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► **To cite this version:**

Alice Letellier, Camille Rolland-Debord, David Luque-Paz, Audrey Milon, Pascaline Choinier, et al.. Prognostic value of serum Krebs von den Lungen-6 (KL-6) levels in COVID-19 pneumonia. *Respiratory Medicine and Research*, 2023, 84, pp.101054. 10.1016/j.resmer.2023.101054 . hal-04505769

HAL Id: hal-04505769

<https://hal.sorbonne-universite.fr/hal-04505769v1>

Submitted on 15 Mar 2024

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Prognostic value of serum Krebs von den Lungen-6 (KL-6) levels in COVID-19 pneumonia

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

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Keywords: COVID-19 pneumonia; SARS-CoV-2; Krebs von den Lungen-6; KL-6; biomarker; pulmonary fibrosis.

1 **Abstract**

2 **Background and Objectives**

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

6 **Methods**

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

11 **Results**

12 Sixty-six COVID-19 patients (average age = 64 ± 14 years ,71.2% males) with KL-6 serum measurement
13 were included. Median KL-6 serum concentration was 409 ± 312 U/mL. KL-6 was significantly higher in
14 men ($p=0.003$), elders ($p=0.0001$) and in patients with greater Charlson's score ($p=0.002$). Higher KL-6
15 concentration was significantly associated with in-hospital mortality (HR: 8.66; 95% CI:1.1-69.2,
16 $p=0.014$), radiological extension of lesions on chest CT scan ($p=0.004$) and higher WHO severity score
17 ($p=0.042$), but not with admission in intensive care unit. In 9 (14%) non-surviving COVID-19 patients,
18 KL-6 serum concentration increased whereas it remained stable or decreased in survivors. At 3
19 months follow-up ($n=48$), DLCO was negatively correlated with the initial KL-6 value ($r=0.47$,
20 $p=0.001$), while FVC, FEV1 and MRC score were not.

21 **Conclusion**

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

25

26 **Introduction**

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

40 Most of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infected people will present
41 a mild form of the Coronavirus disease 2019 (COVID-19). However, some patients may develop
42 pneumonia, leading to a severe hypoxemia for a minority of them (13.8-16%). In worst cases, acute
43 respiratory failure may occur, requiring ventilatory support ranging from nasal oxygen therapy to
44 high flow oxygen therapy, non-invasive ventilation or even mechanical ventilatory support (7,8). The
45 latter leads to death in 30-60% of the cases. At mild term follow-up, although most patients had no
46 significant sequelae, a substantial proportion suffered from persistent symptoms and respiratory
47 impairment or even pulmonary fibrosis lesions (9,10). Identification of prognosis factors that stratify
48 at-risk patients and predict severity and/or mortality may be of great utility for clinical practice. To
49 date, numerous blood-based biomarkers such as D-dimer, C-reactive protein (CRP), lactate
50 dehydrogenase (LDH) and high-sensitivity cardiac troponin I (11) have been associated with COVID-
51 19 worse outcomes. Only few studies have questioned the role of KL-6 in the evaluation of COVID-19

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

54 **Materials and Methods**

55 In order to evaluate the prognostic value of KL-6 serum, we performed a retrospective study (March
56 1st to May 7th, 2020 considering all consecutive adults hospitalized for a suspicion of COVID-19
57 pneumonia in our Chest department. All-cause in-hospital mortality was considered as primary
58 endpoint.

59 ***Study population and data collection***

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

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

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

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

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

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

79 ***Statistical analysis***

80 Results were expressed as mean \pm standard deviation (SD) or medians (IQR, interquartile ranges) for
81 quantitative variables, and n (%) for qualitative variables. Differences between groups were assessed
82 using the Chi-squared test (or Fischer test, as appropriate) for categorical variables and Mann-
83 Whitney test for quantitative variables. For correlation analysis, Pearson correlation was applied. To
84 evaluate risk factors associated with in-hospital mortality, we performed univariate Cox regression
85 analyses. For these survival analyses, quantitative dependent variables were used without any
86 dichotomization, i.e using continuous variables. Results were expressed as hazard ratios (HRs) with
87 95% confidence intervals (95%CI). The proportional hazard assumption was tested using Schoenfeld's
88 test. Kaplan-Meier curves representing survival across strata where survival was assessed by a log-
89 rank test. A p-value less than 0.05 was considered statistically significant. Statistical analyses were
90 performed using GraphPad Prism and R-Studio 2020, Integrated Development for R (R-Studio,
91 Boston, MA, USA).

92 ***Ethical statement***

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

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

99 **Results**

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

101 Among the 221 consecutive patients hospitalized during the study period for a suspicion of COVID-
102 19, 81 met the inclusion criteria among whom 66 (81.4%) had a diagnosis of COVID-19 pneumonia
103 and the other 15 had various non-related COVID-19 respiratory diseases and served as controls
104 (shown in Figure 1).

105 The first KL-6 assay was performed in a mean delay from admission of 1.9 days (± 1.1) in COVID-19
106 and 2.0 days (± 2.3) in controls, and from the first symptoms onset at 9.6 days (± 3.6) in COVID-19 and
107 5.7 days (± 3.5) in controls ($p < 0.001$). KL-6 serial measurements were carried out in 36 (54.5%) COVID-
108 19 patients.

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

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

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

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

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

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

132 Considering the 62 (94%) patients with CT scan at admission (shown in Table 1S supplementary
133 appendix), GGO (95.2%), subpleural and peribronchovascular distribution (54.8%) were the most
134 frequent findings. CT scan involvement was predominantly bilateral (95.1%) and multifocal (64.5%).
135 Compared with controls, COVID-19 patients had more diffuse radiological lesions on CT-scan
136 ($p=0.02$), distributed mostly sub pleural ($p=0.02$), associated with an anteroposterior ($p<0.01$) and
137 craniocaudal ($p=0.01$) gradient. Meanwhile, controls had more lesions of low extent $< 10\%$ ($p=0.046$)
138 compared with COVID-19 patients. Median KL-6 concentration tended to be related to radiological
139 extent on chest CT and was higher when radiological extension was $\geq 50\%$ compared with those with
140 less than 50% radiological extension (529 U/mL [IQR: 380-865] and 354 U/mL [IQR: 221-482],
141 $p=0.0040$; shown in Figure 2A). However, there was no significant association between radiological
142 extension on thoracic CT scan and WHO classes ($p=0.9$).

143 ***KL-6 serum concentrations and COVID-19 pneumonia outcome***

144 Patient median follow-up was 3.6 months. Treatments and outcomes are presented in Table 2. Sixty-
145 six (100%) patients had received antibiotics, 11 (6.7%) antiviral therapy, 3 (4.5%) hydroxychloroquine
146 (including one as a part of his background therapy for rheumatoid arthritis), 19 (29%) hydrocortisone
147 therapy, and 15 (22.8%) tocilizumab. Of the 66 patients, 15 (23%) were recused from ICU admission,
148 9 required high-flow oxygen and 16 were admitted in ICU of whom 12 needed orotracheal
149 intubation. Median KL-6 level at admission was significantly higher in COVID-19 patients in WHO \geq
150 class 6 (532 U/mL [IQR: 383-944]) compared to those in WHO \leq class 5 (387 U/mL [IQR: 246-509];
151 $p=0.046$, shown in Figure 2B). The median time from KL-6 assay to the date of worst WHO was 5 days
152 (IQR: 3-7) in deceased patients.

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

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

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

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

169 Cov2 antibodies in their serum. Twenty-six patients (54.1%) complained of breathlessness, 10
170 (20.8%) of persisting cough, and 16 (33.3%) of chronic fatigue. The initial KL-6 level was not
171 associated with persistence of subjective breathlessness at three months ($p = 0.590$), nor with MRC
172 score value (data not shown). Twenty-seven patients (56.3%) had persistent pulmonary lesions on CT
173 scan, but no patients developed lung fibrosis and we did not find an association with the initial KL-6
174 level and the persistence of radiological abnormalities ($p = 0.947$). By contrast, an inverse correlation
175 was found between the initial KL-6 level and the DLCO value at three months ($r = -0.47$, $p=0.001$, $n=$
176 45, shown in Figure 1S, supplementary appendix), but not with the FVC and FEV1 values. In the
177 COVID-19 group, patients with a history of chronic respiratory disease had a lower DLCO than those
178 without (median of 62% [IQR: 47.5-74] and 76% [IQR: 70.25-89.75]; $p<0.01$). However, the same
179 results were observed when patients with chronic respiratory diseases were excluded ($r=-0.41$;
180 $p=0.0125$). The KL-6 serum level at 3 months remained above 500 U/mL (339.5 U/mL [IQR: 225.5-
181 495.8]) in 12 patients but did not significantly correlate with persistent radiological abnormality or
182 impaired DLCO at this time point.

183 **Discussion**

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

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

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

198 Surprisingly enough, our study did not show any difference in KL-6 levels between the COVID-
199 19 population and our control population. Nevertheless, KL-6 assay was conducted later after onset
200 of symptoms in the COVID-19 group (mean duration of 9.6 days) compared to the control group
201 (mean duration of 5.7 days). This could explain the absence of difference in KL-6 levels between the
202 two groups if KL-6 high point occurs early in disease's evolution. The time between sampling and the
203 onset of symptoms could influence KL-6 dosage (figure 2S supplementary appendix). However, the
204 assays were systematically performed within 72 hours of admission, making our study as
205 homogeneous as possible. Moreover, this emphasizes the relevance of repeated dosing to obtain KL-
206 6 kinetics, which seems to reflect the COVID-19 pneumonia progression, as newly suggested by our
207 study.

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

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

228 On 3-months follow-up analysis in survivors, DLCO values were inversely correlated with
229 baseline serum KL-6 levels, and so despite the exclusion of patients with chronic respiratory diseases.
230 This result is of particular interest as some, although few, patients develop a fibrosing-ILD post SARS-
231 CoV-2 pulmonary infection and is consistent with those reported in ILD in which serum KL-6 level is
232 also negatively correlated with DLCO (31). In a large cohort of SARS-CoV-2 patients that during a 6-
233 month follow-up, DLCO decreased by 22 to 56% and notably more in patients with severe disease. In
234 addition, it was shown that HRCT abnormalities affecting more than 5% of lung parenchyma were
235 present in 52-54% (32). Previous studies have shown that patients with a higher risk of pulmonary
236 fibrosis secondary to COVID-19 are older, male, smokers, and have underlying diseases such as
237 diabetes, pulmonary and cardiovascular diseases as well as, the use of high-flow oxygen therapy,
238 need for mechanical ventilation and the development of ARDS (10). In this context, Milara et al
239 showed that the C-terminal domain of MUC1 has a role in the promotion of pulmonary fibrosis in IPF,
240 and could impact profibrotic gene expression, cell proliferation or senescence through the phospho-
241 Smad3 and act- β -catenin pathway in response to TGF- β (33). Some authors have demonstrated that
242 patients who were severely affected by COVID-19 had an excessive immune response that was partly
243 controlled by TGF- β (34). Testing TGF- β would be interesting to further assess the severity of COVID-
244 19 as a complement to the KL-6 assay, and could argue for a therapeutic target, especially
245 considering TGF- β as the key player in pulmonary fibrogenesis.

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

252 **Conclusion**

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

260 **Declaration**

261 **Consent for publication**

262 Not applicable.

263 **Availability of data and materials**

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

266 **Competing interests**

267 The authors declare that they have no competing interests.

268 **Funding**

269 This work had no external funding source.

270 **Authors' contributions**

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

274 **Acknowledgements**

275 Not applicable.

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Table 1: Baseline characteristics at diagnosis	COVID-19 (n = 66)	Control (n = 15)	p-value
<i>Demographic features</i>			
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
<i>Clinical features</i>			
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
<i>Laboratory findings at diagnostic - median (IQR)</i>			
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)
<i>Treatment</i>	
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)
<i>Outcomes</i>	
ICU admission* – n (%)	16 (31.4)
OTI* – n (%)	12 (23.5)
HFO – n (%)	9 (13.6)
In-hospital mortality – n (%)	9 (13.6)
<i>*Among patients with no LLST</i>	
<i>Abbreviations: ECMO: extracorporeal membrane oxygenation; LLST: limitations on life support techniques; ICU: intensive care unit; OTI: orotracheal intubation; HFO: High flow oxygen.</i>	

Table 3: Univariate analysis for mortality	HR (95%CI)	p-value
<i>Demographic features</i>		
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
<i>Laboratory findings at diagnostic</i>		
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
<i>Abbreviations: BMI, body mass index; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; LDH, Lactate dehydrogenase; PNN, Polynuclear Neutrophil.</i>		

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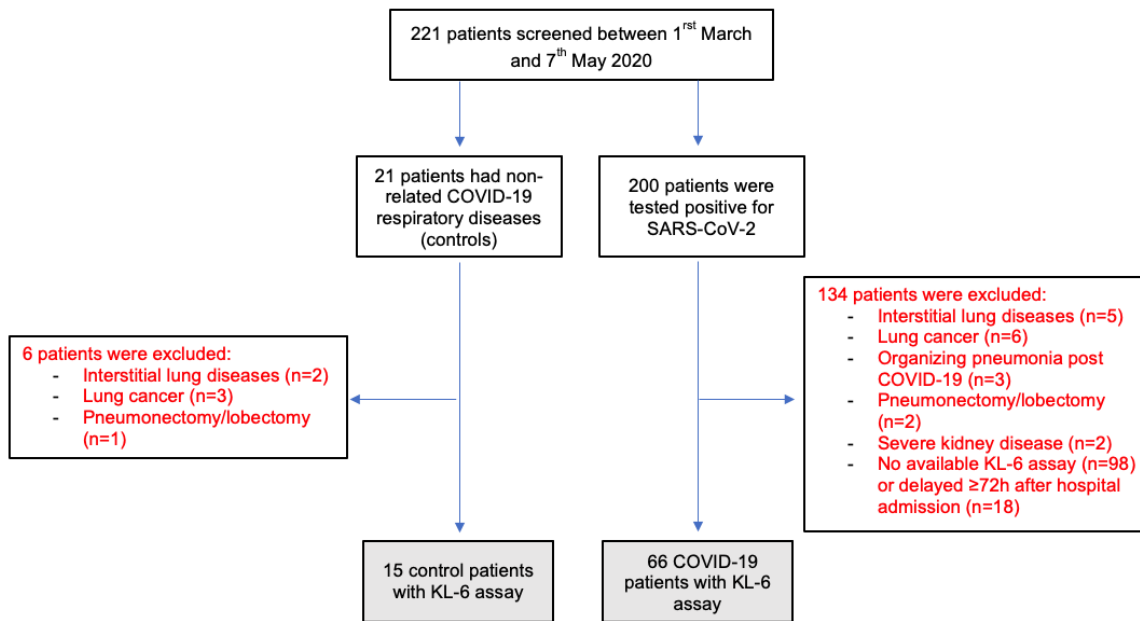
380 Figures:

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)



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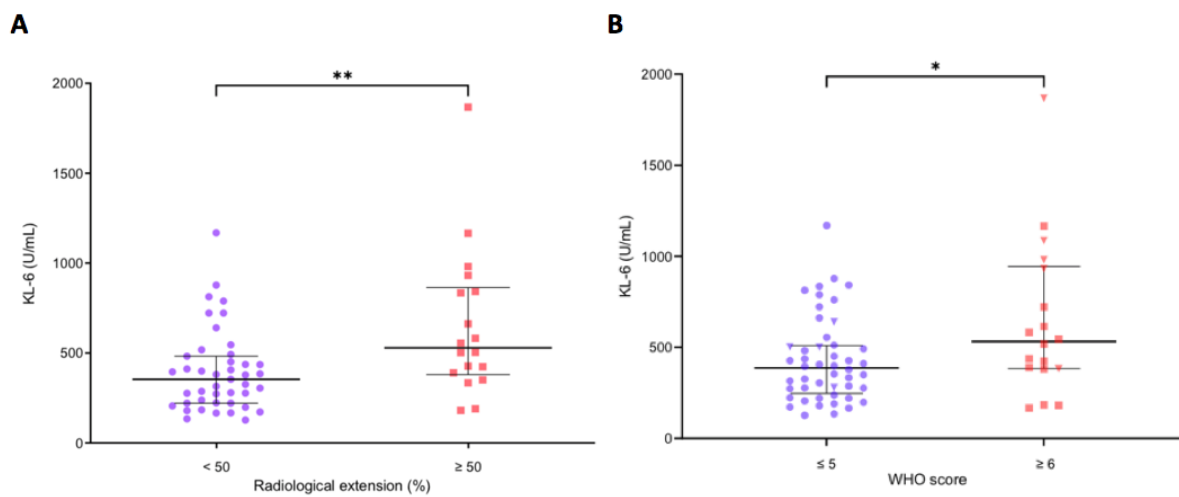
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Figure 4: Flowchart

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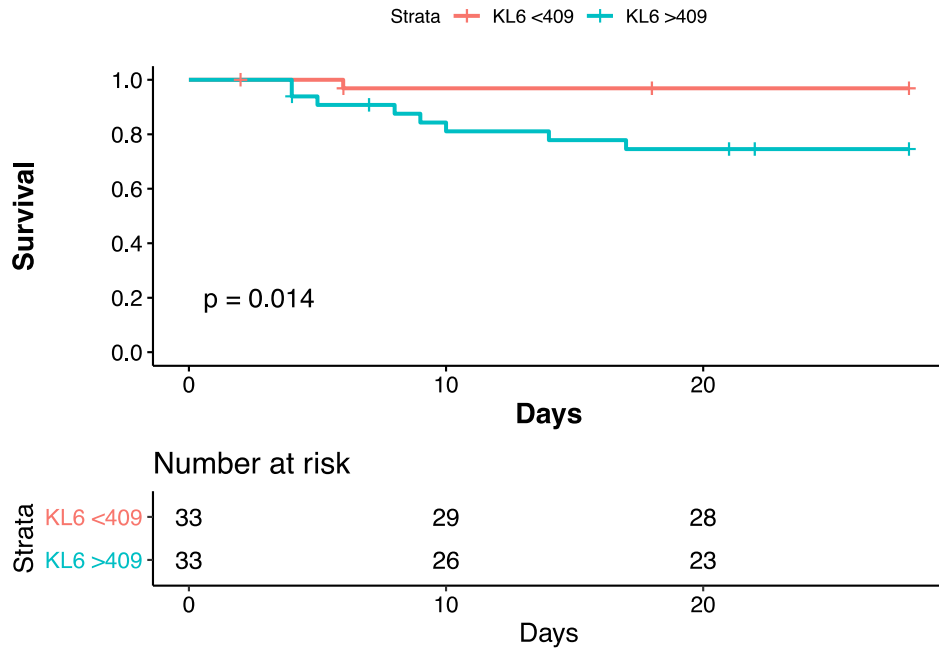
Figure 5: Median KL-6 levels according to radiological extension on chest CT scan (A) and WHO score (B)

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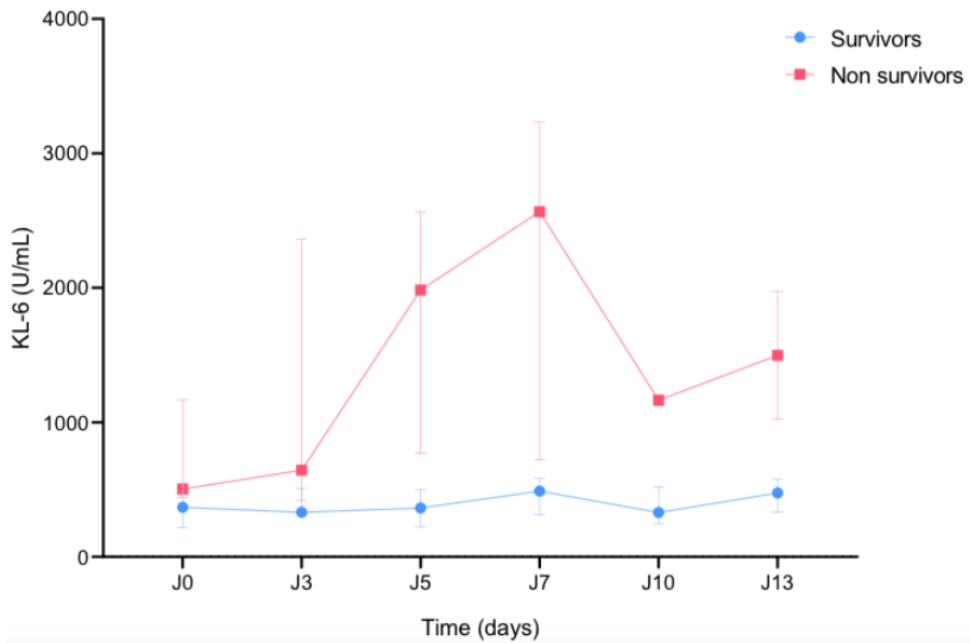


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Figure 6: All-cause mortality at 28 days 28 related to KL-6 levels (in U/mL)

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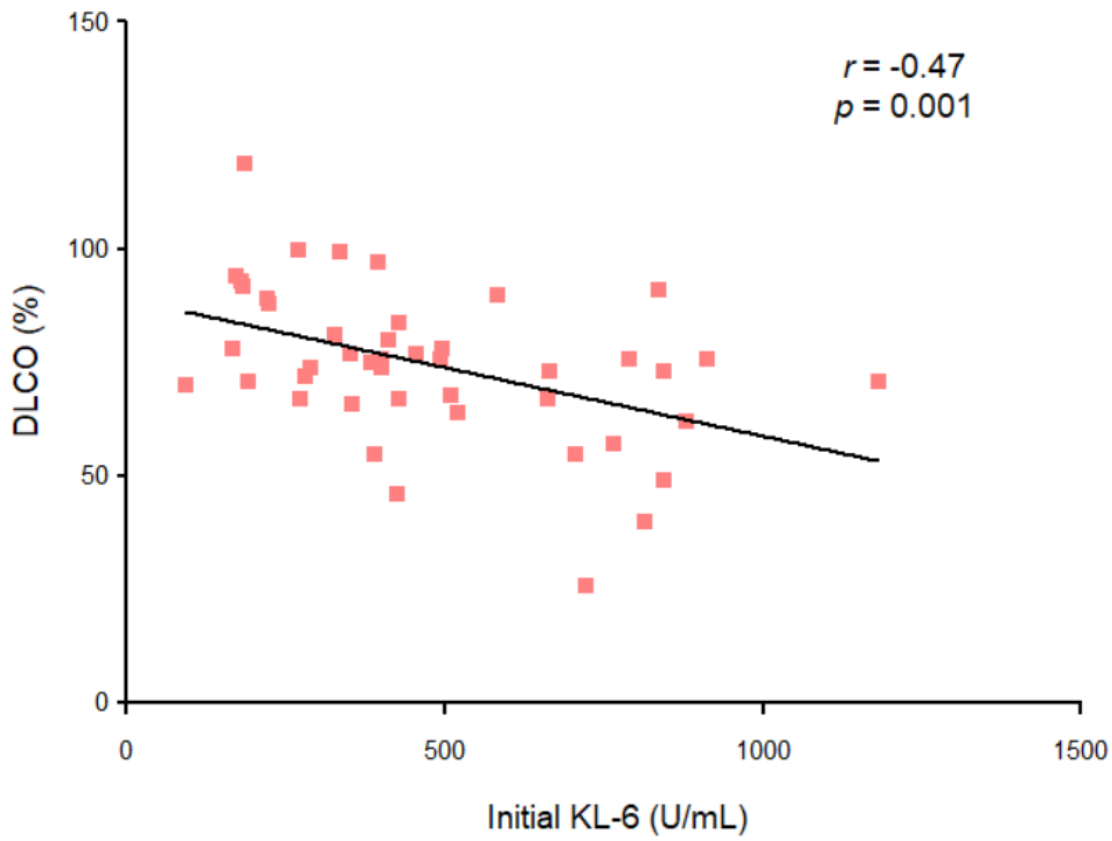
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Figure 7: Dynamic change of KL-6 levels for patients with serial evaluation (n=36)

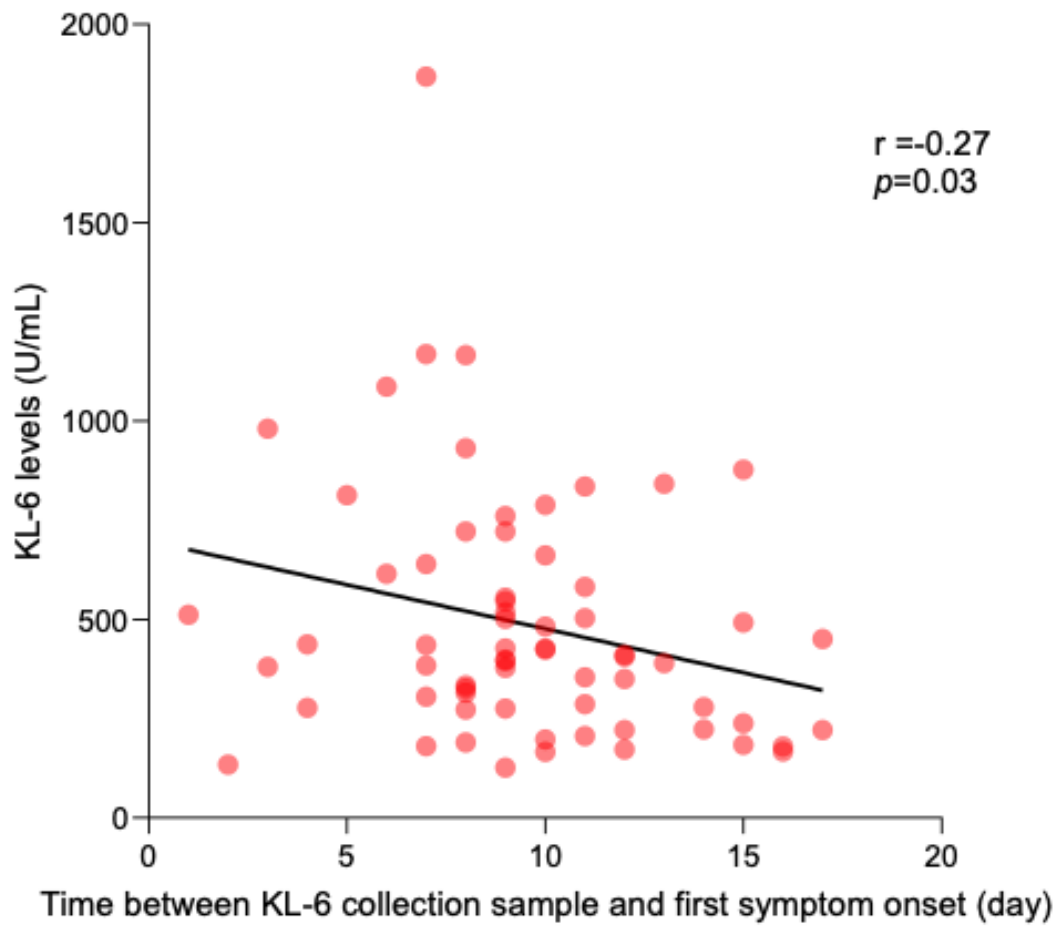
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Figure 8S: Correlation between initial KL-6 level and the DLCO value at three months.



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405 *Figure 9S: Initial KL-6 levels and time between collection sample and first symptoms onset*
406 *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	<i>p</i> -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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