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Short Title: Krebs von den Lungen-6 levels as prognostic marker in COVID-19 pneumonia

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Abstract

Background and Objectives

Krebs von den Lungen-6 (KL-6), expressed by damaged type II pneumocytes, is useful in the diagnosis and severity assessment of many diffuse interstitial lung diseases. The objective of our study was to determine the prognostic value of the initial KL-6 plasma level in COVID-19 pneumonia.

Methods

All patients hospitalized for a suspected COVID-19 pneumonia between March and May 2020 in our Chest department of a French university hospital were included. KL-6 serum concentrations were measured within 72 h of diagnostic suspicion by chemiluminescence enzyme immunoassay Survival analysis was performed using a Cox regression and modeled by a Kaplan-Meier curve.

Results

Sixty-six COVID-19 patients (average age = 64±14 years ,71.2% males) with KL-6 serum measurement were included. Median KL-6 serum concentration was 409±312 U/mL. KL-6 was significantly higher in men (p=0.003), elders (p=0.0001) and in patients with greater Charlson's score (p=0.002). Higher KL-6 concentration was significantly associated with in-hospital mortality (HR: 8.66; 95% CI:1.1-69.2, p=0.014), radiological extension of lesions on chest CT scan (p=0.004) and higher WHO severity score (p=0.042), but not with admission in intensive care unit. In 9 (14%) non-surviving COVID-19 patients, KL-6 serum concentration increased whereas it remained stable or decreased in survivors. At 3 months follow-up (n=48), DLCO was negatively correlated with the initial KL-6 value (r=0.47, p=0.001), while FVC, FEV1 and MRC score were not.

Conclusion

Initial KL-6 serum concentration is significantly associated with in-hospital mortality, unfavorable outcome, and persistent impairment of DLCO at 3 months. Initial KL-6 plasma determination appears as a prognostic biomarker in COVID-19 pneumonia.

Introduction

Transmembrane mucin 1 (MUC1) is a large glycoprotein composed of two domains: N-terminal extracellular domain, and C-terminal domain with a single transmembrane region and a cytoplasmic tail (1). MUC1 is expressed in type II pneumocytes and respiratory bronchiolar epithelial cells. The N-terminal domain of MUC1 (MUC1-N) contains the Krebs von den Lungen-6 (KL-6) epitope, which can be cleaved from the cell surface in response to alveolar cell damage, and may then be detected in blood circulation and bronchoalveolar fluid (2). A murine IgG1 monoclonal antibody was developed to recognize the KL-6 sialylated sugar chain of MUC1-N (3). Furthermore, Ohtsuki et al. reported discontinuous staining of KL-6 in normal lung whereas it was continuous and linear in tissue sections from patients with interstitial lung diseases (ILD) (4). Other studies showed that KL-6 has both pro-proliferative and anti-apoptotic effects on lung fibroblasts, acting similarly to transforming growth factor-beta (TGF-ß) (5). While KL-6 was firstly proposed as a biomarker for lung, breast and pancreatic cancers (3), it has since been considered as a diagnostic and prognostic biomarker in ILD of several causes (6).

Most of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infected people will present a mild form of the Coronavirus disease 2019 (COVID-19). However, some patients may develop pneumonia, leading to a severe hypoxemia for a minority of them (13.8-16%). In worst cases, acute respiratory failure may occur, requiring ventilatory support ranging from nasal oxygen therapy to high flow oxygen therapy, non-invasive ventilation or even mechanical ventilatory support (7,8). The latter leads to death in 30-60% of the cases. At mild term follow-up, although most patients had no significant sequelae, a substantial proportion suffered from persistent symptoms and respiratory impairment or even pulmonary fibrosis lesions (9,10). Identification of prognosis factors that stratify at-risk patients and predict severity and/or mortality may be of great utility for clinical practice. To date, numerous blood-based biomarkers such as D-dimer, C-reactive protein (CRP), lactate dehydrogenase (LDH) and high-sensitivity cardiac troponin I (11) have been associated with COVID-19 worse outcomes. Only few studies have questioned the role of KL-6 in the evaluation of COVID-19

severity (12–23), and correlation with mortality has been scarcely investigated. In the light of these data, the aim of this study was to assess the prognostic role of KL-6 in COVID-19.

Materials and Methods

In order to evaluate the prognostic value of KL-6 serum, we performed a retrospective study (March 1st to May 7th, 2020 considering all consecutive adults hospitalized for a suspicion of COVID-19 pneumonia in our Chest department. All-cause in-hospital mortality was considered as primary endpoint.

Study population and data collection

All patients (older than 18 years old) admitted for a suspicion of COVID-19 infection – confirmed or not, during the study period were consecutively included. Exclusion criteria were: i) KL-6 assay delayed ≥ 72h after, upon admission in our department; ii) patients with a history of ILD, active malignancies (especially lung, breast and pancreatic cancers), or receiving lobectomy or pneumonectomy, chronic renal failure with creatinine ≥ 200µmol/L or needing renal dialysis. Patients with negative SARS-CoV-2 polymerase chain reaction (PCR) testing from nasopharyngeal swab or bronchioalveolar lavage fluid with other diagnosis of respiratory diseases served as controls.

Disease severity was measured according to the Clinical Progression Scale developed by the World Health Organization (24).

Chest high-resolution computed tomography (HRCT) at 1-millimeter slice was performed whenever possible at the time of diagnosis. Radiological severity scores of baseline chest computed tomography (CT) were determined by a thoracic radiologist (AM) as previously reported (25).

Serum KL-6 level was measured with a sandwich-type chemiluminescence enzyme immunoassay (CLEIA) using KL-6 antibodies (LUMIPULSE G600, Fujirebio, Japan). The cut off value of 500 U/ml was

used to distinguish patients with ILDs from healthy subjects (manufacturer's data) (26). For some patients, KL-6 assays were realized at different time of hospitalization.

Three months after hospital discharge, patients underwent, a hospital visit for clinical evaluation, including Medical Research Council (MRC) questionnaire, chest CT scan, pulmonary function test (PFT) and biology.

Statistical analysis

Results were expressed as mean ± standard deviation (SD) or medians (IQR, interquartile ranges) for quantitative variables, and n (%) for qualitative variables. Differences between groups were assessed using the Chi-squared test (or Fischer test, as appropriate) for categorical variables and Mann-Whitney test for quantitative variables. For correlation analysis, Pearson correlation was applied. To evaluate risk factors associated with in-hospital mortality, we performed univariate Cox regression analyses. For these survival analyses, quantitative dependent variables were used without any dichotomization, i.e using continuous variables. Results were expressed as hazard ratios (HRs) with 95% confidence intervals (95%CI). The proportional hazard assumption was tested using Schoenfeld's test. Kaplan-Meier curves representing survival across strata where survival was assessed by a log-rank test. A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism and R-Studio 2020, Integrated Development for R (R-Studio, Boston, MA, USA).

Ethical statement

This study complied with the Declaration of Helsinki and the principles of good practices. This research is a retrospective non-interventional study and KL-6 was measured on samples collected for clinical purposes. Written informed consent was not required and an information sheet stating the right to modify or oppose data collection was provided by the investigators to each patient. The

study was approved by the Institutional Review Board of the French learn society for of respiratory medicine - Société de Pneumologie de Langue Française (CEPRO 2021-055).

Results

KL-6 serum concentrations at admission in COVID-19 patients and controls

Among the 221 consecutive patients hospitalized during the study period for a suspicion of COVID-19, 81 met the inclusion criteria among whom 66 (81.4%) had a diagnosis of COVID-19 pneumonia and the other 15 had various non-related COVID-19 respiratory diseases and served as controls (shown in Figure 1).

The first KL-6 assay was performed in a mean delay from admission of 1.9 days (±1.1) in COVID-19 and 2.0 days (±2.3) in controls, and from the first symptoms onset at 9.6 days (±3.6) in COVID-19 and 5.7 days (±3.5) in controls ($p<0.001$). KL-6 serial measurements were carried out in 36 (54.5%) COVID-19 patients.

Demographic and clinical characteristics of COVID-19 patients and controls are summarized in Table 1. COVID-19 patients differed from controls with higher proportion of obesity. In the COVID-19 group, median time from onset of symptoms to positive SARS-CoV-2 PCR was 7 days (6-10). Upon inclusion, 60 patients (91.9%) were under oxygen therapy.

Laboratory findings showed significantly lower lymphocyte and PNN counts ($p=0.02$ and $p=0.002$ respectively), and higher LDH ($p<0.0001$) in the COVID-19 group compared to control. Other biological parameters (D-dimer, platelet count, CRP) did not significantly differ between the two groups. Finally, median KL-6 value was 409 U/mL [IQR: 275-621] and 362 U/mL [IQR: 172-548] in COVID-19 patients and controls respectively ($p=0.25$). It should be noted that delay between KL-6 assay and onset of symptoms is longer in the COVID-19 group compared with controls (median delay of 9 days [IQR: 8-12] and 5.7 days [4-7.3] respectively, $p<0.001$). Moreover, the time between

sampling and the onset of symptoms seems to negatively influence KL-6 dosage (r=-0.27, p=0.03; figure 2S supplementary appendix).

KL-6 serum concentrations and clinical, biological and radiological characteristics in COVID-19 patients

The KL-6 serial measurement was performed on average within 9.6±3.6 days relative to the onset of the first symptoms and within 1.8±1.1 days relative to hospital admission. Median serum KL-6 concentration at admission was significantly higher in men (p=0.0028), elders (p=0.0001), in patients with high Charlson's score (p=0.0016) and longer delay between symptoms and hospital admission (p=0.0042). There was no difference in KL-6 levels between smokers and non-smokers.

Furthermore, KL-6 values were positively correlated with D-Dimer concentrations ($p < 0.001$; r=0.50) and platelet counts ($p = 0.02$; r=0.29), but not with other biological parameters (LDH, CRP, and lymphocyte count).

Considering the 62 (94%) patients with CT scan at admission (shown in Table 1S supplementary appendix), GGO (95.2%), subpleural and peribronchovascular distribution (54.8%) were the most frequent findings. CT scan involvement was predominantly bilateral (95.1%) and multifocal (64.5%). Compared with controls, COVID-19 patients had more diffuse radiological lesions on CT-scan (p=0.02), distributed mostly sub pleural (p=0.02), associated with an anteroposterior ($p<0.01$) and craniocaudal (p=0.01) gradient. Meanwhile, controls had more lesions of low extent < 10% (p=0.046) compared with COVID-19 patients. Median KL-6 concentration tended to be related to radiological extent on chest CT and was higher when radiological extension was ≥ 50% compared with those with less than 50% radiological extension (529 U/mL [IQR: 380-865] and 354 U/mL [IQR: 221-482], p=0.0040; shown in Figure 2A). However, there was no significant association between radiological extension on thoracic CT scan and WHO classes (p=0.9).

KL-6 serum concentrations and COVID-19 pneumonia outcome

Patient median follow-up was 3.6 months. Treatments and outcomes are presented in Table 2. Sixty-six (100%) patients had received antibiotics, 11 (6.7%) antiviral therapy, 3 (4.5%) hydroxychloroquine (including one as a part of his background therapy for rheumatoid arthritis), 19 (29%) hydrocortisone therapy, and 15 (22.8%) tocilizumab. Of the 66 patients, 15 (23%) were recused from ICU admission, 9 required high-flow oxygen and 16 were admitted in ICU of whom 12 needed orotracheal intubation. Median KL-6 level at admission was significantly higher in COVID-19 patients in WHO ≥ class 6 (532 U/mL [IQR: 383-944]) compared to those in WHO ≤ class 5 (387 U/mL [IQR: 246-509]; p=0.046, shown in Figure 2B). The median time from KL-6 assay to the date of worst WHO was 5 days (IQR: 3-7) in deceased patients.

Finally, among the 66 patients enrolled, 9 (13.7%) died (among them, 7 patients had limitation of life-sustaining treatment (LLST) out of the 15 patients with LLST in total)). Using univariate Cox regression, the median KL-6 value above 409 U/mL (median of the COVID-19 group) was significantly associated with in-hospital mortality at 28 days (8 patients died in group with KL-6 value above median vs 1 patient in group under median, HR: 8.66; 95CI: 1.1-69.2, shown in Figure 3). Similarly, age (HR: 1.09; 95CI: 1.017-1.168) and Charlson score (HR: 1.422; 95CI: 1.051-1.925) were as well, but not to the other laboratory results (CRP, LDH, D-dimer and PNN, platelet and lymphocyte counts; Table 3). There were no association between in-hospital mortality and WHO scale at admission or radiological disease extension (respectively p=0.10 and p=0.60).

For some patients, repeated assessments of serum KL-6 concentration were conducted (the first measurement was performed on average within 9.6±3.6 days after the onset of initial symptoms). Interestingly, for these patients (n=36), KL-6 level increased for the six patients who finally deceased, while it remained stable or decreased in the 30 who survived (shown in Figure 4).

KL-6 serum concentration and COVID-19 pneumonia resolution at 3 months

Among the 57 patients who survived after COVID-19 pneumonia, 48 (84.2%) underwent a clinical, pulmonary function tests (PFT) and chest CT scan imaging at three months. All patients showed SARS-

Cov2 antibodies in their serum. Twenty-six patients (54.1%) complained of breathlessness, 10 (20.8%) of persisting cough, and 16 (33.3%) of chronic fatigue. The initial KL-6 level was not associated with persistence of subjective breathlessness at three months (p = 0.590), nor with MRC score value (data not shown). Twenty-seven patients (56.3%) had persistent pulmonary lesions on CT scan, but no patients developed lung fibrosis and we did not find an association with the initial KL-6 level and the persistence of radiological abnormalities (p = 0.947). By contrast, an inverse correlation was found between the initial KL-6 level and the DLCO value at three months (*r* = -0.47, p=0.001, n= 45, shown in Figure 1S, supplementary appendix), but not with the FCV and FEV1 values. In the COVID-19 group, patients with a history of chronic respiratory disease had a lower DLCO than those without (median of 62% [IQR: 47.5-74] and 76% [IQR: 70.25-89.75]; p<0.01). However, the same results were observed when patients with chronic respiratory diseases were excluded (r=-0.41; p=0.0125). The KL-6 serum level at 3 months remained above 500 U/mL (339.5 U/mL [IQR: 225.5-495.8]) in 12 patients but did not significantly correlate with persistent radiological abnormality or impaired DLCO at this time point.

Discussion

COVID-19 is a rapidly spreading pandemic, with symptoms ranging from common rhinorrhea to ARDS. It is therefore crucial to determine disease severity early on. Our study is the first to investigate the correlation between KL-6 serum level at admission and mortality in hospitalized patients for COVID-19 pneumonia, with additional prospective mid-term follow-up data.

The characteristics of our patients hospitalized for COVID-19 pneumonia were consistent with those reported in previous studies, except for a higher rate of patients with history of chronic respiratory disease (25.7%) due to a “Chest department” center bias recruitment and a lower proportion of patients with comorbidities such as, malignancies, ILDs and severe renal failure which were excluded from our study (27,28). With these exclusion criteria, the mortality rate (13.7%) was also lower than that reported by French epidemiological data during the first epidemic wave of

COVID-19 (29). Although our patients suffered from severe COVID-19 pneumonia with oxygen support in 91.9% of cases and a median lung parenchyma extension of 25-49% on chest-CT scan, median KL-6 serum level was similar to that of controls and within the expected range according to the literature (362U/mL [IQR: 172-548]) (30).

Surprisingly enough, our study did not show any difference in KL-6 levels between the COVID-19 population and our control population. Nevertheless, KL-6 assay was conducted later after onset of symptoms in the COVID-19 group (mean duration of 9.6 days) compared to the control group (mean duration of 5.7 days). This could explain the absence of difference in KL-6 levels between the two groups if KL-6 high point occurs early in disease's evolution. The time between sampling and the onset of symptoms could influence KL-6 dosage (figure 2S supplementary appendix). However, the assays were systematically performed within 72 hours of admission, making our study as homogeneous as possible. Moreover, this emphasizes the relevance of repeated dosing to obtain KL-6 kinetics, which seems to reflect the COVID-19 pneumonia progression, as newly suggested by our study.

Over a mean follow-up of 3.6 months, 9 patients (13.6%) reached the primary endpoint of all-cause mortality. Initial KL-6 level is associated with disease severity (according to the WHO progression scale), extent of radiological injury, and death. It should be noted that KL-6 was the only biological variable to be significantly associated with mortality in univariate analysis in our study. Conversely, KL-6 level was not significantly associated with the rate of ICU admission or orotracheal intubation. This finding could be possibly explained by: i) the limited access to ICU admission during the first wave of COVID-19 epidemic in France, leading to strict patient selection for orotracheal intubation and ii) a large use of high flow oxygen device in conventional unit for patients with limitation of ICU admission. Previous studies have evaluated the role of KL-6 as prognostic marker in COVID-19 pneumonia (12–23). Most of these studies compare patients according to severity scales that differ among countries making results difficult to interpret and direct comparisons between studies problematic. For instance, in these studies, serum KL-6 levels range from 283 IU/mL to 1000

IU/mL on receiver operator characteristics (ROC) curves designed to discriminate between severe and non-severe COVID-19 patients. In addition, none of these studies consider all confounding factors associated with high levels of KL-6, such as patients suffering from ILD or cancer which are also known to be strong confounding factors of mortality. Only two studies chose mortality as primary end-point (22,23), but regardless of possible underlying comorbidities that might have impacted on serum KL-6 levels and altered prognostic outcomes. In our opinion, evaluating KL-6 level as a continuous variable on a 3-months follow-up duration seems more appropriate as a prognostic primary endpoint than using crude mortality.

On 3-months follow-up analysis in survivors, DLCO values were inversely correlated with baseline serum KL-6 levels, and so despite the exclusion of patients with chronic respiratory diseases. This result is of particular interest as some, although few, patients develop a fibrosing-ILD post SARS-CoV-2 pulmonary infection and is consistent with those reported in ILD in which serum KL-6 level is also negatively correlated with DLCO (31). In a large cohort of SARS-CoV-2 patients that during a 6-month follow-up, DLCO decreased by 22 to 56% and notably more in patients with severe disease. In addition, it was shown that HRCT abnormalities affecting more than 5% of lung parenchyma were present in 52-54% (32). Previous studies have shown that patients with a higher risk of pulmonary fibrosis secondary to COVID-19 are older, male, smokers, and have underlying diseases such as diabetes, pulmonary and cardiovascular diseases as well as, the use of high-flow oxygen therapy, need for mechanical ventilation and the development of ARDS (10). In this context, Milara et al showed that the C-terminal domain of MUC1 has a role in the promotion of pulmonary fibrosis in IPF, and could impact profibrotic gene expression, cell proliferation or senescence throw the phospho-Smad3 and act-β-catenin pathway in response to TGF-β (33). Some authors have demonstrated that patients who were severely affected by COVID-19 had an excessive immune response that was partly controlled by TGF-β (34). Testing TGF-β would be interesting to further assess the severity of COVID-19 as a complement to the KL-6 assay, and could argue for a therapeutic target, especially considering TGF-β as the key player in pulmonary fibrogenesis.

There are some limitations in our study such as its small sample size and single-center retrospective nature leading to inevitable selection bias. However, the careful exclusion of confounding factors associated with KL-6 increase (history of ILD, active malignancies, previous lobectomy or pneumonectomy, chronic renal failure…) and the fact that KL-6 level was the only biological variable significantly associated with mortality in univariate analysis are part of the strengths of our study.

Conclusion

Taken together, our results underline that KL-6 not as a diagnostic but rather a prognostic biomarker for COVID-19. This study showed that initial serum KL-6 levels above median in patients with COVID-19 pneumonia appear to predict all-cause mortality and is associated with respiratory severity i.e. WHO scale severity and radiological extension. In addition, we suggest that KL-6 level modulation on early evaluation should better predict the fatal issue. Finally, initial KL-6 level is associated to lower DLCO at 3-months and could be used to select patients to screen for fibrosing complications. These results deserve to be confirmed in a large multi-centered cohort.

Declaration

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors participated as investigators or study coordinators in the trial and were involved in data collection. AL and JC wrote the first draft of the manuscript and made revisions after feedback from co-authors. All authors read and approved the final manuscript.

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Tables:

Table 1: Baseline characteristics at diagnosis	COVID-19 (n = 66)	Control (n = 15)	*p*-value
Demographic features			
Male – n (%)	47 (71.2)	8 (53.3)	0.18
Age (years) - mean (±SD)	64 (±13.6)	64 (±20.4)	0.91
Smoking status – n (%)	25 (37.9)	4 (27)	0.51
Charlson score – median (IQR)	3 (1-4)	3 (2-4.5)	0.86
Diabetes – n (%)	11 (16.7)	2 (13)	
Hypertension – n (%)	27 (40.9)	4 (29)	
Cardiovascular diseases – n (%)	38 (57.6)	5 (33)	
Respiratory disease – n (%)	17 (25.7)	4 (27)	
Obesity BMI> 30 – n (%)	18 (27.3)	0 (0)	0.03
Clinical features			
Number of days between symptom onset and the hospitalization – median (IQR)	7 (6-10)	3 (0-4.8)	<0.01
WHO score at admission – median (IQR)	5 (5-5)	5 (4-5)	0.14
Crepitation – n (%)	58 (87.9)	4 (29)	<0.01
Oxygen therapy – n (%)	60 (91.9)	9 (60)	0.02
Laboratory findings at diagnostic - median (IQR)			
PNN (G/L)	5.21 (3.7-7.2)	8.17 (6.1-10.5)	<0.01
Lymphocytes (/µL)	800 (530-1223)	1260 (790-1970)	0.02
Platelets (G/L)	217 (164-276)	255 (215-280)	0.20
CRP (mg/L)	153 (62-203)	70 (27-311)	0.79
D-dimer (µg/mL)	1285 (759-2134)	1783 (888-4060)	0.44
LDH (U/L)	398 (307-609)	260 (195-303)	<0.01
KL-6 (U/mL)	409 (275-621)	362 (172-548)	0.24

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; KL-6, Krebs von den Lungen-6; LDH, Lactate dehydrogenase; PNN, Polynuclear Neutrophil; WHO, World Health Organization.

Table 2: Treatment and follow-up	COVID-19 (n = 66)
Treatment	
Antibiotics – n (%)	66 (100)
Antiviral drug – n (%)	11 (16.7)
Hydroxychloroquine – n (%)	3 (4.5)
Steroids – n (%)	19 (29)
Tocizulimab – n (%)	15 (22.8)
ECMO – n (%)	0 (0)
LLST on ICU admission – n (%)	15 (23)
Outcomes	
ICU admission* – n (%)	16 (31.4)
OTI* – n (%)	12 (23.5)
HFO – n (%)	9 (13.6)
In-hospital mortality – n (%)	9 (13.6)

**Among patients with no LLST*

Abbreviations: ECMO: extracorporeal membrane oxygenation; LLST: limitations on life support techniques; ICU: intensive care unit; OTI: orotracheal intubation; HFO: High flow oxygen.

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Table 3: Univariate analysis for mortality	HR (95%CI)	*p*-value
Demographic features		
Male	1.41 (0.71-6.78)	0.67
Age	1.09 (1.02-1.17)	0.02
Smoking status	0.21 (0.03-1.65)	0.14
Charlson score	1.42 (1.05-1.93)	0.02
Diabetes	0.66 (0.08 - 5.31)	0.7
Hypertension	0.19 (0.02 - 1.49)	0.11
Cardiovascular diseases	0.21 (0.04- 1.02)	0.05
Obesity BMI> 30	0.33 (0.04- 2.61)	0.29
Laboratory findings at diagnostic		
PNN	1 (0.99-1)	0.28
Lymphocytes	0.9992 (0.997-1.001)	0.32
Platelets	1 (1-1)	1
CRP	1.004 (0.9979-1.01)	0.12
D-dimer	1 (0.9999-1)	0.42
LDH	1.002 (0.999-1.005)	0.21
KL-6	1.002 (1.001-1.003)	0.001

Abbreviations: BMI, body mass index; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; LDH, Lactate dehydrogenase; PNN, Polynuclear Neutrophil.

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380 **Figures:**

381 Figure 1: Flow chart

382 Figure 2: Median KL-6 levels according to radiological extension on chest CT scan (A) and WHO score
383 (B)

384 Figure 3: All-cause mortality at 28 days 28 related to KL-6 levels (in U/mL)

385 Figure 4: Dynamic change of KL-6 levels for patients with serial evaluation (n=36)

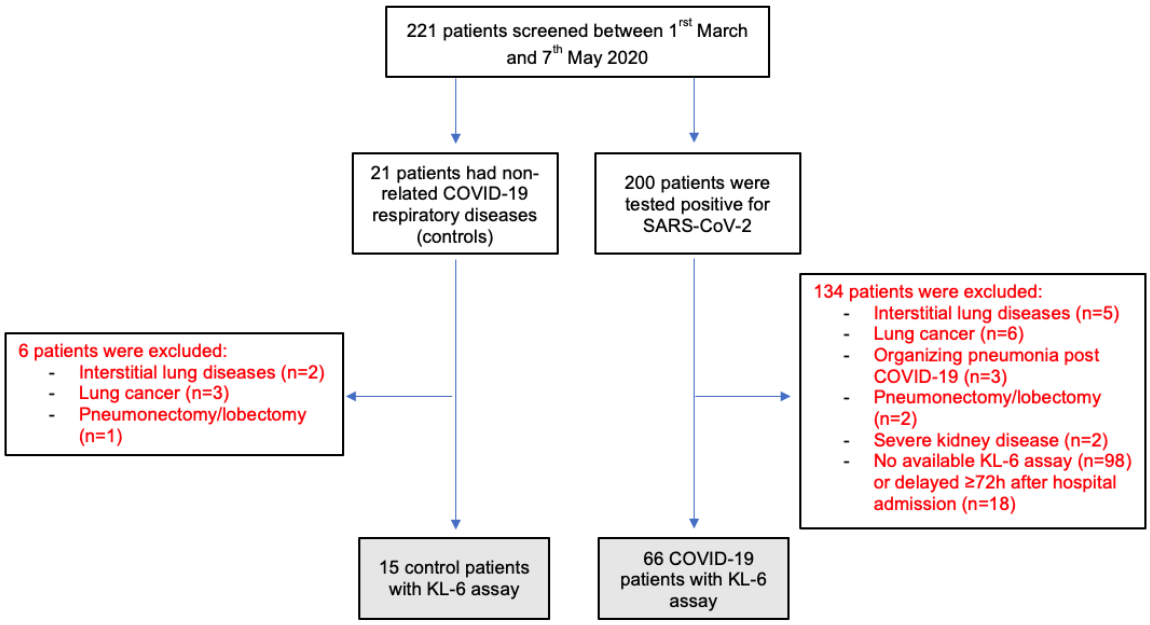


Figure 4: Flowchart

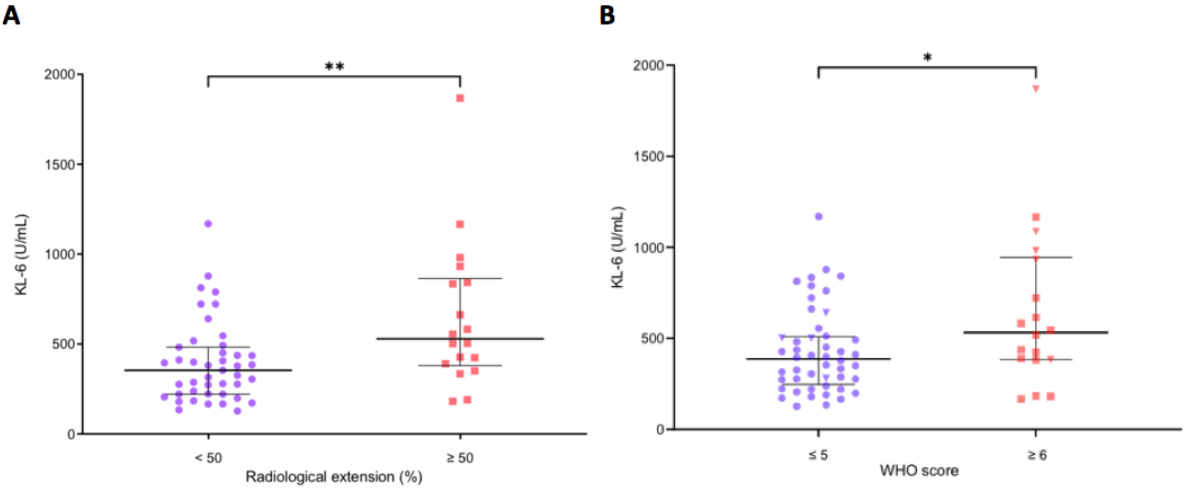
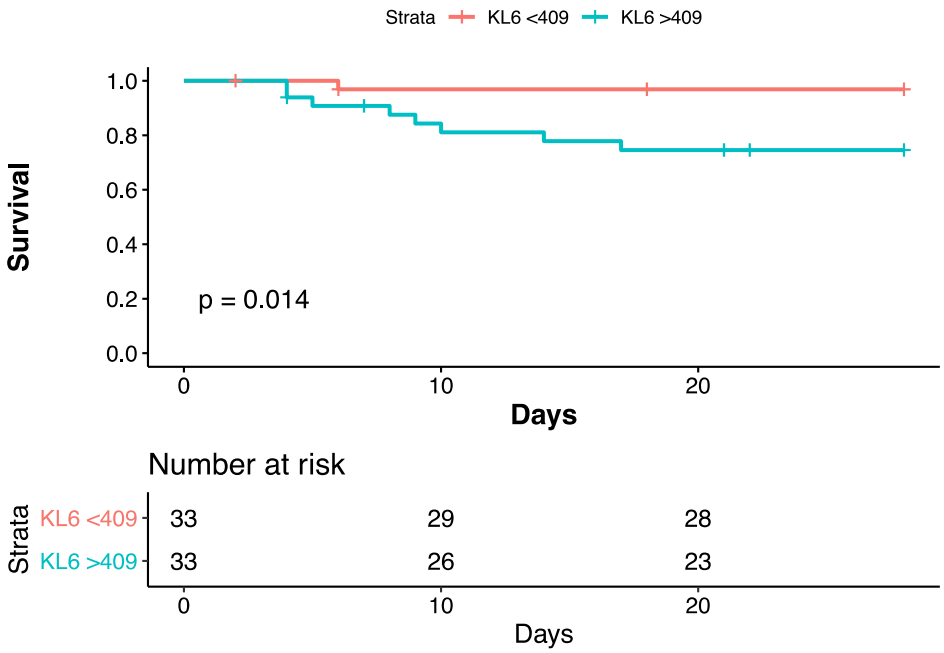


Figure 5: Median KL-6 levels according to radiological extension on chest CT scan (A) and WHO score (B)



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397 ***Figure 6: All-cause mortality at 28 days 28 related to KL-6 levels (in U/mL)***

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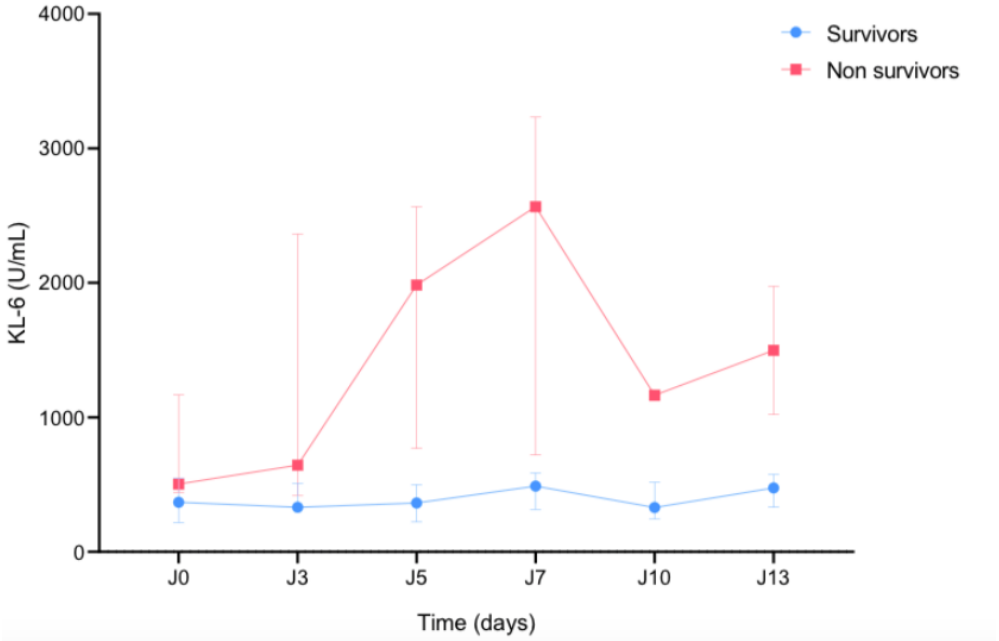

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400 ***Figure 7: Dynamic change of KL-6 levels for patients with serial evaluation (n=36)***

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Figure 8S: Correlation between initial KL-6 level and the DLCO value at three months.

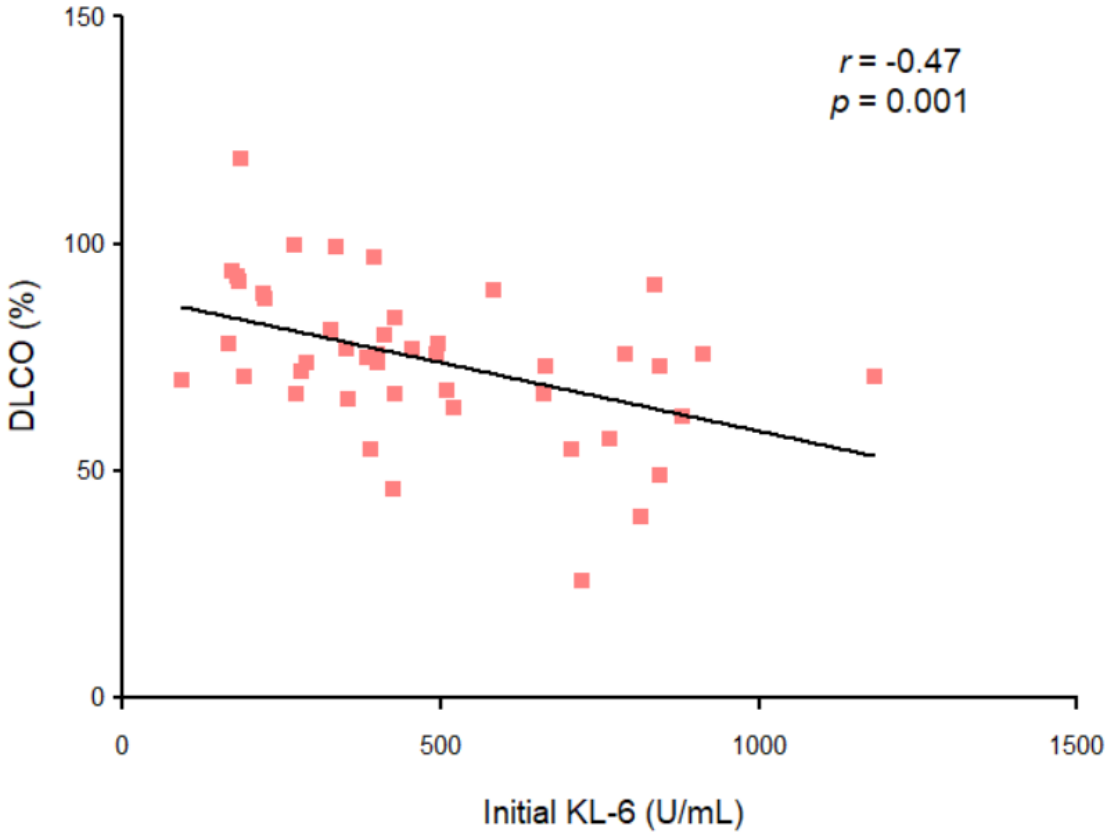

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Figure 9S: Initial KL-6 levels and time between collection sample and first symptoms onset in COVID-19 patients

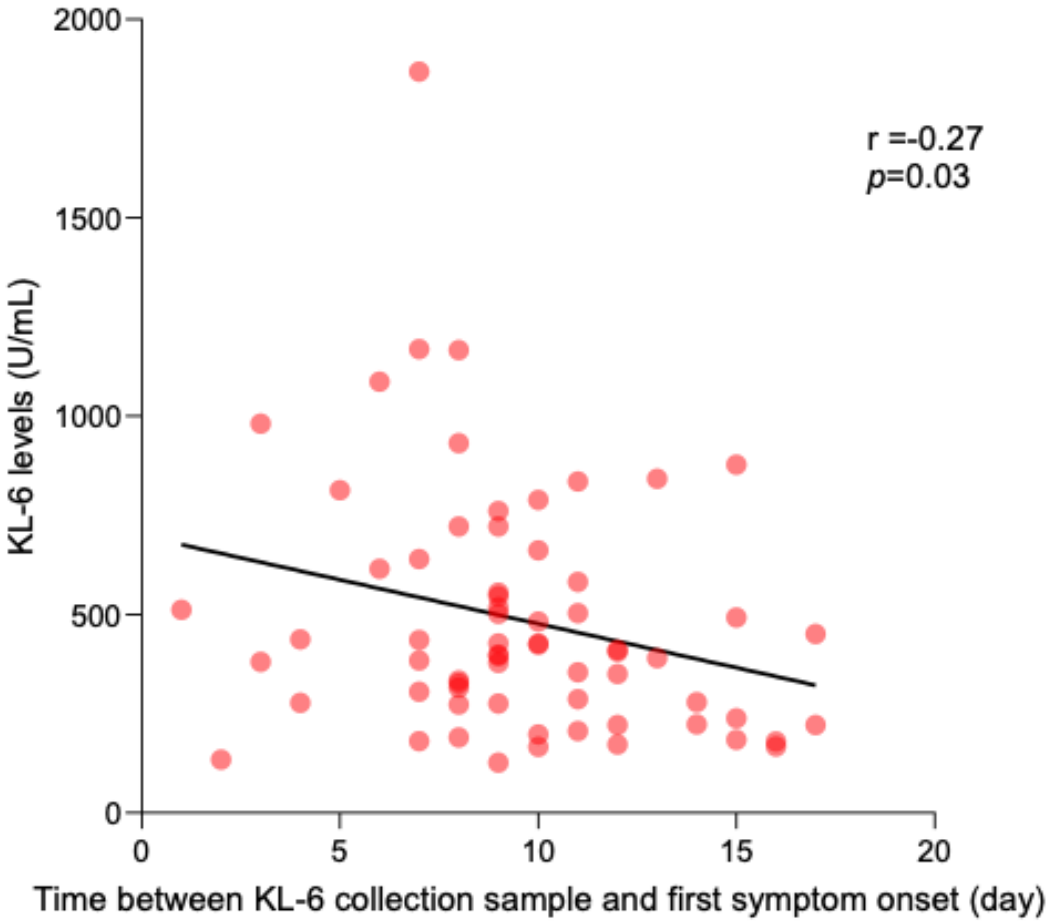

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Table 1S: Baseline CT scan findings of COVID-19 and controls patients	COVID-19 n = 62	Controls n = 15	*p*-value
Lung involvement area – n (%)			
0%	0 (0)	3 (20.0)	<0.01
1-9%	5 (8.1)	5 (33.3)	0.046
10-24%	15 (24.2)	4 (26.7)	>0.99
25-49%	24 (38.7)	3 (20.0)	0.39
50-74%	15 (24.2)	0 (0)	0.12
≥ 75%	3 (4.8)	0 (0)	>0.99
Predominant distribution – n (%)			
Unilateral	3 (4.8)	3 (20.0)	0.11
Bilateral	59 (95.2)	5 (33.3)	0.06
Subpleural	23 (37.1)	0 (0)	0.02
Peribronchovascular	5 (8.1)	1 (6.7)	0.99
Mixt subpleural and peribronchovascular	34 (54.8)	0 (0)	0.05
Anteroposterior gradient	35 (56.5)	0 (0)	<0.01
Craniocaudal gradient	40 (64.5)	1 (6.7)	0.01
Focal	4 (6.4)	7 (46.7)	<0.01
Multifocal	36 (58.1)	5 (33.3)	0.44
Diffuse	22 (35.5)	0 (0)	0.02
Morphologic – n (%)			
Consolidation	47 (75.8)	9 (60)	0.65
Ground glass opacities	59 (95.2)	9 (60)	0.37
Crazy paving	27 (43.5)	1 (6.7)	0.06
Pulmonary fibrosis	0 (0)	0 (0)	>0.99
Pulmonary embolism	5 (8.1)	4 (26.7)	0.10

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