

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Abstract

Lung damage caused by SARS-CoV-2 infection has been described in the literature as resulting from direct cytopathic effects on type II pneumocytes, playing a critical role in the extent and progression of pulmo-nary injury. This study aimed to evaluate the prognostic value of the mucin glycoprotein KL-6 in predicting clinical outcomes in COVID-19 patients. KL-6, a mucin glycoprotein secreted by damaged type II pneumo-cytes, is emerging as a valuable biomarker for assessing pulmonary injury and disease progression in COVID-19 patients. We prospectively enrolled 260 COVID-19 patients admitted to specialized hospitals in Naples under the ASL Napoli 1 Centro between March 20, 2020, and March 31, 2022, when the state of emergency ended. The study cohort had a median age (IQR) of 63 years (54–72), comprising 199 males and 61 females. All patients underwent clinical, radiological, and functional assessments in the emergency department. Serum KL-6 levels were measured at admission, after detecting interstitial lung damage, and during hospitalization. Elevated KL-6 concentrations were observed in patients with pulmonary abnormali-ties, and baseline levels demonstrated good accuracy in identifying those with radiologically documented fibrotic sequelae. In hospitalized COVID-19 patients, serum KL-6 levels also correlated with severe cases requiring mechanical ventilation and predicted the development of fibrotic lung sequelae during follow-up. Finally, KL-6 proved to be an effective predictive marker of disease severity, emphasizing its potential role in guiding clinical management and prognosis. These findings highlight the potential of KL-6 as a valuable biomarker for guiding clinical decisions and monitoring long-term pulmonary outcomes in COVID-19 pa-tients.

Introduction

Mucin 1, or Krebs von den Lungen-6 (KL-6), is a high molecular weight circulating glycopro-tein whose levels are elevated in various interstitial lung diseases (ILDs), including idiopathic pulmonary fibrosis and hypersensitivity pneumonitis [1-3]. It is primarily produced by type-II al-veolar pneumocytes that are damaged or regenerating, and its serum concentrations are thus considered a biomarker of pulmonary and epithelial injury. Specifically, MUC1 (mucin 1), more commonly known as KL-6, is a transmembrane glycoprotein expressed on the surface of alveolar and bronchiolar cells.

The extracellular domains of mucin are released, and serum levels increase in response to mechanical stress, microbial interactions, pH changes, variations in ion concentration or hydra-tion, and particularly to inflammatory stimuli, such as TNFα and neutrophil elastase [4] (Fig. 1).

The prognostic value of peripheral KL-6 in ILDs has been validated, as it has its potential to predict responses to antifibrotic therapies [5,6]. Additionally, KL-6 has been proposed as a bi-omarker for acute respiratory distress syndrome (ARDS) and infectious pneumonia. Since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), KL-6 has also been suggested by several authors as a prognostic marker for this disease [7-10].

The pathogenesis of COVID-19 (the pulmonary disease caused by SARS-CoV-2) remains in-completely understood [11], although it is hypothesized that elevated serum concentrations of pro-inflammatory

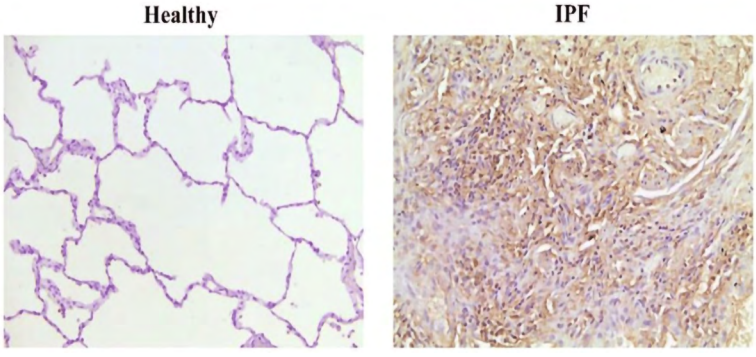

Figure 1. *MUC1 expression in lung tissue from healty and IPF subjects. Immunohistochemistry of MUC1 in lung tissue from healthy and IPF patients. In IPF patients, MUC1 expression is observed at hyperplastic al-veolar type II cells and fiobrotic areas. However, MUC1 is expression is almost undetectable in healthy subjects.* (*Da Ballester et al. Journal of Clinical Medicine. J Clin Med.* 2019 *Sep*; 8(9): 1447.*Published online* 2019 *Sep* 1.*doi*:10.3390/*jcm*8091447

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cytokines, oxidative mediators, and stress signals contribute to lung damage, promoting the onset of acute respiratory syndrome (similar to ARDS) [12]. Host susceptibility and direct cytopathic effects induced by the virus on type I and II pneumocytes are believed to play a critical role in mediating and perpetuating lung injury [13-15].

Our research group was among the first to report elevated serum concentrations of KL-6 in critically ill COVID-19 patients, and our findings were subsequently confirmed. However, no me-ta-analyses are currently available on the role of KL-6 in the follow-up of COVID-19 patients or its predictive value for developing pulmonary fibrotic changes. This study aimed to evaluate serum KL-6 behavior in a population of hospitalized COVID-19 patients within our ASL (Napoli 1 Centro), given our laboratory's role as the metropolitan reference center and a Coronet Lab network component for SARS-CoV-2 molecular diagnostics via rt-PCR in the Campania region.

Between March 20, 2020, and March 31, 2022, when the state of emergency declared by the Italian government ended, we conducted 203,368 rt-PCR molecular tests for COVID-19 on hospitalized patients in ASL Napoli 1 Centro hospitals ("Ospedale del Mare", "San Giovanni Bosco", "Loreto Mare", "San Paolo", "Pellegrini"). Among these, 17,650 tests were positive (8.67%), and from this cohort, we selected 260 patients for our study.

Materials and Methods

Study population

The pandemic required the reorganization of hospital care to admit COVID-19 patients even outside specialized infectious disease units [16-17]. Our ASL network involved five hospitals in Naples ("Ospedale del Mare", "San Giovanni Bosco", "Loreto Mare", "San Paolo", "Pellegrini"). A total of 260 patients (median age: 63 years, IQR: 55-71), including 199 males and 61 females, all hospitalized for COVID-19 in the dedicated units of ASL Napoli 1 Centro, were enrolled in this study. All these patients required admission to intensive care unit (ICU) and mechanical ventila-tion. Blood samples were collected for serum KL-6 evaluation at hospital admission (T0) after emergency room admission and on day 7 of hospitalization (T1). Patients without informed consent or with a previous diagnosis of interstitial lung disease or chronic obstructive pulmonary disease were excluded.

All patients underwent comprehensive evaluations, including physical examination, arterial blood gas analysis, lung ultrasound, and chest CT scans. CT features (fibrotic interstitial abnormali-ties, ground-glass opacities, and air trapping) were assessed by radiologists at the respective hos-pitals. Written informed consent was obtained from all patients for clinical data collection.

KL-6 Assay

Serum samples were obtained at admission (T0), prior to any biological treatment, high-dose intravenous steroid infusion, or invasive ventilation, and during hospitalization (T1). Serum KL-6 concentrations were measured using the KL-6 Immunoassay reagent test (ST AIA PACK KL-6) pro-vided by Tosoh Bioscience, following established protocols [2,3,8,14,18]. The assay is based on antigen-antibody agglutination of sialylated carbohydrate antigen with KL-6 mAb. Absorbance changes were measured to determine KL-6 concentrations, expressed in U/ml, with a cut-off value of 465 U/ml [19]. The automated analyzer (Tosoh Bioscience AIA 360) reads fluorescence and converts it to KL-6 concentrations.

Serum handling

Serum (not heparinized, EDTA, or citrate plasma) was used. Improper centrifugation, fibrin, or particulate matter could yield inaccurate results. Samples were inspected for air bubbles or foam, which were removed prior to analysis. Samples could be stored for up to 7 days at 2-8°C or frozen at 20°C for up to 60 days.

Statistical Analysis

Non-normal data distribution was noted. Results are reported as median (IQR). Univariate and multivariate logistic regression models assessed associations between unfavorable outcomes and high baseline KL-6 levels (>1,000 U/ml) or age. Spearman's correlation tested associations between baseline KL-6 and age, while Mann-Whitney U test compared KL-6 levels between fa-vorable and unfavorable outcomes. The Wilcoxon signed-rank test was used for intra-patient comparisons between T0 and T1. We observed 60 patients with baseline KL-6 > 1,000 U/ml, all of whom died.

Statistical analyses and graphical representations were performed using GraphPad Prism 8.0 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Table 1 summarizes the main characteristics of our COVID-19 population at T0 (hospital ad-mission from the emergency room). Serum KL-6 concentrations, pulmonary function test (PFT) parameters, and arterial blood gas analysis data were collected. The table includes key charac-teristics of these parameters. The incidence of tobacco smoking was also evaluated to assess its potential additive effect on lung damage. It is noteworthy that at different sampling points, the study population did not show significant differences in terms of sex, age, or comorbidities.

Our findings align with those reported by d'Alessandro M. et al. in 2021 [26]. Based on pul-monary CT characteristics, patients were classified according to evidence of fibrotic lung altera-tions, including ground-glass opacities, linear thickening,

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Table 1. Main Characteristics of the COVID-19 population. The table includes demographic and clinical da-ta such as age, sex, smoking habits, BI, arterial blood gas values, and serum KL-6 concentrations. Continu-ous variables are presented as median (IQR), while categorical variables are expressed as count (percent-age).

Parameter	COVID-19 patients (n: 260)
Age (years)	63 (54; 72)
Gender: Male/Female	199 (76.5%) / 61 (23.5%)
Smoker: No/Yes	168 (65%) / 92 (35%)
BMI (Kg/m^2)	26 (25; 28)
SvO_2 (%)	97 (96; 98)
$PaCO_2$ (mmHg)	37 (33; 39)
PaO_2 (mmHg)	86 (79; 92)
HR (bpm)	76 (70; 82)
T0 KL-6 (U/mL)	739 (301; 1,321)

SvO_2: Venous oxygen saturation; $PaCO_2$: Partial Pressure of Carbon Dioxide; PaO_2: Partial Pressure of Oxygen; HR: Heart Rate.

or organized lung areas. The analysis conducted by the authors demonstrated that baseline KL-6 values showed good accuracy in dis-criminating patients with CT evidence of interstitial lung abnormalities (AUC = 85%, standard er-ror 0.108, 95% CI 64–100, p = 0.04).

We investigated potential association between biometric and clinical parameters with base-line KL-6 levels. We found that KL-6 levels displayed a significant correlation with age (r: 0.301, 95% CI 0.169–0.423, p-value < 0.001) (Figure 2).

Next, we stratified the cohort in two subgroups, according to the favorable/unfavorable outcome. We noted that patients with unfavorable outcomes exhibited significantly higher KL-6 levels compared to those with favorable outcomes (p-value: <0.001, Table 2, Figure 3).

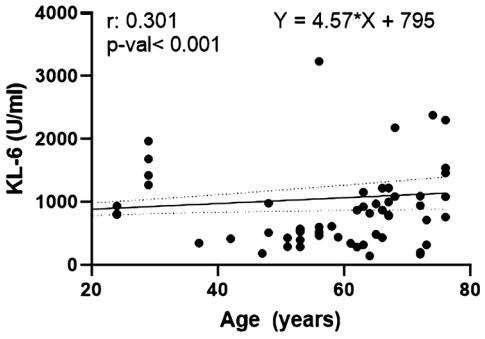

Figure 2. *Linear regression depicting the correlation between basal KL-6 levels and patients' age. Linear regression is represented by the continuous line and its 95% Confidence interval (dotted lines). As it can be seen, higher values of KL-6 are significantly associated with age progression. Figure also reports the re-gression formula and correlation outputs, such as Spearman rho value and its p-value.*

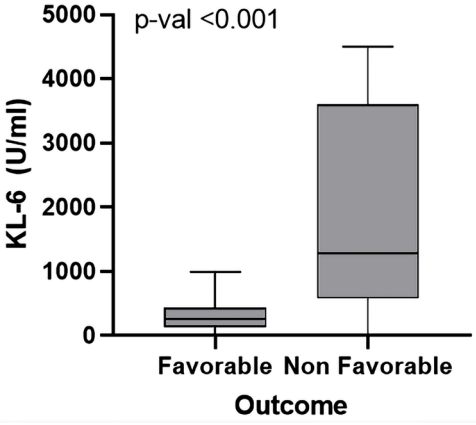

Figure 3. *KL-6 distribution stratified for clinical outcome. Boxplots depicting KL-6 distribution between pa-tients with favorable and unfavorable outcome. The unfavorable cohort displays a strong and significant increase in KL-6 circulating levels compared to the favorable group. P-value for comparison among the two distributions is also showed.*

Table 2. *Measurement of KL-6 levels based on the clinical outcome in the enrolled population. Data are reported as median (IQR); p-values <0.05 are considered statistically significant.*

Laboratory Parameter at T0	Favorabole Outcome (N: 200)	Unfavorable Outcome (N: 60)	p-value
KL-6 (U/ml)	260 (125-421)	1,188 (592-3,608)	<0.001

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Furthermore, stratification based on a KL-6 threshold value of 1,000 U/ml showed a signifi-cant association with negative clinical outcomes (OR: 17, 95% CI: 2.76 – 104.54, p-value: <0.01). This association remained significant even when age was included as covariate (OR: 11.29, 95% CI: 1.04 – 122, p-value < 0.05) (Table 3).

Table 3. Univariate and Multivariate regression analysis among clinical outcomes and characteristics for the COVID-19 population. For each parameter, Univariate and Multivariate Odds Ratios, adjusting for age, are reported as estimate and 95% Confidence Interval. p-values <0.05 are considered statistically signifi-cant.

Parameter	OR (95% CI)	p-value	aOR (95% CI)	p-value
Age >60 years	1.17 (0.28 – 4.83)	0.83	-	-
KL-6 > 1,000 U/ml	17 (2.76 – 104.54)	**<0.001**	11.29 (1.04 – 122)	**<0.05**

Discussion

This study described peripheral KL-6 concentrations in a population of COVID-19 patients at the time of hospital admission from the emergency department and during hospitalization, along-side the evaluation of radiological and functional parameters. It was reported that serum KL-6 concentrations increased significantly and progressively in severe patients requiring intensive care unit admission and prone intubation with mechanical ventilation, but not in mild-to-moderate patients with less severe respiratory failure [8]. This mucin protein has been extensively studied in idiopathic pulmonary fibrosis and patients with ARDS, but there is limited data on its potential prognostic value in viral pneumonia [11-13]. Our interest arose from the observation that KL-6 was associated with prognosis in ILD and ARDS, reflecting damage to type I and II alveolar pneumocytes. The SARS-CoV-2 virus is known to have a specific tropism for alveo-lar epithelial cells, causing interstitial lung damage, epithelial changes, and regenerative process-es, primarily during the acute phase [8]. A significant increase in serum KL-6 concentrations has been demonstrated in critical COVID-19 patients [8, 10], and this was further confirmed in our study.

Conclusions

Based on our experience, it can be affirmed that KL-6 is an important prognostic factor in COVID-19 infection. The concentration of this mucin reflects the extent of lung damage and also correlates with CT findings, as demonstrated by us and several other authors [1, 2, 5-7, 9-12, 15, 20-24]. In our experience, we have confirmed that serum KL-6 concentrations in hospitalized COVID-19 patients can help identify severe cases requiring mechanical ventilation and predict those who will develop pulmonary fibrotic sequelae in the follow-up. Additionally, it is particular-ly noteworthy that KL-6 has proven to be an effective predictive index of infection severity. In our experience, 60 patients with KL-6 levels above 1,000 U/mL all passed away. Therefore, we can conclude that KL-6 can serve as a useful indicator of the severity of lung damage and also as an important prognostic factor for mortality if its concentration exceeds 1,000 U/ml. These find-ings highlight the potential of KL-6 as a valuable biomarker for guiding clinical decisions and mon-itoring long-term pulmonary outcomes in COVID-19 patients.

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Institutional Review Board Statement: The study was conducted in accordance with the Decla-ration of Helsinki. Ethical review and approval were waived for this study due to pandemic emergency reasons. Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, upon reasonable request.

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