ACTA MICROBIOLOGICA BULGARICA Volume 40 / 1 (2024)

Premier Hospital Surabaya Score: COVID-19 Mortality Score Model Based on Blood Levels of Neutrofil-Lymphocite Ratio, C-Reactive Protein, D-dimer, and Interleukin-6

Agatha Efrad Saputri[1], Jusak Nugraha[1,2], Valentinus Besin[1,3], Paulus Budiono Notopuro[2*]

[1]*Premier Hospital, Jl Nginden Intan Barat Blok B, Surabaya, Indonesia*
[2]*Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Jl. Mayjen Prof. Dr. Moestopo, Surabaya, Indonesia*
[3]*Faculty of Medicine, University of Surabaya, Surabaya, Indonesia*

Abstract

In order to effectively prevent COVID-19 mortality, accurate prediction is crucial. To address this, we examined the relationship between patient mortality and important clinical factors (NRL, CRP, D-dimer, IL-6). Our study led to the development of the Premier Hospital Surabaya (PHS) Score, which utilizes the analysis of these parameters to correlate with COVID-19 mortality. In this cross-sectional analytic observational study, data were collected via consecutive sampling from eligible subjects' medical records. The initial measurements of NLR, IL-6, CRP, and D-dimer were recorded. Bivariate analysis assessed all variables, and rank Spearman tests were used for each laboratory parameter. Subsequently, logistic regression was employed for multivariate analysis, creating a mortality prediction score model. The cutoff score for early prediction of COVID-19 mortality was determined through the analysis of the Receiver Operating Characteristic (ROC) curve. The laboratory parameters were used to calculate the PHS Score model as follows: PHS Score = NLR score + CRP score + D-dimer score + IL-6 score. The ROC analysis revealed that the PHS score had an AUC of 71.6%. The optimal cutoff point was determined to be 4.5, with a sensitivity of 74.6% and specificity of 65.9%. A PHS Score of ≥4 is indicative of an increased probability of mortality in patients diagnosed with COVID-19. The PHS score derived from the laboratory parameters can aid in predicting the mortality risk during the initial hospital admission. By utilizing this score, healthcare professionals can enhance the management of COVID-19 patients.

Keywords: neutrophil-lymphocyte ratio, C-reactive protein, D-dimer, Interleukin-6, COVID-19, mortality

Резюме

С цел ефективно предотвратяване на смъртността от COVID-19, точното предсказване е от съществено значение. Проучихме връзката между смъртността на пациентите и ключови клинични фактори (NLR, CRP, D-димер, IL-6). Нашето изследване доведе до разработването на индекс, който да се използва в Премиерната болница в Сурабая (PHS) за анализ на тези параметри и тяхната корелация със смъртността от COVID-19. Данните в това кръстосано аналитично изследване са събрани чрез последователно избиране от медицинските досиета на подходящи субекти. Установени са първоначалните стойности на NLR, IL-6, CRP и D-димер. Бивариантният анализ оцени всички променливи, а за всеки лабораторен параметър са използвани тестове на рангова корелация на Spearman. След това е приложена логистична регресия за многомерен анализ, създавайки модел за предсказване на смъртност. Праговата стойност за ранно предсказване на смъртността от COVID-19 е определена чрез анализ на кривата на работната характеристика на приемника (КРХП). Лабораторните параметри са използвани за изчисляване на модела PHS индекс както следва: PHS индекс = NLR индекс + CRP индекс + D-димер индекс + IL-6 индекс. Анализът на КРХП показа, че PHS индексът има AUC (Площ под кривата) от 71.6%. Оптималната прагова точка беше определена на 4.5, с чувствителност от 74.6% и специфичност от 65.9%. PHS индекс от ≥4 показва увеличена вероятност за смъртност при пациенти, диагностицирани с COVID-19. PHS индексът, извлечен от лабораторните параметри, може да помогне в предсказването на риска от смърт при първоначалното

*Corresponding author: paulus-b-n@fk.unair.ac.id
Acta Microbiol. Bulg. 2024; 40(01). https://doi.org/10.59393/amb24400103

приемане в болница. Чрез използване на този индекс, медицинските специалисти могат да подобрят управлението на пациентите с COVID-19.

Introduction

According to the latest global data on June 28, 2023, the number of reported Coronavirus disease of 2019 (COVID-19) infections reached a staggering 767.518.723 cases, with a significant 6.947.192 reported deaths caused by the disease. These numbers reflect the impact of COVID-19 on a global scale. Additionally, during the initial year of the COVID-19 outbreak worldwide, there were approximately 79 million reported cases of infection and approximately 1.7 million reported deaths attributed to COVID-19 (Ponsford *et al.,* 2022). Shifting the focus to Indonesia, from January 3, 2020, to June 28, 2023, the country recorded 6,811,818 cases of COVID-19 infection and 161,867 deaths due to the disease (WHO, 2023).

Laboratory tests play a crucial role in detecting, diagnosing, assessing the severity, and guiding treatment for COVID-19 infection (Das *et al.,* 2021). Lymphopenia and an elevated neutrophil-lymphocyte ratio (NLR) have been recognized as potential indicators for diagnosing and evaluating the severity of a condition (Samprathi and Jayashree, 2021). C-reactive protein (CRP) and D-dimer are inflammation markers that tend to elevate in COVID-19 infection and may serve as indicators of disease severity. COVID-19 guidelines recommend examining these markers to determine disease prognosis (Garc *et al.,* 2021).

Patients with severe COVID-19 infection should undergo additional laboratory testing, such as measuring interleukin 6 (IL-6) levels (Samprathi and Jayashree, 2021). However, it is worth noting that there have been inconsistencies in the findings regarding its relationship with mortality (Liu *et al.,* 2021; Aljohani *et al.,* 2022; Talwar *et al.,* 2022). Some studies have even reported no correlation between certain markers, such as IL-6 and CRP, with mortality (Aljohani *et al.,* 2022; Liu *et al.,* 2021; Talwar *et al.,* 2022)

The clinical manifestation of COVID-19 infection exhibits a wide spectrum of presentations, ranging from individuals who do not display any symptoms to those who develop severe pneumonia (Das *et al.,* 2021). Most patients experience flu-like symptoms, including cough (59.6%) and fever (46.9%), while a smaller percentage develop pneumonia (2.89%) and acute respiratory distress (0.22%) (Israfil *et al.,* 2021). From March 2020 to February 2022, the COVID-19 pandemic has become a significant global health crisis, resulting in more deaths than diseases like malaria, tuberculosis, HIV, and other tropical infections, as well as non-communicable illnesses such as heart diseases, cancer, chronic lung diseases, and diabetes. As of December 29, 2022, Indonesia has reported the highest number of COVID-19 cases and fatalities in Southeast Asia, ranking second after India in terms of cases across Asia (Dong *et al.,* 2020). By June 29th, 2023, Indonesia's COVID-19 death rate stood at 2.4% (Kemenkes, 2023).

This research was conducted on patients with COVID-19 admitted to Premier Hospital Surabaya from January to December 2021. It involved analyzing laboratory tests such as NLR ratio, CRP, D-dimer, and IL-6 cytokine levels. The study's objective was to determine the relationship between these specific lab tests and the mortality rate in COVID-19 patients. The results would lead to the creation of the Premier Hospital Surabaya (PHS) Score. The study carried out from January 2021 to December 2021, developed a score that predicts the mortality risk of COVID-19 patients using specific laboratory parameters. This scoring system serves as a crucial instrument for initial evaluations of patients upon their admission to the hospital, significantly contributing to the improved management and treatment of COVID-19 cases.

Materials and Methods

Study design

This cross-sectional analytic observational study aimed to investigate the relationship between laboratory examinations (NLR, CRP, D-dimer, and IL-6) and mortality risk scores in COVID-19 patients. The inclusion criteria required patients to have a positive result for RT PCR COVID-19 and complete laboratory tests for NLR, CRP, D-dimer, and IL-6. Exclusion criteria encompassed patients with incomplete laboratory tests, referrals to other hospitals, or discharge against medical advice.

This study included COVID-19 patients who were hospitalized at Premier Hospital Surabaya between January 1, 2021, and December 31, 2021, and met the inclusion criteria. During their hospital stay, 63 patients (15.52%) unfortunately passed away. Age was categorized into two groups: under 50 years (171 patients) and over 50 years (235 patients). The study protocol was approved by the Health Research Ethics Committee of Universitas Airlangga School of Medicine (21/EC/KEPK/

FKUA/2023) and the Ethics Committee of Premier Surabaya Hospital (01/RSPS/KERS/V/2022).

Data collection

This study aimed to gather diverse datasets in order to examine the correlation between laboratory parameters and the risk of mortality in individuals diagnosed with COVID-19. The dataset encompassed various demographic variables, including age and gender, alongside preexisting medical conditions such as hypertension (HT), diabetes mellitus (DM), chronic kidney disease (CKD), dyslipidemia, coronary artery disease (CAD), and stroke. Additionally, we recorded the initial measurements of NLR, CRP, D-dimer, and IL-6 levels at the time of admission. Finally, we documented the occurrence of in-hospital mortality. Ensure the reliability and representativeness of the data; we employed consecutive sampling from eligible subjects' medical records.

Laboratory assessment

The initial laboratory test measurements of NLR, IL-6, CRP, and d-dimer were recorded using specific instruments. NLR was examined using the Alinity-H series and Cell-dyn Ruby. CRP was examined using Architect Ci4100 and TMS premium. D-dimer was examined using Architect C4100. IL-6 levels were examined using Roche with the ECLIA method.

Statistical analysis

The statistical analysis was performed using the SPSS (Statistical Product and Service Solution) version 26.0 software. A bivariate analysis was performed in order to investigate the association between variables. The study evaluated subject characteristics, such as age, gender, and comorbidity from 406 COVID-19 patients, through the utilization of cross tabulation and the chi-square test. The laboratory parameters were analyzed using the rank Spearman test to determine their correlations. Following this, multivariate analysis using logistic regression was performed to develop a mortality prediction score model. The model underwent additional simplification, and logistic regression analysis was applied to all scores. Subsequently, a Receiver Operating Characteristic (ROC) analysis was performed to determine the optimal threshold score for the early prediction of mortality due to COVID-19. A statistical significance level of $p < 0.05$ was deemed to be significant.

Results

Subjects characteristics

The median age of the patients in this study was 54 years. Among the patients, 245 (60.34%) were male, while 161 (39.66%) were female. Based on the analysis, there was a significant correlation found between age and mortality ($p = 0.001$). Table 1 demonstrates that older patients exhibited a stronger positive correlation with mortality compared to younger subjects. In this study, the common comorbidities observed were HT, CAD, stroke, CKD, dyslipidemia, and DM. Among these, HT was the most prevalent comorbidity, affecting 119 subjects, followed by diabetes mellitus in 113 subjects.

By employing cross-tabulation and chi-square tests, we found a significant correlation between HT, CKD, and DM with mortality ($p < 0.05$). Patients with comorbidities demonstrated both clinical and statistical risks for mortality. The odds ratios (OR) were as follows: 14.17 (95% CI: 3.56 - 56.4) for CKD, 2.22 (95% CI: 1.28 - 3.85) for HT, and 3.34 (95% CI: 1.92 - 5.81) for DM. These findings emphasize the increased mortality risk associated with these comorbid conditions (Table 2).

Correlation between laboratory parameters with mortality

The main aim of this study was to examine the association between laboratory parameters (NLR, CRP, D-dimer, IL-6), and in-hospital mortality. These parameters were categorized as normal or abnormal, serving as categorical variables.

Significant correlations were observed between all laboratory parameters and mortality (p-value < 0.001). Higher levels of NLR, CRP, D-dimer, and IL-6 were associated with an in-

Table 1. Subjects' characteristics

Characteristic	Mortality				Total	p
	No	%	Yes	%		
Age						
≤ 50 years	156	91.2%	15	8.8%	171	0.001
> 50 years	187	79.6%	48	20.4%	235	
Gender						
Male	202	82.4%	43	17.6%	245	0.163
Female	141	87.6%	20	12.4%	161	

Table 2. Comorbidity and mortality

Comorbid	Mortality				Total	p	OR (CI)
	No	%	Yes	%			
Hypertension							
No	252	87.8%	35	12.2%	287	0.009	2.215 (1.276 – 3.846)
Yes	91	76.5%	28	23.5%	119		
Coronary Heart Disease							
No	324	84.8%	58	15.2%	382	0.458	1.470 (0.528 – 4.093)
Yes	19	79.2%	5	20.8%	24		
Stroke							
No	333	84.9%	59	15.1%	392	0.170	2.258 (0.685 – 7.437)
Yes	10	71.4%	4	28.6%	14		
CKD							
No	340	85.9%	56	14.1%	396	0.000	14.167 (3.558 – 56.408)
Yes	3	30.0%	7	70.0%	10		
Dyslipidaemia							
No	323	84.1%	61	15.9%	384	0.392	0.530 (0.121 – 2.324)
Yes	20	90.9%	2	9.1%	22		
Diabetes Mellitus							
No	282	89.4%	31	10.6%	293	0.000	3.339 (1.920 – 5.806)
Yes	81	71.7%	32	28.3%	113		

creased likelihood of in-hospital mortality. The correlation between each laboratory parameter and mortality was found to be moderate.

The logistic regression analysis revealed that all laboratory parameters, including NLR (OR= 1.170; 95% CI = 1.072 – 1.278; p=0.000), CRP (OR= 1.006; 95% CI = 1.001 – 1.010; p=0.015), D-dimer (OR= 1.001; 95% CI = 1.0003 – 1.001; p=0.002), and IL-6 (OR= 1.007; 95% CI = 1.003 – 1.012; p= 0.002), were both clinically and statistically significant. These parameters were considered suitable for inclusion in the mortality prediction score model. Based on the significant correlations observed in all laboratory parameters, the PHS Score was formulated as follows:

$$Ln\left(\frac{p}{1-p}\right) = -3.936 + 0.007\,IL6 + 0.157\,NLR + 0.006\,CRP + 0.001\,Ddim$$

The original equation was simplified by converting the coefficients of each parameter into a score. This was done by dividing the (β/SE) value of each parameter by the smallest (β/SE) value. The scores for each parameter are presented in Table 3. These resulting values were used as the scores for the PHS Score model, as follows:

PHS Score = NLR score + CRP score + D − dimer score + IL − 6 score

Table 3. Scoring Predictor Factor

	Simplification
NLR	1
≤ 2	0
> 2	1
CRP	1
≤ 0.499 mg/l	0
> 0.499 mg/l	1
D-dimer	2
≤ 500 ng/ml	0
> 500 ng/ml	2
IL -6	1
≤ 7 pg/ml	0
> 7 pg/ml	1
Total score	0 – 5

Note: Interpretation: A PHS score of ≥ 4 is indicative of a higher mortality rate

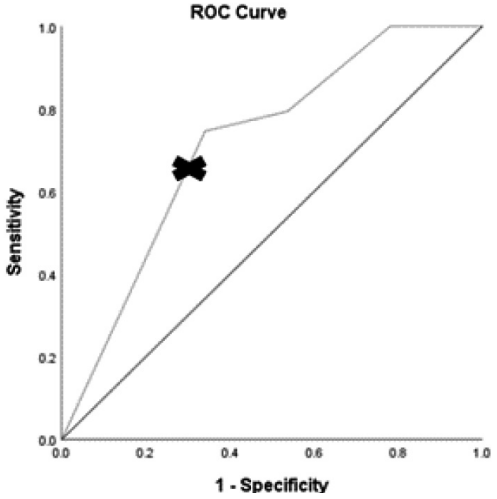
NLR, IL-6, and CRP were assigned a score of 1, while D-dimer received a score of 2 (Table 3). To further explore the association between the total scores of the PHS Score and mortality, a logistic regression analysis was conducted. The findings revealed a statistically significant p-value (< 0.05), suggesting that the total scores of the PHS Score can be utilized as a predictive tool for mortality in

individuals with COVID-19. Following that, an analysis of the receiver operating curve (ROC) was conducted to ascertain the most suitable threshold score for early prediction of COVID-19 mortality. This determination was made by considering the cumulative score of each parameter concerning mortality. The objective of this analysis was to determine the optimal threshold value that maximizes both the sensitivity and specificity of the PHS Score in predicting mortality outcomes.

The ROC analysis yielded an area under the curve (AUC) of 0.716 (95% CI: 0.654 - 0.778) with a significant p-value of 0.000 (Fig. 1).

Fig. 1. Roc curve

The optimal cut-off point on the ROC curve



was determined to be 4.5. This cutoff point yielded a sensitivity of 74.6% and a specificity of 65.9%. In the final analysis, the probability of COVID-19 mortality was calculated based on the total score, as presented in Table 4. Patients with a total score value of 4 or higher are considered to have a higher mortality risk.

Table 4. Probability of COVID-19 mortality based on total score

Total score	Probability	Total score	Probability
0	0.000 (<1.4%)	3	0.067 (6,7%)
1	0.014 (1.4%)	4	0.141 (14,1%)
2	0.031 (3.1%)	5	0.272 (27,2%)

Discussion

The association between mortality and age in COVID-19 patients has been consistently reported in previous studies (Dadras *et al.,* 2022; Rachmawati *et al.,* 2022). This study also revealed a correlation between age and in-hospital mortality in COVID-19 patients, which aligns with previous research findings.

A meta-analysis of 70 studies indicated that the risk of in-hospital and case mortality increases by 5.7% and 7.4% per year of age, respectively, without a specific age threshold identified at which the risk escalates significantly (Starke *et al.,* 2021). Previous studies provided multiple explanations for the association between age and mortality in relation to COVID-19. The age-dependent expression of the Angiotensin-Converting 2 (ACE2) gene, which acts as the receptor for SARS-CoV-2 in humans, is a significant factor (Bunyavanich *et al.,* 2020; Biswas *et al.,* 2021; Rachmawati *et al.,* 2022)

The variation in ACE2 gene expression based on age may account for the lower incidence of COVID-19 in children relative to adults (Biswas *et al.,* 2021; Bunyavanich *et al.,* 2020). Together with the ACE2 gene expression, Glucose-regulated protein 78 (GRP78) serves as a binding partner that increases the entry COVID-19 virus into the host cells (Allam *et al.,* 2020; Carlos *et al.,* 2021; Han *et al.,* 2023). The elevated GRP78 in older patients is related to the poor outcome of COVID-19 infection (Shin *et al.,* 2021, 2022). In addition, aging individuals may exhibit decreased T-cell and B-cell activity, along with an overproduction of type 2 cytokines. These factors may lead to impaired viral replication control and prolonged proinflammatory responses (Zhou *et al.,* 2020; Rachmawati *et al.,* 2022).

Furthermore, elderly patients are more prone to developing critical pneumonia and acute respiratory distress syndrome (ARDS) due to factors such as lung muscle atrophy, reduced airway clearance, decreased lung reserve, and impaired defense barrier function. They may also experience complications such as secondary infections, gastrointestinal symptoms, septic shock, and multiple organ dysfunction syndrome, which are more common in this age group (Aslan *et al.,* 2021; Dadras *et al.,* 2022; Fan *et al.,* 2020).

Our study found no significant correlation between gender and mortality in patients with COVID-19. This finding aligns with a prior study that similarly found no gender-based disparities in COVID-19 patient mortality (Rai *et al.,* 2021; Raimondi *et al.,* 2021). It is noteworthy that various studies have consistently reported a higher mortality rate among male patients in comparison to female patients (Biswas *et al.,* 2021; Rachmawati *et al.,* 2022).

The absence of gender-based disparities in mortality may be influenced by multiple factors. Females typically exhibit a greater concentration of immune-related genes on the X chromosome, po-

tentially resulting in more robust innate and adaptive immune reactions relative to males (Griffith *et al.,* 2020; Rachmawati *et al.,* 2022). Sex hormones may influence immune responses. Male patients may exhibit elevated expression of ACE2, potentially rendering them more susceptible to SARS-CoV-2 infection and leading to unfavorable clinical outcomes (Biswas *et al.,* 2021; Raimondi *et al.,* 2021).

Differences in behavior between men and women may also impact COVID-19 infection rates. Women tend to be more compliant with preventive measures such as wearing face masks, practicing hand hygiene, maintaining social distancing, and seeking timely medical assistance (Galasso *et al.,* 2020; Griffith *et al.,* 2020). As a result, women may be less likely to spread the disease and become infected. However, once severe disease occurs, the mortality risk is similar between genders (Raimondi *et al.,* 2021; Doerre and Doblhammer, 2022).

Our study found that COVID-19 patients with comorbidities such as DM, HT and CKD had a higher mortality risk compared to those without any comorbidities. This finding is consistent with the previous study that reported a significant association between DM (OR = 2.27; 95% CI = 1.69 - 3.04; p = 0.0001), HT (OR = 2.74; 95% CI = 2.03 - 3.68; p = 0.0001), CKD (crude OR = 4.47; 95% CI = 2.54 - 7.88; p = 0.0001), and mortality (Rai *et al.,* 2021; Zhang *et al.,* 2023).

Hypertension is linked to endothelial dysfunction, which plays a significant role in the progression of COVID-19 infection (Puicón-Suárez *et al.,* 2022; Zhang *et al.,* 2023). Another study proposed that the upregulation of the renin-angiotensin system (RAS) and the frequent use of antihypertensive medications, specifically angiotensin receptor blockers (ARBs) and angiotensin-converting enzyme inhibitors (ACE inhibitors), might enhance the overexpression of ACE2. This, in turn, could facilitate the entry of SARS-CoV-2 (Biswas *et al.,* 2021; Bepouka *et al.,* 2022).

The poor outcomes observed in patients with diabetes mellitus may be due to impaired innate immunity or increased coagulation activity in these individuals (Rai *et al.,* 2021). Elevated GRP78 in diabetes also causes severe symptoms through cellular inflammatory responses and viral replication (Shin *et al.,* 2021, 2022). Patients with CKD have an impaired immune system, and those on maintenance hemodialysis may experience poorer outcomes due to multiple comorbidities, frailty, and aging (Biswas *et al.,* 2021; Singh *et al.,* 2022).

In this study initially, bivariate analysis using rank Spearman was conducted to assess the correlation between each laboratory parameter and patients' mortality. Subsequently, multivariate analysis using logistic regression was performed to determine the best predictor of mortality Goodness-of-fit tests were performed to assess the appropriateness of the logistic regression analysis, and all laboratory parameters satisfied the requisite criteria.

Our study confirmed a significant correlation between NLR, CRP, D-dimer, and IL-6 levels and in-hospital mortality in COVID-19 patients. This finding is consistent with previous studies that have reported elevated NLR, CRP, D-dimer, and IL-6 levels as indicators of higher mortality risk (Poudel *et al.,* 2021; Al-Shajlawi *et al.,* 2022; Rahayu *et al.,* 2022).

The NLR, which represents the interplay between innate and adaptive immune responses, has been identified as a diagnostic and prognostic indicator of disease severity and mortality in individuals with COVID-19 (Toori *et al.,* 2021; Regolo *et al.,* 2022). Neutrophil activation and degranulation are significantly increased during SARS infection, resulting in the release of multiple cytokines and effector molecules (Simadibrata *et al*., 2020; Cavalcante-Silva *et al*., 2021; Toori *et al*., 2021). In addition, COVID-19 induces systemic inflammation, which promotes the apoptosis of lymphocytes, impairs cellular immunity, decreases CD4+ cell numbers, and elevates CD8+ suppressor T-lymphocytes, resulting in further suppression of lymphocyte levels (Simadibrata *et al*., 2020; Toori *et al.*, 2021).

Neutrophil elevation is observed not only in the bloodstream but also in the lungs. Moreover, the presence of immature or dysfunctional mature neutrophils plays a role in an imbalanced immune response observed in severe cases of COVID-19. SARS-CoV-2 infection induces the release of neutrophil extracellular traps (NETs), which degrade cytokines and chemokines, leading to a reduction in inflammation. Additionally, they can cause lung injury and microvascular thrombosis, resulting in tissue and endothelial damage (Cavalcante-Silva *et al.,* 2021; Samprathi and Jayashree, 2021; Zhang *et al.,* 2023).

The absence of a universally established threshold for NLR makes it challenging to differentiate between normal and elevated levels, especially in the context of COVID-19 infection. Prior research has documented varying NLR values in different populations, suggesting that the ideal

threshold values may differ among these populations (Simadibrata *et al*., 2020; Sarkar *et al*., 2022).

CRP is an acknowledged prognostic inflammatory biomarker that plays a critical role in pathogen resistance and immune (Sadeghi-Haddad-Zavareh *et al.,* 2021; Smilowitz *et al.,* 2021; Regolo *et al.,* 2022). During COVID-19 infection, cytokine storms, specifically IL-6 and TNF-α, induce hepatocytes to synthesize CRP (Luan *et al.,* 2021; Samprathi and Jayashree, 2021; Montazersaheb *et al.,* 2022). CRP not only enhances phagocytosis and aids in pathogen clearance but also activates the classical pathway of the complement system (Luan *et al.,* 2021; Smilowitz *et al.,* 2021; Montazersaheb *et al.,* 2022).

Elevated levels of CRP in the blood have consistently been linked to the occurrence of cardiovascular events, ARDS, and mortality in individuals infected with the SARS-CoV-2 virus (Luan *et al.,* 2021; Regolo *et al.,* 2022; Li *et al.,* 2023). CRP concentrations in COVID-19 are indicative of disease severity and the extent of acute inflammatory response. Elevated levels of CRP may independently contribute to multi-organ damage in individuals with COVID-19 (Luan *et al.,* 2021; Montazersaheb *et al.,* 2022; Smilowitz *et al.,* 2021).

D-dimer, a product of fibrin degradation, typically increases in older individuals and during pregnancy. It is commonly used for diagnosing and managing thrombotic disorders (Nemec *et al.,* 2021; Poudel *et al.,* 2021). These activities exacerbate atherosclerosis, plaque rupture, local inflammation, and the production of procoagulant factors, thereby heightening the likelihood of ischemia and thrombosis (Rai *et al.,* 2021; Montazersaheb *et al.,* 2022). Severe COVID-19 cases with hypoxia also activate the hypoxia-inducible transcription factor-dependent signaling pathway, further predisposing individuals to thrombosis (Poudel *et al.,* 2021; Montazersaheb *et al.,* 2022).

SARS-CoV-2 infection elicits innate and adaptive immune responses, resulting in the release of IL-6 and other cytokines, heightened vascular permeability, and respiratory failure (Santa Cruz *et al.,* 2021; Montazersaheb *et al.,* 2022). IL-6 production is stimulated by proinflammatory cytokines, particularly IL-1β and TNFα (Sabaka et al., 2021; Montazersaheb et al., 2022). Overexpression of IL-6 is crucial in initiating and perpetuating the "cytokine storm," which involves the release of IL-1, IL-6, IL-8, TNFa, and other inflammatory mediators (Sabaka *et al.,* 2021; Santa Cruz *et al.,* 2021). Increased levels of IL6 contribute to the permeability of lung capillaries, resulting in the development of ARDS. Additionally, elevated IL-6 levels activate the coagulation pathway, which raises the likelihood of microthrombi formation in the lung circulation and the occurrence of thrombotic events (Sabaka et al., 2021; Montazersaheb *et al.*, 2022).

In our study, all laboratory parameters including NLR, CRP, D-dimer, and IL-6 exhibited both clinical and statistical significance in mortality. To facilitate the prediction of mortality risk, these parameters were integrated into the PHS Score model. The ROC analysis of the PHS Score revealed a significant AUC of 71.6%. The analysis identified 4.5 as the optimal cutoff value for the score, which demonstrated a sensitivity of 74.6% and a specificity of 65.9%. The sensitivity of 74.6% is notably efficient, reflecting the PHS Score's capacity to accurately identify patients at a higher risk of mortality from COVID-19. On the other hand, the specificity of 65.9%, while moderate, highlights a need for careful consideration, especially in the context of comorbidities that might affect the specificity of the score.

It is noteworthy that patients with a PHS Score of 4 or higher are identified as having a heightened risk of mortality. Thus, the PHS Score can serve as a valuable tool in predicting the mortality risk upon hospital admission, thereby aiding healthcare professionals in effectively managing COVID-19 patients. By incorporating this score into their decision-making process, healthcare providers can prioritize resources and interventions for individuals who are most susceptible to adverse outcomes.

Acknowledging the limitations of this study is crucial. Laboratory parameters were assessed solely upon admission, making it difficult to ascertain whether the observed elevations were solely attributable to the progression of COVID-19 infection or influenced by comorbid conditions. Additionally, there may be other markers involved in cytokine storms that could affect CRP and IL-6 levels, which were not explored in this study. Further research is needed to explore these aspects and enhance our understanding of the prognostic value of the PHS Score in COVID-19 patients.

Conclusion

This study provides novel findings on the correlation between age, hypertension, diabetes mellitus, chronic kidney disease, NLR, CRP, D-dimer, IL-6, and mortality in COVID-19 patients. Notably, this is the first study conducted in Surabaya Hospital, Indonesia, to develop an equation using these laboratory parameters. The analysis revealed

that a PHS score of ≥ 4 is associated with a higher mortality rate. This PHS score holds potential for predicting mortality risk during the initial examination upon hospital admission, thereby facilitating improved management of COVID-19 patients.

Acknowledgment

We would like to express our gratitude to the Health Research Ethics Committee of Universitas Airlangga School of Medicine and the Ethics Committee of Premier Surabaya Hospital for granting permission to conduct this study. Their support and approval were crucial in ensuring the ethical conduct of the research. We would also like to extend our appreciation to Premier Surabaya Hospital for granting us permission to access and collect patient data.

References

Al-Shajlawi, M., A. R. Alsayed, H. Abazid, D. Awajan, A. Al-Imam, I. Basheti (2022). Using laboratory parameters as predictors for the severity and mortality of COVID-19 in hospitalized patients. *Pharm. Pract. (Granada)* **20**: 1–9. https://doi.org/10.18549/PharmPract.2022.3.2721.

Aljohani, F. D., A. Khattab, H. M. Elbadawy, A. Alhaddad, Z. Alahmadey, Y. Alahmadi, H. M. Eltahir, H. M. H. Matar, H. Wanas (2022). Prognostic factors for predicting severity and mortality in hospitalized COVID-19 patients. *J. Clin. Lab. Anal.* **36**: 1–7. https://doi.org/10.1002/jcla.24216.

Allam, L., F. Ghrifi, H. Mohammed, N. El Hafidi, R. El Jaoudi, J. El Harti, B. Lmimouni, L. Belyamani, A. Ibrahimi (2020). Targeting the GRP78-dependant SARS-CoV-2 cell entry by peptides and small molecules. *Bioinform. Biol. Insights* **14**: 117793222096550. https://doi.org/10.1177/1177932220965505.

Aslan, A., C. Aslan, N. M. Zolbanin, R. Jafari (2021). Acute respiratory distress syndrome in COVID-19: possible mechanisms and therapeutic management. *Pneumonia* **13**. https://doi.org/10.1186/s41479-021-00092-9.

Bepouka, B., H. Situakibanza, M. Sangare, M. Mandina, N. Mayasi, M. Longokolo, O. Odio, D. Mangala, F. Isekusu, J. M. Kayembe, J. Nachega, M. Mbula (2022). Mortality associated with COVID-19 and hypertension in sub-saharan africa. A systematic review and meta-analysis. *J. Clin. Hypertens* **24**: 99–105. https://doi.org/10.1111/jch.14417.

Biswas, M., S. Rahaman, T. K. Biswas, Z. Haque, B. Ibrahim (2021). Association of sex, age, and comorbidities with mortality in COVID-19 patients: a systematic review and meta-analysis. *Intervirology* **64**: 36–47. https://doi.org/10.1159/000512592.

Bunyavanich, S., A. Do, A. Vicencio (2020). Nasal gene expression of angiotensin-converting enzyme 2 in children and adults. *J. Am. Med. Assoc.* **323**: 2427–2429. https://doi.org/10.1001/jama.2020.8707.

Carlos, A. J., D. P. Ha, D-W. Yeh, R. Van Krieken, C-C. Tseng, P. Zhang, P. Gill, K. Machida, A. S. Lee (2021). GRP78 binds SARS-CoV-2 spike protein and ACE2 and GRP78 depleting antibody blocks viral entry and infection in vitro. *J. Biol. Chem.* **296**: 100759. https://doi.org/10.1016/j.jbc.2021.100759.

Cavalcante-Silva, L. H. A., D. C. M. Carvalho, É. de A. Lima, J. G. F. M. Galvão, J. S. de F. da Silva, J. M. de Sales-Neto, S. Rodrigues-Mascarenhas (2021). Neutrophils and COVID-19: the road so far. *Int. Immunopharmacol.* **90**: 107233. https://doi.org/10.1016/j.intimp.2020.107233.

Dadras, O., S. Seyed Alinaghi, A. Karimi, A. Shamsabadi, K. Qaderi, M. Ramezani, S. P. Mirghaderi, S. Mahdiabadi, F. Vahedi, S. Saeidi, A. Shojaei, M. Mehrtak, S. A. Azar, E. Mehraeen, F. A. Voltarelli (2022). Retracted: COVID-19 mortality and its predictors in the elderly: a systematic review. *Heal. Sci. Reports* **5**: e657. https://doi.org/10.1002/hsr2.657.

Das, B., S. Y. Bhatia, P. M. Pal (2021). Evaluation of the role of routine laboratory biomarkers in COVID-19 patients: perspective from a tertiary care hospital in India. *Indian J. Clin. Biochem.* **36**: 473–484. https://doi.org/10.1007/s12291-021-00978-x.

Doerre, A., G. Doblhammer (2022). The influence of gender on COVID-19 infections and mortality in Germany: insights from age- and gender-specific modeling of contact rates, infections, and deaths in the early phase of the pandemic. *PLoS ONE* **17**: 1–20. https://doi.org/10.1371/journal.pone.0268119.

Dong E., H. Du, L. Gardner (2020). An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect. Dis.* **20**: 533–534. https://doi.org/10.1016/S1473-3099(20)30120-1.

Fan, E., J. R. Beitler, L. Brochard, C. S. Calfee, N. D. Ferguson, A. S. Slutsky, D. Brodie (2020). COVID-19-associated acute respiratory distress syndrome: is a different approach to management warranted? *Lancet Respir. Med.* **8**: 816–821. https://doi.org/10.1016/S2213-2600(20)30304-0.

Galasso, V., V. Pons, P. Profeta, M. Becher, S. Brouard, M. Foucault (2020). Gender differences in COVID-19 attitudes and behavior: panel evidence from eight countries. *Proc. Natl. Acad. Sci. USA* **117**: 27285–27291. https://doi.org/10.1073/pnas.2012520117.

García-Gasalla, M., J. M. Ferrer, P. A. Fraile-Ribot, A. Ferre-Beltrán, A. Rodríguez, N. Martínez-Pomar, L. Ramon-Clar, A. Iglesias, I. Losada-López, F. Fanjul, J. A. Pou, I. Llompart-Alabern, N. Toledo, J. Pons, A. Oliver, M. Riera, J. Murillas (2021). Predictive immunological, virological, and routine laboratory markers for critical COVID-19 on admission. *Can. J. Infect. Dis. Med. Microbiol* **2021**: 1–8. https://doi.org/10.1155/2021/9965850.

Griffith, D. M., G. Sharma, C. S. Holliday, O. K. Enyia, M. Valliere, A. R. Semlow, E. C. Stewart, R. S. Blumenthal (2020). Men and COVID-19: a biopsychosocial approach to understanding sex differences in mortality and recommendations for practice and policy interventions. *Prev. Chronic. Dis.* **17**: 1–9. https://doi.org/10.5888/PCD17.200247.

Han, B., Lv, Y., D. Moser, X. Zhou, T. Woehrle, L. Han, A. Osterman, M. Rudelius, A. Choukér, P. Lei (2023). ACE2-independent SARS-CoV-2 virus entry through cell surface GRP78 on monocytes – evidence from a translational clinical and experimental approach. *EBioMedicine* **98**: 104869. https://doi.org/10.1016/j.ebiom.2023.104869.

Israfil, S. M. H., M. M. R. Sarker, P. T. Rashid, A. A. Talukder, K. A. Kawsar, F. Khan, S. Akhter, C. L. Poh, I. N. Mohamed, L. C. Ming (2021). Clinical characteristics and

diagnostic challenges of COVID−19: an update from the global perspective. *Front. Public. Health* **8**: 1–17. https://doi.org/10.3389/fpubh.2020.567395.

Li, F., M. He, M. Zhou, Y. Lai, Y. Zhu, Z. Liu, Y. Wang, Y. Wang (2023). Association of C-reactive protein with mortality in COVID−19 patients: a secondary analysis of a cohort study. *Sci. Rep.* **13**: 1–10. https://doi.org/10.1038/s41598-023-47680-x.

Liu, X., H. Wang, S. Shi, J. Xiao (2021). Association between IL-6 and severe disease and mortality in COVID-19 disease: a systematic review and meta-analysis. *Postgrad. Med. J.* **98**: 871–879. https://doi.org/10.1136/postgradmedj-2021-139939.

Luan, Y. Y., C. H. Yin, Y. M. Yao (2021). Update advances on C-reactive protein in COVID-19 and other viral infections. *Front. Immunol.* **12**: 1–10. https://doi.org/10.3389/fimmu.2021.720363.

Montazersaheb, S., S. M. Hosseiniyan Khatibi, M. S. Hejazi, V. Tarhriz, A. Farjami, F. Ghasemian Sorbeni, R. Farahzadi, T. Ghasemnejad (2022). COVID-19 infection: an overview on cytokine storm and related interventions. *Virol. J.* **19**: 1–15. https://doi.org/10.1186/s12985-022-01814-1.

Nemec, A., L. Radolfová-Křížová, M. Maixnerová, M, Nemec, P. Španělová, R. Šafránková, O. Šedo, B. S. Lopes, P. G. Higgins (2021). Delineation of a novel environmental phylogroup of the genus acinetobacter encompassing acinetobacter terrae sp. nov., acinetobacter terrestris sp. nov. and three other tentative species. *Syst. Appl. Microbiol.* **44**: 126217. https://doi.org/10.1016/j.syapm.2021.126217.

Peta Sebaran tanggal 13 Juli 2023. https://covid19.go.id/id/peta-sebaran

Ponsford, M. J., R. J. Burton, L. Smith, P. Y. Khan, R. Andrews, S. Cuff, L. Tan, M. Eberl, I. R. Humphreys, F. Babolhavaeji, A. Artemiou, M. Pandey, S. R. A. Jolles, J. Underwood (2022). Examining the utility of extended laboratory panel testing in the emergency department for risk stratification of patients with COVID-19: a single-centre retrospective service evaluation. *J. Clin. Pathol* **75**: 255–262. https://doi.org/10.1136/jclinpath-2020-207157.

Poudel, A., Y. Poudel, A. Adhikari, B. B. Aryal, D. Dangol, T. Bajracharya, A. Maharjan, R. Gautam (2021). D-dimer as a biomarker for assessment of COVID-19 prognosis: D-dimer levels on admission and its role in predicting disease outcome in hospitalized patients with COVID-19. *PLoS One* **16**: 1–13. https://doi.org/10.1371/journal.pone.0256744.

Puicón-Suárez, J. B., S. Zeña-Ñañez, V. E. Failoc-Rojas (2022). Association between chronic kidney disease and mortality in patients with a confirmed COVID-19 diagnosis. *PeerJ.* **10**: e13437. https://doi.org/10.7717/peerj.13437.

Rachmawati, M. R., Y. Amiarno, Y. Restuti, W. Guslianti, L. Adriyanti, N. Fajriah (2022). Age and gender as the risk factors for mortality rate in COVID-19 patients. *Bali Med. J.* **11**: 2017–2021. https://doi.org/10.15562/bmj.v11i3.3446.

Rahayu, R., W. Winarto, T. Nasihun (2022). Interleukin-6 and C-reactive protein on admission as predictor of mortality in severe COVID-19 patients: A retrospective cohort study. *Open Access Maced. J. Med. Sci.* **10**: 227–231. https://doi.org/10.3889/oamjms.2022.7968.

Rai, D., A. Ranjan, A. H, S. Pandey (2021). Clinical and laboratory predictors of mortality in COVID-19 infection: a retrospective observational study in a tertiary care hospital of eastern india. *Cureus* **13**: 1–13. https://doi.org/10.7759/cureus.17660.

Raimondi, F., L. Novelli, A. Ghirardi, F. A. Russo, D. Pellegrini, R. Biza, R. Trapasso, L. Giuliani, M. Anelli, M. Amoroso, C. Allegri, G. Imeri, C. Sanfilippo, S. Comandini, E. Hila, L. Manesso, L. Gandini, P. Mandelli, M. Monti, M. Gori, M. Senni, F. L. Lorini, M. Rizzi, T. Barbui, L. Paris, A. Rambaldi, R. Cosentini, G. Guagliumi, S. Cesa, M. Colledan, M. Sessa, A. Masciulli, A. Gavazzi, S. Buoro, G. Remuzzi, P. Ruggenenti, A. Callegaro, A. Gianatti, C. Farina, A. Bellasi, S. Sironi, S. Fagiuoli , F. Di Marco (2021). Covid-19 and gender: lower rate but same mortality of severe disease in women - an observational study. *BMC. Pulm. Med.* **21**: 1–11. https://doi.org/10.1186/s12890-021-01455-0.

Regolo, M., M. Vaccaro, A. Sorce, B. Stancanelli, M. Colaci, G. Natoli, M. Russo, I. Alessandria, M. Motta, N. Santangelo, L. Fiorito, O. Giarrusso, F. Giangreco, A. Arena, P. Noto, C. Ciampi, G. Carpinteri, L. Malatino (2022). Neutrophil-to-lymphocyte ratio (NLR) is a promising predictor of mortality and admission to intensive care unit of COVID-19 patients. *J. Clin. Med.* **11**: 2235. https://doi.org/10.3390/jcm11082235.

Sabaka, P., A. Koščálová, I. Straka, J. Hodosy, R. Lipták, B. Kmotorková, M. Kachlíková, A. Kušnírová (2021). Role of interleukin 6 as a predictive factor for a severe course of COVID-19: retrospective data analysis of patients from a long-term care facility during COVID-19 outbreak. *BMC. Infect. Dis.* **21**: 4–11. https://doi.org/10.1186/s12879-021-05945-8.

Sadeghi-Haddad-Zavareh, M., M. Bayani, M, Shokri, S. Ebrahimpour, A. Babazadeh, R. Mehraeen, E. Moudi, A. Rostami, M. Barary, A. Hosseini, A, Bijani, M. Javanian (2021). C-Reactive protein as a prognostic indicator in COVID-19 patients. *Interdiscip. Perspect. Infect. Dis.* **2021**: 1–5. https://doi.org/10.1155/2021/5557582.

Samprathi, M., M. Jayashree (2021). Biomarkers in COVID-19: an up-to-date review. *Front. Pediatr.* **8**: 1–12. https://doi.org/10.3389/fped.2020.607647.

Santa Cruz, A., A. Mendes-Frias, A. I. Oliveira, D. Dias, A. R. Matos, A. Carvalho, C. Capela, J. Pedrosa, A. G. Castro, R. Silvestre (2021). Interleukin-6 is a biomarker for the development of fatal severe acute respiratory syndrome coronavirus 2 pneumonia. *Front. Immunol.* **12**: 1–10. https://doi.org/10.3389/fimmu.2021.613422.

Sarkar, S., P. Khanna, A. K. Singh (2022). The impact of neutrophil-lymphocyte count ratio in COVID-19: a systematic review and meta-analysis. *J. Intensive Care Med.* **37**: 857–869. https://doi.org/10.1177/08850666211045626.

Shin, J., S. Toyoda, A. Fukuhara, I. Shimomura (2022). GRP78, a novel host factor for SARS-CoV-2: the emerging roles in COVID-19 related to metabolic risk factors. *Biomedicines* **10**: 1995. https://doi.org/10.3390/biomedicines10081995.

Shin, J., S. Toyoda, S. Nishitani, A. Fukuhara, S. Kita, M. Otsuki, I. Shimomura (2021). Possible involvement of adipose tissue in patients with older age, obesity, and diabe-

tes with SARS-CoV-2 infection (COVID-19) via GRP78 (BIP/HSPA5): significance of hyperinsulinemia management in COVID-19. *Diabetes* **70**: 2745–2755. https://doi.org/10.2337/db20-1094.

Simadibrata, D. M., J. Calvin, A. D. Wijaya, N. A. A. Ibrahim (2021). Neutrophil-to-lymphocyte ratio on admission to predict the severity and mortality of COVID-19 patients: a meta-analysis. *Am. J. Emerg. Med.* **42**: 60–69. https://doi.org/10.1016/j.ajem.2021.01.006.

Singh, R., S. Krishnasamy, J. K. Meena, P. Sirohiya, B. Kumar, B. K. Ratre, S. Vig, A. Pandit, H. K. B. Sagiraju, R. Gupta, S. Bhatnagar (2022). Predictors of in-hospital mortality among COVID-19-infected chronic kidney disease patients on maintenance hemodialysis: a retrospective cohort study. *Int. J. Infect.* **9**: e126323. https://doi.org/10.5812/iji-126323.

Smilowitz, N. R., D. Kunichoff, M. Garshick, B. Shah, M. Pillinger, J. S. Hochman, J. S. Berger (2021). C-reactive protein and clinical outcomes in patients with COVID-19. *Eur. Heart. J.* **42**: 2280–2283. https://doi.org/10.1093/eurheartj/ehab169.

Starke, K. R., D. Reissig, G. Petereit-Haack, S. Schmauder, A. Nienhaus, A. Seidler (2021). The isolated effect of age on the risk of COVID-19 severe outcomes: a systematic review with meta-analysis. *BMJ. Glob. Health* **6**: 1–12. https://doi.org/10.1136/bmjgh-2021-006434.

Talwar, D., S. Kumar, S. Acharya, N. Raisinghani, S. Madaan, V. Hulkoti, A. Akhilesh, S. Khanna, D. Shah, S. Nimkar (2022). Interleukin 6 and its correlation with COVID-19 in terms of outcomes in an intensive care unit of a rural hospital: a cross-sectional study. *Indian. J. Crit. Care Med.* **26**: 39–42. https://doi.org/10.5005/jp-journals-10071-24075.

Toori, K. U., M. A. Qureshi, A. Chaudhry, M. F. Safdar (2021). Neutrophil to lymphocyte ratio (NLR) in COVID-19: a cheap prognostic marker in a resource constraint setting. *Pakistan. J. Med. Sci.* **37**: 1435–1439. https://doi.org/10.12669/pjms.37.5.4194.

WHO Coronavirus (COVID-19) Dashboard With Vaccination Data tanggal 13 Juli 2023. https://covid19.who.int/

Zhang, J., X. Dong, G. Liu, Y. Gao (2023). Risk and protective factors for COVID-19 morbidity, severity, and mortality. *Clin. Rev. Allergy Immunol.* **64**: 90–107. https://doi.org/10.1007/s12016-022-08921-5.

Zhou, F., T. Yu, R. Du, G. Fan, Y. Liu, Z. Liu, J. Xiang, Y. Wang, B. Song, X. Gu, L. Guan, Y. Wei, H. Li, X. Wu, J. Xu, S. Tu, Y. Zhang, H. Chen, B. Cao (2020). Clinical course and risk factors for mortality of adult inpatients with COVID-19 in wuhan, china: a retrospective cohort study. *Lancet* **395**: 1054–1062. https://doi.org/10.1016/S0140-6736(20)30566-3.