RESEARCH ARTICLE

Increased Levels of IFN-γ, PAI-1, and NT-proBNP are Associated with the Occurrence of Hypoxemia in COVID-19

Fathur Nur Kholis[1,*], Nur Farhanah[2], Charles Limantoro[3], Nyoman Suci Widyastiti[4], Mochamad Ali Sobirin[5]

[1]Pulmonology Division, Department of Internal Medicine, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Jl. Prof. Mr. Sunario, Semarang 50275, Indonesia
[2]Tropical Infection Division, Department of Internal Medicine, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Jl. Prof. Mr. Sunario, Semarang 50275, Indonesia
[3]Cardiovascular Division, Department of Internal Medicine, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Jl. Prof. Mr. Sunario, Semarang 50275, Indonesia
[4]Department of Clinical Pathology, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Jl. Prof. Mr. Sunario, Semarang 50275, Indonesia
[5]Department of Cardiovascular, Faculty of Medicine, Universitas Diponegoro/Dr. Kariadi General Hospital, Jl. Prof. Mr. Sunario, Semarang 50275, Indonesia

*Corresponding author. Email: drfnkholis@gmail.com

Abstract

BACKGROUND: Acute respiratory distress syndrome (ARDS) is one of the severe complications of Coronavirus disease 2019 (COVID-19) that can lead to the occurrence of hypoxemia. Hypoxemia occurs due to the role of pro-inflammatory cytokines and coagulation factors. Several studies have shown that interferon (IFN)-γ, plasminogen activator inhibitor 1 (PAI-1) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) are biological markers that can be used to evaluate the severity and prognosis of the disease in severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) infection. This study was conducted to evaluate the association between IFN-γ, PAI-1, NT-proBNP and hypoxemia in COVID-19 patients.

METHODS: This was a cross-sectional study of COVID-19 subjects with hypoxemia. Hypoxemia assessment was performed based on arterial blood gas analysis. IFN-γ and PAI-1 were measured with ELISA, while NT-proBNP levels were measured with Roche NT-proBNP.

RESULTS: Fifty-five COVID-19 subjects with hypoxemia were observed. Thirty subjects experiencing moderate to severe hypoxemia and 25 with mild hypoxemia. Levels of IFN-γ, PAI-1, and NT-proBNP were higher in COVID-19 subjects with moderate to severe hypoxemia compared to those with mild hypoxemia (261.14 (121-348.60) pg/mL *vs.* 145.50 (59.90-348.60) pg/mL, *p*<0.001; 5.47 (3.50-8.50) pg/mL *vs.* 3.40 (2.20-9.30) pg/mL, *p*<0.001; 760 (112-34,066) pg/mL *vs.* 71 (48-364) pg/mL, *p*<0.001).

CONCLUSION: Elevated levels of IFN-γ, PAI-1, and NT-proBNP are associated with hypoxemia in COVID-19 patients, suggesting that these markers may be useful in assessing hypoxemia in COVID-19 patients.

KEYWORDS: IFN-γ, PAI-1, NT-proBNP, hypoxemia, COVID-19

Indones Biomed J. 2023; 15(5): 341-50

Introduction

Hypoxemia is closely associated with the severity and outcomes in Coronavirus disease 2019 (COVID-19) patients. Patients having SpO_2 <94% were reported to have more severe disease and higher morbidity compared to those with SpO_2 ≤94%.(1) Severe hypoxemia in COVID-19 patients with pneumonia has been linked to increased mortality rates in hospitals (2), as well as various respiratory

complications and myocardial injuries associated with this hypoxemia, which are one of the leading causes of death in severely ill COVID-19 patients.(3,4) Therefore, research is needed to investigate factors associated with the occurrence of hypoxemia in COVID-19 patients and the use of these factors to identify early signs of worsening hypoxemia, aiding in risk stratification, prognosis determination, and optimal treatment selection.

Hypoxemia, a condition characterized by low levels of oxygen in the blood, can arise as a consequence of acute respiratory distress syndrome (ARDS), a grave complication associated with COVID-19. Within a timeframe of 24 to 48 hours after the onset of symptoms, approximately 19% of individuals diagnosed with COVID-19 were observed to develop ARDS, indicated by a PaO_2/FiO_2 ratio below 300.(5) The occurrence of ARDS is known to be associated with an inflammatory mechanism triggered by increased production of pro-inflammatory cytokines and the accumulation of coagulation factors in the lungs. Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of fibrinolysis implicated in the development of ARDS, which has been found to increase in infections caused by severe acute respiratory syndrome Coronavirus (SARS-CoV) and acute lung injuries.(3,6)

Patients with comorbid factors such as cardiovascular diseases appear to be more susceptible to SARS-CoV-2 infection and have a higher likelihood of experiencing severe symptoms.(7) Mortality rates in patients presenting with myocardial injury have been reported to be up to 10 times higher.(8) Assessing biomarkers of myocardial injury, such as troponin or high-sensitivity troponin (hs-Trop) and N-terminal pro-B-type natriuretic peptide (NT-proBNP), can be useful in screening COVID-19 patients who are more prone to worsening conditions or complications and require intensive care.(7,8) NT-proBNP is considered an independent risk factor for mortality in severe COVID-19 patients, as patients with increased NT-proBNP levels (>88.64 pg/mL) have shown a significant increase in mortality risk.(9) Other inflammatory markers such as D-dimer also show a potential as a biomarker in COVID-19 infection.(10,11)

Studies in various countries have found associations between various inflammatory markers, coagulation dysfunction, myocardial injuries, hypoxemia, and mortality risk in COVID-19 patients, although results have shown variability and may be influenced by local conditions.(2,3,6–9,12,13) Therefore, to provide more optimal management for patients in Indonesia, further research is needed to evaluate the relationship between the inflammatory marker interferon (IFN)-γ, coagulation dysfunction marker PAI-

1, myocardial injury marker NT-proBNP, and hypoxemia in COVID-19 patients. Additionally, the possibility of using these markers in daily clinical practice as biological indicators to aid in disease severity stratification, prognosis determination, and optimal therapy selection for individual patients should be evaluated.

Methods

Study Design and Study Population

An analytical-observational study with a cross-sectional design involving COVID-19 subjects from a single-center at Dr. Kariadi General Hospital, Semarang, was conducted starting from June 1 to December 30, 2020. Subjects diagnosed with COVID-19 based on reverse transcription polymerase chain reaction (RT-PCR) from nasal/throat/bronchial swab samples and exhibiting hypoxemia (SpO_2 <94%) based on arterial blood gas analysis were included in the study. This study has been approved by the Research Ethics Committee of Dr. Kariadi General Hospital, Semarang (No. 574/EC/KEPK-RSDK/2020).

Data Collection

A consecutive sample of 55 subjects was collected. Subject characteristics data were age, gender, smoking history, history of exposure in the last 14 days, body mass index, blood pressure, respiratory rate, heart rate, body temperature, oxygen saturation, clinical symptoms (fever, dry cough, runny nose, sore throat/swallowing pain, shortness of breath, productive cough/sputum production, diarrhea, abdominal pain, nausea/vomit, joint and muscle pain, fatigue/lethargy, and headache), comorbidities for at least 6 months (diabetes mellitus, hypertension, dyslipidemia, chronic lung disease, stroke, coronary artery disease, ascites, malignancy, chronic kidney failure, and heart failure), chest X-ray findings, laboratory results (complete blood count, liver function, kidney function, electrolytes, arterial blood gas, IFN-γ, PAI-1, and NT-proBNP), and clinical outcomes such as conversion time, length of hospital stay, and mortality or survival, which were collected using medical records. SpO_2 of 91-94% are considered mild hypoxemia, where as SpO_2 ≤90% is considered moderate-severe hypoxemia.

Blood Samples Collection and IFN-γ, PAI-1, and NT-proBNP Measurement

Blood samples were collected from COVID-19 subjects through standard venipuncture techniques. EDTA was used to prevent clotting. The level of IFN-γ was measured using Human IFN-γ ELISA Kit (Elabscience, Wuhan, China).

PAI-1 was measured using PAI-1 Human ELISA Kit (ThermoFisher, Waltham, MA, USA). Plasma levels of NT-proBNP were measured using Roche NT-proBNP (Elecsys, West Sussex, UK).

Statistical Analysis
The obtained measurements for IFN-γ, PAI-1, and NT-proBNP were recorded, and the data was analyzed using appropriate statistical methods to assess the levels of these parameters. Analysis of the data was conducted using the Statistical Package for the Social Sciences (SPSS) program version 21.0 for Windows (IBM, Armonk, NY, USA).

Results

Clinical Characteristics and Laboratory Results
There were more female than male subjects in moderate-severe hypoxemia group. Subjects with moderate-severe hypoxemia were older (52 years) compared to those with mild hypoxemia (50 years). The baseline characteristics of the study subjects were shown in Table 1 and Table 2.

Association between IFN-γ, PAI-1, NT-proBNP and Hypoxemia
The associations between IFN-γ, PAI-1, as well as NT-proBNP and hypoxemia in COVID-19 subjects were analyzed using the Mann-Whitney test. The median IFN-γ level was 261.14 (121.80-348.60) pg/mL for subjects with moderate-severe hypoxemia and 145.50 (59.90-348.60) pg/mL for subjects with mild hypoxemia, with a *p*-value of 0.000 ($p<0.05$). The median PAI-1 level was 5.47 (3.50-8.50) pg/mL for subjects with moderate-severe hypoxemia and 3.40 (2.20-9.30) pg/mL for subjects with mild hypoxemia, with a *p*-value of 0.000 ($p<0.05$). The median NT-proBNP level was 760.00 (112.00-34,066.00) pg/mL for subjects with moderate-severe hypoxemia and 71.00 (48.00-364.00) pg/mL for subjects with mild hypoxemia, with a *p*-value of 0.000 ($p<0.05$) (Table 3, Figure 1). Furthermore, no association was found between IFN-γ, PAI-1, NT-proBNP and clinical outcome of subject (Table 3, Figure 2).

Association between IFN-γ, PAI-1, NT-proBNP and Other Clinical Parameters
NT-ProBNP was correlated with PAI-1 (r=0.71, $p<0.001$), IFN-γ (r=0.54, $p<0.001$), PaO_2/FiO_2 ratio (r=-0.50, $p<0.001$), PaO_2 (r= -0.78, $p<0.001$), creatinine (r=0.27, $p=0.040$) and C-reactive protein (CRP) (r=0.31, $p=0.020$), but was not correlated with other parameters. PAI-1 was correlated with IFN-γ (r=0.63, $p<0.001$), PaO_2/FiO_2 ratio (r=-0.34, $p=0.010$), PaO_2 (r=-0.51, $p<0.001$), urea (r=0.26, $p=0.040$) and creatinine (r=0.28, $p=0.030$). IFN-γ was only correlated with PaO_2/FiO_2 ratio (r=-0.38, $p<0.001$) and PaO_2 (r=-0.49, $p<0.001$) (Supplementary 1).

There was no significant correlation found between NT-proBNP and length of stay (r=0.02, $p=0.860$) and duration to conversion (r=0.03, $p=0.790$). PAI-1 was found to be significantly correlated with duration to conversion (r=0.30, $p=0.020$) and length of stay (r=0.29, $p=0.040$). IFN-γ was also found to be significantly correlated with duration to conversion (r=0.20, $p<0.001$) and length of stay (r=0.32, $p=0.020$) (Figure 3).

Discussion

The demographic characteristics revealed that subject age and sex distribution did not significantly differ between the two groups. Although the prevalence of active smokers was slightly lower among those with moderate-severe hypoxemia, this difference lacked statistical significance. Conversely, subjects with moderate-severe hypoxemia showed a notably higher history of recent exposure to COVID-19 patients, which was statistically significant. Body mass index and vital signs exhibited minimal distinctions between the groups. Symptoms analysis showcased that the moderate-severe hypoxemia group tended to experience more severe symptoms like fever, dyspnea, dry cough, and nausea/vomit. In terms of clinical outcome, the length of stay and duration to negative conversion did not vary considerably between the two groups, and the percentages of deaths and recoveries were roughly comparable.

Radiologic findings showed that subjects with moderate-severe hypoxemia exhibited higher proportions of chest X-ray abnormalities, including ground glass opacity, unilateral and bilateral infiltrates, and interstitial abnormalities. Laboratory data revealed distinct trends; subjects with moderate-severe hypoxemia generally manifested more concerning results. Noteworthy differences included lower PaO_2 and PaO_2/FiO_2 ratio, and higher urea and creatinine levels among the moderate-severe hypoxemia group. Electrolyte levels also vary slightly, with lower sodium and higher potassium levels found in subjects with moderate-severe hypoxemia. In conclusion, the results highlighted that subjects with moderate-severe hypoxemia tend to exhibit more severe clinical manifestations, adverse laboratory and radiologic parameters, and potentially worse outcomes compared to their counterparts with mild

Table 1. Clinical characteristics of COVID-19 subjects.

Characteristics	Degree of Hypoxemia		p-value
	Moderate-Severe (n=30)	Mild (n=25)	
Age, median (IQR) (year)	52 (38-88)	50 (23-74)	0.690#
Sex, n (%)			
Male	14 (46.7)	14 (56.0)	0.490$
Female	16 (53.3)	11 (44.0)	
History of smoking, n (%)			
Non-smoker	21 (70.0)	19 (76.0)	0.270$
Active smoker	9 (30.0)	6 (24.0)	
History of contact with COVID-19 patients in the last 14 days, n (%)			
Yes	16 (53.3)	5 (20.0)	0.001$*
No	14 (46.7)	20 (80.0)	
History of exposure from area with known local transmission			
Yes	25 (83.3)	15 (60.0)	0.070$
No	5 (16.7)	10 (40.0)	
Body mass index, median (IQR) (kg/m^2)	24.6 (18.6-30.4)	24.8 (19.9-30.8)	-
Vital sign on hospital admission, median (IQR)			
Systolic blood pressure (mmHg)	130 (100-215)	127 (100-178)	-
Diastolic blood pressure (mmHg)	80 (60-108)	80 (57-124)	-
Respiratory rate (breaths/minute)	24 (20-39)	22 (18-28)	-
Heart rate (bpm)	95 (68-170)	89 (67-113)	-
Temperature (^{o}C)	37.1 (36.4-38.6)	36.6 (35.5-38.9)	-
SpO_2 (%)	88 (75-90)	93 (92-94)	-
Symptoms, n (%)			
Fever	23 (76.7)	7 (28.0)	
Dry cough	16 (53.3)	9 (36.0)	
Runny nose	2 (6.7)	1 (4.0)	
Sore throat/odynophagia	3 (10.0)	2 (8.0)	
Dyspnea	21 (70.0)	9 (36.0)	
Productive cough	8 (26.7)	4 (16.0)	
Diarrhea	4 (13.3)	3 (12.0)	
Abdominal pain	6 (20.0)	4 (16.0)	
Nausea/vomit	10 (33.3)	11 (44.0)	
Joint/muscle ache	8 (26.7)	6 (24.0)	
Weakness	8 (26.7)	5 (20.0)	
Headache	7 (23.3)	3 (12.0)	
Comorbidities, n (%)			
Diabetes mellitus	14 (46.7)	10 (40.0)	
Hypertension	13 (43.3)	10 (40.0)	
Dyslipidemia	3 (10.0)	1 (4.0)	
Chronic obstructive pulmonary disease	1 (3.3)	-	
Stroke	1 (3.3)	-	
Coronary artery disease	1 (3.3)	-	
Liver disease	1 (3.3)	-	
Ascites	1 (3.3)	1 (4.0)	
Malignancy	5 (16.7)	1 (4.0)	
Heart failure	2 (6.7)	1 (4.0)	
Kidney failure	2 (6.7)	1 (4.0)	
Length of stay, median (IQR) (days)	13 (8-40)	13 (7-28)	0.684#
Duration to negative conversion, median (IQR) (days)	10 (7-36)	10 (7-21)	0.125#
Hospital Discharge, n (%)			
Death	7 (23.3)	3 (12)	0.446$
Recovered	23 (76.7)	22 (88)	

#Mann-Whitney test; $Chi-square test; *$p<0.05$; IQR: Interquartile range.

Table 2. Laboratory and radiologic features of COVID-19 subjects.

Variables	Degree of Hypoxemia		*p* -value
	Moderate-Severe (n=30)	Mild (n=25)	
Chest X-ray abnormalities, n (%)			
Ground glass opacity	12 (40.0)	1 (4.0)	
Unilateral infiltrates	7 (23.3)	4 (16.0)	
Bilateral Infiltrates	8 (26.7)	16 (64.0)	
Interstitial abnormalities	3 (10.0)	4 (16.0)	
Laboratory data, median (IQR)			
PaO_2 (mmHg)	52.0 (23.5-69.1)	75.0 (70.6-78.4)	<0.001*
FiO_2 (%)	60.0 (21.0-82.0)	42.0 (21.0-68.0)	0.020*
PaO_2/FiO_2 ratio	84.8 (32.6-263.8)	181.7 (107.4-373.3)	<0.001*
Leukocyte (/mm^3)	10.3 (2.9-21.1)	9.4 (2.0-36.0)	0.560
Lymphocyte (/mm^3)	12.0 (4.0-26.0)	14.0 (6.0-70.0)	0.090
Thrombocyte (/mm^3)	265.0 (60.0-536.0)	226.0 (20.0-569.0)	0.300
Hemoglobin (g/dL)	12.7 (4.1-16.6)	11.5 (6.2-16.2)	0.240
NLR (%)	6.1 (2.4-20.5)	5.1 (0.2-14.8)	0.140
aCL (/μL)	1,035.0 (203.0-4,356.0)	1,688.0 (260.0-10,560.0)	0.420
Other laboratory findings, median (IQR)			
Random blood glucose (mg/dL)	123.0 (82.0-441.0)	136.0 (75-276.0)	0.880
Urea (mg/dL)	43.0 (16.0-95.0)	23.0 (17.0-46.0)	0.040*
Creatinine (mg/dL)	1.3 (0.8-2.3)	1.1 (0.8-1.3)	0.030*
Albumin (g/dL)	3.3 (2.4-4.1)	3.9 (3.5-4.0)	0.890
SGOT (U/L)	49.0 (27.0-159.0)	67.0 (56.0-152.0)	0.730
SGPT (U/L)	42.0 (23.0-65.0)	57.5 (17.0-114.0)	0.940
Total protein (g/dL)	6.6 (6.2-7.5)	6.1 (5.7-7.0)	0.220
Alkaline phosphatase (U/L)	72.0 (35.0-574.0)	117.0 (22.0-731.0)	0.880
Lactate (mmol/L)	4.9 (1.0-12.2)	4.0 (2.3-6.6)	0.780
C-reactive protein (mg/L)	11.4 (0.2-34)	5.7 (0.2-27.9)	0.080
Lactate dehydrogenase (U/L)	815.0 (594.0-2120.0)	638.0 (11.0-1,507.0)	0.160
Ferritin (mg/L)	711.0 (515.6-1,461.5)	1,193.3 (1,117.7-1,269)	0.730
Procalcitonin (ng/L)	0.2 (0.1-9.5)	0.3 (0.1-25.4)	0.320
Total bilirubin (μmol/L)	1.2 (0.4-4.8)	0.65 (0.5-1.3)	0.880
Direct bilirubin (μmol/L)	0.3 (0.1-3.6)	0.35 (0.2-0.6)	0.880
PTT (second)	12.8 (0.7-14.4)	12.2 (11.0-13.4)	0.710
aPTT (second)	34.7 (31.3-54.0)	40.5 (34.2-46.7)	0.480
INR (mg/dL)	1.1 (1.0-1.6)	0.9 (0.9-1.0)	0.150
D-dimer (mg/dL)	3,780.0 (910.0-20,000.0)	3,645.0 (810.0-6,480.0)	0.580
Fibrinogen (mg/dL)	555.9 (188.1-1,091.0)	646.8 (615-678.6)	0.100
Electrolyte, median (IQR)			
Sodium (mmol/L)	136.5 (126.0-146.0)	137.0 (117.0-158.0)	0.280
Potassium (mmol/L)	3.9 (2.8-4.9)	3.6 (3.2-4.6)	0.510
Chloride (mmol/L)	97.5 (92.0-110.0)	94.5 (88.0-105.0)	0.020*
Calcium (mmol/L)	2.1 (1.8-2.5)	2.1 (1.8-2.3)	0.760
Magnesium (mmol/L)	0.9 (0.7-1.3)	0.7 (0.5-1.1)	0.140

*$p<0.05$, Mann-Whitney test; IQR: interquartile range; PaO_2: Partial oxygen pressure; FiO_2: Fraction of inspired oxygen; NLR: Neutrophil-to-lymphocyte ratio; aCL: Anticardiolipin antibodies; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; INR: International normalized ratio; PTT: Partial thromboplastin time; aPTT: Activated partial thromboplastin time.

Table 3. Differences between IFN- γ, PAI-1, and NT-proBNP, degree of hypoxemia and clinical outcome.

Variables	Degree of Hypoxemia			Clinical Outcome		
	Moderate-Severe (n=30)	Mild (n=25)	*p* -value	Death (n=8)	Recovered (n=47)	*p* -value
IFN-γ, median (IQR) (pg/mL)	261.14 (121.80-348.60)	145.50 (59.90-348.60)	0.000*	158.65 (116.10-323.00)	188.60 (59.90-348.60)	0.430
PAI-1, median (IQR) (pg/mL)	5.47 (3.50-8.50)	3.40 (2.20-9.30)	0.000*	3.55 (3.20-8.50)	4.30 (2.20-9.30)	0.340
NT-proBNP, median (IQR) (pg/mL)	760.00 (112.00-34,066.00)	71.00 (48.00-364.00)	0.000*	169.00 (50.00-6,535.00)	218.00 (48.00-34,066.00)	0.630

*$p<0.05$, Mann Whitney test; IQR: interquartile range.

hypoxemia. However, interpretations should be mindful of varying levels of statistical significance across different parameters.

Hypoxemia can occur in COVID-19, and this condition meets the criteria for ARDS without the characteristic features of the syndrome.(14) One of the triggers for hypoxemia in SARS-CoV-2 infection is lung dysfunction related to inflammation.(4) This inflammatory process triggers an immune response mediated by T cells. The immune response relies heavily on the involvement of CD4^{+} and CD8^{+} T cells, which have significant functions.

CD4^{+} T cells are responsible for stimulating B cells to generate antibodies specific to the virus, while CD8^{+} T cells contribute to the elimination of virus-infected cells through inducing their apoptosis.(15,16) In severe cases, pathogenic CD4^{+} T cells also show increased expression of IFN-γ, which plays a role in triggering increased infiltration of immune cells from the intravascular compartment to the alveolar space. Therefore, the elevated cytokine IFN-γ can be used as a biological marker to evaluate the development and severity of lung injury caused by SARS-CoV-2 infection.(3)
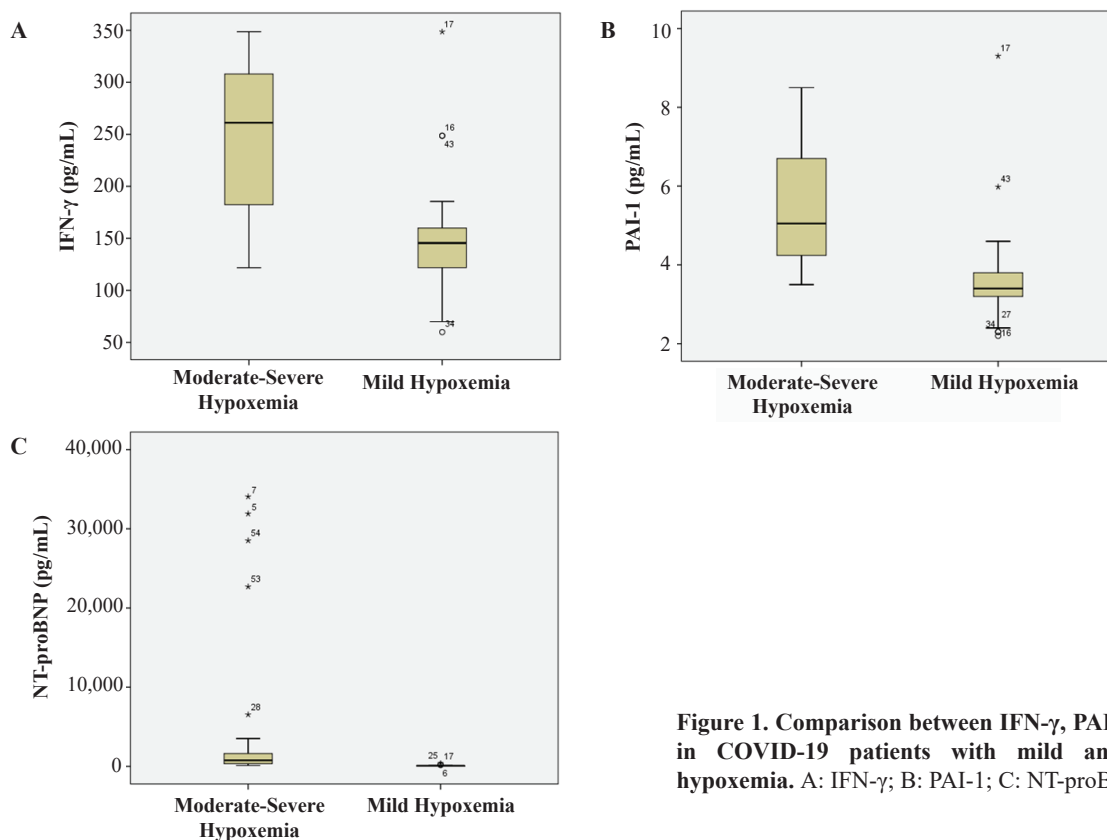
Figure 1. Comparison between IFN-γ, PAI-1, and NT-proBNP in COVID-19 patients with mild and moderate-severe hypoxemia. A: IFN-γ; B: PAI-1; C: NT-proBNP.
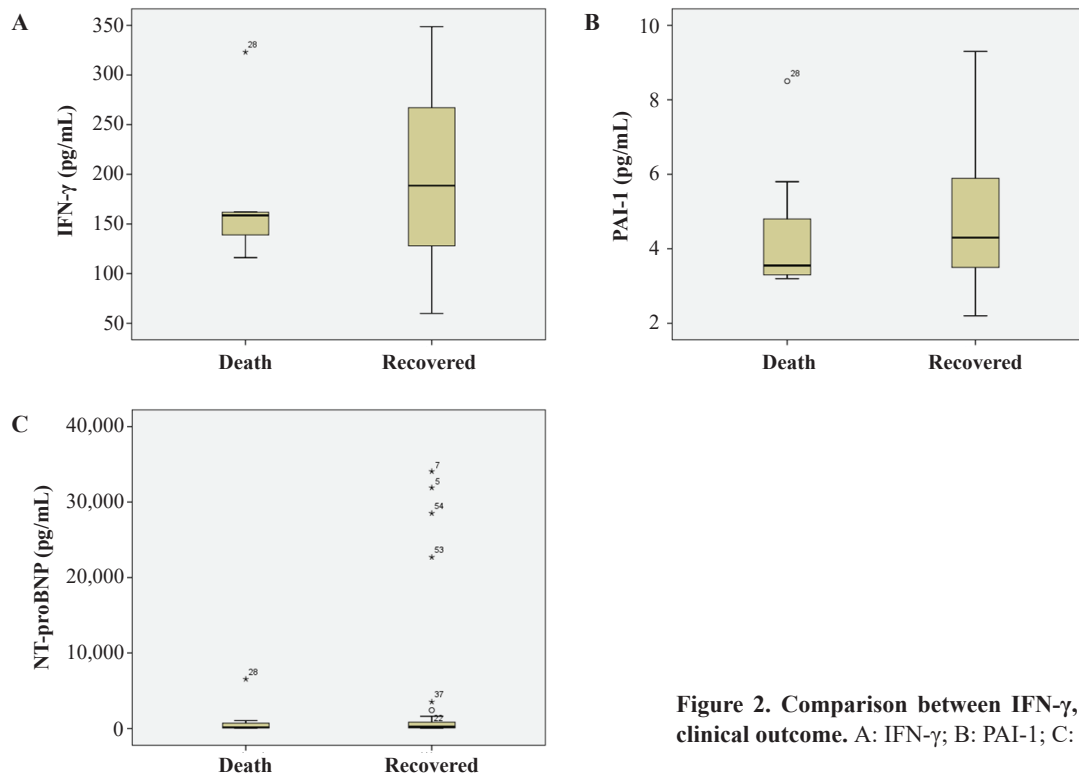
Figure 2. Comparison between IFN-γ, PAI-1, NT-proBNP and clinical outcome. A: IFN-γ; B: PAI-1; C: NT-proBNP.

Our study found an increase in IFN-γ levels in mild, moderate, and severe degrees, with a more significant increase in the moderate-severe degree. Other studies have also shown a significant increase in plasma IFN-γ levels in patients with severe COVID-19 compared to mild cases after 3-4 days of illness onset.(17) Elevated levels of pro-inflammatory cytokines like IFN-γ have been strongly associated with severe lung tissue damage and adverse outcomes in SARS-CoV or Middle East respiratory syndrome Coronavirus (MERS-CoV) infections.(18–21) IFN-γ is a cytokine produced by lymphocytes, particularly T cells and natural killer (NK) cells, upon activation by specific antigens or mitogens. The signaling of IFN-γ serves various functions in cellular processes, encompassing the enhancement of macrophage activity and the mediation of host defense against infections caused by pathogens.(22)

Severe complications of ARDS can be attributed due to the formation of hyaline membranes.(23) These membranes form due to severe and diffuse alveolar damage, hindering gas exchange and lung expansion.(23,24) Fibrin deposits, caused by imbalances in coagulation and fibrinolysis processes, contribute to hyaline membrane formation and alveolar fibrosis in ARDS.(3) Increased levels of PAI-1, an inhibitor of fibrinolysis, have been observed in SARS-CoV infection and acute lung injury,

exacerbating fibrin deposition.(3,25) The coagulation process in the severely ill and critical patients may also lead to thrombocytopenia.(26)

Our research confirmed elevated levels of PAI-1 in COVID-19 particularly in subjects with moderate-to-severe hypoxemia compared to those with mild hypoxemia. Similar findings were observed in another study comparing PAI-1 levels in COVID-19 patients with healthy controls. (27) In ARDS, PAI-1 release from endothelial cells can be triggered by CRP and platelet infiltration leading to increased circulation levels. Higher PAI-1 levels suppress the activity of urokinase (uPA) in bronchoalveolar fluid resulting in abnormal fibrin production in the alveolar spaces.(3) PAI-1 has been reported as a prognostic marker in ARDS cases, with higher levels associated with increased complications.(28)

Our study demonstrated that COVID-19 subjects with moderate-to-severe hypoxemia exhibited a significantly higher levels of NT-proBNP compared to subjects with mild hypoxemia. A study reported a significant correlation between increased plasma NT-proBNP levels and the severity of pneumonia in COVID-19 patients.(29) The study also stated that NT-proBNP levels ≥300 pg/mL indicate a high mortality, with an 8.7% mortality rate.(29) Another study on COVID-19 patients without a history of
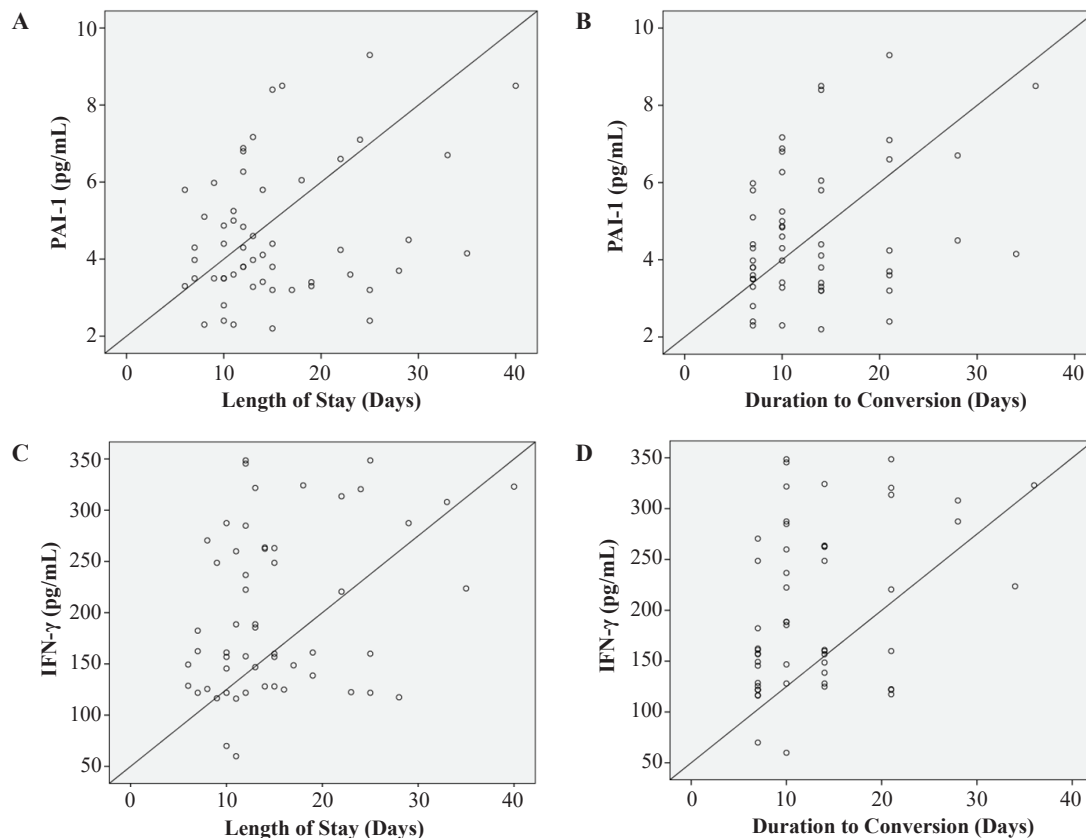
Figure 3. Significant correlation scatter plots between PAI-1, IFN-γ, length of stay and duration to conversion. The correlation was calculated with Spearman's rho test. A: PAI-1 and length of stay; B: PAI-1 and duration to conversion; C:IFN-γ and length of stay; D: IFN-γ and duration to conversion.

heart failure demonstrated that an independent increase in NT-proBNP levels ≥260 pg/mL was associated with in-hospital mortality, with a sensitivity of 82% and specificity of 93%.(30)

NT-proBNP is released in response to myocardial wall stress. In SARS-CoV-2 infection, cardiac injury can result from pro-inflammatory mechanisms, viral invasion of cardiomyocytes via angiotensin converting enzyme 2 (ACE2) binding, reduced myocardial oxygen supply, and cytokine storm conditions.(9) The virus itself disrupts the balance between angiotensin 2 and angiotensin 1-7, leading to elevated NT-proBNP levels.(31)

Although the results emphasize the connections between IFN-γ, PAI-1 and NT-proBNP levels and the severity of oxygen levels in COVID-19 subjects, it is important to mention that the research did not find any significant correlation between these biomarkers and the subjects' clinical outcomes. This suggests that while these biomarkers could be useful in indicating the severity of oxygen levels they may not have an impact, on the overall prognosis of the subjects. Specifically, a more comprehensive analysis is needed to establish clear associations between the severity

of hypoxemia and all relevant parameters of interest. Defining precise threshold values for these markers requires a distinct study design. Hence, we recommend that these markers be subjected to additional evaluation as potential indicators for early assessment in COVID-19 patients. This study adds to our comprehension of the intricate interplay between COVID-19 pathophysiology and disease severity, laying the groundwork for future investigations aimed at enhancing our capability to predict and manage hypoxemia progression in COVID-19 patients.

Conclusion

In conclusion, our study found that increased levels of IFN-γ, PAI-1, and NT-proBNP are observed in COVID-19 patients and associated with the degree hypoxemia, suggesting that these markers may be useful in assessing hypoxemia in COVID-19 patients. While our findings suggest potential clinical implications, it is important to note that further investigation is required before considering these markers as tools for daily clinical practice.

Authors Contribution

FNK, NF and CL were involved in planning and supervised the work, NSW performed the measurements, FNK, NF and MAS processed the experimental data, performed the analysis, drafted the manuscript and designed the figures. NSW and MAS performed the calculations and statistical analysis. FNK, NF and CL aided in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

References

1. Asleh R, Asher E, Yagel O, Samuel T, Elbaz-Greener G, Wolak A, *et al*. Predictors of hypoxemia and related adverse outcomes in patients hospitalized with COVID-19: A double-center retrospective study. J Clin Med. 2021; 10(16): 3581. doi: 10.3390/jcm10163581.

2. Xie J, Covassin N, Fan Z, Singh P, Gao W, Li G, Kara T, Somers VK. Association between hypoxemia and mortality in patients with COVID-19.. Mayo Clin Proc. 2020; 95(6): 1138-47.

3. Whyte CS, Morrow GB, Mitchell JL, Chowdary P, Mutch NJ. Fibrinolytic abnormalities in acute respiratory distress syndrome (ARDS) and versatility of thrombolytic drugs to treat COVID-19. J Thromb Haemost. 2020; 18(7): 1548-55.

4. Geng YJ, Wei ZY, Qian HY, Huang J, Lodato R, Castriotta RJ. Pathophysiological characteristics and therapeutic approaches for pulmonary injury and cardiovascular complications of coronavirus disease 2019. Cardiovasc Pathol. 2020; 47: 107228. doi: 10.1016/j. carpath.2020.107228.

5. Ji HL, Zhao R, Matalon S, Matthay MA. Elevated plasmin(ogen) as a common risk factor for COVID-19 susceptibility. Physiol Rev. 2020; 100(3): 1065-75.

6. Zhou Y, Fu B, Zheng X, Wang D, Zhao C, Qi Y, *et al*. Pathogenic T-cells and inflammatory monocytes incite inflammatory storms in severe COVID-19 patients. Natl Sci Rev. 2020; 7(6): 998-1002.

7. Babapoor-Farrokhran S, Gill D, Walker J, Rasekhi RT, Bozorgnia B, Amanullah A. Myocardial injury and COVID-19: Possible mechanisms. Life Sci. 2020; 253: 117723. doi: 10.1016/j. lfs.2020.117723.

8. Chapman AR, Bularga A, Mills NL. High-sensitivity cardiac troponin can be an ally in the fight against COVID-19. Circulation. 2020; 141(22): 1733-5.

9. Gao L, Jiang D, Wen XS, Cheng XC, Sun M, He B, *et al*. Prognostic value of NT-proBNP in patients with severe COVID-19. Respir Res. 2020; 21(1): 83. doi: 10.1186/s12931-020-01352-w.

10. Ferdian H, Siregar R, Moelyo AG. D-dimer as a potential biomarker of severity in children confirmed with COVID-19. Mol Cell Biomed Sci. 2022; 6(3): 141–6.

11. Tandirogang N, Fitriany E, Mardania N, Jannah M, Dilan BFN, Ratri SR, *et al*. Neutralizing antibody response by inactivated SARS-CoV-2 vaccine on healthcare workers. Mol Cell Biomed Sci. 2023; 7(1): 18–27.

12. Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, *et al*. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020; 180(7): 934-43.

13. Du RH, Liang LR, Yang CQ, Wang W, Cao TZ, Li M, *et al*. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. Eur Respir J. 2020; 55(5): 2000524. doi: 10.1183/13993003.00524-2020.

14. Nitsure M, Sarangi B, Shankar GH, Reddy VS, Walimbe A, Sharma V, *et al*. Mechanisms of hypoxia in COVID-19 patients: A pathophysiologic reflection. Indian J Crit Care Med. 2020; 24(10): 967-70.

15. Xie J, Tong Z, Guan X, Du B, Qiu H. Clinical characteristics of patients who died of coronavirus disease 2019 in China. JAMA Netw Open. 2020; 3(4): e205619. doi: 10.1001/jamanetworkopen.2020.5619.

16. Channappanavar R, Zhao J, Perlman S. T cell-mediated immune response to respiratory coronaviruses. Immunol Res. 2014; 59(1-3): 118-28.

17. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, *et al*. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine. 2020; 55: 102763. doi: 10.1016/j.ebiom.2020.102763.

18. Chien JY, Hsueh PR, Cheng WC, Yu CJ, Yang PC. Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. Respirology. 2006; 11(6): 715-22.

19. Chu H, Zhou J, Wong BH, Li C, Cheng ZS, Lin X, *et al*. Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. Virology. 2014; 454-455: 197-205.

20. Zhou J, Chu H, Li C, Wong BH, Cheng ZS, Poon VK, *et al*. Active replication of Middle East respiratory syndrome coronavirus and aberrant induction of inflammatory cytokines and chemokines in human macrophages: Implications for pathogenesis. J Infect Dis. 2014; 209(9): 1331-42.

21. Kong SL, Chui P, Lim B, Salto-Tellez M. Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients. Virus Res. 2009; 145(2): 260-9.

22. Zhou F. Molecular mechanisms of IFN-gamma to up-regulate MHC class I antigen processing and presentation. Int Rev Immunol. 2009; 28(3-4): 239-60.

23. Wu Y, Wang T, Guo C, Zhang D, Ge X, Huang Z, *et al*. Plasminogen improves lung lesions and hypoxia in patients with COVID-19. QJM. 2020; 113(8): 539-45.

24. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, *et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020; 8(4): 420-2.

25. Gralinski LE, Bankhead A 3rd, Jeng S, Menachery VD, Proll S, Belisle SE, *et al*. Mechanisms of severe acute respiratory syndrome coronavirus-induced acute lung injury. mBio. 2013; 4(4): e00271-13.

26. Prayoga AA, Bintoro SUY, Romadhon PZ, Diansyah MN, Amrita

PNA, Savitri M, *et al*. Increased hs-CRP and sepsis influence the occurrence of thrombocytopenia in severe and critically ill COVID-19 patients receiving anticoagulants. Indones Biomed J. 2023; 15(1): 77–84.

27. Zuo Y, Warnock M, Harbaugh A, Yalavarthi S, Gockman K, Zuo M, *et al*. Plasma tissue plasminogen activator and plasminogen activator inhibitor-1 in hospitalized COVID-19 patients. Sci Rep. 2021; 11(1): 1580. doi: 10.1038/s41598-020-80010-z.

28. El Solh AA, Bhora M, Pineda L, Aquilina A, Abbetessa L, Berbary E. Alveolar plasminogen activator inhibitor-1 predicts ARDS in aspiration pneumonitis. Intensive Care Med. 2006; 32(1): 110-5.

29. Wang L, Chen F, Bai L, Bai L, Huang Z, Peng Y. Association between NT-proBNP level and the severity of COVID-19 pneumonia. Cardiol Res Pract. 2021; 2021: 5537275. doi: 10.1155/2021/5537275.

30. Selçuk M, Keskin M, Çınar T, Günay N, Doğan S, Çiçek V, *et al*. Prognostic significance of N-Terminal Pro-BNP in patients with COVID-19 pneumonia without previous history of heart failure. J Cardiovasc Thorac Res. 2021; 13(2): 141-5.

31. Zhang H, Penninger JM, Li Y, Zhong N, Slutsky AS. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: Molecular mechanisms and potential therapeutic target. Intensive Care Med. 2020; 46(4): 586-90.