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Coronavirus Pandemic

Study of biomarkers to determine severity and lung damages in COVID-19 patients

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Abstract

Introduction: Identifying inflammation and lung damage markers is crucial in reducing morbidity and mortality of coronavirus disease 2019 (COVID-19). This study aimed to examine the validity and reliability of severity and post-infection lung damage and analyse their relationship.
Methodology: This was a prospective analysis study at the Airlangga University Hospital, Surabaya, Indonesia, from March to August 2021. The infection's severity was measured by examining angiotensin-converting enzyme 2 (ACE2) levels and complete blood count. Lung damage was estimated by reviewing Krebs von de Lungen (KL)-6, matrix metalloproteinase (MMP)-9, tissue inhibitor metalloproteinase (TIMP)-1, and MMP-9/TIMP-1. Two-factor confirmatory factor analysis (CFA) and canonical correlation were calculated using Lisrel and SPSS (version 25).
Results: The research sample included 76 patients. The t count loading factor values were calculated: ACE2 (6.00), neutrophils (-0.80), lymphocytes (-0.63), neutrophil-lymphocyte ratio (NLR, 1.27), eosinophils (-1.52), basophils (1.72), monocytes (0.05), platelets (0.53), leukocytes (-0.51), platelet-lymphocyte ratio (PLR, -1.15), KL-6 (10.47), MMP-9 (11.91), TIMP-1 (11.79), and MMP-9/TIMP-1 (-0.24). The t values were: neutrophil covariance error (6.11), lymphocytes (6.12), NLR (6.10), eosinophils (6.08), basophils (6.07), monocytes (6.12), platelets (6.12), leukocytes (6.12), PLR (6.10), ACE2 (0.97), KL-6 (5.63), MMP-9 (2.08), TIMP-1 (2.77), and MMP-9/TIMP-1 (6.12). t value canonical correlation of 7.04 (t count > 1.96) indicated a correlation between the severity of the patient and post-infection lung damage.
Conclusions: The severity was adequately measured through ACE2, IL-6, IL-10, neutrophils, lymphocytes, leukocytes, and NLR. Lung damage was measured with KL-6, MMP-9, and TIMP-1. There was a correlation between disease severity and lung damage.

Key words: COVID-19; infection; biomarkers; lung; severity; pathophysiology.

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Introduction

The coronavirus disease 2019 (COVID-19) pandemic had become a public health concern for almost two years since 2019. COVID-19 is a disorder of bodily functions, specifically attacking the lungs, and is caused by infection with the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. COVID-19 spreads on a massive scale, significantly impacting global life; and infection with SARS-CoV-2 results in increased morbidity and mortality. In the absence of adequate control measures, the high transmission rate, can worsen the quality of global health [2,3].

According to the World Health Organization (WHO) records [4], COVID-19 had two transmission waves until mid-2021 in South Africa. The first wave occurred in mid-2020, and the second wave in mid-2021 [5]. By early August 2021, there were 4.4 million confirmed cases of COVID-19 and more than 68,000 deaths worldwide. Although most cases of COVID-19 were in the mild category, 14% of the cases progressed

to severe COVID-19, and 5% required intensive care unit hospitalization, resulting in more than 50% of hospital patient deaths [6,7].

Various efforts have been made to reduce morbidity and mortality after SARS-CoV-2 infection. The identification of patient laboratory parameters at an early stage of diagnosis is critical to prevent an increase in severity of the COVID-19 symptoms and to anticipate post-infection lung damage [7]. Post-infection severity is often associated with angiotensin converting enzyme (ACE)-2 activity. Excessive ACE2 activity leads to increase in the patient's viral load because ACE2 acts as a gateway for virus entry into the body through interaction with viral spike proteins [8]. Complete blood count is an important indicator to assess the severity of the disease in the patient [9].

The assessment of post-infection lung damage can be made through the levels of Krebs von de Lungen (KL)-6, matrix metalloproteinase (MMP)-9, and tissue inhibitor metalloproteinase (TIMP)-1. KL-6 is a serum fibroblast chemoattractant and plays a role in pulmonary fibrosis [10]. Serum KL-6 can predict the severity of lung injury following COVID-19 [9]. Pulmonary fibrosis is a pathological consequence of acute and chronic interstitial lung disease characterized by collagen deposition and extracellular matrix (ECM) components [10]. The imbalance between TIMP-1 and MMP-9 can increase the risk of pulmonary fibrosis and impair alveolar function [11]. Other studies report that MMP-9 and TIMP-1 can be used as biomarkers of severity and mortality due to sepsis [12].

Identifying post-infection markers of inflammation and pulmonary abnormalities is essential to reduce the risk of patient mortality due to SARS-CoV-2 infection. Previous reports have demonstrated the importance of various parameters for determining the severity of COVID-19 patients and associated lung disorders. However, measurement of all the previously reported indicators will result in high treatment costs. Therefore, this study examines the validity and reliability of ACE2, neutrophils, lymphocytes, neutrophil-lymphocyte ratio (NLR), eosinophils, basophils, monocytes, platelets, leukocytes, and platelet-lymphocyte ratio (PLR) as indicators in determining patient severity. KL-6, MMP-9, TIMP-1, and the ratio of MMP-9 to TIMP-1 are indicators for measuring post-infection lung damage, and may be used for analyzing the relationship between disease severity and post-infection lung damage.

Methodology

Research design

This research was a prospective analysis study. The research population included COVID-19 patients who were hospitalized at the Airlangga University Hospital from March to August 2021 and followed until discharge. Informed consent was obtained from all the patients included in this study. The study complies with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects, 2013). This research was approved by the Ethics Committee of the Universitas Airlangga University (approval number 121/KEP/2021).

The inclusion criteria were patients aged 21–70 years, whose complete medical data for measurement indicators was available. The exclusion criteria were a history of tuberculosis, interstitial lung disease, asthma, chronic obstructive pulmonary diseases (COPD), human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), and pregnancy (in women).

The research sample was a random selection from the population who were selected based on the inclusion and exclusion criteria. The sample size was calculated according to the analytic correlation formula below [13]:

$$n = \frac{(Z\alpha + Z\beta)}{0.5 \ln ((1 + r)/(1 - r))}$$

Where: n = sample size; α = probability of type I error = 5%; β = probability of type II error = 10%; Zα = standard value alpha = 1.64; Zβ = standard value beta = 1.28; r = correlation coefficient determined by researcher = 0.3.

Based on this formula, the minimal sample size needed for the study was 68. The research sample in this study included 76 patients.

Biomarkers and lung damages

The infection's severity was measured by examining ACE2 levels and complete blood count, including neutrophils, lymphocytes, NLR, eosinophils, basophils, monocytes, platelets, leukocytes, and PLR. NLR was caculated by dividing the relative neutrophil percentage by the relative lymphocyte percentage from differential count measures in the complete blood count. PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count.

The complete blood count was measured by the Sysmex XN55 Automatic Hematology Analyzer (Sysmex Coorporation, Kobe, Japan). We also included IL-6, IL-10, and a ratio of IL-6 to IL-10 as severity biomarkers. Lung damage was measured by examining

the KL-6, MMP-9, and TIMP-1 levels, and the ratio of MMP-9 to TIMP-1. ACE2, IL-6, IL-10, KL-6, MMP-9, and TIMP-1 were measured from patients' blood using an enzyme-linked immunosorbent assay (ELISA) reader (iMarkTM Microplate Absorbance Reader, Bio-Rad Laboratories Inc. Hercules, CA, USA) with a wavelength of 450 nm. Blood from the peripheral veins was taken once within 24 hours of admission to the emergency. The blood parameters used were ACE2, IL-6, IL-10, KL-6, MMP-9, and TIMP-1 (Elabscience Biotechnology Inc, Wuhan, Hubei, China). This research followed standard Elabscience protocols [14].

Severity and mortality

The severity of respondents' symptoms was assessed at the time of emergency admission. The patients were divided into four severity categories following the COVID-19 guidelines in Indonesia: (1) mild cases included patients exhibiting signs or symptoms of COVID-19 without evidence of pneumonia or hypoxia; (2) moderate cases included patients with pneumonia confirmed through clinical assessment or imaging, with a blood oxygen saturation level (SpO_2) of ≥ 93% while breathing room air at sea level; (3) severe cases were characterized by severe pneumonia, indicated by a respiratory rate (RR) exceeding 30 breaths per minute, significant respiratory distress, or SpO_2 < 93% while breathing room air at sea level; (4) critical cases included patients experiencing acute respiratory distress syndrome (ARDS), sepsis, and/or septic shock [15]. To simplify the severity classification, we grouped non-severe cases (mild and moderate) separately from severe cases (severe and critical).

This research was prospective; so the participants were followed until discharged from the hospital. Duration of care was measured in length of stay (days). Mortality was assessed when the participants were discharged from the hospital. We divided mortality groups into survivors and non-survivors.

Data analysis

This study focused on two aspects: severity of the COVID-19 infection, and post-infection lung damage, with measurement indicators for each phase. Data was analyzed to test the validity and reliability of each variable in measuring the severity of infection and lung damage in COVID-19 patients. In addition, the relationship between the two aspects—severity of COVID-19 and post-infection lung damage—was also assessed. Statistical analysis included two-factor confirmatory factor analysis (CFA) and canonical correlation, calculated using Lisrel [16].

The t-count value in the analyses was compared with the t-table value for 5% (1.96). An indicator was considered to be a valid measure of the phase (severe or lung damage) if the t-count loading factor value was greater than the t-table (t-value loading factor > 1.96). In addition, an indicator was considered to be a reliable measure of the phase (severity or lung damage) if the t-count error covariance value was greater than the t-table (t-value error covariance > 1.96).

SPSS version 25 (IBM Corp, Armonk, NY, USA) was used for statistical analyses. The data was displayed in tables and figures. Kolmogorov Smirnov was used to analyze the normality of data. *p* value of > 0.05 was considered as normal distribution. The groups' severity and mortality were analyzed using the Mann-Whitney U test if the distribution was abnormal, or independent t-test if the distribution was normal. The area under the curve (AUC) of significant variable(s) was used to predict the severity and lung damage.

Table 1. Patient characteristics.

Characteristics	n = 76	(%)
Gender		
Male	40	52.6
Female	36	47.4
Age (years)	**50.83 ± 12.18**	
20–39 years old	14	18.4%
40–59 years old	15	19.7%
60–70 years old	47	61.8%
Symptoms		
Cough	57	75
Fever	46	60.5
Dyspnea	38	50
Nausea	22	28.9
Heartburn	13	17.1
Vomiting	13	17.1
Anosmia	12	15.8
Rainy Nose	12	15.8
Diarrhea	4	5.3
Sore throat	2	2.6
Comorbidities		
Diabetes mellitus	16	21
Hypertension	15	19.8
Ischemia heart disease	3	4
Stroke	2	2.6
Chronic kidney disease	2	2.6
Severity		
Non-severe	38	50
Severe	38	50
Mortality		
Non-survivor	23	30.3
Survivor	53	69.7
Length of stay (days)	11.82 ± 8.028	

Results

The average age of the patients included in this study was 50.83 ± 12.18 years, and majority (52.6%) were male. Most respondents were 60–70 years old (61.8%). The patients' symptoms were dominated by cough (75%), fever (60.5%), and dyspnea (50%) (Table

Table 2. Comparison of biomarkers and severity groups.

Biomarkers	Non-severe	Severe	*p*
ACE2 (ng/mL)	1.45 ± 0.76	1.23 ± 0.62	0.18
IL-6 (pg/mL)	76.71 ± 123.99	79.75 ± 89.85	0.91
IL-10 (pg/mL)	108.36 ± 61.99	115.30 ± 62.11	0.64
KL-6 (U/mL)	55.36 ± 30.94	46.07 ± 26.16	0.16
MMP-9 (ng/mL)	892.91 ± 830.10	1,682.90 ± 1.937.68	0.02*
TIMP-1 (ng/mL)	5.45 ± 4.54	9.29 ± 9.52	0.03*
Leucocyte (10^3/mL)	8.18 ± 3.53	8.81 ± 4.38	0.49
Neutrophil (%)	75.08 ± 10.11	76.11 ± 11.82	0.68
Lymphocyte (%)	17.55 ± 8.69	20.30 ± 33.18	0.62
NLR	6.34 ± 5.67	8.01 ± 6.78	0.25
PLR	22.59 ± 26.82	25.30 ± 22.75	0.63
Eosinophil (%)	0.35 ± 0.68	0.25 ± 0.58	0.49
Basophil (%)	0.32 ± 0.20	0.27 ± 0.18	0.30
Monocyte (%)	6.67 ±2.57	6.75 ± 3.02	0.90
D-dimer (mg/L)	1.97 ± 3.59	3.16 ± 5.35	0.26
Ferritin (ng/mL)	1,272.40 ± 1,284.64	1,283 ± 1,088.79	0.97
CRP (mg/L)	83.09 ± 78.38	101.58 ± 89.66	0.35
Procalcitonin (mcg/L)	11.00 ± 61.52	3.86 ± 15.09	0.49

* $p < 0.05$ significant, Mann-Whitney test. ACE2: angiotensin converting enzyme 2; CRP: C reactive protein; KL6: Krebs von de Lungen; MMP-9: matrix metalloproteinase; NLR: neutrophil to lymphocytes ratio; PLR: platelets to lymphocytes ratio: TIMP-1: tissue inhibitor metalloproteinase-1.

1). Diabetes mellitus (21%) and hypertension (19.8%) were the most common comorbidities and 30.3% patients died while hospitalized. The mean length of stay was 11.82 ± 8.02 days.

The levels of ACE2, IL-10, KL-6, MMP-9, TIMP-1, neutrophil, eosinophil, basophil, and procalcitonin were higher in the severe groups than in the non-severe groups (Table 2). IL-6, leucocyte, lymphocyte, NLR, PLR, D-dimer, and CRP were higher in the severe groups than in non-severe groups. MMP-9 was significantly higher in severe groups, and TIMP-1 was lower in non-severe groups. Table 3 lists the area under curve (AUC) for biomarkers used to predict severity and lung damage. Only MMP-9 significantly predicted lung damage (Figure 1). The biomarkers ACE2, IL-6, IL-10, neutrophil, NLR, PLR, KL-6, MMP-9, and TIMP-1 were higher in non-survivors than survivors; although this difference was not statistically significant (Table 4).

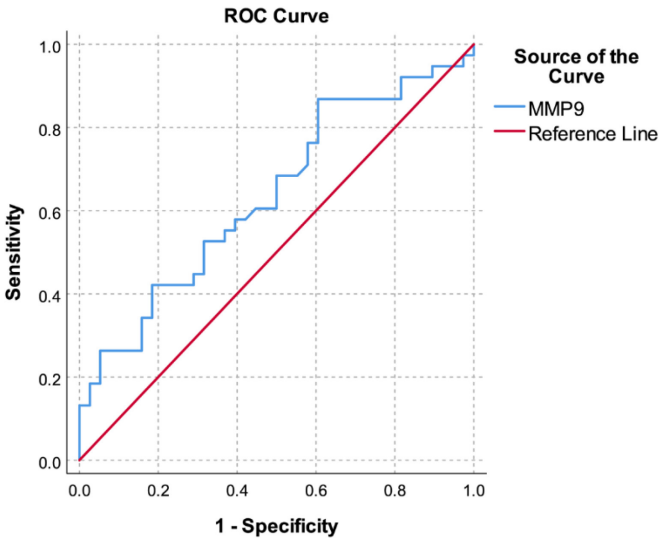
The laboratory results were grouped into severity indicators and lung damage indicators. The levels of ACE2, eosinophils, basophils, monocytes, lymphocytes, and hematocrit were decreased in severe patients. IL-6, IL-10, neutrophil, NLR, CRP, and procalcitonin were increased in severe patients. MMP-9, D-dimer, and ferritin were increased as lung damage indicators. KL-6 and TIMP-1 were decreased as lung damage indicators.

Table 3. Area under curve (AUC) of biomarker in predict severity and lung damage.

Biomarkers	AUC	*p* value
Predictions for Severity		
ACE2	0.415	0.205
IL-6	0.555	0.439
IL-10	0.541	0.553
IL-6/IL-10	0.492	0.913
Leucocyte	0.542	0.526
Neutrophil	0.544	0.513
NLR	0.581	0.224
PLR	0.537	0.575
Lymphocyte	0.581	0.226
Eosinophil	0.569	0.243
Basophil	0.574	0.258
Monocyte	0.541	0.831
Predictions for lung damage		
KL-6	0.583	0.213
MMP-9	0.636	0.041*
TIMP-1	0.612	0.097
MMP-9/TIMP-1	0.585	0.210

p significant < 0.05. ACE2: angiotensin converting enzyme 2; KL6: Krebs von de Lungen; MMP-9: matrix metalloproteinase; NLR: neutrophil to lymphocytes ratio; PLR: platelets to lymphocytes ratio: TIMP-1: tissue inhibitor metalloproteinase-1.

Figure 1. Receiver operating characteristic (ROC) analysis of MMP-9 to predict lung damage in COVID-19.



The area under curve (AUC) R2 = 0.636 with cut-off MMP-9 = 572 ng/mL (Sn = 0.684; Sp = 0.5). Data was analyzed with SPSS version 25.

Analysis of the indicators for measuring the severity of patients showed that the mean values and standard deviation were ACE2 1.34 ± 0.68 (ng/mL); lymphocytes 18.92 ± 24.13 (%); neutrophils 75.59 ± 10.94 (%); NLR 7.17 ± 6.26; eosinophils 0.30 ± 0.63 (%); basophil 0.29 ± 0.19 (%); monocytes 0.29 ± 0.20 (%); leucocyte 8,560 ± 3,930 cell/mL; platelets 259,000 ± 103,520 cells/mL; and PLR 23.95 ± 24.74. Analysis of the indicators for measuring post-infection lung damage in all patients showed that the mean and SD values were KL-6 49.92 ± 27.29 U/mL; MMP-9 1,179.41 ± 1,240.99 ng/mL; TIMP-1 7.20 ± 7.53 ng/mL, and the ratio of means of MMP-9 to TIMP-1 was 168.74 ± 44.59. Patient characteristics and the results for each measurement are summarized in Supplementary Table 1.

The indicator validity test was used to measure patients' severity and the results showed that of the 10 measurement indicators, only ACE2 can be used to measure validity of severity level. ACE2 levels were 6.00 (> 1.96), showing significant validity in measuring severity level. The other nine indicators values were not significantly valid to measure severity: i.e. neutrophils (-80 < 1.96), lymphocytes (-0.63 < 1.96), NLR (1.27 < 1.96), eosinophils (-1.52 < 1.96), basophils (1.72 < 1.96), monocytes (0.05 < 1.96), platelets (0.53 < 1.96), leukocytes (-0.51 < 1.96), and PLR (-1.15 < 1.96).

The results of the reliability test to measure the severity of the patient showed that of the 10 measurement indicators, 9 indicators were not reliable to measure the severity, including neutrophils (6.11 > 1.96), lymphocytes (6.12 > 1.96), NLR (6.10 > 1.96), eosinophils (6.08 > 1.96), basophils (6.07 > 1.96), monocytes (6.12 > 1.96), platelets (6.12 > 1.96), leukocytes (6.12 > 1.96), and PLR (6.10 > 1.96). In contrast, in the case of one indicator the validity was not reliable, namely ACE2 levels (0.97 < 1.96).

The results of the validity test to measure post-infection lung damage showed that of the 4 indicators, 3 indicators were valid to measure post-infection lung damage, including KL-6 (10.47 > 1.96), MMP-9 (11.91 >1.96), and TIMP-1 (11.79 > 1.96). In contrast, one indicator was invalid, namely MMP-9/TIMP-1 (-0.24 < 1.96). The results of the reliability test for measuring post-infection lung damage showed that all four indicators were reliable for measuring post-infection lung damage, including KL-6 (5.63 > 1.96), MMP-9 (2.08 > 1.96), TIMP-1 (2.77 >1.96), and MMP-9/TIMP-1 (6.12 > 1.96). The results of the validity and reliability of indicators on the severity of the patient and post-infection lung damage are presented in Supplementary Table 2.

The results showed that the t-value for the canonical correlation between the severity of the patient and post-infection lung damage was 7.04 > 1.96; therefore, there was a correlation between the severity of the patient and post-infection lung damage.

Discussion

The ACE2 receptor is a negative regulator of pulmonary fibrosis, and SARS-CoV infection reduces ACE2 expression. Therefore, SARS-CoV infection can cause pulmonary fibrosis through various signalling pathways, and TGF-b activation is one of the main contributors [17]. Other studies reported that ACE2 was downregulated during COVID-19 because the spike protein and ACE2 receptors bind on cell surfaces [18]. This study showed that ACE2, neutrophils, lymphocytes, NLR, eosinophils, basophils, monocytes, platelets, leukocytes, and PLR were valid and reliable

Table 4. Biomarkers of mortality.

Biomarkers	Survivor	Non-survivor	*p* value
ACE2	0.69 (0.35)	1.29 (0.25)	0.897
IL-6	81.95 (78.84)	85.65 (100.47)	0.049*
IL-10	53.33 (29.20)	102.26(9.84)	0.048*
Neutrophil	83.85 (9.26)	86.65 (3.32)	0.937
Leucocyte	9,975 (2,665)	8,210 (3,082)	0.937
Lymphocyte	11.40 (5.79)	9.65 (1.90)	0.572
Eosinophil	0.35 (0.49)	0.00 (0.00)	0.800
Basophil	0.35 (0.07)	0.02 (0.00)	0.178
Monocyte	4.05 (3.04)	3.5 (1.41)	0.870
NLR	8.56 (5.22)	9.19 (2.16)	0.583
PLR	38.61 (23.85)	44.85 (26.53)	0.888
KL-6	28.10 (12.42)	35.46 (3.17)	0.713
MMP-9	951.36 (954.52)	1,433.95 (1,712.47)	0.369
TIMP-1	5.81 (5.06)	8.00 (8.45)	0.555

p significant < 0.05, Mann-Whitney test was performed using SPSS. ACE2: angiotensin converting enzyme 2; KL6: Krebs von de Lungen; MMP-9: matrix metalloproteinase; NLR: neutrophil to lymphocytes ratio; PLR: platelets to lymphocytes ratio: TIMP-1: tissue inhibitor metalloproteinase-1.

measures of the severity of COVID-19 patients. A direct linkage between the decrease in ACE2 levels and the severity of the patient may cause this condition. ACE2 plays a role in facilitating SARS-CoV-2 entry into the lungs. A reduction of ACE2 in the lungs is associated with a high viral load. COVID-19 patients in the severe category were found to have a higher viral load when the nasopharynx was swabbed, than patients in the mild category [19]. Patients usually use regular corticosteroids or azithromycin for conditions like asthma, which can predominantly produce interferon (IFN), which helps combat SARS-CoV-2 [20]. This study concluded that ACE2 decreased in all severity groups. The reduction in ACE2 was lower in the severe group compared to the non-severe group, although it was not statistically significant (Table 2).

Higher NLR levels of more than 5.8 can predict mortality of COVID-19 hospitalized patients [21]. Visuddho *et al.* reported that NLR has an accuracy of 0.726 (95% CI 0.641–0.812) to predict worsening outcomes [22]. Increased eosinophils may be beneficial in COVID-19 patients [20]. Increased levels of leukocytes or neutrophils in COVID-19 patients can occur when accompanied by a secondary bacterial infection. The presence of bacterial infection can affect severity and mortality [23]. Other studies have found lymphopenia and eosinophilia in COVID-19 hospitalized patients [24].

PaO_2/FiO_2 and IL-6 have the potential to act as independent risk factors to predict mortality in COVID-19 patients requiring intensive care [25–27]. IL-6 and IL-10 are two cytokine markers that can be used as predictors of a higher risk of severe COVID-19, and, therefore, can be well managed to have a favorable prognosis [28]. In this study, however, IL-6 and IL-10 were higher in severe groups; although statistically nonsignificant (Table 2). We found that IL-6 and IL-10 were significantly ($p < 0.05$) higher in non-survivors compared to survivors (Table 4). These findings support that IL-6 and IL-10 can be used to determine the mortality due to COVID-19, but not the severity.

Interstitial pneumonia was the primary clinical manifestation of COVID-19. Interstitial pneumonia is characterized by edema and inflammatory cell infiltrates in the interstitial spaces (alveolar walls), which are more numerous than in the alveolar spaces. In more advanced stages of the disease, edema and infiltration begin to fill the alveolar spaces, first partially (depicted as ground glass opacity) and then totally (seen as consolidation). COVID-19 interstitial pneumonia has no specific manifestations, and patient determination as positive for COVID-19 cannot be based solely on chest imaging [29]. Chest x-rays have been established as the first diagnostic imaging option for COVID-19 severity assessment and disease monitoring in many healthcare facilities [30].

SARS-CoV-2 infection can cause lung damage. Post-infection lung damage impacts changes in the immune response in expressing pro-inflammatory and anti-inflammatory cytokines. Lung abnormalities of COVID-19 can be assessed using chest x-rays. This imaging modality can be used to determine disease severity [31]. SARS-CoV-2 infection disrupts the endothelial epithelial barrier and endothelial cells of the pulmonary capillaries, including pneumocytes. Pneumocystis destruction, particularly type II pneumocytes, increases KL-6 activity. KL-6 expression was used to identify lung damage. The KL-6 indicator determines pulmonary fibrosis, a lung disorder characterized by fibroblasts in the lungs [32,33]. Andro *et al.* reported that KL-6 has a sensitivity of 79% and a specificity of 86% to predict severe lung injury in COVID-19 [9]. We found that KL-6 decreased in all severity and mortality groups. Suryananda and Yudhawati also reported decreased KL-6 levels in their COVID-19 study in Indonesia [34]. This study's results differ from those of other studies, which stated that KL-6 increased [35,36]. This could be caused by differences in KL-6 kits and genetic variances between countries. We suggest further research using the same KL-6 kit as other studies and comparing the results between populations from different countries.

MMP-9 is another indicator that is used to identify post-infectious lung damage. MMP-9 can be expressed by various cells in the lung, including epithelium, fibroblasts, macrophages, and myofibroblasts. Increased MMP-9 activity is found in multiple lung diseases. MMP-9 activity plays a role by entering leukocytes during inflammation and affects the permeability of the blood-brain barrier, thereby increasing the risk of inflammation [37]. Increased MMP-9 and decreased TIMP-1 were found in COVID-19 patients [38], and our study supports those findings. MMP-9 was significantly higher in severe than non-severe patients (Table 2). Another study reported that alterations of MMP-9 and MMP-2 were associated with COVID-19 mortality [39].

In addition, the identification of post-infection lung damage was characterized by TIMP-1 activity. The role of TIMP-1 is demonstrated in the process of suppressing MMP-9 activities. TIMP can inhibit various MMPs in vitro. TIMP has been shown to mediate angiogenesis through interaction with one integrin. The balance between MMP and TIMP is

responsible for the proteolysis of the ECM. The shift in the balance between the two components results in impaired body functions. The change in the balance with MMP dominance results in increased ECM proteolysis. Meanwhile, a shift in the balance with TIMP dominance resulted in ECM protection and decreased proteolysis [40].

This study showed that KL-6, MMP-9, and TIMP-1 were valid and reliable indicators to measure infectious lung damage. The most severe lung damage in COVID-19 patients is characterized by fibrosis. Pulmonary fibrosis is the result of infection with a poor prognosis. The main histological features of pulmonary fibrosis consist of alveolar septal lesions, abnormal re-epithelialization, fibroblast proliferation, and excessive deposition of ECM macromolecules due to abnormal wound healing and inflammation characterized by the entry of macrophages, neutrophils, and lymphocytes [41].

MMP and TIMP play an essential role in the fibrogenic process. MMP/TIMP imbalance is an indicator used to determine fibrogenic conditions. A high MMP/TIMP ratio in lung tissue indicates a fibrogenic process. MMPs are thought to be involved in releasing and activating profibrotic growth factors and cytokines; therefore, they are responsible for developing fibrogenic conditions [12,41].

The limitations of this study include the limited indicators used to assess the severity of the patient. In addition, lung damage was measured through only one type of TIMP and MMP.

These results may have implications in the future because COVID-19 was a new viral infection resulting in pulmonary disease that spread broadly. These biomarkers may be used in other coronaviral diseases to predict the severity, post-infection damage, and mortality; and, clinicians can use those biomarkers to prevent the progression of the conditions. Research can be continued in the form of therapy based on biomarkers.

Conclusions

Severity of the COVID-19 hospitalized patients can be measured based on ACE2, IL-6, IL-10, neutrophils, lymphocytes, and NLR levels. In our study, there was a decrease in ACE2, lymphocyte, KL-6, and TIMP-1 levels in severe COVID-19 group. Other biomarkers that increased in COVID-19 patients, included IL-6, IL-10, neutrophil, NLR, CRP, procalcitonin, MMP-9, D-dimer, and ferritin. Lung damage can be measured with KL-6, MMP-9, or TIMP-1. Based on our results, MMP-9 cut off value of 572 ng/mL can predict lung damage due to COVID-19. There were correlations between severity and lung damage due to COVID-19.

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Ethics approval and consent to participate.

This research was approved by the Ethics Committee of the Universitas Airlangga with the number 121/KEP/2021. All patients received an explanation of the study and signed informed consent before participating.

Author contributions

ANR, ADP: conceptualization, project administration, methodology, investigation, data curation, formal analysis, visualization, and writing–original draft; HWS, WIE: data curation, formal analysis, and software; AB, IAM: investigation, and data curation; ADS, JN, MA: conceptualization, data curation, and supervision.

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Availability of data and materials

Data is available from the corresponding author upon reasonable request.

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Annex – Supplementary items

Supplementary Table 1. Laboratory results.

	Mean	Std dev	Reference value	Results
Severity indicators				
ACE2 (ng/mL)	1.34	0.68	9–7	Decrease
IL-6 (pg/mL)	77.41	107.21	< 7	Increase
IL-10 (pg/mL)	111.62	61.04	< 13.68	Increase
Leucocyte (10^3/mL)	8.56	3.93	6–12	Normal
Eosinophils (%)	0.30	0.63	1–4	Decrease
Basophil (%)	0.29	0.19	0.5–1	Decrease
Neutrophil (%)	75.59	10.94	40–60	Increase
Monocytes (%)	0.29	0.20	2–8	Decrease
Lymphocyte (%)	18.92	24.13	20–40	Decrease
Platelets (10^3/mL)	259.986	103.177	150–440	Normal
NLR	7.17	6.26	3.5	Increase
PLR	23.95	24.74		
Hematocrit (%)	39.72	4.90	40–50	Decrease
CRP (mg/L)	92.214	84.068	< 10	Increase
Procalcitonin (mcg/L)	7.481	44.902	< 0.5	Increase
Lung damage indicators	**Mean**	**SD**		
KL-6 (U/mL)	49.92	27.29	131–363	Decrease
MMP-9 (ng/mL)	1,179.41	1,240.99	48–211	Increase
TIMP-1 (ng/mL)	7.20	7.53	30–537	Decrease
MMP-9/TIMP-1	168.74	44.59		
D-dimer (mg/L)	2.562	4.559	< 0.5	Increase
Ferritin (ng/mL)	1,277.748	1,117.46	24 - 336	Increase

ACE2: angiotensin converting enzyme 2; NLR: neutrophil to lymphocytes ratio; PLR: platelets to lymphocytes ratio. Std dev: standard deviation.

Supplementary Table 2. Results of validity and reliability of indicators on patient severity and infection lung damage.

	t-value loading factors	t-value error covariance
Severity indicators		
ACE2	6.00*	0.97
Neutrophil	-0.80	6.11*
Lymphocyte	-0.63	6.12*
NLR	1.27	6.10*
Eosinophils	-1.52	6.08*
Basophil	1.72	6.07*
Monocytes	0.05	6.12*
Platelets	0.53	6.12*
Leukocytes	-0.51	6.12*
PLR	-1.15	6.10*
Lung damage indicators		
KL-6	10.47*	5.63*
MMP-9	11.91*	2.08*
TIMP-1	11.79*	2.77*
MMP-9/TIMP-1	-0.24	6.12*

*t-value > 1.96. ACE2: angiotensin converting enzyme 2; KL6: Krebs von de Lungen; MMP-9: matrix metalloproteinase; NLR: neutrophil-lymphocyte ratio; PLR: platelet-lymphocyte ratio; TIMP-9: tissue inhibitor metalloproteinase-9.