



# Exploratory analysis of serum Krebs von den Lungen-6, blood gas analysis & Brixia score in determining COVID-19 severity & mortality

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**Background & objectives:** Krebs von den Lungen-6 (KL-6) is primarily expressed by the damaged type II pneumocytes. In this context, the relationship of KL-6 with blood gas analysis (BGA) parameters and Brixia score is still limitedly discussed. This study aims to analyze the correlation of KL-6, BGA and Brixia scores to the severity and mortality of COVID-19.

**Methods:** A cross-sectional study was conducted in adult COVID-19 positive individuals at Universitas Airlangga Hospital, Surabaya, East Java, Indonesia, from March to August 2021. KL-6, BGA, and Brixia scores were compared according to severity (severe vs. non-severe) and mortality (non-survivor vs. survivor). The receiver operating characteristic (ROC) analysis was also performed to define the optimal cut-off, sensitivity, as well as the specificity of KL-6, BGA and Brixia scores to determine the COVID-19 severity and mortality.

**Results:** Total 35 severe and 20 non-severe COVID-19 positive individuals were enrolled in this study. Of those, there were 22 non-survivors. No significant difference in serum KL-6 levels was observed in the severity and mortality groups. KL-6 and  $\text{HCO}_3^-$  had positive correlation in the severe group ( $r=0.37$ ). KL-6 and Brixia scores showed a significant negative correlation among COVID-19 positive individuals ( $r=-0.283$ ;  $P=0.036$ ). KL-6 and Brixia scores together served as the best severity markers in the current study [AUC 0.809 (0.697–0.920); Sn/Sp=0.686/0.900], followed by KL-6 and P/F ratio [AUC 0.800 (0.637–0.963); Sn/Sp=0.971/0.750].

**Interpretation & conclusions:** The findings of this study suggest that KL-6 has the potential to be a useful adjunct laboratory parameter to the BGA and Brixia score representing COVID-19 severity and mortality.

**Key words** Biomarker - blood gas analysis - brixia - COVID-19 - KL-6 - mortality - SARS-CoV-2-severity

Coronavirus disease 2019 (COVID-19) is a pandemic viral infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The associated clinical spectrum varies, ranging from

asymptomatic to critical cases, with a case fatality rate of 6.5 per cent, or even higher to 9.1 per cent in Indonesia<sup>1,2</sup>. During the disease progression, 20.7–31.4 per cent reportedly may develop into severe cases and 4.9–11.5 per cent may need ventilatory support<sup>3</sup> in context of this disease progression many biomarkers are still needed to explore the severity and mortality of COVID-19, including Krebs von den Lungen-6 (KL-6) and blood gas analysis (BGA). KL-6 (*MUC1* or Mucin-1) is a sialoglycoprotein expressed by damaged type II alveolar cells<sup>1,4</sup>. SARS-CoV-2 replication has been shown to increase serum KL-6 levels, representing the damaged lung area<sup>5,6</sup>. Circulating KL-6 in the the bloodstream can induce inflammatory processes, which may lead to organ failure and mortality<sup>1,5,6</sup>. Furthermore, it is thought that SARS-CoV-2 infection downregulate its own port entry, through the angiotensin-converting enzyme 2 (ACE2)<sup>7</sup>. This process disrupts the acid-base equilibrium by upregulating the classic renin-angiotensin system (RAS) pathway. It interferes with the renal balancing mechanism, which can be reflected in the BGA results<sup>7</sup>. Silent hypoxemia, fever, multi-organ inflammation, thrombogenesis and carotid body suppression may disturb the pulmonary vasculature eventually leading to alveoli or damage through acute respiratory distress syndrome (ARDS)<sup>8</sup>.

Radiologic assessments are also vital in determining COVID-19 severity and mortality. Brixia score, a semi-quantitative assessment, is a simple assessment of the chest X-ray (CXR)-based scoring in COVID-19<sup>9</sup>. This scoring is designed to rank the pulmonary involvement area and characteristics through a total 18-point severity scale<sup>9,10</sup>. The CXR is divided into upper, middle and lower zones for each lung, then scored 0–3 for each zone, with 0 as ‘no lung abnormalities’ and 3 as ‘visible interstitial and alveolar infiltrate’<sup>9</sup>.

Due to the quick deterioration of COVID-19, some laboratory parameters and radiological assessments are essential to determine its severity and mortality. Furthermore, with a hypothesis that KL-6, BGA and Brixia scores correlate with each other and the COVID-19 severity and mortality it was envisioned that such a study could provide novel knowledge regarding its pathophysiology. Currently, no study discusses their relationship with COVID-19. Hence, this study aimed to analyse the relationship between KL-6, BGA and Brixia scores in the severity and mortality of Indonesian COVID-19 positive individuals

in Indonesia. Their sensitivity and specificity were also determined.

## Material & Methods

*Study design and setting:* This cross-sectional study was conducted on adult hospitalized COVID-19 positive individuals enrolled from March to August 2021 at Universitas Airlangga Hospital, Surabaya, Indonesia. All clinical and laboratory data were collected from hospital medical records. A blood examination was conducted at the same hospital. This study was approved by the ethics committee of Universitas Airlangga Hospital.

*Eligibility criteria:* The inclusion criteria in this study: (i) adults  $\geq 18$  yr, COVID-19 positive confirmed by reverse transcription polymerase chain reaction (RT-PCR) of nasopharyngeal swab; (ii) undergoing CXR examination and evaluated for Brixia score; (iii) individuals with KL-6 and BGA results; and (iv) participants who provided their written informed consents. The exclusion criteria were individuals with pre-existing or history of lung tuberculosis (TB), interstitial lung disease (ILD) and/or chronic obstructive pulmonary disease, pregnant women and individuals with human immunodeficiency virus infection.

*Clinical data and laboratory assessment:* Age, gender, length of stay, respiratory rates (RR) and comorbidities were recorded as the clinical data. The Charlson Comorbidity Index (CCI) were calculated and classified them into three groups based on the scores: 0–1; 2–3; and  $\geq 4$ <sup>11</sup>. Complete blood count, liver function test, renal function test, electrolyte, anion gap, D-dimer, ferritin, interleukin-6 (IL-6), procalcitonin (PCT), C-reactive protein (CRP), KL-6, BGA and PaO<sub>2</sub>/FiO<sub>2</sub> ratio (P/F ratio) were also collected. KL-6 were measured using the Bioassay Technology Laboratory KL-6 ELISA reagent kit (BT-Lab, Cat. No E1980Hu) and interpreted using iMark<sup>TM</sup> Microplate Absorbance Reader (Bio-Rad Laboratories Inc., Hercules, CA). CXR was scored using the Brixia system<sup>12,13</sup>, first interpreted by a radiologist followed by a pulmonologist and then by the senior radiologist to confirm the interpretation. The Brixia score results were then classified into four groups of severity: score 0 as normal; score 1–6 as mild; score 7–12 as moderate; and score 13–18 as severe<sup>13</sup>.

The study participants were categorized into four severity levels based on the Indonesian COVID-19 guideline. Those who had any signs and/or symptoms of COVID-19 without any proof of pneumonia or hypoxia were grouped as 'mild'; those who had pneumonia proven by clinical assessment or imaging with  $\text{SpO}_2 \geq 93\%$  on room air at sea level were grouped as 'moderate'; those who had severe pneumonia indicated by RR  $>30$  breaths/min, severe respiratory distress, or  $\text{SpO}_2 < 93\%$  on room air at sea level were grouped as 'severe'; and those who had ARDS, sepsis and/or, septic shock were grouped as critical<sup>13,14</sup>. The severity classification was simplified into non-severe (mild and moderate) and severe (severe and critical). Furthermore, samples were also classified into survivors and non-survivors.

*Statistical analysis:* The results were presented as mean $\pm$ standard deviation (SD) and median (interquartile range [IQR]). We analyzed the comparison between groups, either severe vs. non-severe or non-survivor vs. survivor. Mann–Whitney *U* and Chi-square tests were performed for ordinal and nominal data, respectively. Fisher's exact test was also performed if the conditions of Chi-square test were not met. For the continuous data, an independent *t*-test or Mann–Whitney *U* test was applied depending on the Shapiro–Wilk normality test. An independent *t*-test was selected if  $P \geq 0.05$ ; otherwise, the Mann–Whitney *U* test was chosen. Pearson's correlation was performed to assess the correlation of parameters. We also analysed the receiver operating characteristics (ROCs) curve and its components, including area under the curve (AUC), sensitivity, specificity and optimal cut-off (Youden's index). Statistical analyses were conducted using IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, IL).  $P < 0.05$  was considered significant. Furthermore, uni- and multivariate logistic regressions were performed to obtain odds ratios (ORs) if  $P < 0.05$ . Missing values were imputed by aggregating five iterations of multiple imputations by chained equations imputation method into their mean values<sup>15</sup>. Multiple imputations were only conducted if the missing values on a variable were  $< 5\%$  to avoid result bias<sup>16</sup>.

## Results

A total of 55 COVID-19 positive individuals were included (49.1% were male) and their severity and mortality parameters were compared (Table I and Supplementary Table I). The RR was significantly different in the severe group ( $30.5 \pm 5.1$  vs.  $24.2 \pm 3.5$ ;

$P < 0.001$ ) and in non-survivors ( $30.7 \pm 5.7$  vs.  $26.6 \pm 4.8$ ;  $P = 0.005$ ) compared to each opposing group. Multivariate logistic regression (Table II) showed that RR was significantly higher in the severe group (OR 1.567 [1.130–2.174];  $P = 0.007$ ), but not in the non-survivors (OR 1.094 [0.904–1.325;  $P = 0.355$ ]) as compared to each opposing group. The two most common comorbidities in this study were diabetes mellitus and hypertension (each 18.2%). In the CCI, a score of 0-1 was the most common among all participants (63.6%). However, none of the groups showed significantly different numbers of comorbidities and CCI scores. Missing values were present for D-dimer, ferritin, IL-6, PCT and CRP (Supplementary Table II). A significantly higher platelet ( $P = 0.011$ ) and D-dimer ( $P = 0.002$ ) levels were observed in the severe groups. Red blood cells, serum glutamic oxaloacetic transaminase, blood urea nitrogen, potassium, D-dimer and IL-6 were significantly higher in the mortality groups ( $P < 0.05$ ). However, according to the multivariate logistic regression, all these factors had similar values, proven by insignificant ORs in Table II.

*Diagnostic value of KL-6, BGA and Brixia score in COVID-19 severity and mortality:* Table I provided the KL-6, BGA parameters and Brixia score. No significant differences in the severity and mortality groups were observed in KL-6,  $\text{pCO}_2$ , BE,  $\text{HCO}_3^-$  and  $\text{SaO}_2$ . However, alveolar-arterial oxygen gradient,  $\text{FiO}_2$  and Brixia score were significantly different in the severity and mortality groups. We also performed multivariate logistic regression (Table II). The result showed that only Brixia score was significantly higher in severe participants as compared to the non-severe ones (OR 1.401 [1.032–1.903];  $P = 0.031$ ). There was no significant correlation between KL-6 and several BGA parameters in COVID-19 severity (Fig. 1A-E) and mortality (Fig. 2A-E). Nevertheless, the strongest KL-6-BGA correlations were observed between KL-6 and  $\text{HCO}_3^-$  in the severe group ( $r = 0.370$ , Fig. 1D) and between KL-6 and  $\text{pCO}_2$  in the survivor group ( $r = 0.224$ , Fig. 2B). Interestingly, KL-6 and Brixia scores had a significantly negative correlation overall among all COVID-19 positive individuals ( $r = -0.283$ ;  $P = 0.036$ ), as shown by the black line in Figs. 1F and 2F. In separated analysis, KL-6 and Brixia score were significantly correlated in the COVID-19 survivors ( $r = -0.347$ ;  $P = 0.048$ , Fig. 2F), but not in the non-severe group ( $r = -0.285$ ;  $P = 0.223$ , Fig. 1F).

**Table 1.** COVID-19 individuals characteristics based on the severity and mortality groups.

Parameters	Study participants (n=55)	Severe (n=35) (Severe: n=17; Critical: n=18)	Non-severe (n=20) (Mild: n=2; Moderate: n=18)	p-value	Non-survivor (n=22) (Non-severe: n=3; Severe: n=19)	Survivor (n=33) (Non-severe: n=17; Severe: n=16)	P-value
Age (yr)	50.9±11.6 52 (45, 59)	51.2±10.4 52.0 (46.0, 59.0)	50.3±13.7 52.0 (43.5, 58.8)	0.784 <sup>a</sup>	52.4±11.4 52.5 (47.8, 59.0)	49.9±11.8 52.0 (43.5, 58.0)	0.440 <sup>a</sup>
Male, n(%)	27 (49.1)	16 (45.7)	11 (55.0)	0.508 <sup>b</sup>	11 (50.0)	16 (48.5)	0.912 <sup>b</sup>
KL-6 (U/ml)	48.0±26.1 40 (32.4, 56.7)	42.7±20.4 37.7 (32.4, 48.2)	57.3±32.4 44.1 (32.4, 82.5)	0.130 <sup>c</sup>	43.7±22.1 40.4 (32.2, 50.5)	50.9±28.4 39.1 (32.1, 71.7)	0.757 <sup>c</sup>
Blood gas analysis							
pH	7.4±0.1 7.4 (7.4, 7.4)	7.4±0.1 7.4 (7.4, 7.4)	7.4±0.1 7.4 (7.4, 7.5)	0.958 <sup>c</sup>	7.4±0.1 7.4 (7.3, 7.4)	7.4±0.1 7.4 (7.4, 7.5)	0.003 <sup>c</sup>
pO <sub>2</sub> (mmHg)	91.4±29.9 86.6 (66.8, 117.1)	86.8±27.6 83.8 (62.1, 101.3)	99.6±32.8 95.2 (73.6, 133.4)	0.129 <sup>a</sup>	96.2±30.9 99.9 (68.4, 122.5)	88.2±29.3 83.0 (65.3, 100.8)	0.337 <sup>a</sup>
pCO <sub>2</sub> (mmHg)	32.8±12.2 32.2 (25.9, 37)	34.5±14.6 32.6 (25.9, 40.9)	29.9±5.5 32.2 (26.0, 33.6)	0.248 <sup>c</sup>	35.4±16.6 33.2 (24.9, 43.3)	31.1±8.0 32.1 (26.2, 34.5)	0.390 <sup>c</sup>
BE (mEq/L)	-3.7±4 -2.9 (-5.6, -1.2)	-3.2±4.2 -2.5 (-5.2, -0.4)	-4.7±3.5 -4.2 (-6.2, -2.4)	0.192 <sup>a</sup>	-4.3±4.8 -3.3 (-7.3, -1.0)	-3.4±3.4 -2.7 (-4.8, -1.3)	0.378 <sup>a</sup>
HCO <sub>3</sub> <sup>-</sup> (mEq/L)	19.9±4.8 20.3 (17.9, 22.8)	20.6±21.4 21.4 (18.3, 24.6)	18.7±3.5 19.2 (17.8, 21.6)	0.161 <sup>a</sup>	19.9±5.8 20.8 (15.7, 23.9)	19.9±4.1 20.3 (18.3, 22.2)	0.965 <sup>a</sup>
AaDO <sub>2</sub> (mmHg)	325.2±201.5 369.5 (130.1, 485.3)	403.3±158.5 439.1 (269.5, 539.4)	188.6±198.9 82.2 (17.4, 401.9)	<0.001 <sup>a</sup>	424.0±148.5 477.9 (300.2, 543.1)	259.4±207.1 259.6 (54.5, 421.8)	0.003 <sup>c</sup>
SaO <sub>2</sub> (%)	94.9±5 96.5 (92.7, 98.5)	94.4±5.0 96.3 (91.7, 97.9)	95.8±5.0 97.4 (95.2, 99.0)	0.115 <sup>c</sup>	94.5±6.0 97.3 (90.4, 98.8)	95.2±4.4 96.3 (94.1, 97.7)	0.777 <sup>c</sup>
FiO <sub>2</sub> (%)	58.5±21 61.0 (33.0, 81)	65.4±20.0 81.0 (50.0, 81.0)	46.5±17.0 45.0 (33.0, 53.0)	0.002 <sup>c</sup>	69.1±18.1 81.0 (59.0, 81.0)	51.5±19.9 53.0 (33.0, 69.0)	0.001 <sup>c</sup>
P/F Ratio (mmHg)	182.4±109 163.7 (106.9, 223.9)	142.9±54.2 129.1 (102.5, 171.9)	251.5±143.6 234.9 (153.8, 325.3)	0.001 <sup>c</sup>	150.3±71.7 144.9 (102.2, 170.3)	203.8±124.5 175.7 (108.6, 256.0)	0.080 <sup>c</sup>
Brixia score	12.3±4.8 13.0 (8.0, 17.0)	14.1±4.2 15.0 (12.0, 18.0)	9.1±4.3 10.0 (6.5, 12.0)	<0.001 <sup>c</sup>	14.0±4.3 15.5 (11.0, 18.0)	11.2±4.9 12.0 (7.5, 15.0)	0.032 <sup>c</sup>
Normal CXR, n(%)	1 (1.8)	0 (0.0)	1 (5.0)	<0.001 <sup>c</sup>	0 (0.0)	1 (3.0)	0.026 <sup>c</sup>
Mild changes, n(%)	6 (10.9)	2 (5.7)	4 (20)		2 (9.1)	4 (12.1)	
Moderate changes, n(%)	19 (34.5)	7 (20.0)	12 (60.0)		4 (18.2)	15 (45.5)	
Severe changes, n(%)	29 (52.7)	26 (74.3)	3 (15)		16 (72.7)	13 (39.4)	

Continuous variables are shown as mean±SD and median [interquartile range (IQR)]. The comparison between two groups, either severe vs. non-severe and non-survivor vs. survivor, was performed using an independent  $\chi^2$ -test, <sup>a</sup>Mann-Whitney *U* test, or Fisher's exact test. AaDO<sub>2</sub>, alveolar-to-arterial oxygen pressure gradient; BE, base excess; CXR, chest X-ray; FiO<sub>2</sub>, fraction of inspired oxygen; HCO<sub>3</sub><sup>-</sup>, bicarbonate ion concentration; KL-6, Krebs von den Lungen-6; P/F ratio, PaO<sub>2</sub>/FiO<sub>2</sub> ratio; pCO<sub>2</sub>, partial carbon dioxide pressure; pO<sub>2</sub>, partial oxygen pressure; SaO<sub>2</sub>, arterial oxygen saturation; AaDO<sub>2</sub>, alveolar-to-arterial oxygen pressure gradient.

**Table II.** Univariate and multivariate logistic regressions in the COVID-19 positive individuals

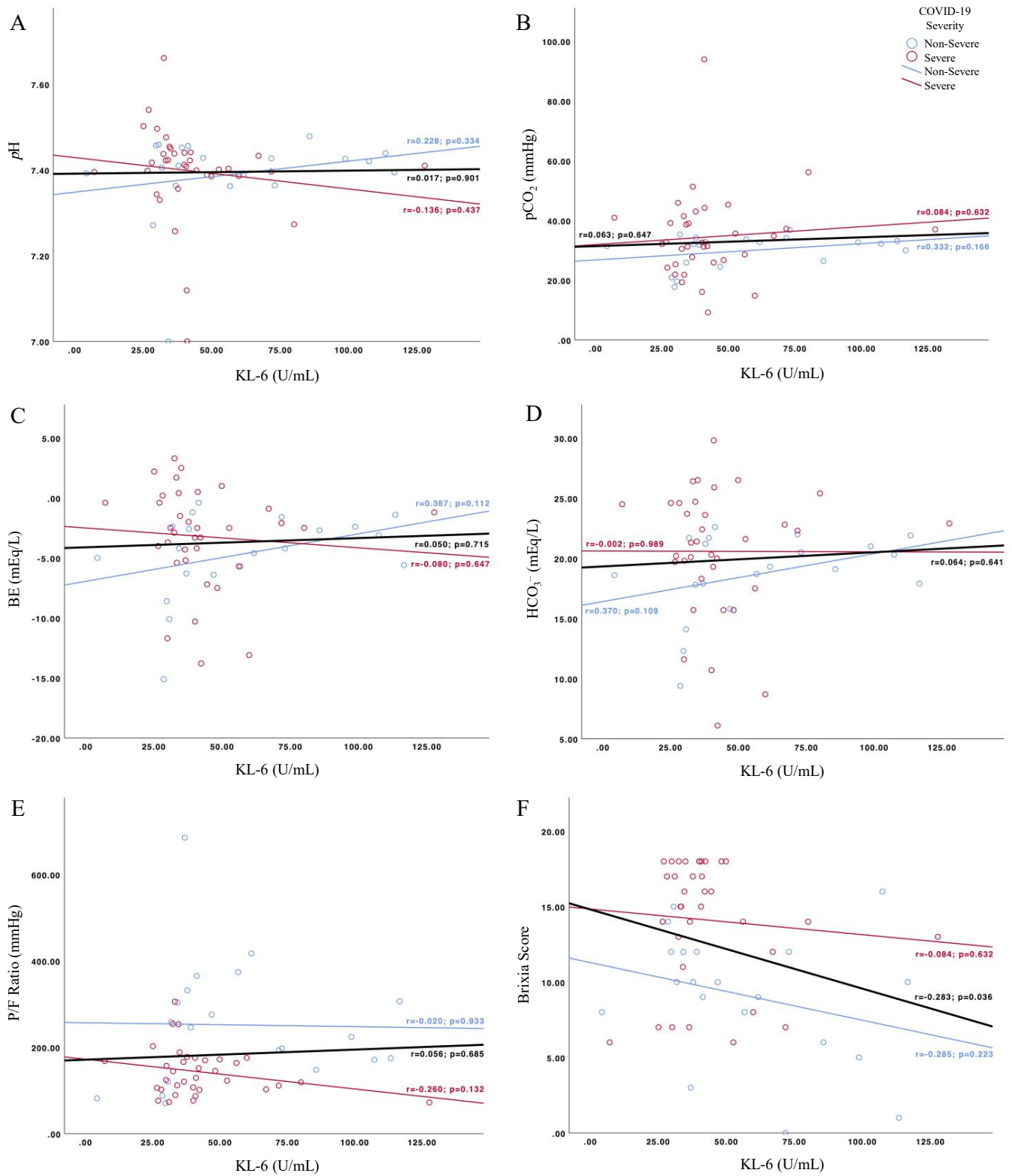
Parameters	Severe vs. Non-severe				Non-survivor vs. Survivor			
	Univariate		Multivariate		Univariate		Multivariate	
	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)
Respiratory rate (breaths/min)	<0.001	1.420 (1.179–1.71)	0.007	1.567 (1.130–2.174)	0.011	1.171 (1.037–1.323)	0.355	1.094 (0.904–1.325)
Red blood cell (10 <sup>6</sup> /ml)					0.036	2.532 (1.062–6.039)	0.077	3.614 (0.869–15.037)
Platelet (10 <sup>3</sup> /ml)	0.034	1.007 (1.001–1.013)	0.105	1.011 (0.998–1.025)				
SGOT(U/L)					0.107	1.010 (0.998–1.022)		
BUN (mg/dl)					0.025	1.087 (1.010–1.169)	0.208	1.080 (0.958–1.217)
Potassium (mmol/L)					0.004	6.672 (1.847–24.101)	0.236	3.291 (0.459–23.613)
D-Dimer (mcg/ml)	0.07	2.831 (0.918–8.732)			0.259	1.074 (0.949–1.215)		
Interleukin-6 (pg/ml) (N/A=13)					0.060	1.006 (1–1.012)		
C-reactive protein (mg/L)					0.011	1.010 (1.002–1.018)	0.114	1.011 (0.997–1.024)
pH					0.113	0.008 (0.000–3.159)		
AaDO <sub>2</sub> (mmHg)	0.001	1.006 (1.003–1.010)	0.362	1.003 (0.997–1.010)	0.005	1.005 (1.001–1.008)	0.422	1.002 (0.997–1.008)
FiO <sub>2</sub> (%)	0.002	1.050 (1.018–1.083)	0.736	1.014 (0.937–1.096)	0.003	1.048 (1.015–1.081)	0.091	1.043 (0.993–1.095)
P/F ratio (mmHg)	0.002	0.986 (0.978–0.995)	0.720	0.997 (0.981–1.013)				
Brixia score	0.001	1.294 (1.111–1.507)	0.031	1.401 (1.032–1.903)	0.037	1.149 (1.008–1.310)	0.473	1.093 (0.857–1.393)

BUN, blood urea nitrogen; CI, confidence interval; OR, odds ratio

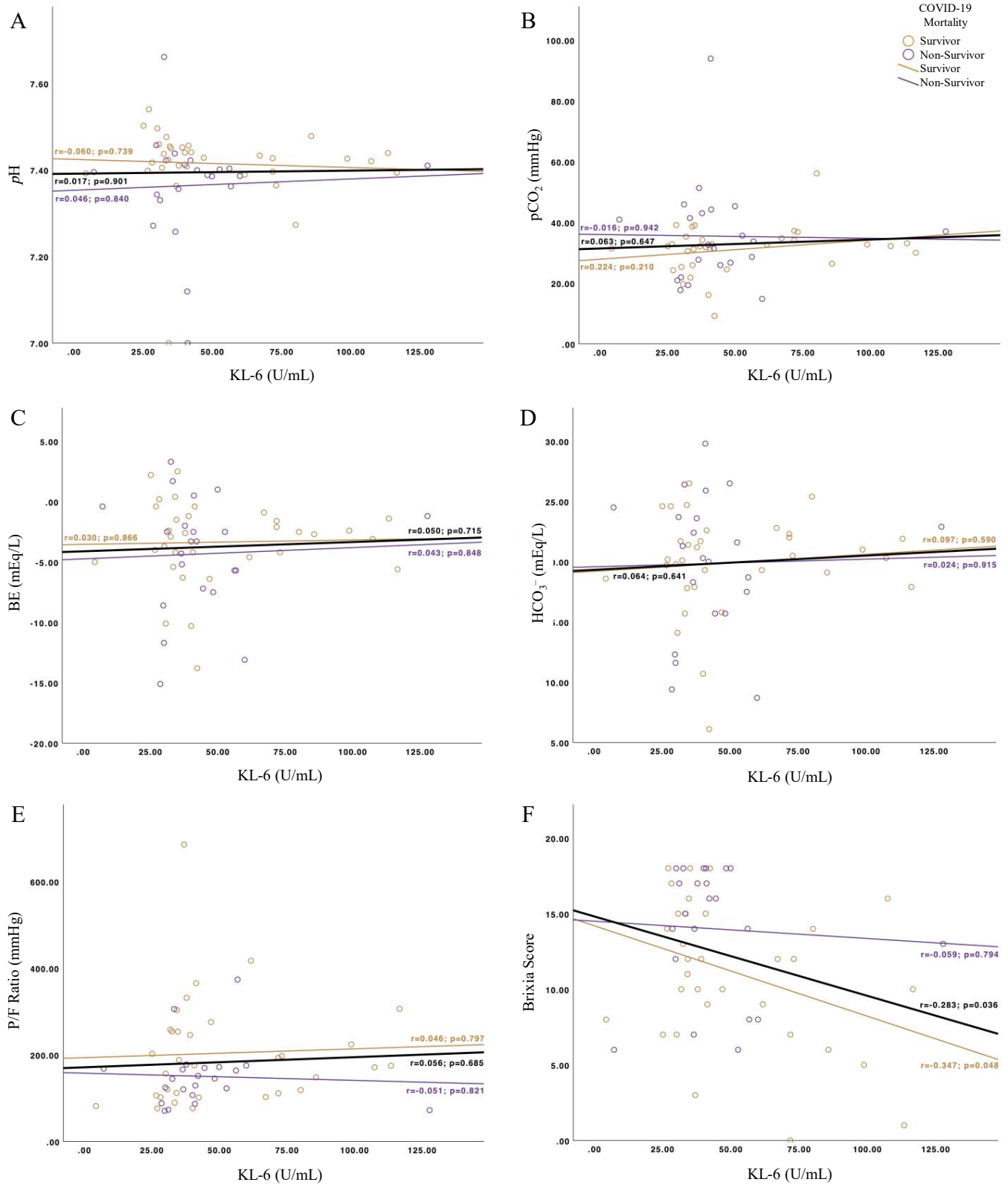
ROC analyses to assess the diagnostic value of KL-6, P/F ratio and Brixia score in COVID-19 severity and mortality are shown in Figure 3 and Table III. In this study, the AUCs of P/F ratio were 0.764 (0.614–0.915) in the severe group and 0.64 (0.491–0.79) in non-survivors. Even though KL-6 had a low AUC in determining COVID-19 severity (0.376 [0.212–0.541]) and mortality (0.475 [0.320–0.630]), KL-6 might still be beneficial to improve the AUC of P/F ratio. The AUC increase was found when adding KL-6 to the P/F ratio analysis, either in severe participants (0.764–0.8) or in non-survivors (0.640–0.652). Interestingly, adding KL-6 to P/F ratio showed an increase in specificity in the ‘severe’ group from 0.35 to 0.75 while also increasing its

sensitivity (0.886–0.971), but not in non-survivors. Furthermore, the Brixia score had the highest AUC in the severe group [0.801 (0.685–0.917)] and non-survivors [0.671 (0.522–0.82)], with both having a similar optimal cut-off ( $\geq 12.5$ ). We analysed the AUC of the Brixia score using our pre-defined cut-off ( $\geq 7$ ) according to the previously stated classification in the methods section and Table III. The AUC increase of Brixia score was only observed in the severity analysis, but not in the mortality. Of those analyses, KL-6 and Brixia score together served as the best severity markers in this study (AUC 0.809 [0.697–0.920]; Sn/Sp=0.686/0.900), followed by KL-6 and P/F ratio (AUC 0.800 [0.637–0.963]; Sn/Sp=0.971/0.75).

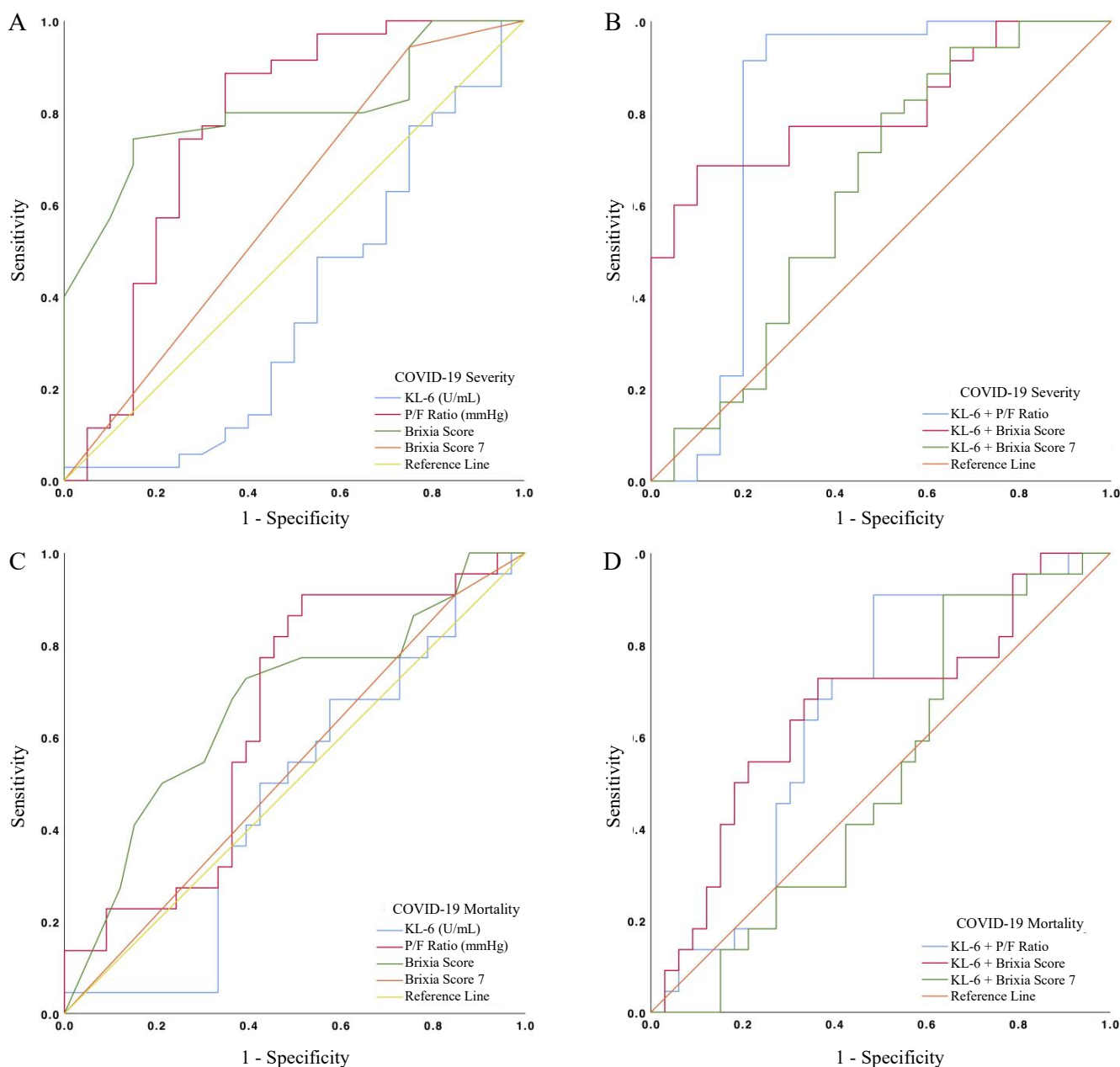




**Fig. 1.** Correlation strengths between KL-6 and several COVID-19 severity parameters of BGA and CXR. (A) pH, (B)  $pCO_2$ , (C) BE, (D)  $HCO_3^-$ , (E) P/F ratio and (F) Brixia score. BE, base excess; BGA, blood gas analysis; COVID-19, Coronavirus disease 2019; CXR, chest X-ray; KL-6, Krebs von den Lungen-6; P/F ratio,  $PaO_2/FiO_2$  ratio;  $pCO_2$ , partial carbon dioxide pressure.



**Fig. 2.** Correlation strengths between KL-6 and several COVID-19 mortality parameters of BGA and CXR. (A) pH, (B) pCO<sub>2</sub>, (C) BE, (D) HCO<sub>3</sub><sup>-</sup>, (E) P/F ratio and (F) Brixia score.



**Fig. 3.** ROC curve of KL-6, P/F ratio, Brixia score and their combinations in COVID-19 severity (A, B) and mortality (C, D). ROC, receiver operating characteristic.

**Discussion**

So far, the authors believe that this is the first study from Indonesia to report the significance of KL-6 and its correlation with BGA and Brixia score and their ROC analyses in COVID-19 severity and mortality. Although no significant difference in KL-6 was found in severe *vs.* non-severe participants or non-survivors *vs.* survivors. However, in the multivariate analysis, we found that RR and Brixia scores were significantly higher in the severe patients compared

to the non-severe patients. In the KL-6-BGA analysis, a positive correlation was found between KL-6 and  $\text{HCO}_3^-$  in the severe group and KL-6 and  $\text{pCO}_2$  in the survivor group. KL-6 expression and Brixia scores were inversely correlated in all the samples. Despite its non-significant difference and its low AUC in both severity and mortality, KL-6 was found to increase the P/F ratio diagnostic value in determining COVID-19 severity or mortality. KL-6 and Brixia score only had a noticeable increase of diagnostic value in COVID-19



**Table III.** The performance of serum KL-6 levels determines the severity and predicts the mortality of COVID-19 individuals

Parameters	AUC (95% CI)	P-value	Optimal cut-off	Sensitivity	Specificity	Youden's Index
COVID-19 severity (severe)						
KL-6	0.376 (0.212–0.541)	0.13	≥5.732	1.000	0.050	0.050
P/F ratio	0.764 (0.614–0.915)	0.001	≤190.515	0.886	0.350	0.536
Brixia score	0.801 (0.685–0.917)	<0.001	≥12.500	0.743	0.850	0.593
Brixia score ≥7	0.596 (0.434–0.758)	0.238	N/A	0.943	0.250	0.193
KL-6+P/F ratio	0.800 (0.637–0.963)	<0.001	N/A	0.971	0.750	0.721
KL-6+Brixia score	0.809 (0.697–0.920)	<0.001	N/A	0.686	0.900	0.586
KL-6+Brixia score ≥7	0.630 (0.463–0.797)	0.111	N/A	0.800	0.500	0.300
COVID-19 mortality (non-survivors)						
KL-6	0.475 (0.320–0.630)	0.757	≥28.388	0.955	0.152	0.106
			≥35.681	0.682	0.424	0.106
P/F ratio	0.640 (0.491–0.79)	0.080	≤182.850	0.909	0.515	0.394
Brixia score	0.671 (0.522–0.82)	0.033	≥12.500	0.727	0.606	0.333
Brixia score ≥7	0.530 (0.375–0.686)	0.705	N/A	0.909	0.152	0.061
KL-6+P/F ratio	0.652 (0.504–0.799)	0.059	N/A	0.909	0.515	0.424
KL-6+Brixia score	0.663 (0.512–0.813)	0.043	N/A	0.727	0.636	0.496
KL-6+Brixia score ≥7	0.51 (0.356–0.663)	0.904	N/A	0.909	0.364	0.273

AUC, area under curve

severity, but not in mortality. These findings suggested that KL-6 might still be used in reflecting the extent of COVID-19 severity and mortality.

This study found that KL-6 expression were not significantly different, between the COVID-19 severity and mortality groups. Moreover, in this study KL-6 expression was lower than in the previous studies<sup>17,18</sup>. Several reasons might explain both results. The probable explanation was the different reagents used. We conducted the serum KL-6 investigation using a similar ELISA reagent kit as used by Suryananda and Yudhawati<sup>5</sup>. KL-6 levels were similar, ~40–50 U/ml. Two studies by Awano *et al*<sup>17</sup> and Xue *et al*<sup>18</sup> were observed using two different reagents, resulting in a higher serum KL-6, ~200–600 U/ml.

Several previous studies<sup>19,20</sup> found that KL-6 might also be related to the individual's ethnicity and genotype. Horimasu *et al*<sup>19</sup> found that KL-6 was higher in the German population than in the Japanese. Furthermore, cases of individuals with ILD serum KL-6 was higher among those with A/G and G/G genotypes compared to the A/A genotype. The allele variation also influenced this difference in some individuals<sup>19</sup>. Those with the *MUC1* rs4072037 CC genotype might have higher KL-6 due to the disequilibrium abundance of tandem repeats in *MUC1*<sup>19,20</sup>. Thus, we might assume that the survivors and non-severe participants

probably have this single nucleotide polymorphism, which leads to a higher serum KL-6, even in healthy conditions. Furthermore, Suryananda and Yudhawati<sup>5</sup> stated similar results as ours, indicating that Indonesian people certainly had a low serum KL-6.

Strict COVID-19 management might reduce lung injury sequelae and be related to low KL-6. It might also be used as a successful indicator of aggressive interventions<sup>21</sup>. Gender differences might also affect KL-6 results. Even though sex was not significantly different in our study, 55 per cent of males had non-severe COVID-19, resulting in a higher level of KL-6 expression. This was similar to the study by Suryananda and Yudhawati<sup>5</sup>, with 61.3 per cent of the participants being males. As mentioned earlier, KL-6 levels might change dynamically due to the affected lung area combined with the possibility of a genetic polymorphism<sup>18,20</sup>. In this study, the onset of the disease, the time of blood samples taken, the severity during admission and the disease progression rates might have lead to an earlier treatment; due to which serum KL-6 levels were lower than in other studies.

This study showed that KL-6 was not significantly correlated with the BGA. This might be caused by the aggressive COVID-19 treatment, which affected KL-6 and BGA<sup>21</sup>. Another study suggested that KL-6 levels tended to remain unchanged during mild lung

injury compared to other biomarkers, indicating that KL-6 only increased when there was severe alveolar damage and an increase in alveolar permeability; thus, might create a discrepancy between KL-6 and BGA<sup>22</sup>. Nevertheless, this study demonstrated a strong correlation between KL-6 and  $\text{HCO}_3^-$ . The  $\text{HCO}_3^-$  levels remained unchanged in severe conditions for 90 days. Interestingly, persistent KL-6 levels in the severe participants could be observed until 56 days. This might explain our finding related to KL-6 and  $\text{HCO}_3^-$ <sup>18,23</sup>. We also found a significant negative correlation between KL-6 and Brixia score. However, to date, no studies have discussed KL-6 and Brixia scores. This finding might be affected by the contradictory low serum KL-6 and high Brixia score in our study population.

Our results contradicted the previous studies conducted in East Asia and Europe, where KL-6 was shown to have a high diagnostic value in evaluating COVID-19 severity (AUC 0.824–0.850) and mortality (AUC 0.849)<sup>1,4,17,24,25</sup>. Their optimal cut-off values varied from 278.3 to 406.5 U/ml, while the cut-offs in this study ranged from 5.7 to 35.7 U/ml. These values were expected due to our study's lower mean of KL-6 as compared to other studies. Again, we speculated that the differences in reagent kits, population characteristics and racial ethnicities might cause the discrepancy in the KL-6 diagnostic performance for COVID-19 severity and mortality. Thus, more extensive studies involving a specific type of population are needed to test this hypothesis and confirm our findings.

Unlike KL-6, our ROC analysis on the P/F ratio and Brixia score aligned with earlier research. Gu *et al*<sup>26</sup> found that the P/F ratio had a high performance in predicting COVID-19 mortality in the intensive care ward, while Sinatti *et al*<sup>27</sup> revealed that the P/F ratio was reliable for identifying severe COVID-19 individuals with oxygen support. For predicting COVID-19 in-hospital mortality, Gatti *et al*<sup>28</sup> found that the Brixia score had an AUC of 0.81. However, combining KL-6 did not seem to benefit its diagnostic value, neither using our optimal cut-off nor the pre-defined cut-off. This was proven by our significant multivariate analysis, which showed that the Brixia score is an independent factor in determining COVID-19 severity. The most apparent AUC increase of the P/F ratio was observed when incorporating KL-6 into the severity analysis. Nevertheless, we encourage more research evaluating the KL-6 combination with P/F ratio and Brixia score, especially in East Asia and Europe, given that KL-6 in these populations was shown to have a high diagnostic value for COVID-19.

This study had several limitations. First, a single-centred cross-sectional study with limited participants, might affect the statistical analysis. Second, some missing values <5 per cent on several variables were replaced using multiple imputations. This still could not eliminate or even reduce the issue around the small sample size. We also did not achieve this method for variables with missing values  $\geq 5$  per cent to avoid further bias in the analysis. Third, there was a difference in the time interval between the hospital admission, the blood sampling and the time of CXR taken for each participant. At this interval, the participants received different therapeutic approaches with extra length of time to maintain their best clinical conditions. This might have affected the KL-6 levels, BGA results and Brixia scores.

In conclusion, KL-6 has the potential to be an adjunct laboratory parameter to BGA and Brixia scores representing COVID-19 severity and mortality. KL-6 and P/F ratio could be used as promising diagnostic tools since their combination yielded high sensitivity and specificity, either in COVID-19 severity or mortality analyses. KL-6 also had the strongest correlation with  $\text{HCO}_3^-$ , which might both be maintained at a certain level to depict COVID-19 lung injury. Furthermore, even though combining KL-6 did not seem to benefit the diagnostic value of the Brixia score, it still served as the best COVID-19 severity marker in this study. Future studies involving a larger population are needed to confirm the relationship between KL-6, BGA and Brixia scores. We also suggest that more future research is needed to analyze the comparison between different reagent kits of KL-6 since they may affect the values of KL-6.

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**Supplementary Table I.** Other clinical parameters of COVID-19 individuals based on the severity and mortality groups.

Parameters	All patients (n=55)	Severe (n=35) (Severe: n=17; Critical: n=18)	Non-severe (n=20) (Mild: n=2; Moderate: n=18)	p-value	Non-survivor (n=22) (Non-severe: n=3; Severe: n=19)	Survivor (n=33) (Non-severe: n=17; Severe: n=16)	p-value
Length of stay (days)	12.3±7.32 11.0 (8.0, 17.0)	12.8±8.3 11.0 (8.0, 17.0)	11.5±5.3 11.0 (8.3, 12.8)	0.667 <sup>a</sup>	10.3±7.5 8.5 (5.3, 16.3)	13.7±7.0 11.0 (10.0, 17.0)	0.037 <sup>a</sup>
Respiratory rate (breaths/min)	28.2±5.5 28.0 (24.0, 31.0)	30.5±5.1 30.0 (28.0, 33.0)	24.2±3.5 24.0 (22.0, 24.0)	<0.001 <sup>a</sup>	30.7±5.7 30.0 (28.0, 34.0)	26.6±4.8 24.0 (24.0, 30.0)	0.005 <sup>a</sup>
Comorbidities, n(%)							
Diabetes mellitus	10 (18.2)	8 (22.9)	2 (10.0)	0.297 <sup>b</sup>	7 (31.8)	3 (9.1)	0.070 <sup>b</sup>
Hypertension	10 (18.2)	8 (22.9)	2 (10.0)	0.297 <sup>b</sup>	5 (22.7)	5 (15.2)	0.498 <sup>b</sup>
Chronic kidney disease	3 (5.5)	2 (5.7)	1 (5.0)	1.000 <sup>b</sup>	2 (9.1)	1 (3.0)	0.557 <sup>b</sup>
Cerebrovascular accident	1 (1.8)	0 (0.00)	1 (5.0)	1.000 <sup>b</sup>	1 (4.6)	0 (0.0)	0.400 <sup>b</sup>
CCI, n(%)							
Score 0–1	35 (63.6)	22 (62.9)	13 (65.0)	0.983 <sup>a</sup>	12 (54.55)	23 (69.70)	0.218 <sup>a</sup>
Score 2–3	19 (34.6)	13 (37.1)	6 (30.0)		9 (40.91)	10 (30.30)	
Score ≥4	1 (1.8)	0 (0.0)	1 (5.0)		1 (4.55)	0 (0.00)	
Complete blood count							
Haemoglobin (g/ dl)	13.7±1.9 13.5 (12.3, 15.1)	13.4±1.8 13.3 (12.3, 14.2)	14.2±2.0 14.1 (12.5, 15.9)	0.123 <sup>c</sup>	13.9±2.2 13.4 (12.4, 15.6)	13.5±1.7 13.6 (12.2, 15.0)	0.488 <sup>a</sup>
Red blood cell (10 <sup>6</sup> /ml)	4.8±0.7 4.7 (4.3, 5.3)	4.80±0.7 4.6 (4.2, 5.3)	4.8±0.7 4.8 (4.4, 5.3)	0.947 <sup>c</sup>	5.0±0.7 5.1 (4.6, 5.5)	4.6±0.7 4.6 (4.2, 5.1)	0.030 <sup>a</sup>
Haematocrit (%)	39.7±4.9 39.4 (35.7, 43.1)	39.1±5.0 38.7 (34.5, 42.2)	40.8±4.8 41.2 (36.4, 44.4)	0.231 <sup>c</sup>	40.6±5.5 39.5 (36.9, 43.7)	39.1±4.5 39.4 (35.2, 42.8)	0.283 <sup>a</sup>
Platelet (10 <sup>3</sup> /ml)	265.8±113.2 254.0 (183.0, 331.0)	291.3±124.9 286.0 (191.0, 381.0)	221.1±72.1 217.0 (159.3, 262.0)	0.011 <sup>c</sup>	246.5±102.6 198.0 (178.0, 332.5)	278.7±119.5 256.0 (184.0, 348.0)	0.497 <sup>a</sup>
White blood cell (10 <sup>3</sup> /ml)	8.7±4.0 8.1 (6.0, 10.3)	9.3±3.4 9.3 (6.6, 10.6)	7.6±4.7 6.5 (5.5, 8.6)	0.139 <sup>c</sup>	9.8±5.1 10.0 (5.5, 11.2)	7.9±2.9 7.5 (6.0, 9.4)	0.084 <sup>c</sup>
Neutrophil count (10 <sup>3</sup> /ml)	6.9±3.6 6.7 (4.5, 8.4)	7.6±3.1 7.3 (5.1, 9.3)	5.8±4.2 4.9 (3.1, 7.1)	0.087 <sup>c</sup>	8.2±4.5 8.2 (3.9, 9.7)	6.1±2.6 5.4 (4.6, 7.4)	0.057 <sup>a</sup>
Lymphocyte count (10 <sup>3</sup> /ml)	1.5±2.9 1.0 (0.7, 1.6)	1.7±3.7 1.0 (0.6, 1.6)	1.2±0.6 1.2 (0.8, 1.6)	0.286 <sup>a</sup>	1.1±0.5 1.0 (0.7, 1.6)	1.8±3.8 1.0 (0.8, 1.6)	0.514 <sup>a</sup>
Eosinophil count (10 <sup>3</sup> /ml)	0.01±0.03 0.00 (0.00, 0.01)	0.02±0.03 0.00 (0.00, 0.01)	0.01±0.02 0.00 (0.00, 0.01)	0.465 <sup>a</sup>	0.01±0.02 0.00 (0.00, 0.00)	0.01±0.03 0.00 (0.00, 0.01)	0.460 <sup>a</sup>
Basophil count (10 <sup>3</sup> /mL)	0.02±0.01 0.02 (0.01, 0.03)	0.02±0.01 0.02 (0.01, 0.03)	0.02±0.01 0.02 (0.01, 0.03)	0.489 <sup>a</sup>	0.02±0.01 0.02 (0.01, 0.02)	0.02±0.01 0.02 (0.01, 0.03)	0.460 <sup>a</sup>
Monocyte count (10 <sup>3</sup> /ml)	0.5±0.3 0.5 (0.3, 0.7)	0.5±0.3 0.5 (0.3, 0.7)	0.5±0.4 0.4 (0.3, 0.6)	0.462 <sup>a</sup>	0.5±0.4 0.5 (0.3, 0.6)	0.5±0.3 0.5 (0.3, 0.7)	0.966 <sup>c</sup>
NLR	7.9±6.5 6.2 (4.0, 9.7)	9.1±7.4 7.9 (4.4, 10.7)	5.7±4.0 4.7 (2.1, 8.9)	0.050 <sup>a</sup>	9.5±7.7 8.0 (4.4, 10.2)	6.8±5.5 5.0 (2.7, 9.7)	0.112 <sup>a</sup>
PLR	299.8±234.4 221.4 (150.0, 362.6)	344.2±268.6 244.1 (165.8, 448.6)	221.9±130.6 184.0 (127.0, 325.1)	0.104 <sup>a</sup>	284.0±190.6 196.6 (144.7, 376.5)	310.3±262.0 223.9 (154.1, 349.1)	0.959 <sup>a</sup>

Contd...

Parameters	All patients (n=55)	Severe (n=35) (Severe: n=17; Critical: n=18)	Non-severe (n=20) (Mild: n=2; Moderate: n=18)	p-value	Non-survivor (n=22) (Non-severe: n=3; Severe: n=19)	Survivor (n=33) (Non-severe: n=17; Severe: n=16)	p-value
<b>Liver function test</b>							
SGOT (U/L)	73.3±66.7 57.0 (37.0, 83.0)	80.9±76.1 64.0 (38.0, 88.0)	60.2±44.8 55.5 (37.0, 68.0)	0.198 <sup>a</sup>	93.7±88.0 72.5 (51.0, 103.5)	59.8±44.2 48.0 (35.0, 70.5)	0.025 <sup>a</sup>
SGPT (U/L)	61.3±93.9 40.0 (27.0, 63.0)	56.8±41.8 44.0 (27.0, 73.0)	69.1±147.8 33.5 (24.0, 47.3)	0.132 <sup>a</sup>	54.3±37.6 46.0 (24.5, 79.3)	65.9±117.9 38.0 (27.5, 56.5)	0.514 <sup>a</sup>
<b>Renal function test</b>							
BUN (mg/dl)	20.4±17.9 14.6 (11.8, 22.8)	22.1±17.7 15.7 (11.8, 24.7)	17.4±18.3 13.5 (11.5, 15.5)	0.090 <sup>a</sup>	29.4±25.3 20.1 (12.9, 41.9)	14.4±5.3 13.7 (11.5, 15.8)	0.019 <sup>a</sup>
Creatinine (mg/ dl)	1.4±1.92 1.0 (0.72, 1.25)	1.41±2.31 0.85 (0.66, 1.29)	1.25±0.96 1.03 (0.85, 1.21)	0.358 <sup>a</sup>	1.88±2.95 1.05 (0.65, 2.05)	0.99±0.40 0.93 (0.73, 1.20)	0.577 <sup>a</sup>
<b>Electrolyte</b>							
Natrium (mmol/L)	134.5±6.1 135.0 (132.0, 138.0)	134.7±7.0 135.0 (132.0, 139.0)	134.3±4.2 134.5 (131.3, 137.0)	0.875 <sup>c</sup>	135.6±4.4 136.0 (132.5, 139.3)	133.7±7.0 135.0 (132.0, 137.5)	0.270 <sup>c</sup>
Potassium (mmol/L)	4.1±0.6 4.0 (3.7, 4.5)	4.1±0.6 4.0 (3.7, 4.5)	4.1±0.5 4.2 (3.7, 4.4)	0.685 <sup>c</sup>	4.4±0.6 4.4 (4.0, 4.8)	3.9±0.4 3.8 (3.7, 4.3)	0.001 <sup>c</sup>
Chloride (mmol/L)	103.6±5.2 104.0 (102.0, 106.0)	103.7±5.5 104.0 (102.0, 106.0)	103.5±4.7 104.0 (100.0, 107.0)	0.915 <sup>c</sup>	104.6±3.0 104.5 (102.0, 106.3)	103.0±6.2 104.0 (101.0, 106.5)	0.273 <sup>c</sup>
Anion gap (mmol/L)	15.1±5.5 14.2 (11.7, 18.9)	14.4±6.1 14.0 (8.5, 19.3)	16.3±4.2 15.3 (13.8, 17.5)	0.236 <sup>c</sup>	15.6±5.9 15.3 (11.2, 19.9)	14.7±5.3 14.0 (11.5, 17.5)	0.587 <sup>c</sup>
D-dimer (mcg/ml)	2.8±4.9 1.2 (0.7, 1.9)	3.9±5.9 1.3 (1.0, 4.1)	0.9±0.6 0.7 (0.5, 1.3)	0.002 <sup>a</sup>	3.8±4.4 1.6 (1.1, 5.2)	2.2±5.2 1.0 (0.6, 1.4)	0.009 <sup>a</sup>
Ferritin (ng/ml) (N/A=18)	1481.7±1220.7 1043.0 (714.4, 2090.5)	1541.3±1243.3 1043.0 (823.4, 2173.5)	1357.5±1216.3 1129.0 (375.1, 2128.0)	0.581 <sup>a</sup>	1867.3±1417.8 1423.0 (874.5, 3004.5)	1153.9±940.5 1001.0 (393.4, 1398.5)	0.113 <sup>a</sup>
Interleukin-6 (pg/ ml) (N/A=13)	408.9±1694.1 48.9 (13.5, 168.0)	191.8±359.4 65.2 (12.8, 215.3)	761.7±273.1 25.5 (13.2, 114.1)	0.816 <sup>a</sup>	917.1±2622.2 126.6 (59.9, 341.21)	63.3±108.3 18.8 (11.0, 65.2)	0.003 <sup>a</sup>
<b>Procalcitonin, ng/ml, n (%)</b>							
<0.05	16 (29.1)	9 (25.7)	7 (35.0)	0.322 <sup>a</sup>	6 (27.3)	10 (30.3)	0.130 <sup>a</sup>
0.05–<0.5	30 (54.6)	19 (54.3)	11 (55.0)		9 (40.9)	21 (63.6)	
0.5–<2	5 (9.1)	4 (11.4)	1 (5.0)		3 (13.6)	2 (6.1)	
2–<10	1 (1.8)	1 (2.9)	0 (0.0)		1 (4.5)	0 (0.0)	
≥10	3 (5.5)	2 (5.7)	1 (5.0)		3 (13.6)	0 (0.0)	
C-Reactive protein (mg/L)	103.9±86.0 77.9 (43.6, 169.1)	112.8±88.5 84.4 (46.6, 178.9)	88.3±81.3 67.5 (30.2, 96.7)	0.377 <sup>a</sup>	142.7±107.7 138.6 (45.3, 210.1)	78.0±56.1 65.4 (35.2, 108.2)	0.041 <sup>a</sup>

Continuous variables are shown as mean±SD and median (interquartile range [IQR]) The comparison between two groups, either severe vs. non-severe and non-survivor vs. survivor, was performed using (a) Mann–Whitney *U* test (b), Fisher's exact test (c), independent T-test, BUN, blood urea nitrogen; CCI, Charlson Comorbidity Index; N/A, not available; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; RBC, red blood cell; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; WBC, white blood cell.



**Supplementary Table II.** Details of missing data and multiple imputation status.

Variable	Missing, n(%)	Multiple imputation status
D-dimer	1 (1.8)	Imputed
Ferritin	18 (32.7)	Not imputed
Interleukin-6	13 (23.6)	Not imputed
Procalcitonin	1 (1.8)	Imputed
C-reactive protein	1 (1.8)	Imputed